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Algal Farming Systems

From Production to Application for a Sustainable Future




Editors
Jeyabalan Sangeetha
Devarajan Thangadurai

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Edited by
Jeyabalan Sangeetha, PhD
Devarajan Thangadurai, PhD

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Nature is that entity composed of three main internally interactive components: biodiversity–human–environment. These are triangularly related aspects of life science, and the interdependency of these three reflects the crucial need of maintenance of equilibrium in their interaction. Both human and biodiversity are two interactive phases of environment that provide a necessary platform for interaction. Being part of biodiversity, human life is almost dependent on biodiversity and its products. This is the point where the human–biodiversity interaction fluctuates due to overexploitation of biodiversity, where humans take more than the basic needs of human life. Over the past few centuries, this fluctuation in interaction has caused dramatic depletion of biodiversity and thus a drastic change in environment. This has now boomeranged on human life significantly. This variation in the interaction triangle has already travelled the long path of time. Until now, conservationists and life scientists are thinking of restoring the equilibrium in the interaction triangle by taking innovative steps in conserving biodiversity and protecting the environment.

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Gathering and monitoring information about biodiversity, conservation, and environment is a crucial need of biologists. Surveys from biological survey agencies, establishment of bio-inventories, modern tools and technologies to monitor the biodiversity and collection of data, and strengthening the scientific networks to make awareness, to generate the data, and for the accumulation of both traditional and scientific knowledge about biodiversity conservation and environmental management have been achieved in the recent decades. Conservationists need technological innovations to resolve the threats to biodiversity and environment that are now within reach.

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ABBREVIATIONS

ACE	angiotensin-converting enzyme
AFA	*Aphanizomenon flos-aquae*
AIV	avian influenza virus
ALA	α-linolenic acid
ALV-J	avian leukosis virus J
AML	acute myeloid leukemia
Ap	*Arthrospira platensis*
ARA	arachidonic acid
BFGF	basic fibroblast growth factor
BHA	butylated hydroxyanisole
BHT	butylated hydroxytoluene
BIM	building information modeling
BOD	biological oxygen demand
CBL	*Cystoseira barbata* laminaran
CEH-MS	coupled enzymatic hydrolysis and membrane separation
CGF	chlorella growth factor
CMC	carboxymethyl cellulose/Critical micellar concentration
CNT	carbon nanotube
CO_2	carbon dioxide
COD	chemical oxygen demand
COPD	chronic obstructive pulmonary disease
COX-2	cyclooxygenase-2
CRGs	carrageenans
CSIR	Center of Scientific and Industrial Research
CVDs	cardiovascular diseases
DCLU	direct changes in land use
DENV	dengue virus
DHA	docosahexaenoic acid
DIT	di-iodothyronine
DM	diabetes mellitus
DMAPP	dimethyl allyl pyrophosphate
DSS	dextran sulfate sodium
DW	dry weight
EAA	essential amino acids

EAE	enzyme assisted extraction
EFA	essential fatty acids
EGFR	epidermal growth factor receptor
EPA	eicosapentaenoic acid
EPS	exopolysaccharides
ERK	extracellular signal-regulated kinase
EU	European Union
FACS	fluorescence-activated cell sorting
FAO	Food and Agriculture Organization
FAS	fatty acid synthase gene
FC	free cholesterol
FL	Fogh and Lund
FP	flat panel
FSANZ	Food Standards Australia New Zealand
FSV	feline sarcoma virus
GAG	glycosaminoglycan
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GGPP	geranylgeranyl pyrophosphate
GIP	glucose-independent-insulinotropic polypeptide
GIS	geographic information system
GLP-1	glucagon-like-peptide-1
GLUT	glucose transporter
GMP	good manufacturing practices
GR	glutathione oxidoreductase
GSH	glutathione
HACCP	hazard analysis and critical control points
HBV	hepatitis B virus
HCMV	human cytomegalovirus
HDL	high-density lipoprotein
HIV	human immunodeficiency virus
HME	high moisture extrusion
HPV	human papillomavirus
HSV	herpes simplex virus
ICAM	intercellular adhesion molecule
IHD	ischemic heart disease
IPP	isopentenyl pyrophosphate
JEV	Japanese encephalitis virus
LA	linoleic acid
LDLC	low-density lipopolysaccharide cholesterol

LEDs	light-emitting diodes
LK	leukotrienes
LMW	low molecular weight
LNCaP	lymph node carcinoma of the prostate cell line
LPS	lipopolysaccharide
MAAs	mycosporine-like amino acids
MAE	microwave-assisted extraction
MAPK	mitogen-activated protein kinase
MEF	mouse embryonic fibroblasts
MFE	membrane filtration extraction
MIPP	microalgae industrial production plant
MMP-1	matrix metalloproteases-1
MPPP	microalgae pilot production plant
MUFAs	monounsaturated fatty acids
NAXA	Natural Algae Astaxanthin Association
NDV	newcastle disease virus
NEAA	non-essential amino acids
NK	natural killer
NO	nitric oxide
NP	nanoparticles
NPK	nitrogen, phosphorus, and potassium
NRPS	non-ribosomal peptide synthase
NSAIDs	nonsteroidal anti-inflammatory drugs
NTA	nitrilotriacetic acid
O/W	oil in water
ORAC	oxygen radical absorbance capacity
OSE	organic solvent derived extraction
PARP	poly (ADP-ribose) polymerase
PBRs	photobioreactors
PC	polycarbonate
PCL	poly ε-caprolactone
PEF	pulsed electric field
PEG	polyethylene glycol
PG	prostaglandins
PGE2	prostaglandin E2
PLE	pressurized assisted extraction
PMBC	peripheral blood mononuclear cells
PSS	propylene glycol alginate sodium sulfate
PTLC	preparative thin layer chromatography

PUAs	polyunsaturated aldehydes
PUFA	polyunsaturated fatty acids
R&D	research and development
RAAS	renin-angiotensin-aldosterone-system
RDI	reference daily intake
ROS	reactive oxygen species
RP-HPLC	reversed-phase high performance liquid chromatography
RSV	respiratory syncytial virus
SA	spirulina algae
SCFAs	short-chain fatty acids
SFAs	saturated fatty acids
SFE	supercritical fluid extraction
SHRs	spontaneously hypersensitive rats
SOD	superoxide dismutase
SPs	sulfated polysaccharides
STDs	sexually transmitted diseases
SWE	subcritical water extraction
T3	triiodothyronine
T4	thyroxin
TBHQ	tert-butyl hydroquinone
TC	total cholesterol
TCGA	the cancer genome atlas
TE	trolox equivalent
TGs	triglycerides
TNF-α	tumor necrosis factor-alpha
TNF-β	tumor necrosis factor-beta
TSC	total serum cholesterol
TTB	tuberatolide B
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labeling
TX	thromboxanes
UAE	ultrasound-assisted extraction
UNU	United Nations University
UV	ultraviolet
VEGF	vascular endothelial growth factor
VLDL	very-low-density lipoprotein
WHO	World Health Organization
WNV	West Nile virus
YFV	yellow fever virus

PREFACE

The potential renewable and sustainable energy resources are generally considered by three major features: cost, safety, and environmental impact. A country's economic growth and development depend increasingly on the sustainable utilization of reliable energy resources. Even though the potential of algal biomass has been recognized, still significant in-depth understanding is still needed in the field of algal production for various applications.

Mass production of microalgal biomass was primarily aimed at the extraction of nutritious food supplements and nutraceuticals. Marine algae have the potential ability to concurrently fuel vehicles and recycle carbon dioxide. Microalgal farming on a large scale is very limited in most developing countries due to several technological barriers and regulatory measures. A comprehensive mapping, characterization, up-scaling of the production volumes, and marketing strategies are the major aspects to boost and support the growth of algal farming. In the near future, World's blue bioeconomy will depend on the algal industry.

The farming of algal biomass on a large scale for various purposes is converting the production to a commercial scale. During this crucial stage, institutional framework supports and commercialization could kindle the development of the algal industry as a promising source of renewable fuels, high-value protein, and low-cost drugs. The assistance programs supported by local governments and international bodies need to focus more on algae production and commercialization. Such initiatives and support are imperative for emerging algal-based industries to encourage investments, build basic and advanced infrastructure, share technical experience, and create markets.

Algal Farming Systems: From Production to Application for a Sustainable Future consolidates the latest research in the field together with market potential and policy considerations. This book is dedicated to enthusiastic researchers, academicians, entrepreneurs, policymakers, and anyone interested in the status and future possibilities of algae commercialization. The book consists of four parts. Part I includes a chapter on phycotechnology and highlights the current trend and future scope of algal technology. Part II consists of three chapters and provides comprehensive information on algal culture conditions and cultivation strategies. Algal production, marketing strategies, and their commercialization are discussed in Part III. In Part IV, five chapters are extensively devoted to industrial applications of algae and focus mainly on nutraceutical, pharmaceutical, and cosmeceutical applications of microalgae and macroalgae. This section also highlights the green synthesis of nanoparticles from algae for various commercial applications.

The contributing authors have comprehensively illustrated the concepts. All the chapters in the present book were prepared and authenticated by eminent researchers and entrepreneurs of algal farming. We greatly appreciate all the people who have shared their valuable suggestions and guidance to complete this book.

—Editors

PART I

PHYCOTECHNOLOGY

CHAPTER 1

ALGAL TECHNOLOGY: CURRENT TREND AND FUTURE SCOPE

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ABSTRACT

Nature's produce has been the lone most productive source in the development of new pharmaceuticals and nutraceuticals. Interest for natural products has revived with great consideration in the last few decades. Their efficacy with the least toxicity to humans has increased their affinity in the research community. One such variant is algae which are known to produce a wide range of secondary metabolites of human interest. A few examples of secondary metabolites produced by algae include peptides, phenolic compounds, carotenoids, polysaccharides, unsaturated fatty acids, phycobiliprotein pigments etc. These compounds are demonstrated to possess biological activities like anticancer, antioxidant, antimicrobial against bacteria and virus. Moreover, many algal species are equipped with the potential to produce an enormous amount of target molecule viz. proteins, lipids, carbohydrates, pigments of commercial scale. Therefore, hyphenated extraction processes such as solvent derived extraction, enzyme-assisted extraction for secondary metabolites is under great consideration. Given the side effects and growing resistance towards the existing drugs, the potential of different algal species in nutraceuticals and pharmaceuticals are the need of the hour.

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Jeyabalan Sangeetha, Devarajan Thangadurai (Eds.)

1.1 INTRODUCTION

Nature's produce has been the lone most productive source in the development of new pharmaceuticals and nutraceuticals. It harbors the majority of the active ingredients used in these industries. This is the sole approach for drug discovery in bygones age before the advent of the proficient screening processes. Interestingly, natural products or their derivatives constitute more than 80% of drug compounds (Sneader, 1997). Arguably, this is still the fact that almost 50% of the drugs approved since 1994 are nature based (Newman and Cragg, 2007). Butler (2008) has reported 13 natural product based drugs that were approved between 2005 and 2007. Out of them, 5 constituted the first candidate for the new classes of drugs. Interest for natural products has revived with great consideration in the last few decades. Their efficacy with the least toxicity to humans has increased their affinity in the research community. One such variant is algae, the photosynthetic organisms adapted to survive in extreme and complex environments. Algae coincide with plants in the production of a wide range of secondary metabolites (Cardozo et al., 2007). The substances do not participate directly in the growth and development processes of the host organism. Interestingly, they possess a great array of activities because of that it proved to be a boon to mankind. The production of these metabolites is limited either towards the end of the growth phase or under stressed environmental conditions which leads to metabolic alteration. A few examples of secondary metabolites produced by algae include peptides, phenolic compounds, carotenoids, polysaccharides, unsaturated fatty acids, phycobiliprotein pigments etc., (Shalaby, 2011). Vis-à-vis, all these compounds are demonstrated to own biological activities like anticancer, antioxidant, antimicrobial against bacteria, virus (El-Baroty et al., 2007). Moreover, many algal species are equipped with the potential to produce an enormous amount of target molecule viz. proteins, lipids, carbohydrates, pigments of commercial scale (Priyadarshani and Rath, 2012). The key focus of this chapter is to apprehend the algal farming and extraction processes such as solvent derived extraction, enzyme-assisted extraction with special emphasis on secondary metabolites holding bioactivities. Given the side effects and growing resistance towards the existing drugs, the potential of different algal species in nutraceuticals and pharmaceuticals that includes antioxidant, anticancerous, antihypertensive are among the major highlights.

1.1.1 BASICS

Algae are plants like organisms inhabiting rivers, seas, ponds, and lakes. They can be broadly categorized into two types based on their size – macroalgae and microalgae. Macroalgae, very commonly known as seaweed, is a multicellular, marine organism that can be seen with naked eyes. They include brown, red, and green algae. Microalgae, the microscopic counterpart of macroalgae, are largely comprised of diatoms, dinoflagellates, and blue green algae or cyanobacteria. Additionally, based on their pigments, algae are classified into seven groups inhabiting either freshwater or marine ecosystems. They are euglenoids, golden-brown algae, fire algae, green algae, red algae, brown algae, and yellow-green algae. Being the primary producer of the ecosystems, organisms at different strata are highly dependent on algae (Hanson et al., 2010). This indicates that the energy requirements of the organisms of the food web are fulfilled by algae. Fascinatingly, the marine habitat which rarely disturbed and majorly remains unexplored is believed to be a reservoir of bioactive compounds. Hence, it is reasonable to contemplate that algae bear a wide range of secondary metabolites of great importance.

1.1.2 BACKGROUND

Post 1970, three areas of aquatic natural products have emerged namely chemical and physical ecology, toxins, and bio-products. So far more than 2100 compounds are identified. Considering the by-products it produces, pharmaceutical research has given major emphasis to the algal research. To survive the extreme environment, both marine and freshwater algae produce a plethora of compounds of structural and chemical diversity to strengthen their defense mechanism. The exploration of these compounds has revealed numerous prototypes for the discovery of novel components that jolted industries to use the best extraction techniques. Hence, there is a need to accelerate the process of extraction and identification of pure components of economical grade. Hence, both primary and secondary metabolisms are studied as a prelude for future pharmaceutical and economic advancement. The primary metabolism provides major intermediates for the synthesis of vital molecules whereas secondary metabolism is found to be more restricted. Body of literature provides countless works on algae but only a few studies describe about biosynthetic pathways. Majorly reports found to focus on the secondary metabolites containing structurally and chemically diverse groups. The diversity is the result of the modification and combination of secondary metabolites with different molecules of primary metabolism down the line. Majorly, the sectors influenced by algae are pharmaceuticals (Agyei and Danquah, 2011) and nutraceuticals (Chacón-Lee and González-Mariño, 2010). As a reservoir of biomolecules like lipids, bioactive peptides, algae has gained significant interest among the scientific community (Cavalier-Smith, 1999). Many of the isolated peptides are found to mimic hormones (Samarakoon and Jeon, 2012). This is an indication that the peptides could play an important role in the physiological system. For instance, their use to target specific cells or receptors gives a proclaim image of the mechanism (Fitzgerald and Murray, 2006; Kitts and Weiler, 2016). Moreover, many species have reached beyond nutritional properties and depicted activities similar to bio-proteins viz. antioxidants (Karawita et al., 2007), anticancer (Sheih et al., 2010), immune-modulatory (Morris et al., 2007). These factors collectively lead to a spike in the number of algal studies to uncover "yet to be known" novel molecules.

1.2 ALGAL FARMING

Several thousand tons of algae are farmed every year for nutraceuticals and pharmaceutical industries. The great demand owes to the wide range of bioactivities it performs/bioactive molecules it possesses. China is the largest producer of algae and supports approximately half of the production. The other half is farmed in India, Japan, United States, Taiwan, and Australia. A small stake of algal farming is devoted to foods of zooplanktons and rotifers. A few commonly farmed species are *Chlorella*, *Haematococcus*, *Spirulina*, *Dunaliella*. Often algae are used in the food industries due to the wide range of metabolites present in it. Later, the interest has been shifted to farming than wild harvest so that the desired molecules can be produced in a large scale. However, the algal toxins accumulated in the ecosystem poses an alarming threat for the other animals sharing the ecosystems (Baden, 1993). Hence, an efficient extraction procedure followed by separation of toxin residue is of prime importance (Keijola et al., 1988). This certainly allows us to framework the scenario for isolation of bioactive components that could be probably entering the drug industry. Hence, the analysis of the isolated bio-elements from the algae and their possible components would lead us to establish and platform for future therapeutic drugs (Spolaore et al., 2006).

Therefore it would benefit humanity by providing various pharmaceuticals and nutraceuticals.

1.2.1 BASIS OF ALGAL FARMING

Algal farming is undoubtedly the solution for large scale production of desired biomolecules. The process requires standard protocols and requirements beforehand. Suitable land topography, temperature range, and water bodies are a few examples that facilitate the accuracy of production. Some algae grow in brackish water, thus eliminates the requirement of freshwater. Many universities namely the University of California, University of Texas have the pilot plant (Murphy and Allen, 2011). It takes years for the establishment of a large scale algal production. With the advancement of technology and control over the process and harvesting, farmers reap benefits like cost effectiveness, improved production (Resurreccion et al., 2012). Further benefits could be obtained by using wastewater, residual nutrients, excess heat that resulted in effective farming (Yun et al., 1997; Zhou et al., 2012). Algal farming is commonly done in greenhouses, photobioreactors, ponds, and a hybrid system that combine the pond with photobioreactor. For example, *Dunaliella* requires deep saline ponds. On the other hand, *Spirulina* needs shallow water along with compressed air that allows the culture to move and remains on the surface to ensure proper sunlight absorbance. Then harvesting is done by filtering, centrifugation, or flocculation.

1.2.2 BASIC REQUIREMENTS

Algal farming is dependent on some basic prerequisites (Hochman and Zilberman, 2014). One major need is the high temperature that sustains the algal growth. Proper sunlight should reach the cells in order to carry out photosynthesis. Water requirement is large when farming involves a pond. Moreover, flat surface and clay soil is an important ingredient for setting up ponds. Yet another important constituent is the continuous supply of carbon dioxide required to continue the photosynthesis process. The culturing process is reliably begun in photobioreactors with a 1–2% amount of expected algae biomass. This is because adding a small algal culture in the large pond won't produce the desired result. These cultures were then transferred to the pond for large scale production.

1.2.3 APPROACH FOR ALGAE FARMING

Today algae farming is done in open raceways as they are cost effective to build and operate (James and Boriah, 2010). Areas with high temperatures, low rainfall rely on ponds that heighten productivity. However, outdoor ponds often lead to lower productivity due to temperature variation and contamination. One added difficulty is predators like rotifers, amoeba, fungi, zooplankton, etc. that devour on algae biomass leaving it for no use (Wang et al., 2013). To address these issues, research is going on to introduce an effective automated system. One of the modern concepts includes community gardens that are the new farming ground for algae (Gardiner et al., 2014). Even, shipping containers are revamped with controlled conditions for algae farming. They are cost effective with easy insulation and can be placed anywhere. Algae can also be harvested in pods, popularly known as podponics, often comes with led light and well controlled conditions that use less than 90% water than the greenhouses, zero pesticides, and fast harvesting rate (Juracsek, 2013). Broadly farming process involves photobioreactors and ponds (Figure 1.1). Photobioreactors are preferred for small scale farming and ponds are chosen for large scale biomass production. Photobioreactors are closed, self-contained system that flow water through tubes or plastic bags continuously to bath algae. They are exposed to light through the LED bulbs.

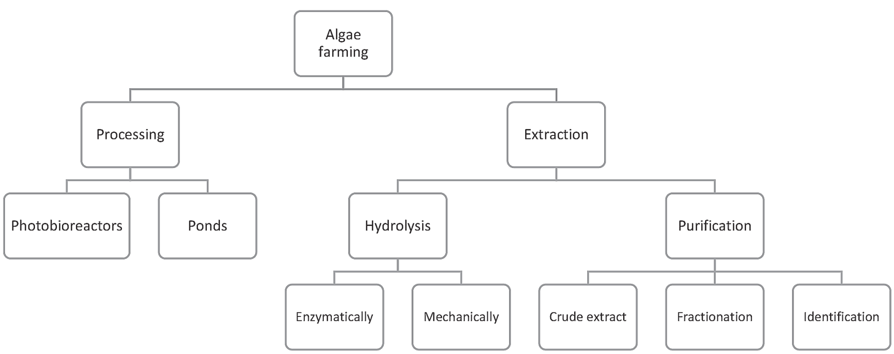

FIGURE 1.1 Generalized procedure of algae farming to identify novel compounds.

1.2.4 CHALLENGES FACED IN FARMING

Harvesting algae is not as easy as it seems. Several technological challenges need to be addressed. According to the estimation, everyday 20–40% of the biomass has to be harvested (Hannon et al., 2010; Vassilev and Vassileva, 2016). Moreover, the cultures have to be sufficiently concentrated. Chemical additives required for augmenting cell flocculation (Smith and Davis, 2012). However, its minimal and careful application is suggested to ensure no contaminants in the fuel produced. The biggest challenge is to finalize a cost effective, effective harvesting system. However, to retrieve the final products, biomass has to pass through the appropriate extraction procedures. The final oil obtained has to be further processed before use it.

One major technical issue into which scientists ponder is that gene mutation. The brief scenario is that the algal culture basically starts with one or a few cells which multiply and reaches billions in number at the end of the process. The main concept is about gene mutation which prevailed in due course of the multiplication process. It is obvious and questions the effectiveness and safeguard of nutrients and oil content. Moreover, proper screening is highly recommended before the introduction of algal cells in the pond as a single invasive species can substantially affect its productivity.

1.2.5 EXTRACTION

Standardizing new analytical methods are of concern in order to identify more and more ingredients from algae with pharmaceutical and nutraceuticals importance. Arizona Center for Algae Technology and Innovation, USA, actively grappled in the research of bioactivities of algae. Indeed, extraction and isolation are of prime importance when we deal with the biomolecules to ensure the safety and effectiveness of the end-products. Biological activities are highly dependent on confirmation which in turn relies on the surrounding environment (Cheng and Rossky, 1998). Hence, hyphenated techniques like supercritical CO_2, ultrasonic based extraction are preferred to ensure the usability of the final product. More emphasis is given for the extraction of algae biomolecules intended to be used as drugs or pharmaceuticals. Hence, the best possible extraction process is finalized based on important criteria. For example, the process should be efficient to draw all the important secondary metabolites, at the same time ensures no existence of toxic residues. Based on the potentiality, two extraction processes are dominating – organic solvent derived extraction (OSE) and enzyme-assisted extraction (EAE). EAE has superseded the other counterpart due to its higher yield potential and purity (Hahn et al., 2012) (Hardouin et al., 2016). Moreover, the major limitation associated with the OSE is the traces of toxic residue recovered with the target molecules.

Briefly, the processing of macroalgae involves 3 steps, i.e. preparation, hydrolysis, and purification (Hammed et al., 2013). As a part of the preparation step, algae are stored in the opaque vials to avoid degradation of UV-light sensitive molecules immediately after harvesting. While handling, algae are thoroughly washed to remove all the debris and associated shrubs. Then the samples are dried, and ground into a powder and processed for hydrolysis. However, the preparation is different in the case of microalgae. They are initially cultured in the lab that mimics its natural habitat followed by harvesting and isolating the target species. Then they are mass cultured in polycarbonate bottles for 14 days. The biomass is then isolated through centrifugation or filtration process. Like microalgae, they are powdered and processed for hydrolysis. The essential components present inside the algal cell are accessed through the enzymatic break down of the cell wall (Gerken et al., 2013). Mechanical techniques like ultrasound sonication and pulverization of lyophilic components are also in use. Commonly hydrolysis is done by three methods for recovering the biomolecules from the inner cell wall. They are as follows:

- Digestive enzymes from animals
- Proteolytic enzymes from microorganisms and plants
- Proteolytic microorganisms during fermentation

The physico-chemical character of protein should be scrutinized thoroughly while carrying out hydrolysis. Temperature and pH are the major factors which if not maintained under the suitable range would cause the degradation of proteins. The next step is the purification of the crude extract obtained from cells to identify the molecule. Currently, fractionation uses ultra-filtration and sodium-dodecyl gel electrophoresis. These enable the accurate separation of protein hydrolysates based on their molecular mass. Further purification is done using chromatographic techniques like ion-exchange, gel filtration, reverse-phase, etc. (Figure 1.2). Additionally, techniques like LC-MS and MS-MS are frequently being in use for characterizing the structure of the hydrolysates.

1.3 USE OF ALGAE IN PHARMACEUTICALS

The use of algae in treating medical conditions or as a preventive measure can be traced back to the ancient era. Their popularity in Asian countries was correlated to the activities pertaining to seas that have led to the discovery of beneficial characteristics of the algal community. They have well emerged

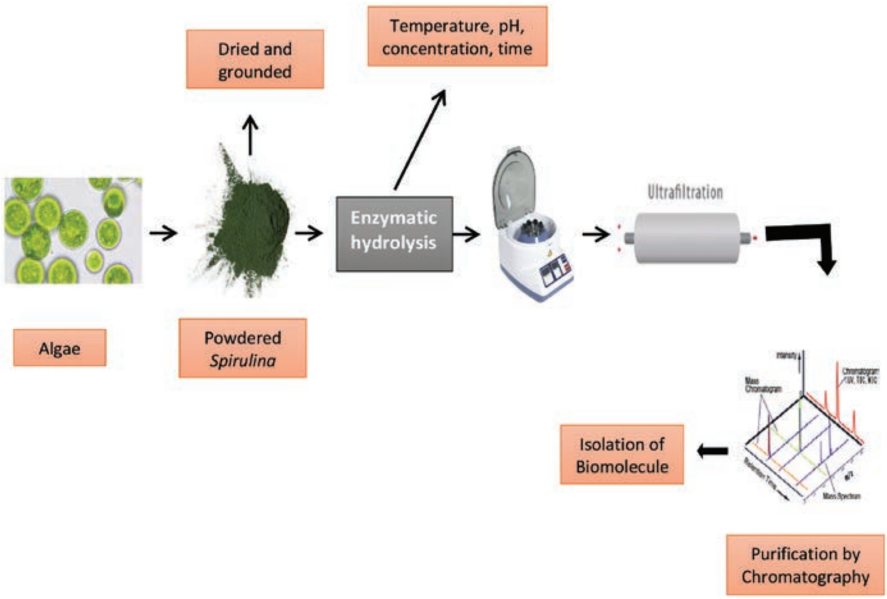

FIGURE 1.2 Isolation of bioactive molecules from algae using enzyme assisted extraction methodology.

Source: Modified from Selvamuthukumaran & Shi (2017).

as a phycocolloids, polysaccharides derived from seaweeds, which later paved the way for finding its associated bioactivities. However, their involvement in the drug industry is reported. Carrageenan, one of the major phycocolloid, is chiefly involved in cough syrup emulsions, and the makings of dental molds. Another phycocolloid, agar is used as an anticoagulant, binding agent for the tablets. Seldom, the algal based polysaccharides are used in non-habit forming laxatives too. Later, the algal industry has seen a significant expansion due to bioactivities like antiviral, anti-helminthic, antibiotics, anticancerous, anti-hypertensive, etc. For example, microalgae such as *Spirulina*, *Dunaliella*, and *Chlorella* are substantiated to possess bioactivities like antitumor, antimicrobial, anti-inflammatory, antiviral, anti-allergy, antioxidant, etc. (El-Baky and El-Baroty, 2013). Noaman et al. (2004) have further added to the efficiency of production by quoting the important factors like temperature of incubation, pH of the culture medium, incubation period, medium constituents, and light intensity that would form the base in the drug industries. The detailed insight into the major pharmacological values of algae is discussed in Table 1.1.

TABLE 1.1 Pharmacological Properties of the Biomolecules Isolated from Few Major Algae Species

Name of Algae	Classification	Bioactivity	Active Compound	References
Ecklonia cava	Brown macroalga	Antioxidant, anticancer, antihypertensive, anti-inflammatory, immunostimulant	Phenolic compounds, sulfated polysaccharides, galactofucan, fucoidan, phlorotannins, peptides	Heo et al. (2003); Ahn et al. (2008); Kim & Bae (2010); Kim & Himaya (2011); Ahn et al. (2015)
Scytosiphon lomentaria	Brown macroalga	Antioxidant	Phenolic compounds	Heo et al. (2003)
Ishige okamurae	Brown macroalga	Antioxidant, anti-inflammatory	Peptides, polyphenols, galactofucan, fucoidan	Heo & Jeon (2008); Kim et al. (2009)
Scytosiphon lomentaria	Brown macroalga	Antioxidant	Polysaccharides	Ahn et al. (2004)
Palmaria palmata	Red macroalga	Antioxidant	Phenolic content	Wang et al. (2010)
Spirulina platensis	Blue-green microalga	Antioxidant, anticancer, antihypertensive, increasing intestinal lactobacilli, reduces nephrotoxicity, lowers cholesterol	C-phycocyanin, peptides	Belay et al. (1993); Bhat & Madyastha (2000); Suetsuna & Chen (2001); Wergedahl et al. (2004); Wang et al. (2007)
Ulva fasciata	Green macroalga	Antioxidant	Sesquiterpene	Chakraborty & Paulraj (2010)
Laminaria japonica	Brown macroalga	Antioxidant	Fucoidan	Wang et al. (2008)
Cystoseira barbata	Brown macroalga	Antioxidant	Alginates	Sellimi et al. (2015)
Polysiphonia urceolata	Red macroalga	Antioxidant	Phenolic content	Duan et al. (2006)
Dunaliella salina	Green microalga	Antioxidant	β-carotene	Zhu & Jiang (2008)
Chlorella vulgaris	Green microalga	Anticancer, antihypertensive, immunomodulating	Peptides, glycoprotein peptide	Suetsuna & Chen (2001); Morris et al. (2007); Sheih et al. (2009); Chen et al. (2011)
Sargassum pallidum	Brown macroalga	Anticancer	Sulfated polysaccharides	Ye et al. (2008)

TABLE 1.1 *(Continued)*

Name of Algae	Classification	Bioactivity	Active Compound	References
Porphyra vietnamensis	Red macroalga	Anticancer	Porphyran	Venkatpurwar et al. (2011)
Saccharina japonica and *Undaria pinnatifida*	Brown macroalga	Anticancer	Fucoidan	Vishchuk et al. (2011)
Gelidium cartilegineum	Red macroalga	Antiviral	Polysaccharides	Gerber et al. (1958)
Cladosiphon okamuranus	Brown macroalga	Antiviral	Fucoidan	Hidari et al. (2008)
Gracilariopsis lemaneiformis	Red macroalga	Antihypertensive	Peptide	Cao et al. (2017)
Porphyra yezoensis	Red macroalga	Antihypertensive, anticoagulant, immunomodulating, anti-hyperlipidemic	Peptide, polysaccharide, sulfated polysaccharide	Yoshizawa et al. (1995); Tsuge et al. (2004); Qu et al. (2010)
Codium fragile	Green macroalga	Anticoagulant	Sulfated proteoglycans, polysaccharides, proteoglycans	Jurd et al. (1995); Athukorala et al. (2007)
Sargassum horneri	Brown macroalga	Anti-coagulant	polysaccharides, proteoglycans	Athukorala et al. (2007)
Codium pugniforme	Green macroalga	Anti-coagulant	Sulfated polysaccharides, proteoglycan	Matsubara et al. (2000)
Codium intricatum	Green macroalga	Anti-coagulant	Fibrinolytic enzymes	Matsubara et al. (1998)
Gracilaria sp.	Red macroalga	Anti-inflammatory	Peptides	Almeida et al. (2011); Chen et al. (2013)
Porphyridium sp.	Red microalga	Anti-inflammatory	Polysaccharides	Talyshinsky et al. (2002)

TABLE 1.1 *(Continued)*

Name of Algae	Classification	Bioactivity	Active Compound	References
Polyopes affinis	Red macroalga	Anti-inflammatory	Phenolic compounds	Lee et al. (2011)
Ulva sp.	Green macroalga	Anti-inflammatory	Sulfated polysaccharides	Jin et al. (2006); Margret et al. (2009)
Caulerpa mexicana	Green macroalga	Antinociceptive, Anti-inflammatory	Lectin, sulfated polysaccharides	Silva et al. (2011); Coura et al. (2012)
Ulva rigida	Green macroalga	Immunomodulating	Carrageenan, sulfated polysaccharides	Ogata et al. (1999); Leiro et al. (2007)
Digenea simplex	Red macroalga	Antihelminthic	Kainic acid	Moo-Puc et al. (2008)
Chondria armata	Red macroalga	Antihelminthic	Domonic acid	Higa & Kuniyoshi (2000)
Sargassum natans	Brown macroalga	Antihelminthic	Sulfated polysaccharides	Orhan et al. (2006)
Laurencia dendroidea	Red macroalga	Antihelminthic	Sesquiterpene	Veiga-Santos et al. (2010)
Undaria pinnatifida	Green macroalga	Differentiation of osteoblast cells, protection of gastric mucosa against regular acids, inhibit UV-B induced MMP-1	Fucoidan	Moon et al. (2008); Cho et al. (2009); Choi et al. (2010)
Ulva pertusa	Green macroalga	Anti-hyperlipidemic, sequestration of bile acids	Sulfated polysaccharide	Lahaye (1991); Pengzhan et al. (2003)
Fucus sp.	Brown algae	Anticancer	Fucoxanthin	Kotake-Nara et al. (2001)
Haematococcus pluvialis	Red microalga	Antioxidants	Astaxanthin	Capelli et al. (2013)

1.3.1 ANTIOXIDANT PROPERTIES

Antioxidants play a major role in the human system by keeping a check on the antioxidative reactions thus reducing the reactive oxygen species (ROS). The in-build antioxidants like catalase, glutathione peroxidase, superoxide dismutase, selenium, vitamin C often responsible for maintaining homeostasis (Ahn et al., 2004). However, conditions like environmental pollution, alcohol, high-fat diet, chemical smoke, ultraviolet radiation cause imbalances which often lead to an increase in ROS accumulation in the body tissues. ROS is a major underlying culprit behind several diseases like cancer, hypertension, diabetes mellitus, cardiovascular ailments, inflammatory conditions, neurodegenerative diseases, and aging (Valko et al., 2007). This could be reasoned since ROS attacks the major macromolecules, viz. carbohydrate, protein, lipids of our body. Many commercial antioxidants used in pharmaceutical industries namely butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl gallate (PG) function to retard the rate of oxidation and peroxidation reactions. However, strict regulations are implemented on their use due to possible health hazards. Hence, an alternative that could address these issues needs to be introduced. In this regard, the research community has been galvanized towards natural resources. The marine algae provide the best solution which largely remains untouched. This is an environment of extreme environmental condition which increases ROS production in algae (Ngo et al., 2011). However, it is found that they efficiently quench the ROS and protect themselves from the oxidative damage (Guedes et al., 2011). The mechanism and the metabolites involved in the process presents an astonishing ray to combat the life threatening ailments. Secondary metabolites including peptide, polysaccharides, polyphenol compounds, carotenoids, etc. are responsible for these activities. Among all, the antioxidant property of algae is well studied (Heo et al., 2005, 2006; Kim et al., 2006; Karawita et al., 2007). It is established that covalent bonds in certain proteins are associated with antioxidant properties (Sheih et al., 2009). The antioxidant property of algae is substantiated by a large number of works found in the literature. A survey conducted about a decade earlier, has documented the prominent antioxidant properties of both micro and macroalgae employing DPPH, hydrogen peroxide, superoxide anion scavenging methods (Ngo et al., 2011). Another report evaluated the antioxidant activities of seven species, viz. *Ecklonia cava*, *Ishige okamurae*, *Sargassum fulvellum*, *Sargassum horneri*, *Sargassum coreanum*, *Sargassum thunbergii*, and *Scytosiphon lomentaria* of brown seaweed. The enzymatic extracts of the algae were reported to significantly reduce the DNA damage compared to the commercial antioxidants

(Heo et al., 2003). Among these, enzymatic extracts of *E. cava* have been reported to show the best antioxidant property with thermal stability. It is found that majorly hydrogen peroxide activity is affected by the enzymatic extracts of algal species (Heo and Jeon, 2008). The efficacy of protease extract over carbohydrase extract in displaying hydrogen peroxide scavenging activity is one of a kind result. In his work, Ahn et al. (2004) have established another brown alga *Scytosiphon lomentaria* with potent ROS scavenging activity. Similar work with *Palmaria palmata*, red algae, has reported antioxidant activity of proteases over carbohydrase against peroxyl radicals (Wang et al., 2010). An isolate of *Spirulina platensis*, C-Phycocyanin has effectually inhibited lipid peroxidation induced by CCl_4 in vivo condition with rat liver cells (Bhat and Madyastha, 2000). *Ulva fasciata*, a green alga, were also found to possess free radical scavenging activities due to sesquiterpenes (Chakraborty and Paulraj, 2010). Even blue-green algae *Spirulina platensis* has gained considerable attention for possessing a number of health benefits including antioxidant properties (Piero Estrada et al., 2001). While surveying through the literature it is majorly seen that algal peptides are ahead in the race. However, a review was looked to emphasize on the sulfated polysaccharides (SP) extracted from the marine algae (Wijesekara et al., 2011). Beyond their well-known functions, SPs of marine origin are also found to possess antioxidant properties that increase the thrust to unveil more aspects of it. SPs like fucoidan, alginates proves it all (Wang et al., 2008; Sellimi et al., 2015). Most of the polyphenols are extracted from micro and macroalgae and show the antioxidant property. Approximately 8000 different polyphenols belonging to 10 groups are identified to date. They are known to exist in variable conformations ranging from simple structures (e.g. phenolic acids) to complex groups (e.g. phlorotannins). Studies reported that antioxidant activities largely dependent on the polymerization of polyphenols. The less the complexity of polymerization the greater antioxidant potential is exhibited. The species *Polysiphonia urceolata*, a red alga, portrays the positive correlation between antioxidant capacity and the phenolic content it possesses (Duan et al., 2006). Other candidate carotenoids, the naturally occurring pigment, are known to possess antioxidant properties and regarded as an active component against cancer, cardiovascular ailments, and muscular degeneration too (Cooper et al., 2009). Vis-à-vis, many algal species are known reservoirs of carotenoids. For instance, the microalga *Dunaliella salina* can accumulate large amounts of β-carotene when cultivated under specific conditions (Zhu and Jiang, 2008). The statement is further substantiated by the work of Higuera-Ciapara et al. (2006) who have proved the antioxidant property of astaxanthin, a well-known carotenoid.

1.3.2 ANTICANCER/ANTI-TUMOR ACTIVITY

Cancer is one of the leading causes of death in both developed and developing nations (Ezzati et al., 2002). It is found that genetic cancers constitute about 10–15% of all the cases whereas the remaining 85–90% is grappled by the epigenetic factors. This includes alcohol, smoke, environmental pollution, fat-rich diet, sedentary lifestyles (Anand et al., 2008). The characteristic, uncontrolled cell division is often induced by the oxidative free radicals. Hence, knowledge and awareness about cancer-causing factors and their early diagnosis help to reduce the risks. However, the majority of the cases are detected at the progressive stage where the use of chemotherapy is the most promising approach available now in order to treat the disease (Sheih et al., 2010). However, reports on resistance development pose a great challenge to pharmaceutical industries. Hence, much attention is given to identify bioactive molecules from the natural sources that have intended to reduce morbidity and mortality. Marine based seaweeds and microalgae have been a reservoir of potent components to be added in the cancer treatment. For example, sulfated polysaccharides have actively reduced carcinogenesis in humans (Rocha De Souza et al., 2007). During multistage cancer, normal cells proceeds through three phases, i.e. initiation, promotion, and progression. The problem can be checked by the naturally occurring components which manipulate the cancer cells with no side effects. Hence, exploring and identifying novel natural bioactive components holds a ray of hope for future cancer treatment. Proteases derived from marine algae show significant health benefits. One such work conducted by Chen et al. (2011) has shown that proteases isolated from *Chlorella vulgaris* effectively inhibited the UV-b induced MMP-1 in skin fibroblast cells. The MMP 1 degrades collagen and resulted in photoaging (Ågren et al., 2015). *C. vulgaris* derived peptides have effectively downregulated the MMP1 and other players in the trophic pathway, viz. CYR61 and MCP-1 (Chen et al., 2011). Moreover, a glycoprotein (ARS-2) eluted with hot water from *C. vulgaris* was expressed by the toll like receptors 2 against the anti-tumor activity (Hasegawa et al., 2002). The scientists have explained that interleukin production in ARS-2 treated rat spleen cells was TLR 2 dependant and not TLR 4. Wang et al. (2007) have reported the anti-tumor activities of C-Phycocyanin (C-PC) isolated from *Spirulina platensis*. The scientists have expressed β-subunit of C-PC that have effectively inhibited the proliferation of four different cell lines and induced their apoptosis. The mechanism of action is linked with the interaction between C-PC/β with β-tubulin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Moreover, they resulted in the depolymerization of microtubules and actin-filaments. It is noted that the C-PC/β treatment has resulted in the cell cycle arrest at G_0/G_1 phase, upregulation of caspases mainly C-3 and C-8. The decrease in the level of GAPDH indicates cell cycle arrest thus suggesting C-PC/β as a promising anti-cancer candidate. Several reports claim the anti-proliferative actions of sulfated polysaccharides in vitro (Ye et al., 2008). They also possess anti-metastatic activity by blocking the interaction between cancer cells and their basement membrane (Parish et al., 1987). A report by Yamamoto et al. (1986) has documented the effectiveness of seaweeds in reducing the incidence of carcinogenesis when administered orally. In an in vitro study, Porphyran induced the death of cancer cells through apoptosis without affecting the growth of normal cells (Venkatpurwar et al., 2011). Another SP isolated from *Ecklonia cava* induced cell death in vitro and showed a potential antiproliferative effect in a human lymphoma cell line (Ahn et al., 2015). Fucoidan also shows anti cancerous activity which is closely related to the sulfated content of the same (Vishchuk et al., 2011). Often anti-cancerous property of algal components is seen to be associated with their antioxidant property. This is justified by the work of Lee et al. (2012) who have reported the positive correlation between ROS generation and cancer induction on metal exposure. Moreover, continuous production of ROS causes severe damage to the genomic DNA and often leads to carcinogenic conditions (Aykin-Burns et al., 2009). One major issue faced by drug industries is that many of the drugs associated with the ROS generation can be proclaimed through miRNAs expression (Fayyaz and Farooqi, 2013). Correlated to this, the upregulation of miRNA-34a was reported in the cells treated with ferric nitrilotriacetate. The involvement of ROS and miRNA became prominent in cancer when the downregulation of miRNA-34a by siRNA was reported in HeLa and MCF7 cells. Additionally, it is seen that representative of red, green, brown and blue-green algae shows anticancerous property (Khan et al., 2005; Kwon and Nam, 2007; Zandi et al., 2010; Ermakova et al., 2011). Hence, components of natural origin would help the drug industries to design prototypes for cancer treatment.

1.3.3 ANTIVIRAL PROPERTIES

Viral infections are age-old reality and have caused havoc to mankind with regard to their life. Influenza A and B are responsible for flue like conditions in human and have affected many lives. A report by Gerber et al. (1958) has demonstrated the antiviral potential of polysaccharides extracted from *Gelidium cartilagineum* that tested against virus infested embryonic eggs.

Polysaccharides with antiviral properties are found to be highly sulfated (Wijesekara et al., 2011). Moreover, molecular mass, sugar groups, are the vital factors that highly influence the antiviral properties. Simultaneously, the degree of sulphation and distribution of sulfate groups in the polysaccharides are directly correlated with antiviral activity. It is reported that low sulfate content polysaccharides are inactive against the virus (Damonte et al., 2004; Adhikari et al., 2006). The SPs have actively inhibited the replication and growth of flavivirus, togavirus, orthpoxvirus, herpesvirus, etc. (Witvrouw and De Clercq, 1997). Acquired immunodeficiency syndrome caused by HIV-1 has affected millions of lives and the most deadly condition next to cancer. The first generation of anti-AIDS treatment has severely failed with only a 50% success rate (Artan et al., 2008). The major reason for failure is the resistance development in the virus. Moreover, the virus changes its confirmation quickly that makes it more difficult to design a drug against it. Therefore, launching a new generation of potent drugs that actively retard the growth of the virus is a need of the hour (Singh et al., 2005). This can be addressed by exploring natural bioactive agents and their derivatives as a new line of therapeutics against the disease with no side-effects. Sulfated polysaccharides extracted from *Mimosa scabrella* were reported with anti-viral activity and have negatively affected yellow fever virus, HIV, and flavivirus both in vivo and in vitro testing (Talarico et al., 2005). To date, no specific licensed vaccine is available for the dengue virus. However, yellow fever virus belonging to the same family Flaviviridae has a vaccine. Over time, we have discussed the phenomenon of "resistance development." Hence, finding a vaccine candidate from the natural resources would be an appropriate solution. Hidari et al. (2008) have demonstrated that Fucoidan, SP isolated from *Cladosiphon okamuranus*, has efficiently impeded dengue virus type 2 infection. Their detailed investigation has revealed that fucoidan interacts directly with the enveloped glycoprotein of dengue virus 2 particles. This proves the effectiveness of this against dengue virus type 2. This leaves us with the hope that unveiling other SP molecules from algae will eventually control other types of dengue virus. Another study has thrown light into the efficacy of SP molecules isolated from several algae against the herpes simplex virus I and II (Harden et al., 2010). The SPs were found very effective at the first hour of viral infection however after that their effect has diluted. Moreover, macroalgal sulfonic polysaccharides are frequently used in the vaginal antiviral formulation. Their use is promoted for being safe without inferring with the normal function of vagina lining cells and the bacterial flora (Béress et al., 1993). The major challenge is the selection of the most promising antiviral

SP compound from the whole reservoir. Low cost, safety, least toxicity, and broad spectrum properties are the advantages of SPs that make it a favorable drug candidate with further studies required to approve the best one. The resistance development of the pathogenic microorganisms against the available antibiotics is a major concern that leaves mankind in vain. This raises the need to identify new antibiotics of natural origin as they are least toxic to humans. Among all, marine derived antimicrobial peptides offer promising results with broad spectrum antimicrobial activities. No such antibiotics are in use currently. But they can make up a novel future drug line against the pathogenic microorganisms. For example, the Covid-19 pandemic against which the whole world is battling is a virus borne disease. Though a vaccine is a need of the hour but resistance development with time is an expected phenomenon. Hence, to deal with such difficulty finding out a novel natural solution would be the best cure. Recently, Council of Scientific and Industrial Research (CSIR), India, has announced its upcoming drug trial schedule with phytopharmaceutical ACQH against covid-19. Moreover, favipiravir, another plant based drug, entering the clinical trial is an indication of future prospects of algal based biomolecules in curbing the deadly disease.

1.3.4 ANTI-HYPERTENSIVE

Cardiovascular diseases are among the high-risk factors affecting and claiming millions of lives every year (Kearney et al., 2005). Their incidence is more likely connected with pre-existing conditions like high blood pressure, hypertension, and aging which resulted in ROS accumulation (Archer et al., 2008). Hypertension often leads to other medical complications like stroke, arteriosclerosis, myocardial infarction later. This a major cause of death in the developed as well as developing nations. These conditions are often managed by a strict diet and lifestyle management that keep a check in the blood pressure. Nevertheless, natural resources with ACE-1 inhibitory potential can bring new hope in the pharmaceutical for treating the condition (Wilson et al., 2011). Blood volume and its pressure in humans are maintained by the Renin-angiotensin-aldosterone system (Fitzgerald et al., 2011). Angiotensin I gets readily converted to Angiotensin II by Angiotensin I converting enzyme (Zhao and Xu, 2008). Moreover, the enzyme is also known to inactivate the vasodilator bradykinin. Angiotensin II is the vasoconstrictive molecule. Hence, targeting ACE-I with biomolecules of natural origin would immensely help (Riordan, 2003). Renin is another candidate whose blockage can give the drug industry suitable results as they catalyze the formation of Angiotensin I from Angiotensinogen. Interestingly, the association of ACE in the degradation of neuropeptides like substance P, neurotensin, encephalin, which ensure the proper functioning of the cardiovascular system, provides a suitable target in drug development. The drug industry depends on synthetic molecules to treat hypertension. Majorly, chemically synthesized drugs like captopril, alcacepril, enalapril, lisinopril are in use to treat cardiovascular disease. Aliskerin, a renin inhibitor, was discovered almost a decade ago but it was banned due to harmful effects. The naturally occurring ACE inhibitor has been isolated from snake venom. However, it reported numerous side effects like cough, skin rash, angioneurotic edema, taste disturbance. Henceforth, the greater interest is devoted to finding novel components from the algal community that can inhibit the ACE-I without posing a negative effect on human health. The foundation of the natural ace-I inhibitor was laid almost four decades ago (Oshima et al., 1979). The first inhibitor peptide was reported from the food protein hydrolyzed by the digestive proteases. Later on, the efficacy of marine organisms in the treatment of mild hypertension patients by inhibiting ACE-I converting enzymes was testified (Lee et al., 2005; Guang and Phillips, 2009). Gradually, ACE inhibitory peptides are isolated and reported from algae (Suetsuna and Chen, 2001; Cao et al., 2017). The peptides work either by binding to the active site or to the inhibitor site which eventually alters the structure of the substrate. This doesn't allow enzymes to bind to the substrate molecule and thus inhibit the ACE-I enzyme. It is seen that some peptides opt for the non-competitive mechanism (Qian et a., 2007). Algal peptides have efficiently acted as ACE-I inhibitor and that is well understood (Samarakoon and Jeon, 2012; Cao et al., 2017). The efficacy of the anti-hypertensive effect of seven different brown algae namely *Ecklonia cava*, *Ishige okamurae*, *Sargassum fulvellum*, *Sargassum horneri*, *Sargassum coreanum*, *Sargassum thunbergii*, and *Scytosiphon lomentaria* was tested. Among all, *Ecklonia cava* has shown the best ACE inhibitory activity (Heo et al., 2003). Chao et al. (2000) have compared the potential of ACE inhibitory enzymes derived from green and brown algae. Results indicated that brown algae are superior to green algae. In a study by Sheih et al. (2009) ACE-I inhibitory activity was analyzed for the peptide isolated from the *C. vulgaris* protein waste. It was hendecapeptide and found to exhibit high ACE-I inhibitory activity with additional benefits of pH and heat stability against GI tracks enzymes. This provides a suitable example of waste utility. *Porphyra yezoensis*, edible red algae, has significantly inhibited ACE-I in a hypertensive rat (Qu et al., 2010). The active compound isolated from the species was albumin, gliadin, and glutelin. Marine based bioactive peptides have the potential for use as functional ingredients in pharmaceuticals due to their effectiveness in both the prevention and treatment of hypertension. Moreover, cost effective and safe natural health products can be produced from marine bioactive peptides. Hence, further studies are needed with clinical trials to commercialize the anti-hypertensive peptides.

1.3.5 ANTI-COAGULATION OR ANTI-HYPERLIPEMIA ACTIVITIES

Blood coagulation is essentially a complex process which is a key for maintaining homeostasis. Blood borne coagulation factors stop bleeding and repair the injured blood vessels. Abnormal vascular condition is also causes bleeding which in turn induces the coagulation pathway. The whole pathway involves almost thirteen different plasma serine proteases often regarded as coagulating factors. They are the major player of the clotting mechanism all originated independently in response to the stimulus and progress towards the ultimate common pathway (Gailani and Renné, 2007). However, anti-coagulants often hinder or restrict the coagulation process due to numerous extrinsic or intrinsic factors and cause blood coagulation process to be continued for a long time or stopped altogether (Jung et al., 2002). This provides an important tool to study the coagulation cascade thoroughly. A few anticoagulants, viz. heparin, warfarin, coumarin are commercially available since a long time (Wardrop and Keeling, 2008). Their use is promoted for treating thrombic disorders and in the manufacturing of clinical equipments like renal tube, blood transfusion, test tubes, etc. However, their use has reported major side effects like hemorrhage and thrombocytopenia, inability to inhibit the binding of thrombin to fibrin, ineffectiveness in congenital or acquired antithrombin deficiencies (Pereira et al., 2002). Moreover, the source of heparin is pig intestine which harbors very less amount. Hence, the need of finding an alternative source of anticoagulant has arisen with great demand. The natural based molecules are known for their bioactivities and least toxicity. In this regard, anticoagulants of algal origin hold prime interest. This ensures a safer future application on humans. Though the field is relatively naïve but studies have reported that proteoglycans and sulfated polysaccharides isolated from algae show anticoagulant activity (Jurd et al., 1995). The work of Athukorala et al. (2007) has checked the efficacy of 22 different species of brown and green algae in Jeju, South Korea. Among all, two species namely *Codium fragile* and *Sargassum horneri* holds the best anti-coagulant property in activated partial thromboplastin time and prothrombin time. The result shows that the polysaccharides with high molecular weight were the key player behind the bioactivity and concluded that the effect is due to carbohydrates and proteoglycans. Another report (Matsubara et al., 2000) on *Codium pugniforme* and their anticoagulant effect on human plasma were tested and an comparison was made with the widely used commercialized anticoagulant heparin. The efficacy of the alga was correlated with the amount of sulfated polysaccharides, proteoglycan, and protein polysaccharide. Moreover, marine based peptides of algal origin such as *Codium intricatum* (Matsubara et al., 1998) and *Porphyra yezoensis* (Indumathi and Mehta, 2016) have also shown anti-coagulant activity. The studies hold a promising future of algal peptides and polysaccharides to be used in drug industries. The clinically approved anticoagulant majorly target anti-thrombin instead of thrombin and initiate clot formation. Nevertheless, algal based components directly targeting thrombin could provide a novel class of anticoagulants.

1.3.6 ANTI-INFLAMMATORY ACTIVITY

Inflammation commonly occurs in body tissues and is a mode of response to harmful external agents. They often lead to cell deaths and reported to pave ways for deadly diseases. It is noted that oxidative stress plays important role in lung disease (Rosanna and Salvatore, 2012), endothelial dysfunction (Schramm et al., 2012), gastrointestinal dysfunction (Kim et al., 2012), and all of them involve inflammatory reactions. The commonly used anti-inflammatory drugs are aspirin, ibuprofen, naproxen, acetaminophen, etc. that constitute age-old Nonsteroidal anti-inflammatory drugs (NSAIDs). Unfortunately, these drugs often lead to strong side effects that question their use. For instance, aspirin can cause bleeding in the stomach, acetaminophen may lead to liver damage, Vioxx and Celebrex (Cox-3 inhibitors) cause heart problems. Moreover, many NSAIDs have been reported to claim several deaths every year. These factors should be addressed collectively and the existing anti-inflammatory drugs need to be replaced. The natural products seemed the best solution here as well. This led to the finding of novel anti-inflammatory compounds from marine algae. It is hypothesized that the naturally grown algae can withstand extreme stresses and hence could be a reservoir of novel compounds that can trigger immunological responses in man. As discussed in the antioxidants property, many marine algae species are known to contain natural antioxidants and hence believed to possess anti-inflammatory properties too (Abad Martinez et al., 2008; Wang et al., 2012). Two species of *Gracilaria* namely *G. verrucosa* and *G. textorii* have been reported to possess anti-inflammatory properties (Almeida et al., 2011). *G. tenuistipitata*, another species, has significantly reduced inflammation caused by the virus (Chen et al., 2013). *Porphyridium* extracted polysaccharide was involved in the suppression of retrovirus replication (Talyshinsky et al., 2002). Another study has revealed the antiviral properties of ethanol derived extract of the red alga, *Polyopes affinis*, against the reactions that occurred in asthma (Lee et al., 2011). The scientists worked on the *Ulva conglobata* and *U. lactuca* have revealed their anti-inflammatory effects against murine hippocampal and microglial cells (Jin et al., 2006) and rat model (Margret et al., 2009). Interestingly, some green algae also possess antinociceptive activities other than anti-inflammatory. For instance, *Caulerpa mexicana* extracted with methanol represents both the activities in nociceptive models (Bitencourt et al., 2011) (Da Matta et al., 2011). Lectin (Silva et al., 2010) and sulfated polysaccharides (Coura et al., 2012) were found as the major factor behind this function. The efficacy of brown algae as an anti-inflammatory agent is also studied. For example, the ethanolic extract of *Ecklonia cava* (Kim and Bae, 2010) and *Ishige okamurae* (Kim et al., 2009) shows an anti-inflammatory effect. Studies revealed that sulfated polysaccharides like galactofucan (Sarithakumari et al., 2013), fucoidan (Kawashima et al., 2012) are responsible for the anti-inflammatory activity. Moreover, pigments like fucoxanthin (Heo et al., 2010) and phlorotannins (Kim and Himaya, 2011) derived from *Myagropis myagropides* and *Ecklonia cava* respectively possess the bioactivity. The reports are enough to draw the conclusion about the anti-inflammatory potential of algae. However, further research and clinical trials are needed before finalizing the component. Above all, the bioactivities of the algae present a promising future in the development of anti-inflammatory drugs that can address all the issues faced while using NSAIDs.

1.3.7 IMMUNOMODULATING ACTIVITY

Macrophages are an important component of the innate immune system. It is unique as it changes its function according to the tissue it resides and thus helps in maintaining the homeostasis. They are the precursor of the major inflammatory factors that mark the inflammation. It is proposed that chronic inflammation often leads to cancer development (Karin et al., 2006). Many immunomodulating drugs are available in the market but their side effects are a major drawback. Therefore, research on algal species to uncover the biomolecules initiated. Red algae namely *Porphyra yezoensis* and *Gracilaria verrucosa* were found to stimulate phagocytosis and respiratory burst in mouse macrophages both in vivo and in vitro (Yoshizawa et al., 1995). Red algae derived carrageenan is an efficient anti-inflammatory agent which initiates the formation of tumor necrosis factor-α in mice when exposed to bacterial lipopolysaccharides (Ogata et al., 1999). Additionally, it is seen that carrageenan can both activate (Nacife et al., 2004) and inhibit the function of macrophages (Rooijen and Sanders, 1997). Moreover, it is found that SPs have a potential application in stimulating the immune system or in controlling macrophage activity to reduce negative effects (Leiro et al., 2007). *Ulva rigida* extracts containing SPs stimulate two-fold increase in the expression of several interleukins and chemokines. It also induces nitrite production which in turn releases macrophage derived prostaglandins and facilitates an increase in COX-2 and nitric oxide synthase 2. This indicates their potential in the clinical industry where they can be used to modulate the macrophages in diseased conditions (Leiro et al., 2007). *C. vulgaris* derived peptides have displayed both innate and acquired immune responses in undernourished Balb/c mice (Morris et al., 2007). Moreover, hemopoiesis and functional activities of macrophages are among the other beneficial characters which were found to increase. In a report, *E. cava* isolated protease enzymatic extract reported an immunostimulant effect on murine in vitro. Results showed that the treated groups reported with a boosted proliferative rate of monocyte, granulocyte, and lymphocyte. The number of $CD4^+$, $CD8^+$T cells and CD45R/B22O$^+$ B cells also increased compared to the untreated group. Moreover, Th-1 cytokine was downregulated whereas Th-2 cytokine upregulated (Ahn et al., 2008). Studies stated that the sulfated polysaccharides have efficiently modulated the macrophages thus indicates its efficacy in immune modulation (Wijesekara et al., 2011). Moreover, proteins and protein derived peptides also reports the same characters (Samarakoon and Jeon, 2012). These provide a great possibility of its use in future grafting and transplantation procedures.

1.3.8 ANTI-HELMINTHIC OR VERMIFUGES

The concept of the anti-helminthic activity of seaweeds exists since ancient times (Stein and Borden, 1984). They are majorly seen in traditional medicines that connected with the knowledge of tribal and ethnic groups. It is largely recorded in the tropical and sub-tropical regions where the helminthic diseases are prevalent and hit the poor most. However, this class of disease often remains neglected. To gain interest in the same and encourage researches for these severe diseases, as it is often overlooked by the drug developer, WHO developed a list of neglected anti-helminthic diseases including Leishmaniasis, Chagas disease. Currently, medicines like ivermectin, albendazole, mebendazole are in use to treat helminthic diseases. However, side effects like headache, fever, myalgia, distension, vertigo, jaundice, alopecia, thrombocytopenia, tachycardia are often experienced by patients (Shenoy et al., 1992; Teggi, et al., 1993). Hence, there is a strong need to have a replacement that can expel the parasitic population without posing any side effects. The use of algae has seemed an intelligent move that could offer anti-helminthic activity. The pharmacological potential of algae as a source of the anti-helminthic drug was first proven by the tropical species *Digenea simplex* which has been known for more than a 1,000 years (Watase et al., 1958; Moo-Puc et al., 2008). The major component is the kainic acid that leads to neuromuscular obstruction in the worm. A dose of 5–10 mg effectively expels the *Ascaris* worm from the patients without any side effects. Additionally, Domonic acid extracted from the *Digenea simplex* and *Chondria armata* is used in Japan for ages as an anti-helminthic agent (Higa and Kuniyoshi, 2000). Research showed that many seaweeds such as *Durvillaea*, *Sargassum*, *Corallina officinalis*, and *Ulva* spp. displays vermifuges activity (Anand et al., 2016). The positive results have gained the attention of the research community and their thrust for secondary metabolites to be examined for their efficacy against various helminthic diseases. With this view, several studies on marine algae are initiated. The exploration of compounds requires fast and effective bioassay and their availability ensures the proper identification of the compounds (Sykes and Avery, 2013). The anti-helminthic effect of ethanolic extracts of many Turkish macroalgae viz. *Dictyota dichotoma*, *Halopteris scoparia*, *Posidonia oceanica*, *Scinaia furcellata*, *Sargassum natans*, and *Ulva lactuca* was analyzed (Orhan et al., 2006). All the extracts were found effective against *T. brucei rhodesiense* and *S. natans* was the best. Moreover, all except *H. scoparia* showed leishmanicidal activity against the amastigote forms. *U. lactuca* and *P. oceanica* showed the strongest leishmanicidal potential. However, none of the algal extracts could elicit a fatal effect against trypomastigotes of *Trypanosoma cruzi*. In his report, Spavieri et al. (2010) have evaluated the anti-helminthic efficacy of 21 algal extracts against *T. brucei rhodesiense*, *T. cruzi*, and *L. donovani.* Although, 21 extracts had exhibited leishmanicidal activity, most active extracts were isolated from *Halidrys siliquosa* and *Bifurcaria bifurcata*. Another group of scientists have isolated sesquiterpenes namely elatol, obtusol, triquinane from the red algae

Laurencia dendroidea and assessed their anti-helminthic potential (Veiga-Santos et al., 2010). The sesquiterpenes displayed antiprotozoal activity without any signs of cytotoxicity to mammalian cells. Elatol was the most active compound of the species and posed anti-helminthic activity against amastigotes and trypomastigotes of *T. cruzi*. It was also tested and showed a positive effect against *Leishmania amazonensis* (Santos et al., 2011). A deeper evaluation of the mechanism of action of the compound in *T. cruzi* has shown their involvement in increased production of superoxide anion and mitochondrial depolarization (Lopes et al., 2012). The accumulation of free radicals is considered as the cause of parasitic death. Numerous references discussed above and many more existing in the literature claim that natural products like algae offer the best anti-helminthic activity with the least side effects. Henceforth, it should be encouraged to enter the pre-clinical trial now so that its commercialization becomes possible and these neglected diseases can be treated with more effective agents.

1.3.9 OTHER BIOLOGICAL ACTIVITIES

Among a handful of components with the potential activity we discussed, fucoidan has been the one with diverse roles. A study on *Undaria pinnatifida* derived fucoidan has been reported to involve actively in the differentiation of osteoblast cells (Cho et al., 2009). Hence, its use in bone supplements can form an effective new generation of drugs in the future. Moreover, they have effectively protected the gastric mucosa against the regular acids and pepsin which opens up the door to be developed as a potential anti-ulcer agent (Choi et al., 2010; Raghavendran et al., 2011). The group of scientists has worked on the potential of fucoidan against UV-B induced Matrix metalloproteases-1 (MMP-1) that degrades or stops the synthesis of the collagenous matrix in connective tissue (Moon et al., 2008). The result showed that fucoidan has effectively inhibited the UV-B in human fibroblasts by reversing the ERK pathways. This indicates it's potential to be used in the dermatological formulations. Cholesterol level maintenance is a new area of clinical management system due to the risk factors associated with it. For the same, the use of dietary fibers that too with ion-exchange properties is encouraged (Guillon and Champ, 2000). In his work Pengzhan et al. (2003) has attempted to uncover the effectiveness of Ulvan, a sulfated polysaccharide, isolated from *Ulva pertusa.* The result showed a significant reduction in the level of serum triglycerides, Low Density Lipoprotein–Cholesterol and increase in High Density Lipoprotein–Cholesterol. This proves the anti-hyperlipidemic potential of Ulvan. Sequestration of bile acids through the uronic acids and sulfate moieties are other activities of Ulvan (Lahaye, 1991). Another aspirant is porphyran isolated from red algae *Porphyra yezoensis* also possess anti-hyperlipidemic potential (Tsuge et al., 2004). Porphyran is further studied to see its effect on lipid synthesis in human liver derived cells. Results showed that it reduces the lipid synthesis which eventually would reduce the apolipoprotein B100 secretion (Inoue et al., 2009). The apolipoprotein B100 level is very important for very low density lipoprotein and its amount in the blood is positively correlated with cardiovascular diseases (Huff and Burnett, 1997). Hence, it is established that the hypolipidemic activity of the porphyran is correlated with the sulfate moieties present in it. Moreover, *Spirulina* is reported to possess a range of activities that are positively correlated with human health. A review by Belay et al. (1993) has enlisted its beneficial activities like cholesterol reduction, increasing intestinal lactobacilli. Moreover, it helps to reduce drug and heavy metals mediated nephrotoxicity and also provides protection against the harmful radiation. The algae derived bioactive peptides are also found to reduce the risk of cardiovascular diseases by lowering the level of cholesterol (Wergedahl et al., 2004). Carotenoids like fucoxanthin or astaxanthin are extracted from numerous algal species. Fucoxanthin is the main pigment found in brown algae. The oxygenated carotenoids are an effective inhibitor of cellular growth and promote apoptosis (Kotake-Nara et al., 2001; Capelli et al., 2013). Hence, it makes a channel to be used in the treatment of cancer. They also hold anti-inflammatory (Shiratori et al., 2005), antidiabetic (Maeda et al., 2007), and antioxidant activities (Sachindra et al., 2010). Astaxanthin, another pigment, has been procured from different marine organisms but *Haematococcus pluvialis* is the major producer. The pigment constitutes approximately 5% of its dry weight (Yuan and Chen, 2000). The positive results indicate and encourage to explore the naïve areas of the marine community with the view of more novel molecules can be discovered.

1.4 ALGAE AS NUTRACEUTICALS

In many countries, the food industries use a wide range of algae due to the high share of vitamins, minerals, fibers, antioxidants present in it (Korhonen and Pihlanto, 2006). Nutraceuticals have emerged as a major section of the pharmaceutical industries (Figure 1.3). This is mainly due to the malnutrition and modern sedentary lifestyle that prevails globally. The marine borne seaweeds are considered as the most promising agent for nutraceuticals

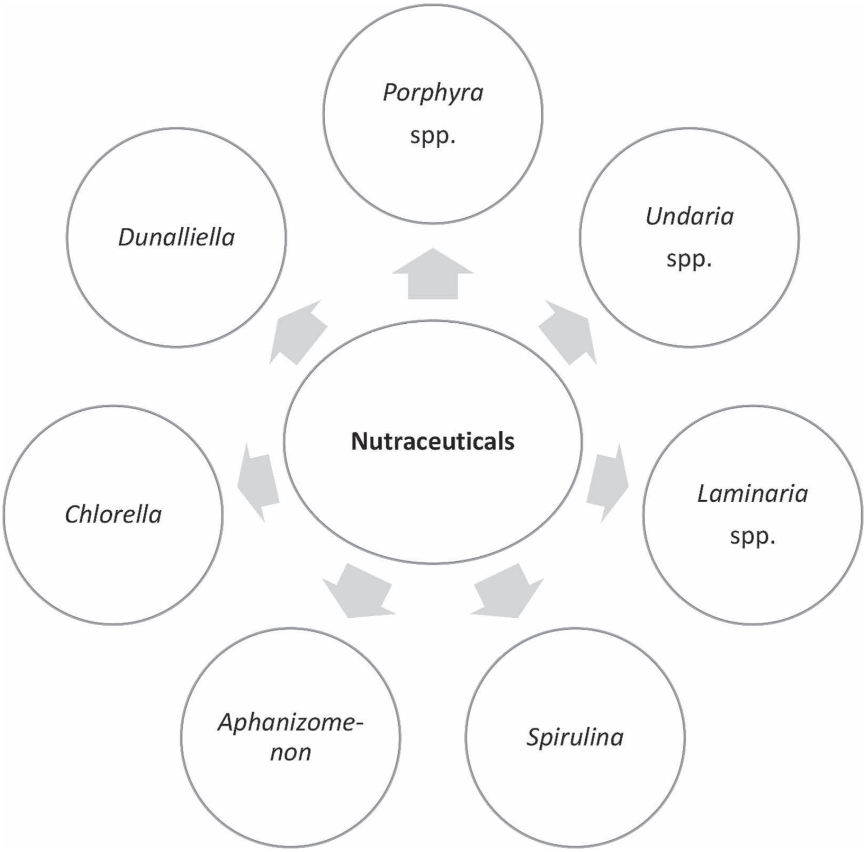

FIGURE 1.3 Major algal species used as nutraceuticals.

(Mohamed et al., 2012). Asia and the Pacific consume seaweeds for centuries due to its wide range of health benefits. However, its importance is realized recently across the globe with an increase in awareness and due to information transfer technology. The use of seaweeds as raw material is often seen in the manufacturing units of jam, cheese, tea, noodles, etc. The European countries specifically use it for health benefits like food industries and pharmaceuticals (McLachlan, 1992). Moreover, many species of seaweeds are nutritionally rich and can be used either raw or after processing (Kumar et al., 2008). One of the blessed species is *Porphyra* spp., red algae, which is a reservoir of protein, vitamins, minerals, and dietary fibers (Senevirathne et al., 2010). Moreover, it contains iodine and numerous bioactive molecules of therapeutic use (Rao et al., 2007). Incorporation of fucoxanthin and fucosterol, derived from *Undaria pinnatifida*, in pasta enhances the antioxidant properties and so the value of pasta is another example (Prabhasankar et al., 2009). Many algae including seaweeds are an exceptional source of dietary fibers, minerals. They are widely used to treat problems like vitamin deficiency, weight gain, etc. Seaweeds are used in foods for several reasons namely color, flavor, texture, and value. They are an excellent source of protein constituting about 20–25% of the dry weight. Moreover, they are a brilliant source of vitamin which includes A, D, B1, B12, E, riboflavin, niacin, pantothenic acid, and folic acid (Croft et al., 2006). Interestingly, the level of vitamin C is exceptionally high in many seaweed species that matches the level of citrus fruits. They also contain trace elements essential to human consumption (Macartain et al., 2007). It is estimated that the genera *Porphyra*, *Undaria* and *Laminaria* together contribute to a value of US$ 1 billion. The nutritional value of algae is not only restricted to seaweeds, microalgae too contribute hugely in nutraceuticals. They are well known for their unique proteins that constitute almost 40–70% of the total dry weight depending on the species. They are a piece of protein machinery capable of producing all the amino acids and can serve humans and animals with the essential ones (Guil-Guerrero et al., 2004). They also possess carbohydrates that exist in the form of glucose, starch, sugars, and polysaccharides. The algal cells contain lipid in the range of 1–70% of dry weight which is likely to increase up to 90% under the influence of certain conditions (Metting, 1996). Moreover, they possess ω-3 and ω-6 fatty acids that are important sources of all vitamins (Ryckebosch et al., 2012). Microalgae are also a rich reservoir of pigments like chlorophyll, carotenes and phycobiliproteins. Green algae possess chlorophyll which is structurally similar to heme. The only difference between the two is that hemoglobin is formed around iron whereas chlorophyll has a central magnesium atom. In this regard, haem can be built in our body using chlorophyll by exchanging the central magnesium ion with the iron. The mechanism explains the importance of consuming chlorophyll rich foods that ultimately improves blood condition and poor tissue oxygenation. It is seen that *Spirulina*, *Chlorella*, and *Aphanizomenon* are used in China and Japan as prescription nutraceuticals (Jensen, 2001). UK has started the consumption of *Aphanizomenon flos-aquae* (AFA) as a nutraceutical for a wide range of health benefits including boosting immune function, protective against heart diseases and general well-being (Kushak et al., 2000). Moreover, algae have a unique ability to cleanse and purify and simultaneously it nourishes and rebuilds the tissues. *Aphanizomenon flos-aquae* is reported to bear most concentrated of all the chlorophyll food sources on earth and constitutes about 3–6% of it (Benedetti et al., 2004). Hence, it exhibits cleansing and detoxifying action. Moreover, its high protein content, i.e. 60% of its dry weight makes it an evident pool for various amino acids. Algae are the reservoir of alkalizing compounds and known to be the most alkalizing of all the natural foods. It maintains the internal environment in an alkaline condition that explains its credibility in the treatment of cancer (Coleman and Colman, 1981). Algae are well known for their pigments and their bioactivities. One of the major pigments is phycocyanin produced abundantly by *Spirulina* and AFA aids in the process of linking amino acids. They are the vital components of neurotransmitters that maintain the coordination between the cells. Furthermore, AFA is the only source of phenylalanine that is reported to alleviate depression and help to uplift the mood. Hence, it is a promising candidate for the treatment of neurological imbalances, learning difficulties and attention related disorders. Hence, intake of AFA is related to the improved concentration, mental focus, mental stamina and sleep quality. One added benefit of AFA is its diuretic potential that aids in maintaining internal homeostasis. AFA can also modulate the immune system by increasing the release of NK cells that forms the first line of defense against viruses. AFA directly changes the immune cell trafficking thereby immune surveillance is increased without stimulating the immune system. AFA possesses the unique ability to boost the release and migration of stem cells from the bone marrow (Jensen et al., 2007). It is unique in being the only natural component able to stimulate stem cells. Henceforth, its use in the treatment of Alzheimer's disease, Parkinson's disease, multiple sclerosis, diabetes, regeneration is likely to give intended results. Phycocyanin, a pigment, isolated from AFA possesses antioxidant and anti-inflammatory potential. The pigment has effectively inhibited the growth of cell lines in vitro thus provides a ray of hope in the cancer treatment (Gardeva et al., 2014). *Spirulina*, *Chlorella*, and *Dunaliella* are rich sources of vitamin A and a source of numerous micro and macronutrients. They are widely used as nutraceuticals after its safe effect on human consumption studies (Tang and Suter, 2011). The main group of nutraceuticals comes from the species *Spirulina*. It contains relatively a high percentage of protein (68%) and other nutritive properties (Spolaore et al., 2006). Additionally, it is associated with the alleviation of high serum glucose, safeguards against renal failure, protection against hypertension, and promoting the growth of *Lactobacillus* in the intestine (Vílchez et al., 1997; Liang et al., 2004). It contains 0.7–1.1% of chlorophyll which makes it mild and tonifying than other species. *Spirulina* has efficiently stopped the replication of HIV in human peripheral mononuclear cells, T cells, and Langerhans cells (Qureshi et al., 1996; Ngo-Matip et al., 2015). Hence, its use is encouraged worldwide to strengthen the immune system. Moreover, it is analogous to AFA in promoting mental concentration. It is largely due to the presence of phycocyanin. The walls of the species are coated with mucopolysaccharides that effectively strengthen the cardiovascular system against deadly vascular diseases. A study conducted with the random, placebo-controlled, geriatric patients revealed that *Spirulina* has reduced the LDL to HDL ratio significantly (Park et al., 2008). Hence, it is considered as a cholesterol lowering supplement. Moreover, AFA and *Spirulina* have been promoted by doctors to treat patients exposed to radiation due to its high chlorophyll content (Soheili and Khosravi-Darani, 2016). Its application in topical ointments quickly heals the ulcers that have become gangrenous. The next important microalga is *Chlorella*, a very popular nutraceutical. It is next to *Spirulina* in its protein content that constitutes around 51–60% of its dry weight. The most unique characteristic of *Chlorella* is its outer cell wall that firmly binds with pesticides, carcinogens, and heavy metals. Thus its uptake assures the detoxification and gentle removal of toxins out of the body. This made their acceptance easy in chemotherapy which uses heavy metals like Carboplastin, Cisplatin. β 1-3 present in it is an active immunostimulator, free radical scavenger. Moreover, it is consumed to ease a number of health issues like constipation, wounds, gastric ulcers, hypercholesterolemia, atherosclerosis (Yamaguchi, 1996; Jong-Yuh and Mei-Fen, 2005). The growth factors commonly known as Chlorella Growth Factor (CGF) are derived from its genetic material and play a major role in cellular repair and regeneration process. It would prove as an effective supplement as the damaged causes by the nonspecific sources of the environment is more prevalent as we age. It is noteworthy that Dr. Scultze's superfood comprises of these three algae, a blend of approximately 90–95%. The other 5–10% includes spinach leaf, acerola cherry, dulce, beet tops, palm fruit, rose hips, lemon, and orange peel. It is one of the different blends of chlorophyll rich food available internationally. A detailed insight into the bioactivities of algal species and their possible incorporation as nutraceuticals agents is discussed below.

1.4.1 PHLOROTANNINS

Marine seaweeds such as *Ecklonia cava*, *Hizikia fusiformis*, *Sargassum thunbergii*, *Eisenia bicyclis* gather a variety of polyphenols known as phlorotannins. They exist in different conformations with low, moderate and high molecular weight. They are hydrophobic and contain phenyl as well as phenoxy units. Pholorotannins are widely used as nutraceuticals due to health benefits like antioxidant properties (Li et al., 2011).

1.4.2 SULFATED POLYSACCHARIDES

Algae contain unique sulfated polysaccharides and their amount and constituent vary in different algal groups. The active SPs of marine algae are fucoidan and laminarans (from brown algae), carrageenan (from red algae), ulvan (from green algae). SPs have made their way to the nutraceuticals industries in the last few years. The use of carrageenans in the manufacturing of food additives like stabilizers, or thickeners represents the best example (Campo et al., 2009). Moreover, ulvan (Lahaye and Robic, 2007) and fucoidans (Li et al., 2008) are used as nutraceuticals and as functional foods respectively due to their nutritional values.

1.4.3 FUCOXANTHIN AND ASTAXANTHIN

Fucoxanthin and astaxanthin are famous carotenoids and synthesized by algae, plants, microorganisms, and fungi. These pigmented compounds are highly demanded due to its association in preventing deadly human diseases like hypertension, cancer, and many other chronic diseases. Brown seaweeds are the largest source of fucoxanthin and produce more than 10% of total carotenoid production in nature (Chuyen and Eun, 2017).

1.4.4 LECTINS

Lectins, the glycoprotein, are isolated from bacteria to animals and serve as the recognition molecules in cell-cell interactions. Suddenly, their demand increased in the biomedical industries due to several bioactivities (Cardozo et al., 2007). Sato et al. (2000) have reported that seaweeds constitute a major source of lectins. Algal lectins are drawing major attention due to the characteristic features like thermal stability, monomeric forms, low molecular weight and metal-independent hemagglutination (Hori et al., 1990). Moreover, phytolectins are highly specific and interact with the targeted glycoprotein. Hence, its journey in the development of antibiotics would be a successful one (Rogers and Hori, 1993).

1.4.5 MYCOSPORINE-LIKE AMINO ACIDS (MAAs)

Recently, a group of intracellular compounds known as MAAs is identified. They are recorded to protect various aquatic organisms against UV light. Mycosporine-glycine (Suh et al., 2003) and mycosporine-taurine (Zhang et al., 2003) belonging to the family of oxocarbonyl-MAAs showed to work against the cellular damage caused by oxidative stress (Arbeloa et al., 2010).

1.4.6 PROTEINS AND PEPTIDES

The protein content varies in different seaweed species. The green and red seaweed (10–45% of the dry weight) contains a higher share of protein than the brown seaweeds (3–15% of the dry weight). With regard to high protein or peptide contents of algae, red algae have emerged as a promising source of food proteins that can be developed as nutraceuticals (Fleurence et al., 2018). Isolation of peptides solely depends on the enzymatic degradation along with the suitable range of temperature and pH of the solution. The appropriate extraction process fulfills the demand for novel biopeptides with antioxidant, anticoagulant, and antihypertensive activities (Harnedy and Fitzgerald, 2011). This explains the nutritional value of algal proteins and their future as a possible nutraceutical. Currently, the algae based nutraceuticals are widely used and they have a high market value. The basic nutraceuticals include food and diary supplements, value-added processed foods and non-food supplements viz. tablets and soft gels (Ansorena and Astiasarán, 2013). Omega 3 polyunsaturated fatty acids (PUFA), β-carotene, astaxanthin, carotenoids, etc. are the prime compounds extracted from algae. PUFA is of great interest for its role in major metabolisms. Algal groups namely Chlorophyta, Bryophyta produce many of the essential fatty acids. It also produces N-6 PUFA that is an important diet rich fatty acids. Microalgae are in use for their nutraceuticals properties which is positively correlated with the protein content in the host algae. For instance, the vitamin pool found in *Haematococcus* species makes it a more interesting candidate for nutraceutical development. Natural Algae Astaxanthin Association (NAXA) set to identify the unique benefits of astaxanthin. They are produced from carbon precursors and commercialized as a potent food supplement.

1.5 CONCLUSION

Algae have been a matter of interest to the scientific community for ages. Though the freshwater ecosystem is quite closer to mankind but marine algal community harbors (embrace) the largest unexplored reservoir of components of biological importance. Unfortunately, based on the area to study ratio, we see only a few reports on the same in the literature. Therefore, this chapter endeavors to gather the reported functional groups, like proteins, sulfated polysaccharides, fatty acids, pigments and putting the beads in the string. Since the extraction processes have a large influence on the final product, hence an elaborated section on sample processing and harvesting are discussed. In this regard, major emphasis is given to the organic solvent derived extraction and enzyme-assisted extraction processes. Furthermore, the algal components were elaborately discussed with regard to their uses to serve the recent pharmaceutical and nutraceuticals needs. Interestingly, the functional groups can act as a molecular drug. The bioavailability and target specificity are among a few traits that make them a wise choice. The list continues with low mammalian toxicity, least accumulation in the body tissues that makes its acceptance easier. Taken together, the shreds of evidence suggested that the biological function associated with the algae open door in future nutraceuticals and pharmaceutical industries. The major constrain so far is the studies that are mainly focused on the in vitro system or maximally reached to a mouse model. With the purpose of increasing its usage, prototypes should find its way to preclinical studies followed by its validation as a commercialized drug or nutraceuticals.

KEYWORDS

- **algae**
- **biological activities**
- **extraction**
- **farming**
- **nutraceuticals**
- **pharmaceuticals**
- **secondary metabolites**

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PART II

CULTURE CONDITIONS AND CULTIVATION STRATEGIES

CHAPTER 2

ALGAE CULTIVATION STRATEGIES: AN OVERVIEW

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ABSTRACT

Algae are important biological forms that have been used in its raw or processed state in a variety of sectors. Different types of microalgae and macroalgae were identified still, whereas the complete exploitation of algal species is restricted due to few concerns as unawareness of mass cultivation systems, specific culture methods, culture conditions or growth parameters. On the other hand, the examination of the potential of various algae for value-added products is necessary at the moment to ensure sustainable development. Hence, the present chapter discusses the strategies that can be admirable for the cultivation of algae for its extensive utilization. The choice

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Jeyabalan Sangeetha, Devarajan Thangadurai (Eds.)

of cultivation system like open or closed or dark system could be based on the scale and purpose of cultivation. Among the closed type systems, continuous systems and photobioreactors are more preferable for large-scale cultivation. There are different methods that can be accessible for algae cultivation as autotrophic, mixotrophic and heterotrophic modes. Media selection is another criteria, where the selection could be done according to the nature of habitat of algae. In addition, diverse isolation techniques, nutrient requirements, growth parameters and sterilization techniques feasible for the implementation of specific algae cultivation schemes are discussed in detail.

2.1 INTRODUCTION

Algae are significant group of life forms that are widely inhabited in ocean, river, lake, ponds, brackish water etc., since watery or moist environment is preferred for the growth development of the algae. They are photosynthetic, unicellular or multicellular organisms possess lack of a well-defined body. Both sexual and asexual mode of reproduction is found in algae, where the mode of reproduction is varied with species to species. Generally, algae range from unicellular microalgae (*Chlorella*) to multicellular macroalgae (Brown algae). Although, the thriving characteristics of various micro and macro algae throwed interest in many scientists and engineers to work with novel aspects and exploration of algae for socio-economic development, for the time being. However, a deep understanding on algae cultivation techniques is necessary at the moment to explore more algal species with economic importance along with its absolute utilization (Li et al., 2011). Hence, this chapter discusses about the techniques available for the cultivation of algae with respect to various algae culturing methods, growth influencing parameters and culturing systems.

2.1.1 CLASSIFICATION OF ALGAE

Based on the cellular makeup of algae, they are broadly categorized into two types: (i) microalgae; and (ii) macroalgae. Microalgae are small unicellular plant like organisms that invade in sea, river, pond and lake. They are also called as phytoplankton since they contain photosynthetic pigments and accessory pigments that assist photosynthesis. Microalgae are classified into two groups namely Diatoms and Dinoflagellates. The classification of microalgae and macroalgae are depicted by the Figure 2.1. Dinoflagellates (Pyrrhophyta) are marine, single-celled, eukaryotic algae with biflagellate structure, which belongs to the phylum Pyrrhophyta. Diatoms or

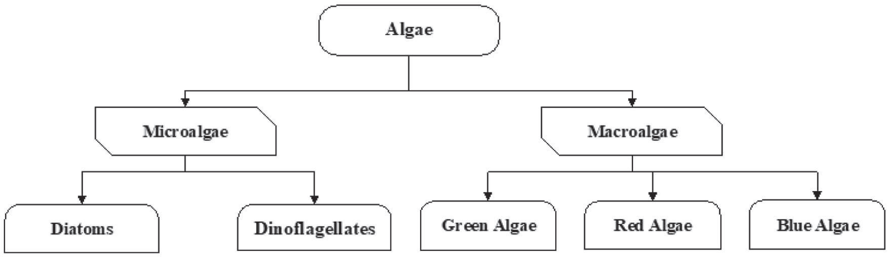

FIGURE 2.1 Classification of algae.

Bacillariophyta are the single-celled, eukaryotic algae found in different shapes. Microalgae sometimes cause algal blooms, as reported in many areas. There are about 12000 known species of Diatoms reported still. Few of them were reported to have efficiency in the production of economically important value-added products (Chen et al., 2018).

The second class of algae is macroalgae, which are large, multicellular organisms commonly called as seaweeds, which are mainly found with sea origin. They are again grouped in to red algae (Rhodophyta), green algae (Chlorophyta) and brown algae (Phaeophyta) (Borowitzka, 1999). There are about 6000 to 8000 species of green algae were identified, of which most of them where from fresh water habitat rather than marine habitat. In the case of red algae, around 4000 to 5000 species were explored and among that 90% belongs to marine origin. Agar and carrageenan are the most commercially important products obtained from red algae. Many stunning seaweeds are coming under red algae and most of them were known for their pharmacological importance. Hence red algae possess much industrial importance that are used worldwide to derive diverse economically important products. The third category is brown algae, which are commonly called as seaweeds. Around 1500 to 2000 species of brown algae were established and many of them have potential for producing pharmaceutical compounds. Most of them are edible, such brown algae getting much attention in food industries for the usage of its raw or processed form. The current trend of algae is the production of economically important products as biodiesel, biogas, alcohol, etc. Furthermore, the processed residue is used as good fertilizer.

2.1.2 IMPORTANCE OF ALGAL CULTIVATION

Nowadays, the importance of algae increased worldwide due to its diverse promising properties explored through various studies. However, the advancement in cultivation techniques are desired at the moment in order to eliminate various constraints associated with algae cultivation process. A deep understanding on algae cultivation techniques such as diverse cultivation methods, parameters involved and cultivation systems employed, is necessitate to assure the usage of apposite method for culturing of different types of algae. Although, the choice of cultivation media is primarily depending upon the type/species of algae to be cultivated. The open cultivation systems like ponds or lakes, tanks and raceway ponds have been found feasible for the cultivation of microalgae. Likewise, closed systems like bioreactors are also desirable for algae cultivation. Along with the cultivation systems, selection of culture vessels and media formulation directly influences the growth of algae.

Algae are rich with magnesium, calcium, potassium, iodine, iron and zinc that are necessary micronutrients, hence they are used as food supplements. For instance, a frequently used algae in smoothies is spirulina. Although, the presence of various functional compounds that has anticancer, antiviral, antioxidant and anti-inflammatory properties were reported among most of the algae identified. Due to their multi-nutritional characteristics, algae are used as organic fertilizer to enhance the growth of crop plants. The biofuel production capacity of algae are promising (>10–20%) than that of any other energy crops identified for the purpose (Mata et al., 2010; Ndimba et al., 2013). The existing applications of algae are presented in the Figure 2.2.

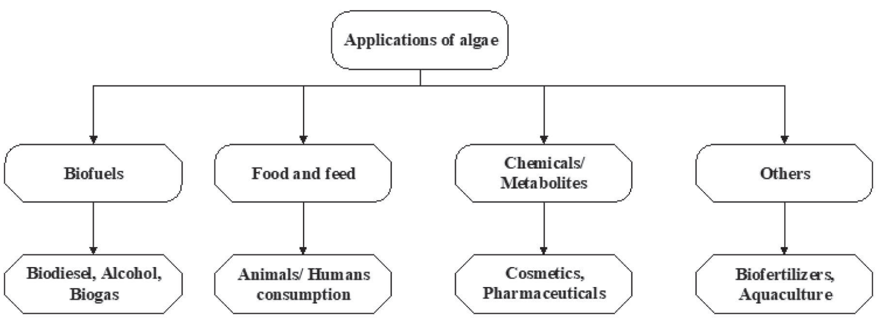

FIGURE 2.2 Diverse economical applications of algae.

2.2 CONVENTIONAL ALGAL CULTURING METHODS

2.2.1 HISTORICAL BACKGROUND

A German-born scientist Ferdinand, who had grown an algae *Haematococcus* (Chlorophyceae) in his laboratory for a short time and referred this practice as "cultivation." Followed by another scientist Miquel was the first one who had isolated freshwater and marine diatoms and established its pure or axenic cultures. Another study reported that non-toxicity study using *Spirogyra* was effective for the culturing of fresh water based algae since elements like copper showed negative impact on their growth (Lin 2005). Consequently, several novel isolation methods are introduced by Ward. He tried sterile, nutrient media of plaster of paris to grow algae and found that few hardy algae supported the growth on the hardened media. Another experiment done with enriched media, silica jelly, lime-water and carbon dioxide with the principle of formation of calcium carbonate, was also showed some algae exhibited growth. Ward also used first time a stencil to create patterns of algal growth on solid platform. This experiment was conducted by covering the inoculated plate with nontransparent sheet and made a clear shape of an alphabetic letter on the sheet. During the cultivation period, light entered into the dish through clear zone only, hence, obtained green growth in the area of illuminated zone as looking like an alphabetic letter on the solid media (Lin 2005).

2.2.2 ALGAL CULTIVATION IN 19TH AND 20TH CENTURY

A well-established algal cultivation technique was introduced by Zumsteinin in the 19th Century, who cultivated *Euglena gracilis* without any bacterial contamination through pure culture isolation using capillary pipette and acidic medium. Followed by Pringsheim worked on the same aspect to reduce bacterial contamination by single cell isolation technique with the aid of capillary pipette. In 1910, Allen and Nelson made cultures of *Chaetoceros*, *Skeletonema*, and *Thalassiosira* for the purpose to use it as provender to marine larvae. In the year 1912, Pringsheim also explored the function of soil extract and minerals supplemented media for algal cultivation to attain good growth. Russians used mineral salt supplemented media to obtain pure cultures of *Volvox* in course to prevent precipitation (Lin, 2005; Singh et al., 2015). In the following years, advanced centrifugation techniques were introduced to isolate algae and thereby to develop pure cultures. Dense laboratory cultures was achieved by Warburg (1919) who established microalgae (*Chlorella*) growth in Berlin among dense cultures by using the centrifugation principle (Borowitzka, 1999).

In 1949, Ketchum flourished the algae cultivation through semi continuous culture model and obtained good yield (Lin 2005). The scientists Myers and Clark employed a larger gadget for culturing *Chlorella* in the University of Texas through continuous culture method (Myers and Clark, 1944). Until 1950s, natural habitats were the typical source to harvest seaweeds. Advanced cultivation methods were established in the subsequent years. The concept of synchronous cultures were first developed by Tamiya in Tokyo (Burlew, 1953). The extensive cultivation of *Porphyra* had been started in the seventeenth century in Tokyo Bay, is the seaweed that mainly used as food in coastal regions of southeast Asia and Pacific Ocean basin began (Santelices, 1999). Open raceway ponds were reported as the primarily used platform to cultivate microalgae. Followed by, large-scale cultivation of microalgae and macroalgae were initiated in the following years (Johnson et al., 2018).

2.3 CULTIVATION SYSTEMS

There are two types of systems generally used for algal cultivation at present. They are open systems and closed systems. A highly advanced closed system for algae cultivation is dark systems, where an oxygen free environment is maintained. An overview of common algal cultivation systems is presented in the Figure 2.3. A detailed description of both the types of systems are given below. Open cultivation systems include all the outdoor natural or artificial sources like pond, lake, etc. Closed systems are tightly closed where the algal growth is restricted for some area in a tank, for instance, photobioreactors (Dębowski et al., 2012).

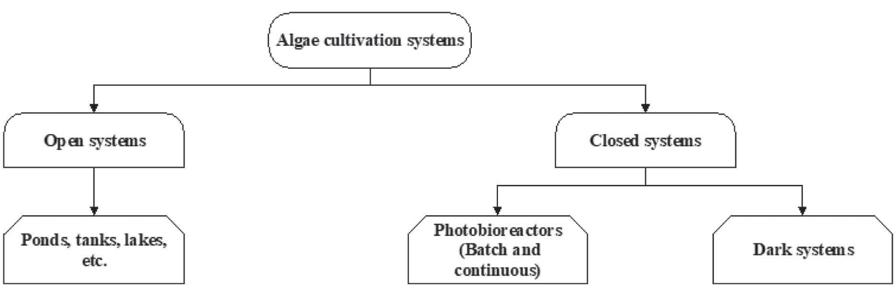

FIGURE 2.3 Algal cultivation systems.

2.3.1 *OPEN SYSTEMS (OPEN-WATER CULTIVATION TECHNIQUE)*

The open systems may be naturally occurring or artificially created space where algae can be cultivated in an open area at normal environmental conditions. Such systems include traditional water sources as ponds, ground or concrete structures with a surface area of about 250 hectare and a depth of around 0.5 m, raceway ponds and cascade type ponds (Borowitzka, 1999). Mechanical stirring or stirring using paddle agitator is preferred for such systems to assure maximum algal growth. Where, the addition of microelements is desirable to ensure better nutrition and thereby to avoid the deficiency of various micronutrients in case of open natural systems. There are several advantages and disadvantages noted for open systems in contrast to closed system, are tabulated in the Table 2.1 (Mata et al., 2010).

TABLE 2.1 Significance and Drawbacks Associated with Cultivation Systems

Type of Systems	Open Systems	Closed Systems
	Round/Raceway Ponds	Vertical/Plate/Horizontal Tubular Photobioreactors
Significances	• Easy to use • Cost-effective • Easy cleaning • Large-scale cultivation • Provide large-surface area	• Good mixing • Ensures sterility • Good productivity • Controlled conditions/ parameters • Reduced photoinhibition • Algal immobilization possible
Drawbacks	• Low productivity • Limited culture species • Affects external factors • Uncontrolled conditions • Difficult long-term cultivation • Chances of contamination	• Cost-expensive • Risk of fouling • Small-surface area exposure • Causes hydrodynamic stress • Skilled person needed to operate • Maintenance difficult

The significance of open platform is simple, inexpensive and no need of skilled person. Open systems are user friendly, easy to clean and it provides a larger area for the free growth of the algal species cultivated. Apart from that, lower cost compared to highly sophisticated closed system, is another significant of open systems. In some extent, open systems are preferred for mass cultivation, for both domestic and commercial purposes. However, it is difficult to maintain the growth parameters mentioned as evaporation, temperature, etc. in open pond system (Mata et al., 2010). Although, some disadvantages reported in case of open systems are low production rate, uncontrolled environmental factors, high chances of contamination and promotes selective species only. The other disadvantages such as high water loses as a result of evaporation, susceptibility of specific algae to infections were also reported in some cases. In case of racetrack ponds, a small paved portion or channels (1000 to 5000 m^2) constructed by PVC or concrete or other materials could be used to grow algae, in fact, the medium to be continuously supplemented to attain maximum growth in such cases. The cultivated biomass can be collected from the device located at the end of the loop before the paddle wheel.

2.3.2 CLOSED SYSTEMS

Closed systems are purely constructed closed by means of cultivating algae in course of controlling various parameters to ensure higher yield. There are two types of closed systems commonly used, which are photobioreactors and dark systems. In closed systems, algal inoculums can be cultured under controlled condition in a restricted area. Such systems provide real-time monitoring and control of various growth parameters such as temperature, light, contaminants and other rate-limiting factors. This type system also possesses some advantages as well as disadvantages. The significance of such cultivation includes better productivity, controlled parameters, ensures sterility and proper stirring along with reduced photoinhibition of algal growth. However, expensive nature, fouling characteristic, hydrodynamic stress and small surface area are the drawbacks reported by the system (Dębowski et al., 2012). The closed systems are further categorized on the basis of working mechanism, as follows:

- Batch systems
- Continuous systems
- Fed-batch culture systems
- Immobilized culture systems (entrapment)

2.3.2.1 BATCH SYSTEMS

Usually, microalgal cells are cultivated through this method. In this type cultivation, a specific amount of culture medium and inoculum are poured in to the chamber and cultivate the algae until that reach its maximum growth under incubation with suitable conditions. One-time addition of inoculum and one-time removal of cultivated biomass is offered by such systems, hence, which supports cultivation for one batch culture at a time. Once the cell density reaches its threshold, the resulted biomass is separated and harvested from the medium. These kinds of systems are easy to operate than continuous cultures, since this is a most preferred method for viable cultivation of algae. The exchange of nutrients and gases during incubation period can be accomplished by gentle or mechanical agitation. Both small-scale (simple conical flask) and large-scale production (fermenter) cultivation can be furnished through batch cultivation method. In course of autotrophic or mixotrophic culture, carbon dioxide (CO_2) enriched air is supplied to the system once there is its deficiency occurs. Likewise, either natural illumination or artificial light sources can be opted according to the type of cultivation desires (Javanmardian and Palsson, 1991; Muller-Feuga, 2000; Ugwu et al., 2008). Moreover, there is low requirement for complete sterilization whilst its cleaning process requires much time.

2.3.2.2 CONTINUOUS SYSTEMS

In continuous systems, a constant inflow and outflow of culture medium is maintained during cultivation, where always maintains the algal growth rate close to the maximum. There is a continuous addition of culture media required to meet the nutrient requirements of homogeneously mixed culture. Whenever the algae reach its exponential phase, culture is removed and harvested constantly. This kind of cultivation is based on the principle of substrate depletion and products accumulation occurred during the growth. Due to the continuous cultivation process, the growth promoting substrates to be added eventually to avoid cell depletion along with growth inhibitory products to be removed during the cultivation period. However, continuous system is more economic due to the fact that which ensures continuous production of biomass and there is no need for system cleaning after every batch culture (Borowitzka, 1999).

2.3.2.3 FED-BATCH CULTURE

The fed-batch or semi-continuous systems work with the principle of continuous addition of culture or media while partial reception of biomass. In such systems, a portion of the growing culture is occasionally picked out from the culture and fresh culture medium is continuously added to the system. Thus, the concentration of media is not persistent during cultivation. The biomass concentration and culture parameters could be varied according to the fed-batch cycle maintained. Fed-batch culture is preferred extensively in industrial algal biomass production.

2.3.2.4 IMMOBILIZED CULTURES (ENTRAPMENT)

In this method, natural polysaccharide gels or synthetic polymers are used to entrap microalgae cells (Moreno-Garrido, 2008). Calcium alginate is the most frequently used chemical for the entrapment of all kind of cells. The alginate beads offer a protective and steady micro environment for their growth with exact growth rate under specific conditions. This method is selective and was effectively applied for the cultivation of *Haematococcus* spp. in a high shear bioreactor at a temperature of 32°C (Satpati and Pal, 2018).

2.3.3 PHOTOBIOREACTORS

Photobioreactor is a closed system used for culturing a wide variety of algal species under controlled conditions. Horizontal tubular photobioreactors, biocoil type reactors, continuous/semi-continuous big bag systems and flat-plate photobioreactors are the various types of photobioreactors available for the purpose. Photobioreactors that can operate under different climatic conditions are also available at the moment with apposite control measures. The first closed photobioreactor attempt was made with a big gags photobioreactor, which was fabricated by large sterile plastic bags with an adjusted aeration system. Glass or polycarbonate (PC) was used to construct most of the tubular reactors where the supply of culture medium and gases accomplished through pumps or airlift system. Such additional fittings may be done in the horizontal, vertical or inclined style. The airlift system also assists mixing of the contents in the bioreactor at apposite time intervals. However, poor temperature control during culturing is noted as the main difficulty associated with this kind of bioreactors. Biocoil sort photobioreactor is an example of tubular photobioreactor, which is usually fabricated with transparent plastic tube with a screw-wrap, where the temperature may be controlled manually or automatically (Concas et al., 2016). Such type reactor designs pledge constant mixing and thereby to reduce the tendency of cells to adhere on the inner surface of the system. The drawbacks of photobioreactors are it requires all process control measures and skilled personnel which make it as an expensive system.

2.3.4 DARK SYSTEMS

These are also closed type system and the cultures obtained through this method are also termed as heterotrophic cultures. In closed systems, the culture medium could be fed to the bioreactors in which acetate or glucose can be supplemented as a sole source of carbon. There are certain criteria required for maintaining heterotrophic cultures which includes pH, temperature, agitation and nutrient composition. There is no light requirement for dark systems. A temperature range of 26–28°C and pH between 6.1 and 6.5 is needed for algal cultivation in dark systems. Moreover, the desired agitation range is 200–480 rpm. The nutrients as carbon:nitrogen:phosphorous present in the medium should be in the ratio of 9:1.25:1.25. Hence the maintenance of constant composition of carbon, nitrogen and phosphorous source is very essential. Usually, glucose or acetate (20 g/l) is preferred as carbon source for algal growth in dark systems. Closed photo-bioreactors are considered as a better tool for biofuel generation from biomass at current scenario (Lehr and Posten, 2009).

2.4 DIVERSE ALGAE CULTIVATION METHODS

Microalgae follow diverse metabolic pathways to retain their maximum growth using different raw materials. Based on the type of nutrition they follow, algae can be cultivated by three methods. They are autotrophic/phototrophic/photo-autotrophic cultivation, mixotrophic cultivation and heterotrophic/photo-heterotrophic cultivation. Various features of different cultivation methods are tabulated in the Table 2.2 (Chen et al., 2011).

TABLE 2.2 Features of Various Algal Cultivation Schemes (Chen et al., 2011)

Cultivation Conditions	Energy Source	Cultivation Systems	Cell Density	Economic Feasibility
Phototrophic	Light source	Open pond/ photobioreactor	Lower	Less expensive
Heterotrophic	Organic source	Fermenter	High	Average cost
Mixotrophic	Light/organic source	Closed photobioreactor	Medium	Highly expensive
Photoheterotrophic	Light source	Closed photobioreactor	Medium	Expensive

2.4.1 AUTOTROPHIC/PHOTOTROPHIC/PHOTO-AUTOTROPHIC CULTIVATION

It is the most commonly employed method for microalgae cultivation. It is done in open ponds or photobioreactors. In autotrophic metabolism, light serves as energy source and inorganic carbon serves as carbon source. As a result, chemical energy is formed by the conversion of light energy through the biological process photosynthesis. Such autotrophic algae require the raw materials light, CO_2, water, nutrients yielding lipids, proteins and sugar rich compounds to carry out the process. Light availability is essential to ensure good growth of such photosynthetic algae. Sometimes, cellular self-shading decreases the light availability which restricts total biomass production, as noted in many cases. Since, low biomass concentrations are preferred while growing autotrophic cultures. Another constraint is the requirement of gas-liquid transfer that is necessary to evade oxygen (O_2) accumulation occurred during photosynthesis in the liquid culture. A CO_2-rich environment is favored to enhance biomass productivity throughout the process. During photo-autotrophic cultivation, light penetration decreases with increasing the turbidity of the medium. Hence it is essential to control biomass concentration by timely biomass removal. The design of photobioreactors affects photo-autotrophic cultivation in such a way that which affects fluid dynamics and total biomass productivity (Concas et al., 2016).

2.4.2 MIXOTROPHIC CULTIVATION

The mixotrophic cultivation requires a combination of autotrophic metabolism and heterotrophic metabolism where oxidation of reduced carbon source occurred in the medium. Mixotrophic cultivation enhances biomass production by improving the availability of different energy sources. The main substrates used for mixotrophic microalgae cultivation are glucose, ethanol or other cheaper waste products such as acetate or glycerol. In this mode, light and organic carbons are instantaneously provided hence such cultures follow both heterotrophic and autotrophic metabolism. As a result, the total biomass productivity could be higher due to simultaneous metabolisms. Moreover, the necessity of gas–liquid exchange in mixotrophic cultivation is lower than that of other type cultures.

The photosynthetic rate is not strictly depending upon the quantity of light availed but it depends upon the accurate intensity of light provided. Growth reduction due to photo-inhibition of mixotrophic cultures are reported in earlier, which could be reduced by avoiding too low or too high illumination. Mixotrophic cultivation is advantageous due to the fact that which improves growth rate, reduce biomass loss through pure respiration in dark hours and enhance biomass productivity. High lipid productivity obtained through this cultivation shows its promising prospect in biodiesel production. Moreover, CO_2 obtained through aerobic respiration during mixotrophic cultivation could be employed for photosynthesis, is another environmental benefit attainable form this method by offering carbon credit (Wang et al., 2014).

2.4.3 HETEROTROPHIC CULTIVATION

In heterotrophic cultivation, organic compounds serve as both energy and carbon source. There is no requirement of light hence the issues associated with photo limitation is not a part of it and also higher biomass productivity can be achieved. In heterotrophy, a wide range of organic compounds including organic acids can be adopted for algal growth. Few studies reported that cheaper sources like corn powder hydrolysate and glycerol are more affordable for the purpose. A constraint behind heterotrophic cultivation is that this type cultivation requires large quantity of organic compounds in order to compensate light utility along with bioreactors, that makes this method so expensive when compared to photo-autotrophy (Chen et al., 2011; Lowrey et al., 2015).

Another type of heterotrophic cultivation is photo-heterotrophic cultivation, in which light serve as energy source instead of organic source. Closed photobioreactors are used for photo-heterotrophic cultivation. Medium cell density is maintained during such type of cultivation. However, high production cost is reported as a constraint behind this kind of culturing (Chen et al., 2011).

2.5 IMPORTANCE OF ALGAL CULTIVATION

2.5.1 MICROALGAE CULTIVATION AND ITS SIGNIFICANCES

Open system or open-water cultivation method or pond cultivation technique is the most frequently used method for cultivation of microalgae. At first, microalgae were grown in open systems to increase their biomass while after recognizing their lipid production potential, batch systems are used (Sharma et al., 2012). By photosynthesis, most of the microalgae can synthesize all the biochemical components required for their growth. Hence microalgae cultivation requires light, water, carbon dioxide, nutrients and trace elements. Microalgae possess various important properties and most of them are known for diverse commercial applications as presented in the Table 2.3.

TABLE 2.3 Commercial Importance of Various Microalgae

Algae	Sources
Spirulina	γ-Linoleic acid, vitamins
Dunaliella	β-carotene, glycerol
Chlamydomonas	Polysaccharides
Chlorella	Special growth factor, food supplements
Porphyridium	Polysaccharides, arachidonic acid
Haematococcus	Astaxanthin (red pigment)
Blue-green algae	Ammonia

2.5.2 MACROALGAE CULTIVATION AND ITS ADVANTAGES

Nowadays, macroalgae or seaweeds are employed for the production of animal feed, fertilizers, biofuel, cosmetics and pharmaceuticals. Seaweeds have been used directly as food in many parts of the world for long years. It is also used as texturing agent in the processed form. Due to their gelling properties (agar), these are preferred in pharmaceutical, cosmetic, textile and food industries. Some macroalgae were directly adopted as soil conditioners in large extent. Certain red algal species are substantial for paper production and the resulted waste products can be used as raw material for bioethanol production. A green algae species *Ulva* spp. reported to have high levels of a polysaccharide Ulvan, which is extensively used for ethanol and methane production (Morand and Merceron, 2005; Adams et al., 2009; Kraan, 2013). The economic properties of macroalgae are primarily allied with the chemical constituents present in it. The chemical composition of various macroalgae in comparison with microalgae are presented in the Table 2.4 (Chen et al., 2015).

Some macroalgae species are cultivated in natural open water while some are easily cultivated in tanks and ponds (Rajkumar et al., 2014). For example, tank-based cultivation was effective for the species *Chondrus crispus* (Canada) and *Eucheuma* spp. (Florida), raceway pond-based cultivation was feasible for *Gracilaria* (Israel) and man-made ponds were suitable for

TABLE 2.4 Typical Chemical Components of Macroalgae in Comparison with Microalgae (Chen et al., 2015)

Microalgae	Macroalgae		
	Red Algae	Green Algae	Brown Algae
• Starch	• Carrageenan	• Starch	• Laminarin
• Arabinose	• Cellulose	• Cellulose	• Cellulose
• Fucose	• Lignin	• Ulvan	• Mannitol
• Galactose	• Agar	• Mannan	• Alginate
• Glucose	• Glucose	• Glucose	• Glucose
• Mannose	• Galactose	• Mannose	• Galactose
• Rhamnose	• Agarose	• Uronic acid	• Uronic acid
• Ribose	• Lipids	• Lipids	• Glucan
• Xylose	• Proteins	• Proteins	• Lipids
	• Ash	• Ash	• Proteins
			• Ash

various algal species in China, Taiwan, and Thailand. However, appropriate site selection, design formation, information on specific species metabolism, efficient strains, control of optimum parameters, agitation, nutritional addition, density management are the challenges reported for macroalgae cultivation (Morand and Merceron, 2005; Rajkumar et al., 2014; Montingelli et al., 2015). In some kind of cultures, cultivation techniques involve different stages. For instance, *Porphyra* cultivation includes four major steps such as culture of conchocelis, formation of conchospores followed by sporelings and harvesting.

2.6 STAGES OF ALGAL CULTIVATION

The cultivation of algae can be accomplished through diverse phases. Those are collection of sources, isolation, culturing, mass cultivation and harvesting (Figure 2.4). Collection of algae inhabiting source is the primary step that determines the species of algae to be cultivated. Subsequently, isolation, culturing and harvesting is being carried out. Where, algal isolation is an important step that require more attention; for that specific culture media, optimum parameters and culture conditions are to be provided. Once the culture parameters are optimized, large-scale mass culturing can be done by means of its feasible cultivation method. The important algae harvesting techniques are filtration, flocculation, flotation and sedimentation. The choice of harvesting method is depending upon the category of algae (microalgae/macroalgae), mode of cultivation and purpose of cultivation. Post-harvesting methods may vary according to the purpose of cultivation which includes direct processing, cell disruption, lipid extraction/conversion, anaerobic decomposition, alcoholic fermentation/biodiesel production, biofertilizer formulation, etc.

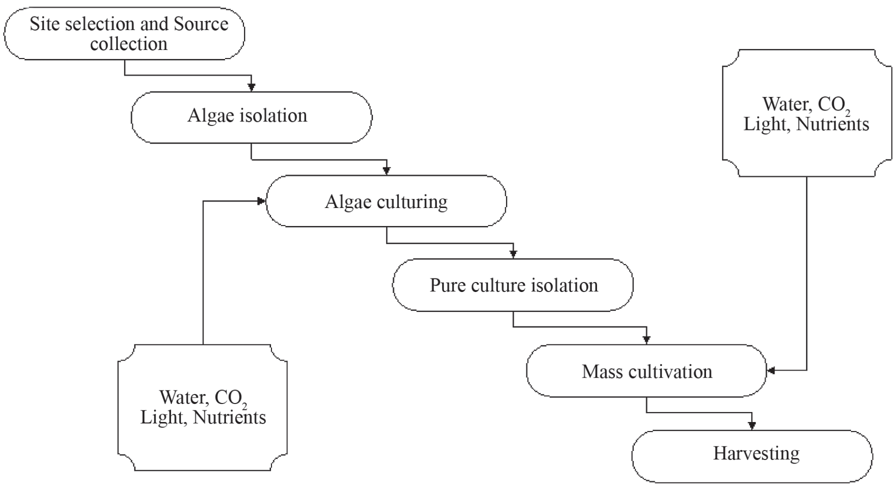

FIGURE 2.4 Different stages of algal cultivation.

2.6.1 ISOLATION OF ALGAE

2.6.1.1 TRADITIONAL ISOLATION METHODS

The isolation and culturing of algae is mainly allied with the culture conditions present in their natural habitats. The cultivation under laboratory conditions of each algae depending on their different habitats. Since, most of the algae are susceptible to unfavorable culture conditions (Brennan and Owende, 2010). Usually, the isolation procedure of algae from its natural environment includes sampling, serial dilution, plating and culturing.

2.6.1.1.1 Sampling and Single Cell Isolation

Microalgae are habituated in different environmental conditions like ice, fresh water, marine water, waste water, coastal areas, rocks, soil, etc. The first step for isolation of algae is the collection of samples from apposite natural sites. The sampling can be done using syringe, scraping, brushing or inverted petri dish method. The abiotic factors of the habitat such as light, water contents, temperature, dissolved O_2/CO_2, nutrient concentration, pH and salinity should be noted, which are obligatory to provide favorable condition in the laboratory while cultivation. Likewise, sterile conditions should be maintained during isolation process in order to avoid contamination (Mutanda et al. 2011). Single cells are picked from the sample using a micropipette under microscopic observation. For isolation, single cells or algal filaments from the sample are to be diluted primarily and then transferred to the suitable media. This technique requires skilled expertise since the cell fragments may be damaged by the shear stress caused by micropipette.

2.6.1.1.2 Serial Dilution and Plating

Serial dilution is the first step of algal isolation in which the collected samples are serially diluted to obtain algal monocultures. For isolation, sterilized agar plates to be prepared and inoculation can be done using streak plate technique in order to obtain pure strains from a mixture of microorganisms. After inoculation, the plates have to be incubated under optimum environmental conditions similar to that of their actual habitat. The incubation period required may vary from few days to several months in accordance to soil to fresh water and marine species. After isolation, single cells can be further sub cultured using plate or broth cultivation in order to obtain pure cultures for further use (Singh et al., 2015).

2.6.1.1.3 Density Centrifugation and Enrichment

Centrifugation is the commonly employed separation technique that practiced to separate microalgal cells among crude sample, which work with the principle of separating cells based on cell size. It is well reported that density gradient centrifugation using silica sol and percoll were effective microalgae separation methods. For algal enrichment, certain substances are added to the medium for the enrichment of cultures. The enrichment materials used for the purpose includes desired nutrient medium, soil extract, other nutrients as nitrate, phosphate and trace metals (Mutanda et al., 2011). Specific pH is essential that avoid the possibility of bacterial contamination.

Other enrichment sources identified were yeast extract and casein, which also provided good results.

2.6.1.2 ADVANCED ISOLATION TECHNIQUES

Few advanced methods are developed to accomplish algal isolation, which are micromanipulation and automated techniques like fluorescence-activated cell sorting.

2.6.1.2.1 Micromanipulation

It is a practice used for the isolation of microalgal cells, which supports culturing of single cells. Traditionally, micro-manipulation was used to target microscopically identified cells. While modern technique with micromanipulator, stereomicroscope and optical tweezers helps accurate separation of cells, which makes it as an idyllic model for single cell isolation. Microcapillary tubes made up of glass with a diameter of 1 mm is desirable for this technique (Mutanda et al., 2011; Singh et al., 2015).

2.6.1.2.2 Automated Technique

An automated method employed for quick isolation and purification of microalgae is flow cytometry coupled with fluorescence-activated cell sorting (FACS) (Singh et al., 2015). FACS is based on the principle of light scattering through laser beams. Where the cells present in the exposed material absorb the light and further emit fluorescence. The resultant fluorescence gives information about cell size, density and photosynthetic characteristics of the algae. This method helps to produce axenic cultures directly since that avoid bacterial contamination in large-extent (Pereira et al., 2011). This method provides high throughput screening and whereby cells can be characterized and sorted. However, the efficacy of cell sorting is allied with the original sample and its abundance, cell size, shape, and hardiness. The technique is restricted in course of categorization of algae occur as aggregates; which could be overcome by sonication process (Doan and Obbard, 2012). This method was also useful for the identification of desirable mutants among a mixed population instead of using antibiotic resistance or morphology analysis.

2.7 MEDIA FOR ALGAE CULTIVATION

The nutrients such as nitrogen, carbon, phosphorus, vitamins and trace metals are to be supplemented in algal culture media since which are very essential for algal metabolism. Based on the nature of habitat where algae isolated (fresh-water or marine origin), two types of media are available – fresh water culture media and marine water culture media. The type culture medium, classification and its chemical components are tabulated in the Table 2.5. The culture media can be used either in liquid or solid form. The commercially available typical algae cultivation media are TMRL medium, Schreiber's medium, F/2 Medium and Conway's or Walne's Medium. Where TMRL medium is ideal for the mass cultivation of diatoms and Schreiber's medium is used for the cultivation of nanoplanktons. The solidified culture medium for algae cultivation was first introduced by Klebs (1896) and Tischutkin (1897). Agar used as a solidifying agent to produce solid media, is a chemical component that made up of agarose and agaropectin. Where a small inoculum of cells speeded on the surface of the solidified media and the cells started to utilize media and finally obtained good biomass (Lin, 2005).

Apart from chemical components, there are certain criteria should be followed to employ apposite culture vessels for media preparation. Glassware's like beakers, flasks, pipettes, glass rods, petri dishes, funnels, syringe, stirring rods, etc. are required for preparation of culture media. Although, the culture vessels used for media preparation should be chemically inert, transparent and resistant to sterilization. Vessels with large surface area are adequate for effortless growth of the algae. The vessel materials that cannot lead leaching or inert materials are desirable for algal culturing. Teflon, polystyrene, borosilicate, glass, etc. are the frequently used culture vessel materials while the choice of culture vessels is based on the algal species to be cultivated. The culture vessels such as glass or polycarbonate Erlenmeyer flasks/tubes and sterilized polystyrene flasks are successfully implemented for culturing. Nowadays, heat-resistant borosilicate glasswares such as pyrex and duran are better choice for culturing. Moreover, for the preparation of trace metals or silicate stock solutions polyethylene, polycarbonate or teflon-lined plastic vessels are desirable.

2.7.1 FRESH-WATER CULTURE MEDIA

Fresh-water culture media are classified in to three categories, they are synthetic media, enriched media and soil water media. This kind of media are preferred for the culturing of algae those possess fresh-water origin.

TABLE 2.5 Types of Culture Media and Its Chemical Composition

Types of Culture Medium	Classification	Chemical Components
Fresh-water culture media	Artificial media Enriched media Soil-water media	• Macronutrients • Trace metals • Soil extract • Vitamins • Chelators • Agar
Marine water culture media	–	• Macronutrients • Water source • Trace metals • Soil extract • pH buffers • Vitamins • Chelators • Agar

2.7.1.1 SYNTHETIC MEDIA

Synthetic media is the artificially defined media that used for the maintenance of algal strains. For instance, Bold's Basal medium, BG-11 medium, WC medium, and V medium are the chemically defined media used for the purpose of various algae cultivation. For the preparation, the stock solution of required nutrients and specific buffer to be dissolved in desired amount of distilled water. After adjusting pH, the prepared media to be sterilized and can be used for cultivation. Both the liquid and solid forms of this media are used for the cultivation, which depends upon the purpose of cultivation.

2.7.1.2 ENRICHED MEDIA

Enriched media are usually prepared by adding nutrients to a natural source (natural lake/stream water) or to a synthetic medium for enriching the media, where, soil extract, plant extract or yeast extract can be used as enrichment source. For such media, the stock solution containing macronutrients, microelements, vitamins, and extracts to be mixed and organic nutrients to be supplemented to it. All nutrients to be liquified with 80–90% of the desired volume of distilled water and adjust the pH as appropriate and then autoclaved. For instance, Walne medium and the Guillard's $F/_2$ medium are the two commercially important enrichment media that extensively used for algae cultivation purpose.

2.7.1.3 SOIL WATER MEDIA

Soil media is prepared by placing 1–2 cm of garden soil in to the bottom of a vessel and followed by adding distilled or deionized water up to is $3/4^{th}$ volume of the vessel. After complete mixing, glassware to be sealed with a cotton plug or screw cap and sterilize it for 1 hour on 2 successive days before cooling it for 24 hours. The sterilized media can be reserved in refrigerator until further use (Lin, 2005).

2.7.2 PREPARATION OF FRESH-WATER CULTURE MEDIA

The quality of the chemical components employed for the preparation of fresh water culture media is an important factor that affects whole cultivation process. High quality chemicals are generally prepared for the preparation of fresh water culture media. However, the quality is allied with the designation and grade of specific chemical provided by the manufacturer. A well-equipped lab with basic laboratory infrastructures such as weighing machine, autoclave, pH analyzer, membrane filters, magnetic stirrer, ultrasonic washer, Laminar air flow, incubation unit and refrigerator are necessary for algal cultivation. General purpose agar can be preferred for such algae cultivation except in course to sensitive algae, whereas a preliminary washing is obligatory to eliminate the impurities from agar and thereby to avoid contamination.

Soil extract is another component used for culturing algal species especially is a known component of the enrichment media, which is also referred as solid particulate soil. The selection of suitable soil is an important task since, soil contains heavy clay content not reliable for application. Soils from gardens or greenhouses were commonly employed for the purpose, in fact that such soils have not been exposed to chemicals. Another choice is grasslands soil, which has not been tilled or grazed. However, it is recommended to choose soil from the habitat where they naturally grown. Usually, soil extract is prepared by adding 1-part soil to 2-parts distilled water followed by pasteurizing or autoclaving it for about 2 hours.

The working stocks of macronutrients, trace elements, and vitamins are to be prepared separately by mixing the desired quantities of each component in distilled water and diluting up to a final volume. The prepared stocks should tightly seal and kept in refrigerator until use, in order to prevent chemical alterations. For instance, a stock solution with 100- to 1000-fold final concentration of macronutrients are used in most of the cases. There are three vitamins such as vitamin B_1 (thiamine), vitamin B_{12} (cyanocobalamin), and vitamin H (biotin) are commonly required for microalgae cultivation. Moreover, it is reported that Na_2EDTA (disodium ethylene diamine tetra acetic acid) was used in some freshwater media as a chelator (Moreno-Garrido, 2008).

2.7.3 PREPARATION OF MARINE WATER CULTURE MEDIA

Natural seawater is usually invaded with 50 recognized elements and a huge number of organic components. Since preparation of marine water culture media requires the addition of a marine water source along with macronutrients, vitamins, trace metals and soil extracts that are used in the fresh water culture media. Apart from that, pH buffers and chelators are adequate for maintaining the constancy of the medium. Oligotrophic water is idyllic for algae cultivation due to its favorable nutrients and trace metal composition. Also, low sediment content and very few phytoplankton are the characteristic features and its filtration is very easy. Water from blooms are usually not be preferred due to its toxic nature. The collected sea water should be filtered through 0.2 to 0.45 mm membrane filters by removing organic fractions, whereas, activated charcoal mediated adsorption can be done to remove dissolved organics present in it. After filtration, sterilization using an autoclave at 121°C for a time of 15 minutes is recommended. Followed by autoclaving, media allowed to equilibrate by keeping at room temperature for 24 hours.

The main requirements for marine culture media are reagent grade chemicals and salts. The equipments and glasswares used for the preparation of marine water culture media are similar to that of used for fresh water culture media. Stock solution of each of the macronutrients, vitamins and trace metals are to be prepared separately. The macronutrients such as nitrogen, silicon and phosphorus are used for the preparation of this media, which are required in a ratio of 16:16:1, respectively (Parsons, 1961; Zhiliang et al., 2006). Nitrate and phosphate are normally added in the form of $NaNO_3$ and $NaHPO_4 \cdot H_2O$. An alternative nitrogen source ammonia can also be added to the media in the form of ammonium chloride. Silicate is added in the form of $Na_2SiO_3 \cdot 9H_2O$.

The trace metals such as Zinc, Cobalt, Manganese, Selenium and Nickel are to be added as their chloride or sulfate salts, whereas iron is prepared and stored in a separate vessel in order to avoid precipitation. Vitamins like vitamin B_{12}, thiamine, and biotin are used for marine water culture media formulation. Whilst the addition of vitamins done after autoclaving in order to maintain their maximum potency. Likewise, buffers such as tris and glycylglycine are used to prevent or reduce precipitation. EDTA is the most known chelator used in the form of disodium salt ($Na_2EDTA.2H_2O$) in such media. Nitrilotriacetic acid (NTA) and citric acid were used in few cases as chelators (Brand et al., 1986). Soil extract is another component of marine water culture media, whereas the selection of suitable soil is important.

2.8 GROWTH PARAMETERS OF ALGAE

2.8.1 ALGAL GROWTH CHARACTERISTICS

The growth rates of algae in the culture can be measured to justify its growth improvements. The biochemical constituents of the culture vary with increase in growth rate, which can be measured in terms of physiological studies. Growth can be interpreted as any changes occurred in the total biomass of algae in a culture. However, certain parameters are used to follow growth enhancement of algae as a static percentage of the total biomass per unit time. Usually, one of the parameters cell number is directly proportional to the cell growth of the culture. Hence, measurement of cell density provides great understanding about different growth stages. The growth characteristics and parameters influenced for batch or continuous cultures are different.

In continuous cultures, fresh medium is frequently added to the culture at the same proportion of the medium removed. This technique maintains the cell concentration in a steady-state, which also offers continuous production and harvesting of biomass. The cell density of continuous culture can be analyzed by appropriate methods. The chemostat or turbidostat were effective for the determination of population growth in the continuous culture. The following relation can be used to determine the population growth of continuous culture. Where, 'F' represents the flow rate of medium to and from the chamber, 'V' denotes the volume of the culture vessel and 'D' represents dilution rate (1).

$$\mu = FV = D \quad (1)$$

Batch cultures are commonly adopted technique for small-scale cultivation, which possess much significance such as low expense, easy processing and less amount of media required. Although, it is imperative to realize the growth characteristics of batch culture. Generally, growth rate measurement in batch cultures takes much time, hence biomass rate measurement is time consuming. The common methods used to determine the progress of growing cells include *in vivo* fluorescence based biomass analysis and optical density analysis. The chemical components as chlorophyll, protein, carbohydrate, and lipid present in the culture can also be used as a proxy measure in some cases. Such calculations can be done on the basis of cell number or biomass analysis. The algal growth rate is greatly implied with various physical and nutritional conditions, which are explained below. The different physical and nutritional factors that affect the growth of algae during culturing are briefly demonstrated in the Figure 2.5.

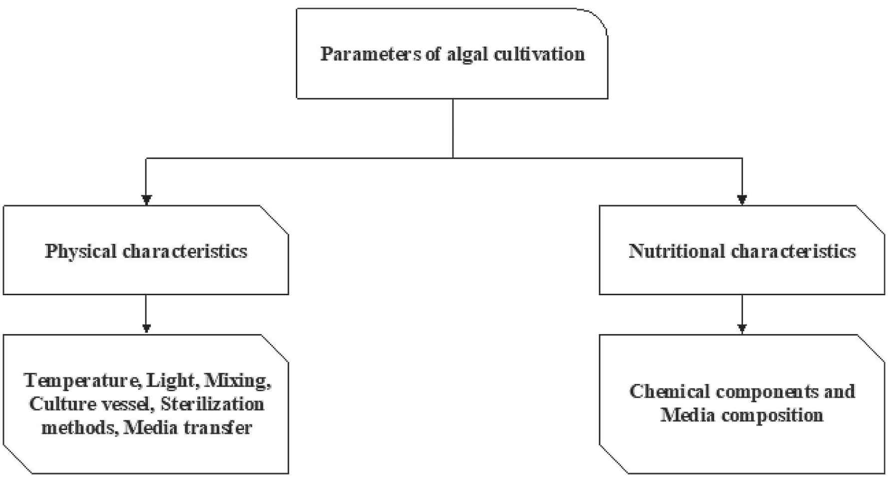

FIGURE 2.5 Growth parameters that affect algal culturing.

2.8.2 PHYSICAL CHARACTERISTICS

The physical parameters that influence algal growth during its culturing are temperature, light stirring, culture vessels, sterilization methods and media transfer methods.

2.8.2.1 TEMPERATURE

The temperature obligatory for algae is vary with species to species. An optimal temperature for algal cultures is established in the range of 20–24°C. Beyond this, temperature below than 16°C and higher than 35°C will drastically affect biomass production. If temperature increases, the addition of cold water or flowing of refrigerated air to the vessel is necessary.

2.8.2.2 LIGHT

Light is an important constraint that affects culturing process. Like plants, algae involved in photosynthesis, where light plays an imperative role. Since light intensity is a significant factor that control algal growth, although, the light requirement is usually based on the density of the culture media. The light intensity needed for culture with high density is higher when compared to the lower density. For example, 1,000 lux intensity is apposite for Erlenmeyer flasks while 5,000–10,000 lux is necessary for higher volumes. Either natural light source or fluorescent tubes can be employed to provide light required for the cultivation. Even though, photo-inhibition reported in some cases due to excessive penetration of light. The desired rate of artificial illumination is about 18 hours per day.

2.8.2.3 MIXING

Mixing or stirring of culture is necessary during incubation since that avoid sedimentation of biomass. It also ensures good light penetration, nutrient circulation and gas exchange throughout the medium, which also avoid the possibility of thermal stratification. Mixing can be achieved by manual stirring every day (for test tubes and Erlenmeyer's flasks) or by mechanical method using paddle wheels and jet pumps (for ponds).

2.8.2.4 STERILIZATION TECHNIQUES

Sterilization is the process of removal of microbial forms from the cultivation media that avoid the risk of contamination. A number of sterilization methods are accessible but the choice of methods is depending upon the purpose and the material used for cultivation. There are different sterilization methods are available for algal cultivation. Those are gas sterilization, moist heat sterilization, dry heat sterilization and radiation.

- Gas sterilization (ethylene oxide gas is applicable for sterilization of disposable plastic).
- Moist heat sterilization (Steaming through autoclave (121°C temperature) or pressure cooker is used for sterilization under steam or pressure.
- Dry heat sterilization (Oven sterilization is mainly used for sterilization of glassware for at least 2 hours at 160°C temperature or Flame sterilization is applicable for sterilization of incandescence metal).
- Radiation (UV-ray/X-ray radiation is preferred for preparing chambers or media that used for cultivation process).

2.8.2.5 MEDIA TRANSFER

The media or transfer should be done in laminar flow cabin. Where, a clean working surface could be maintained with 70% alcohol. Although, hands should be cleaned with disinfectant followed by 70% alcohol. Clean, sterile pipettes and autoclavable tips for repeating micropipette use are recommended to avoid contamination and thereby to keep sterile environment and for media transfer. The frequency of culture transfer depends on the algal species and culture conditions. Accordingly, the culture should be transferred when population growth decreases or before the stationary phase. Culture transfers can be done either by direct pouring or transfer with pipette.

2.8.3 NUTRITIONAL CHARACTERISTICS

The chemical components of the media and media formulation are the two important nutritional aspects that affects overall algal growth. These nutritional requirements are reported in detail below.

2.8.3.1 CHEMICAL CONSTITUENTS

The chemical constituents required in large quantities for algal growth are considered as nutrients, which are mainly added in the form or composition of various elements in the culture media. There are three groups of elemental composition usually preferred, they are group A, group B and group C. The elements present in the culture media with respect to different elemental groups are presented in the Table 2.6. The concentrations of group A constituents have no distinction in sea water, hence, high algal biomass cannot deplete them easily. These nutrients to be added to the deionized water while making artificial sea water media, but required to add in artificial media. Group B nutrient composition also have similar concentrations in seawater. Whereas, Group C nutrient is required for microalgae, which are present in natural seawater with quite small quantities. Even as all these nutrients are essential to generate significant microalgal biomass during cultivation.

Some microalgal species requires vitamin B_{12} for neutralizing toxic elements such as arsenic, mercury, tin, thallium, etc. While some species prefer thiamine and some others need biotin. Therefore, these vitamins are usually supplemented to the seawater media in order to assure good metabolism and growth. In some cases, soil extract provides all nutrients, where chelators desired in apposite quantities. The trace metals are also essential for the growth of all kinds of algae. Of which the concentrations of Fe, Mn, Zn, Cu and Co is important and its deficiency limits algal growth in natural water. Sometimes, a constant range of free ionic metal causes toxic effect on algal growth. For example, some metals like Cu caused toxic effect when there is no proper chelation occurs, whilst Fe causes precipitation and becomes inaccessible to phytoplankton. Hence chelators play an important part in algal culturing. Citrate is sometimes used as an alternative chelator instead of typical chelator EDTA.

TABLE 2.6 Elements Present in Different Groups of Culture Media

Group A	Group B	Group C
• Sodium	• Bromide	• Nitrate
• Potassium	• Fluoride	• Phosphate
• Magnesium	• Iodate	• Ferric ion
• Calcium	• Lithium	• Zinc
• Chloride	• Rubidium	• Manganese
• Sulfate	• Strontium	• Cupric ion
• Bicarbonate	• Barium	• Cobalt
• Borate	• Molybdate	• Orthosilicate
	• Vanadate	• Nickel
	• Chromate	
	• Arsenate	
	• Selenate	

2.8.3.2 MEDIA COMPOSITION AND PREPARATION

The quality of water used for media preparation is an imperative factor since the quality of water is often vary from different sources and that is unpredictable, especially in the case of temperate and polar regions. Anthropogenic pollution and release of toxic metabolites are identified as the major causes for this phenomenon. Ageing of water for a few months improved water quality, followed by autoclaving or filtering is required. Artificial seawater is also available, which is prepared by adding various salts into deionized water, for example, ESAW medium.

The control of pH is essential to provide specific ionic concentration for their better growth. The pH of natural seawater is around 8.0. The addition of bicarbonate or Tris or glycylglycine buffers assists to maintain desirable pH, which also avoid precipitation during culturing. In the case of marine water culture media, salinity should be checked at regular intervals to assure constant growth. Reagent grade chemicals and double distilled water are pleasant for media preparation. During the preparation of a mixed stock solution, dissolve each component with minimal amount of water at first and then dilute to final volume. This provides homogenous mixing of various components in the media.

Pure or axenic culture can be obtained by isolating single cells from the primary culture with a micropipette and inoculating the same into fresh culture media. This method is useful to eliminate bacteria attached to the primary culture and provide bacteria-free cultures. The presence of antibiotics like penicillin, streptomycin and gentamycin decreased the chances of contamination in the media. The toleration rate is differing with species to species with respect to varied concentrations of antibiotics ranging from 50–500 mg/l. Moreover, chloramphenicol, tetracycline, and bacitracin also showed positive effect on controlling contaminants. Such antibiotic solutions can be made by dissolving filter-sterilized compounds in to distilled water and further stored in frozen condition until use. The absence of bacterial growth ensures that the culture is axenic.

2.9 IMPORTANT MEDIA STERILIZATION TECHNIQUES

The media used for algae cultivation should be free of all kinds of germs and spores, since microbial contamination is reported as the major concern of algal research. Four common methods frequently employed for the sterilization of algae cultivation media are autoclaving, pasteurization, ultraviolet radiation and filtration (Figure 2.6).

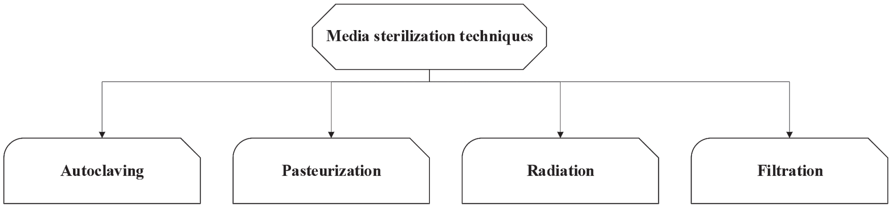

FIGURE 2.6 Methods used for algal culture media sterilization.

2.9.1 AUTOCLAVING

It is the typical method used for sterilization of most of the culture media, offer devastation of all microbes including viruses. Autoclaving is the most effective method for algal culture media sterilization where the media is subjected to steam under pressure at 121°C (250°F) for around 15–20 minutes. The time of exposure is depending upon the density of the contents. The media should not be more than ¾ volume of the flask and the vessel should be plugged with cotton wool and covered with aluminum foil. After autoclaving, the pressure release valve can be opened once the temperature has released below 80°C.

2.9.2 PASTEURIZATION

It is a process of heating culture media for 30 minutes at 90–95°C in Teflon or polycarbonate bottles. This is an alternate method used for algal media sterilization, which completely remove all kinds of contaminants including most of the spores. However, pasteurization is not at all effective for seawater culture media since some bacterial spores survived even after pasteurization process. Improved sterilization efficiency was reported while heating the media at higher temperature (90–95°C) for 30 minutes followed by cooling for two successive days and the process is referred as tyndallization.

2.9.3 ULTRAVIOLET (UV) RADIATION

The sterilization by UV rays is applicable for the sterilization of working area and algae culture media. It is mainly used to sterilize seawater base of the media, but very high intensities of radiation are needed for absolute sterilization. Such highly intensive UV light may lead to accumulation of free radicals and other toxic compounds generated in the algal cells. After UV irradiation, the media to be stored for some days in order to decrease the level of toxic chemical formed in it.

2.9.4 *FILTRATION*

Filtration is the feasible method for sterilization of seawater without varying its chemical nature. While the sterilization efficiency is less than that of heat sterilization. Membrane filters with a pore size of 0.2 μm are generally used for the purpose, where the time required for filtration of large size of culture media is very long. Mostly vitamins are sterilized by filtration method because heat sterilization causes denaturation of such compounds.

2.10 GROWTH OPTIMIZATION AND GROWTH RATE ANALYSIS

2.10.1 *ALGAL GROWTH OPTIMIZATION*

The algal biomass production can be augmented by optimizing various growth conditions. Their maximal production is also depending on the ideal cell concentration, since regulation of cell density is very important. The environmental conditions such as light and temperature are difficult to control. Contamination is another major issue reported for over few decades. In open systems, cross-contamination is reported, hence, the product obtained are not uniform in such cases. Even as, the laboratory level cultivation also resulted contamination in many cases. Such issues can be rectified by selecting appropriate sterilization methods or by optimizing exposure time. The selection of algal species should be based on certain criteria. The desirable characteristics to choose algae for cultivation are fast growth rate, high tolerant characteristics, good proteins, lipids or carbohydrates content and easy harvesting.

2.10.2 *GROWTH RATE ANALYSIS*

The analysis of growth rate is necessary to understand the growth characteristics of a growing culture. There are several methods used to analyze algal growth rate as microscopic cell counting, electronic cell counting, turbidity analysis and dry-weight measurement (Figure 2.7).

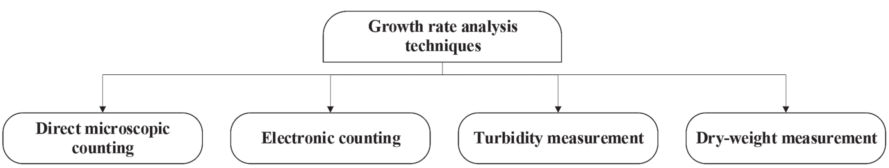

FIGURE 2.7 Techniques employed for growth rate analysis.

2.10.2.1 DIRECT MICROSCOPIC COUNTING

This is a traditional method used to detect algal growth rate of a growing culture, where inverted microscope is utilized for counting algal cells. The sampling, dilution, chamber filling and microscope magnification have to be done with adequate attention. The counting should be clean before every use. After slide preparation, the cells should be allowed to settle for 4–6 minutes prior to counting. Then samples can be examined at low magnification followed by high magnification to ensure reasonable distribution of the cells.

2.10.2.2 ELECTRONIC COUNTING

It is an advanced method for counting algal cells by electronic measurement. In this method, diluted algal suspension is pumped through a small orifice between two compartments filled with fluid. Where, the electrodes present in each compartment measures the electrical resistance of the system. The orifice is enough to ensure that its electrical resistance is high. When a sample entered through the orifice, the conductivity and resistance increase than that of the medium. The variation of resistance is recorded as electrical pulse which being further counted. Two main disadvantages of using this method are which do not offer daughter cell separation and orifice clogging.

2.10.2.3 TURBIDITY MEASUREMENT

This is a quick, non-destructive method used for algal cultivation. Turbidity measurement method or light-scattering method is frequently used to identify the growth of pure cultures. This type measurement mainly gives information about biomass rate (dry weight) but not about the number of cells. The laboratory equipment's such as calorimeter or spectrophotometer is used to measure turbidity in qualitative manner. At low turbidities, number of cells present in the light path will be lower. The intensity of the unscattered light reduces exponentially according to the number of cells increases.

2.10.2.4 DRY WEIGHT MEASUREMENT

Measurement of dry weight of the cells is a direct method to evaluate the changes in biomass production. In this method, the aliquots of algal culture are taken and filtered through membrane filter or by centrifugation to separate the cells. The parted cells are then washed with buffer to remove the salts and other contaminants. The resultant cells are dried and measured to found the corresponding cell weight. This can be performed at regular intervals to trace the changes in cells occurred during algal culture.

2.11 CONCLUSION

Algal biomass possesses much attention at current scenario due to their diverse application in the field of food, chemicals, pigments, energy production and waste-water treatment. In fact, of this potential application, a deep knowledge on algae cultivation and maintenance techniques are essential to perform more research in this area and thereby to explore the potentiality of more algal species. The present chapter deliberated the important facets of algal cultivation with respect to isolation, cultivation methods, culture media, cultivation systems, various parameters, optimization and growth analysis methods. Moreover, a profound understanding of these aspects would provide a great insight for researchers or scientist for exploring the promising characteristics of algae in the meantime.

KEYWORDS

- **algae**
- **cultivation systems**
- **filtration**
- **growth media**
- **growth rate analysis**
- **isolation**
- **optimization**
- **parameters**
- **photobioreactors**
- **sterilization**

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CHAPTER 3

GEOGRAPHIC POSITION AND LOCAL CLIMATE AS KEY FACTORS IN THE IMPLEMENTATION OF MICROALGAE-BASED PROCESSES

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ABSTRACT

Microalgae comprise a diverse group of microorganisms that are present so much in the tropical territory how much in the Antarctic. These microorganisms are a source of various products such as unicellular protein, pigments, and polyunsaturated fatty acids. In light of this, there is a growing effort among researchers and entrepreneurs to make microalgae a regular source of consumer products. Some studies, in particular, have shown that climate plays a key role in directing appropriate locations for commercial-scale microalgae cultivation. Taking into account that most commercial microalgae production is outdoors. However, holistically, besides the climate, there are other parameters of a local nature that also need to be strategically considered before implementing any microalgae-based process. Having said that, the chapter proposes to provide an overview of the geographic position and the local climate as key factors in the implementation of microalgae-based processes. Directly related to the geographic position, the local climate, the availability of natural resources such as water, nutrients, and land, as well

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as logistics and transport considerations are discussed in their main aspects. Approaches to targeting appropriate sites for large-scale microalgae culture are also addressed.

3.1 INTRODUCTION

The microalgae comprise a diverse group of microorganisms with about 30,000 cataloged species (Kapoore et al., 2018). These microorganisms have a rich global biogeographic distribution based mainly on temperature and are present so much in the tropical territory how much in Antarctic (Zamaloa and Tell, 2005).

Currently, the microalgae are becoming a new platform of food and energy, and there is a growing effort among researchers and entrepreneurs to make these microorganisms a regular source of consumer products (Katiyar et al., 2017; Torres-Tiji et al., 2020). In this sense, some studies have shown the important role of the climate in the targeting of appropriate places for large-scale microalgae culture (Banerjee and Ramaswamy, 2017; Huesemann et al., 2017; Siqueira et al., 2020). Notoriously, the environmental regime to which the microalgae are exposed influences the production and biochemical composition of microalgae biomass (Teoh et al., 2013). Although few studies have evaluated the spatial and temporal variation of the climate and its impact on cultures, this topic has emerged in recent years as a key factor for the implementation of facilities and processes for microalgae-based feedstocks.

Of the elements, that make up the climate, the temperature and solar radiation are the most studied. These elements vary according to the geographical position, which, based on the influence of climatic factors, such as altitude and latitude, results in the climatic diversity of the terrestrial globe. Bearing in mind that the climate plays a fundamental role in the commercial cultivation of microalgae, the choice of a location stand up many questions, such as quantity and quality of solar radiation, daily and seasonal fluctuations of temperature, and duration of the growing season. Undoubtedly, these issues is relevant to the economic viability of an industrial unit (Schade and Meier, 2019).

Holistically, besides the climate, the land availability, inputs, and access to transport infrastructure are parameters of local nature that also need to be considered when locating suitable geographical positions for microalgae culture. The strategic selection of locations taking into account all these parameters can considerably reduce the capital and operational costs of production (Ruiz et al., 2016).

Geographically, the implementation of microalgae-based processes faces restrictions that unfeasible the standardization of photoproduction on a global scale. Therefore, this chapter aims to provide an overview of the geographical position and the local climate as key factors in the implementation of microalgae-based processes. Besides, directly related to the geographical position, the availability of resources such as land, water, nutrients, carbon dioxide, as well as access to transport infrastructure, will be reviewed in its main aspects. The main approaches used in targeting appropriate locations for microalgae culture also will be presented and discussed.

3.2 MICROALGAE AND THE IMPORTANCE OF THE LOCAL CLIMATIC ELEMENTS

Microalgae can be cultivated in closed and open environments in photoautotrophic, heterotrophic, mixotrophic, and photoheterotrophic modes. Today, the most share of the commercial microalgae production is photoautotrophic and takes place outdoors in sunlight (Sanghamitra et al., 2020). The daily and seasonal variations associated mainly with sunlight and temperature significantly influence the growth of microalgae and may result in losses in productivity and changes in the microalgae biochemical composition (Moreno-Garcia et al., 2019). Therefore, it becomes important to identify locations with favorable environmental conditions for microalgae culture. In particular, in order not to overload the construction and operating costs, are geographical positions preferable, which do not require the implementation of thermal regulation systems (Kang et al., 2020).

The use of thermal regulation systems has been widely proposed for temperature control (Nwoba et al., 2019). Although it is relevant to control the temperature to reach maximum productivity, the constant control is uneconomical. Besides overloading construction and operating costs, it has a negative impact on the environment, due to the high demand for water in cooling systems (Huang et al., 2019).

In this context, studies related to the spatial and temporal variation of the climate have become a tendency and aims at locating conducive locations to the economic implementation of microalgae-based processes (Sheets et al., 2014). The identification of an appropriate place for the cultivation of microalgae is extremely crucial, being necessary to analyze the prevailing climatic conditions, as the amount of solar radiation received, temperature, evaporation rate, and frequency of precipitation pluviometric. All these conditions/phenomena that make up the climate of a given location affect the microalgae productivity, being it relevant to locate regions whose climate favors the cultivation and harvesting of microalgae throughout the year (Boruff et al., 2015).

Natural light conditions affect the photosynthesis kinetics of microalgae (Khoeyi et al., 2012). In other words, the capture of sunlight feeds all the microalgae processes performed photosynthetically (Sivakaminathan et al., 2020). Insufficient or excessive light intensities can result in a lower rate of cell growth (Kim et al., 2020). Namely, the cell growth is affected by the level of light intensity that falls into one of the three categories: limitation, saturation, or inhibition of light. When the light intensity is low, the increase this parameter improves the photosynthetic efficiency of the cells, up until the photosynthetic apparatus becomes saturated. The excessive light intensity can cause photoinhibition and result in damage to the photosynthetic apparatus. It can be emphasized that artificial light has great advantages over the regulation of light intensity, spectrum, and photoperiod. However, there is a high cost involved in implementing it in an industrial unit (Baer et al., 2016).

Noteworthy, as cells grow and the culture becomes denser, it becomes increasingly difficult for the incident light to penetrate deeply into the cell culture. Most cells can suffer from light limitation, due to the lack of light energy necessary for photosynthesis. In such cases, although there may be additional energy and maintenance costs, continuous biomass harvesting is recommended (Suparmaniam et al., 2019).

Photosynthesis of microalgae occurs in the light and dark phase, also called the photoperiod (Taghavi et al., 2013). During a long period of darkness, cells adapt to conditions and continue their metabolic activities using storage nutrients until they are exposed to light again (Oncel et al., 2011). In this context, geographical positions with prolonged periods of light, with a daytime period bigger than that of the nocturnal, are more favorable from the performance of cell growth rates.

With regard to temperature, the daily and seasonal variations can interfere with the microalgae growth and, therefore, affect production efficiency. Intrinsically, each species present an ideal growth range (Subhash et al., 2014). In other words, an increase in temperature below the ideal range can improve growth rates; however, high temperatures above the ideal range can be harmful (Singh et al., 2015). Considering that, for systems exposed to the outdoors, the temperature is a key climatic element for the bioprocesses performance, it is essential to identify locations with an ideal temperature range, according to the needs of the interest culture, for that there are no additional costs with the maintaining of the temperature (Ras et al., 2013).

Concomitant, the local average temperature, as well as solar radiation, relative humidity, and wind speed are directly related to water loss by evaporation. The climate has a significant impact on evaporation rates. In hot climates, the evaporation rates are more high, which can require demand greater of water for cultivation (Doucha et al., 2009; Farooq et al., 2015). However, the high demand for water can affect the economy and sustainability of cultivation (Guieysse et al., 2013).

Still, prolonged periods of cloudiness can cause variations in solar radiation during cultivation days. In this context, sunny days offer a better distribution of light in external cultivation, unlike cloudy days, where there is a certain level of shading. In addition, a high level of precipitation pluviometric increases the chances of diluting the microalgae cells in open systems. Consequently, an increase in cell dilution tends to increase harvest costs (Kumar et al., 2015). However, places with elevated pluviometrics index can exploit this resource and use it in cultivation.

In general, depending on the geospatial location, there may be marked variations in light, temperature, photoperiod, rainfall, cloudiness, and evaporation. These climatic elements have a direct impact on the performance of microalgae cultures (Siqueira et al., 2020). Regarding the seasonal variations, according to the latitude of the geographical position, there is a noticeable difference between summer (characterized by high light intensity and long days) and winter (characterized by low light intensity and short days). In this context, equatorial and tropical areas are considered better than temperate areas for microalgae economic cultivation, due to more stable seasonal temperatures and more hours of light throughout the year (Chiu et al., 2016). Theoretically, the equatorial and tropical areas show less variation between the four seasons, while the temperate area shows a marked variation, having the four seasons well defined. Taking this into account, in the tropical and equatorial areas there is the possibility of cultivation without an off-season.

Finally, the environmental conditions influence the growth rates of microalgae, and the key climatic elements for the success of a productive system are temperature and light. More specifically, temperature affects all metabolic processes, and light is the driving force of photosynthesis and an indispensable element for photoautotrophic cultures (Wahidin et al., 2013).

3.3 GEOSPATIAL VARIATION OF THE CLIMATE

The climate, throughout history, has played a crucial role in maintaining life on earth. Climatologically, the climate can be defined as a set of atmospheric conditions that characterize a given location. On the earth's surface, the climate is not uniform and differs significantly from one place to another. The spatial and temporal variation of the climate determines the geographical distribution of microalgae biodiversity (Adey and Steneck, 2001; Sharma and Rai, 2011).

Present in all ecosystems on earth, the microalgae are governed by climate. In commercial cultures, besides the temperature, all other climatic elements that make up the climate of a location are essential and interfere in the performance of the bioprocesses. These elements presented characteristic daily and seasonal patterns that vary spatially (Wigmosta et al., 2011). In the biotechnology field, the spatial variation of the climate is being assessed to identify places whose climatic conditions favor the performance of the microalgae cultures.

There are some implications involved in climate geospatial variation that refers to the inability to standardize microalgae photoproduction on a global scale. Typically, the economic cultivation of microalgae is carried out outdoors, with little or no control over the prevailing climatic conditions, and the production rates of metabolites remain subject to daily and seasonal variations of the local climate (Barceló-Villalobos et al., 2019). Therefore, the identification and mapping of locations with favorable climates for large-scale microalgae cultivation facilities are now in the focus of researchers from around the world.

3.3.1 IMPACT OF THE CLIMATE CHANGE ON MICROALGAE CULTURE

The global environment is going through a period of significant changes in the climate due to anthropic actions. These actions have resulted in an increase in the carbon dioxide atmospheric levels (CO_2), temperature, and UV-B radiation. Historically, the pace at which these changes are observed is unprecedented, and their impact on biological systems has been investigated. In particular, many studies have analyzed the implications of climate change in photosynthesis and microalgae growth. These microorganisms form the basis of the food chain, and any drastic changes in the climate can unbalance the entire ecosystem (Häder and Barnes, 2019).

Regarding CO_2, the measure that the atmospheric CO_2 levels increase in the troposphere, an increase in oceans acidification has been observed, challenging marine life to face an uncertain future. Simultaneously, the elevated levels of CO_2 have shown a high probability of stimulating photosynthesis and the growth of photoautotrophic microalgae (Li et al., 2016). In the current juncture, these microorganisms have been seen as a promising technology for the CO_2 sequestration and climate change reduction (Patidar and Mishra, 2017). Notoriously, each species present different responses to the inorganic carbon concentration and clearly will respond distinctly to the increasing change in atmospheric CO_2.

In the last decades, coincident with the CO_2 increase in the troposphere, there has been an increase in global warming. The temperature is an environmental parameter that affects the metabolism, growth, and survival of the microalgae. Inherently, each species of microalgae has a tolerance range and physiological responses distinct to temperature variations. Some species are eurythermal, tolerating a wide temperature range, but there are those that, besides the ideal temperature, present limitations (Yong et al., 2016). Consequently, the increase of global temperature can result in the proliferation of some microalgae and alteration of the species composition of a phytoplankton community (Nagai et al., 2011). In outdoor commercial cultures, the increase of the global warming rates may require the need to implement technologies for the management of the temperature and the consequence preservation of the cultivation system efficiency. Curiously, long-term projections have shown concrete changes in temperature and, presupposing that the CO_2 emissions do not cease, are foreseen alterations in local and regional climates. Climate change can occur more quickly in a scenario of rapid economic growth and launch of emerging technologies (Djamila and Yong, 2016). Noteworthy, it is predicted that the increase of the global temperature will broaden the limit of biogeographic distribution of some species of microalgae towards the poles (Cahill et al., 2014).

About the increasing incidence of UV-B radiation in the troposphere were observed alterations in marine systems, how much to species composition, and microalgae biomass production (Xue et al., 2005). The increase of the UV-B radiation is due to the use of atmospheric pollutants, such as chlorofluorocarbons, which reduces the stratospheric shield of ozone. In microalgae, the UV-B radiation tends to damage cellular machinery, and the destiny of the cells is to adapt by developing repair mechanisms or cell death

(Rastogi et al., 2019). Recent studies on the effect of the UV-B radiation have shown severe damage to the chloroplast ultrastructure and alterations in the photosynthetic pigment content of the microalgae (Zhang et al., 2015; Singh et al., 2019).

In general, there is still little data on the microalgae resilience to stress by UV-B radiation. However, a better understanding of the deleterious effects of the UV-B radiation that imitate current and future global climate conditions could be useful to assess its impact on the growth and biochemical composition of microalgae. Since the microalgae dependent on the climate, climate change may harm the large-scale industrial production of microalgae. Therefore, strategies aimed at improving the resilience of the microalgae to climate change must be anticipated.

3.4 RESOURCES AVAILABILITY IN THE GEOGRAPHICAL CONTEXT

Considerable efforts are being made to overcome the technical challenges associated with implementing microalgae-based processes. Among the countless key factors related to the geographic position, besides the local climate, pertinent issues such as the availability of natural resources such as water, nutrients, and energy, as well as the assessment of locations and feasibility of access, have become crucial elements to be considered for the consolidation of a microalgal industrial plant concept (Branco-Vieira et al., 2020).

3.4.1 WATER RESOURCE

The basis of a microalgae-based process consists of upstream processing, which refers mainly to the cultivation of microalgae. However, it is widely known that microalgae culture has a high demand for water resources (Yin et al., 2020). Estimates indicate the expenditure of 1000 kg of water per kg of microalgae biomass produced (Murphy and Allen, 2011). Thus, as in any other biological system, a sufficient supply of water for microorganisms is necessary.

Thereby, putting geographic mapping into practice can be an effective method to help researchers study the locations of implementation of microalgae-based systems (Yuan et al., 2020). Given these aspects, the choice of a location where there is high availability of water is a key factor.

According to the available literature, microalgae use freshwater as the main natural resource for their growth. Some species are able to use alternative resources such as wastewater, brackish, saline, underground sources, in addition to seawater (Xu et al., 2020). However, disadvantages may be associated with these alternative sources. For example, seawater cannot be used economically in areas far from the ocean. Likewise, the composition of groundwater is highly variable and requires improved matching, requiring pre-use corrections (Venteris et al., 2013).

Alongside, it is essential to be aware that even in the case where there is the substitution of freshwater for other alternative resources, freshwaters will continue to be a demand in the later stages unit (Yang et al., 2011). This is because in addition to being added regularly to compensate for water losses and avoiding salt build-up due to the evaporation rates must also be accounted for freshwater demand used in cleaning equipment and steps processing of biomass (Venteris et al., 2013).

Inherently, the instruction on basic meteorological data, given the geographic position and the corresponding local climate, can significantly influence the water demands of the processes. This because key parameters such as solar irradiance, air temperature, wind speed, precipitation, and relative humidity are directly related to the water availability of local domains. In view of this context, the establishment of microalgal culture is known to represent greater water requirements in arid climates than in tropical climates. Proving this statement, studies assessing water demand under different climates suggest that tropical climates require about 4.59 $m^3/m^2/yr$, while arid climates require values up to 6.39 $m^3/m^2/yr$ (Guieysse et al., 2013).

From the same point of view, the rainfall indexes for each specific local climate can represent eminent advantages in choosing the location. In short, rainwater represents a natural resource that is easy to collect. Thus, places with high rainfall levels can explore this alternative in the utilization of this element as a resource in microalgal installations. In addition, studies report that the composition of rainwater, different geographic domains, presented representative samples of trace elements such as iron, manganese, zinc, beyond copper. These essential elements are responsible for assisting to increase cellular productivity. Moreover, the advantages do not stop, some economic studies suggest that the use of rainwater for the dilution of cultures can represent a cost reduction of up to 60% (Xie et al., 2019). However, maximum attention should be paid to places where there is an incidence of acid rain, because, until the date, the relationship between these microorganisms and the tolerance to these compounds is still poorly understood, but resulting in adverse effects on the microalgae cells (Bhadra and Jhung, 2020). Therefore, to establish compensation prioritizing geographic locations where the ratio of water availability and costs become economically viable, it seems to be by far the most attractive alternative. To this end, the availability of sustainable water supplies will provide significant challenges in terms of choosing the location for microalgae-based processes.

Associated with these aspects, the utilization of natural resources such as water can be a major environmental indicator before the depletion of natural resources. According to the basic water footprint concept, this parameter is an element of one of the crucial aspects behind a favorable environment (Hossain et al., 2019). Given this, the water footprint assigned to microalgal processes can offer advantages about the demand and utilization of the high amount of water required. This is because, under an environmental character, these microorganisms allow the use of the three main types of water: blue, green and gray. In short, the first is specific of places where there are high rainfall levels. The second, in turn, refers to the surface water available and the last is attributed to wastewater from industrial processing. Thus, if the locations where the microalgal processes are implemented make it possible to integrate wastewater into cultures, it is estimated that about 90% of the total green and blue footprint values can be reduced (Yang et al., 2011).

3.4.2 NUTRIENTS RESOURCE

The essential macronutrients of microalgae, that is, carbon, nitrogen, and phosphorus play a notable role in the growth of these microorganisms (Nguyen et al., 2020). Biologically, carbon is a limiting factor in microalgae culture. This is because of its high supplementation in the culture medium allows a significant increase in microalgal biomass. Similarly, concentrations of nitrogen and phosphorus act as secondary building blocks (Zhuang et al., 2018). Cellular nitrogen is used mainly to build proteins, amino acids, and nucleic acids, while phosphorus is mainly a constituent of nucleic acids and phospholipids, and therefore increasing rates of cell growth (Bougaran et al., 2011).

Controversially, it is reported that stress conditions due to nutrient deprivation seem to be a potential strategy for stimulating the production of specific products, which usually occurs at the expense of productivity (Mandal et al., 2020). However, independent of this strategy, the demands for these nutrients represent a high economic influence during the cultivation stage (Gao et al., 2019). In addition, the choice of plant location can drastically influence the monetary deficit. This is because the supply of these resources can result in increased costs according to the distance and the logistics of defining the microalgal plant.

One of the most attractive alternatives can be established by choosing a location co-located with one-time emitters of greenhouse gases. A highly common example is the use of industries such as coal plants as suppliers of carbon dioxide (residual product), as a carbon supply for microalgae cultures. This measure aims to reduce concomitant costs of supplying CO_2, as well as mitigating greenhouse gases, assisting to reduce impacts related to global warming (Molitor et al., 2019). However, the integration of gaseous effluent emissions systems with microalgal culture represents a great challenge so far. Firstly, the exhaust gases used contain high concentrations of oxides of nitrogen and sulfur and can inhibit microalgal growth. In addition, the high temperatures of the gases represent a risk factor for the cells. For this, it is necessary to have cooling and gas separation structures so that the capture and storage systems are integrated. However, the units in operation present expensive techniques, resulting in cost-effective relations, to date, costly (Elrayies, 2018).

Likewise, wastewater recovery strategies containing less desirable nitrogenous substances appear viable only because of a combination of various wastewater that can lead to better growth of microalgae (Javed et al., 2019). In theory, nitrogen compounds can be found in countless ways in wastewater. However, its main assimilation by microalgae microorganisms is given as inorganic substrates, preferably in the forms of ammonium nitrate, nitrites and organic nitrogen (Sakarika et al., 2020). Under similar conditions, the reuse of phosphorus compounds present in wastewater was also considered a promising way to reduce nutrient costs and their environmental burden.

This is because although widely available in the ecosystem, the synthetic process of producing the nutrients needed for the cultivation of microalgae is a regime that consumes a lot of energy. It is estimated that the global demand for energy from the production of the main fertilizers, that is, ammonia and phosphates, resulting from chemical synthesis processes, requires approximately 78.23 MJ/kg and 17.50 MJ/kg, respectively (Gellings and Parmenter, 2004), contributing about 40% of the indirect energy needed for microalgae-based processes (Nagarajan et al., 2020). Thus, under environmental aspects, these fertilizers contribute substantially to the environmental burden related to the categories of acidification potential, eutrophication and global warming potential (de Siqueira-Castro et al., 2020). Associated with these impacts, depending on the location of the installation, these adverse effects on the ecosystem can result in monetary credit in the milder cases, or even in the closure of industrial facilities at more severe levels.

Therefore, the strategy to reconcile microalgae installations close to industrial effluents from agro-industrial systems seems to be potentially promising. In general, agroindustries play an important global economic role. Associated with this, the expressive volume demanded (about $10m^3$/kg of product), results in high volumes of wastewater (Jacob-Lopes and Franco, 2013; Rodrigues et al., 2014). These, in turn, present considerable amounts of carbonaceous, nitrogenous and phosphate compounds, derived from a raw material and are suitable to support the growth of microalgae. In addition, these residues have low chemical risk and are potentially available on a large scale and can generate, concomitant with wastewater treatment, a rich biomass for technological bioexploration (Solovchenko et al., 2019).

3.4.3 LAND USE RESOURCE

Despite recent progress, information about more in-depth geographic data on land use and land cover for microalgal facilities still lacks references in the literature (Lozano-Garcia et al., 2019). Thus, greater awareness needs to be spent before locating and installing process designs based on microalgae.

As widespread, microalgae emerge as potential raw materials for the competition from arable cultures, once are widely recognized for not needing arable lands. However, in order to choose the local geographical position, it is necessary to meet the specific peculiarities required by these facilities. In view of this context, prior measures are essential to assess the availability of land. These pro-analyzes require the identification of available land areas where the facility can be located by applying restrictions related to land characteristics (Boruff et al., 2015).

Among the main desirable features, it is assumed that the land slope is restricted by an upper limit of 2%. This measure aims to reduce the costs of pumping water, as well as excavating the soil. In this sense, it is assumed that microalgae-based process installations require mainly flat land areas to avoid excessive soil excavation (Langholtz et al., 2016). However, from the point of view of environmental protection, it is imperative to state that the locations chosen are exempt from land-use changes. Thus, it is crucial to evaluate and quantify direct changes in land use (DCLU), to estimate the environmental burden on the life cycle of microalgae systems and products. Otherwise, ignoring indicators such as DCLU represents a discrepancy in the assumptions of hypothetical scenarios in view of the consolidation of microalgae plants (Arita et al., 2016).

At the same time, there is a concept in force that any activity or new implementations values and has as its main impulse the mitigation of climate change. As a result of this concept, deforestation and the occupation of agricultural land can occur in the excessive search for sustainable systems. In practice, the location and land required for microalgal facilities often require a higher level of access to transport, causing expansion of land use. As a result, the expansion of these impacts, as well as direct and indirect changes, may threaten the balance of the ecosystem and, consequently, food supply and security (German et al., 2011).

Besides, although some measures make it possible to reduce environmental impacts related to the choice of installation locations, it is common knowledge that any construction and modification of the original soil results in an adverse effect on the environment (Serra et al., 2020). Not unlike, large-scale installations generally involve the removal and displacement of solid outer layers of earth. This procedure can drastically increase the potential for erosion, soil compaction, as well as hardening and even increase the geological risks since it will alter the structure and the physical and chemical properties of the soil. Likewise, the biodiversity of the site can be severely degraded, since the porosity and soil structure depend on the development of plant roots (Zhu et al., 2015).

Given this scenario, it is of interest, not only to the decision-makers responsible for the plant but also from a sustainable point of view, that the elected lands have inadequate characteristics for agricultural activities. This selection makes it possible to avoid competition with food production and, beyond, it can be seen as a beneficial advantage for microalgae. Also, it is suggested that areas close to urban regions, wetlands, protected areas such as parks, as well as a transport network, be disregarded as potentially available territories due to the competitiveness of places that are already used for the most diverse purposes (Kang et al., 2020).

Concurrently, geographical domains located in arid and semi-arid regions, seem to be ideal places for the cultivation of microalgae (Lan et al., 2018). This premise is based mainly on the abundance of natural resources such as high light incidence. Besides, another relevant factor is the lower land cost. However, desert locations are recognized for having adverse conditions. In general, drought, high salinity, wide temperature variations, usual wind, and sand storms, can interfere with microalgal installations (Langholtz et al., 2016). On the other hand, authors such as Schade and Meier (2019), argue that these regions, despite the bottlenecks/challenges, are exposed to greater solar radiation and higher temperatures tended to have lower rates of land use and, therefore, had higher productivities biomass.

In the end, regardless of the place of selection, the importance of checking and studying the location to be installed the microalgal plant becomes essential to identify the soil location as well as the quality of the terrestrial domain. Because only after a previous delineation, the risks susceptible to occasional setbacks such as droughts, floods, earthquakes, availability of infrastructure, interruptions in supply, and temporary availability can be avoided.

3.4.4 LOGISTICS AND TRANSPORT CONSIDERATIONS

It is evident that the geographical location has a high influence on commercial logistics and the transport of raw materials from microalgae facilities. Thus, it becomes explicit that the variability in the choice parameters related to the natural resource indicators described previously needs clarity and caution for the integrated definition of correlated factors. For this reason, developments in microalgal plant projects are generally known to have predominantly non-linear process characteristics (Kosinkova et al., 2015).

In this sense, the impetus for the ideal geographical position of microalgae-based processes consists of the identification of possible uncertainties diagnosed beyond the predicted time period (Coleman et al., 2014). Notoriously, decision-makers evaluate the installation by studying time projections so that the industrial operating system remains operational without any occasional problems, that is, such as the unexpected lack of water or nutrients. However, the choice of the access location must take into account the distances necessary for the supply of nutrients or even the transport of the product for commercialization (Gerbens-Leenes et al., 2014).

Among other points, the flexibility of the supplements required by microalgae, as well as the chain of products generated, allows different types of transport modes to be used, being the most used rail and road transport (Arabi et al., 2019). Among all modes of transport, the road is perhaps the most adequate for the transport of commodities, whether internationally for export or import, or national transport, as well as short and medium distance travel. Road transport is strongly encouraged for the transport of commodities with high added value. However, this modal loses much of its competitiveness for agricultural products in bulk, since its value is very low, where it ends up making its final cost more expensive. Among the main advantages stand out the agility and speed in the delivery of the goods in short spaces to travel, the cargo unit reaches the goods, while in the other modes the merchandise must meet the cargo unit, beyond the goods can be delivered directly to the customer without having to search them. However, its charter cost is more expressive than other competitors with next characteristics, its cargo traction capacity is quite reduced, the vehicles used for traction have a high degree of pollution to the environment, in addition to which additional costs can be included since the road network must be constantly under maintenance or construction (Yi and Kim, 2018).

Alternatively, the railway modal is characterized, especially, by its capacity to transport large volumes, with high-energy efficiency, mainly in cases of displacements over medium and long distances. It also presents greater security concerning the road modal, with a lower accident rate and a lower incidence of theft and robbery. Otherwise, among its restrictions are weak flexibility, that is, network limitations, expensive operations, and the need for transfers (Berger, 2019).

Therefore, the smart choice is a crucial parameter for the development of logistical networks that encompass each required, aiming to meet the maximum demands required for microalgae installations. Besides, is evidence that a robust and economically viable plant must provide the possibility of integrating other established units to simplify the requirements (Klein et al., 2018).

Finally, evaluating trade-offs to determine the ideal capacity of a project as a function of location requires the analysis of infinite combinations to be performed. Thus, the possibility of evaluating the requirements of a robust modeling system based on advanced software design principles, allows these results to assist in the choice of scenarios for high application performance of microalgae plant installations.

3.5 APPROACHES FOR THE TARGETING OF LOCATIONS FOR LARGE-SCALE MICROALGAE CULTURE

The cultivation systems productivity is directly related to a number of factors that affect the growth performance of large-scale microalgae culture.

Such factors are dependent on the elements of a given geographic location, including climatic conditions, such as temperature and irradiance, land availability, accessibility of inputs, including water, CO_2, and nutrients (nitrogen and phosphorus) (Maeda et al., 2018). In addition, the physical adequacy of the region, accessibility, and political, social, environmental, and economic issues must also be considered (Figure 3.1).

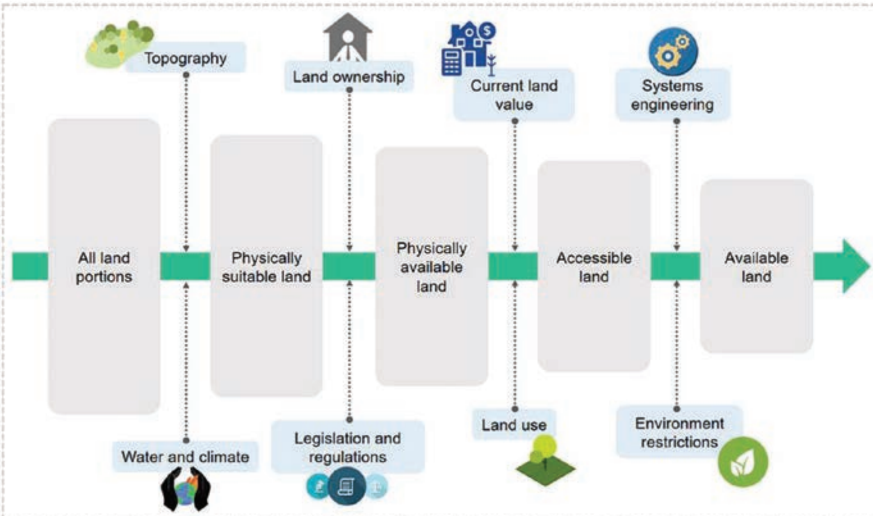

FIGURE 3.1 Main controlling elements to be considered in targeting the identification of locations for microalgae cultivation.

Source: Adapted from Borowitzka et al. (2012).

For the microalgae cultivation, the identification of areas that present adequate topography must be considered first and, the availability of water and favorable climatic conditions. Second, land ownership and legislation and regulations, environmental restrictions, and systems engineering must be addressed, taking into account aspects of the current land value and output expenses. Additionally, the choice of the optimum location for the microalgae culture can be changed according to the particular needs of the strain used, cultivation system, and desired final product. However, for each element considered, there are several factors that must be carefully selected and evaluated, as shown in Figure 3.2.

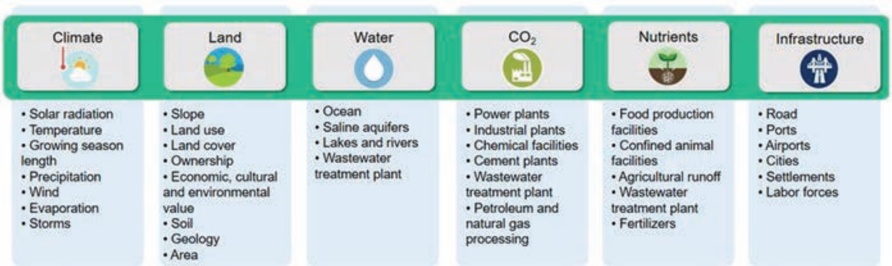

FIGURE 3.2 Factors for consideration in location targeting for microalgae cultivation.

Source: Adapted from Borowitzka et al. (2012)

The climate is considered one of the factors with the greatest impact on the implementation of microalgae-based processes. Solar radiation (qualitative and quantitative aspects), thermal amplitude, duration of the growing season, pluviometric index, evaporation rate, the intensity of winds and storms should be considered whenever possible. Concerning land requirement, this factor must be evaluated from the perspective of soil viability, being topographically broad, affordable purchase price, and free from environmental and cultural restrictions. On the other hand, all types of water are desirable sources for cultures. But the aspects of viability, quantity, composition, and regulations on the withdrawal of natural water resources, as in the case of seawater, must be analyzed. As it is an essential requirement for cultivations, the CO_2 contribution must be facilitated through the integration of sources close to the installation and available abundantly at a low cost. The nutrients supply must also follow the availability rule in an economically viable way. Finally, the infrastructure factor is essential to provide access to resources and services input (demands) and output (export market) in the microalgae processing. However, this factor is not accounted for in most studies of location selection (Boruff et al., 2015).

Given the above, there is a research trend nowadays focused on identifying suitable sites for the successful implementation of microalgal cultures. Approaches that guide the selection of the best region and provide spatial information for the establishment of new microalgae facilities are key factors towards scaling (Bravo-Fritz et al., 2015). For this reason, some approaches have been used to assist in the identification of the location potential for microalgae cultivation. Many studies have use software tools, while others use mathematical models from the literature or develop their own models.

The most used software tool for location selection of microalgae-based processes is the geographic information system (GIS). This computer-based analytical tool allows and facilitates the collection, manipulation, integration, and analysis of geographic data and the phenomena that occur in it. In other words, it uses principles of geography, cartography, and remote sensing methodology to calculate and describe information relevant to the space in question and perform sophisticated evaluations of data referenced to a known coordinate system. All of these highly accurate information can be translated into thematic maps of the study area (Wang et al., 2019). Also, there are many GIS-based software platforms, many of which are open-source, while others are paid. ArcGIS software is the most widely used in microalgae processes.

In terms of application, GIS can be used for a variety of purposes, including local and regional planning, environmental monitoring, agriculture, and infrastructure maintenance. However, the GIS-based tool has motivated many researchers in the field of microalgae processes to put into practice studies that determine the mapping of the sites capable of producing them. The selection of the ideal site is a strategy that interferes with the decision-making of the microalgae commercialization sector. This is because there is great spatial variability in the identification of locations and, therefore, in the estimates of the final product. These two criteria are dependent on the resources available in the area and the geographic characteristics (Sharma et al., 2015).

Increasingly studies have been conducted to identify the optimal location for the implementation of microalgae installations using GIS tool. Table 4.1 shows a compilation of surveys to this extend. Although not shown in Table 4.1, the precursor study on the use of GIS for the selection of microalgae production locations was Maxwell et al. (1985), carried out in the United States. The main factors they considered were land (use, cover, and slope) and climate (temperature, solar radiation, rainfall, and evaporation). Afterward, numerous surveys emerged, especially in the past 10 years. One of the most robust and recent assessments GIS-based tools for targeting microalgae culture sites is the Lozano-Garcia et al. (2019). They addressed as many environmental and physical factors as possible applied for potential biodiesel production in Mexico.

Another computer tool used in microalgae processes is building information modeling (BIM), which has been integrated with the GIS tool. BIM is a digital representation of the physical information on sustainable construction projects. Yet, it has very specific applications, for example,

TABLE 3.1 Targeting Different Microalgae Culture Locations Using GIS-based Tool

Goal	Study Location	Factors	References
Analysis of the potential of resources for the microalgae biofuels production in the country and to assist decision-makers.	India	CO_2, climate, nutrients, water, and land.	Milbrandt and Jarvis (2010)
Investigation of the potential of microalgae productivity in locations with land availability and slope criteria.	United States	Land use, land cover, slope, solar radiation, temperature, nutrients, and CO_2.	Quinn et al. (2012)
Investigation of the potential of microalgae productivity with a CO_2 resource availability.	United States	CO_2, light, temperature, and nutrients.	Quinn et al. (2013)
Assess the viability of microalgae biodiesel production as a commercial option using locally available resources.	United Kingdom	Water, CO_2, and land use.	Umar (2014)
Analysis of factors for selection of microalgae cultivation areas on a large scale.	Chile	Natural resources, road infrastructure, geographic characteristics, and industrial activities.	Bravo-Fritz et al. (2015)
Identify geographic locations capable of cultivating large-scale microalgae for biofuel production.	Western Australia	Topography, climate, CO_2 and nutrients availability, soil workability, land use, labor, and infrastructure.	Boruff et al. (2015)
Analyze area requirements using international policies and targets to identify locations for the microalgae cultivation and assess the global availability of inputs.	European Union, United States and Brazil	Land use, climate, nutrients availability, CO_2, water, and political boundaries.	Speranza et al. (2015)
Analysis of a two-stage model for the design and planning of a microalgae-based biodiesel supply chain.	Iran	Land use, slope, road, power plant, wastewater treatment plant, water, solar radiation, temperature.	Mohseni et al. (2016)
Identification of areas for microalgae cultivation and biomass production in the country.	Mexico	Land use, slope, temperature, evaporation, solar radiation, vegetation, water and CO_2 sources, wastewater treatment plants, rivers, lakes, cities, roads, natural protected areas, historical sites, airport, and geological faults.	Lozano-Garcia et al. (2019)
Analysis of a three-stage structure for the design of an economical microalgae biofuel supply chain.	United States	CO_2, water, nutrients, climate, and land.	Kang et al. (2020)

in the elaboration of microalgae systems parameters inserted in buildings or photovoltaic panels (Castro-Lacouture et al., 2014; Dutt et al., 2017). It is worth mentioning, however, that any computer software tools are very expensive, require the entry of a massive amount of data, becoming prone to costly errors, and, consequently, mistaken assumptions, and it has a relative loss of image resolution (FAO, 2015).

On the other hand, studies have been conducted without the aid of software for mapping candidate sites for microalgae culture. In general, the research applies mathematical modeling to predict the growth and production of biomass in different cultivation systems based on the interaction of parameters and desirable factors (Huesemann et al., 2016). Thus, modeling can be done with equations already defined or from the development of their own models. Also, regarding input data, they can be accounted for from theoretical or empirical models. Table 4.2 summarizes a range of studies found in the scientific literature on assessments of the potential for microalgae production in different locations using predictive data modeling.

The benefit of using this approach is concerning the possibility of optimizing data from laboratory-scale to pilot or demonstration-scale. This is because there is an enormous challenge in guaranteeing the satisfactory performance of the cultures when projected at larger scales. It is practically impossible to make such an extrapolation to determine the cultivation conditions and identification of the appropriate place without taking into account predictive data models. Besides, the modeling offers an opportunity to judge the viability of the proposed microalgae installation in a particular location. However, a problem often attributed to these studies is the lack of specific information and how they are argued. Many surveys compute for generic data; that is, the information is based on experiments that do not match practical situations. This can lead to significant uncertainties in the assumptions (Slegers et al., 2011).

Finally, regardless of the approach adopted to identify potential geographic locations for the cultivations, one must carefully evaluate which criteria, parameters, and factors should be considered in the study. Both software tools and mathematical models will help – even partially – in planning and decision-making estimates for commercial microalgae production.

TABLE 3.2 Studies Using Models to Predict the Local Potential for Microalgae Cultivation

Goal	Study Location	Model Description	Factors	References
Biomass production of two different microalgae species in flat-plate photobioreactors under effect of location, varying light conditions, and reactor design.	Netherlands, France, and Algeria	Theoretical estimation	Light, temperature, nutrients, CO_2, and water.	Slegers et al. (2011)
Potential use of microalgae for biofuel production considering the monthly variation in biomass yield for two climatic conditions.	India	Theoretical estimation	Light, temperature, humidity, precipitation, evaporation, land topography, nutrients, CO_2, and water.	Sudhakar et al. (2012)
Investigation of the annual productivity of biomass and lipids from microalgae and carbon sequestration in five regions of the country under study.	Ethiopia	Theoretical estimation	Light, temperature, humidity, precipitation, evaporation, land topography, nutrients, CO_2, and water.	Asmare et al. (2013)
Optimization of operational criteria for the microalgae growth in raceway ponds under the influence of environmental variables.	France	Theoretical estimation	Light, temperature, and nutrients.	Muñoz-Tamayo et al. (2013)
Investigation of biomass growth and productivity of three microalgae species in outdoor raceway ponds.	United States	Empirical estimation	Light and temperature.	Huesemann et al. (2016)
Performance evaluation of microalgae in open ponds using a biophysical model of site-specific conditions.	India	Empirical estimation	Light, temperature, and topography.	Behera et al. (2018)
Evaluation of biomass productivity and microalgae CO_2 sequestration based on site-specific climatological variables at a power plant.	India	Empirical estimation	Light, temperature, topography, and CO_2.	Behera et al. (2019)
Evaluation of outdoor microalgae productivity under different seasonality.	Germany	Empirical estimation	Light, preciptation, and cloudiness.	Holdmann et al. (2019)
Annual growth evaluation of different microalgae in outdoor cultivation in a pilot plant bubble column photobioreactor.	Italy, Spain, Israel, and Netherlands	Empirical estimation	Light and temperature.	Mazzelli et al. (2020)

3.6 CONCLUSION

The implementation and standardization of microalgae-based processes face geographical restrictions. The climate, followed by physical adequacy and availability of inputs, are crucial factors to be considered in a geographic exam. The approaches to identify geographic positions with potential for microalgae cultivation still lack a robust data matrix to predict suitable cultivation locations, taking into account the requirements of the species in question. The targeting on a global scale of the adequacy of geographic positions for implementing cultivation facilities would be useful for the large-scale planning production.

KEYWORDS

- **algae**
- **bioproducts**
- **cultivation potential**
- **geographic information**
- **photoproduction**
- **strategic locations**

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PART III

PRODUCTION, MARKETING, AND COMMERCIALIZATION

CHAPTER 4

A ROAD TO THE MICROALGAE MARKET: ALLMICROALGAE CASE STUDY

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ABSTRACT

Due to their most diverse taxonomy, biochemical composition, and bioactivities, microalgae are associated with tremendous potential applications in distinct markets. Food and feed currently take most of the microalgae biomass globally produced, but other markets start arising, such as cosmetics, agriculture, biomedical, and pharmaceutical. Allmicroalgae is a Portuguese company, producing microalgae for various markets. The company has been operating for more than a decade, dealing with scientific, biotechnological, business, and market limitations. Allmicroalgae had found the solution in research and development activities to face the various challenges encountered. Those activities are essential to optimize production processes and innovate product portfolio constantly. This chapter focus on the European

Algal Farming Systems: From Production to Application for a Sustainable Future.
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microalgae market and its tendencies, introducing Allmicroalgae as a case study. The difficulties faced since the company's first steps are described, as well as the approaches adopted to overtake them. The microalgae producing process as carried out in the first semester of 2020 is described, however, later adjustments may have been performed. This document intends to demonstrate that resilience and innovativeness are crucial in this business segment.

4.1 INTRODUCTION

Microalgae represent an immense biodiverse resource with a wide range of applications. The large number of chemical compounds biosynthesized, together with the genetic diversity provided by different taxonomic groups, make them potential key players in many industries, such as food and animal feed, nutraceuticals, cosmetics, pharmaceutical, chemical, and bioenergy, biofertilizers, and carbon dioxide (CO_2) sequestration (Vieira 2016; Olaizola and Grewe, 2019). The low production volumes – 30,000 ton/year of biomass (Olaizola and Grewe, 2019) – and the high production costs of microalgae, especially when compared with traditional food commodities, encourage the use of high-value compounds rather than the whole-cell dried biomass (Vigani et al., 2015). Thereby, it is recommended and eventually mandatory a huge reduction in the production costs, through an increase in the production volumes (Vigani et al., 2015) and the use of alternative nutrient and energy sources. Nevertheless, according to the National Center of Biotechnology Information, by 2050, microalgae may account for 18% of protein sources across the world market (Anon., 2020a). The global microalgae-based products market was evaluated at €3,025 million in 2019 and it is expected to grow at a compound annual growth rate of roughly 2.9% over the next five years, reaching €3388 million in 2024 (Anon., 2020b). Nowadays, microalgal biomass generates a global annual turnover of €641 million (Olaizola and Grewe, 2019), and it is estimated that worldwide sales of microalgae will exceed €68 million in revenues by the end of 2026 (Anon., 2020c). The investment, by several countries and union of countries, on bioeconomy-based strategies is expected to shortly boost the microalgae-based product´s market (Anon., 2020d). A myriad of different European funding programs had been available for the period between 2014 and 2020 to strengthen the bio-based economy in Europe: €70 billion for research and innovation, more than €100 billion for rural development, maritime investments, and fisheries, and €66 billion for environmental projects and Trans-European transport connections (Weerdmeester et al., 2014). Governments and companies have been largely investing in research and the development of genetically modified microalgae with high expectations on biofuel production, biochemicals, and other microalgae-derived bioproducts (Anon., 2020d). Furthermore, consumer´ growing demand for natural and organic products is strongly contributing to the microalgae market growth. Food and beverage are the current leading segments in terms of revenues for microalgae application (Anon., 2020d).

From 2000 to 2010, microalgae production for food and feed suffered a 5-fold increase (Ismail, 2010). These organisms have been marketed as a "superfood," and *Spirulina* was even proclaimed as "the best food for humanity in the 21st Century" by the World Health Organization (Anon., 2020e) and "the most ideal food for mankind" by the United Nations (Piccolo, 2012). *Spirulina/Arthrospira* sp. (Cyanobacteria) and *Chlorella* sp. (Chlorophyta) are the most representative microalgae in the market, with 18000 tons and around 95000 tons of biomass produced every year, respectively (Olaizola and Grewe, 2019). Microalgae-based high-value molecules have smaller production volumes, but larger market value than dried algae (Vigani et al., 2015). The market value of a particular microalgae depends on four main variables: its chemical composition, purity level, application, and formulation (Vieira, 2016). Based on these parameters, Voort et al. (2015) built a microalgae value pyramid. In the base of the pyramid are commodity products, like biofuels and biofertilizers. These low-value products are not economically feasible yet (Rahman, 2020). Regarding biofuels, besides the very energy-intensive dewatering and drying processes, the extraction of the lipid fraction of microalgal biomass is also a very expensive processing step. To decrease the global production costs and the consequent market price of the microalgae-derived biofuels, a technological revamping on biomass processing steps and an increase in the dimension of microalgae cultivation farms are needed (Enzing et al., 2014; Rahman, 2020). An economy of scale is essential to lower the prices (Olaizola and Grewe, 2019). Besides, a biorefinery approach, in which all the biomass fractions are valued into different products to reach different market sectors, will raise the revenue streams, allowing the commercialization of biofuels and other low-value products at competitive market prices (Vigani et al., 2015; Rahman, 2020). The top of the value pyramid contains the added-value products extracted from microalgae (Vigani et al., 2015). High-value extracts include proteins (e.g., phycocyanin), lipids (e.g., omega-3 fatty acids, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA)), carbohydrates (alginates), pigments (e.g., astaxanthin), antioxidants (e.g., β-carotene), and vitamins (tocopherol) (Rahman, 2020). Given their high market value, the commercialization of these products is already economically viable. However, they are still more expensive than the same compounds produced by alternative sources, reinforcing the importance of the biorefinery concept.

The first industrial-scale microalgae production facility was established in Japan in the 1960s. By the 1980s, other large-scale production facilities were established in Asia, India, the US, Israel, and Australia (Enzing et al., 2014). Currently, the Asian Pacific region is a major hub of many international players across the world. Many microalgae manufacturers operate in China, India, and Australia (Anon., 2020d). Europe has a share of about 5% of the global microalgae production for food and feed products (Enzing et al., 2014). Due to its unfavorable climatic conditions, especially the countries outside southern Europe, the yields are, on average, 50% of the other locations. Thus, the bulk production of low-value microalgae products is not a competitive industry for European organizations (Enzing et al., 2014; Vigani et al., 2015). Many of them stepped into the microalgae-based high-value molecules market, existing a few companies producing whole dried algae (Enzing et al., 2014; Vigani et al., 2015). However, it was estimated that, from 2015 to 2025, Europe could become the market leader in microalgae-based products for the food and feed markets (Enzing et al., 2014).

4.2 CURRENT TRENDS AND FUTURE PERSPECTIVES IN MARKETED MICROALGAE

Globally, there are more than a hundred commercial producers of microalgae, in which the Asia and Pacific regions embrace the majority with an annual estimated production of 5,000 tons/year of dry biomass (Sathasivam et al., 2019). Microalgal products are mainly positioned into various market segments, such as nutraceuticals, food, feed, biomedical, pharmaceutical, and agricultural. Nevertheless, several dynamic factors can affect the sustainability of the microalgal industry, which comprises the use of robust strains, low-cost cultivation methods, social awareness and acceptability of the microalgal products, policies, and regulations (Enzing et al., 2014; Nethravathy et al., 2019).

The food, feed, and cosmetic are the main market segments on which microalgae play a role. All these market segments belong to the microalgae isolated compounds market, which is mainly ruled by pigments but is growing for polyunsaturated fatty acids (PUFAs), proteins, and oils in the short-term. This interconnected market has an independent behavior and the top companies are based in business-to-business transactions.

4.2.1 FOOD – NOVEL FOODS

Microalgae had been consumed by humans worldwide, mainly in Asia, since ancient times (Mobin et al., 2019). Microalgal large-scale cultivation for food usage in Europe started with the production of *Chlorella vulgaris* (Nethravathy et al., 2019). As food safety is the main concern of different regulatory agencies, shifting to new food sources is progressively more recognized as an essential resolution to feed the world's growing population (Parodi et al., 2018). Microalgae appear well-positioned to meet this need and replace the plant and animal-based traditional sources. Microalgae are generally classified as "other dietary supplement" both when using intact microalgae or isolated compounds incorporated in processed food, such as cakes, drinks or crackers (Nethravathy et al., 2019). Scientific investigations related to the nutritional profiling of diverse microalgae had been performed to unravel their specific application (Enzing et al., 2014; Nethravathy et al., 2019).

The microalgae biomass for human consumption is available in dry powder and tablets, capsules, or liquid. Microalgae can be supplemented to processed food, such as pasta, snacks, drinks, and others. Those are now getting more attention from the food industry, which implements microalgae as a nutrient-enriched source in new food formulae (Priyadarshani and Rath, 2012; Grubišić et al., 2019). The most commercially explored microalgae are *C. vulgaris*, *Haematococcus lacustris* (formerly *Haematococcus pluvialis*), *Dunaliella salina* (Chlorophyta), *Aphanizomenon flos-aquae*, *Nostoc* sp., and *Arthrospira* sp. (mainly, *Arthrospira platensis*) (Cyanobacteria), used mainly for nutritional supplements (Spolaore et al., 2006; Priyadarshani and Rath, 2012; Grubišić et al., 2019). Microalgae are exploited as human food resources because of their bioactive compounds and high nutritional value, such as the inherent high protein and mineral content (potassium, iron, magnesium, calcium, and others). The biomass is composed of remarkable compounds that are important for human nutrition, such as carotenoids, PUFAs, astaxanthin, β-carotene, sterols, pigments, and vitamins A, C, B_1, B_2, B_6 (Grubišić et al., 2019; Nethravathy et al., 2019).

Microalgae are typically used as a supplementation of the conventional food preparations by improving their nutritional value (Spolaore et al., 2006; Priyadarshani and Rath, 2012). The best example and one of the most exploited microalgae for food supplement is *A. platensis* (*Spirulina*), used in human nutrition due to the high protein content and exceptional nutrient profile (Spolaore et al., 2006). It also has linolenic acid, an EFA that is not synthesized by humans (Milledge, 2011). *A. platensis* extract is used in pasta, biscuits, and other processed food products, mainly as simple microalgae powder. The main health benefit of this microalga is related to the function of the digestive tract and the maintenance of the intestinal microbiome (Pulz and Gross, 2004).

The industry and market have been adapting to the increasing demand for microalgae-based products (Nova et al., 2020). *Chlorella* sp. is the second most explored microalgae in the food industry, with 5,000 tons of dried biomass produced per year. These productions are expected to grow considering the increasing demand for novel foods that can alleviate the pressure on the traditional food forms (García et al., 2017; Nethravathy et al., 2019). The nutritional value (100 g of dry weight) in *Chlorella* sp. have mild calories rating (medium of 410 KCa, 21% of the Reference Daily Intake (RDI) for adult person); low content in carbohydrates (medium of 23.2 g; 8% of the RDI) as the same of fats (9.3 g, 14% of the RDI; where the mainly is PUFAs). However, the relevant nutritional importance of *Chlorella* sp. is the high protein content (58.4 g, 117% of the RDI) and high dosage of vitamins, such as vitamin A, Thiamine, Riboflavin, Niacin, vitamin B6 (Anon., 2020f).

4.2.2 FEED

Microalgae are the base of the food chain in aquatic ecosystems, being the primary feed source for various species. However, for others, microalgae are used as a feed supplement due to their high nutritional value, such as the quality of protein, minerals, vitamins, and PUFAs (Sirakov et al., 2015). Microalgae play a key role in aquaculture (mariculture), being the natural food source to various molluscs, crustaceans, and fish larvae forms.

The most used microalgae in aquaculture are from the genera *Chlorella*, *Tetraselmis* (Chlorophyta), *Isochrysis*, *Pavlova* (Haptophyta), *Phaeodactylum*, *Chaetoceros*, *Skeletonema*, *Thalassiosira* (Bacillariophyta), and *Nannochloropsis* (Ochrophyta, Eustigmatophyceae) (Spolaore et al., 2006). Microalgae are employed in aquaculture as live feed for all growth stages of bivalve and molluscs, for the juvenile stages of abalone, crustaceans, and even some fish species. The use of microalgae as a feed additive result in gaining weight and enhancement of triglyceride and protein deposition in muscle, the animal digestibility, starvation tolerance, and carcass quality by the animals (Sirakov et al., 2015; Madeira et al., 2017). Some microalgae species, such as *Dunaliella salina*, *H. lacustris*, and *A. platensis*, can be used as a source of natural pigments for the culture of prawns, salmonid fish, and ornamental fish (Sirakov et al., 2015).

A. platensis is also applied in other animal feed for cats, dogs, birds, horses, cows, and breeding bulls (Spolaore et al., 2006). In poultry feeds, the product Algrow®, produced by the Germanic company Institute für Getreideverarbeitung, containing *Chlorella* sp. and *Arthrospira* sp., showed to modify the coloring of broiler skin, egg yolk, and shanks. However, dosage higher than 10% of the microalgae-based feed provoked adverse effects in broiler (Pulz and Gross, 2004). It was proven that microalgae can be used as a feed supplement and not as total feed replacement. Animal feed currently represents nearly 30% of the current global microalgae segment application, and it is estimated to grow mainly due to the high cost of the fish meal in fish aquaculture (Sirakov et al., 2015; Madeira et al., 2017)

4.2.3 COSMETIC

Cosmetics is the third major market segment for microalgae application and the most rapidly growing one. In the last decade, organic and sustainable tendencies in the cosmetic market have led to the investment in research considering new high value, innovative, and natural formulations for new products by the cosmetics companies and start-ups (Nethravathy et al., 2019). Microalgae, contrary to macroalgae, are not very exploited in the cosmetic field. Nevertheless, microalgae and their added-value compounds had been incorporated in diverse skin and hair products, with a wide range of functions, such as excipient (stabilizer or emulsifier) or active ingredients. Besides, microalgae have been used for a long time as colorant for diverse cosmetic products (Grubišić et al., 2019).

Microalgal amino acids and PUFAs have proven to improve moisturization (efficient water restoration properties of a cream). For example, *Thalassiosira*, *Nannochloropsis* genus, and *Monodus subterraneus* (Ochrophyta, Xanthophyceae) were exploited for this purpose (Guedes et al., 2011; Couteau and Coiffard, 2018). These natural compounds from microalgae can substitute the principal moisturizers used in cosmetic, hydroxyl acid-based compounds from animals and plants, which are limited and have a high cost for the companies (Guedes et al., 2011; Couteau and Coiffard, 2018). *Chlorella* and *Arthrospira* represent other moisturizer sources, and those are already in the market by the cosmetic producer Nykaa (Joshi et al., 2018). The squalene isolated from *Thraustochytrium*, *Aurantiochytrium*, and *Schizochytrium* (*Labyrinthulomycetes*) is applied in cosmetics as emollients, skin softener, or soother (Joshi et al., 2018). Most cosmetics use synthetic ingredients with these properties. Therefore, microalgae can represent a natural and sustainable alternative (Nethravathy et al., 2019).

Other important segments are anti-aging and photoprotection. There has been a substantial increment in this market during the last decade. The products in this selected market intend to contradict the natural skin aging, for example, gradual loss of elasticity and dullness of skin, due to harsh environmental (e.g., ultraviolet (UV) radiations, which promote the production of reactive oxygen species (ROS) (Pallela et al., 2010). ROS and the loss of skin fibers, elastin, and collagen, are the major causes of skin aging (Lan et al., 2019). The main anti-UV radiation segment is the sunscreen (Lan et al., 2019). The principal compounds isolated from microalgae integrating these products are the carotenoids (β-carotene, astaxanthin, lutein, zeaxanthin, and canthaxanthin), mycosporine-like amino acids, and polysaccharides, which are already commercialized products (Conde et al., 2004; Oren and Gunde-Cimerman, 2007; Carreto and Carignan, 2011; Wada et al., 2015; Nethravathy et al., 2019). Astablank, a Japanese cosmetic product for skin wrinkles and spots, uses the carotenoid astaxanthin, isolated from *H. lacustris*, as an ingredient in the cosmetic formulation (Nethravathy et al., 2019). Fucoxanthin extracted from microalgae is regularly used as cosmeceuticals to reduce melanogenesis (Thomas and Kim, 2013). *Scenedesmus* and *Chlorella* genus are used to extract lutein, which plays a protective role against UV-induced skin damage. Mycosporine-like amino acids from *Dunaliella* and *Chlorella* are used mainly as anti-UV radiation sunscreen (Conde et al., 2004; Oren and Gunde-Cimerman, 2007; Carreto and Carignan, 2011; Wada et al., 2015; Nethravathy et al., 2019). The polysaccharide-enriched extract of *Porphyridium* sp. (Rhodophyta) is an active ingredient in the Alguard™ cosmetic product by Frutarom, with the principle of stimulating the elastin synthesis (Martins et al., 2014). Cosmetic companies from Germany and Switzerland, Ocean Pharma and Penthapharm, respectively, are known for including microalgae extracts in their cosmetic formulations. Other commercial products include protein-rich extracts from *Arthrospira* sp. and *C.*

vulgaris as anti-aging active agents (Exsymol S.A.M. and Codif companies, respectively). *Arthrospira* sp. and *C. vulgaris* extracts proved to have anti-aging activity, more specifically skin regeneration, collagen production, and wrinkle formation reduction (Stolz and Obermayer, 2005; Ryu et al., 2015; Rizwan et al., 2018). Some multinational cosmetic companies, like LVMH and Daniel Jouvance, have their microalgal production system, producing themselves the microalgae they use (Spolaore et al., 2006; Rizwan et al., 2018). Extracts from *Chlorella* genus have proven to be a potential source for new anti-aging formulas, by enhancing the collagen synthesis, due to the tissue regenerating and wrinkle decreasing activity (Wang et al., 2015). Other cosmetic segments are now interested in microalgae bioactivities and can open new opportunities for microalgae, such as hair and skin-whitening cosmetic segment.

4.2.4 COMPOUNDS ISOLATED FOR NUTRACEUTICAL AND OTHER APPLICATIONS

The number of added-value compounds isolated from microalgae is exponentially growing and has gained more attention in the last decade. These compounds are used in various industries and fields, being natural substitutes of other natural equivalents or synthetic sources. For example, *Schizochytrium* (Labyrinthulomycetes) oils and *Chlorella* lipid-rich fraction have been explored for food and feed (García et al., 2017). Pigments are one of the key group of compounds and have multiple industry applications, such as animal feed (mainly, aquaculture), human food, nutraceutical, pharmaceutical, cosmetic and, in the biomedical industry for possible labels and colorants for marking antibodies and receptors in diverse types of medical assays (Grubišić et al., 2019).

4.2.4.1 CAROTENOIDS

One of the most attractive class of compounds extracted from microalgal biomass are the photoprotective pigments carotenoids. The carotenoid abundance is normally low (0.1 to 2%), however, several methodologies can be applied to enhance its concentrations. For example, *Dunaliella* sp. cultivated under stress can accumulate up to 14% β-carotene. β-carotene is used in food supplementation. The specific market is estimated in US$ 285 million with a tendency to grow at 1.8% annual rate (Nethravathy et al., 2019). Astaxanthin is another explored carotenoid, with a high antioxidant activity, approved by the Food and Drug Administration as a nutritional supplement (Shah et al., 2016). Besides being *H. lacustris* a novel food-approved source of astaxanthin, other species can also produce this pigment. Those species are *Botryococcus braunii*, *Chlamydomonas nivalis*, *Scenedesmus* sp., *Chlorococcum* sp., *Neochloris wimmeri*, *Protosiphon botryoides*, *Coelastrella oocystiformis* (formerly *Scotiellopsis oocystiformis*) (Chlorophyta), and *Trachelomonas volvocina* (Euglenozoa) (Rajesh et al., 2017). In addition, a requirement of stress is necessary to stimulate the accumulation of those pigments, such as nitrogen depletion, high light intensity, salinity, or ion stress. *H. lacustris* astaxanthin represents 1% of the worldwide market, and its market value is around US$ 2,000/kg (Hu et al., 2018). Feed market, mainly aquafeed, is the main market for this compound (Nethravathy et al., 2019).

Lutein is another pigment that can be extracted from various microalgae species, such as *Chromochloris zofingiensis* (formerly *Chlorella zofingiensis*), *Muriellopsis* sp., *Tetradesmus almeriensis*, *Coccomyxa acidophila*, and *Auxenochlorella protothecoides* (formerly *Chlorella protothecoides*) (Chlorophyta). Besides marigold being currently the conventional source of lutein, microalgae have the advantages of yield and not competing for arable land. However, lutein production based in microalgae is still under development (Lin et al., 2015).

Canthaxanthin is another commercially available microalgal carotenoid, used as an animal feed supplement to improve pigmentation, for example in chicken, poultry, and aquatic animals (Abe et al., 2007; Sathasivam et al., 2019). *C. zofingiensis* is the main microalgae source of canthaxanthin (Nethravathy et al., 2019).

4.2.5 OTHER APPLICATIONS

Other markets are being explored for the application of microalgae, for example, agriculture, pharmaceuticals, and biofuels, which are mainly under investigation.

4.2.5.1 PHARMACEUTICAL

This market segment is still under development. Microalgae extracts show a wide range of bioactive effects, such as antimicrobial, antibacterial, antiviral, anti-inflammatory, antitumor, antimalarial, immunosuppressant, and anti-human immunodeficiency virus (Dittmann et al., 2001; Gademann and Portmann, 2008; Volk, 2008; Wase and Wright, 2008; Mayer et al., 2009; Jha et al., 2017). For example, microalgae toxins are being investigated for application as potential antibiotics (Jha et al., 2017; Zahra et al., 2020), turning the attention to the microalgae secondary metabolites (Jha et al., 2017).

Academic and Research and Development (R&D) groups are actively searching to find and develop new microalgae-based drugs. The most considerable efforts are directed to new drugs with anti-cancer potential (Kharkwal et al., 2012; Jha et al., 2017). *Ochromonas* (Ochrophyta, Chrysophyceae), *Prymnesium* (Haptophyta, Coccolithophyceae) genus, and Cyanobacteria are in the pipeline (Beyatli et al., 2012).

4.2.5.2 AGRICULTURE

Due to the current agrochemical restrictions, mainly in the European Union (EU), microalgae had been pointed as a substitute to the synthetic products commonly used (Ronga et al., 2019). Microalgae have the potential to be used as either biofertilizers or biostimulants. Biofertilizers are products that enhance soil biotic and abiotic quality, plant growth, and restore soil fertility. Biostimulant is an organic-based product that is applied in low concentrations, capable of promoting growth, and/or pathogen-resistance (Ronga et al., 2019). These two typologies are considered eco-friendly and cost-effective substitutes for synthetic crop enhancers (Kawalekar, 2013).

Microalgae possess a complex diversity of macro- (Nitrogen, Phosphorus, and Potassium (NPK)) and micronutrients essential for crop development and growth. As organic slow-release fertilizers, microalgae promote gradual crop growth, preventing groundwater contamination and lixiviation of soils (Coppens et al., 2016). Besides, microalgae release plant growth-promoting molecules, such as auxins, cytokines, betaines, amino acids, vitamins, and polyamines (Spolaore et al., 2006; Gebser and Pohnert, 2013; Stirk et al., 2013a,b).

Arthrospira spp. dry biomass demonstrated an excellent NPK ratio, with 6.7, 2.47 and 1.14% in the dried biomass (Aly and Esawy, 2008), with lower content of calcium and lead, appropriate to use as a biofertilizer. Protein hydrolysates from microalgae are considered active plant biostimulants, and their foliar application enhances plant biological activity against abiotic and biotic factors, enabling the development and growth of the crops under stress factors (Calvo et al., 2014). Spirufert®, based in *Arthrospira* spp., is a foliar fertilizer with demonstrated potential at low concentrations to increase the aubergine fruits yield. On the other hand, when surpassed the recommended concentration, the biostimulant negatively impacts the crop (Dias et al., 2016). The American company Del Monte Fresh has investigated the potential of microalgae (mainly Cyanobacteria species) biofertilizers at the Arizona's raw deserts, with promising results (Edwards, 2016). The biggest obstacle to the development of this market is the microalgae cost production that, at this moment, is not competitive with the traditional NPK fertilizers.

4.2.5.3 BIOFUELS

The negative impacts of fossil fuels are conducting research teams into alternative fuels. Diverse companies, such as Algenol, Sapphire Energy, and Seambiotic, use microalgae to produce bioethanol at a commercial level (Khan et al., 2018). However, the companies in this segment are developing new technologies to improve biofuel production, mainly from *Botryococcus braunii* and other microalgae species and strains with high lipid content. This high lipid content maximizes the bioethanol, biogas, and biodiesel production yield and lowers the cost associated with the biorefinery (Suparmaniam et al., 2019; Rajesh Banu et al., 2020). *Chlamydomonas reinhardtii* had also been indicated as a potential source of bio-hydrogen (Zhan et al., 2017).

4.3 ALLMICROALGAE CASE STUDY

4.3.1 HISTORY AND TIMELINE (FROM R&D TO INDUSTRIAL PRODUCTION)

To find new solutions to mitigate CO_2 emissions, Secil group, one of the biggest cement industries located in Portugal, initiated, in 2007, in Pataias, Portugal, a research project focused on the cultivation of microalgae using the produced CO_2. This innovative carbon fixation technology intended to be economically viable and sustainable through equilibrium between the reduction of CO_2 emissions and the sales of microalgal biomass. A timeline resuming the milestones on the company's history is represented in Figure 4.1. In 2008, the first phase of the project, a pilot production plant was constructed, commissioned, and begun to be operated. This microalgae pilot production plant (MPPP) is the current research and development (R&D) unit that is located nearby the production plant. In 2009, MPPP won the

FIGURE 4.1 Milestones of Allmicroalgae's history.

European Environmental Press Award at Polytec, in Paris. Given the promising results obtained up to then, at the end of 2010, Secil moved on to the second phase: the construction of a microalgae industrial production plant (MIPP), nowadays known as the Allmicroalgae facility. At that time, this unit was the largest microalgae production plant under closed systems in Europe. After two years of designing, consigning, and construction, the start-up initiated at the end of 2012 and in 2013 the production began.

Between 2013 and 2015, a balance between the produced biomass, sequestered CO_2, production costs, and sales obtained, fell short regarding the target objectives initially established, leading to reformulation on the mindset and directions of the project. At the end of 2015, new management and technical approaches, where fermentation had been strategically included in the process of microalgae production to boost productivity, was culminated with the creation of a new company – Allmicroalgae Natural Products S.A. In 2015, Allmicroalgae was 100% owned by Secil Group aiming to explore microalgae production and further commercialization. Initially, the production was focused only on the microalga *C. vulgaris*, under the brand Allma®, targeting the human and nutritional supplementation markets. *C. vulgaris* is a protein-rich species approved by the EU as a food ingredient. In 2016, the company started to produce also *Nannochloropsis oceanica* (Ochrophyta, Eustigmatophyceae), an omega-3 rich strain marketed mainly for the aquaculture and feed sector. From 2017 on, Allmicroalgae's portfolio opened up with the commercialization of *Tetraselmis chui* for human and feed nutrition as well as *Tetradesmus obliquus* (formerly *Scenedesmus obliquus*) (Chlorophyta) and *Phaeodactylum tricornutum* (Bacillariophyta) for the feed and biofertilizer sectors. Recently, Allmicroalgae expanded towards new markets, such as feed, cosmetics, and biofertilizers/biostimulants, with the creation of new brands, namely Allvitae® and Allfertis® for feed and fertilizer markets, respectively. At the beginning of 2017, the fermentation unit was designed, commissioned, and started producing under batch conditions. By that time, Allmicroalgae reformulated the production mode where the inoculum of *C. vulgaris* began to be produced in heterotrophic conditions, to reach high biomass concentration, and thereafter inoculated in the outdoors photobioreactors (PBRs) to reach the protein and high-value components that are mainly dependent on the sun. This strategy was crucial for Allmicroalgae to achieve new production capacities that, without the fermentation, would never be achieved.

The participation of Allmicroalgae in research projects like AlgaCO_2 and AlgaValor, and its promotion in the case of AlgaValor (P2020), demonstrates the company´s commitment to the investigation/demonstration of the technical and economic viability of sequestering CO_2 through the cultivation of microalgae. Thus, despite the aforementioned change of mindset, which led to Allmicroalgae´s economic viability, the development of solutions for the CO_2 emissions problem continues in the company's list of priorities. In October 2019, the French microalgae extraction expert Altinat, owner of Greentech and GreenSea, joined the ownership of Allmicroalgae together with the Secil group. This partnership aimed to enormously enlarge the sales volume and open new exportation routes, expanding the company's business.

4.3.2 LARGE-SCALE MICROALGAE PRODUCTION

4.3.2.1 AUTOTROPHY

Allmicroalgae has the biggest closed reactor microalgae production plant in Europe and one of the biggest in the world, with a total production volume capacity of 1300 m^3. The farm occupies about 1 ha of ground area and has 19 horizontal tubular PBRs with a photic stage of 330 km of acrylic tubes that are horizontally oriented and vertically stacked, as can be seen in Figure 4.2. The stacked parallel tubes are connected, at the ends, by two manifolds. This configuration allows microalgae cultivation on a very large scale since it reduces the head losses and oxygen accumulation in the culture (Torzillo and

FIGURE 4.2 Algafarm upper view.

Zittelli, 2015). Thus, at Allmicroalgae's farm, there are 19 PBRs, ranging from 11 to 170 m^3 of total volume:

- Five 11 m^3 PBRs
- Five 40 m^3 PBRs
- Eight 115 m^3 PBRs
- One 170 m^3 PBR

Allmicroalgae cultivate and commercializes both marine and freshwater microalgae. Its commercial portfolio includes *C. vulgaris*, *N. oceanica*, *P. tricornutum*, *T. chui*, and *T. obliquus*; however, other species/strains can be grown and supplied upon demand. Such species diversity enables a year-round production that adds up to 60 tons of dried biomass per year. However, the seasonality of some of the cultivated species obliges to consider it when planning year productions. Besides, in the R&D unit, several other species are continuously being tested and optimized to assess their commercial potential and the viability of the industrial-scale cultivation, such as *Halochlorella rubescens* (formerly *Scenedesmus rubescens*), *Chlorococcum amblystomatis* (Chlorophyta), *H. lacustris* (Chlorophyta), *Pavlova* spp. (Haptophyta, Pavlovophyceae), and the Cyanobacteria *A. platensis*.

The scale-up process depends on the microalgae cultivated. For the marine species and *T. obliquus*, the process is exclusively photoautotrophic, but for *C. vulgaris* there is an initial fermentation stage. All axenic microalgae cultures are kept in master cell banks cryopreserved in liquid nitrogen.

Those cell banks are used to inoculate sequentially bigger flask reactors (50 to 250 mL), which seed 5 L reactors (fermenters or flasks, depending on the scale-up process used). In the exclusively photoautotrophic scale-up process, the cultures grow in 5 L flask reactors at the culture room, under controlled conditions (Figure 4.3). When the dry weight (DW) reaches about 1 g L^{-1}, each 5 L reactor is divided into other five 5 L reactors, and so on (Barros et al., 2019). Twenty 5 L reactors are then used to seed one 1000 L vertical flat panel (FP) grown in a greenhouse under controlled temperature and light conditions (Figure 4.3). From this stage on, the process is very flexible and depends on the size of the PBR that will be inoculated. If the aim is to seed a 10 m^3 PBR, three FPs are usually enough, depending on culture concentration. The volume of inoculum required always depends on the species/strain being cultured, its DW, and the capacity of the PBR that will be inoculated, and those calculations are a result of extensive cultivation experience. In general, for the outdoor reactors, a sequential scale-up is carried out in which the smaller reactors are used to inoculate the bigger ones with a minimum 0.2–0.4 g L^{-1} DW, depending on the cultivated species/strains. An alternative and more productive scale-up process is used for *C. vulgaris*.

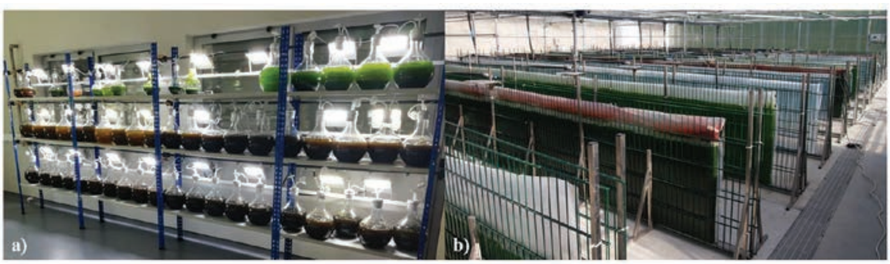

FIGURE 4.3 (a) Culture room; and (b) greenhouse.

For photoautotrophic cultivation, besides culture media, Allmicroalgae uses groundwater from a local underground stream. Marine species need further supplementation of refined sodium chloride, naturally concentrated micronutrients, and other marine salts solution. When chemically and biologically possible, permeates obtained from the processing of marine cultures are treated and reused, accomplishing a circular economy practice that has been implemented for years in the company, but which is currently being studied and refined. So far, only food-grade CO_2 has been used, both as the carbon source and the culture's pH control system. Allmicroalgae has a self-designed formula of a nutritive synthetic medium used to cultivate regular species/strains. Another medium, an organic one, is also used to produce organic certified *C. vulgaris*. To the best of our knowledge, the company is the only European large-scale manufacturer producing such organic certified biomass. Despite being more challenging due to complexity, the development of organic media to grow marine species is also in the pipeline of the company's projects. Allmicroalgae farm is situated in Pataias, Leiria, about 6 km far from the Atlantic Ocean. This location has typical Mediterranean weather, with high sunlight exposition throughout the whole year. During summer, the radiation reaches values up to 1000 W m^{-2}. In days of intense radiation and high temperatures, a fully automated thermoregulation system cools the tubes of the outdoor reactors. The greenhouse has a mobile roof, which opens when the inside temperature is higher than the setpoint established. When the indoor temperature is too high for the cultured microalgae, a thermo-retractable shadowing system covers the FPs.

4.3.2.2 HETEROTROPHY

Since 2017, when the fermentation setup was installed, the scale-up process of the microalga *C. vulgaris*, the company's core business, was reformulated. Taking advantage of the alga ability to grow using either its photoautotrophic or heterotrophic metabolism, Allmicroalgae had optimized a heterotrophic cultivation stage prior to the photoautotrophic cultivation, which is used to produce highly concentrated inoculum for the photoautotrophic PBRs. This way, the photoautotrophic stage is mainly used to promote the biosynthesis of the proteins and photosynthetic pigments, being the overall growth not as susceptible to uncontrolled variables as before. Initially, the culture is grown in one 5 L fermenter, reaching up to 174.5 g L^{-1} DW (Barros et al., 2019). Then, those 5 L of highly concentrated culture are used to seed a 200 L fermenter that further directly inoculates the 5000 L fermenter, which can, by itself, inoculate all the 19 PBRs of the farm, given the very high concentrations (≈120 g L^{-1}) attained (Barros et al., 2019). This was a tremendous breakthrough in the overall cultivation process of *C. vulgaris* in the company once it allowed significantly decreasing the duration (5-fold) and occupancy area (12-fold) of the scale-up process (Barros et al., 2019). Besides, it enabled to deliver reproducible high-quality inoculum, avoiding the build-up of contaminants and culture collapses (Barros et al., 2019). The lower total production time and the more homogeneous inoculum also results in the obtention of a highly reproducible, homogeneous final product.

Beyond the great improvements introduced in the scale-up process, fermentation enabled to increase the volume capacity of production and expand the company's range of products. Alternative *C. vulgaris* products with a smooth taste and lighter colors were launched in the market, such as the Smooth *Chlorella* and Honey *Chlorella*, and intensive research and development have been done to diversify even more the portfolio of products offered. Non-genetically modified strain improvement techniques have been used to obtain *C. vulgaris* mutants with low chlorophyll and improved protein contents, with great potential to be feedstocks for the development of novel food supplements and foods, such as the commercialized Honey *Chlorella* (Schüler et al., 2020). The fermentation of other species is another short-term target for the company, which has the R&D department intensively working on it.

4.3.2.3 MIXOTROPHY

In a photoautotrophic growth regime, light is the energy source for microalgae growth and for this reason, this kind of production mode is highly dependent on environmental conditions. Industrial heterotrophic cultivation of *C. vulgaris* was implemented by Allmicroalgae as a solution for the seasonal weather variations; however, not all microalgae species/strains can carry both an auto- and heterotrophic metabolism. Alternative solutions to promote marine species productivities have been explored, such as the mixotrophic growth cultivation mode. This growth regime enables overcoming light variations, ensuring the growth and expression of photosynthetic pigments (Barros et al., 2017). In the exponential growth phase, light energy is the main energy source, whereas as the culture ages and cell concentration increases, creating a mutual shading effect, organic substrates are the main source of energy (Barros et al., 2017). Notwithstanding, mixotrophic microalgae growth has been demonstrated to increase with light (Barros et al., 2017). Before implementing the fermentation unit in the company, *C. vulgaris* was grown mixotrophically (Barros et al., 2017). Nowadays, a mixotrophic cultivation mode is used for *P. tricornutum* and *T. obliquus*. However, Allmicroalgae has been testing the viability of using organic wastes and industrial by-products as alternative and sustainable carbon sources for mixotrophic cultivation, supporting the circular economy policy implemented.

4.3.3 R&D

Allmicroalgae has an R&D unit (Figure 4.4) fully committed to developing new solutions applicable to the industrial production of microalgae and solving the problems of the current industrial processes. The R&D team is daily focused on the optimization of microalgae growing conditions and processes. A study performed in the department involving *Tetraselmis* sp. biomass recovery by changing the medium salinity allowed an effective recovery of 97% of the total biomass, significantly decreasing the harvesting costs (Trovão et al., 2019). Furthermore, a comparison between *N. oceanica* growth in two different pilot-scale raceway pond configurations (Cunha et al., 2020) allowed deciding regarding the suitable design for industrial cultivation. Bioprospection of new potential large-scale cultivated species is also one of the main challenges of R&D. The heterotrophic cultivation of *Aurantiochytrium* sp. for further use as a source of DHA had been addressed

FIGURE 4.4 Upper view of the R&D unit.

(Trovão et al., 2020). Also, a new protein-rich strain of *C. amblystomatis* was isolated from a lagoon located near the production plant. Adapted to the local environmental conditions, the strain can grow in industrial media and reactors (Correia et al., 2020). However, and because several microalgae species are already being industrially cultivated, innovative applications for those microalgae are continuously being approached. A recent study revealed that the EPA supplied by *N. oceanica* is a great natural source of rumen-protected omega-3 for ruminants (Alves et al., 2018). Furthermore, the department is involved in several national, such as AlgaCO_2, AlgaValor, and ValorMar (Anon., 2020g) and international research projects, such as SIMBA (Anon., 2020h), MAGNIFICIENT (Anon., 2020i), and PROFUTURE (Anon., 2020j). All the referred projects together with the constant new applications, allow the company to collaborate with other experts in complementary topics. Through its R&D department, the company maintains a close relationship with Portuguese universities, hosting around 10 master and doctoral students every year.

4.3.4 SCALE-UP

Aiming at improving the sustainability and viability of microalgae production process, Allmicroalgae is currently investing in open PBRs. Industrial raceway ponds, three 1000 m^2 and one 4000 m^2 of ground area, are currently being constructed. These bioreactors are energetically more efficient than the existing and operating tubular PBRs. With this new technology, it is expected to decrease considerably the operational costs (OPEX) and use the CO_2 release from the adjacent cement factory, not compromising the quality of the biomass produced. Besides, it will significantly increase the annual production capacity beyond the current 60 tons/year, allowing the company to serve new customer segments in which the analytical requirements are not as strict, mainly in the feed and biostimulants markets. This boost in the production capacity will enable the commercialization of biomass at much more competitive prices, allowing Allmicroalgae to enter new markets, such as the Asian market, the current leader of the global production capacity. The success of microalgae production in raceways will be assessed regarding productivities, analytical and biochemical quality of the produced biomass, energetic efficiency, and new products released to the market. A second scale-up, which could vary between 10 and 50 ha, is a possibility considering the balance of the described parameters. Such an expansion would represent a €10 million of sales potential for the company, being the feed market the main aim. In addition to the diversification of microalgae production technologies, current PBR and fermentation technologies are under constant optimization. A comprehensive technical and scientific diagnosis that has pointed out the critical points in the existing reactors will allow the conception and development of new solutions aiming for technological revamping and OPEX reduction. The most relevant parameters addressed focused on the light distribution to reduce the self-shadowing, mass and CO_2 transfer, nutrient supply, and hydrodynamics of the reactors. The improvements achieved will be applied in the construction of two new outdoor PBRs and another 5000 L fermenter. To follow up with a higher production capacity, a new and improved processing unit is also being designed. The increase in the nominal production and number of reactors available will provide the company with higher flexibility in production management enabling the simultaneous production of seven different microalgae species. This, together with the higher versatility of production systems available, will still allow the production of tailor-made biomass with the same premium quality as before.

4.3.5 DOWNSTREAM PROCESSING

After being harvested from the PBRs, the culture passes through sequentially smaller sized pore filters (250 and 130 μm) to prevent the occlusion of the diafiltration membranes in the next processing step. The membranes system represents the first dewatering stage in downstream processing, concentrating the biomass from 1–2 g L^{-1} to up to 100 g L^{-1} DW. The biomass is then subjected to a high-temperature-short-time process, which decreases the bacterial load and eliminates other unwanted contaminants from the concentrated culture. Then, there are two possible routes, which will depend on the intended biomass final formulation, either powder or paste. If the aim is to obtain a paste as the final product, another dewatering step is carried, centrifugation, in which the microalgae suspension is further concentrated, attaining values up to 280 g L^{-1} DW. The paste is then packed in plastic bags and stored at –20°C up to delivery. However, when the aim is to attain a microalgae powder, the microalgae concentrated solution is spray-dried, passing from 90–95% to 3–5% water content, and then packed in opaque plastic bags, with an oxygen adsorbent to prevent the oxidation of the powdered biomass. Spray drying is a suitable drying process since the microalgae concentrated solution suffers a flash high-temperature exposure, preserving the thermal sensitive biomass constituents, such as pigments, and maintaining the original biomass premium characteristics. Furthermore, it is a technique that assures the stability of the biomass during a long period. Nonetheless, the spray-drying process is under scrutiny due to the high energetic consumption, considerable biomass losses throughout the process, and long cleaning and maintenance times associated. Alternatives to prevent the loss of the dried powder from the dryer to the packaging are under study.

4.3.6 COMMERCIALIZED PRODUCTS

Food and human supplements are currently the main segments addressed by Allmicroalgae, which markets its products under the brand Allma®. The company sells a variety of *C. vulgaris* powders, such as Organic *Chlorella* Powder (Figure 4.5), its most popular product. Taste and color represent the bottleneck of this organic sun-grown *Chlorella* version. Thus, alternative fermented products have been developed, namely Smooth *Chlorella* (Figure 4.5) and Honey *Chlorella*, with a smooth taste and lighter colors (lime and yellow color, respectively). With a creamy soft texture, 30–40% protein content, and high quantities in fiber, these products have been a good alternative to eggs, gluten flours thickeners formulations, among others, especially for the vegetarian and vegan segments, but not exclusively. Furthermore, the company also sells Algaessence®, a complementary blend of micro and macroalgae organic certified product, produced together with the macroalgae producing partner ALGAplus. In addition to these powdered food products, Allmicroalgae is also offering *Chlorella* supplements in the form of tablets and capsules.

Besides, a partnership with local producers launched a range of healthy vegan food products. These include three different Super Powders (mix of

FIGURE 4.5 Allma products: (a) organic *Chlorella* powder; (b) smooth *Chlorella* powder; and (c) vegan sea crackers.

superfoods and natural ingredients with several nutritional and functional benefits), the *Chlorella* Bars, the Vegan Sea Crackers (Figure 4.5), which have *T. chui* as highlight product due to the great seafood-like taste, and the Lemon *Chlorella* Cookies. These products contain a high and complete protein content with the full essential amino acid profile at the ratios required for human consumption, which does not appear in any other plant-source protein. Allmicroalgae is not only active within the food and supplements markets. It offers high-value biomass, namely the *N. oceanica*, *T. chui*, *T. obliquus*, and *P. tricornutum*, which are available either as a powder or as frozen paste, for the most varied applications. The company also invests in aquafeed, animal nutrition, and pet food, under the brand Allvitae®, biostimulants, fertilizers, and pesticides (Allfertis®), and cosmetic markets. The recently made official joint venture between Allmicroalgae and the French microalgae extraction expert Altinat, owner of Greentech and Greensea, will not only increase the biomass production and extraction of biocompounds capacities, but also enable offering the client a larger number of products and an increase on the flexibility in terms of product development and overall service.

4.3.7 VISION INTO THE FORTHCOMING

Regarding the perspectives of Allmicroalgae, the company is working to reinforce its presence in the feed market. Despite being already selling microalgae concentrates for the aquaculture sector, the aim is to develop a range of specialized products with proven functionalities, different from all the market available options. The entrance into the pet food sector is also an objective. Regarding the agriculture sector, Allmicroalgae intends to diversify its offer through the production of biostimulants. Here, the focus is, as well, to prove the functionality of the biomass to sell a differentiated product.

4.4 FUTURE PERSPECTIVES FOR THE MICROALGAE MARKET

There is still a long road to go to fully exploit the microalgae worldwide by the companies and R&D units. Only few finite applications are known, mainly food and feed products. Other microalgae applications have been highlighted, such as the cosmetic and agriculture. The algae sector in the EU has been steadily growing in the past years and is currently moving to upscale and commercialization; however, there is no specific legislation to this business area, yet. At the beginning of the year, the European Committee for Standardization developed EN 17399:2020 'Algae and algae products – Terms and definitions,' the first European standard for algae and algae products. Those standards and regulations represented a critical first step, forming the basis for developing specific legislation. Indeed, specific legislation is needed for the algae sector to grow, develop, and professionalize. Besides the fast growth in microalgae-based food products, the need to invest in product development, safety, and achieve good sensory characteristics (from the consumer point of view) without dismissing the health and nutritional side is still strongly needed. The feed market is being slowly occupied with processed microalgae feeds and R&D has been focused on microalgae bioactive compounds to guarantee the best positive impact. The cosmetic and pharmaceutical markets are still very experimental. Researchers are finding new bioactive compounds and describing their action mechanisms to understand the potential of the isolated compounds. However, maintaining the cultivation conditions stable to produce the same molecules at the intended quantities is very challenging (Jha et al., 2017; Nethravathy et al., 2019). Microalgae's genetic modification can be a hypothesis to produce recombinant proteins for therapeutic applications because microalgae possess posttranslational modification systems similar to the mammalian cells lines, unlike yeast and bacteria, the traditional sources of therapeutic proteins (Nethravathy et al., 2019). Microalgae-based agriculture and biofuel markets are still in their infancy. Substantial investment in research and technology is still needed, however, those are promising. Nowadays, the reduced market integration is the major problem in microalgae industry. It is related with the low cost-effectiveness of the production process and the irregular biochemical profile of the microalgal biomass. However, Allmicroalgae have surpassed those difficulties and implemented new ways of producing microalgae based on scientific and technological developments.

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KEYWORDS

- **Allmicroalgae**
- **bioeconomy**
- **bioenergy**
- **cosmetics**
- **feed**
- **high-value bio-compounds**
- **microalgae**
- **novel foods**
- **pharmaceutics**
- **sustainability**

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CHAPTER 5

MICROALGAL BIOTECHNOLOGY: FROM CULTIVATION TO INCORPORATION IN A RANGE OF FOODS

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ABSTRACT

Nowadays the demand for foods with increased nutritional value has increased. Consumers are more interested in food that provides well-being and helps to prevent diseases. In this context, microalgae biotechnology focused on biomass production for food development has increased. Studies focusing on microalgae cultivation and scale up to a commercial level showed that these microorganisms are promising. Previous research highlighted that during the cultivation, parameters such as nutrients, light, and temperature can influence the amount and type of compounds present in their biomass. Enriched foods such as snacks, pasta, and bakery products, can be developed with microalgae biomass. However, during food development, the microalga strain and the amount of biomass used in the formulation should be carefully

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evaluated to not compromise sensory and physical properties. Therefore, this chapter aims to report the latest research and potential commercial applications of microalgae and their biomolecules in different foods. Besides, aspects of the cultivation conditions, main challenges and future trends will be discussed.

5.1 INTRODUCTION

The consumption of nutritionally rich foods plays an important role in health promotion and disease prevention. Consumers all over the world are increasingly aware of this fact. Therefore, the demand for functional foods has been increased (Kuster-Boluda and Vidal-Capilla, 2017). In this way, the market has been remodeling itself to meet this growing demand. Among the new trends in the healthy food market, the nutritional enrichment of conventional foods stands out from the addition of microalgae biomass or its biomolecules (Niccolai et al., 2019a). In addition to the health aspect, microalgae can also play a structuring role in food due their rheological properties (Bernaerts et al., 2017). Within the foods enriched with microalgae, there are snacks (Lucas et al., 2018), pasta (Fradinho et al., 2020; Grahl et al., 2020), fermented (Barkallah et al., 2017) and bakery products (Lafarga et al., 2019a, Diprat et al., 2020), among others.

Microalgae are photosynthetic microorganisms composed of proteins, carbohydrates, lipids, vitamins, pigments, among other relevant compounds (Song et al., 2018). The proportion of each of these biomolecules varies depending on the microalgal species as well as the cultivation conditions. One of the advantages of using microalgae as a nutritional source is the possibility of stimulating the biomolecules accumulation under different growing conditions (temperature, pH, luminosity, salinity, nutrients, among others) (Roleda et al., 2013; Luangpipat and Chisti, 2017; Duarte et al., 2019). Another great advantage of microalgae is the possibility of cultivation in non-arable lands, thus not competing with agricultural lands used to produce other food crops (Hannon et al., 2010).

Microalgae can have a protein content (~60%) higher than foods recognized as high protein, such as meat, yeast, egg, and soy (Koyande et al., 2019). The microalga *Spirulina*, for example, accumulates up to 70% (w w^{-1}, dry basis) of proteins in its biomass (Dillon et al., 1995). Besides that, microalgae have essential amino acids that cannot be endogenously synthesized by humans and, therefore, must be provided in the diet (Koyande et al., 2019). Bioactive peptides obtained from microalgal proteins have potential in the management of some diseases, such as hypertension, inflammation, diabetes, and oxidative stress (Ejike et al., 2017; Barkia et al., 2019).

Among microalgae carbohydrates, polysaccharides have a hypocholesterolemic effect. Moreover, they can assist in the activation of the immune system and act as an antioxidant (Villarruel-López et al., 2017). Lipids are rich in polyunsaturated fatty acids important for the human diet (omega-3 and omega-6). Among omega-3 fatty acids, eicosapentaenoic acid (EPA) has anti-inflammatory effects, while docosahexaenoic acid (DHA) is more used for neurological and cognitive effects (Weiser et al., 2016).

The pigments present in microalgae, such as chlorophylls, carotenoids, and phycobiliproteins, are mainly used as a dye in the food industry, besides having the great advantage of promoting health. As an example, carotenoids have pro-vitamin A activity, and their consumption is associated with an improved immune system and in the control of chronic degenerative and cardiovascular diseases and some types of cancer (Rodriguez-Concepcion et al., 2018; Lafarga et al., 2020). Within this context, this chapter aims to report the latest research and potential commercial applications of microalgae and their biomolecules in different foods. Also, aspects of the cultivation conditions, main challenges and future trends will be discussed.

5.2 MICROALGAE BIOTECHNOLOGY

5.2.1 CULTIVATION CONDITIONS FOR FOOD APPLICATION

The ideal cultivation conditions vary according to the microalgae species and with the desired final product. Some of the main factors that must be taken into consideration to grow microalgae are the type of reactor, culture medium, temperature, pH, light, local climate, possible contaminating organisms, among others (Ras et al., 2013; Di Caprio, 2020; Izadpanah et al., 2020; Zhou et al., 2020).

Temperature is a key factor in microalgae growing. In general, most species have their optimum growth between 20°C and 30°C (Ras et al., 2013). In photoautotrophic cultivations, artificial or natural lights can be used as an energy source. Type, intensity, spectral, and photoperiod are some of the variables that directly influence the growth and composition of microalgae and that must be studied and optimized for each species (Suparmaniam et al., 2019). Generally, the temperature and artificial light in closed systems are controlled throughout the cultivation. On the other hand, in open systems these variables are not controlled and may vary depending on the regional climate. In the latter case, it is interesting to use microalgae that tolerate these fluctuations and take into account the climate when choosing the ideal place for cultivation. Other important parameter is the pH of cultures. The optimum pH of most microalgae is in the 6–9 range (Khan et al., 2018). The use of microalgae that resist alkaline culture medium (pH ~ 11), such as *Spirulina* and *Dunaliella*, is an interesting strategy to prevent the spread of contaminating microorganisms that do not grow in this pH range (Panahi et al., 2019).

In heterotrophic cultivations, closed bioreactors made of stainless steel are often used. These bioreactors have a reduced chance of contamination and yields of products of interest reaching 100 times more than those obtained in photoautotrophic cultures. However, this type of system is rather expensive, and may make its use unfeasible depending on the product to be obtained (Torres-Tiji et al., 2020). Although, the photoautotrophic cultures can be carried out in closed or open bioreactors. Flat plate (Sun et al., 2016), tubular (Cheng et al., 2019), and column photobioreactors (López-Rosales et al., 2019) are examples of closed bioreactors frequently used for photoautotrophic cultivations. These systems allow better control of the cultivation parameters, such as temperature and light, and high biomass productivity can be obtained. There is also less chance of contamination compared to open systems. However, contamination by zooplankton, bacteria and viruses can occur if there is no appropriate sterilization of the water and gases used in the cultivation (Wang et al., 2013).

Most of the large-scale photoautotrophic cultivation intended for food are still carried out in open systems, more specifically in raceway tanks (Torres-Tiji et al., 2020). These photobioreactors have the advantages of low construction and operation costs (Banerjee and Ramaswamy, 2017). Besides that, sunlight is used as an energy source in these systems, which also contributes to lowering costs. However, depending on the region where the microalgae are grown, climate oscillations may affect the productivity of the product of interest (Banerjee and Ramaswamy, 2017). Open systems are more easily contaminated by unwanted organisms. Therefore, different strategies can be applied during cultivation in order to obtain a safe and contaminant-free biomass. Transparent film greenhouses can be used to prevent the entry of possible contaminants present in the surrounding environment. Filtration and chemical addition can also be applied to reduce the unwanted microbiological load (Wang et al., 2013). Authors suggest adding a heating step after cultivation, such as the pasteurization process, for example, commonly used for other types of food (Grossmann et al., 2019).

Allied to this, applying Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Points (HACCP) throughout the production process is highly recommended to ensure safe biomass without danger to the consumer (Matos, 2017).

5.2.2 STRATEGIES TO PRODUCE PROTEIN-RICH BIOMASS

The accumulation of proteins in photoautotrophic systems depends on the nitrogen source and can vary from 0.15 to up to 0.5 g g^{-1} in microalgae biomass (Soto-Sierra et al., 2018). Guccione et al. (2014) reported a 23% increase in protein content of *Chlorella vulgaris* when changing the nitrogen-poor medium to a nitrogen-rich medium. This result is expected, since most microalgae, under nitrogen deprivation, degrade proteins to serve as a source of nitrogen (Senevirathne and Kim, 2012; Soto-Sierra et al., 2018). According to Tan et al. (2016), if microalgae are grown in a medium rich in nitrogen, there is an increase in protein biosynthesis, which is required for cell division. On the other hand, in microalgae cultivation conducted in a nitrogen-poor culture medium, cell division decreases. Hence, protein production is also reduced, and the synthesis of compounds rich in energy, such as polysaccharides or lipids, begins.

Heterotrophic conditions generally result in reduced protein yields and biomass productivity compared to photoautotrophic cultivation (Soto-Sierra et al., 2018). Nevertheless, heterotrophic fed-batch cultivation of *Chlorella vulgaris* strain has proved to be an efficient alternative to maximize protein productivity, making biomass of this microalga a viable source of proteins for commercial application in the food industry (Doucha and Lívanský, 2012).

Increasing the nitrogen to carbon ratio has been a strategy to maximize the protein content per gram of biomass in photoautotrophic systems (Brennan and Owende, 2010). The availability of nitrogen is the most relevant factor in the accumulation of proteins. Depending on the source and amount of nitrogen in the medium, protein accumulation varies from species (Soto-Sierra et al., 2018). Most microalgae grown with concentrations < 450 mg L^{-1} of nitrogen such as $NaNO_3$ (sodium nitrate), KNO_3 (potassium nitrate), $(NH_4)_2CO_3$ (ammonium carbonate), CH_4N_2O (urea), and yeast extract result in protein accumulation (Arumugam et al., 2013; Grossman et al., 2019). Kim et al. (2016) showed that *Tetraselmis* sp. cultivated with yeast extract produced a higher concentration of protein (up to 50%) and biomass density (up to 10.4 g L^{-1}) when compared to ammonium, nitrate, glycine and urea.

While the relationship between the nitrogen source and protein synthesis is well understood, the effect of phosphorus on protein accumulation still unknown. Most microalgae increase protein production under conditions of phosphorus concentration up to 5 mg L^{-1} (Grossmann et al., 2019). However, some studies report that this phosphorus-rich culture medium contributes to reducing the protein content in microalgae (Markou, 2012; Juneja et al., 2013; Trautmann et al., 2016).

Carbon makes up more than 90% of the microalgae nutrient medium (Soto-Sierras et al., 2018). Yeh and Chang (2012) found a protein yield around 0.2 g g^{-1} for *Chlorella vulgaris* cultivated with CO_2 or glucose, and 0.15 g g^{-1} when using $NaHCO_3$ as a carbon source. Besides, protein (0.9 g L^{-1}) and biomass (4.5 g L^{-1}) concentrations were also significantly higher in CO_2 cultivation. Higher protein productivity with CO_2 or glucose as a carbon source was achieved due to higher biomass accumulation compared to the use of $NaHCO_3$ as a carbon source. Regardless of the potential for obtaining high protein productivity, the cost of organic carbon (glucose) must be considered. The glucose cost can reach 80% of the total average cultivation costs and would impact the economic viability of microalgae protein products (Soto-Sierra et al., 2018). The mixotrophic cultivation of *Chlorella vulgaris* using agro-food by-products rich in carbon represented an innovative approach to increase the biomass concentration of microalgae of interest for protein production (Salati et al., 2017).

Parameters of the cultivation process can also influence the production of compounds by microalgae. Prates et al. (2018) observed an increase in protein content for the experiment where *Spirulina* was cultivated with light-emitting diodes (LEDs) when compared to fluorescent light as an energy source. Colla et al. (2007) verified the influence of temperature on the production of proteins in *Spirulina platensis*. In cultivation with nitrogen supply (1.875 g L^{-1} of $NaNO_3$), the authors observed an increase of 10% in protein content when the temperature varied from 30°C to 35°C. Rodrigues et al. (2011) studied the effect of ammonium feeding time and the addition or not of CO_2 to control pH in the cultivation of *Spirulina platensis*. From this study, 38% protein was reached when microalga was grown in mini-tanks reactors at 30°C, with ammonium feed for 7 days, without pH control of the culture medium. However, in the context of the application of the macromolecule on a larger scale for food production, more research is needed to improve the protein content in microalgal cultures under uncontrolled conditions of temperature and light.

5.2.3 STRATEGIES TO PRODUCE LIPID-RICH BIOMASS

Nitrogen and phosphorus limitation are strategies used to increase the content of lipids in microalgae. *Chlorella* is a microalga with potential for implementation in industrial installations since it presents around 30% of polyunsaturated fatty acids (PUFAs) (Ferreira et al., 2019). Under low nitrogen concentration, *Chlorella* sp. MACC-438, *Chlorella minutissima* MACC-452 and *Chlorella* sp. MACC-728 produced maximum concentration of lipids (Ördög et al., 2016). *Botryococcus* can produce up to 70% of lipids under nitrogen stress conditions. When comparing nitrogen and phosphorus deprivation, the former usually leads to a higher accumulation of lipids (Ferreira et al., 2019). The lipid productivity increase is hampered by compensation between lipid synthesis and cell growth (Park et al., 2019). In this way, to maximize the production of biomass together with the lipid accumulation, the two-stage strategy emerges. The first stage involves microalgae cultivation with sufficient nutrient conditions to stimulate growth. In the second stage, the microalgal cells are collected and exposed to nutrient deprivation for lipid accumulation (Kumar et al., 2019).

In addition to nutritional stress, environmental conditions such as temperature, salinity, pH, and light intensity also affect the productivity of lipids in outdoor growing systems (Hindersin et al., 2014; Chen et al., 2017a; Ferreira et al., 2019; Park et al., 2019). *Porphyridium purpureum* is known to produce considerable concentrations of arachidonic acid (ARA). The production of ARA reached up to 40% of the total fatty acids when the microalga was grown under stress conditions (increased salinity, and limited nutrients) (Shanab et al., 2018). Temperature is an essential factor in the accumulation of PUFAs. The synthesis of PUFAs by microalgae is induced at low temperatures, as these compounds help to maintain the fluidity, flexibility, and functionality of cell membranes, which is a strategy to support cell metabolism at such temperatures (Willette et al., 2018; Morales-Sánchez et al., 2020). However, the combined effect of low temperature and nitrogen deprivation on the lipid composition of the membrane is not fully understood yet and requires further studies (Morales-Sánchez et al., 2020). In a study by Schulze et al. (2019), *Chlamydomonas malina* showed high levels of PUFAs when grown at 8°C compared to 15°C. The decrease in temperatures can increase the PUFAs content in microalgae by two to three times (Katyar and Arora, 2020).

Scharff et al. (2017) evaluated the effect of the photoperiod on the fatty acid profile of *Chlorella vulgaris* and *Scenedesmus obliquus*. According to the authors, longer photoperiods (24:0, 22:2, 20:4) can reduce the synthesis of α-linolenic acid and induce the synthesis of linoleic acid. Chandra et al. (2017) observed that the content of linoleic and α-linolenic acids in *Scenedesmus obtusus* enhanced with the increase in light intensity from 50 to 150 μmol m^{-2} s^{-1}. Morales-Sánchez et al. (2020) reported high productivity of biomass (527 mg L^{-1} d^{-1}), lipids (161.3 mg L^{-1} d^{-1}), and PUFAs (85.4 mg L^{-1} d^{-1}) in *Chlamydomonas malina* RCC2488 cultivation with a salinity of 17.5, and the light intensity of 250 μmol photons m^{-2} s^{-1}. Cells cultured at ≤35 salinities synthesized twice the polar lipids (60–68.5 mg g^{-1}) than cells cultured at salinity 80 (30.2 mg g^{-1}). The maximum total PUFA productivity of 83.8 mg L^{-1} d^{-1} was found at the light intensities of 120 and 250 μmol m^{-2} s^{-1} (Morales-Sánchez et al., 2020).

Under heterotrophic growth conditions, large accumulations of DHA and EPA were observed in the strains of *Nannochloropsis*, *Phaeodactylum*, *Schizochytrium* and *Thraustochytrium* (Kumar et al., 2019). The main enzymes and metabolic pathways involved in lipid biosynthesis have been elucidated (Chen et al., 2017a; Zhao et al., 2019) and can be manipulated by genetic engineering to improve lipid production (Ferreira et al., 2019; Park et al., 2019; Sun et al., 2019). Induced mutagenesis is widely used to develop strains with the necessary characteristics. A study on the UV-induced mutant strains of *Pavlova lutheri* showed an increase of more than 30% in the dry weight of the contents of DHA and EPA (Chauton et al., 2015). Besides, CO_2 supplementation in microalgal culture can also increase compounds of high value in lipid fractions, such as omega-3 PUFAs. Carvalho and Malcata (2005) observed an increase in the content of PUFA and monounsaturated fatty acids (MUFAs) in *Pavlova lutheri*, with an increase in CO_2 concentration from 0 to 2%. Therefore, the use of CO_2 for the cultivation of microalgae is not only desirable from an environmental point of view but also to improve the yield of the product (Ferreira et al., 2019).

5.2.4 STRATEGIES TO PRODUCE PIGMENT-RICH BIOMASS

The microalgae pigment content can be improved through environmental stress parameters, such as light, temperature, availability of nutrients such as nitrogen and salt stress (Gong and Bassi, 2016). The limited production of biomass under stress conditions can be overcome by applying a multi-stage microalgal growth strategy (Hodgson et al., 2016). The two-stage cultivation strategy has been commonly applied, as it allows for high biomass productivity coupled with pigment accumulation (Wan et al., 2014; Wichuk et al., 2014).

Light is a critical factor affecting the production of carotenoids and phycobiliproteins (Gong and Bassi, 2016). To overcome this, LEDs are characterized by having specific wavelengths. Thus, they can be used in microalgae cultivation (Figure 5.1) to stimulate the synthesis of pigments (Duarte and Costa, 2018; Prates et al., 2018) to increase energy absorption efficiency, making them an ideal tool for growing microalgae. Due to the chromatic adaptation mechanism in microalgae, blue or red lights can be used to improve the production of phycocyanin, while the green light contributes to phycoerythrin synthesis (Hsieh-Lo et al., 2016). On the other hand, Mishra et al. (2012) reported that the use of a yellow LED stimulates phycocyanin synthesis due to its wavelength overlap with red light. In another study, the phycocyanin content in *Spirulina platensis* obtained using red light was 5–7 times higher compared to blue light mixed with a red light (Lima et al., 2018). However, for lutein production by microalga *Scenedesmus obliquus*, the entire spectrum of white light is more favorable than monochrome LED light sources (Ho et al., 2014).

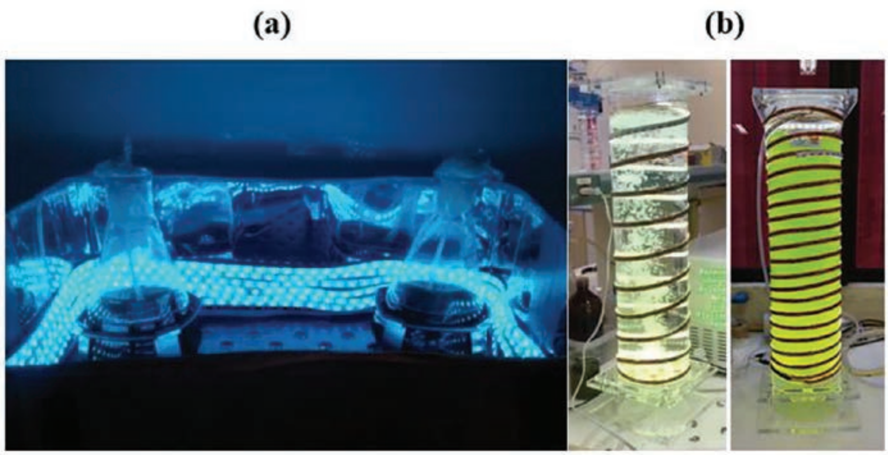

FIGURE 5.1 Illustrations of microalgae cultivation conducted with the application of LEDs in different designs of reactors, such as: (a) Erlenmeyer flasks; and (b) tubular.

Since phycocyanin is a source of intracellular nitrogen, its content can be reduced under conditions limited by this nutrient (Hsieh-Lo et al., 2016). Therefore, the synthesis of this pigment is strongly affected by the nitrogen source in the culture medium. The reduction of nitrate (NO_3^-) can lead to a reduction of phycocyanin, while the excess of NO_3^- can lead to inhibition of such pigment (Manirafasha et al., 2018). Moreover, nitrogen depletion also results in the astaxanthin accumulation (Hu et al., 2018). Fu et al. (2014) reported that the interaction between NO_3^- and salinity had a significant contribution to lutein biosynthesis in *Dunaliella salina*. Through a modeling study, pH = 9 and 20% sodium chloride benefited the production of β-carotene in *Dunaliella salina* (Çelekli et al., 2014).

Temperature interferes in carotenoid biosynthesis and also controls the growth rate of microalgae. Higher temperatures are favorable for cell growth and lutein accumulation (Gong and Bassi, 2016). Fernández-Sevilla et al. (2010) demonstrated that 28°C is the optimal temperature to produce lutein by *Muriellopsis* sp., considering cell growth rate. The effects of day and night temperatures were studied by Wan et al. (2014) to improve the astaxanthin production in outdoor cultivations of *Haematococcus pluvialis*. The authors concluded that raising daytime/dark temperature stimulates astaxanthin accumulation at night until 28°C. Zhang et al. (2016) modeled the attenuation of temperature and nitrogen sources and concluded that 27°C and 4.4 mM nitrate would be optimal for the production of astaxanthin by *Haematococcus pluvialis*.

Besides, microalgae also produce carotenoids under heterotrophic conditions (Gong and Bassi, 2016). The accumulation of lutein in heterotrophic cultures can be triggered by the induction of oxidative stress with the proper addition of chemicals such as hydrogen peroxide, sodium hypochlorite, and ferrous ions (Hu et al., 2018). The microalga *Chlorella zofingiensis* has the potential to produce astaxanthin when cultivated in the dark (Liu et al., 2014) using as the carbon source, glucose, fructose, and sucrose (Liu et al., 2012). Another way to improve pigment accumulation is to use random mutagenesis and isolate high-yielding mutants from an existing commercial microalgae strain (Hu et al., 2018). Random mutagenesis was applied to improve the production of lutein in *Chlorella sorokiniana* (Chen et al., 2017b), and astaxanthin in *Haematococcus pluvialis* (Gómez et al., 2013).

5.2.5 BIOMASS HARVEST AND RECOVERY

The microalgae harvest refers to the separation of its biomass produced from the culture medium. This step is challenging since the densities of cells and water are very close and because the microalgae surface charge is negative (Wibisono et al., 2019). The harvesting of microalgae can represent about 20–30% of the total biomass production costs (Grima et al., 2003; Hattab et al., 2015). Within the techniques used for this purpose, centrifugation (Molina-Miras et al., 2019), filtration (Zhao et al., 2020), flocculation, flotation (Shi et al., 2017), sedimentation (Tan et al., 2019) and electrolytic process (Luo et al., 2017) stand out. When choosing the ideal harvesting method, the characteristics of the microalgae (cell size, density, among others) and the culture medium (as salinity and reuse) must be considered, as well as the possibility of large-scale application, preservation of the biomass quality, time process, cost, toxicity and destination of the biomass produced (Okoro et al., 2019).

The commonly applied methods in harvesting microalgal biomass are mechanical, such as centrifugation and filtration. Centrifugation is one efficient technique of separating biomass (yields up to 98%) in a short time. Besides, it applies to the vast majority of species and culture medium (Molina-Miras et al., 2019). However, the main disadvantage is the high-energy expenditure involved, which increases operating costs, and also the possibility of damage to cells due to gravitational and shear forces during the process (Morais Junior et al., 2020).

Likewise, filtration is also a relatively expensive process since it involves high energy consumption due to the operation of the vacuum pump. Furthermore, high operating costs are also associated with frequent replacement of filter membranes (Dragone et al., 2010). The filtration technique is appropriate for microalgae cells higher than 70 μm. On the other hand, processes such as microfiltration or ultrafiltration are applied in cells with a smaller size (Petrusevski et al., 1995). The genera *Dunaliella* and *Arthrospira* are examples of microalgae that can be harvested through the membrane filtration process (Cuellar-Bermudez et al., 2019; Monte et al., 2020). In general, the filtration process is ideal for cultivation volumes below 2 m^3 and may be economically viable when compared to centrifugation (Brennan and Owende, 2010). On the other hand, the filtration takes longer than centrifugation.

An alternative to make mechanical methods more efficient and reduce operating costs is the combination of them with previous flocculation or flotation steps, for example (Grima et al., 2003). The main advantages of these methods are speed, ease of operation, applicability on a large scale, not damaging cells, applicable for a wide variety of species, and reduced costs (Okoro et al., 2019).

Flocculation eliminates the repulsive force between the microalgae cells due to its negative surface charge, causing the biomass to clump (Wibisono et al., 2019). Among the alternatives to chemicals frequently used in the flocculation process, which can be expensive and toxic, autoflocculation obtained by regulating the pH of the cultures stands out (Golueke and Oswald, 1970). As an example, Tran et al. (2017) used photosynthetic carbon uptake to increase pH and induce autoflocculation of *Nannochloropsis oculata*. Autoflocculation started at pH 9.5 and reached a maximum flocculation efficiency of 90% at pH 10.4. The use of flocculation induced by microorganisms (bioflocculants) is also promising (Tran et al., 2017), as well as bio-polymer based flocculants (Mohd Yunos et al., 2017). Moreover, flotation is also a method of harvesting biomass that can be used in combination or not with mechanical methods. The method involves the injection of air microbubbles dispersed in the culture medium, where the cells will be adsorbed and dragged to the surface of the culture medium (Brennan and Owende, 2010).

After the harvesting step, when the desired final product is dehydrated biomass, a drying step is necessary. Drying has the purpose of preserving the biomass, by reducing the moisture content and, consequently, enzymatic and microbial activities (Agbed et al., 2020). Moreover, drying the biomass facilitates its insertion in different types of food. Drying requires high energy (800 kcal for 1 kg of water), and the final concentration of biomass must be at least 90%, which results in high process costs (Chen et al., 2015). Among the main drying processes used for microalgae biomass, solar drying (Agbed et al., 2020), convective drying (Nakagawa et al., 2016), spray drying (Durmaz et al., 2020), and freeze drying (Ansari et al., 2018) stand out. When choosing the ideal drying process, it is generally considered the preservation of the biomass quality, final destination, process time, costs, and other aspects.

In products intended for food, the most used drying processes are convective drying, spray drying, and freeze-drying (Oliveira et al., 2010; Chen et al., 2015). Convective drying is considered a popular biomass drying process, usually performed in a convective hot air drying (40–55°C) (Chen et al., 2015). The costs involved in this process are lower when compared to the spray drying and freeze drying. However, the disadvantages are the drying time, which can often affect the quality of the final biomass. Oliveira et al. (2010) reported 37% of losses in the phycocyanin content of *Spirulina* sp. when the biomass was dehydrated in convective drying.

Spray drying is one of the most used industrial processes and has the advantages of easy process control, speed, and preservation of biomass quality (Palabiyik et al., 2018; Durmaz et al., 2020). The main disadvantage is the high energy and capital cost. However, in this process it is possible to use biomass directly from photobioreactors, not needing the harvesting step (as filtration and centrifugation), which can make its use economically advantageous (Biz et al., 2019). Like the spray drying process, freeze-drying also has the advantage of preserving the quality of biomass and is used when the final product is a living cell or thermolabile system (Khanra et al., 2018).

However, it is considered a longer process than the spray-dryer, and scaling up is also considered expensive. The energy consumption of industrial freeze-drying for obtaining microalgal biomass requires 38.88 Wh g^{-1} (Ratti, 2001).

In the processes aiming to extract some microalgal intracellular compounds, pre-treatments are applied to disrupt the cell wall. For these processes, wet or dehydrated biomass can be used, depending on the goal. The different types of methods can be classified into chemical, physical (mechanical), or biological (enzymatic). The chosen method will depend mainly on the microalgae morphology, the product of interest, and the recovery efficiency (Morais Junior et al., 2020). Besides that, due to the oxidative properties of some bioactive compounds, the choice of the ideal method needs to take into account the processing time as well as control of temperature, pressure and light. Chemical methods have the advantage of low energy cost and the possibility of scaling up. Nonetheless, some solvents can be toxic, in addition to the possibility of contamination and reduced stability of bioactive compounds. Biological methods are less toxic than chemical ones, however, they are expensive, time-consuming and not ideal for industrial scales. Physical methods are preferred for a large scale, being non-toxic and fast, despite the high-energy cost (Molino et al., 2020). Among the methods frequently reported in the literature for cell rupture, soxhlet, maceration, freeze-thawing, ultrasound, sonication, microwave-assisted, pressurized liquid, supercritical fluid extraction, pulse electric field stand out (Ventura et al., 2017; Mansour et al., 2019; Vernès et al., 2019; Molino et al., 2020).

Lastly, for some products, a purification step is necessary to isolate the biocompound of interest from other compounds and contaminants. The purity grade varies according to the product destination. Combined methods are generally used to increase the purification degree of biomolecules. The most reported methods in the literature for this purpose are precipitation, two-phase aqueous system, membrane separation, chromatographic methods, among others (Chang et al., 2018; Lee et al., 2019; Amarante et al., 2020).

5.3 FOOD DEVELOPED WITH MICROALGAE BIOMASS

5.3.1 SNACKS

Snacking is part of our daily lives due to the advantage of being foods of practical consumption. Snacks as the extrudates can be made within a variety of colors, shapes, and flavors, by using the extrusion process. During last years, it was observed increases in the development of convenience foods with better nutritional properties such as higher content of fibers (Potter et al., 2013), protein (Lucas et al., 2018), and bioactive compounds (Kosińska-Cagnazzo et al., 2017). Ingredients as fruits, vegetables, and microalgae have been added to the cereal-based formulations to achieve such nutritional improvement. Developing enriched snacks with good physical characteristics as well as a higher content of nutrients and bioactive constituents is considered a challenge. Usually, researches investigate different concentrations of microalgae incorporation in snacks, and during the last years, studies are focusing on the microalga *Spirulina* (Figure 5.2).

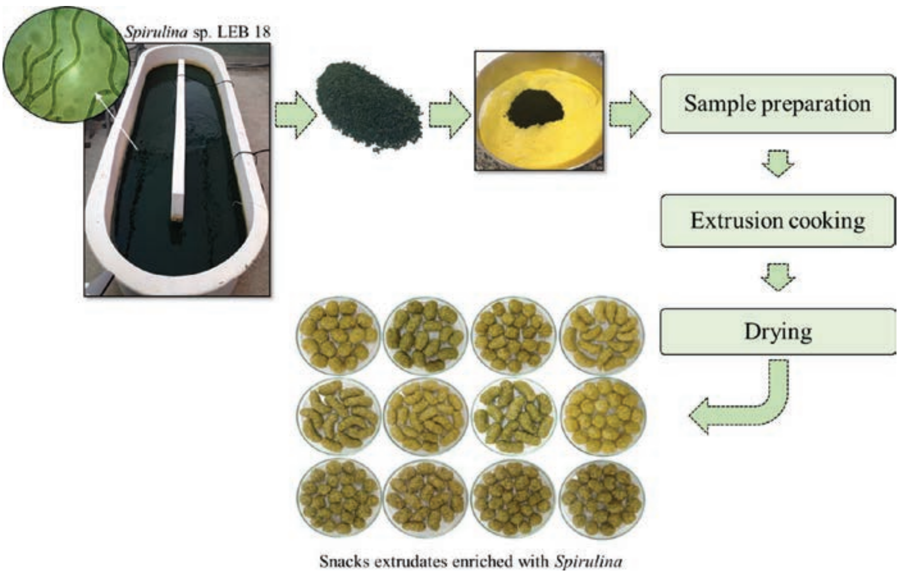

FIGURE 5.2 Schematic representation of the *Spirulina*-enriched extrudates snacks development.

Lucas et al. (2017) investigated the effect of *Spirulina* sp. LEB 18 addition (from 0.4 to 3.2%) in the formulation of snacks extrudates made from organic rice and corn flours. Different levels of feed moisture and the temperature in the last zone of the extruder were also evaluated. The authors observed that higher concentrations of microalga resulted in snacks with high protein content, however with more compact and harder structures. In the end, the concentration of 2.6% of *Spirulina* was chosen as the best one to obtain extrudates. With this concentration, the snacks presented high protein content and adequate physical properties. In another study, Lucas et al. (2018) evaluated the cereal-based snacks enriched with 2.6% of *Spirulina* regarding nutritional, physicochemical, and sensory properties. Microalga addition resulted in increases of 22.6% of protein and 46.4% of minerals. The extrudates enriched with *Spirulina* presented an expansion index of 4.57 cm cm^{-1}, structure with thin cell walls (mean: 18.50 μm), a total color difference of 30.50, and high acceptability index (82%). Furthermore, those snacks showed no significant differences regarding sensory attributes (flavor, texture, taste, and overall acceptance) compared to the control (cereal-based snacks) besides presented stability (textural and microbiological) with 12 months of storage.

Joshi et al. (2014) produced extrudates with maize flour and *Spirulina* (5, 7.5, 10, 12.5, and 15%). The optimized product chosen was 7.5% *Spirulina* and presented 14.63% of protein content and 138.83 mg kg^{-1} of total carotenoids. Vijayarani et al. (2012) produced extrudates with wheat and corn flours and 5, 10, and 15% of *Spirulina*. The extruded product incorporated with 5% of *Spirulina* powder showed higher overall acceptability (4.96) compared to the other formulations. Morsy et al. (2014) developed snacks using corn flour and *Spirulina* (2.5, 5, 7.5, 10 and 12.5%). The authors obtained 18.11% of the protein in the extrudates containing 10% of *Spirulina*, higher than the content in commercial (9.28%), and control snacks (9.43%). The addition of 12.5% of *Spirulina* resulted in lower scores for the sensory properties besides lower overall acceptability (59.88), whereas snacks with 2.5% of *Spirulina* showed the highest score for overall acceptability (89.79).

Tańska et al. (2017) developed extrudates with corn grits and *Spirulina* powder (up to 8%) and observed that a high concentration of microalga in the formulation resulted in increases of protein (34%), ash (36%), and fiber (140%), compared to the control. Moreover, the sample incorporated with 8% of *Spirulina* presented 6.8 mg $100g^{-1}$ of carotenoids content, higher than 0.5 mg $100g^{-1}$, obtained for the control sample. The total color difference between those samples was 26.2, with increased greenness in the trial with 8% of microalga. Regarding sensory characteristics, the scores for aroma, taste, crispness, and color slightly decreased with the increase of *Spirulina* in the formulation. Despite that, the sensory evaluation classified the extrudates with up to 8% of microalga as acceptable. The overall score for microalga-extrudates ranged from 4.0 to 4.7 on a 5-point scale.

Snack bars enriched with *Spirulina* aiming for schoolchildren feeding were developed by Lucas et al. (2020). Two concentrations of *Spirulina* sp. LEB 18 (2 and 6%) were used and resulted in increases of 11.7 and 29.9% of protein content, respectively, compared to the control. *Spirulina* worked as a natural colorant in the cereal bars, where the microalga addition led to greener color. The snack bars (0, 2, and 6% *Spirulina*) presented texture, color, and microbiological stability during 30 days of storage. On sensory evaluation with children (n = 50), appearance, flavor, and taste of the snack bars (6% of *Spirulina*) presented no significant difference compared to the control. The attribute taste showed mean scores between "like" and "like a lot" for all samples. The study demonstrated that those *Spirulina*-incorporated snack bars are promising alternatives for the improvement of the diet of school children.

Based on the presented in this topic, we can point out that *Spirulina* (in a range of concentrations) is suitable for the development of snacks made from different cereals. Furthermore, *Spirulina* showed the potential to be used as a natural colorant besides enhancing the nutritional quality of ready-to-eat snacks. The results were considered promising for commercial purposes.

5.3.2 PASTA

Pasta is one of the most consumed food worldwide, which offers high amounts of carbohydrate content and advantages like easy preparation, low cost, and long shelf life (Chillo et al., 2008; Prabhasankar et al., 2009; De Marco et al., 2018; Monteiro et al., 2019; Fradinho et al., 2020; Gupta et al., 2021). Monteiro's study revealed that consumers (82%) have an interest in eating nutritionally enriched pasta (Monteiro et al., 2019). In this context, several pasta formulations have been developed using healthy ingredients to improve nutritional quality (De Marco et al., 2014; Fradinho et al., 2020). Different raw materials as quinoa protein isolate (Gupta et al., 2021), grape peels (Ungureanu-Iuga et al., 2020), fish (Monteiro et al., 2019), seaweed (Prabhasankar et al., 2009), and microalga (Fradique et al., 2010; Fradinho et al., 2020) were already incorporated to enrich pasta formulations and resulted in acceptable sensory properties. When developing enriched pasta, study on the concentration of raw material applied for the enrichment have great importance. Adding an excessive concentration of microalga or other ingredients aiming pasta enrichment could result in losses of physicochemical quality and sensory acceptance compared to the control (Monteiro et al., 2019; Fradinho et al., 2020; Ungureanu-Iuga et al., 2020).

Fradique et al. (2010) incorporated *Spirulina maxima* and *Chlorella vulgaris* biomass to enrich fresh spaghetti and reported increases in raw pasta firmness. Cooked pasta enriched with *Spirulina* (0.5, 1.0, and 2%) presented a significantly higher protein content (from 7.1 to 8.6%) compared with the control (4.7%). Pasta elaborated with both microalgae showed cooking losses lower than 5%, besides both microalgae acted as colorant due to the presence of pigments (chlorophylls and carotenoids). Pasta enriched with 2% of *Spirulina* showed lower color losses after cooking ($\Delta E = 7.15$). On sensory evaluation, positive scores (>3) were observed in all parameters for all samples, however, the sample containing *Chlorella* (orange) had the greatest score for global appreciation. Another interesting finding was that samples containing higher contents of microalgae (2%) had the color preferred among the panelists. In another study, Fradique et al. (2013) incorporated 0.5, 1.0 and 2 g $100g^{-1}$ of *Isochrysis galbana* and *Diacronema vlkianum* biomass as a source of PUFAs to pasta (spaghetti) formulations. The addition of microalgae biomass (*Isochrysis galbana* or *Diacronema vlkianum*) increased the EPA and DHA in the pasta, while in the control formulation those PUFAs were not detected. All pasta formulations presented cooking loss inferior to 6%. Sensory evaluation revealed that panelists preferred the control pasta. Among the enriched samples, the ones with 0.5 g $100g^{-1}$ of microalga biomass were preferred.

De Marco et al. (2014) incorporated *Spirulina* biomass (5, 10, and 20 g $100g^{-1}$) in dried pasta formulation and observed an increase in protein concentration, phenolic content, and antioxidant activity. The authors reported 23.74 g $100g^{-1}$ of protein content, total phenolic content of 1.05 mg GAE g^{-1}, and antioxidant activity (FRAP) of 4.24 μMoles of Trolox $100g^{-1}$ in pasta with the highest concentration of *Spirulina* (20 g $100g^{-1}$), while the control assay (without microalgae) presented 13.09 g $100g^{-1}$, 0.24 mg GAE g^{-1}, and 1.16 μMoles of Trolox $100g^{-1}$, respectively. The authors found that there were no losses of biological activity during the pasta cooking process. In another study, De Marco et al. (2018) developed dry pasta with wheat flour and the microalga *Nannochloropsis* sp. biomass (10, 20, 30, and 40%). The authors revealed significant increases in omega-3 (EPA fatty acid), protein, and ash contents, compared to the control. Quality parameters (as the optimal cooking time, cooking loss, and elasticity) of the pasta were maintained when added up to 30% of the microalga. In this concentration (30 g $100g^{-1}$), pasta presented 236.7 mg $100g^{-1}$ of EPA, while in the control formulation this fatty acid was not detected. Moreover, the authors reported for this formulation, protein and ash contents of 28.1 g $100g^{-1}$ and 2.0 g $100g^{-1}$, respectively, while the control sample presented 15.3 g $100g^{-1}$ and 0.6 g $100g^{-1}$, respectively. However, the authors observed that consumer acceptance decreased with increases in microalga concentration.

Gluten-free pasta, pasta filled with extrudate, and pasta incorporated with microspheres were within the last developments of pasta containing microalga. Recently, Fradinho et al. (2020) evaluated the effect of two *Spirulina* strains (biomass dried by different methods), on gluten-free pasta incorporation. *Spirulina* added on concentrations of 4, 5, and 10% difficult the manufacturing process of the pasta dough, as the lamination step, besides having a fishy odor. The addition of up to 3% of *Spirulina* biomasses to pasta was chosen for the pasta formulation and resulted in contents of protein (up to 8.9%) and phycocyanin (up to 69 mg $100g^{-1}$), higher than the control sample. Consequently, the antioxidant activity in *Spirulina*-enriched samples was higher and achieved a DPPH radical scavenging capacity of 70.33%, while control showed 50.93%. On sensory evaluation, the buying intention results revealed that more than half of consumers (~58%) reported "would probably buy" or "would certainly buy" the pasta enriched with 2% *Spirulina* biomass (lyophilized).

Grahl et al. (2020) produced pasta (semolina-based) filled with *Spirulina*-soy-extrudate (50% *Spirulina* powder and 50% soy protein concentrate) in the concentrations of 10, 30, and 50%. The consumer's study (conducted in 3 countries) showed that the participants preferred pasta filled with a lower concentration of extrudate (10%). Furthermore, the authors revealed lemon-basil as the preferred flavor to mask the natural flavor from the microalga (undesirable). Zen et al. (2020) prepared pasta formulations with *Spirulina* (microencapsulated or not) and observed greener color in both formulations containing microalga. Samples with encapsulated microalga showed greater firmness than the control. The acceptability index was 88.2% and 83.9% in pasta with microencapsulated *Spirulina* and free *Spirulina*, respectively. Therefore, *Spirulina* applied in both forms resulted in acceptable pasta. Furthermore, the authors observed that after heat treatment, microencapsulated *Spirulina* showed lower losses of antioxidant potential (losses ~ 21%) compared to free *Spirulina* (losses of ~ 35%).

From the results presented in this topic, we observe that different types of pasta showed interesting results of technological and sensory properties after microalga incorporation. Therefore, pasta can be considered one of the most promising food matrices when aiming to enrich with microalgae. The range of microalgae that can be applied to pasta development is impressive. Another important observation is that microalga biomass can be introduced to pasta by different forms (e.g.: powder, microencapsulated, extrudate) resulting in a high-quality product.

5.3.3 FERMENTED FOOD

Fermented foods, such as yogurt and cheese, are highly consumed around the world. These products are included in the diet of people of different ages. Therefore, the incorporation of natural resources into fermented food is a promising opportunity to improve nutrient and bioactive compounds intake (Gahruie et al., 2015). Among the natural ingredients, microalgae biomass has demonstrated potential to increase the nutritional and functional value of food products. Furthermore, the incorporation of microalgae biomass can stimulate the development of probiotic bacteria in yogurt and fermented milk (Nova et al., 2020), besides resulting in acceptable products by the consumers (Barkallah et al., 2017; Patel et al., 2019; Yamaguchi et al., 2019).

Patel et al. (2019) developed probiotic yogurt enriched with *Spirulina*. Fresh biomass (85–90% moisture content) was incorporated in milk to obtain an emulsion. The probiotic yogurt was developed with a biomass concentration of 6, 7, 8, and 9%. Yogurt with 7% *Spirulina* showed a set consistency and obtained a rating of 'Like extremely' by 78% of the consumers. The addition of 7% of fresh *Spirulina* significantly increased the carotenoid (0.407 ± 0.018 mg g^{-1}) and chlorophyll content (0.235 ± 0.016 mg g^{-1}). This sample was chosen for storage study, which showed an increase in the *Lactobacillus acidophilus* count to 8.83 ± 0.11 log CFU mL^{-1}, on day 3, and retention of 71.5% of total carotenoids, by the end of storage period (18 days). Barkallah et al. (2017) also produced yogurt incorporated with *Spirulina*, but in the powder form. The product with the lowest concentration of *Spirulina* (0.25%) showed the highest scores for color, flavor, taste, mouth texture, and overall acceptability. This treatment improves significantly the protein, chlorophyll, carotenoid, and phycocyanin content, as well as the antioxidant activity, compared to the control. Yogurt with 0.25% *Spirulina* presented significantly better water holding capacity and lower syneresis than the unfortified one, besides showed color stability during 28 days of storage.

Yamaguchi et al. (2019) evaluated the effects of *Spirulina* sp. LEB 18 (1.6 g biomass per 100 g reconstituted yogurt) in lyophilized yogurts, with addition before fermentation and after lyophilization. The chemical characteristics of the yogurts and the lactic acid bacteria count remained stable after lyophilization. Moreover, the study found that the addition of the microalgae provided significant antioxidant activity and increased concentration of lyophilized yogurt protein. This product enriched with *Spirulina* showed an acceptability index of 82.4%, proving that it meets the consumer's needs and brings nutritional and health benefits, besides without the need for refrigeration during storage.

Beheshtipour et al. (2012) investigated the addition of two species of microalgae (*Arthrospira platensis* and *Chlorella vulgaris*) in yogurt. Among the concentrations of biomass studied (0.25, 0.5, and 1%), 0.5% presented the viability of the probiotic microorganisms higher than 10^7 CFU mL^{-1} during 21 days of storage. On sensory properties, there was no significant difference between formulations containing 0.25 and 0.5% of the biomass. However, differences were observed for flavor, oral texture, and appearance when compared to the control. The addition of *Arthrospira platensis* had slower pH decline, faster acidity increase, longer incubation time, and greater final titrable acidity, which can contribute to the starter bacteria development. Therefore, these studies demonstrated the potential of microalgae biomass to contribute to the functional and nutritional properties of yogurt.

Recently, the incorporation of *Chlorella vulgaris* (2, 4, and 6%) into processed cheese was carried out by Tohamy et al. (2018). The addition of 4% of microalga increased the content of the minerals selenium, zinc, iron, potassium, and magnesium. Regarding sensory proprieties, the scores for firmness, gumminess, flavor, saltiness of cheese produced with 2% biomass showed no difference compared to the control. The overall preference score was 4 for the product with microalga and 4.5 for the control sample. In another study, fermented Spanish "chorizo" sausages were prepared with different protein sources, including *Chlorella*, and *Spirulina* in a concentration of 3% (Thirumdas et al., 2018). The samples developed with microalga biomass had a protein content (34.9%), similar to the control (35.6%), being higher than sausage with bean (31.8%) and lentil (30.6%). The ratio of essential and nonessential amino acids of products with *Chlorella* was higher than the other formulations. The authors observed a decrease in the redness of the meet and a complete greenness color in the microalgae-added chorizo. The incorporation of *Chlorella* and *Spirulina* increased the hardness compared to the soy-enriched formulation (control).

5.3.4 BAKERY PRODUCTS

During the last years, bakery products have been largely studied and enriched with natural ingredients to improve the nutritional content and the bioactive constituents in these products. Bread (Diprat et al., 2020), biscuits (Singh et al., 2015), cookies (Vieira et al., 2020), crackers (Lafarga et al., 2019a), and crostini (Niccolai et al., 2019b) have already been enriched with microalgae. Generally, those products succeed in presented good physical properties as well as sensory acceptance. However, depending on the type and concentration of microalga used, sensory characteristics may be positively or negatively affected.

Cookies have been largely used as food matrix to microalga incorporation. Morais et al. (2006) elaborated chocolate cookies with 0, 1, 3, and 5% of *Spirulina platensis*. The authors observed that the formulation with 5% of microalga presented as an advantage the highest protein improvement (7.7% higher than the control), besides showing no significant differences in sensory attribute scores (as color and crispness), compared to the control sample. Regarding purchase intention, the formulation with 1% of *Spirulina* scored higher than other concentrations. Aiming to provide natural color to traditional cookies, Gouveia et al. (2007) added the microalga *Chlorella vulgaris* to formulation in the concentrations of 0.5, 1, 2, and 3%. The authors obtained microalga-added cookies with attractive appearance, greener, and darker color compared to the control, where, the higher the *Chlorella* addition the lower was the lightness (L*). The color of the cookies was stable over 3 months of storage. Regarding texture, the microalga addition resulted in cookies with higher firmness.

Singh et al. (2015) incorporated the microalga *Spirulina platensis* (from 1.63 to 8.36 g $100g^{-1}$) in biscuits made from sorghum and wheat flour and observed increases in antioxidant activity (% DPPH and ABTS inhibition) with increases in *Spirulina* content. On sensory evaluation, samples with the highest amount of *Spirulina* (8.36 g $100g^{-1}$) scored the highest for color intensity (9.7). However, the highest score for flavor (9.02) and sweetness (8.12) was obtained with the lowest content of microalga (1.63 g $100g^{-1}$). After optimization, the authors suggest using 7g $100g^{-1}$ of *Spirulina* on biscuits formulation. Batista et al. (2017) developed cookies enriched with 2 and 6% of microalgae biomass (*Arthrospira platensis*, *Chlorella vulgaris*, *Tetraselmis suecica*, and *Phaeodactylum tricornutum*). Microalgae-added cookies showed attractive appearance and significantly higher protein content and antioxidant capacity, compared to the control. All cookies presented color stability during 8 weeks of storage. Regarding hardness, all formulations with 2% of microalgae showed no differences compared to the control. Cookies with 6% of *A. platensis* presented the highest phenolic content (0.90 mg GAE g^{-1}), however, showed increased hardness. Among *Arthrospira platensis* and *Chlorella vulgaris* formulations, the cookie with 2% *Arthrospira platensis* presented the highest scores for global appreciation.

Şahin (2020) evaluated the effect of 1 and 2% microalga biomass (*Spirulina platensis* or *Dunaliella salina*) in cookies formulation. The author reported that 2% of *Spirulina* addition to cookies formulation resulted in higher protein content (16.66%), compared to the control (14.98%). Furthermore, *Spirulina*-added cookies showed a harder texture, greener, and darker colors. The enrichment of cookies with *Dunaliella* biomass resulted in color with significantly higher redness and yellowness values. Cookies (2% *Dunaliella*) also presented the highest content of total phenolic content (3 mg GAE g^{-1}) and both formulations containing *Dunaliella* showed greater overall acceptance, compared to *Spirulina*-added cookies and with the control. Recently, research applying microalga encapsulated extract to cookies development showed advantages compared to the enrichment with raw biomass. Vieira et al. (2020) developed 3D-printed cookies incorporated with *Arthrospira platensis* in three forms (2% of whole dried biomass; freeze-dried antioxidant extract, and encapsulated antioxidant extract). The authors observed that the addition of the raw biomass in the cookie dough replacing the wheat flour led to increases in the dough firmness and hardness, while the encapsulated extract resulted in lower dough viscosity and hardness. All cookies presented low total color difference (ΔE) after 30 days of storage, while the formulation with encapsulated extract showed the lowest color variance along the storage period. Moreover, the authors also observed significantly higher antioxidant activity (ORAC) in this sample, compared to the other cookies.

Breads are also baked goods widely consumed that have been incorporated with different microalgae during the last years. The existing literature showed promising results as increased nutrient content, good texture, and sensory acceptance (Lafarga et al., 2019a; Diprat et al., 2020). Figueira et al. (2011) incorporated *Spirulina platensis* biomass (2, 3, 4, and 5%) on the enrichment of gluten-free bread made with rice flour. With 5% of *Spirulina* addition, protein increased 39% compared to the control bread. Moreover, breads incorporated with *Spirulina* showed an essential amino acid increase, besides darker and greener color, compared to the control. Regarding hardness, up to 4% of microalga addition resulted in no changes on this property. On sensory evaluation, no significant differences were found among the samples incorporated with 3 or 5% of microalga. Graça et al. (2018) developed bread containing wheat flour and *Chlorella vulgaris* biomass (1 to 5 g $100g^{-1}$). The authors reported concentrations up to 3 g $100g^{-1}$ of microalga incorporation as favorable for bread dough characteristics, recommending this concentration to produce enriched bread. Lafarga et al. (2019a) analyzed the potential of *Nannochloropsis* and *Tetraselmis* biomass as ingredients in baked goods (breads and crackers) and reported satisfactory results. Concentrations of 1, 2, and 3% of microalgae were incorporated into bread, while 1.25, 2.5, and 3.75% of microalgae were used in crackers development. Sensory evaluation revealed an acceptability index higher than 70% for all bread and crackers developed. For microalga-added bread, purchase intention scored above 3 for all samples, while for crackers similar behavior was observed. Bread samples with 2% of *Tetraselmis* or 1% of *Nannochloropsis* as well as crackers containing microalgae (2.5%) were selected based on sensory outcomes. For those selected samples, the authors observed color stable during the storage period as well as increased total phenolic content and antioxidant capacity, compared to the control. Recently, Diprat et al. (2020) enriched gluten-free bread formulation with microalga *Chlorella sorokiniana* biomass (2.5 and 5 g $100g^{-1}$) and reported increases in protein, carotenoid content, and omega-3 fatty acids, compared to the control. Protein content increased 26% in bread developed with 5% of *Chlorella*. The authors observed that depending on the baking process, carotenoid content can vary. High amounts of lutein (>40 µg g^{-1}), as well as total carotenoids (>90 µg g^{-1}), were obtained for formulations with 5% *Chlorella*, in both baking conditions tested (180°C and 220°C). Among the microalgae enriched breads, the one with 2.5% *Chlorella sorokiniana* presented the highest acceptance rate (>70%).

5.3.5 MEAT PRODUCTS AND ANALOGS

Meat products are frequently developed with partial replacement of the animal protein by vegetable protein sources, aiming health benefits and cost reduction. In this way, recent studies investigated the incorporation of microalgal biomass in meat products and analogs (Parniakov et al., 2018; Zugcic et al., 2018). Zugcic et al. (2018) elaborated hamburgers with different protein sources (1%), including *Spirulina* and *Chlorella* biomass. No significant changes were found regarding protein content (approximately 18%) compared to the control (soy protein). Furthermore, the addition of *Spirulina* or *Chlorella* increased the concentrations of all amino acids. Color parameters (L*, a*, b*) were significantly affected by incorporating microalgae. In the study, the taste profile was determined from the free amino acid profile and did not present significant changes between the different hamburger formulations. The amino acids correlated to the umami taste (aspartic acid and glutamic acid) and lysine were predominant. Similar results were observed by Parniakov et al. (2018), who investigated the partial replacement of animal proteins by plant and microalgae-based proteins in chicken roti. The microalgae studied were *Spirulina* and *Chlorella* at a concentration of 1%. However, concerning the sensory evaluation, the formulation with microalgae does not present positive results for acceptability and preference. According to the authors, these results could be associated with the characteristic taste of the microalgal biomass as well as the increase of amino acids content correlated to umami and bitter taste.

Microalgae were also used as an ingredient in canned fish burger (Atitallah et al. 2019). Formulations with *Chlorella minutissima* (0.5%), *Isochrysis galbana* (1%), or *Picochlorum* sp. (1%) showed better texture and scores for sensory attributes, compared to the control (microalgae-free burger). The addition of microalgae in fish burgers significantly improved the content of pigments (carotenoids, chlorophylls, and phycocyanin), resulting in a positive correlation with antioxidant activity. DPPH-radical scavenging activity increased from 40% in the control sample to 98% in the burger containing 0.5% *Chlorella minutissima*. Marti-Quijal et al. (2019) also found an increase in the total and essential amino acid content, changes in color and texture parameters in pork sausages made with *Spirulina* (1%) or *Chlorella* (1%).

Meat analogs were produced by high moisture extrusion (HME) using a combination of lupin and *Spirulina* as protein sources (Palanisamy et al., 2019). Increasing the microalga concentration from 15 to 50% improved the content of total phenolic, flavonoid concentration as well as Trolox equivalent (TE) antioxidant activity. The results for the sample with the highest concentration of microalga were 8.47 mg GAE g^{-1}, 1.83 mg EC g^{-1}, and 9.99 μmol TE g^{-1}, respectively. The in vitro digestibility was improved with the high moisture extrusion process, compared to the raw extrusion mixtures, however, it was reduced with the increase in *Spirulina* concentration. The authors highlighted that changes in the extrusion parameters could improve the physical properties of the meat analogs with microalgae.

5.3.6 OTHER FOODS DEVELOPED WITH MICROALGAE

Because of their content of nutrients and bioactive compounds, microalgal biomass has been investigated as an ingredient in other food formulations, such as soup, ice cream, shake, and products for athletes (Santos et al., 2016; Moreira et al., 2018a; Lafarga et al., 2019b; Durmaz et al., 2020). Lafarga et al. (2019b) incorporated *Spirulina* sp., *Chlorella* sp., or *Tetraselmis* sp. biomass in broccoli soup. This food was chosen by the authors due to its natural green color, which could contribute to the acceptability of the product elaborated with microalgae. Increasing the microalgae concentration from 0.5 to 2.0% improved the total phenolic content and antioxidant activity (DPPH and FRAP) when compared to the control soup. It was also observed an increase in the phenolic compounds after in vitro gastrointestinal digestion. The sensory acceptability of the soups containing microalgae was higher at the lower concentrations of biomass (0.5 and 1%). For these soups, the acceptability index ranged between 67.9% and 82.2%. Based on these outcomes, the authors suggested that *Spirulina* sp., *Chlorella* sp., or *Tetraselmis* sp. could be used as an innovative ingredient in broccoli soup, at concentrations of 0.5% and 1%. In another study, a dehydrated soup was developed using 15% *Spirulina platensis* (Los et al., 2018). In this formulation, the protein and fiber contents increased 4.6 and 12 times, respectively, compared to the control. Moreover, the content of total phenolic compounds and antioxidant activity increased when *Spirulina* was incorporated. The scores for acceptability was lower in the soup with *Spirulina* (4.17) than in the control formulation (6.83). However, according to the authors, the acceptability could be greater in the market niche that values functional foods.

Shake-type powdered food could be a convenient way to provide nutrients and bioactive compounds for specific population groups, such as the elderly population and athletes. Besides, this type of food is easy to prepare. In this context, Santos et al. (2016) developed chocolate flavor shake-type powdered food with *Spirulina* sp. LEB 18 (750 mg 100 g^{-1}), for the elderly population. The protein content of the shakes with and without *Spirulina* (control) did not significantly differ. Regarding the acceptance test, both formulations showed higher scores, 7.68 ± 1.27 and 7.97 ± 0.96, respectively, than the commercial product (6.89 ± 1.59). The same concentration of *Spirulina* sp. LEB 18 (750 mg 100 g^{-1}) was incorporated in a meal replacement shake and a high-calorie food (Freitas et al., 2019). The meal replacement shake with microalgal biomass developed by Freitas et al. (2019) showed a reduction of 8.6% in carbohydrate content and an increase of 29.3% in lipid concentration, compared to the formulation without *Spirulina*. This could be favorable since the biomass of microalgae is a source of essential fatty acids (Becker, 2013). Furthermore, both products were accepted by the target public with acceptance above 70% (Freitas et al., 2019).

The addition of *Spirulina* in foods as energy gels contributes to the development of innovative products for athletes (Carvalho et al., 2018; Moreira et al. 2018a). Moreover, *Spirulina* sp. LEB 18 (0.5%) was incorporated in energy gels destined for practitioners of physical activities without affecting the sensory characteristics of the product (Moreira et al., 2018a). Carvalho et al. (2018) developed six different supplements (electrolyte replenisher, muscle enhancer, and recovery supplement), without and with *Spirulina* sp. LEB 18 (500 mg 100 g^{-1}). In this study, the mineral content of the electrolyte replenisher with *Spirulina* was higher than the product without microalga. *Spirulina* biomass afforded an increase in the carbohydrate content of muscle enhancers developed. However, biomass addition did not influence the nutritional composition of muscle recovery.

In the study of Durmaz et al. (2020), ice cream was formulated with spray-dried *Nannochloropsis oculata*, *Diacronema vlkianum* or *Porphyridium cruentum* at different concentrations (0.1, 0.2, and 0.3%). Ice cream produced with *Porphyridium cruentum* had an attractive color due to the red pigment of this microalga. The samples with *Porphyridium cruentum* were preferred more than the other products, for all sensory properties (color, texture, taste, odor, resistance to melting, mouthfeel, and overall acceptability) with scores about 4 (using a 5-points hedonic scale). Furthermore, the increase of microalga concentration had a positive effect on the total phenolic content (from 160 to 220 mg GAE kg^{-1}) of the ice cream.

Hlaing et al. (2019) developed a functional crispy chocolate bar containing 5% of microcapsules of *Scenedesmus obliquus*. The emulsion containing freeze-dried biomass was encapsulated which helped to slow down the process of oxidation compared to the chocolate containing free cell *S. obliquus*. Sensory evaluation showed no significant difference in the texture attribute of both the samples. However, the panelists preferred the flavor of the chocolate crispy bar without microalga.

Microalgal biomass was incorporated in oil-in-water food emulsions, such as mayonnaise (Gouveia et al., 2006). The study aimed to evaluate the use of *Chlorella vulgaris* (from 0.25 to 2%) and *Haematococcus pluvialis* (from 0.05 to 2%) as a colorant in food emulsions stabilized by a pea protein. The results showed color instability for emulsions with concentrations of microalgal biomass above 1%. The authors evidenced an improvement in the resistance to oxidation by emulsions containing microalgae.

5.4 BIOMOLECULES FROM MICROALGAE ADDED TO FOOD

Biomolecules extracted from microalgal biomass, as pigments, fatty acids, and peptides, have potential as an ingredient for the food industry.

These compounds could be applied in the development of functional foods, for example, due to their antioxidant properties (Becker, 2013). Moreover, some of them can improve the technological properties and nutritional value of foods (Lane et al., 2014; Rodrigues et al., 2020), besides replacing synthetic colorants (Becker, 2013; Mezquita et al., 2014).

Among the various fatty acids present in microalgal biomass, the most important ones nutritionally and commercially are linoleic acid, β-linolenic acid, arachidonic acid, EPA, and DHA. These compounds cannot be synthesized by humans, and animals, and must be obtained from the diet, therefore, they are known as essential fatty acids (Becker, 2013). Lane et al. (2014) aimed to evaluate the effect of nanoemulsion technology in the bioavailability of omega-3-rich microalgal oil incorporated in strawberry yogurt. Bioavailability of the omega-3 polyunsaturated fatty acids was investigated in vivo study with 11 volunteer participants. The consumption of yogurt elaborated with nanoemulsion of microalga oil increased blood levels of omega-3 PUFA. The levels were 1.78 and 1.62 times higher than the control, 4 and 6 h after ingestion, respectively. Another study evaluated the potential of yogurt as a food vehicle for polyunsaturated fatty acids from microalga (Robertson et al., 2016). The addition of lipid extract from *Pavlova lutheri* at 0.25 or 0.5% concentration significantly increased the content of omega-3 PUFA in the yogurt. The incorporation of microalga extract did not influence the techno-functional properties. However, both concentrations of microalgal oil were negatively correlated with all sensory attributes evaluated. As highlighted by the authors, further research is necessary to improve the sensory quality of the product.

Rodrigues et al. (2020) investigated the application of an aqueous extract of *Spirulina* containing phycocyanin as an emulsifying and stabilizing agent in ice cream. The formulations were prepared by replacing ingredients with stabilizing and emulsifying activity by 190 mL of the extract containing 13 mg mL^{-1} of phycocyanin. The O/W (oil in water) activity in the formulations with the extract was higher (87.5–94.8 Units of Emulsification, UE) than the control ice cream (83.1 UE). The substitution of ingredients for phycocyanin extract did not significantly affect the W/O (water in oil) activity. These results demonstrated the emulsifying activity of phycocyanin in ice cream. Concerning sensory evaluation, the replacement of the emulsifier for the biocompound did not cause a significant difference for all attributes when compared to the control.

Recently, Mohammadi-Gouraji et al. (2019) evaluated the effects of phycocyanin extracted from *Spirulina platensis* on the antibacterial and physicochemical properties of yogurt. The product was prepared with 2, 4, and 8% of phycocyanin powder. Their physicochemical and microbiological properties were evaluated during 21 days of storage. The authors reported decreased syneresis, color stability, and no pathogen growth for the yogurts containing the biocompound. In terms of sensory evaluation, only the yogurt with 4% phycocyanin not showed negative effects for all attributes, since there was no significant difference compared to the control product. Mezquita et al. (2014) developed yogurt incorporated with astaxanthin extracted from *Haematococcus pluvialis*. In the study, astaxanthin was used as a colorant to simulate the apricot color in yogurts with different fat content. No significant difference was observed in color parameters and astaxanthin concentration during 4 weeks of storage, indicating the stability of this pigment natural in the protein-lipid matrix of the yogurts.

5.5 MARKET AND CONSUMER PERCEPTION TOWARD MICROALGAE AS FOOD

Microalgae and their extracts play an important role in the nutraceutical and pharmaceutical industry, and bioproducts as astaxanthin, beta-carotene, and fatty acids have shown a rising market (Nova et al., 2020; Rahman, 2020). Most of the commercial products obtained from microalgae are available on the market as pills and capsules form (Caporgno and Mathys, 2018). However, a trend has been observed concerning the incorporation of the bioproducts or the whole biomass as an ingredient in food formulation (Lafarga, 2019), since consumers' demand for natural and healthy products is increasing (Vigani et al., 2015).

Thereby, foods developed with microalgae are very promising, given the nutritional content, functional properties, and sustainability of microalgae cultivation, which could improve market opportunities for these products (Vigani et al., 2015). To meet consumer demand for healthier foods, some companies have been developed different product categories with microalgae biomass incorporation (Nova et al., 2020) and an increase in the number of these foods on the market has been observed, mainly products knowing as traditional food (Lafarga, 2019). Examples of products currently commercialized are pasta, soup, snack bar, biscuits, crackers, cookies, cake, and chocolate tablets, being *Arthrospira platensis* and *Chlorella vulgaris* the most used (Lafarga, 2019; Nova et al., 2020). This sector has seen growth, mainly dominated by the United States, Asia, and Oceania (Rahman 2020). According to Vigani et al. (2015), Europe has the potential to significantly improve its share in the global market of food and feed with microalgae (about 5%).

The development of novel foods must take into account the opinion of consumers given the proposed innovations to obtain a successful introduction to the market (Moons et al., 2018). The investigation about consumer expectations and perceptions could indicate which products should be developed as a prototype and sensory tested (Moons et al., 2018). Thereby, recent researches identified some factors that motivate consumers to purchase foods developed with *Spirulina* (Grahal et al., 2018; Moons et al., 2018; Grahl et al., 2020).

Recently, Grahl et al. (2020) aimed to understand consumer preferences concerning filled pasta with *Spirulina*-soy-extrudate in three countries (Germany, Netherlands, and France). The results revealed that the consumers' foreknowledge about *Spirulina* contributed significantly to increase the acceptance of pasta. That indicates the importance of considering the consumers' familiarity with the product of interest. However, food neophobia affected negatively the product acceptance. Food neophobia could influence the acceptance of innovative foods. It was defined as a reluctance to eat unfamiliar food and/or a tendency to avoid novel foods (Pliner and Hobden, 1992).

Another study carried out consumer research about three innovative products with *Spirulina* biomass (Grahal et al., 2018). The acceptance of pasta filled with *Spirulina*, maki-sushi filled with *Spirulina*, and *Spirulina* jerky was evaluated for 1035 consumers from Germany, Netherlands, and France. In general, pasta was most liked in the three countries. This study showed that consumers tend to prefer products that they are more familiar with.

A quantitative study conducted with 1325 Belgium participants investigated the motivational drivers and barriers to the adoption of foods with *Spirulina* in the diet (Moons et al., 2018). The potential early adopter consumers of food with *Spirulina* identified were sporting individuals, vegetarians, and foodies. For these consumer groups, the health consciousness was the main motivator to acceptance of the products, while environmental concern was not a significant motivator to try *Spirulina*-enhanced food, even after they were informed about the environmental benefits of *Spirulina* production. Therefore, these researches could help producers and marketers to set strategies for commercializing food incorporated with microalgae.

5.6 CHALLENGES

The main obstacles to the industrial use of microalgae for the food field are the high cost for biomass obtaining and the small scale of current production systems (Rahman, 2020). Consequently, less than 20,000 t of biomass are produced worldwide, at a cost above €5/kg (Borowitzka, 2013). The low production capacity limits the inclusion of microalgae biomass in food processing. For this, an alternative is to develop more robust and efficient production systems. Moreover, the costs of the process must be reduced by increasing production capacity (Garrido-Cardenas et al., 2018). The biorefinery strategy has been used to reduce processing costs while increasing the sustainability of microalgae production (Camacho et al., 2019). Concerning production technology, different types of reactors have been proposed. In this context, the main challenge is to maximize the performance of strains to provide adequate uncontrolled cultivation conditions at minimum cost (Garrido-Cardenas et al., 2018). Providing ideal conditions on a small scale is possible using different reactor designs. However, when increasing the size of the reactor, it is generally not possible to maintain such conditions. The main disadvantages in large reactors are related to the inability to control temperature, inadequate mixing, excessive energy consumption, and low mass transfer capacity (Fernández et al., 2012; Acien et al., 2013; Garrido-Cardenas et al., 2018).

Regarding the biomass application on food formulation and consumer acceptance, one of the biggest challenges is to find the balance between the biomass content incorporated to contribute with nutrients and bioactive compounds, without compromising the physical properties and sensory quality of the product. A food product can have several health benefits, however, if it is not tasty and attractive, few consumers will be willing to buy it (Nova et al., 2020). Besides, microalgae manufacturing processes must comply with a series of regulations and standards. Labeling issues exacerbate the problem, as they vary by country and can increase the overall cost of manufacture for application in food for commercialization (Borowitzka, 2013; Camacho et al., 2019). In this context, legislative measures are essential to ensure adequate monitoring programs, correct labeling of food products, and the establishment of portions that are safe for consumers (Van der Spiegel et al., 2013; Cherry et al., 2019). Solutions for microalgae to contribute to food security may include the use of improved technologies that can remove possible contaminants and the generation of more mechanistic evidence to support health claims (Cherry et al., 2019).

5.7 FUTURE TRENDS AND OPPORTUNITIES

Foods prepared with microalgae biomass are recognized as innovative and healthy (Pina-Perez et al., 2019). The development of several products with the addition of microalgae is an excellent opportunity to open a new segment of highly nutritious and healthy food products (Ścieszka and Klewicka, 2019). Lafarga et al. (2019b) demonstrated new commercial opportunities for the use of microalgae as an ingredient in vegetable soups, allowing the addition of a "modern" ingredient, which also promotes health.

In addition to their functions related to health aspects, microalgae also demonstrate a structuring role in food, as a texturizing ingredient. Microalgal biopolymers such as proteins and polysaccharides possibly alter the product's rheological properties. Microalgae biomass can be used as a thickening agent that would reduce the need for additional texturizing ingredients (Bernaerts et al., 2019). Thus, the diversity of microalgae available and their functionality contributes to expanding the use of biomass as specialized ingredients in food formulations (Grossman et al., 2019).

The green color of microalgae such as *Spirulina* can be an opportunity to innovate and satisfy the demands of consumers, selling green food and drinks. Besides, previous studies have suggested that the aroma and flavor of some microalgae could be an opportunity to develop innovative fish-based foods (Fradique et al., 2013). Furthermore, microalgae have the potential to be used as natural preservatives during cold storage. According to Takyar et al. (2019), *Spirulina* and *Chlorella* extracts can be used on an industrial scale to delay the oxidation of lipids in fish and fish products. With this, the authors reported that the sensory properties of the foods are maintained or even improved. Moreover, the shelf-life of products can be extended with microalgae extracts.

Nanotechnology is expanding in several areas, mainly in the food industry. Nanomaterials present a high surface area and a controlled release of the encapsulated compounds. Therefore, there is a trend to the application of nanomaterials in food formulations to encapsulate several bioactive ingredients, with emphasis on those with antioxidant properties (Moreira et al., 2018b). Furthermore, recent studies showed that nanofibers of microalgal origin could have antioxidant activity as well as acting on food preservation, being considered another innovative and promising trend (Gonçalves et al., 2017; Moreira et al., 2019). As a result, there is a growing investment in research on nanotechnology, which could benefit both food producers and consumers (Moreira et al., 2020).

5.8 CONCLUSIONS

The search for healthier foods has increased research efforts aiming to develop enriched foods by adding microalgae biomass. Many different genera have been evaluated for this purpose; however, *Spirulina* and *Chlorella* stand out. The choice of species recognized as safe for human health and, besides, the adoption of quality control practices throughout the process is fundamental for obtaining safe biomass. Nutrients and culture parameters (temperature, pH, light and salinity) are the main factors studied for the accumulation of biomolecules of interest, such as proteins, lipids and pigments. The choice of the best methods to be applied in downstream processes is fundamental for efficient product yield and also for reaching higher economic viability.

Foods such as snacks, pasta, yogurt, cheese, bread, burger, among others, have been enriched with microalgae biomass over the last years. In general, these enriched foods showed an increase in the content of proteins, minerals, pigments, phenolic substances, among other essential compounds for human health. In most studies, the highest consumer acceptability indexes were obtained in foods enriched with a low biomass concentration, mainly due to microalgae fishy taste and flavor. Biomass can modify some important food characteristics, such as firmness, hardness and color. Typically, biomass enriched products show long shelf life. Last but not least, the microalgae consumer audience is still very select, the majority being sports, vegetarian and foodie people. Therefore, improving marketing strategies is crucial to win over consumers of all groups in society.

KEYWORDS

- ***Chlorella***
- **consumer acceptance**
- **food enrichment**
- **healthy food**
- **microalgae consumption**
- **microalgae cultivation**
- **microalgal-based**
- ***Spirulina***

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CHAPTER 6

EXPLORING ALGAL VARIETIES TO VALUE-ADDED PRODUCTS FOR COMMERCIALIZATION

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ABSTRACT

In recent decades, the requirement of natural, sustainable, and novel nutritional products is on great interest for the nourishment of growing population. Being eco-friendly, algae are promising alternative sources to produce a variety of value-added rich products. These bioactive products extracted from algae are not only defensive for human and animal health but also fulfilling the food demand of the large population. The application of algae in pharmaceutical and nutraceutical sectors is stimulating the defense mechanism in living beings as well as also attracting the people towards the use of more natural products instead of chemical products for their health and nutrition. So, the production and application of new algal species should be explored and grown at a large scale. Conversion of algae into bio-active products should be improved at the commercial level so that the large fraction of the world population could use it as immunity booster or enhancer. This chapter discussed and evaluated the use of various algae derived nutraceutical and pharmaceutical value-added rich products in health improvement and also in disease treatment (such as diabetes, cancer etc).

Algal Farming Systems: From Production to Application for a Sustainable Future.
Jeyabalan Sangeetha, Devarajan Thangadurai (Eds.)

6.1 INTRODUCTION

Algae generates diverse group of natural compounds that can be used as medicine in pharmaceuticals area, as nutrition in nutraceuticals area, and as beauty products in cosmeceuticals area. It can also be used as food as well as energy source. It has an enormous power to mitigate CO_2 from the environment. So it can be considered as the cleaner and air purifier of the surroundings. The biomass of algae consists of basically carbohydrates, proteins, carotenoids, lipids, and vitamins that can act as healthy nutrition for humans and animals. Algae and its components are studied for their medicinal properties in various areas, including anti-inflammatory, anticancer agents, antioxidants, cardiovascular health, antimicrobials, anti-obesity, and antidiabetic activities (Figure 6.1) (Dominguez et al., 2013).

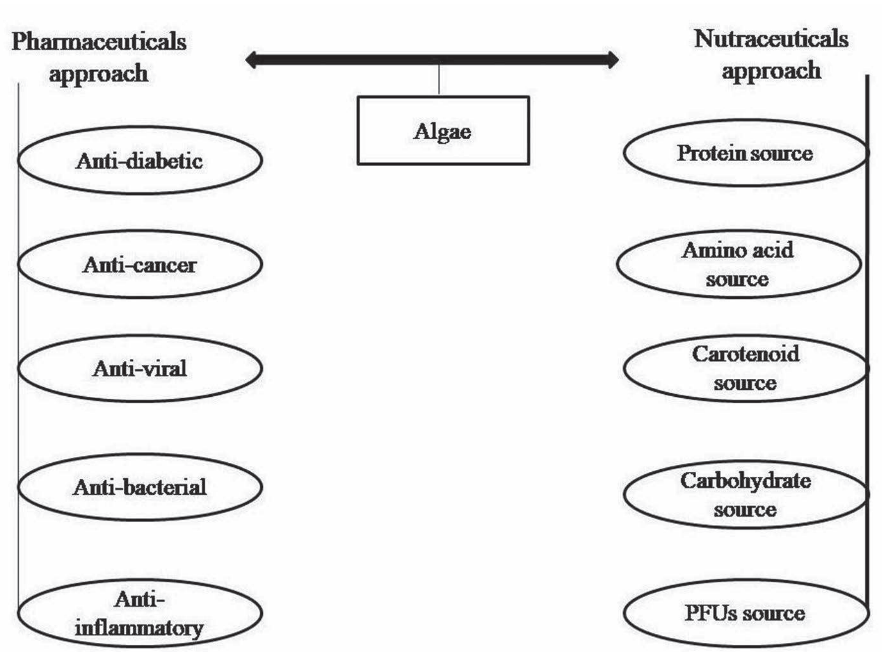

FIGURE 6.1 Approach of algae in pharmaceutical and nutraceutical sector.

Algae are found as unicellular (*Chlorella*) and multicellular (brown algae) in the aquatic environment. It belongs to *Protista* (Kingdom) due to having more characteristics of plants and also placed in *Monera* (Kingdom) of prokaryotic organisms owing to have some resemblance with bacteria.

Microalgae efficiently utilize sunlight for improving algal biomass production rate and value-added product formation rate in comparison to other higher plants. Interestingly, microalgae were used as nutritional supplements for a long time. The large population of Chad has been cropping *Spirulina* and using it as food for centuries. Aztecs utilized *Arthrospira maxima*, and *Arthrospira platensis* from Texcoco lake in Mexico. The *N. punctiforme*, *N. commune*, and *N. flagelliforme* are consumed on a large scale in South America, China, and Mongolia. *Oedogonium* and *Spirogyra* are filamentous algae and consumed as a dietary food in India, Thailand, Burma, and Vietnam. So, having all these old backgrounds of using algae, a new era for new technology began for cyanobacterial and microalgal development in the 1940s. *Spirulina* is considered as best food by the United Nations World Food Conference of 1974. While the United Nations World Health Organization (WHO) declared that algae (*Spirulina*) can be used as attractive food because it is in a rich source of protein and other trace elements (Garcia et al., 2017). *Spirulina* and *Chlorella* have been extensively harvested for their immense potential in the production of various high value-added product formation, for example, vitamins, pigments, protein, phenolic compounds, sterols, peptides, polyunsaturated fatty acids, amino acids, and pigments (Andrade et al., 2018). Lipophilic vitamin as an essential vitamin was observed in the edible algae *Corallina elongata*, *Cystoseira barbata*, *Jania rubens*, *Laurencia obtusa*, and *Sargassum vulgare*. At the same time, other vitamins such as K1 and K2 were also detected in the extract of respected algae (Caf et al., 2019). Some edible algae, for example, *Porphyridium*, *Chlorella*, *Parietochloris*, *Spirulina*, *Chlamydomonas*, *Nostoc*, *Crypthecodinium*, *Scenedesmus*, *Haematococcus*, *Anabaena*, *Botryococcus*, and *Synechococcus* have been used for a variety of macro and micronutrients production such as sodium, magnesium, phosphorus, calcium, nitrogen, iodine, selenium, molybdenum, copper, cobalt, and zinc, *etc.* (Mata et al., 2010; Adarme-Vega et al., 2014). It is being exhausted on a large scale for a variety of valuable product formation like enzyme, food, β-carotene, protein, medicines, lipids, omega-3 fatty acids, oil, carbohydrate, and also for bio-gas and bio-fuel generation, *etc.* Hence, algal biomasses are used for extracting various bioactive metabolites at the industrial level (Liu et al., 2016). The algae have shown its impact as pharmaceutical and nutraceutical approach in Figure 6.1. Algae produce a variety of several nutritional ingredients that participate in healing and curing of various dreadful diseases (heart disease, cancer, *etc.*) along with improving the immune system (Bishop et al., 2012). Algae are also known to be as a producer of a structurally different form of exopolysaccharides (EPS) which is extensively used as thickeners and gelling additives in the food and pharmaceutical industry (Liu et al., 2016). It has many pharmacological properties, such as anticoagulation, antibacterial, antioxidant, antihyperlipidemic, and antitumor activities (Liu et al., 2016). Some other bioactive compounds extracted from microalgae are sterols. The steroid compounds are widely on interest for their quality to diminish LDL cholesterol and improve cardiovascular strength. Steroids have anticancer, anti-inflammatory, antioxidative, antiatherogenic activities. It also protects from Alzheimer's disease and autoimmune encephalomyelitis (Luo et al., 2015). The objective of the recent research is to discuss the variety of algae and their participation as pharmaceutical and nutraceutical agent.

6.2 PHARMACEUTICAL APPROACHES OF ALGAE

6.2.1 ALGAE AS AN ANTIDIABETIC AGENT

Diabetes mellitus is considered an endocrine disorder and is a type of chronic degenerative disease. It is a metabolic disorder that disrupts the protein, carbohydrate, and fat metabolism (Shan et al., 2016). Elevation in blood glucose levels (hyperglycemia) consequently causes hypercholesterolemia, cardiovascular diseases, and hypertriglyceridemia. Furthermore, it also instigates cardiovascular disease, renal failure, blindness, *etc.* worldwide. The problem of diabetes mellitus developed due to deficiency or low secretion of pancreatic insulin as well as negligible or low sensitivity of tissue for insulin. According to the World Health Organization (WHO), more than 220 million people worldwide suffer from diabetes mellitus. Currently, various physical and therapeutic strategies have been in use for controlling this disease like regular exercise, proper diet, insulin therapy, and oral hypoglycemic drugs. Oral antidiabetic agents such as sulphonylurea and biguanides led to adverse side effects (Aboulthana et al., 2018) on health. Hence, the undesirable side effects of these drugs have directed the scientist towards searching for natural and sustainable products to overcome the problem of these side effects of chemically synthesized medicines. Furthermore, various research for curing diabetes problems from natural products is being conducted, so algae owing to natural source is being targeted here as a burgeoning area for this problem. Natural compounds from these algae have immense activity to overcome the problem of many diseases due to having various pharmacological active ingredients that lead to reduce the root cause problem of diabetes without harming any healthy cells or organs. In case of diabetes, two key enzymes named *α*-amylase and *α*-glucosidase act together for breaking down and absorption. The conversion of carbohydrate to glucose is supported by pancreatic *α*-amylase and absorption of this simple sugar (glucose) is supported by intestinal α-glucosidase. Thus, inhibition of these two enzymes would delay the breaking down and adsorption of carbohydrate that can minimize or control the problem of postprandial hyperglycemia (Zinjarde et al., 2011). The pharmacological bioactive compounds from algae have proved its affectivity in the inhibition of *α*-amylase and *α*-glucosidase (Lee et al., 2009). So, discussing with respect to the natural source, here two algae such as *Sargassum polycystum* and *S. wightii* have shown variety of bioactive compounds such as flavonoids, alkaloids, lipids, phenols, carbohydrates, glycosides, and proteins from its extract. These compounds have antioxidant properties and were tested for antidiabetic properties. It was found from the test that the extract of *Sargassum polycystum* and *S. wightii* has shown effective results as an inhibitor of *α*-amylase and *α*-glucosidase due to having natural active compounds that bind the active site of the enzyme and alter the activity of the enzymes (Unnikrishnan et al., 2015). Layam et al. (2006) studied the effect of different doses of *Spirulina* (15 mg/kg body weight, 5 mg/kg body weight, and 10 mg/kg body weight) in streptozotocin-diabetic rats. They concluded that the administration of *Spirulina* (15 mg/kg body weight) was significantly effective in the treatment of diabetic rats in comparison to the other two doses. Diabetic rats treated with *Spirulina* decreased the glucose-6-phosphatase activity and increased the hexokinase activity. Madhumita et al. (2019) investigated the influence of *Spirulina platensis* on blood glucose in diabetic rats (induced with alloxan). They suggested that the oral administration of *Spirulina platensis* significantly reduced the blood glucose level ($P<0.01$). Thus, *Spirulina platensis* seen to be very effective against diabetic rats (induced with alloxan) and used in the future against type 1 diabetes. Simon et al. (2018) explored the effect of *Spirulina fusiformis* in diabetic rats (induced with streptozotocin). They found that it has a defensive response, *i.e.* reducing blood sugar level against diabetic rats. Algae with different bioactive compounds have shown its impact in Figure 6.2.

6.2.2 ALGAE AS AN ANTICANCER AGENT

From the last two decades, a dreaded disease (cancer) is severely affecting the life cycle of the world's population. Cancer is gradually increasing the

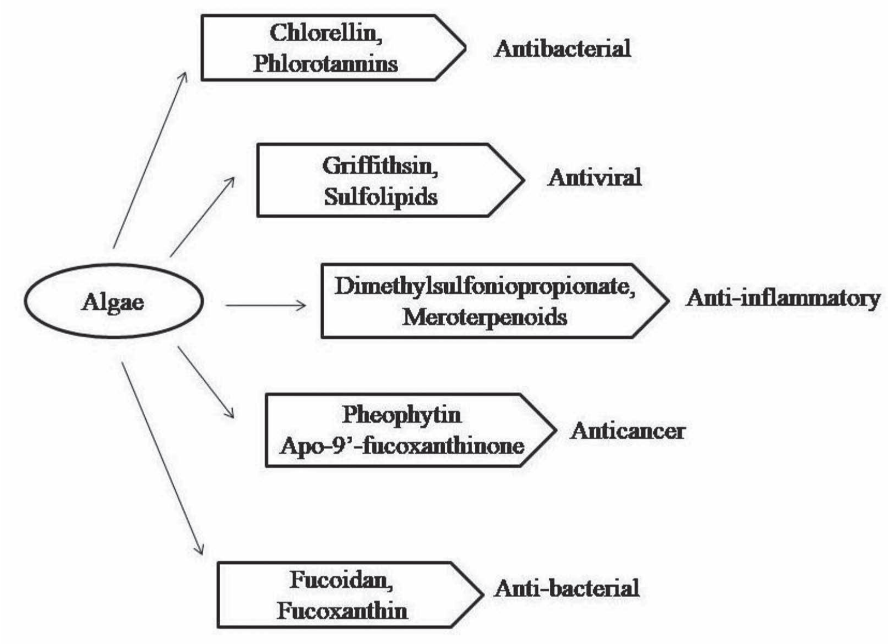

FIGURE 6.2 Role of algal active components in pharmaceutical sector.

mortality rate in various forms such as stomach, breast, colorectal, liver, ovarian, and thyroid cancer. Millions of new patients are diagnosed with cancer in every year due to busy life schedule, improper nutrition, less physical exercise, intake of fast food, intake of pesticides grown food and also due to stress or hypertension. It means that it is spreading gradually in population and destroying defensive mechanism of the population. Various chemically synthesized medicines are used in the treatment of cancer but inversely it is destroying the cancerous cell along with the healthy cell. Hence, discovery toward the use of natural products specially extracted from algae are on boom due to having life saving various effective bioactive compounds.

Polysaccharides or fucoidans bioactive compounds from algae have been attracted in the research field of cancer due to its enormous medicinal properties. Polysaccharides from *Spirulina platensis* has been in attention owing to have scavenging activities on hydroxyl and DPPH radicals and act as immune booster along with improving DNA repair system and inhibiting cancer cell in human beings (Kurd et al., 2015). The anticancer and anti-proliferative effects of *S. platensis* derived tetrapyrrolic bioactive compounds was examined on the cell line of human pancreatic cancer. *S. platensis* has proved (*in vitro* and *in vivo*) that its tetrapyrrolic compound is anti-proliferative and successfully inhibited the growth of pancreatic cancer (Konickova et al., 2014).

Fucoidan is also referred as fucosan, sulfated fucan, and fucan. Fucoidan constitutes a variable percentage of sulfated ester groups, L-fucose, D-xylose, D-galactose, and D-mannose. This active biochemical compound (fucoidan) has been showing as a boon for the research area in various previous studies (Alekseyenko et al., 2007) owing to confirm as an inhibitory response against a variety of cell lines (Teruya et al. 2007). The yield of isolated fucoidan was 4.51 ± 0.24% and also consisted of carbohydrate (38.76 ± 0.26%), sulfate (22.35 ± 0.23%), uronic acid (3.9 ± 1.8%), and protein (4.7 ± 0.43%). The fucoidan of *Sargassum polycystum* contained fucose (46.8%), galactose (14.3%), xylose (13.2%), glucose (11.5%), rhamnose (8.6%), and mannose (5.6%) (Palanisamy et al., 2017). It concluded that fucose and sulfate was the primary bioactive compound of the *Sargassum polycystum* that has shown inhibition against MCF-7 cell line (Palanisamy et al., 2017).

Two polysaccharides, LlF and ScF (fucoidans) were investigated from *Laminaria longipes* and *Saccharina cichorioides* algae respectively. Both the LlF and ScF (fucoidans) were sulfated polysaccharides (32% and 36% respectively). The percentage composition of various fucoidan differs according to brown algal species. The ScF (fucoidan) has an average molecular weight of 237.7 kDa while LlF(fucoidan) has a molecular weight of 254.1 kDa and 838.7 kDa owing to be varied forms. Both the fucoidans were explored for anticancer activity against colorectal cancer HT-29, melanoma SK-MEL-28, and breast cancer MDA-MB-231 cells. These fucoidans executed a noticeable inhibition against the proliferation of colon and melanoma cancer cells (Usoltseva et al., 2019). These compounds have performed comparable assessments regarding radiosensitizing activity on colony formation of cancer cells along with the significant reduction in colony number of breast and colon cancer cells. Water-soluble glutathione oxidoreductase (GR), isolated from *Arthrospira platensis* (*Ap*) was found responsible for cancer cell lines death. The antioxidant enzyme (GR) is a type of scavenger that protects the cell from the harmful effect of oxidative enzymes by reducing glutathione (GSH) from its oxidized form (GSSG). The glutathione oxidoreductase (GR) with short peptide sequence (^{39}GGTCVIR-GCVPKKLM^{53}) from *Arthrospira platensis* named GM15 is investigated. It has reduced oxidative stress by destroying radicals, *i.e.* hydroxyl and superoxide radicals (by *in vitro* cell-free assays). The application of GM15 as scavenger tested in oral carcinoma (KB) cells, and it exhibited potent cytotoxicity against oral carcinoma (KB) cells (Sannasimuthu et al., 2019). Recently, various anticancer active compounds, *i.e.* steroids, resins, amino acids, alkaloids, phenols, tannins, terpenes, saponines, flavones, and proteins were detected in extract of *Spirulina platensis* by GC mass analysis (Fayyad et al., 2019). These compounds have shown a positive impact on MCF7 and L20B human cancer cell lines treatment. Jayshree et al. (2016) expressed in their study that the crude extract concentration (23–46 µg/ml) of *C. vulgaris* and *C. reinhardtii* displayed an excellent reduction in cell viability by 50%. In this study, flavonoids observed as an anticancer agent, and crude extract of *C. vulgaris* constitute high flavonoid content than that of *C. reinhardtii*. Therefore, *C. vulgaris* expressed good anticancer activity against breast cancer cell line in comparison to *C. reinhardtii*.

6.2.3 ALGAE AS AN ANTIVIRAL AGENT

Complicated unique structure and life cycle of viruses have attracted the scientists towards the discovery of antiviral drugs against viral infections. Since, half century has been passed doing research in the field of vaccine for the protection from viral infections. Still, no definite vaccine was produced regarding various viral infections such as human papilloma viruses (HPVs), herpesviruses (HSV-2), respiratory-tract viruses (HSV-1), HIV type 1 (HIV-1) (Li et al., 2012). So, the researchers are focusing in a new area (algal bioactive compounds) for treating the viral infections and it is proved in one study that approximately 9% of pharmaceutical compounds have been extracted from different algae and was successful against viral infections (Jha et al., 2004). Algae can produce enormous active compounds such as vitamins, acetogenins, halogenated compounds, fatty acids, and polysaccharides that can be used directly or indirectly as natural medicines (Goss et al., 2010).

Algae-derived "glycans" known as polysaccharides that plays a significant role as an antiviral agent. These polysaccharides include carrageenan, rhamnan sulfate, galactosyl glycerol, proteoglycans, laminaran agar, fucoidan, alginate, *etc.* Carrageenan (anionic sulfated polysaccharide) has been extracted in large quantities from *Gigartina*, *Eucheuma*, *Hypnea*, and *Chondrus* (red algae). Carrageenans are classified in three forms like *ι*-, *κ*-, *λ*-based on sulfate group's allocation in the chemical structure, and these different forms (ι-, κ-, λ-) act as antiviral agents against the various foreign viruses. Carrageenans work as an inhibitor by prohibiting the binding of the virus to the host cell. In one of the studies, it is predominantly capable of inhibiting the initial stage infection of HPV (*in vitro*) and remarkably able to inhibit sexually transmitted HPV that induces cervical cancer. Some other imported fucoidans (SHAP-2 and SHAP-1) with molecular weights of 5.89×10^5 and 6.55×10^5, respectively, were extracted from *Sargassum henslowianum* and showed excellent antiviral effect against Herpes simplex viruses (HSVs). Both the SHAP-1 and SHAP-2 fucoidans constitute an adequate amount of galactose and fucose residues as main sugar content and a lesser amount of sulfate content. Both SHAP-1 and SHAP-2 were successful for HSV-1 and HSV-2 inhibition in a dose-dependent manner. A sample dose of 10 µg/mL concentration showed influential inhibition of HSV-1 infection (~95%) and HSV-2 infection (~90%) (Sun et al., 2020). Avian Leukosis Virus Subgroup J (ALV-J) is a type of retrovirus (oncogenic) that leads to acute losses to the poultry industry. A sulfated polysaccharide was isolated from *Sargassum fusiforme* and presented an effective result in vitro and exerted potent antiviral (ALV-J) activity *in vivo*. The *in vivo* results proved that the chickens infected with ALV-J (virus) were relieved by the strong antiviral effect of polysaccharides that was extracted from *Sargassum fusiforme* (Sun et al., 2019). Sulfated glucuronorhamnan, a type of polysaccharide that is isolated from *Monostroma nitidum. The* sulfate group in the polysaccharide is positioned at rhamnose sugar. The polysaccharide as a potential agent displayed strong antiviral effects against EV71 (human Enterovirus species) that cause diseases, *i.e.*, hand foot and mouth disease. Sulfated glucurono rhamnan was successful in inhibition of EV71 infection by focusing PI3K/Akt pathway. The introduction of sulfated glucuronorhamnan (100 µg/mL) significantly reduced the expression of EV71 RNA after the post-infection level (Wang et al., 2020). *Nizamuddinia zanardinii* (brown algae), a marine polysaccharide exhibited inhibitory effects against *E. coli*, *P. aeruginosa*, and strong antiviral effect against HSV-2 (Herpes simplex virus-2) infection (Alboofetileh et al., 2019). Sulfated rhamnan, polysaccharide (PML) extracted from *Monostroma latissimum* inhibited EV71 infection by targeting EGFR/PI3K/Akt pathway (Wang et al., 2018) and the viral capsid protein. Thus, it concluded that it might help in the inhibition of EV71 replication and proved as a novel antiviral agent against EV7 infection.

6.2.4 ALGAE AS AN ANTI-INFLAMMATORY AGENT

Inflammation is a part of non-specific protective response of the body to harmful stimuli that include damage to tissues, pathogens, specific disease conditions, and toxic chemicals. The teleological purpose of inflammation is to counteract the masses against the requirements as mentioned above by clearing the dead cells and stimulate the tissue repair mechanisms (Zitvogel et al., 2010). Inflammation is classified into two phases as acute and chronic. The acute phase is associated with the accumulation of fluids, high blood flow, increased vascular permeability, and an increase in the number of leukocytes and inflammatory mediators. In contrast, chronic inflammation is associated with the progression of specific humoral and cellular immune responses (Feghali et al., 1997). Inflammation mediated by a complex system of soluble factors is categorized into several groups based on their sources and chemical compositions; (1) inflammatory lipid metabolites include platelet-activating factor and various types of arachidonic acid derivatives such as eicosanoids, prostaglandins, leukotrienes and lipoxins; (2) plasma protein systems related with kinin, the complement and the clotting/fibrinolysis systems that include thrombin, fibrinopeptides, plasmin, and several other proteins; (3) nitric oxide synthesized in vascular endothelial and macrophages which induce vasodilatation and act as a cytotoxic agent on pathogenic microorganisms and neoplastic cells; (4) pro-inflammatory cytokines such as interleukins, tumor necrosis factor-alpha (TNF-α) and beta (TNF-β), chemokines, colony-stimulating factor 2 and 3 (Larsen et al., 1983; Feghali et al., 1997). Most of these regulatory proteins and other inflammatory mediators are produced in activated cells, such as macrophages, fibroblasts, mast cells neutrophils, eosinophils, monocytes, lymphocytes, and endothelial cells (Martin et al., 2005). The inflammatory response is crucial to counteract infection. Nevertheless, these effects of inflammation, especially chronic conditions could ultimately cause detrimental health issues, including multiple sclerosis, cancer, inflammatory arthritis, atherosclerosis, coronary artery diseases, obesity dermatitis, migraines, interstitial cystitis, irritable bowel syndrome, insulin resistance and an array of other disease conditions (Coussens et al., 2002; Theoharides et al. 2004; Hansson 2005). Thereby anti-inflammatory compounds play a pivotal role in the treatment of inflammatory diseases. Significant advances made in the recent decade are discovering new anti-inflammatory agents (Laufer et al., 2012). Similar to most therapeutic agents, anti-inflammatory drugs also cause complications and severe side effects (Fernando et al. 2016) on health. Therefore, the discovery of novel anti-inflammatory drugs from algae could bring new insight into the field of biomedical research and industry.

Three assays such as albumin denaturation, proteinase, and membrane stabilization are used for the anti-inflammatory activity of the microalgal extract. Protein deformation is a cause of biological activities after denaturation. As in concern with anti-inflammatory activity, the extract that is employed for inhibiting protein denaturation exerted successful inhibition with 83.72% at 500 μg/mL concentration. Tissue damage induced during inflammation can be minimized only by proteinase inhibitors. Furthermore, the extract proved as antiproteinase with effective inhibition of 69.48% at 500 μg/ml concentration. Lysosomal release causes various disorders during inflammation. So membrane stabilization assay was effective in lowering the inflammation by inhibiting the release of lysosomal constituents. The extract showed potent anti-inflammatory activity with 78.84% inhibition at a higher concentration of 500 μg/ml (Ali et al., 2019). A study stated regarding anti-inflammatory agents (algae) that potent polysaccharides (CLGP4) from *Caulerpa lentillifera* (green algae) displayed a robust anti-inflammatory role in LPS-induced HT29 cell by suppressing the mRNA expression of IL-1β and TNF-α. TNF-α (pro-inflammatory cytokine) is generated during inflammation (acute inflammation) and responsible for the activation of other cytokines like IL-1β, IL-6, and IL-8. The TNF-α is found in low concentration in HT29 cell, but the activity of TNF-α is increased significantly by LPS stimulation. Lipopolysaccharide (LPS) is a type of endotoxin and is used as a potent stimulator of inflammation (Sun et al., 2020). IL-8 is a type of cytokines (pro-inflammatory) that causes various inflammatory diseases. In healthy cells, it is expressed in reduced form and induced inflammation signal that proceeds through TNF-α (cytokine) and LPS (bacterial endotoxin). So, inhibition of IL-8 in an activated cell through induction of *Sphaerococcus coronopifolius* (red algae) was a potential and therapeutic strategy for inhibiting inflammation (Salhi et al., 2018). The application of Tuberatolide B (TTB) is only proven as an anticancer activity so far, and now the effect of TTB as an anti-inflammatory is confirmed. Here, the TTB isolated from *Sargassum macrocarpum* (brown algae) arrested the expression of pro-inflammatory cytokines by targeting the suppression of MAPKs and NF-κB signaling pathway in LPS-stimulated RAW264.7 cells and zebrafish model (Kim et al. 2019).

The anti-inflammatory activity of the *Dictyota menstrualis* algae is measured by the quantification of nitrite production in LPS induced RAW 264.7 cells. Nitric oxide (NO) is associated with various physiological processes like inflammation, mitochondrial respiration, neurotransmission, and gastric motility. Nitric oxide (NO) overexpression participates in inflammatory processes by stimulating leukocyte infiltration, and vascular permeability (Do Nascimento Ávila et al., 2019). Song et al. (2019) separated polysaccharides from *Blidingia minima* (BMP) that constitute xylose, rhamnose, and glucose. It exerted an anti-inflammatory effect on intestinal epithelial cells (IPEC-J2 cells) treated with dextran sulfate sodium (DSS) and alleviated colitis problems in mice (DSS-induced). Induction of BMP was successful in repairing the infiltration, colonic dysfunction, colonic morphology problems, and BMP supplementation also inhibited the expression of pro-inflammatory cytokines like AKT, NF-κB, and IκB-α in colonic tissue.

6.2.5 ALGAE AS AN ANTIBACTERIAL AGENT

Antibacterial resistance towards the chemically synthesized drug has emerged a search towards natural compounds to be used as antibacterial against various bacterial infections (gram positive or gram negative) (Vairappan et al., 2001). Many synthetic compounds were used in the treatment of *S. aureus* but last 20 to 30 years' have been worrying decades when *S. aureus* started to be resistant towards penicillin and other antibiotics. The phenomenon of antibacterial resistance has left the world population in danger from the last 20 years. So, search towards bioactive compounds from algal extract has emerged as new area for bacterial infection treatment. These bioactive compounds are phenolic compounds, cyclic polysulfides, terpenoid, fatty acid, polyketides, acryl acid, alkenes, aromatic organic acids, quinones, halogenated furanones ketone, polyacetylenes, alkanes, antifeedant, glycerols, lipids, shikimic acid, ketones, phlorotannins, alcohols, polysaccharides, hydroquinones, alkaline, indole alkaloids, terpenes, peptides, aldehydes, polyketides, and steroid (Saravanakumar et al., 2008; Cabrita et al. 2010)

Antibacterial activity of *Arthrospira platensis* against human pathogenic microbes was highly effective and responsive (Sassi Aydi et al., 2020). Depolymerized fucoidan of *Laminaria japonica* displayed highly potent antibacterial activity against *Escherichia coli* than *Staphylococcus aureus*. Depolymerized fucoidan interacts to the membrane protein of *Escherichia coli* and *Staphylococcus aureus* and finally lead to cell disruption and cell death due to polyanionic property along with the decrease in cell number viability (Liu et al., 2017). A recent study also supports the effect of depolymerized fucoidan (*Sargassum tenerrimum*) as an antibacterial drug (Ashayerizadeh et al., 2019). A therapeutic agent fucoidan fraction-2 from *Sargassum polycystum* was evaluated as a potent antibacterial activity against *Pseudomonas aeruginosa* at 50 μg/ml (Palanisamy et al., 2019). Polysaccharide (Laminaran) extracted from brown seaweed *Cystoseira barbata* laminaran (CBL) significantly showed potent antibacterial activity against pathogen *E. coli*, *S. enterica*, *M. luteus*, *S. typhimurium*, and *K. pneumonia* (Sellimi et al., 2018). *Gracilaria verrucosa* algae performed moderate antibacterial activity against fish pathogenic microbes *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, and as well as weak activity against *Vibrio harveyi* to support fish farming protection (Kurniawati et al., 2016). β-amyrin active compound from *Laurencia microcladia* exhibited intense antibacterial activity against pathogens *Bacillus subtilis* (32 ± 1.5), followed by *Staphylococcus aureus* (27 ± 2.0), *Escherichia coli* (19 ± 0.34), *Salmonella typhi* (18 ± 2.3), and *Pseudomonas aeurginosa* (16 ± 0.55) (Abdel-Raouf et al. 2015). Application of various algae in pharmaceutical sector has been explored in Table 7.1.

TABLE 6.1 Application of Algae in Pharmaceutical Sector

Algae	Application	References
Spirulina maxima	Antidiabetic	Pandey et al. (2011)
Spirulina versicolor	Antidiabetic	Hozayen et al. (2016)
Enteromorpha prolifera	Antidiabetic	Yan et al. (2019)
Sargassum confusum	Antidiabetic	Yang et al. (2019)
L. papillosa	Anticancer	Omar et al. (2018)
Spirulina maxima	Anticancer	Oh et al. (2011)
Laminaria japonica	Antiviral	Cao et al. (2016)
Caulerpa mexicana	Anti-inflammatory	Carneiro et al. (2014)
Red and blue-green algae	Antiviral	Takebe et al. (2013)
Fucus evanescens	Antiviral	Krylova et al. (2020)
Sargassum henslowianum	Antiviral	Sun et al. (2020)
Laminaria japonica	Antibacterial	Liu et al. (2017)

6.3 NUTRACEUTICAL APPROACHES OF ALGAE

6.3.1 ALGAE AS POLYUNSATURATED FATTY ACIDS SOURCE

Polyunsaturated fatty acids (PUFA) are in demand due to having various health benefits, *i.e.* as an integral role in metabolism, essential intrinsic part in all the organelle membrane, and perform a necessary act in improving and maintaining the life of well beings. Its other importance includes to preserve the fluidity of the membrane, signal transduction mediation, and to help in the attachment to the membrane-bound specific enzymes. PUFAs are being exploited for the formation of various active biomolecules, like resolvins, eicosanoids, prostaglandins, and leukotrienes. It is, directly and indirectly, involved in anti-arrhythmic, anti-inflammatory, and anti-aggregatory effects as well as in cardiovascular health improvement. Interestingly, specific PUFAs can enhance memory and eye function in newly born babies and even in adults. Two PUFAs, specifically docosahexaenoic acid (DHA; 22:6 *n*-3) and arachidonic acid (ARA; 20:4 *n*-6) were (Helland et al., 2001) used for infants food. PUFAs can also participate in the alleviation of some chronic bowel disorder, Alzheimer's disease, and some cancers, *etc.* So, PUFAs are on attention owing to have various nutritional values in clinical medicine (Ratledge, 2013). PUFAs are subdivided into two categories, *i.e.* omega-3 fatty acids and omega-6 fatty acids. Omega-3 fatty acids participate in the protection of heart problems by limiting blood clot formation and decreasing blood triglycerides, arrhythmias as well as by improving the arteries' function. The omega-6 fatty acid has an enormous potential role in controlling high blood pressure, allergy, osteoporosis, breast cancer, diabetic neuropathy, multiple sclerosis along with improving human health development such as, regulating metabolism, skin and hair growth, maintaining bone health (Udayan et al. 2017). Some of the algal oils from *Cryptheconidium cohnii*, *Ulkenia* sp., and *Schizochytrium* sp. were found rich in long-chain omega-3 fatty acids and was approved for human intake as passing through safety assessment procedures and can be used as food fortification and key ingredients in food supplements (Ansorena et al., 2013). Algae *Schizochytrium*, *Thraustochytrium*, *Nannochloropsis*, and *Phaeodactylum* were extensively studied for high DHA contents while *Schizochytrium* as well as *Thraustochytrium* supported the previous study (Adarme-Vega et al., 2014) for reporting high DHA content (30–40% of total fatty acids). Food Standards Australia New Zealand (FSANZ) monitors the application of new food products, i.e. algae. FSANZ has permitted the use of algae *Schizochytrium* as it is rich in omega-3 long-chain polyunsaturated fatty acid (DHA) according to the Food Standard Code (Garcia et al. 2017). A new green algae *Monoraphidium* sp. CCALA 1094 has produced PFUs more than 60% of the total fatty acids (Rezanka et al., 2017) and was able to provide value-added products at low temperature at a large scale. *Phaeodactylum tricornutum* improved the production of DHA and EPA content using birch and spruce hydrolysates (Patel et al., 2019). The output of PUFAs by fish is unsustainable to fulfill the need of large population. So, the attention towards PUFAs production from algae has been targeted for human health improvement. Role of algae with various biochemical components in nutraceutical sector has been shown in Figure 6.3.

6.3.2 ALGAE AS CAROTENOIDS SOURCE

Carotenoids are included in the category of natural pigments which is widely dispersed in nature and extensively observed in microorganisms, plants, and animals. Humans cannot synthesize carotenoids themselves, and they are dependent on plants and algae for carotenoids (pigments) consumptions. Chemically, carotenoids are characterized into two groups (carotenoid hydrocarbons or carotene carotenoids and oxygenated carotenoids or xanthophyll carotenoids). Some carotenoids such as β-carotene and lycopene are examples of carotene carotenoids. While lutein, astaxanthin, canthaxanthin, violaxanthin, zeaxanthin, capsorubin are examples of xanthophyll carotenoids (Schweiggert et al., 2017). In animals and humans, these bioactive compounds displayed a vital precursor for vitamin A (pro-vitamin A activity), and retinoid compounds played a significant role in morphogenesis and embryonic development. Carotenoids have importance in the food and feed industry being natural colorants and nutraceutical properties, and in addition to these, it also has anti-inflammatory as well as anticancer effects.

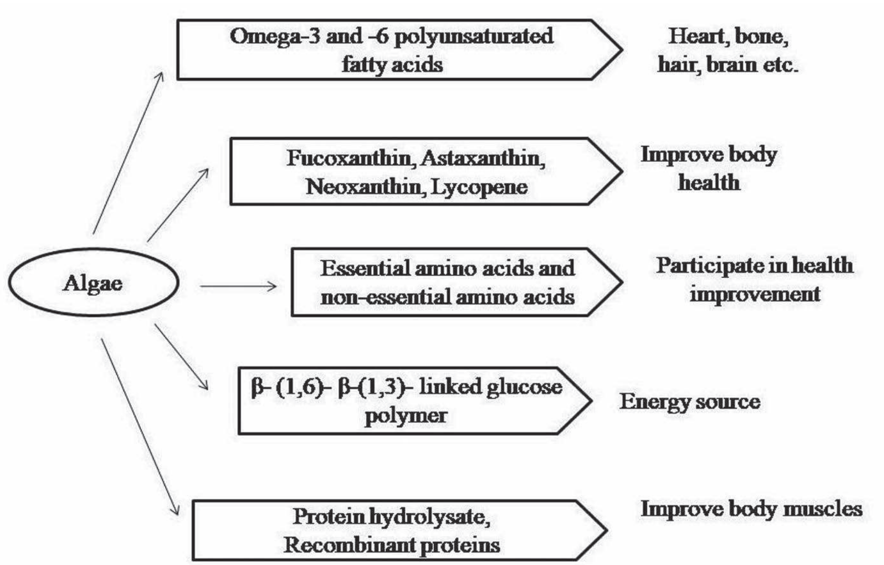

FIGURE 6.3 Role of algal active components in nutraceutical sector.

Carotenoids (lutein and β-carotene) are essential for survival as they are a vital ingredient of cellular apparatus. Inadequate lutein and β-carotene function are performed by the incorporation of carotenoids, e.g., adonixanthin astaxanthin, and canthaxanthin. Several algal strains are being noted for their valuable carotenoid production such as lutein from *Chlorella protothecoides*, astaxanthin from *Chlorella zofingiensis*, β-carotene from *Dunaliella salina*. Some of the explored carotenoids in terms of food additive and health prospective so far are β-carotene, astaxanthin, canthaxanthin, lycopene, fucoxanthin, lutein, violaxanthin, zeaxanthin (Liu et al., 2014).

Importantly, β-carotene involves the incorporation of isopentanyl pyrophosphate for its synthesis. Isopentanyl pyrophosphate (IPP), a joint precursor compound for the formation of all isoprenoids from G3P. Acetyl CoA participates in IPP synthesis. IPP with mevalonic acid and dimethyl allyl pyrophosphate (DMAPP) synthesize geranylgeranyl pyrophosphate (GGPP). Two GGPP molecules react to each other to form phytoene which go through under a series of various biosynthesis reactions to form lycopene that participates in β-carotene synthesis (Singh et al., 2019). *Eustigmatos* cf. *polyphem* was explored for accumulating a noticeable amount of *β*-carotene content, reaching 6% of dry biomass weight and yield 450 mg/L (Li et al., 2012). *Spirulina* algae (SA) are considered as an excellent source of carotenoids. So, 40 mg SA/Kg for young chicken flocks and 160 mg SA/Kg for elder has been included as the diet and the result was promising for improving performance in chick body weight, fertility, and hatchability (El Iraqi, 2018). Inhibition in autophagic activity of *Chlamydomonas reinhardtii* CC-124 mediated overproduction of β-carotene content (23.75 mg/g DCW). In *Chlamydomonas reinhardtii* CC-124, ATG1 kinase regulates the initial step in autophagy induction, and ATG8 is a fundamental key for the downstream formation of autophagosome membranes. These two genes TG1 and ATG8 were silenced with artificial microRNA and finally reduced the mRNA expression of TG1 (84.4%), and ATG8 (74.3%) in *Chlamydomonas reinhardtii* CC-124 (Tran et al., 2019).

6.3.3 ALGAE AS A PROTEIN SOURCE

Algae used as a food and a high protein source for centuries owing to have a variety of nutritious ingredients. Japan was the first in the world to produce *Chlorella* sp. for consumption at a commercial level in the 1960s (Vigani et al., 2015). AlgaVia®, a protein-rich algae (gluten and allergen-free), is being sold by USA company (Khanra et al., 2018).

Protein yield is maximized in microalgae *Chlamydomonas reinhardtii* by optimizing some of the key factors such as harvesting time and pre-treatment. Autolysin was effective in biomass treatment for the separation of proteins (Sierra et al., 2017). Protein supplement in the form of protein hydrolysates is given to the individuals who suffer from the problem of cystic fibrosis and digestibility. Protein hydrolysates can also be used as protein supplements in drinks and food. So, large scale production of *S. obliquus* for protein hydrolysates has been achieved by enzymatic hydrolysis using papain, pepsin, and trypsin. For fulfilling the protein intake of the large population, it should be dealing with alternative sources such as microalgal hydrolysates production at a large scale as it is an emerging and promising area in various studies (Afify et al., 2018). The need for protein hydrolysates as a nutraceutical bioactive compound is a burgeoning area in the improvement of health-related value-added products as they have more bioactivities in comparison to their parent (Valdez-Flores et al., 2016). Besides, various studies have been directed to analyze biologically active peptides available in protein hydrolysates (Cian et al., 2013). Nutrient-rich defatted microalgae meal prepared from *Haematococcus pluvialis* has shown an influential impact on the growth of shrimp by replacing of 50% fishmeal protein. As concern over the conclusion that *Haematococcus pluvialis* is proved as an alternative protein source for other crustacean's growth and development as proved in the case of shrimp (Ju et al., 2012).

Recombinant proteins also played a vital role in the nutritional, industrial, and medical fields. The green photosynthetic algae are easy to grow, scalable, safe, and genetically easy to modify using the transformation method. Hence, the algae have been targeted for recombinant protein production to meet the protein requirement of humans as well as the animal's population. Among the different genetically transformable algae, *Chlamydomonas reinhardtii* as a host is extensively used for recombinant protein expression. Algae from the genera of *Chlorella* and *Dunaliella* also presented the potential for protein production like antibodies, hormones, subunit vaccines, and colostral protein for gastrointestinal health (Rasala et al., 2015).

Red algae explored its total protein and reported 32.3% in *Ceramium rubrum* var. *barbatum* G. Feldmann-Mazoyer, 19.5% in *Phyllophora crispa* (Hudson) P.S. Dixon, 36.72% in *Batrachospermum gelatinosum* (Linnaeus) De Candolle, 27.84% in *Lemanea fluviatilis* (Linnaeus) C. Agardh (Akgul et al., 2015). Red seaweeds have been reported to have a significant level of protein in comparison to other conventional protein-rich sources. Protein, peptides, and amino acids derived from red seaweed have an influential approach in the nutraceutical field and should be included as a protein source in human health improvement (Pangestuti et al. 2015).

6.3.4 ALGAE AS AN AMINO ACID SOURCE

Amino acids are the essential components of all proteins. The quality of protein is based on the existence of, especially essential amino acids. The World Health Organization (WHO), the United Nations University (UNU), and the Food and Agriculture Organization (FAO) have been engaged for the fulfillment of nutritional and energy requirements of the large population including the amino acid demand. The study regarding amino acid demand in humans has been consistently reviewed since the 1950s based on the previous research FAO/WHO report (1973); the requirement of essential amino acid for humans was reported by WHO/FAO/UNU (1985). The distribution of total amino acids profile has been analyzed in Chlorophyta, Cyanophyceae, Phaeophyta, and Rhodophyta group. Red seaweed *P. palmata* and freshwater blue-green alga *S. platensis* displayed the best amino acid profiles. Products from *Spirulina* genus showed the highest participation of all EAAs (essential amino acids) in RDI (Misurcova et al., 2014). Taurine is a type of amino acid that was found high in some algae such as *Porphyra* spp. (1·22 mg/g DM), red algal species: *Mazaella* spp. (4·11 mg/g DM), and *Chondracanthus* spp. (6·28 mg/g DM) (McCusker et al., 2014). 32 amino acids were identified, calculated and determined in algae such as *Ceramium rubrum* var. *barbatum* G. Feldmann-Mazoyer, *Phyllophora crispa* (Hudson) P.S. Dixon, *Batrachospermum gelatinosum* (Linnaeus) De Candolle, *Lemanea fluviatilis* (Linnaeus) C. Agardh. Research in all these respective algae published the report regarding the existence of nine free non-essential amino acids, eight free essential amino acids, and the other fifteen amino acids. Among different content of the amino acids, aspartic acid was the dominant constituent in all the samples and the percentage of amino acids varies algae to algae (Akgul et al., 2015). Seaweeds being algae are focused on a protein source and exerted that its protein is a source of all amino acids, especially aspartic acid, glycine, arginine, alanine, glutamic acid, and proline. The amount of protein in seaweeds ranges from 5% to 47% of its dry mass, and its value is based mainly on species types. The algal protein profile shows close similarity to the egg protein profile and essential amino acids (EAAs) perform approximately half of total amino acids while red, brown, and green seaweeds contain non-EAAs in equal amounts. Red seaweed is highly on demand owing to have a good source of protein (47%). The problem of protein malnutrition forces keen research to identify a novel, cheap and sustainable source for fulfilling the protein demand. Algae owing to have a high content of nitrogen compound might help to produce a large quantity of protein at massive scale. Algae are being focused on large scale production due to having valuable ingredients with high nutritional quality (Cerna et al., 2011). The brown algae *Saccharina latissima*, *Laminaria digitata*, and *Ascophyllum nodosum* were studied for total nitrogen content and amino acid composition. These algae have been targeted for functional foods production, drug production as well as also for mono- and polysaccharides production. The basic content of protein exhibits a variety of properties like antihypertonic, radioprotector, immunostimulating, and antioxidant. The *Laminaria digitata* and *Saccharina latissima* algae are regarded as raw material for producing amino acid. Still, the processing for obtaining these valuable bioactive compounds from algae is inadequately studied (Bogolitsyn et al., 2014). The amino acid profiling in biomass of freshwater algae *Scenedesmus quadricauda*, *Chlorella* sp., *Chlorella kessleri* and blue-green algae *Spirulina platensis* (Samek et al. 2013) has been evaluated by various methods. Chicken rotti fortified with seaweed protein exerted the highest content of total amino acid and presented the best proportion of essential and non-essential amino acids (EAAs and NEEAs) (Parniakov et al., 2018).

6.3.5 ALGAE AS CARBOHYDRATE SOURCE

Polysaccharides as an energy source are important for all types of living cells' function. The total content of polysaccharides in algae varies species to species, and also varies based on optimized factors of the growth medium. It can vary such as 4 to 76% in *Ascophyllum*, *Porphyra*, *Palmaria* and 65% in *Ulva* (Holdt et al., 2011).

Furthermore, polysaccharides are seen abundantly in the form of cellulose and starch. Cellulose is found in the cell wall while starch is stored in the form of reservoir. These polysaccharides turn to monosaccharide or simple sugars after the process of saccharification. Polysaccharides are identified in various forms in algae such as fucoidan, alginate, laminarin, carrageenan *etc.* Fucoidans are the polysaccharides that are found in the cell wall of brown macroalgae. These fucoidans protect the algae from the harsh environmental conditions. These fucoidans are also called fucose that contains sulfated polysaccharides which is formed of (1→3)-linked α-l-fucopyranosyl, and (1→4)-linked α-l-fucopyranosyl residues (Garcia-Vaquero et al., 2017).

Alginates contain α-L-guluronic acid (G) units linked by 1–4 glycosidic bonds and β-D-mannuronic acid (M). Alginates can be extracted from variety of algae with effective yield (45 to 55%) such as *Macrocystis pyrifera* and *Durvillaea potatorum* (Lorbeer et al., 2017). Laminarins are composed of

1,3-linked β-D-glucose monosaccharides and it is used as energy storage for new cell growth. It is found in highest yield in *Laminaria* sp. (vary up to 32%) while also observed in *Undaria* sp., *Ascophyllum* (Holdt et al 2011). Carrageenans (CRGs) is composed of α-1,3-linked D-galactosyl residues and β-1,4-linked D-galactosyl residues. It is observed 71% in *Chondrus crispus* and 88% in *Kappaphycus* sp. (Holdt et al., 2011).

The phycocolloid (polysaccharides) from macroalgae such as agar is widely used in food industry as well as in animal industry as emulsifiers, stabilizers and thickeners (Hayes et al., 2015). A recent research has analyzed and evaluated the carbohydrate composition in *Nannochloropsis*, *Rhodomonas* and flagellated *Isochrysis*. The compositional analysis of carbohydrate in *Rhodomonas marina* revealed maximum content of monosaccharides (Fernandes et al., 2017). Application of different algae in nutraceutical sector has been explored in Table 7.2.

TABLE 6.2 Application of Algal Bioactive Components in Nutraceutical Sector

Algae	Bioactive Compounds	References
Amphidinium carterae, *Peridinium aciculiferum*, *Cystodinium* sp.	Long-chain PFUs, phosphatidylcholine, triacylglycerols, and phosphatidic acid	Rezanka et al. (2017)
C. muelleri	DHA, linoleic acid, EPA, and other long-chain PUFAs	De Jesus-Campos et al. (2020)
Anabaena doliolum	Carotenoid	Dash et al. (2013)
Dunaliella salina	β-carotene	Zhu et al. (2018)
Chlamydomonas reinhardtii, *Chlorella vulgaris*	Carotenoids	Varaprasad et al. (2019)
Gracilaria sp.	β-carotene	Kavalappa et al. (2019)
Dunaliella salina	β-carotene	Rammuni et al. (2019)
Nannochloropsis sp., *Tetraselmis suecica*, and *Dunaliella tertiolecta*	High protein content	Norzagaray-Valenzuela et al. (2017)
Porphyra columbina	Protein source	Cian et al. (2014)
Kappaphycus alvarezii and *Ulva lactuca*	Protein source	Abirami et al. (2011)
Spirulina sp.	Aspartic acids and glutamic acids	Dewi et al. (2016)
U. lactuca, *G. salicornia*	Amino acids	Tabarsa et al. (2012)

Note: DHA: Docosahexaenoic acid; EPA: Exopolysaccharides; PUFAs: Polyunsaturated fatty acids.

6.4 CONCLUSION

In current decades, algae are known for their influence in food, bio-fuel, and medicine field. The exploration of algae is on-demand in the area of technology and scientific research. Algae derived pharmaceutical and nutraceutical value-added products possess vast bioactive compounds that can play a keen role in declining malnutrition and other disease-related issues in developing countries. The affluence of nutritional properties like protein, pigments, amino acids, enzymes, PFUs, carbohydrates, steroids, and medicinal properties like antidiabetic, antibacterial, antiviral, anticancer, and anti-inflammatory in algae can promote the commercialization of algal-based pharmaceutical as well as nutraceutical industries at large scale. The main hurdle in marketing is due to the high cost of algal harvesting, costly extraction process of valuable products from algae, and low awareness about its food and health-related benefits. So, by eradicating all these obstacles, the application of algal-based rich, valuable bio-products should be enhanced and commercialized in all fields.

KEYWORDS

- **algae**
- **antibacterial agent**
- **anticancer agent**
- **antidiabetic**
- **anti-inflammatory**
- **antiviral agent**
- **bioactive compounds**
- **nutraceutical product**
- **pharmaceutical product**
- **polyunsaturated fatty acids**

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PART IV
INDUSTRIAL APPLICATIONS

CHAPTER 7

NUTRACEUTICAL POTENTIAL OF MARINE MACROALGAE

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ABSTRACT

Macroalgae or seaweeds contribute a significant role in the marine ecosystem. Seaweeds generally have a high growth rate, CO_2 sequester potential, and biomass composition and also do not demand freshwater, arable land, fertilizers for growth which makes them an attractive alternate bioresource for food, pharmaceuticals, nutraceuticals, cosmeceuticals, fertilizer, biochemicals, and biofuel applications. In many countries, seaweed has been used as food for decades, because of its health and nutritional benefits. Seaweeds contain several bioactive components like macronutrients, micronutrients, proteins, vitamins, sulfated polysaccharides, and antioxidants with proven nutraceutical and biological properties which are found useful against diseases such as type 2 diabetes, cancer, obesity, and other disorders. Additionally, these environmentally friendly natural products derived from macroalgae have also been recognized for immune-stimulating and anti-microbial properties. In recent years, food industries have begun to use seaweed components to

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prevent food contamination and thus ensure food safety. Seaweeds have been thoroughly investigated for nutraceutical applications and efforts are still ongoing to derive new products with a variety of health benefits or as a natural remedy for therapeutic application. This chapter provides detailed information on seaweeds and their potential applications, bioactive components of seaweeds with nutritional potentials, and future prospective.

7.1 INTRODUCTION

The marine environment is a natural trove with numerous aquatic species having precious features. Seaweeds, one of the major bioresource of the marine ecosystem, are eukaryotic, multi-cellular, and photosynthetic algae which are mainly categorized as brown (Phaeophyta), red (Rhodophyta), green (Chlorophyta) algae (Jiang et al., 2016; Shannon and Abu-Ghannam, 2019). Around 620 species of seaweed are present in India with a potential of 77,000 tons (wet weight) per annum have been reported. The red seaweeds contribute 27.0%, brown seaweeds 0.2%, and others contribute 72.8% (Pal et al., 2014). Global seaweed production has been surpassed tripled during the period of 2000 (10.6 million tons) to 2018 (32.4 million tons) (FAO, 2020). The Asian country, China has a lion's share in the production of seaweed followed by other countries like Indonesia, Japan, Korea, Malaysia, and the Philippines (FAO, 2018).

Seaweeds are having many advantages over other photosynthetic species such as terrestrial plants. Seaweed exhibit 6–40 times more biomass production capacity as compared to terrestrial plants (Ditchburn and Carballeira, 2019). The seaweed biomass production or cultivation does not require freshwater, arable land, and fertilizers and could also lower eutrophication (Fei, 2004). It also aids in the prevention of acidification by sequestering CO_2 (carbon dioxide) (Zacharia et al., 2015) and surpasses the terrestrial plants in CO_2 sequestration (Tsai et al., 2017). The world has a coastline of 11.63 million kilometers which provides tons of natural seaweed biomass.

Seaweeds are most predominantly used for gelling, and thickening purposes as it is a source of alginate, carrageenan, and agar (Qin, 2018). Apart from this application seaweed could be utilized for many purposes which include as a food source for humans (Wendin and Undeland, 2020), feed for the animal (Morais et al., 2020), fertilizer (Nedumaran, 2017), cosmetics (Nurjanah et al., 2016), pharmaceuticals (Othman et al., 2018), nutraceuticals (Ganesan et al., 2019), biofuels (Ashokkumar et al., 2017), environmental protection (Sadhukhan et al., 2019), bioplastic production (Sudhakar et al., 2020).

Several Asian countries, especially East Asian countries such as Japan use seaweed as an essential component of their food. More than a hundred seaweed species are used as food in Japan which includes *Laminaria japonica*, also called as kombu is a liked snack in the pickled and dried form, *Porphyra*, and *Pyropia* species (*Porphyra tenera*) known as nori used as a sushi wrap, *Undaria pinnatifida* known as wakame used in soups, and salads. Other seaweed such as *Ulva lactuca* or *Ulva pertusa* known as sea lettuce are used as vegetables (Fleurence et al., 2018; FAO, 2020).

Edible seaweeds are a treasure of many beneficial components for human health. These components include proteins, *Porphyra tenera* or *Palmaria palmata* contains 10–26% protein of its dry weight with most of the amino acids (essential and non-essential) also have more protein percent compared to pulses like soya bean. Other green seaweeds such as *Ulva* and *Enteromorpha* have protein content close to that of common vegetables (Galland-Irmouli et al., 1999; Fleurence et al., 2018). It also contains dietary fibers (Praveen et al., 2019), polyphenols (Gómez-Guzmán et al., 2018), fucoxanthin (Rajauria et al., 2017), macro, and micronutrients (Fe (iron), I (iodine), Ca (calcium), Mg (magnesium), Na (sodium), K (potassium), Mn (manganese), Zn (zinc), Cu (copper)) (Banu and Mishra, 2018; Ganesan et al., 2020), vitamins (mainly vitamin A, C, and B12) (Škrovánková, 2011), polyunsaturated fatty acids (PUFAs) (Kumari et al., 2010), and many other bioactive compounds.

Owing to the presence of bioactive components, seaweeds possess properties such as antioxidant (Ismail and Hong, 2002; Ganesan et al., 2008), antimicrobial, anti-inflammatory (Boonchum et al., 2011), anti-cancer (Gutiérrez-Rodríguez et al., 2018), anti-diabetic (Chin et al., 2015), antihypertensive, and anti-obesity compounds (Seca and Pinto, 2018). Seaweed holds many health benefits along with therapeutic potential against diseases, mainly type 2 diabetes. Flavonoid-rich fraction of the aqueous-ethanol extract of *Enteromorpha prolifera* was found to be effective against type 2 diabetes (Yan et al., 2019), cardiovascular-related disorders (Gómez-Guzmán et al., 2018), metabolic syndrome (Rico et al., 2018), and many more health conditions.

Seaweeds possess many bioactive components, including vitamins, proteins, macro, and microelements, but the unrestricted consumption should be screened as it leads to the consumption of salts, iodine, and heavy metals more than the required quantity. Though the concentration of heavy metals in edible seaweeds is below the toxic level, its excessive consumption could lead to the bioaccumulation of arsenic-like heavy metals. These scenarios may lead to detrimental health effects. Hence, though seaweed is beneficial, we should not consume it beyond the limit (Cherry et al., 2019a). To resolve this complication the required bioactive components from seaweed could be extracted to utilize for health benefits, the term for this is called nutraceuticals. The term "nutraceutical" is a combination of "nutrient," and "pharmaceutical" which means the component of the food with specific health benefits, and potential to reduce disease conditions (Sasi, 2017).

Seaweed nutraceuticals could be the essential future health care component when precise components of the seaweed will be studied for specific health benefits or therapeutic purposes. This chapter enlightens about the importance, applications, and different components of the seaweeds along with the existing seaweed nutraceuticals available in the market.

7.2 POTENTIAL APPLICATIONS OF SEAWEEDS

Seaweeds are abundant sources of polysaccharides (20–76% of dry weight), vitamins, proteins (15–40% of dry weight), mineral ions (36% of dry weight), lipids, and trace elements (Kumar and Brown, 2013). The compounds existing in seaweeds along with the metabolites produced protect them from environmental damage and have the vast preponderance of applications in nutraceutical (functional foods), pharmaceutical (therapeutics), cosmeceutical (cosmetics), agricultural, and fuel industry (Cotas et al., 2020). It is ecologically and economically viable since ancient times to the future contributing significant benefits to mankind.

7.2.1 FOOD POTENTIAL OF SEAWEEDS

Consumption of seaweeds in Japan and China can be dated back to the 4th and 6th centuries, respectively. The demand for functional food is expanding worldwide with China, Japan, and the Republic of Korea being the largest primordial consumers of seaweeds (Kilinç et al., 2013). Species of seaweeds like *Porphyra*, *Monostroma*, and *Ulva* are directly consumed in several Asian countries. High nutritional properties and low-fat content make seaweed desirable functional food for human consumption. Edible seaweeds include wakame (*Undaria pinnatifida*), sea spaghetti (*Himanthalia elongata*), sweet kombu (*Laminaria saccharina*), nori (*Porphyra umbilicalis*), and hiziki (*Hizikia fusiforme*) which are most commonly consumed (Baweja et al., 2016). Seaweeds are also used in salads, soups, and side dishes. Nori is used for preparing nori sheets used in sushi rolls or crispy snacks, and also in desserts like Spanish nougats. Besides having the anticancer property, pigments like carotenoids are used in food industries as color enhancers (Leandro et al., 2020).

Furthermore, seaweed phycocolloids like carrageenan (κ, λ, ι) are utilized as thickener, stabilizer, and emulsifier to prepare yogurts, flans, jellies, ice creams, and hams. Agar extracts of *Gracilaria* sp., *Gelidium* sp., and *Pterocladiella* sp. are used in food products as thickening agents (Leandro et al., 2020). Together with the gelling property, alginate also can act as a stabilizer in yogurt, cream, cheese, ice cream, a thickener, and an emulsifier in sauces, dressings, and jams. Among the amino acids (valine, lysine, leucine, etc.) owned by the seaweeds, aspartic, and glutamic acid exists in higher concentrations making macroalgae an exciting potential source of food (Baweja et al., 2016). Vitamins generally required for the body are present in *Porphyra umbilicalis*, *Gracilaria changii*, *Codium fragile*, and *Gracilaria chilensis* seaweeds that can mitigate the consequences of several other diseases. Likewise, the mineral requirement of an adult human can also be fulfilled by seaweeds (Ganesan et al., 2019).

7.2.2 NUTRACEUTICAL POTENTIAL OF SEAWEEDS

Various lifestyle diseases in this modern world have attracted attention to natural bioactive compounds with potential health benefits. Seaweeds are an excellent source of healthy compounds such as omega-3 fatty acids, polysaccharides, essential amino acids, vitamins, and minerals with low caloric content that are globally recognized for their nutritive value. Edible seaweeds and their compounds have beneficial impacts on several maladies. Beneficial bioactive compounds obtained from seaweeds include PUFAs, polysaccharides, proteins, enzymes, pigments, phenolics, and others. Iodine in seaweeds (*Laminaria japonica*, *Gracilaria confervoides*, *Sargassum kjellmanianum*, *Codium fragile*, *Monostroma nitidum*, *Ulva pertusa*, and *Dictyopteris divaricata*) can curb thyroid goiter (Couteau and Coiffard, 2016). Non-digestible seaweed polysaccharides like agar, carrageenan, and alginate are considered as dietary fibers because they bind to toxic compounds and obstruct their movement within an organism. Besides, species of *Laminaria*, *Porphyra*, and *Ulva* produce storage polysaccharides which can also be used as dietary fibers. Soluble fibers, on the other hand, lower blood cholesterol, and glucose levels (Baweja et al., 2016). *Ecklonia stolonifera* exhibits alpha-glucosidase inhibitory, and anti-hyperglycemic activity, reducing the absorption of glucose from the gut (Mohapatra et al., 2013). Phycobiliproteins of *Porphyridium cruentum* and *Palmaria palmata* show antioxidant property which could be beneficial in treating cardiovascular and neurodegenerative diseases by neutralizing the detrimental effects of free radicals in body cells, reducing oxidative stress. The existence of alpha and gamma tocopherols in seaweeds facilitates the production of nitric oxide and alleviates the activity of nitric oxide synthetase, contributing towards averting cardiovascular diseases (Baweja et al., 2016). Antioxidant activity of the lipid content (omega-3 and omega-6 fatty acids) of seaweeds (*Ulva* sp., *Acanthophora* sp. and *Gracilaria* sp.) helps in averting several cardiovascular diseases (Ganesan et al., 2019). Nevertheless, a recent study on human intestinal cell line (Caco-2) showed a positive impact of *Gracilaria coronopifolia* symbiotic prepared by using *G. coronopifolia* (red algae) as a source (prebiotics) with probiotics (*Lactobacillus* sp. and *Bifidobacterium* sp.) to improve intestinal health (Li et al., 2019).

7.2.3 PHARMACEUTICAL POTENTIAL OF SEAWEEDS

Seaweeds have been known for their medicinal properties for centuries but are recently been commercially exploited for pharmacologically active compounds to prohibit several diseases. Several studies have exhibited antimicrobial and cytotoxic properties of bioactive compounds obtained from seaweeds (Gomez-Zavaglia et al., 2019). Seaweeds confront huge infectious and surface-fouling bacteria in oceans making them more resistant. Hence, the bioactive compounds produced by them like phlorotannins, polysaccharides, and peptides can be used to develop effective antibiotics (Shannon and Abu-Ghannam, 2016). Certain potential pharmacological effects like antiviral, antitumor, anti-inflammatory, and other immunogenic effects are also observed in the bioactive compounds. For example, methanolic extracts of *Cystoseira barbata*, *Dictyota dichotoma*, *Corallina officinalis*, *Cladostephus spongiosus*, *Ulva rigida* and *Halopteris filicina* were found effective against several Gram-positive (*Micrococcus luteus*, *Enterococcus faecalis*, *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Enterobacter aerogenes*) bacteria (Taskin et al., 2007; Leandro et al., 2020). Among polysaccharides, alginate with anticoagulant and antitumor activity are used in drug formulations, and also as excipients. Similarly, agar is also used as an excipient in tablets and capsules (Leandro et al., 2020). Fucoidan, a type of sulfated polysaccharide, restricts the binding of viral particles to the host cells, thereby acting as a potent antiviral agent for Respiratory Syncytial Virus (RSV), Herpes Simplex Virus (HSV), and Human Immunodeficiency Virus (HIV). Besides, fucoidan also shows anti-inflammatory, anticoagulant, and antithrombotic activity (Mohapatra et al., 2013). Fucoxanthine procured from macroalgae like *Laminaria japonica* has demonstrated against melanogenesis and tyrosinase activity (Thomas and Kim, 2013). Extracts of phlorotannins from *Ascophyllum nodosum* and *Ecklonia cava* have reported antidiabetic and antioxidant properties (Leandro et al., 2020). Mannitol with its anti-oxidant property can also be used in diabetic foods and tablets due to its low affinity to water with a relative sweetness of 40–50% (Baweja et al., 2016).

7.2.4 COSMECEUTICAL POTENTIAL OF SEAWEEDS

Cosmeceutical applications utilizing macroalgae-derived bioactive compounds are in the growing demand for their beneficial effect on the skin. Various active ingredients such as vitamins, proteins, enzymes, antioxidants, and other phytochemicals are employed for formulating cosmeceutical products. The rationales behind the usage of seaweeds comprise skin cell regeneration capability, degradation of free radicals, rehydration, oxygenation, and detoxification of skin cells, and natural cleansing property. Phycolloids are used as additives in cosmetics for retaining healthy skin, while fucoidan and polyphenols have good antioxidant and anti-aging properties. Abundant cosmeceutical products such as body wraps, soaps, shampoos, creams are likewise prepared and commercialized due to their effective outcomes on the skin. Seaweeds like *Ulva lactuca*, *Ascophyllum nodosum*, *Laminaria* sp., *Alaria esculenta*, and *Chondrus crispus* are mostly found in cosmetics (Baweja et al., 2016). Terpenoids have an excellent photoprotective mechanism, while carotenoids act as ultraviolet (UV) radiation filters with strong antioxidant attributes. Unsaturated fatty acids can filter UV, rejuvenate the skin, and prevent wrinkles. Face masks and body washes are formulated using alginates for hydrating and repairing the skin and are moreover used as a gelling agent and stabilizer. Apart from alginates, carrageenans can also be used as a gelling agent with an additional thickening property. Similarly, agar can be used as an emulsifier as well as a gelling agent (Agatonovic-Kustrin and Morton, 2013). Furthermore, seaweed extracts are used in the Spa and Thalasso products as wraps, pastes, and detox baths (Mohapatra et al., 2013). Seaweed compounds are incorporated into cosmetics for optimizing their properties, stabilizing, and preserving the products (Gomez-Zavaglia et al., 2019). Products like CODIAVELANE® prepared from *Codium tomentosum* and ASPAŔAGE™ prepared from *Asparagopsis armata* are marketed as cosmeceutical products (Leandro et al., 2020).

7.2.5 AGRICULTURAL POTENTIAL OF SEAWEEDS

The rising demand for the population for food has intensified the rate of production of agricultural products. Seaweeds have been used chronologically as a soil amendment to enrich crop productivity and reclamation of alkaline soil (Nabti et al., 2017). The components of seaweeds include macronutrients (Ca, P, Mg, and K), micronutrients (Fe, Zn, Cu, Mn, B, Co, and Mo), vitamins, amino acids, and growth regulators (cytokinins, auxins, and gibberellins) (Kumar and Sahoo, 2011; Baweja et al., 2016). Seaweed extracts utilized as biofertilizers are prepared in three forms: liquid, powder, and manure form, and are used either by blending in soil or as foliar sprays. Brown seaweeds, the most commonly preferred genera for the use of biofertilizers, include *Ascophyllum nodosum*, *Durvillaea potatorum*, *Ecklonia maxima*, *Laminaria* sp., *Macrocystis pyrifera*, *Sargassum* sp., and *Fucus* sp. (Baweja et al., 2016). A study conveyed an increase in plant biomass due to the existence of zinc, derived from the micronutrients of seaweeds (Nabti et al., 2017). Seaweed biofertilizers can be an alternative to chemical fertilizers as they are easy to handle, have an extended shelf life, have good water-holding potential, contain more organic manure, and are inexpensive. Apart from functioning as soil conditioners or enhancers, seaweed biofertilizers further stimulate plant growth and vigor, improve nutrient uptake, increase lateral root formation, promotes the growth of beneficial microbes, and enhance plant defense mechanism against pests and diseases. It also elicits a tolerance mechanism against various environmental stresses like heat tolerance, drought tolerance, salt tolerance (Baweja et al., 2016). Biofertilizers derived from seaweeds are environment-friendly, biodegradable, and non-toxic.

7.2.6 OTHER MISCELLANEOUS APPLICATIONS

Seaweeds contain a massive amount of carbohydrates to be used as a source of bioethanol with brown seaweeds being the principal feedstock for bioethanol production. The cell wall of seaweeds is comprised of sulfated polysaccharides (alginate, agar, carrageenan, ulvan, porphyrin, etc.), mannitol, laminarin, fucoidan, starch, cellulose, and hemicelluloses which are required for the production of bioethanol. *Ulva pertusa*, *Ulva lactuca*, *Sargassum fulvellum*, *Sargassum sagamianum*, *Gelidium amansii*, *Gelidium elegans*, *Saccharina japonica*, *Laminaria hyperborea*, *Saccharina latissima*, *Laminaria japonica*, *Nizimuddinia zanardini*, *Laminaria digitata*, *Gracilaria salicornia*, *Eucheuma* sp. and *Gracilaria verrucosa* are recently reported seaweeds used for ethanol production. Extraction of sugars from polysaccharides thereupon proceeds for fermentation by the respective microbial strains to produce bioethanol. The wastes of the biorefinery industries that utilize algal biomass for the generation of energy can be used as raw materials for manufacturing ethanol. Bioconversion of algal materials into bioethanol is an environmental procedure that can further serve as a good source of revenue (Milledge et al., 2014; Baweja et al., 2016).

Seaweeds have played a significant role in treating wastewater by reducing the number of nutrients before getting discharged into rivers and oceans. They can remove nutrients like phosphorus and nitrogen, which are primarily responsible for eutrophication, from domestic sewage and wastewater effluents. *Gracilaria verrucosa*, a type of red seaweed, can regulate the level of Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD), however, green seaweed (*Ulva fasciata*) is more efficient in the removal of ammonia. Some seaweeds possess the capacity to absorb maximum nutrients inhibiting eutrophication (Pati et al., 2016).

Furthermore, seaweeds are also gaining interest as feedstock for cattle, poultry, fish, and other farm animals since they are rich in macro-and micronutrients. Several countries (Iceland, France, and Norway) use seaweeds as domestic feed (Craigie, 2011). Norway was the first country to produce seaweed animal feed (Kilinç et al., 2013). Seaweeds have several beneficial effects including the ability to reduce the rate of mastitis, and cow fever, enriching the fat level, and iodine content in milk along with improving the fertility in animals. For example, *Gracilaria* sp., *Gelidiella* sp., *Sargassum* sp. and *Hypnea* sp. are used as fish feed (Pati et al., 2016). Kelps like *Ascophyllum nodosum*, *Fucus* sp., *Laminaria* sp., *Macrocystis* sp. are used for livestock feeding (Craigie, 2011).

As the manufacturing of paper employs wood as raw materials and protection of trees from deforestation is important for a clean and green environment, hence seaweeds can be a substitute for wood as they contain endofibers (also known as rhizoidal filaments) which can be utilized to make paper after bleaching. Red seaweeds of the family *Gelidiaceae* and green seaweed *Cladophora* are mainly used for the manufacturing of paper (Baweja et al., 2016).

7.3 NUTRACEUTICAL POTENTIAL OF SEAWEED POLYSACCHARIDES

Polysaccharides are the derivates of complex carbohydrates which constitutes most of the organic carbon source on our planet, profusely known to occur naturally in distinct structure and forms. The glycosidic linkage between numerous monosaccharides is key to responsible for its complex structural glycobiology (Venkatesan et al., 2015). The polysaccharides are mainly classified as homopolysaccharides and heteropolysaccharides. Synthesis of the algal or seaweed polysaccharide is largely dependent upon species and ecological abiotic factors. These complex carbohydrate derivatives play a pivotal role in protecting seaweeds from various environmental stresses (Cotas et al., 2020). About 76% of the total polysaccharides constitute the dry weight of seaweeds. Polysaccharides extracted from algal species are broadly classified as sulfated and non-sulfated polysaccharides (Shannon and Abu-Ghannam, 2019). The presence of sulfated polysaccharides in the algal cell wall forms an intracellular matrix to protect the thalli deformation, improve rigidity, and elasticity with the help of cationic salts and enhance ion exchange capability which makes algae different from terrestrial plants (Hentati et al., 2020). Sulfated polysaccharides extracted from macroalgal biomass are classified depending upon phyla as Red seaweed or Rhodophyceae have carrageenan and agaran, Green seaweed, or Chlorophyceae have ulvan and Brown seaweed or Phaeophyceae have fucoidans. Apart from fucoidans, the brown seaweed also possesses major non-sulfated polysaccharides such as alginate. These seaweed polysaccharides exhibit distinct biological activities such as: antiviral, antibacterial, antitumor or anticancer, antiaging, immunomodulating, anticoagulant, antilipidemic, etc. activities and due to their presence, the seaweeds have been demonstrated to exhibit widespread applications in the field of nutraceuticals (Venugopal, 2019).

7.3.1 CARRAGEENAN

7.3.1.1 STRUCTURE

The carrageenan is an anionic sulfated polysaccharide obtained from Rhodophyceae (red seaweed) (Venugopal, 2019) that occurs when galactan units bind with 4-linked α-galactose residues of D-conformation. The composition of repeating galactose units and 3,6-anhydro-galactopyranose copolymer, i.e. carrabiosis (sulfated dioside) by alternating 4-linked α-D-galactopyranose and 3-linked ß-D-galactopyranose construct the structural backbone of D-galactans (Jiao et al., 2011; Hentati et al., 2020). The three major structural forms of carrageenan are kappa (κ), lambda (λ), and iota (ι) which are most relevant to the industrial application (Venugopal, 2019). Apart from these, the other sub-forms includes: mu (μ)-carrageenan, alpha (α)-carrageenan, beta (ß)-carrageenan, gamma (γ)-carrageenan, psi (ψ)-carrageenan, omega (ω)-carrageenan, nu (ν)-carrageenan, theta (θ)-carrageenan, delta (δ)-carrageenan, and xi (ξ)-carrageenan (Tanna and Mishra, 2019). However, carrageenan naturally occurs in hybrid forms like κ and ß-hybrid, κ and μ-hybrid, κ and ι-hybrid or ν and ι-hybrid influenced by the environmental (biotic and abiotic) factors and developmental cycle of species (Jiao et al., 2011; Cotas et al., 2020). Carrageenan contains sulfate (or ester-sulfate) groups which can range up to 40%. The different compositions of 3,6-anhydro-D-galactopyranose unit and sulfate groups classify the different structural forms of carrageenan (Venugopal, 2019) which determines the property and functionality (Cotas et al., 2020). Carrageenan is obtained from various sources such as κ-carrageenan from *Kappaphycus alvarezii* (*Eucheuma cottonii*), λ-carrageenan extracted from *Gigartina*, and *Chondrus* genera, and ι-carrageenan from *Eucheuma denticulatum* (*Eucheuma spinosum*) (Jiao et al., 2011; Cunha and Grenha, 2016). However, *Chondrus crispus* produces the hybrid form (κ and λ)-carrageenan. Other few species which also serve as sources of carrageenan are *Agardhiella*, *Furcellaria*, *Iridae*, and *Hypnea* (Cunha and Grenha, 2016; Hentati et al., 2020).

7.3.1.2 PHYSICOCHEMICAL PROPERTY

The carrageenan has a molecular weight distribution ranging from 10^5–10^6 kDa (Venugopal, 2019) but the industrially accepted molecular weight relies on the average of 200–800 kDa (Cotas et al., 2020) and its water or aqueous solubility depends upon several factors: sulfate and hydroxyl group content, chemical composition (pH and ionic strength of medium), associated cations (K^+, Na^+, Ca^{2+}) presence. Mostly the presence of sulfate and hydroxyl groups reflects the hydrophilicity whereas, the hydrophobicity is influenced by 3,6-anhydro-galactopyranose units that classify the water or aqueous solubility in different forms of carrageenan. Therefore, carrageenan with more sulfate groups and less 3,6-anhydro-galactopyranose units are more soluble in water and vice versa. The hydrophilicity of carrageenan follows the series (from less to highly soluble): κ-carrageenan < ι-carrageenan < λ-carrageenan.

However, the alteration in solubility results due to the occurrence of cations that influence the aggregation within carrageenan helices (helix-helix).

The κ-carrageenan and ι-carrageenan in the form of Na^+ salt easily solubilize in cold water however, K^+ salt form requires hot water for solubilization. Only λ-carrageenan can easily solubilize in both hot and cold water but in the form of salts. However, in organic solvents carrageenans are highly insoluble (Cunha and Grenha, 2016). The carrageenan is extracted industrially using two processes: gel press technique, and alcohol extraction technique (Cotas et al., 2020). The natural rheological properties of carrageenan make them good thickening (thickener) or gelling agents and stabilizers which make them an essential component in industrial applications. Only λ-carrageenan is widely used as thickeners due to its weak or no gel-forming ability even with high K^+ ions. However, κ- and ι-carrageenan along with the presence of cations (K^+ and Ca^{2+}) can exhibit gelling characteristics on cooling (Calvo et al., 2019). Carrageenans are polymers that have a high tendency to undergo viscous solution which is largely dependent on temperature, concentration, solute presence, molecular weight, and forms of carrageenan. However, excess temperature and low pH may lead to its functionality loss (Necas and Bartosikova, 2013).

7.3.1.3 NUTRACEUTICAL APPLICATION OF CARRAGEENAN

The carrageenan is a hydrocolloid with no nutritional value for humans due to the lack of enzymes for metabolizing them in our gastrointestinal or digestive tract. However, they strongly act as dietary fibers, and also have huge applications in food industries. They are considered as one of the safe natural additives for preserving and texturing food and dairy products (Cotas et al., 2020). The commercial recognition of carrageenan according to European legislation is distinguished into two categories i.e. refined and semi-refined carrageenan having additive numbers E407 and E407a respectively (Hotchkiss et al., 2016). The carrageenans have good polysaccharide stabilizing and protein binding properties which are not only used by the meat processing industries to manufacture low-fat meat but are also used as thickening agents by the dairy industries (Tanna and Mishra, 2019). Also, κ-carrageenan is known to enhance cheese firmness, emulsification of milk fat molecules, coagulation of whey proteins, fermentation of curd, and production of low-fat cheese. It is also used as cheese analogs (Mozzarella analog, tofu, and cheese sauce) for the vegans (sensitive to lactose) but with the low melting property, however, can be improved by adding Na^+ salts (Błaszak et al., 2018). The antioxidant property of carrageenan (κ-carrageenan) majorly depends on the structural constitution and an extraction method has been currently identified which inhibits the formation of the superoxide radicals increasing the shelf-life of food (Noor, 2018). The carrageenan has been demonstrated with a good prebiotic activity which confers a benefit to human health by improving diet-host-microbe interaction. In murine models, carrageenan extracts from *Chondrus cripsus* inhibited the growth of pathogen microbes (*Clostridium septicum* and *Streptococcus pnemoniae*) with the simultaneous increase in probiotic microbe (*Bifidobacterium breve*), propionate, butyrated short-chain fatty acids (SCFAs), and acetate. Several bacteria such as *Bacteroides xylanisolvens* and *Escherichia coli* possess ß-carrageenase activity which on hydrolyzing κ-carrageenan stimulates the synbiotic activity by cross-feeding the probiotic microbes (Cherry et al., 2019). The insoluble dietary fibers sometimes behave as prebiotics by fermenting the complex polysaccharides with the help of gut microbiota. The prebiotic activity of low molecular weight (LMW) λ-carrageenan derivatives enhances the immunomodulatory and anticancer properties of seaweeds. The presence of floridean starch and S-galactans in carrageenan has been reported to improve the functionality of gut microbiota, colon histo-morphology, water holding capacity of stool, and immune response (Raposo et al., 2016). The carrageenan obtained from *Kappaphycus alvarezii* increases the SCFAs production and probiotic bacterial growth with the decrease in the population of *Clostridium coccoides* and *Eubacterium rectale*. The in vivo studies of *Chondrus crispus* polysaccharides reported reduced colonization of several pathogenic bacterial genera (*Sutterella*, *Holdemania*, *Legionella*, *Shewanella*, *Streptococcus*, *Agarivorans*) (Lopez-Santamarina et al., 2020).

Apart from food, the superoxide radicals are also known to induce reactive oxygen species (ROS) in living cells which destroy essential biomolecules required for metabolism causing an impact on human health. All three common carrageenan forms (κ, ι, λ) exhibit effective antioxidant activities. However, λ-carrageenan has shown more potentiality compared to the other two carrageenan forms (Hentati et al., 2020). Food grade carrageenan does not have any side effects on human consumption and even does not degrade in the stomach, and small intestine having pH ranging from 4.5–6.7 but on artificial acid hydrolysis of carrageenan produces two products d-carrageenan (degraded carrageenan) and poligeenan (PNG) which have been identified to exhibit potent toxicological effects causing ulcerative colitis, inflammation, and lesions in the digestive tract when administered in animal models, and therefore designated as unsafe for human consumption (McKim et al., 2019). The industrial carrageenan polysaccharides have been identified to exhibit different antiviral activity depending upon the occurrence of sulfate groups and molecular weight. The ι-carrageenan shows a better effect in inhibiting the infection caused by human papillomavirus (HPV) and dengue virus (DENV) compared to κ-carrageenan and λ-carrageenan. The O-acetylated polysaccharides of carrageenan undergo sulfation and depolymerization which boosts the antiviral-HIV activity. However, κ-carrageenan having 3kDa molecular weight has a good in-vivo antiviral effect against the influenza virus (Wang et al., 2012). Positive results have also been identified in using ι-carrageenan as a nasal spray in treating patients suffering from the common cold (Necas and Bartosikova, 2013). The κ-carrageenan, λ-carrageenan, ν-carrageenan, μ-carrageenan, and ι-carrageenan from *Gigartina skottsbergii* can possess an active antiviral effect on both type 1 and 2 HSV. Additionally, carrageenan lubricant gels can be used for treating distinct sexually transmitted diseases (STDs) i.e. genital warts and gonorrhea (Tanna and Mishra, 2019). The negative charge of carrageenan resembles glycosaminoglycan (GAG) of mammalian cell membranes which exhibits biological activity by inhibiting cancer cells to form matrices (Calvo et al., 2019). The anti-metastatic and anti-proliferative property of carrageenan has shown different positive outcomes both *in-vitro* and *in-vivo* (murine models) but their mode of action is not known (Necas and Bartosikova, 2013). For example, λ-carrageenan extracted from *Chondrus ocellatus* has both anti-tumor and immunomodulatory activities (Tanna and Mishra, 2019). However, depolymerized carrageenans (κ, ι, λ) with LMW have high anti-tumor activity. κ-carrageenan extracted from *Hypnea musciformis* exhibited a positive effect by resisting the proliferating activity of SH-SY5Y and MCF-7 cancer cell lines (Hentati et al., 2020). Moreover, purified κ-carrabiose on treating LM2 tumor cells shows positive results by inducing cytotoxicity against cancer cells that can be used as an anti-cancer agent (Calvo et al., 2019). Carrageenan obtained from *Gigartina* and *Tichocarpa* genera help to increase the secretion of anti-(IL-10) and pro-(IL-6 and TNF-α) inflammatory response for balancing the cytokine response according to the dose administered. The number and characteristics of O-glycosidic linkages, distribution, and positions of sulfate groups with the main carbohydrate backbone as well as the monosaccharide composition reflect the immunomodulatory activity of carrageenan (Hentati et al., 2020). The different forms of carrageenan also act as compounds for treating several cardiovascular diseases (CVDs) and their threats or risk factors (obesity, hypertension, dyslipidemia, and overweight). Diet supplemented with *Gigartina pistillata* and *Kappaphycus alvarezii* reduces dyslipidemia involves: total triglycerides (TGs), total cholesterol (TC), and low-density lipopolysaccharide cholesterol (LDLC). Ischemic heart disease (IHD) patients are recommended for short-term supplements of carrageenan to reduce the level of plasmatic TC. *Gigartina tenella* and *Chondria crassicaulis* supplemented diet helps in the treatment of hypertension by targeted angiotensin-converting enzyme (ACE)-I inhibition. Carrageenans are often identified to cure atherosclerotic-plaque formation and progression-related issues (Cardoso et al., 2015).

7.3.2 AGARAN

7.3.2.1 STRUCTURE

Agaran is another sulfated polysaccharide of red seaweeds also known as agarocolloids. In contrast to carrageenan, the agaran forms when sulfated galactan units bind with 4-linked-α-galactose residues (galactopyranose) of L-conformation. The agarans broadly categorized into two distinct groups depending upon their structure and gelling property are agar and agaroids (Jiao et al., 2011; Hentati et al., 2020). Agar comprises a chemical structure with a combination of two polymers such as agarose and agaropectin which varies with different species. The agarose being a neutral polysaccharide exhibits a double helical structure with water holding capacity, having alternated 1,4-linked 3,6-α-L-anhydro-galactopyranose and 1,3-linked ß-D-galactopyranose. However, the additional presence of pyruvic acid (pyruvate), uronic acid (uronate), sulfonic acid (sulfate), and methyl groups in the same backbone structure of agarobiose give rise to another complex polymer i.e. agaropectin. More than 700 genera and 6000 species of red seaweeds have been identified for possible extraction of agar (also known as agar-agar, Japanese isinglass, and vegetable gelatin). However, commercially, agar is extracted from the species with rich agar sources such as *Gelidium*, *Gracilaria*, and *Pterocladiella* seaweeds. The sulfate (or ester-sulfate) group content in agaran can range up to 5% (Venugopal, 2019) which is mainly found in the agaropectin polymer of agar. The *Gracilaria gracilis*, *Gracilaria bursapastoris*, and *Gracilaria cervicorvis* have 1.34%, 1.87%, and 5.3% charged sulfate groups respectively. Additionally, different species of red seaweeds have different sulfated agar compositions (Chen et al., 2020). On the other hand, *Gelidium* sp. is well known for the agar extraction includes: *Gelidium allanii*, *Gelidium johnstonii*, *Gelidium koshikianum*, *Gelidium longipes*, *Geledium crinale*, *Gelidium pusillum*, *Gelidium capillaceum*, *Gelidium acerosa*, *Gelidium linoides*, *Gelidium tenuifolium*, *Gelidium isabelae*, *Gelidium microdonticum*, *Gelidium bernabei*, *Gelidium pluma*, and many more which are present ubiquitously throughout the marine world are widely used for agar extraction (Boo et al., 2014). Agaroid is the second classified form of agaran which is further subdivided into two forms i.e. funoran and porphyran. Funoran exhibits a structural backbone comprising repeated ß-galactopyranose-6-O-sulfate and 3,6-α-L-anhydro-galactopyranose-2-O-sulfate (Hentati et al., 2020). On the other hand, the porhyran structure is constructed with alternated 3-linked ß-D-galactopyranose and 4-linked α-L-galactopyranose-6-sulfate or 3,6-anhydro-α-L-galactopyranose. The commercialized form of porphyran is largely obtained from *Porphyra capensis*, *Porphyra umbilicalis*, and *Porphyra haitanensis.* The sulfation occurs by O-linked substitution of sulfate at the C-6 position (backbone structure) along with methyl and xylosyl groups in agaran (mostly agaroids) as a result contains xylose and sulfated xylose residues in a small proportion (Jiao et al., 2011). The 3,6-anhydrogalactopyranose and ester-sulfate groups in porphyrans range up to 11% and 19%, respectively. However, porphyran 3,6-anhydrogalactopyranose uniquely known to exhibit both D- and L-conformation, therefore, can be classified as both carrageenan (if a 4-linked residue is in D-conformation) and agaran (if a 4-linked residue is in L-conformation) (Bhatia et al., 2010).

7.3.2.2 PHYSICOCHEMICAL PROPERTY

Both groups of agaran i.e. agar and agaroid resemble close physical structures with little difference in their chemical properties. The agar is a phycocolloid with a good gelling activity where 1.5% of agar forms strong clear and transparent gel between 32°C and 43°C and melts only above 80°C to liquefied form which makes it unique from other gelling agents at similar concentrations. Contrasting to carrageenan, the gelling activity of agar requires only simple water without the addition of any other cationic solutes (K^+ and Ca^{2+}). However, the addition of sugar at a small amount increases the strength of the aqueous agar gel, commonly known as sugar reactivity. Agar can withstand a broad range of pH (5–8) and high temperatures (above 100°C) without any hindrance. The gelling activity of agar is mainly due to the presence of agarose which has the greater potentiality to form gels compared to agaropectin. It is known to exhibit high thermo-reversible (liquid gel) properties without losing its physicochemical properties. The agar can be used as a flavoring and fragrant-fixing agent without using any additional additives with them. The agar gels can be easily colored to increase the attractiveness of the product. Additionally, agar is known to exhibit strong gelling activity compared to agaroids. However, the potentiality to form gels depends on the presence of 3,6-anhydrogalactose (or 3,6-anhydrogalactopyranose) residues and sulfate groups (Gioele et al., 2017; Venugopal, 2019). The agar extracted from *Gracilaria* exhibits high sulfate, methoxy, and pyruvate groups along with high order of sulfation comparing to agar obtained from *Gelidium* and *Pterocladiella*. The *Gracilaria* agar exhibits low gelling ability which on alkali pretreatment can be modified for converting α-L-galactopyranose-6-sulfate to 3,6-anhydro-α-L-galactopyranose residues whereas *Gelidium* agar being better quality can be simply isolated by hot water extraction. Compared to agar, agaroids tend to exhibit pale-yellow color along with fibrous material on autoclaving (hot-water extraction) (Torres et al., 2019) thus making it complex and translucent gel. The chemical modification agaroids result in sulfate group displacement at the C-6 position (where 3,6-anhydro-L-galactopyrananose residues are converted to 3,6-anhydro-galactopyranose residues) on alkali treatment (a type of double hydrolysis) which improves the gelling property (Zhang et al., 2005). Primarily the modification of the sulfate groups was done by using reductive and oxidative hydrolysis methods which exhibited limitation during the absence of consecutive agarobiose units and were laborious with low sensitive results respectively therefore the alkaline treatment was introduced to effectively determine the polysaccharide residues (Bhatia et al., 2010). This displacement of the sulfate residues makes the modified agaroid to exhibit higher gelling capability compared to the natural or crude agaroid.

7.3.2.3 NUTRACEUTICAL APPLICATION OF AGARAN

Like carrageenan, the agarans (agar and agaroids) are also indigestible in humans due to the lack of enzymes in the digestive system, however, can be metabolized by gut microflora (bacteria). Agar is mostly recognized due to its industrial and food applications as gelling, stabilizing, thickening, and cryoprotecting agents. Food grade agar is commercially recognized with an additive number i.e. E406 (Torres et al., 2019). The prebiotic activity either from insoluble macroalgal polysaccharides or oligosaccharides improves gut and colon morphology and ecology by increasing the beneficial bacterial colonization, organic acids, and SCFAs production which in return lowers the pH eliminates the growth of pathogenic bacteria (*Escherichia coli* and *Salmonella*), improves mineral absorption, and gut immunology. The agaran polysaccharides from *Porphyra* and *Gracilaria* exhibit prebiotic activity (Raposo et al., 2016). The increase in SCFAs production stimulates the proliferation of *Lactobacillus* and *Bifidobacterium*. The sulfated polysaccharide extracted from *Gracilaria birdiae* does not exhibit any improvement of the gut microbiota population in rat models. However increased the gut metabolic activity thus preventing damage from naproxen. Polysaccharide from *Gracilaria rubra* increases the population of *Bacteroides*, *Prevotella*, and *Phascolarctobacterium* with the decrease in *Fusobacteriaceae* and *Lachnospiraceae* which demonstrated a significant increase in the gut essential metabolites (acetic acid, propionic acid, SCFAs, and isobutyric acid) (Lopez-Santamarina et al., 2020). The in vitro study of porphyran demonstrated a lack of SCFAs production ability, however, stimulates the proliferation and growth of *Lactobacillus* and *Bifidobacterium*. Apart from *Bifidobacterium bifidum*, *Bifidobacterum longum*, *Bifidobacterium adolescentis*, *Bifidobacterium breve*, and *Bifidobacterium infantis* possess the ability to ferment the porphyran from dried *Porphyra yezoensis* commonly known as Nori (Cherry et al., 2019). Food industries widely use agar (1–2%) as texturing and stabilizing agents of food products (confectionaries, meat production, and bakery products). It can behave as natural dietary fiber which can be used to treat constipation (as a laxative) and also used to substitute the gluten content of the wheat products. Commercial agar and agar-based products (agarose) are widely used in molecular biology, microbiology, biotechnology, immunology, and pharmaceutical fields (Venugopal, 2019). Apart from these applications agar also exhibits nutraceutical potential with effective biological properties. The agar is known to have antiviral, antibacterial, antioxidant, anti-inflammatory, and anticoagulant activity (Rosemary et al., 2019). Agar-based aerogels and nanoparticles are widely used as drug delivery, and antimicrobial agents respectively (Gioele et al., 2017). The agar exhibits potential activity by stimulating the apoptosis of tumorous cells making it is an effective anti-cancer agent (Cotas et al., 2020). Agar reduces the blood glucose level which is effective for patients suffering from diabetes. The sulfated agar oligosaccharide and polysaccharide have also demonstrated antioxidant and immunomodulatory activity respectively (Tanna and Mishra, 2019). The application of agar towards nutraceuticals is still less known in comparison to other polysaccharides available. On the other hand, the sulfated agaran is less commonly used for food applications due to the ubiquity of sulfate groups that reduce the gelling ability and viscosity compared to its application towards nutraceuticals. The gelling activity of agaran can be improved by chemical modification.

However, among agaran, porphyran (agaroid) shows enormous nutraceutical activities as well as mostly used. The anti-allergic activity of porphyran reduces hypersensitive reaction by inhibiting histamine secretion. The immunomodulating activity of porphyran is induced by gut bacteria which not only stimulates the Ig (immunoglobulin) M and macrophage response, but also balances the IgE, IgG 2a, and cytokine responses. However, in murine models, sulfated porphyran stimulates the production of macrophages, T-lymphocytes (CD4+), dendritic cells, IL factors, and tumor necrosis factors. Porphyran extracted from *Porphyra yezoensis* shows potent toxicity on dose-dependent against cancerous cells and restricts either G0/G1 or G2/M checkpoints of cancer or tumor cell cycle. The porphyran polysaccharide lowers the serum lipid concentration and platelet aggregation as an anticoagulant to overcome thrombosis. The anti-coagulation activity increases with elevation in sulfate group distribution and sulfation degree. Macroalgae are known to generate free radicals during oxidative stress which functions as antioxidants with scavenging activity against radicals. *Porphyra tenera* and *Porphyra haitanensis* exhibit antioxidant properties when the polysaccharides are extracted by aqueous or methanol extracts and degradation procedures respectively. LMW porphyran has been demonstrated to exhibit improved antioxidant activity. It can also act as an antioxidant enzyme that stimulates total antioxidant ability by lowering lipid peroxidation which reflects its anti-aging potentiality. It is also used as an anti-viral agent against HIV by restricting reverse transcriptase activity as well as against HSV, human cytomegalovirus (HCMV), DENV, influenza virus, and RSV by inhibiting cellular attachment. The porphyran oligosaccharide exhibits protection against Parkinson's disease (by regulating the P13K/Akt/Bcl-2 pathway) and acute renal malfunction. Apart from these, porphyran is largely used by nutraceutical companies for producing health supplements that exhibit antihyperlipidemic, hypoglycemic, hypotensive, and hypocholesterolemic activities by reducing cholesterol absorption in the gut, and TC, free cholesterol (FC), TGs as well as a phospholipid in the liver (Bhatia et al., 2010; Liu et al., 2019; Venkatraman and Mehta, 2019).

7.3.3 ULVAN

7.3.3.1 STRUCTURE

Ulvan is the charged sulfated polysaccharide obtained from Chlorophyceae (green seaweeds) which comprises 29% of cellular dry weight (Venugopal, 2019). However, ulvan along with three other cell wall polysaccharides (cellulose, glucuronan, and xyloglucan) constitute about 45% of cellular dry weight (Kidgell et al., 2019). The polysaccharide composition of ulvan includes rhamnose (45%), xylose (9%), glucuronic acid (23%), iduronic acid (5%), galactopyranose (20%), and glucose (6.4%). The sulfate content in the ulvan ranges up to 19% (Cunha and Grenha, 2016; Kidgell et al., 2019; Hentati et al., 2020). Similar to other polysaccharides the structural properties of ulvan are dependent upon the species and extraction techniques. The ulvan is also known as sulfated xylorhamnoglycuronan (Hentati et al., 2020) which exhibits two distinct types of aldobiouronic acid with the same oligosaccharide residue i.e. rhamnose or rhamnopyranose (aldobiose) such as type A-ulvanbiouronic acid-3-sulfate (A3s) contains glucuronic acid and type B-ulvanbiouronic acid-3-sulfate (B3s) contains iduronic acid. Rhamnose is mainly sulfated either in the C2 position or both C2 and C3 positions (Cunha and Grenha, 2016). Therefore, the structure of ulvan is commonly made up of the repeating units of either 4-ß-D-glucuronic acid-1, 4-linked-α-L-rhamnopyranose-3-sufate-1 or 4-α-L-iduronic acid-1,4-linked-α-L-rhamnopyranose-3-sulfate-1 units (Jiao et al., 2011). Apart from rhamnose, xylose, and sulfated xylose containing disaccharide structural backbone are commonly known as ulvanbioses, commonly designated as type U is classified into two forms ulvanbiose acid-3-sulfate (U3s) which comprises ß-D-xylose-1,4-linked-α-L-rhamnopyranose (rhamnose)-3-sulfate, and ulvanbiose acid-2,3-sulfate (U2´s,3s) have ß-D-xylose-2-sulfate-1,4-linked-α-L-rhamnopyranose-3-sulfate units (Kidgell et al., 2019). The ulvan is extracted from two Chlorophyta genera (commonly known as Ulvales) such as *Ulva* (*Ulva rigida*, *Ulva lactuca*, *Ulva reticulata*, *Ulva conglobata*, *Ulva arasakii*, *Ulva armoricana*, *Ulva compressa*, *Ulva fasciata*, *Ulva gigantea*, *Ulva intestinalis*, *Ulva linza*, *Ulva ohnoi*, *Ulva pertusa*, *Ulva prolifera*, *Ulva rotundata*, *Ulva scandinavica* etc.), *Enteromorpha* (*Enteromorpha compressa*, *Enteromorpha prolifera*, etc.) (Jiao et al., 2011; Kidgell et al., 2019; Tanna and Mishra, 2019; Venugopal, 2019). *Ulva* is mostly used for the extraction of ulvan compared to *Enteromorpha*.

7.3.3.2 PHYSICOCHEMICAL PROPERTY

The ulvan comprises the average molecular weight ranging from 189–8200 kDa (Tanna and Mishra, 2019). Nonetheless, the ulvan extracted from *Ulva pertusa*, *Ulva conglobata*, and *Enteromorpha prolifera* exhibits the molecular weight ranging from 530–3.6×10^3 kDa (Cunha and Grenha, 2016). The degree of sulfation in ulvan may range up to 40%. The physicochemical properties of ulvan are largely dependent upon the sulfation degree and molecular weight. However, the alteration in the molecular structure can be achieved by depolymerization and alteration in ester-sulfate (or sulfate) groups (Kidgell et al., 2019). Based on physicochemical properties, ulvan are categorized into two i.e. water-soluble ulvan and insoluble cellulose-related compounds (Venugopal, 2019). The extraction of ulvan is obtained with hot water which is enhanced by the incorporation of acidic, alkali, or cationic (Ca^{2+}) chelating agents. Moreover, freeze-drying and ethanol precipitation ameliorate the purity of the material extracted (Venugopal, 2019). The optimal conditions required for ulvan extraction include temperature (90°C), pH (4.5), and duration (3 hours) to increase the yield, selectivity, and minimum degradation. Additionally, a temperature above 90°C, and pH below 2, increases the degree of depolymerization compared to the degree of extraction of ulvan (Kidgell et al., 2019). The ulvan extraction conditions vary with the species. Ulvan extracted from *Ulva lactuca* exhibits pseudoplastic characteristics with increased shear rate and decreased viscosity which determines its structural and rheological property. However, ulvan extracted from *Ulva rigida* has been demonstrated with high thermoreversible characteristics. Additionally, incorporation of cationic ions, as well as high pH, ameliorates the gel-forming ability from viscous solvent (Cunha and Grenha, 2016).

7.3.3.3 NUTRACEUTICAL APPLICATION OF ULVAN

Ulvan exhibit enormous biological activity towards nutraceutical applications such as antiviral, antioxidant, anticoagulant, and immunomodulating activities (Venugopal, 2019). The ulvan has been demonstrated to stimulate the *Lactobacillus* and *Bifidobacterium* population growth that helps in the fermentation of the dietary polysaccharide in the in-vitro fecal study model. However, in murine models polysaccharides from *Enteromorpha* species exhibited increased growth of *Firmicutes* and *Actinobacteria* with a significant decrease in Bacteroidetes and Proteobacteria (Cherry et al., 2019). In contrast to red, and brown seaweed, the prebiotic activity of green seaweed polysaccharides is less known. The prebiotic activity of the green seaweed polysaccharide in murine (mice) models stimulated the growth of beneficial gut bacteria along with SCFAs production, however simultaneously reduced pathogenic bacterial growth, histopathological inflammatory lesions in the distal colon, and expression of diabetes-related genes. Polysaccharide extract from *Enteromorpha clathrata* inhibits the growth of *Enterobacter*, *Staphylococcus*, *Streptococcus*, *Peptococcus*, *Rikenellaceae*, and *Alistipes* with a significant increase in the population of *Bacteroides*, *Alloprevotella*, *Blautia*, *Ruminococcaceae*, and *Akkermansia muciniphila.* Contrastingly, this polysaccharide stimulates the growth of *Preptococcus* and *Eubacterium* with the decrease in cancer-related *Helicobacter* populations in male mice, however, in female mice an increase in *Odoribacter*, *Clostridium IV*, *Oscillibacter*, and *Alistipes* populations were observed with the decrease in the growth of beta-proteobacteria (Lopez-Santamarina et al., 2020). Ulvan possesses dose-dependent and strains specific antiviral and inhibitory activity on several human viruses (Cunha and Grenha, 2016) such as Feline Sarcoma Virus (FSV), Newcastle Disease Virus (NDV), West Nile Virus (WNV), Japanese Encephalitis Virus (JEV), DENV, Yellow Fever Virus (YFV), Avian Influenza Virus (AIV), and H1N1 (Influenza virus) by modulating the viral entry and replication cycle into the cells of human and model animals (Kidgell et al., 2019). LMW ulvan is known to exhibit high antioxidant activity (Venugopal, 2019).The ulvan extracted from *Ulva pertusa* has been demonstrated to have high antioxidant activity due to sulfate content against superoxide radical by producing superoxide dismutase and glutathione peroxidase observed in murine models (Hentati et al., 2020). The ulvale species increases the antioxidant enzyme activity by amplifying the potential activity of transcriptional enzymes such as Nrf2, AP-1, AP-2, NF-κB, C/EBP, and Sp1. Numerous studies have reported the anticancer activity of ulvan which lowers proliferation by increasing apoptosis of cancer cells. It shows effective activity against murine sarcoma cancer cell lines, cell lines of human, hepatocellular carcinoma, breast cancer, gastric or stomach cancer, colon cancer, and cervical cancer. Additionally, the sulfation degree improves the anticancer and antitumor activity of ulvan (Kidgell et al., 2019). The antilipidemic or antihyperlipidemic activity of ulvan reduces the total serum cholesterol (TSC), TGs, TC, LDL-cholesterol, and high-density lipoprotein (HDL)-cholesterol. The LMW ulvan effectively reduces TGs and HDL-cholesterol however TSC and LDL-cholesterol can be controlled by high molecular weight ulvan (Cunha and Grenha, 2016). These antilipidemic activities of ulvan are well observed in murine models (Tanna and Mishra, 2019). Ulvan extracted from *Ulva clatharata*, *Ulva lactuca*, *Ulva prolifera*, *Ulva fasciata*, and *Ulva nematoidea* exhibits effective anticoagulant activity by amplifying the anticoagulating factors such as serine proteases and glycoproteins by both intrinsic and extrinsic pathways. The sulfation degree of ulvan improves anticoagulation activity. However, the correlation between molecular weight and activity remains unclear (Kidgell et al., 2019). For example, sulfated ulvan polysaccharides from *Ulva conglobata* exhibits high rhamnose and sulfate group content which inhibits the thrombin, and heparin cofactor II modulation (Jiao et al., 2011). Moreover, the ulvan immunomodulating activity differs with species. Several animal models have been demonstrated with inflammatory activities with the ulvan application. It stimulates both the inflammatory and anti-inflammatory responses depending upon the cytokine release. Moreover, the improved immunomodulating activity helps to control and stimulate the function of other biological activities of ulvan obtained from distinct ulvale sources such as *Ulva intestinalis*, *Ulva ohnoi*, *Ulva amoricana*, *Ulva rigida*, and *Ulva pertusa* (Kidgell et al., 2019). Apart from nutraceutical applications ulvan is less commonly used in food industries due to its weal to gelling capacity with low structural stability, however, can be consumed as dietary fibers as it possesses excess water retention behavior as well as remain indigestible due to the inadequacy of enzymes in the human digestive tract (Tanna and Mishra, 2019).

7.3.4 FUCOIDAN

7.3.4.1 STRUCTURE

Fucoidan (negatively charged), naturally obtained from brown algae, is a fucose supplemented cell wall sulfated polysaccharide having large binding affinity to other biomolecules. In contrast to other seaweed polysaccharides, the molecular structure along with the presence of sulfate groups in fucoidan differs within the species. Sulfated fucan backbone is composed of distinct molecules such as minor saccharide constituents (glucose, rhamnose, arabinose, xylose, galactose, and mannose), fucose (L-conformation), acetyl groups, and uronic acids (glucuronic acids). The sulfated backbone exhibits complex branching which distinguishes the fucoidan molecules. The fucoidan homofucose containing backbone is majorly differentiated into two types: type-1 demonstrates a repeated unit of 1,3-linked-α-L-fucopyranose and type-2 demonstrates alternated and repeated unit of 1,3-, and 1,4-linked-α-L-fucopyranose (Luthuli et al., 2019; van Weelden et al., 2019). The sulfate groups addition in the structural backbone of fucoidan occurs either in C2, C3, and C4 or distributed in C2 and C4 position of fucopyranose by interlinked-O-glycosidic linkages (O2 and/or O4 position) (Tanna and Mishra, 2019; Hentati et al., 2020). Additionally, 2,3-O-disulfate ($2SO_3$) fucose has also been identified in some fucoidans (van Weelden et al., 2019). The distinct fucoidan structure obtained from different brown seaweeds demonstrates inconsistent backbone structural stability (Cunha and Grenha, 2016). Fucoidan was first extracted in the year 1913 by Kylin (Jiao et al., 2011) from the different species of Phaeophyta such as *Fucusvesiculosus*, *Laminaria digitata*, and *Ascophyllum nodosum* (Luthuli et al., 2019). Therefore, the fucoidan classification depends on the type and sources which include: type-1 fucoidan can be extracted from *Laminaria saccharina*, *Laminaria digitata*, *Analipus japonicus*, *Cladosiphon okamuranus*, and *Chorda filum*, and type 2 fucoidan is extracted from *Ascophyllum nodosum* and *Fucus* species (Jiao et al., 2011). Few other species which are also the sources of fucoidan are *Fucus serratus*, *Dictyota menstrualis*, *Fucus distichus*, *Hizikia fusiformis*, *Laminaria hyperborea*, *Padina gymnospora*, *Macrocystis pyrifera*, *Kjellamaniella crassifolia*, and *Caulerpa racemosa* (Luthuli et al., 2019).

7.3.4.2 PHYSICOCHEMICAL PROPERTY

The chemical composition of the sulfated polysaccharides reflects both the physicochemical and biological properties which are dependent upon distinct parameters i.e. species variety, abiotic factors, and ecology. The brown seaweeds are largely distinguished into three types: fucoidans, ascophyllans, and sulfated glycuronofucogalactans. The improved potentiality of fucoidan can be achieved by structural modulations (oversulfation, desulfation, acetylation, and benzoylation) (Cunha and Grenha, 2016). The molecular weight of the water-soluble polysaccharide ranges from 20–2300 kDa. The average sulfate content of fucoidan ranges from 5 to 38% (Dobrinčić et al., 2020). The extraction of fucoidan is achieved through various techniques such as microwave heating, hot water, or hot acid extraction (Wang et al., 2019). Conventional fucoidan extraction was done by the hot acid extraction method which resulted in good polysaccharide isolation with improper purification (alginate precipitation using Ca^{2+} ions) forming alginate which is water-soluble but alcohol insoluble (Ale and Meyer, 2013; Dobrinčić et al., 2020). However, in present days mostly hot water extraction (60–70°C) is used to extract the fucoidan (soluble in both water and alcohol) from brown seaweeds which is effective compared to a hot acid extraction. The crude fucoidan from hot water extraction can be purified effectively using biophysical (chromatography) and chemical precipitation treatments (cetyl trimethyl ammonium hydroxide or cationic cetyl pyridinium chloride) (Venugopal, 2019). Moreover, other advanced technologies used in the extraction of fucoidan are ultrasound extraction, pressurized liquid extraction, and enzymatic extraction. The different extraction parameter affects the physical and chemical stability including the viscosity of the solvent (Dobrinčić et al., 2020). The solubility of fucoidan is dependent on the presence of sulfate groups and branched chains. Several fucoidans from various species have been demonstrated to exhibit dynamic stable viscosity with pseudoplastic behavior however lesser applications in food industries due to weak gelling capability (Cunha and Grenha, 2016).

7.3.4.3 NUTRACEUTICAL APPLICATION OF FUCOIDAN

Similar to ulvan, the weak gelling ability of fucoidan exhibits minimum applications in food industries compared to nutraceutical applications. However, among seaweed polysaccharides, fucoidan is mostly used due to its enormous biological, or nutraceutical activities such as anticancer, antiviral, anticoagulant, antioxidant, immunomodulatory, and antilipidemic activity (Venugopal, 2019; Dobrinčić et al., 2020). The fucoidan exhibits the prebiotic activities which on interacting with gut microbiota such as bacteria, archaea, protozoa, and virus either stimulate or inhibit growth and proliferation. Several bacterial families such as *Bacteroidaceae*, *Pervotellaceae*, *Rikenellaceae*, *Ruminococcaceae*, and *Lachnospiraceae* are essential for the regulation of a healthy gut with the inhibition of pathogens (Lopez-Santamarina et al., 2020). The limited prebiotic effect of fucoidan depends on structure-dependent fermentation. Fucoidan extracted from *Laminaria japonica* stimulates the growth of *Lactobacillus* and *Bifidobacterium* during in vitro study and promotes the proliferation of *Lactobacillus* and *Ruminococcaceae* when administered in the mice or murine models (Cherry et al., 2019). Fucoidans obtained from *Undaria pinnatifida* ferments in the distal colon vessel by the intestinal microbes. Fucoidan consumption increase SCFAs concentration which not only serves as an energy source of the host within the colon but also increases the acidity of the intestine which inhibits the proliferation and multiplication of pathogens (O'Sullivan et al., 2010). The anticancer activity of fucoidan depends upon several parameters, i.e. the health of the cancer patient, carcinoma location site, cancer stage, and dosage used. The inhibition of cancer cell proliferation is mainly achieved by apoptosis. The combined therapy with different anticancer agents ameliorates the efficacy of fucoidan potentiality (Luthuli et al., 2019). This sulfated polysaccharide has also been identified to modulate the cancer signaling pathways like P13K/AKT (phosphatidylinositol-4,5-bisphosphate-3-kinase/protein kinase B) pathway, caspase (cysteine aspartic proteases) pathway, and MAPK/ERK (mitogen-activated protein kinase/extracellular signal-regulated kinase) pathway. The fucoidan is known to suppress the P13K/AKT and MAPK/ERK pathway activity which further affects the caspase pathway (van Weelden et al., 2019). Fucoidans extracted from *Undaria pinnatifida*, *Sargassum hemiphyllum*, and *Fucus vesiculosus* possess potential anticancer or antitumor activity against the cancerous cell and cell lines (Hentati et al., 2020). The fucoidans are also endorsed for their efficient antiviral activities against HSV, HCMV, hepatitis B virus (HBV), influenza virus, canine distemper virus (CDV), and HIV. The antiviral activity of fucoidan is well studied in murine models with numerous positive outcomes. *Kjellmaniella crassifolia* fucoidan exhibits antivirulence potentiality against the influenza virus (type A) by interacting with neuroaminidase and epidermal growth factor receptor (EGFR) pathway which resists the viral infection and cellular adsorption with minimum toxicity. Fucoidan obtained from *Fucus evanescens* with a molecular weight ranging from 130–400 kDa can act as adjuvants to improve the immunogenic response of the HBV vaccine. Fucoidans as anti-HBV either activate the ERK signaling pathway or MAPK/ERK1/2 pathway to suppress the viral replication or production of viral biomolecules respectively (Luthuli et al., 2019). The anticoagulant property of fucoidan majorly depends on the saccharide content, branched chains, molecular weight, and sulfate groups (Cunha and Grenha, 2016). The fucoidans with high molecular weight exhibit greater anticoagulant activity compared to the LMW fucoidans. However, the presence of the sulfate group is required to achieve long chains for effective anticoagulant activity. The anticoagulant activity of fucoidans seems to be dynamic concerning distinct factors. *Fucus vesiculosus* was the first brown seaweed in fucoidan extraction which exhibits both anticoagulant and antithrombotic activities (Jiao et al., 2011). *Enteromorpha cava* fucoidan improves AT-III mediated coagulation factor inhibition in murine models. Heparin-mediated anticoagulant and antithrombotic activity of fucoidan mostly occur due to the presence of sulfate groups (Tanna and Mishra, 2019). However, the antithrombotic activity is dependent upon fucoidan anticoagulant activity. The sulfated fucoidan polysaccharide is also known as the ROS scavenging agent that imparts its antioxidant activity. The molecular weight of fucoidan is essential for the determination of its antioxidant ability. Moreover, the chemical modification of fucoidan enhances the antioxidant activity. Contrasting to anticoagulant activity high molecular weight fucoidan imparts minimum antioxidant potentiality compared to its lower molecular weight (Wang et al., 2019). Commercial antioxidants (α-tocopherol and butylated hydroxyanisole) possess less antioxidant activity compared to fucoidans in nitric oxide (NO) scavenging. The immunomodulating activity of fucoidans can improve the host immune defense mechanism to resist tumor or cancer cell proliferation. The cytolytic activity of natural killer (NK) cells increases with fucoidan interaction which induces the activity of macrophage-interposed immunogenic signaling molecules. In murine models, it shows a positive effect on the increase in phagocytic cells and cytokine release (Hentati et al., 2020). Fucoidan extracted from *Fucus vesiculosus* is well known to control diabetes by inhibiting α-glucosidase activity. It influences the AMPK signaling and glucose transporter (GLUT) activity to ameliorate glucose tolerance. It is also known to reduce the lipid content in murine models which demonstrates fucoidan antilipidemic characteristics (Luthuli et al., 2019).

7.3.5 ALGINATE

7.3.5.1 STRUCTURE

The alginate is the non-sulfated anionic polysaccharide obtained naturally from the cell wall of brown seaweed as alginic acid. Few bacteria such as *Azotobacter* and *Pseudomonas* are also known to possess alginic acid. The alginate is derived from the alginic acid which is commercially available in the form of potassium (E402), sodium (E401), calcium (E404), and ammonium (E403) salt other than commercial alginic acid (E400) (Szekalska et al., 2016; Parreidt et al., 2018). Alginate is a mixture of two units M (D-mannuronic acid) and G (L-guluronic acid). The alginate exhibits a linear non-repeating biopolymeric structure of (1, 4)-ß-linked-M and (1,4)-α-linked-G residues (Venugopal, 2019). The residues of the alginate are either arranged in consecutive homogenous block i.e. polymeric-G (GGGGGG) and polymeric-M (MMMMMM) residues or/and alternative heterogeneous block patterns i.e. polymeric-MG (GMGMGM) residues (Lee and Mooney, 2012). The composition of the G blocks is essential as it is not only involved in the intramolecular cross-linking with the cations (Ca^{2+}) termed as an egg box which imparts the gelling capability, and cellular rigidity but also reflects the physicochemical characteristics of the alginates. Alginates are also responsible for the cell wall flexibility and their composition in seaweeds is more in the turbulent water compared to the still water (Afonso et al., 2019). The alginates are extracted from brown macroalgal species such as *Laminaria hyperborea*, *Laminaria digitata*, *Laminaria japonica*, *Macrocystis pyrifera*, *Ecklonia maxima*, *Ascophyllum nodosum*, *Sargassum* sp., etc. (Lee and Mooney, 2012; Parreidt et al., 2018).

7.3.5.2 PHYSICOCHEMICAL PROPERTY

The alginate exhibits molecular weight up to 200 kDa with molecular formulae of $(C_6H_6O_6)_n$ where n ranges between 80 and 83 (Venugopal, 2019). Distinct parameters such as ecology, species, seaweed type, seaweed age, and harvesting time affect the quantity and nutritional quality of the alginates. The harvesting time is dynamic even with the same species which depends upon the geographical location including the season (Łabowska et al., 2019). The G and M residues of the alginate are mostly responsible for the viscosity and gelling capability which also describe its rheological properties. Alginates are also known as polyuronide as it exhibits ion exchanging characteristics which on binding or crosslinking with bivalent cations (Ca^{2+}, Sr^{2+} (strontium), Ba^{2+} (barium)) except magnesium (Mg^{2+}) tends to form a good gel, binding with divalent cations (Pb^{2+} (lead), Cd^{2+} (cadmium), Ni^{2+} (nickel), Zn^{2+}, and Mn^{2+}) form a weak gel-like substance (gelatin), and on interacting with monovalent cations forms viscous solvent (Parreidt et al., 2018). On the other hand acid precipitation also tends to form good alginate gels that occur at low pH below the dissociation constant (pKa) (Ching et al., 2017). Alginate solubility and viscosity are dependent on the pH, G:M ratio, and molecular weight. The low pH (3–3.5) and higher molecular weight accelerate the protonation of hydrogen ions and physical properties respectively (Lee and Mooney, 2012). The homogenous block alginates (either polymeric G/M) are less soluble compared to heterogeneous block (polymeric MG) at low pH. However, the alginic acid is insoluble both in aqueous and organic solvents, only the monovalent salt-derived alginates are soluble in water (Szekalska et al., 2016). Alginates with high G blocks will exhibit dense rigid gels compared to the M blocks which exhibit porous flexible gels (Parreidt et al., 2018). Therefore, most of the sodium (Na^+) form of alginates is highly soluble even in cold water (Fertah et al., 2017). The alginates are also known to exhibit mucoadhesive properties that impose electrostatic and hydrogen bonding of the polymer due to the occurrence of free COOH (carboxyl) groups (Szekalska et al., 2016). The conventional alginate extraction from brown seaweeds is a long process with many steps: (a) Pre-treatment of the seaweeds, b) extraction with an alkaline solvent or reagent, (c) precipitation using cationic ions, (d) bleaching the precipitate into alginic acid, and, (e) conversion of the alginic acid into desired alginate salts. The pre-treatment procedure is more important compared to the extraction process regarding the bioavailability of alginate from seaweed tissues. This procedure is done using physical and chemical (formaldehyde) treatment (Łabowska et al., 2019). However, the novel advanced technologies used in the extraction of alginates are microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), pressurized assisted extraction (PLE), and enzyme assisted extraction (EAE) which depends on extraction factors (time, temperature, sample to solvent ratio, and power) (Dobrinčić et al., 2020).

7.3.5.3 NUTRACEUTICAL APPLICATION OF ALGINATE

The alginates are mostly used in biomedical, food, and few nutraceutical applications due to their dynamic gelling and coating characteristics. In food industries, alginate microgels function as functional foods that are mainly used for modification of food texture, digestive tract targeted delivery, encapsulation, and fat substitution (Ching et al., 2017). The alginate has been demonstrated to modulate the population of gut microbiota for a long time. The supplementation of alginate oligosaccharides improves the growth and proliferation of *Lactobacillus* and *Bifidobacterium* with a decrease in the *Enterobacteriaceae* and *Enterococcus* proliferation. The guluronic and mannuronic oligosaccharides on alginate hydrolysis induce due to the elevated production of propionate, acetate, and total SCFAs. The intestinal microflora possesses genes for CAZymes which on expression break down the macroalgal saccharides (Cherry et al., 2019). Alginate extracted from *Ascophyllum nodosum* stimulates the growth of *Lactobacillus delbrueckii* and *Lactobacillus casei* similar to the commercial prebiotics. Additionally, in rat models, the alginate from *Ascophyllum nodosum* has been demonstrated to increase the butyrated SCFAs, propionate, and acetate. The healthy gut is responsible for maintaining body weight which improves the fermentation of food by beneficial microbes with the elimination of *Clostridium*, *Streptococcus*, and *Enterobacter* (Lopez-Santamarina et al., 2020). The alginates are also used as bio-sorbents which exhibits divalent cations binding affinity which helps in interacting with the heavy metal ions from the intestine by replacing the Ca^{2+} ions allowing detoxification of heavy metals from the human body. Moreover, the mixing of activated charcoal in alginate will allow the adsorption of metal ions simultaneously (Trica et al., 2019). Alginates are also used as plasticizers as additives for coating food products. The addition of glycerine, sodium lactate, sorbitol, glycerol, PEG (polyethylene glycol), palmitic acid, glycerol monostearate, and ß-cyclodextrin impose different parameters to alginate depending on the requirements in the preparation of the coating material of foods (Parreidt et al., 2018). Alginates also have been demonstrated as stabilizing and thickening agents in the production of distinct food products. Several low pH food products (syrups, sherbets, and sauces) are treated with propylene glycol alginate due to their solubility, emulsifying, and thickening ability even at low pH (Venugopal, 2019). The microencapsulation using sodium alginates has been widely used in the preparation of synbiotic (mixture of prebiotic growth substance and probiotic microorganisms) products which improves the shelf life of the probiotics with reduced damage in cell viability and integrity. The sodium-carboxymethyl cellulose (CMC) based microencapsulated mucoadhesive alginate beads have demonstrated good probiotic entrapment and mucoadhesive properties, but comparatively less than calcium-CMC based alginate beads in encapsulating *Lactobacillus sporogenes* (Solanki and Shah, 2016). The alginate-based drink has been identified in lowering the cholesterol level, glucose uptake, and overweight in individuals on consuming it for a short duration with long term effects exhibit antilipidemic and antidiabetic characteristics (Brown et al., 2014). Sodium-alginates are widely used as a food additive that can lower blood cholesterol and weight. In contrast, 2% of calcium-alginates have exhibited positive results in reducing plasma cholesterol on oral administration to murine models (rats) by stimulating the synthesis of bile acids (Idota et al., 2016). There are several biomedical applications of alginates due to their hydrogel-forming ability. Different lower molecular weight small chemical drugs are incorporated in alginate gels for simultaneous drug delivery. The hydrophobic drugs (theophylline) are loaded in the amphiphilic alginate beads either made up of poly ε-caprolactone (PCL) and carbon nanotube (CNT). Chitosan-alginate and amoxicillin/metronidazole-loaded chitosan-alginate beads are also used for the effective treatment of *Helicobacter pylori* infection by oral administration in mouse models. Alginate encapsulation is a widely used protein delivery system that resists the denaturation of the protein before releasing it to the targeted sites in vivo models. Alginate hydrogels are used in the delivery of basic fibroblast growth factor (BFGF) and vascular endothelial growth factor (VEGF). High pI proteins (lysozyme and chymotrypsin) are delivered using encapsulated ion cross-linked alginate microspheres. However, a mixture of hydrogels and microspheres alginate is used in the targeted delivery of transcriptional activator-heat shock protein 27 (TAT-HSP27). Alginate gels/hydrogels are utilized as wound dressings because of their moisture imparting capability and reduced pathogenic invasion at the dry wound site which replaced the conventional gauze. On the other hand, it also has wider biomedical applications in cell culture, and tissue regeneration studies (Lee and Mooney, 2012). Alginates are also identified with limited nutraceutical activities. Propylene glycol alginate sodium sulfate (PSS) are mostly utilized as an anticoagulant, antihyperlipidemic, antihypotensive for effectively treating ischemic cardio-cerebrovascular diseases and reduction of blood viscosity. The high molecular weight PSS increases anticoagulant activity. The sulfated groups of PSS also play a crucial role in anticoagulant activity. Lower molecular weight fragments of PSS (FPs) exhibited inhibition of platelet aggression along with the antithrombotic activity in mice models (Xin et al., 2016). The alginate oligosaccharides exhibit effective treatment in the eradication of Alzheimer's disease, Chronic Obstructive Pulmonary Disease (COPD), cystic fibrosis, bacterial (*Pseudomonas*, *Proteus*, *Acinetobacter*, and *Escherichia*), and fungal infections. However, the joint application of sulfated polysaccharide and alginate/alginic acid-fractions possess antiviral activity against flavivirus, togavirus, rhabdovirus, herpesvirus, HIV, and HBV due to the interaction of sulfated algal polysaccharide and cationic charge of the host cell (Szekalska et al., 2016). The alginate oligosaccharides such as mannuronate and guluronate oligosaccharides which are extracted by oxidative degradation and acid hydrolysis respectively induce immunomodulatory activity by activating the macrophage stimulated NO, TNF-α, ROS production and signaling pathways (NF-κB and MAPK) (Xu et al., 2014). The guluronate and mannuronate oligosaccharides produced by the enzymatic depolymerization of alginate exhibits radical scavenging activity thus reflecting its antioxidant characteristics by lowering the production of NO, ROS, secretion of IL-1, and IL-2, NO synthase, cyclooxygenase 2, and macrophage induced signaling pathways. The additional presence of ascorbic and glucuronic acid stimulates the antioxidant activity of the alginate oligosaccharides. Sodium alginates are commonly used for the extraction of these alginate oligosaccharides. These oligosaccharides are widely utilized as natural antioxidants in the dairy and food industries apart from nutraceuticals to increase the shelf life or longevity of food (Falkeborg et al., 2014; Szekalska et al., 2016).

7.3.6 NUTRACEUTICAL POTENTIAL OF SEAWEED PROTEINS

7.3.6.1 COMPOSITION, SOURCES, AND EXTRACTION

Seaweeds are also known as a valuable source of amino acids and proteins ranging from 5–47% and 42–48% on a dry weight basis respectively which are identified to exhibit several beneficial nutraceutical potentialities essential for human health (Shannon and Abu-Ghannam, 2019). The algal proteins are mostly identified as glycoproteins (conjugation of protein and carbohydrates) that occurs in seaweed or macroalgal cell wall (Admassu et al., 2018). The protein constitution of a few macroalgal seaweeds is similar to that of the traditional or classical sources of protein like egg, meat, milk, and terrestrial plant sources (legumes, lentils, and soybean). Moreover, microalgae also exhibit similar or higher protein content up to 71% dry weight compared to soybean and wheat having protein content up to 70% and 80% dry weight respectively (Kumar and Brown, 2013). The digestibility and the composition of amino acid regulate the nutritional quality of proteins (Bleakley and Hayes, 2017). Similar to other biomolecules the protein composition or content in seaweeds differs with the ecology, species, and abiotic factors. Among the macroalgae, the red seaweeds have been identified with high protein content in comparison to green and brown seaweeds, and thus follows the series (from high to low protein content): red seaweeds (20–35% dry weight) > green seaweeds (10–20% dry weight) > brown seaweeds (less than 10% dry weight) (Ganesan et al., 2019). The biological importance of the seaweed protein relies on the presence and ratio of essential amino acids (EAA) and non-essential amino acids (NEAA) (Cotas et al., 2020). The similar EAA found in macroalgae and animals is valine, threonine, leucine, isoleucine, methionine, tryptophan, phenylalanine, lysine, arginine, and histidine (Kumar and Brown, 2013). The essential amino acids may range up to 50% dry weight of total amino acids in seaweeds differs within the species (Cotas et al., 2020). In contrast to the animal and macroalgal proteins, the presence of insoluble polysaccharides in plants makes their proteins less digestible

(Bleakley and Hayes, 2017). Few other amino acids for example lysine, tryptophan, isoleucine, cysteine, and leucine are found comparatively in fewer amounts in seaweeds depending upon the algal species. However, other than glutamic acid, essential amino acids, and aspartic acid are profusely present in the seaweeds. Moreover, the biological and structural properties of seaweed amino acids and proteins are still unclear as well as less documented (Cian et al., 2015). Few amino acid-like compounds such as chondrine, citrulline, aminobutyric acid, D-homocysteic acid, laminine, baikiain, N,N,N-trimethyltaurine, pyrrolidine-2,5-dicarboxylic acid, 3,5-diiodotyrosine, 3,5-diiodothyronine, pipecolic acid, N-methylmethionine sulfoxide, rhodoic acid, taurine, ornithine, and kanic acid are also identified in few seaweeds (Harnedy and FitzGerald, 2011). The macroalgal seaweed amino acid and protein sources include red seaweeds (*Chondrus crispus*, *Porphyra tenera*, *Porphyra columbina*, *Palmaria palmata*, *Pyropia acanthophora*, *Laurencia*, and *Hypnea* species), green seaweeds (*Ulva pertusa*, *Ulva amoricana*, *Ulva lactuca*, *Ulva fasciata*, *Cladophora rupestris*, *Enteromorpha intestinalis*, etc.), and brown seaweeds (*Laminaria hyperborea*, *Laminaria digitata*, *Saccharina latissima*, *Alaria esculenta*, *Ascophyllum nodosum*, *Fucus vesiculosus*, *Himanthalia elongata*, etc.) (Harnedy and FitzGerald, 2011; Cian et al., 2015; Lafarga et al., 2020). Other than essential amino acids, the glutamic and aspartic acids are profusely present in the red and green seaweeds such as *Codium* sp., *Caulerpa* sp., *Ulva* sp., *Acanthophora spicifera*, *Agalothamnion uruguayense*, *Gracilariopsis tenuifrons*, *Cryptonemia seminervis*, and *Laurencia flagellifera*. The protein content of the seaweeds also differs with the different extraction procedures and techniques which makes the determination of the exact protein composition difficult (Lafarga et al., 2020). Various conventional algal protein extraction procedures mostly include physical extraction methods (mechanical grinding, ultrasonication, and osmotic shock) and enzymatic hydrolysis methods (polysaccharide-mediated hydrolysis). However, both the conventional extraction methods are now not being used commercially due to several limitations such as time-consuming, laborious, and proteases release which imparts a negative effect on protein structure and stability. However, current algal protein extraction is done using various new advanced technologies involving pre-treated algal cell wall disruption method which improves the protein bioavailability are: UAE, MAE, subcritical water extraction (SWE), supercritical fluid extraction (SFE), pulsed electric field (PEF), and membrane filtration extraction (MFE) (Bleakley and Hayes, 2017). After the extraction procedure, the functional peptides are isolated by hydrolyzing the crude proteins. These functional peptides are further purified by using different chromatographic techniques (gel-permeation chromatography, ultrafiltration membrane chromatography, mass-mass spectrophotometry, and liquid chromatography-mass spectrophotometry) and coupled enzymatic hydrolysis and membrane separation (CEH-MS) method to obtain the specific bioactive peptides and amino acids of interest classified based on molecular weight (Admassu et al., 2018). The bioactive peptides of seaweeds with less knowledge are majorly known to exhibit antioxidant and antihypertensive properties as well as identified as an essential component in the treatment of other metabolic syndromes.

7.3.6.2 NUTRACEUTICAL APPLICATION OF PROTEINS

The seaweed bioactive peptides exhibit antioxidant properties commonly known as antioxidant peptides i.e. carnosine and glutathione which not only arrest the oxidative stress but also demonstrate a positive outcome on the anticancer treatments (Lafarga et al., 2020). The antioxidants scavenge the ROS which is responsible to generate oxidative stress widely associated with causing metabolic diseases which are caused due to various maladaptive functions such as vasoconstriction, atherosclerotic plaque development, endothelial dysfunction, and low-density lipoprotein development (Cheung et al., 2015). Several commercialized enzymes like Protamex, Alcalase, Neutrase, and Flavourzyme are used in the antioxidant protein hydrolysates production from *Ishige okamurae*, *Scytosipon lomentaria*, *Ecklonia cava*, and several *Sargassum* species exhibited high oxygen scavenging potentiality which is purified by chromatographic techniques (Lafarga et al., 2020). The extracted protein substrates of *Palmaria palmata* hydrolyzed with Corolase and Alcalase exhibited higher antioxidant potentiality on generating hydrolysates from alkaline and combined alkaline-aqueous treatment which demonstrated increased oxygen radical absorbance capacity (ORAC) assay values in contrast to the aqueous hydrolysates with no antioxidant activity (Harnedy and FitzGerald, 2013). The chemical antioxidants like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propylgallate, tert-butyl hydroquinone (TBHQ), etc. although possess the higher antioxidant property but impose chemical toxicity to human health in comparison to seaweed peptides. The antioxidant property of the bioactive peptides depends on the molecular weight and structural stability (Admassu et al., 2018). The highest antioxidant property or potentiality of seaweed peptide was identified against hydrogen peroxide (H_2O_2) which was even greater than chemical antioxidants. LMW pepsin hydrolysate from *Costaria costata*, *Enteromorpha prolifera*, *Grateloupia*, and *Porphyra tenera* is also known to exhibit antioxidant potentiality (Harnedy and FitzGerald, 2011). The seaweed proteins have been demonstrated to exhibit antihypertensive activity which counteracts or inhibits the ACE activity as a result acts as a potential therapeutic approach in regulating the blood pressure to prevent CVDs. The ACE-inhibitory peptides are isolated from seaweeds either by hot water extraction or using proteolytic enzymes which restrict the angiotensin-I conversion to angiotensin-II (Pangestuti and Kim, 2013). The seaweed bioactive peptides are also known to counteract the enzymatic activity of renin, ACE-I along with the enzymes that take part in renin-angiotensin-aldosterone-system (RAAS). The stability and functionality of the ACE-I inhibitory peptide hydrolysates are temperature-dependent (Lafarga et al., 2020). Protein hydrolysate from *Palmaria palmata* has been identified to exhibit several renin inhibitory peptides that have high ACE counteracting potentiality. These inhibitory bioactive peptides isolated using Alcalase, Colorase, and Flavourzyme from the alkaline, aqueous, and combined alkaline-aqueous treatment exhibits ACE-I inhibiting activity to prevent hypertension. Several murine models have been identified to demonstrate positive results on bioactive peptide administration concerning angiotensin inhibition. For example, hot-water extracted bioactive inhibitory peptides from *Undaria pinnatifida* exhibited ACE inhibition along with reduced blood pressure against spontaneously hypersensitive rats (SHRs) on oral dosage administration. Diabetes mellitus (DM) is a type of metabolic syndrome or disorder that mainly occurs due to the lack of insulin production as a result causes dysregulation in the blood glucose level (Admassu et al., 2018). Long-term hyperglycemia may end up with various severe complications and metabolic dysfunctions such as renal failure, diabetes, Alzheimer's disease, etc. The DM is majorly categorized into two types i.e. type-1 and type 2 DM. The non-insulin-dependent type 2 DM is related to the activation of RAAS which elevates the blood pressure causing complications in the patients with acute hyperglycemic conditions. Therefore, by arresting the RAAS activity the hyperglycemic conditions can be prevented (Gunathilaka et al., 2020). Moreover, enzymes like α-glucosidase and α-amylase are involved in the hydrolysis of glucose molecules which are further absorbed by our body system causing hyperglycemia. Thus inhibiting the activity of this enzyme by introducing glucose-independent-insulinotropic polypeptide (GIP) and glucagon-like-peptide-1 (GLP-1) which has been demonstrated to regulate the glucose level in the blood (Admassu et al., 2018). Other than GLP-1 and

GIP, the Alcalase and Corolase enzyme-treated protein hydrolysate extracted from *Palmaria palmata* with alkaline solvents exhibited dipeptidyl peptidase IV (DPP IV) inhibitory activity which is essential in regulating insulin production (Harnedy and FitzGerald, 2013). The bioactive peptides from other marine organisms or sources are known to possess enormous nutraceutical properties such as anti-diabetic properties, antimicrobial, antiviral, antihypertensive, immunomodulatory, anticarcinogenic, and antioxidant activities (Cheung et al., 2015). However, the bioactive peptides and peptide hydrolysates from seaweeds remain less explored in comparison to polysaccharides with little knowledge in antioxidant, antihypertensive, antidiabetic properties.

7.3.7 NUTRACEUTICAL POTENTIAL OF SEAWEED LIPIDS

The lipid profile of seaweed depends on species, geographical location, temperature, light intensity, salinity, nitrogen level in the marine environment as well as the season and does not surpass more than 5% of dry weight (w/w) (Hentati et al., 2020). Generally, lipid in seaweeds is composed of fatty acids with an even number of carbon atoms in a linear chain with one or more double bonds (Chandini et al., 2008b). Fatty acids are mainly categorized into three classes: (i) Saturated fatty acids (SFAs), (ii) Monounsaturated fatty acids (MUFAs), and (iii) PUFAs. Palmitic acid and myristic acid are the principal saturated fatty acids possessed by seaweeds (Shannon and Abu-Ghannam, 2019). SFAs and MUFAs are synthesized by the human body; however, PUFAs which cannot be synthesized by humans are commonly known as essential fatty acids (EFA) and has to be obtained from the food (Mišurcová et al., 2011). PUFAs of macroalgae include eicosapentaenoic acid (EPA), C20:5 n-3; docosahexaenoic acid (DHA), C22:6 n-3; linoleic acid (LA), C18:2 n-6; α-linolenic acid (ALA), C18:3 n-3; arachidonic acid (AA), C20:4 n-6; and octadecatetraenoic acid (SDA), C18:4 n-3 (Hentati et al., 2020). EFA is the PUFAs having the first double bond on C3 and C6 carbon atom from the methyl-end based on which they are categorized into ω-3 (n-3) and ω-6 (n-6) fatty acids. The primary precursor of ω-3 fatty acid is ALA and that of ω-6 fatty acid is LA (linoleic acid) (Mišurcová et al., 2011). Comparatively, lipid content is less than 1% in warm water seaweeds than cold water seaweeds with 1.6% lipid content. Warm water seaweeds comprise more of SFAs. Unsaturated fatty acids, particularly PUFAs are present in an enormous amount in cold water seaweeds (Chandini et al., 2008b). Macroalgae are exclusively an abundant source of long-chain PUFAs due to the presence of specific enzymes employed for the desaturation process (Cotas et al., 2020). Since seaweeds are underexploited, they can emerge as a favorable substitutional source of ω fatty acids while considering the primary sources (higher plants and marine organisms) to be a limited future bioresource due to over-harvesting (Jayasinghe et al., 2018). Along with the consumption of EFAs, it is important to maintain the ratio of ω-6:ω-3 as the mammalian cells lack the enzyme ω-3 desaturase required for conversion of ω-6 to ω-3 fatty acids. Nowadays, the balance between ω-3 and ω-6 fatty acids gets disturbed due to the increase in the intake of ω-6 fatty acids from the diet as it worked together to maintain a healthy metabolic profile, preventing several chronic inflammatory diseases. An ideal ω-6:ω-3 ratio is 1. However, there is an excessive ω-6 fatty acid ingestion in Western countries with an imbalanced ratio of 20:1. The ω-6:ω-3 fatty acid ratio in macroalgal seaweeds lie within 2.5:1 and 4:1, preventing chronic diseases and serving as an excellent source of dietary lipids (Mišurcová et al., 2011; Shannon and Abu-Ghannam, 2019). According to WHO guidelines, the ratio of n-6:n-3 in the diet must be lower than 10 (Hanjabam et al., 2017). Among seaweeds, brown seaweeds contain an abundant amount of PUFAs followed by green, and red in the order: brown seaweed > green seaweeds > red seaweeds (Ganesan et al., 2019). The species of red macroalgae belonging to the order Gigartinales, Corallinales, and Gracilariales contain a large diversity of EFAs (Cotas et al., 2020). Nevertheless, brown and red seaweeds like *Undaria pinnatifida*, *Cryptonemia undulata*, *Rhodymenia sonderi*, *Gracilaria corticata*, *Pyropia* sp., *Halymenia* sp., *Polysiphonia* sp., *Acanthophora* sp., *Gelidium* sp., and *Gelidiella* sp. produce a huge quantity of long-chain PUFAs such as EPA and AA while species of green seaweeds like *Ulva*, *Monostroma*, *Bryopsis*, *Caulerpa*, *Udotea*, and *Acrosiphonia* produce high levels of ALA (C18:3 n-3) (Kumari et al., 2013). Apart from the above, red seaweeds also produce an oxygenated derivative of fatty acid, oxylipins (e.g., eicosanoids), which is accountable for regulating cell differentiation, homeostasis, and immune responses (Baweja et al., 2016). Along with *Undaria* and *Ulva* which contain high levels of stearidonic (ω-3) and hexadecatrienoic (ω-6) acids, species of *Ulva*, *Acanthophora*, and *Gracilaria* seaweeds are also rich sources of ω-3 fatty acids with *Ulva* containing the precursor of ALA, EPA, and DHA (Baweja et al., 2016; Ganesan et al., 2019). Several previous studies have reported that *S. wightii*, *S. turbinaria*, *K. alvarezii*, and *U. lactuca* possess a considerable amount of DHA, LA, and AA with LA being the most abundant one in all the species (Jayasinghe et al., 2018). Various lipid extraction methods are employed for the extraction of PUFAs like supercritical fluid extraction, enzyme-assisted extraction, microwave-assisted extraction, and ultrasound-assisted extraction. Industrially, lipids are extracted using mechanical stress and hexane leaching process (Cotas et al., 2020).

Sterols are amphipathic lipids from a subgroup of steroids with an –OH (hydroxyl) group at the C3 position and a branching chain at the C17 position of a carbon atom. Cholesterol, fucosterol, isofucosterol, and clionasterol are the main nutritional sterols present in macroalgae (Hentati et al., 2020). Cholesterol is a major constituent of red seaweeds like *Chondrus crispus* (Irish moss), *Sargassum* spp., *Laurencia papillosa*, *Polysiphonia brodiei*, *Chondria collinsiana*, *Ceramium* spp., *Corallina* spp., *Bangia fuscopurpurea*, *Gracilaria salicornia*, and *Hypnea flagelliformis* (Kumari et al., 2013). Among the derivatives of cholesterol like cholesta-4,6-dien-3-ol, desmosterol, and cholest-5-ene-3,7-diol, desmosterol is present in a considerable amount in *Porphyra* sp. and *Palmaria* sp. (Kumari et al., 2013; Cotas et al., 2020). Fucosterol is a major sterol in brown seaweeds like *Laminaria digitata*, *Ascophyllum nodosum*, *Fucus serratus*, *Laminaria ochroleuca*, *Undaria pinnatifida*, *Himanthalia elongata*, *Fucus spiralis*, *Cystoseira* spp., *Cladostephus spongiosus*, *Stypocaulon scoparium*, *Padina gymnospora*, *Hormophysa triquetra*, *Asparagopsis armata*, *Plocamium cartilagineum*, and *Osmundea pinnatifida*. Green seaweeds have isofucosterol as dominant sterol in *Ulva* spp. and *Enteromorpha* spp., while clionasterol is a dominant sterol in *Bryopsis plumosa*, *Caulerpa* spp., and *Spyridia filamentosa* (Kumari et al., 2013). The extraction process for sterol involves conventional solid-liquid extraction or the advanced supercritical CO_2 extraction methods. The solid-liquid extraction method using a single solvent, or solvent mixtures (chloroform-methanol, hexane, methyl chloride, and acetone) is used only for free sterols. Though the advanced extraction process using supercritical CO_2 is not frequently used, however, it carries several advantages of being non-toxic, chemically inert, cost-effective, and readily available (Lopes et al., 2013).

7.3.7.1 NUTRACEUTICAL APPLICATION OF LIPIDS

The absence of the enzyme Δ^{12} and Δ^{15} desaturases in the human body compels humans to obtain ALA from the diet. Since EFA of long-chain PUFAs is a mandatory dietary intake for the efficient activity of the human body, seaweeds could be a better source of EFAs (EPA, DHA, AA) with low caloric content. A balanced dietary intake of ALA and LA is vital not for their ratio but for conversion to EPA and DHA and is required for the maintenance of homeostasis. Inhibition of the enzyme cyclooxygenase-2 (COX-2) by EPA and DHA prohibits the synthesis of prostaglandin E_2 (PGE_2), responsible for cancer initiation (Mišurcová et al., 2011). Apart from immunomodulation and brain development, EPA and DHA moreover play a significant role in cellular signaling, regulating transcription factors, preventing cardiovascular disease, cancer, neurodegenerative and autoimmune diseases (Cotas et al., 2020), hypertension, Alzheimer's disease, and improve coronary artery disease. DHA also exists in the cell membrane of brain tissue and retina (Mišurcová et al., 2011). Both DHA and AA form crucial components for the brain and eye in human infants. For maintaining a good metabolic profile in the human body, a balanced ω-6:ω-3 fatty acid ratio is vital for decreasing cardiovascular mortality rate, arthritis-associated inflammation, and cancer. PUFAs, representing ω-3 and ω-6 fatty acids own several health benefits from regulating blood clotting and blood pressure to averting chronic diseases (diabetes, arthritis, and obesity) and modulating microglia signaling (Barbalace et al., 2019). PUFAs present in *Ulva* also reflects strong antioxidant activity. Species of *Ulva*, *Acanthophora*, *Gracilaria* with abundant ω-3 fatty acids have reported anti-hypertensive, anti-hyperlipidemic, anti-inflammatory effects. Nevertheless, they also curb the action of ACE responsible for converting angiotensin I to angiotensin II (Ganesan et al., 2019). Polyunsaturated ω-3 fatty acids have cardioprotective effects due to their stimulating effect on concentrations of lipoprotein, the function of membrane enzymes, and receptors, the fluidity of biological membranes, regulation of blood pressure, and metabolism of minerals. It also exists as ligands of peroxisome proliferator-activated receptors (PPAR-α) that are supposed to decrease lipogenesis, the formation of very-low-density lipoprotein (VLDL), and elevate reverse transport of cholesterol. Prostaglandins (PG), thromboxanes (TX), leukotrienes (LK), and prostacyclins are the eicosanoids derived from ω-3 and ω-6 fatty acids showing antagonistic effects (Mišurcová et al., 2011). Sterols are the membrane components that act as precursors of plant and animal hormones. Seaweed sterols can be used in nutraceutical products as anti-cholesterol additives. Several compounds of sterols have biological properties like antioxidants, antiviral, anti-cancer, anti-obesity, anti-fungal, anti-bacterial, antitumoral, and cardioprotective ability which can be utilized in nutraceutical products (Cotas et al., 2020). Similarly, fucosterol prohibits adipocyte differentiation and lipid formation.

7.3.8 NUTRACEUTICAL POTENTIAL OF OTHER SEAWEED BIOACTIVE COMPONENTS

Nutraceuticals are an essential component of functional foods that are recommended for daily food consumption and have nutritional and medicinal benefits. Seaweeds are highly nutritious and are a good resource of numerous bioactive compounds. Around 25,000–30,000 species of seaweed have been reported with a wide range of shapes and sizes (Charoensiddhi et al., 2017), and are classified into various classes based on their pigmentation (Chandini et al., 2008a). Across the globe, approx. 150 species of seaweeds are used as a source of food and more than 100 species for the extraction of phycocolloid (Chandini et al., 2008b). A wide range of bioactive compounds are present in seaweeds, many of which have commercial uses in the pharmaceutical, nutraceutical, food, cosmetic, medical, and agricultural industries. Over 15,000 novel compounds, many of which have bioactive properties have been isolated from these seaweeds (Kelman et al., 2012). In comparison to cultivated vegetables, seaweeds are very good sources of bioactive compounds like carotenoids, antioxidants, fibers, proteins, vitamins, essential fatty acids, minerals, and also various primary and secondary metabolites such as phycobilins, sulfated compounds, phenolic compounds, non-starch polysaccharides, vitamins, and certain trace elements (Chandini et al., 2008b; Jaswir et al., 2011).

7.3.8.1 ANTIOXIDANTS

Oxygen radical species like superoxide, peroxyl radicals, and hydroxyl are formed by endogenous factors in human body tissues, and externally cause substantial oxidative stress, which results in cancer, aging, and a broad range of other human diseases (Chandini et al., 2008a), to get rid of these harmful free radicals, the inherent defense mechanism, containing many antioxidants, has been developed to combat oxidative stress in different species. The idea – "antioxidant can be used exogenously to improve the internal defense mechanism in the fight against oxidative stress" has shown the new path in oxidative stress-mediated disease therapy. Even when the amount of the substance is less than the substance to be oxidized, the antioxidants effectively delay or prevent the adverse effect caused due to free radicals. Now a day's synthetic antioxidants are being used nevertheless, it can be assumed that these chemically synthesized antioxidants can cause several harmful effects such as liver damage and carcinogenesis. Because of these adverse effects, consumers are becoming more aware of fitness and researchers are discovering natural antioxidant alternatives that are safe and effective for use in medicinal materials and nutraceuticals, which can substitute the synthetic antioxidants (Munir et al., 2013). Natural antioxidants are essential bioactive compounds that play a significant role in fighting against different diseases and slowdowns aging processes by protecting cells from oxidative harm (Kelman et al., 2012). Numerous genera of algae have been described to produce antioxidants, including *Polysiphonia*, *Ahnfeltiopsis*, *Colpomenia*, *Hydroclathrus*, *Gracilaria*, *Halymenia*, *Laurencia*, *Padina*, and *Turbinaria* (Sonani et al., 2017). The antioxidants obtained from seaweeds have been shown in Figure 7.1.

7.3.8.1.1 Nutraceutical Application of Antioxidants

Among the compounds present in macroalgae, compounds with antioxidant activity have very good potential for use in the foodstuffs industry as well as in the pharmaceutical companies, these compounds mitigate some damage caused by free radicals. Because of the existence of these compounds, macroalgae have other health benefits too and for that reason, they may be used in functional foods or as nutraceuticals. Because of the growing demand by the nutraceutical and pharmaceutical companies to develop natural bioactive compounds such as anti-aging and anti-cancerous compounds that display quantifiable benefits for health. The activity of the antioxidant compounds has become an interesting subject and a topic of rigorous study, several species of seaweed have scavenging potential for hydrogen peroxide (Vijayabaskar et al., 2012), also these have been claimed to have advantageous health functions for delaying aging and preventing inflammatory, cardiovascular, neurological diseases, as well as carcinogenic damage (Wang et al., 2010). It has also been observed by some experiments that antioxidant compounds have antiatherogenic properties which help in lowering cholesterol levels in rabbits, hamsters, and rats and in preventing oxidative damage and apoptosis in cardiomyocytes (Shannon and Abu-Ghannam, 2019). As antioxidants and free radical scavengers, sulfated polysaccharides which have been extracted from a member of Phaeophyta play an important role in thwarting oxidative damage to living organisms (Vijayabaskar et al., 2012), whereas deterioration of food is also prevented by antioxidant-rich polysaccharides (de Souza et al., 2007). Recently, particular interest has been developed and attention

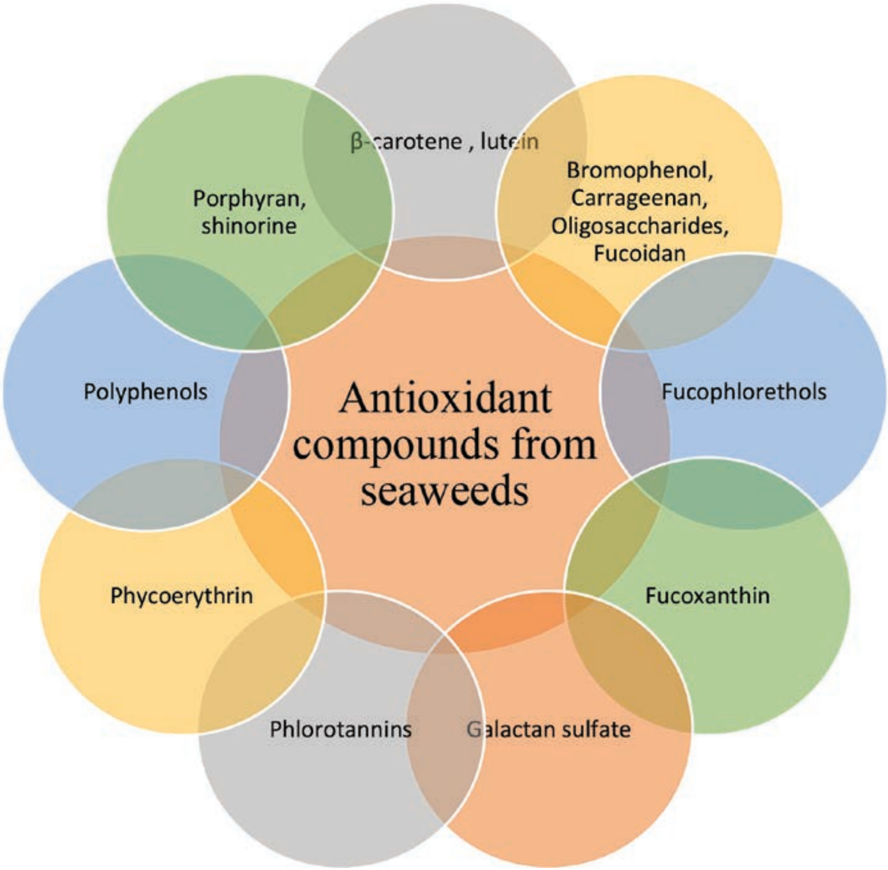

FIGURE 7.1 Antioxidant compounds reported from seaweeds.

has been paid to various antioxidant properties of carotenoids which can reduce the chances of a few chronic disorders. Carotenoids also defend cells from oxidative damage by quenching singlet oxygen damage using various mechanisms (Michalak and Chojnacka, 2015). In cosmetics or food industries, natural antioxidant constituents are very important due to their ability to minimize free radical-mediated degradation of cells and tissues in living organisms (Wang et al., 2010).

7.3.8.2 MINERALS

In the lifecycle of macroalgae, minerals play a very important role as the minerals are biosynthesized by the macroalgae but rather these are absorbed from the external environment, depending on various factors such as salinity, pH, temperature, and light. Seaweeds are generally known to have high mineral content as compared to vegetables, they have 10–100 times rich mineral content as compared to traditional vegetables due to their superb bio-absorption and bioaccumulation power (Holdt and Kraan, 2011; Salehi et al., 2019). Based on phylum, season, environment, geography, physiology, and harvesting season the mineral composition of seaweeds get changed (Chandini et al., 2008b; Hamed et al., 2015; Salehi et al., 2019). Ash of marine algae contains different kinds of minerals absorbed by them which constitutes more than 30%-55% of the dry weight of seaweed (Holdt and Kraan, 2011). For that reason alone, all the necessary minerals and trace elements needed for the human diet occur in macroalgae and this is considered a valuable functional food. These seaweeds are rich in the contents of Ca, Mg, P, K, Zn, Na, and Fe (Chandini et al., 2008b; Becker, 2013; Salehi et al., 2019). The daily intake of certain trace minerals (Fe, Zn, Mn, and Cu) and major minerals (Na, K, Ca, and Mg) also known as dietary supplements were obtained from seaweeds *viz.* some Phaeophyceae (*Sargassum* and *U. pinnatifida*), and some Rhodophyta (*Gracilariopsis* and *Chondrus crispus*) (Chandini et al., 2008b; Hamed et al., 2015), the total amount of Na, Mg, Ca, and K ranges from 6.6 to 8.9% in Chlorophyceae; 9.7 to 20.7% in Phaeophyceae; and 7.8 to 8.9% in Rhodophyceae (Salehi et al., 2019). There are also relatively high levels of Na and K, although the Na:K ratios are typically below 1:5 (MacArtain et al., 2007). Seaweeds have a higher Ca content as compared to that of P, which is similar to *Aloe vera* and sage content (Salehi et al., 2019). The extraordinary K level of all seaweeds is very close to our normal plasma level (Chandini et al., 2008b).

7.3.8.2.1 Nutraceutical Application of Minerals

The iodine content is very high in these seaweeds as compared to other higher plants (MacArtain et al., 2007; Chandini et al., 2008b; Hamed et al., 2015), also it have been found that in comparison to marine fish like cod the iodine content is thousand times higher in various seaweeds. In brown algae, iodine content reached about 1.2% of its dry weight. Right mineral consumption is essential for maintaining a nutritionally sound diet. Thus, it reduces the chances of illnesses that may be caused due to nutritional deficiency. Seaweeds are also a good source of di-iodothyronine (DIT) which is a precursor to essential thyroid hormones thyroxin (T4) and triiodothyronine (T3) *Laminaria japonica* is a very good source of iodine which has been used as the dietary supplement of iodine in China for centuries to avoid goiter and has been used for medicinal and nutritional purposes in China, Japan, and Korea for over 1,500 years. For the extraction of iodine in Ireland the kelps were used as a source of raw material since the seventeenth century. Among all living systems, species belonging to the genus *Laminaria* are excellent iodine accumulators, which can accumulate iodine up to 30,000 times more as compared to the surrounding environment. In some countries where marine algae are commonly used as foods, excessive intake of seaweeds may cause hypothyroidism, goiter, and Hashimoto's thyroiditis, while iodine-induced thyrotoxicosis tends to be uncommon after intake of seaweed-containing nutritional supplements (Holdt and Kraan, 2011).

In marine algae, the trace elements such as zinc are found, and these have harmful health effects, such as arsenic. Further examination of the speciation in the case of arsenic, suggests that the kind of arsenic is important in the assessment of toxicity; as certain forms of arsenic can not be metabolized, they do not cause health-related issues (Holdt and Kraan, 2011). During pregnancy and lactation, Calcium plays an important role in shaping the bones and keeping the bones solid, as in these stages the demand for calcium is high. Calcium is also important for the proper growth and preservation of calcium-containing dental tissues. If Calcium requirements are not fulfilled by dietary consumption, many diseases such as hypocalcium symptoms and osteoporosis would be caused by the lack of this mineral (Hamed et al., 2015). The content of Mn varies notably among the species in three algae groups with the highest content in red seaweed (*Agarophyton vermiculophyllum* 392 mg/kg d.w.); green seaweed (*Ulva intestinalis* 180 mg/kg DW); and brown seaweed (*Fucus serratus* 150 mg/kg DW). Bromine is an important element in tissue growth and the building of the basement membrane and has recently been recognized as an essential element in tissue development (Salehi et al., 2019). The level of heavy metals is naturally below food safety limits for the majority of seaweeds (MacArtain et al., 2007). Due to the higher capacity of accumulation of environmental elements present in surrounding and increasing the pollution-related problems in the marine environment because of the increasing concentration of unwanted elements such as arsenic, cadmium, mercury, etc. it is accepted to restrict the use of these seaweeds in applications as nourishing ingredients (Salehi et al., 2019).

7.3.8.3 VITAMINS

As photosynthesis can be carried out by several marine algae, they can synthesize all the vitamins that are synthesized by terrestrial plants (Chandini et al., 2008b). Vitamins are generally required by the human body for various vital biochemical and physiological functions essential for several life processes (Hamed et al., 2015). Microalgae biomass represents an important source of both essential water- and fat-soluble vitamins (MacArtain et al., 2007; Becker, 2013; Hamed et al., 2015). The vitamin composition is generally determined by several factors which include species, geographic area, season, environmental parameters, post-harvesting treatment, and method for drying (Norziah and Ching, 2000; Chandini et al., 2008b; Becker, 2013; Hamed et al., 2015). Several reports confirmed that 100 g seaweed provides more than the daily requirement of essential vitamins such as vitamins A, B2, B12, and nearly two-thirds of the vitamin C requirement (Hamed et al., 2015). Red seaweeds such as *Palmaria palmata* and *Porphyra tenera* contain large amounts of provitamin A and considerable quantities of vitamins B1 and B2 (Chandini et al., 2008b). Vitamin B12 (cobalamin) is majorly present in higher concentrations in Chlorophytes and Rhodophytes than in Phaeophytes (Hamed et al., 2015). One unique difference between terrestrial plants and marine algae is that the latter contains a relatively more quantity of vitamin B12. Significantly, in *Porphyra* the content of vitamin B12 is highest as compared to other seaweed (134 μg B12/100 g DW) (Chandini et al., 2008b; Hamed et al., 2015). Vitamin B12 must be assumed to be adsorbed or absorbed by the bacteria closely associated with or grown on algae (Becker, 2013). Vitamin B12 is recommended for the treatment of aging, chronic, anemia, and fatigue syndrome (Hamed et al., 2015).

Vitamin C is present in all species of seaweeds (Hamed et al., 2015). High levels of vitamin C have been observed in certain brown seaweeds (Chandini et al., 2008b). Historically, red algae (*Porphyra*) have been used to prevent scurvy, a disease which is caused due to deficiency of vitamin C. Other benefits of this vitamin include health benefits such as radical scavenging, boosting immunity, and anti-aging activity (Hamed et al., 2015). Other than that, vitamins such as vitamin K, vitamin E (a mixture of tocopherols) as well as metabolic intermediates are found in almost all algal forms (Becker, 2013; Hamed et al., 2015). The brown algae contain higher vitamin E levels than the green and red algae.

7.3.8.3.1 Nutraceutical Application of Vitamins

Vitamins have a range of biochemical roles, including their role as cell signaling mediators, hormones, antioxidants, cell and tissue growth, and differentiation regulators (Holdt and Kraan, 2011). Vitamin D deficit causes many complications, such as infant rickets and adult osteomalacia (Hamed et al., 2015). It has been reported that Nori is 1.5 times richer in vitamin C content as compared to oranges (Salehi et al., 2019). All red, brown, and green marine algae contain vitamin C (ascorbic acid). Historically, red algae (Porphyra) have been used to avoid scurvy caused due to deficiency of vitamin C. Moreover, vitamin C has other health benefits too, which are radical scavenging, immune system strengthening, and anti-aging activity (Munir et al., 2013). Seaweeds are also the source of vitamin B12 available in the form of seaweed vegetables (MacArtain et al., 2007). Vitamin B12 contains cobalt-tetrapyrrole which is related to chlorophyll and heme molecule. Cobalamin is an enzymatic cofactor and deficiency of cobalamins, such as neuropsychiatric disorders and megaloblastic anemia, can cause severe health disorders. To treat the symptoms of chronic fatigue syndrome, aging, and anemia, vitamin B12 is recommended (Munir et al., 2013). Members of Phaeophyceae contain a large amount of Vitamin B whereas brown seaweeds are being used for treating conditions such as thyroid, goiters (Salehi et al., 2019). A mixture of tocopherols is a kind of vitamin E. α-tocopherol is the only known type to present in all seaweed species. There are also β- and γ-tocopherols in Phaeophyceae (Chandini et al., 2008b; Hamed et al., 2015). These vitamins are known for the action of its antioxidants, also α- and γ-tocopherols are known to prevent CVDs. Some microalgae, like Spirulina, make them particularly suitable as nutritional supplements for vegetarian individuals as they are rich in vitamin B12 and iron content (Munir et al., 2013).

Many seaweed companies manufacture different products used for various purposes such as medicine, food, and cosmetics. Companies such as Acadian Seaplants Limited supply nutrient enrich foods and many other companies along with their products, source of product, and uses of product is mentioned in Table 7.1.

7.4 CONCLUSION

In the modern scientific world, seaweeds have occupied the most attractive sources of natural bioactive components by exhibiting huge potentiality in the field of functional foods and nutraceutical applications (Cotas et al., 2020). The extraction and usage of these seaweed bioactive components is a feasible technological approach (Torres et al., 2019). The most available seaweed component is a polysaccharide (sulfated and non-sulfated) having enormous bioactive properties with huge structural diversity whose occurrence and composition are dynamic depending upon species, ecological, and abiotic factors. However, very few commercial products are available with poor clinical evidence. Most of the components are either tested in vitro or in vivo (animal models) with very less knowledge of human clinical trials (Jönsson et al., 2020). Several research papers have discussed the disease counteracting ability of seaweed polysaccharides. In the future, the polysaccharides potentiality should be studied in vivo with proper human clinical trials. The increase in research must reflect good commercialization. Apart from nutraceuticals, seaweed polysaccharides can be extensively used in the field of pharmaceutics including biomedical applications and cosmeceuticals (Tanna and Mishra, 2019).

Seaweeds have been identified to exhibit protein content with nutraceutical properties. The protein content of the seaweeds differs within their families. The presence of different bioactive peptides in the seaweed proteins makes their importance in nutraceutical potentialities. The disadvantage of these peptides extraction relies on extracting procedures. However, several novel advanced extraction procedures have been developed to overcome the limitation which limits the consumption of heat or solvent during extraction (Bleakley and Hayes, 2017). Administration of the seaweed bioactive peptides into the foods will not only confer nutraceutical potentialities but will also help to regulate metabolic diseases/syndromes. The lack of seaweed protein utilization relies on the poor knowledge of its complex structural and molecular diversity which must be overcome (Admassu et al., 2018).

Seaweeds altogether contribute to a low-fat diet with enriched nutritive value required for a healthy metabolism. The presence of PUFAs in seaweeds can act as a replacement for fish oils required for obtaining ω-6 fatty acids. Since fish oils have an undesirable taste and oxidative nonstability, seaweeds could be a boon for vegetarians to obtain a balanced ω-6 diet (Mišurcová et al., 2011). Macroalgae could be a good source of low-fat foods with a high level of essential PUFAs and EPAs. An increase in the utilization of macroalgae to formulate PUFA rich nutraceuticals should be encouraged as it would reduce the dependency on traditional sources. Seaweed consumption, either directly or as nutraceuticals, should be encouraged among individuals to prevent several cardiovascular dysfunctions and

TABLE 7.1 Global Companies Manufacturing Seaweed-based Nutraceuticals

Name of Company	Available Product	Source of Product	Uses of Product	Source
Acadian Seaplants Limited	Seaweed vegetable	*Chondrus crispus*	Nutrient enriched food, low in calories and rich in iron, magnesium, and fiber.	https://www.acadianseaplants.com/
Cargill Incorporated	Carrageenan	Red seaweeds from the Gigartinales group mainly *Chondrus crispus*.	A texturizer used in various applications such as dairy, confectionery, and personal care products.	https://www.cargill.com/
DuPont de Nemours, Inc.	Hydrocolloids: carrageenan and alginates.	Seaweeds	Many favorite foods, including ice creams, yogurts, and cottage cheese, were thickened using these.	https://www.dupont.com/
Irish Seaweeds	Kelp seaweed capsules.	*Laminaria* sp. and *Fucus vesiculosus*.	Source of iodine.	https://irishseaweeds.com/product/emerald-rma-rest-of-world/
	Triple blend seaweed capsules.	Bladderwrack, sea spaghetti, and dulse.	Triple blend seaweed consists of three seaweeds, sea spaghetti, bladderwrack, and dulse contain vitamin C, D, E, iron, and omega 3.	https://irishseaweeds.com/product/emerald-rma-europe/
	Dulse capsules	Red seaweeds	All the rarer nutrients like B12 and zinc, natural vitamins, minerals, selenium, proteins, antioxidants, essential phenols, fatty acids, enzymes, trace elements, amino acids.	https://irishseaweeds.com/product/dulse-capsules/
	Hamper, seaweed bath, seaweed facial wrap, seaweed powder bath soak and body.	Seaweeds	The benefits of a seaweed bath can be beneficial for psoriasis, acne, muscle pain, back aid, rheumatism relief, arthritis, and circulation. Seaweed baths are useful for hair and skin, leaving a fresh "glowing feeling."	https://irishseaweeds.com/product/christmas-hamper-seaweed-bath-facial-edible-dulse-herbs-triple-blend/
	Irish Moss (*Chondrus crispus*) and Irish Spirulina Capsules (*Ulva lactuca*, *Spiralis*)	*Chondrus crispus* and *Ulva lactuca*	Rich source of calcium, B12, iron, iodine, zinc, and magnesium.	https://irishseaweeds.com/product/irish-moss-irish-spirulina-capsules-*chondrus-crispus*-and-lactuca-spiralis/

TABLE 7.1 *(Continued)*

Name of Company	Available Product	Source of Product	Uses of Product	Source
Beijing Leili Marine Bioindustry Inc. (Leili Group)	Fertilizers	Seaweeds	Seaweed fertilizer, potassium humate, humic acid, amino acid, microelements.	https://leilichina.en.china.cn/
Mara Seaweed	Mineral nutrients	*Palmaria palmata*, *Laminaria digitata*, *Saccharina latissima*	Iodine, calcium, potassium, magnesium, fiber, iron, protein in form of flakes.	https://maraseaweed.com/
Qingdao Gather Great Ocean Algae Industry Group (GGOG)	Various industrial products, seaweed food, and fertilizers.	Seaweeds	Sodium alginate, seaweed food, fertilizers, mannitol, iodine, alginic acid, agar-agar, carrageenan.	http://en.judayang.com/

metabolic syndromes, and also to increase its commercialization (Kumari et al., 2013).

Major bioactive compounds providing benefits to the human body are provided by marine resources. They can be used in many industries, such as the food, cosmetics, and drug industries. As they are readily available and have the potential to prevent and cure some diseases, functional foods can easily be produced from marine products. Various kinds of seafood are consumed as nutritionally beneficial food as these are low in calories and have a high concentration of minerals, vitamins, proteins, and indigestible carbohydrates. The sea provides an immense resource for discovering new compounds and is known to be the largest remaining repository of natural molecules that can be used in the food industry as functional ingredients. Consequently, efforts should be made to develop marine functional foods responsibly, since seaweeds can be considered as futuristically promising plants forming one of the important marine living resources of high nutritional value also their consumption could result in the decrease of the occurrence and gravity of chronic diseases and have high nutritional value.

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- **functional foods**
- **health**
- **macroalgae**
- **nutraceuticals**
- **seaweed bioactive components**
- **seaweed polysaccharides**

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CHAPTER 8

NUTRACEUTICAL APPLICATIONS OF MARINE MACROALGAE TOWARDS DNA INTEGRITY PROTECTION

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ABSTRACT

Marine macroalgae have been consumed as feed and food for centuries, mainly in East Asian countries. Accordingly, epidemiological studies highlighted a linkage between marine macroalgae consumption and lower incidence of diet-related disorders, such as osteoporosis, diabetes, metabolic syndrome and related cardiovascular disorders, or cancer. For this reason, marine macroalgae have been currently defended as functional foods or ingredients. One of the reported beneficial properties is related with the ability to protect the DNA molecule. Indeed, marine macroalgae have been continually described to possess antioxidant activity, showing also

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antigenotoxic, antimutagenic and antiproliferative actions. The beneficial properties of macroalgae towards the integrity of DNA have been linked to their rich and diverse phytochemical composition. Particularly, sulfated polysaccharides, polyunsaturated fatty acids, phytosterols, pigments, minerals, vitamins, phlorotannins, as well as mycosporine-like amino acids have been associated with those properties. Moreover, the phytochemical profile of macroalgae, and respective bioactive properties, is known to vary due to growing conditions. In this sense, algal farming arises as a controlled and secure growing system and with potential to develop specimens showing higher genoprotective properties.

8.1 INTRODUCTION

The first record of macroalgae usage by man for consumption dates back 14,000 years in Monte Verde, Chile (Dillehay et al., 2008). In certain areas of Europe, mainly in the north, coastal populations were consuming marine macroalgae far back into history, particularly littoral communities suffering from famine. Furthermore, in Norway, *Palmaria palmata* has been used for human food since the Viking Era and in Ireland, *P. palmata*, *Chondrus crispus*, *Mastocarpus stellatus* and *Porphyra umbilicalis* were consumed by the coastal communities. Moreover, records confirm the consumption of macroalgae in Scotland and Wales around the 19th and 20th centuries. Paradoxically, the generalization of the non-native potato cultivation in all European countries decreased the risk of famine for those populations, but as a result, macroalgae use as vegetables in human nutrition became marginal. On the contrary, this is not the case in East Asian countries, where marine macroalgae consumption have been part of their diet since prehistoric times and remains extremely high (Fleurence and Levine, 2016). For example, in Japan, remains of marine algae were found mixed with shellfish and fish in relics of aborigines on prehistoric archaeological sites (Nisizawa et al., 1987). In South Korea, fragments of brown macroalgae have been found in fossilized meals dating back 10,000 years (Pérez, 1997). Nowadays, a great variety of macroalgae is consumed daily by South Korean populations. For instance, South Korean newly mothers consume sea mustard (*Undaria pinnatifida*) soup after childbirth, since it is believed that its content in calcium and iodine improves mothers' milk (Ii, 1999; Fleurence and Levine, 2016). The history of the utilization of marine macroalgae in China is one of the longest and most extensive of any country. Although the actual time they began to be consumed in China may not be identified, there are written records estimating their consumption for over 2,000 years (Xia and Abbott, 1987). Along with Japan and South Korea, China completes the top 3 countries of the largest consumers of marine macroalgae as food (McHugh, 2003). Globally, human consumption of marine macroalgae [of about 150 species (Tiwari and Troy, 2015)] includes raw products, such as in salads, soups, and main dishes, including sushi, as well as in processed form, namely food additives and flavorings, such as in chips and snacks (Fleurence and Levine, 2016).

8.2 MARINE MACROALGAE AS FUNCTIONAL FOODS WITHIN THE CONTEXT OF ANIMAL AND HUMAN NUTRITION

Despite the historical usage and vast research regarding marine macroalgae beneficial properties (Noda, 1993; Patarra et al., 2011; Liu et al., 2012; Yende et al., 2014; Cornish et al., 2015; Fleurence and Levine, 2016), their classification as functional food is relatively recent (Plaza et al., 2008; Holdt and Kraan, 2011; Mohamed et al., 2012; Wells et al., 2017). In this context, various epidemiological studies highlighted an association between marine macroalgae consumption and lower incidence of certain illnesses, namely frequently diet associated diseases as osteoporosis, diabetes, metabolic syndrome and related cardiovascular disorders, and cancer (Fleurence and Levine, 2016).

Backing up these data, recent studies applying *in vivo* and *in vitro* trials disclosed additional beneficial properties of marine macroalgae (Mohamed et al., 2012; Fleurence and Levine, 2016). Accordingly, marine macroalgae extracts or isolated compounds have also shown great neuroprotective activity against neuropsychiatric, as well as neurodegenerative diseases, neuroinflammation, epilepsy or general pain (Mohamed et al., 2012; Fleurence and Levine, 2016). Additionally, several studies have reported the macroalgae properties on organ or tissue regeneration (Mohamed et al., 2012) and their immunomodulatory properties, namely through antiallergic, anti-inflammatory and immunostimulant actions, as well as antimicrobial and antiparasitic activities (Mohamed et al., 2012; Fleurence and Levine, 2016). In addition to those properties, macroalgae can play a crucial role on modulating endocrine system, namely the thyroid activity, due to their high iodine content, which may have repercussions on different endocrine functions, besides being an important source of micro and macronutrients, minerals, and vitamins, essential for growth and well-being (Mohamed et al., 2012; Fleurence and Levine, 2016).

Similarly, although the concept of functional food in the context of animal feed is relatively underapplied and undervalued, it seems reasonable that marine macroalgae could be applied on the livestock, aquaculture, and pet nutrition fields, to enhance animals' general health status. The producers' attention is focused on the animals' growth and feed utilization, as well as the general health condition that can, ultimately, affect production. In fact, there is a growing interest in the market for functional feeds, based on the principle of added-value linked to the health benefits of livestock (Tiwari and Troy, 2015). Recently, research findings showed that animals' diet supplementation with macroalgae, as a source of macronutrients (carbohydrates, protein and fat) and micronutrients (as minerals and vitamins) may bring many benefits apart from the nutritional improvement (Tiwari and Troy, 2015). Specifically, aquaculture is one of the most promising areas regarding the inclusion of marine macroalgae as animal feed. Indeed, some of the aquatic animals raised in aquaculture eat macroalgae naturally (Tiwari and Troy, 2015) and they have been defended as an alternative therapeutic source for aquatic disease management (Thanigaivel et al., 2016). Hence, several studies have demonstrated that the incorporation of marine macroalgae on fish diet may improve immune system, tolerance against diverse stress factors and resistance to diseases. For example, the red sea bream (*Pagrus major*) diet supplementation with the macroalgae *Ascophyllum nodosum*, *Pyropia yezoensis*, or *Ulva pertusa* at 5% of the fish meal resulted in increased body weight, feed utilization, and muscle protein deposition in all three groups (Mustafa et al., 1995). Moreover, 5% incorporation of *Ulva* meal improved growth performance, feed efficiency, nutrient utilization, and body composition of Nile tilapia (*Oreochromis niloticus*) (Ergün et al., 2008). Recent studies showed that the supplementation of European seabass (*Dicentrarchus labrax*) with *Ulva* spp., *Fucus* spp. and *Gracilaria* spp. up to 7.5% have no impact on growth performance, but generally improved the immune system and antioxidant responses (Araújo et al., 2016; Peixoto et al., 2016). These authors also demonstrated that *O. niloticus* can accept *Ulva* spp. in its diet up to 10% without influencing the growth performance or flesh organoleptic properties, and enhances their innate immune response (Valente et al., 2016). Another study showed that feeding *Sparus aurata* with a supplementation of 10% of *Pterocladiella capillacea* or 5% of *U. lactuca* enhanced fish growth performance, feed utilization, nutrient retention and survival, and 10% of *P. capillacea* also improved fish stress response after a 5-min anoxia (Wassef et al., 2005).

8.3 DNA INTEGRITY PROMOTING ACTIONS

The importance of genome integrity and stability is undeniable, since the DNA molecule carries the genetic information of each living cell and, thus, its fidelity plays a major key role in the balance between health and disease. However, the DNA molecule is under constant attack from both endogenous and exogenous (or environmental) sources of damage.

Interestingly, nutrition may simultaneously be a source of genotoxic substances (O'Brien et al., 2006), as well as antigenotoxic compounds (Izquierdo-Vega et al., 2017). In this context, marine macroalgae have been described as a potential source of genoprotective substances. In fact, they have shown to possess antioxidant, antiproliferative, antigenotoxic and antimutagenic properties in various studies (Table 8.1) (Okai and Higashi-Okai, 1994; Athukorala et al., 2006; Yuan and Walsh, 2006; Celikler et al., 2008; Celikler et al., 2009a; 2009b; Zubia et al., 2009a; 2009b; Valentão et al., 2010; Osuna-Ruiz et al., 2016). An enzymatic extract of *Ecklonia cava*, as well as its crude polysaccharide and crude polyphenolic fractions showed antioxidant and antiproliferative activity on various cancer cell lines (Athukorala et al., 2006). Moreover, a crude extract of *U. rigida* showed antigenotoxic/anticlastogenic potential against chromosome aberration, sister chromatid exchange and micronuclei induced by the mutagenic agent mitomycin-C (Celikler et al., 2008). The same research group demonstrated that an ethanolic extract of the same alga enhanced anti-hyperglycemic and antigenotoxic capacities in diabetic rats (Celikler et al., 2009a), as well as the antigenotoxic ability on hypothyroid rats (Celikler et al., 2014). In addition, these authors also demonstrated that a crude ethanolic extract of *Codium tomentosum* efficiently reduced the genotoxic effects of mitomycin-C, ethyl methanesulfonate and H_2O_2 (chromosome aberrations, sister chromatid exchange and micronuclei) in human lymphocytes *in vitro* (Celikler et al., 2009b). A dried powder of *F. vesiculosus* depicted strong antioxidant activity on different *in vitro* antioxidant assays (Díaz-Rubio et al., 2009) and the pre-treatment of cultured human lymphocytes with an extract of *F. vesiculosus* revealed genoprotection properties against chromosome aberrations and DNA fragmentation induced by doxorubicin (Leite-Silva et al., 2007). Additionally, aqueous and ethanolic extracts of 16 marine macroalgae collected

TABLE 8.1 Summary of DNA Integrity Related Properties Attributed to Different Extracts/Fractions or Whole Macroalgae Species, Achieved Using Multiple Testing Methods, both *In Vitro* and *In Vivo* Studies

Bioactive Property	Sample	Testing Method	In Vitro or In Vivo Testing?	References
Antioxidant	*Ulva lactuca* hydroethanolic extract	Glutathione (GSHt) and lipid peroxides (LPO) contents, and glutathione peroxidase (GPx), glutathione reductase (GR) and superoxide dismutase (SOD) activities.	*In vivo* (arthritic rats)	Ahmed et al. (2017)
Antiproliferative	*Hydropuntia cornea* and *Gracilariopsis longissima* aqueous extracts.	MTT assay	*In vitro* (RAW 264.7 cell line)	Álvarez-Gómez et al. (2019)
Antioxidant	*Gracilaria corticata* and *G. edulis* hydromethanolic extracts.	DPPH$^•$, ABTS$^{•+}$ and NO$^•$ radical scavenging and iron reducing power assays.	*In vitro*	Arulkumar et al. (2018)
Antioxidant	*Ecklonia cava* enzymatic extract and polysaccharide and polyphenolic fractions.	DPPH$^•$, $^•O_2^-$, H_2O_2 and $^•OH$ radical scavenging, reducing power and hemoglobin-induced linoleic acid system assays.	*In vitro*	Athukorala et al. (2006)
Antiproliferative		MTT assay	*In vitro* (CT-26, THP-1, B-16, U-937 and V79-4 cell lines).	
Antigenotoxic [against mitomycin-C (MMC)]	*U. rigida* ethanolic extract.	Chromosome aberration, sister chromatid exchange and micronucleus tests.	*In vitro* (human lymphocytes).	Celikler et al. (2008)
Antigenotoxic	*U. rigida* ethanolic extract.	Micronucleus test.	*In vivo* (diabetic rats).	Celikler et al. (2009a)
Antioxidant	*Codium tomentosum* ethanolic extract.	Lipid- and water-soluble antioxidant capacity assays.	*In vitro*	Celikler et al. (2009b)
Antigenotoxic [against MMC, ethyl methanesulfonate (EMS) and H_2O_2]		Chromosome aberration, sister chromatid exchange and micronucleus tests.	*In vitro* (human lymphocytes)	
Antigenotoxic	*U. rigida* ethanolic extract.	Micronucleus test.	*In vivo* (hypothyroid rats)	Celikler et al. (2014)

TABLE 8.1 *(Continued)*

Bioactive Property	Sample	Testing Method	In Vitro or In Vivo Testing?	References
Antioxidant	*G. changii* extracts (different solvents).	DPPH· radical scavenging, ferric reducing power (FRAP) and β-carotene-linoleic acid bleaching (BCB) assays.	*In vitro*	Chan et al. (2015)
Antioxidant	*Porphyra rosengurttii*, *Gelidium corneum* and *Ahnfeltiopsis devoniensis* mycosporine-like amino acids (MAAs) fractions.	$ABTS^{\cdot+}$ and $\cdot O_2^-$ radical scavenging and BCB assays.	*In vitro*	Coba et al. (2009)
Antioxidant	*Himanthalia elongata*, *Undaria pinnatifida* and *P. umbilicalis* aqueous-organic (methanol and acetone) extracts.	$ABTS^{\cdot+}$ radical scavenging and FRAP assays.	*In vitro*	Cofrades et al. (2010)
Antioxidant	*G. rubra* sulfated polysaccharide fractions.	DPPH·, $ABTS^{\cdot+}$, $\cdot O_2^-$ and ·OH radical scavenging, iron chelating activity and lipid peroxidation assays.	*In vitro*	Di et al. (2017)
Antioxidant	*Laminaria ochroleuca*, *P. umbilicalis* and *G. corneum* sulfated polysaccharide fractions.	DPPH· and $ABTS^{\cdot+}$ radical scavenging assays.	*In vitro*	Díaz et al. (2019)
Antiproliferative		MTT assay.	*In vitro* (RAW 264.7 cell line; HTC-116, MCF-7, G-361 and U-937 cell lines for *L. ochroleuca*).	
Antioxidant	*Fucus vesiculosus* aqueous-organic (methanol and acetone) extract.	$ABTS^{\cdot+}$ radical scavenging, FRAP and oxygen radical absorbance capacity (ORAC) assays.	*In vitro*	Díaz-Rubio et al. (2009)
Antioxidant	16 marine macroalgae (rhodophyta, chlorophyta and ochrophyta species) aqueous and ethanolic extracts.	DPPH· radical scavenging, iron chelating activity, reducing power and liposome model system assays.	*In vitro*	Farvin & Jacobsen (2013)

TABLE 8.1 *(Continued)*

Bioactive Property	Sample	Testing Method	In Vitro or In Vivo Testing?	References
Antigenotoxic [against streptonigrin (SN) and endogenous challenges]	Whole *P. umbilicalis* and *Grateloupia turuturu*	Somatic mutation and recombination test (SMART)	*In vivo* (fruit fly)	Ferreira et al. (2019)
Antioxidant	*Sargassum dentifolium* water-soluble polysaccharide fractions.	ORAC assay	*In vitro*	Gamal-Eldeen et al. (2013)
Antigenotoxic [against cyclophosphamide (CP)]		Chromosome aberration and micronucleus tests, and comet assay.	*In vivo* (mice)	
Antioxidant	26 Rhodophyta species aqueous and methanolic extracts.	DPPH$^\bullet$, $^\bullet O_2^-$, H_2O_2 and $^\bullet OH$ radical scavenging assays.	*In vitro*	Heo et al. (2006)
Antioxidant	*P. yezoensis* porphyran fraction.	$^\bullet O_2^-$ and $^\bullet OH$ radical scavenging assays.	*In vitro*	Isaka et al. (2015)
Antioxidant	21 marine macroalgae (rhodophyta, chlorophyta and ochrophyta species) lipid fractions.	DPPH$^\bullet$ radical scavenging assay.	*In vitro*	Ito et al. (2018)
Antioxidant	*G. livida* ethanolic extract and fractions (different solvents).	DPPH$^\bullet$ and $^\bullet OH$ radical scavenging and BCB assays.	*In vitro*	Jiang et al. (2013)
Antioxidant	*Bifurcaria bifurcata*, *H. elongata* and *Saccharina latissima* aqueous-organic (methanol and acetone) and aqueous extracts.	ABTS$^{\bullet+}$ radical scavenging, photochemiluminescence (PCL; for $^\bullet O_2^-$ radical scavenging) and FRAP assays.	*In vitro*	Jiménez-Escrig et al. (2012)
Antigenotoxic (against doxorubicin)	*F. vesiculosus* aqueous extract.	Chromosome aberration test and comet assay.	*In vitro* (human lymphocytes).	Leite-Silva et al. (2007)

TABLE 8.1 *(Continued)*

Bioactive Property	Sample	Testing Method	In Vitro or In Vivo Testing?	References
Antigenotoxic (against SN and endogenous challenges)	Whole *U. rigida*, *F. vesiculosus*, *G. vermiculophylla* and *G. gracilis.*	SMART	*In vivo* (fruit fly)	Marques et al. (2018)
Antigenotoxic (against oxytetracycline, formalin and CP)	Whole *U. rigida*, *F. vesiculosus* and *G. gracilis* (mix).	Erythrocytic nuclear abnormalities (ENA) assay and erythrocyte maturity index (EMI).	*In vivo* (gilthead seabream)	Marques et al. (2019)
Antigenotoxic (against SN and endogenous challenges)	Whole *U. rigida*	Comet assay	*In vivo* (fruit fly)	Marques et al. (2021)
Antioxidant	*Cystoseira crinita*, *C. sedoides* and *C. compressa* aqueous extracts.	DPPH• radical scavenging and FRAP assays.	*In vitro*	Mhadhebi et al. (2014)
Antiproliferative		MTT assay.	*In vitro* (MDCK, rat fibroblast, A549, MCF7 and HCT15 cell lines)	
Antioxidant	*Gracilaria* sp., *F. vesiculosus* and *U. rigida* extracts (different solvents and extraction methods).	DPPH• and ABTS$^{•+}$ radical scavenging assays.	*In vitro*	Monteiro et al. (2020)
Antimutagenic (against several mutagens)	*Hizikia fusiforme* aqueous extract and polysaccharide and non-polysaccharide fractions (high- and low-molecular-weight fractions).	*umu* test	*In vitro* (*Salmonella typhimurium* TA 1535/pSK 1002 strain)	Okai & Higashi-Okai (1994)
Antimutagenic [against tryptophan-P-1(Trp-P-1)]	8 marine macroalgae (rhodophyta, chlorophyta and ochrophyta species) methanolic extracts.	*umu* test	*In vitro* (*Salmonella typhimurium* TA 1535/pSK 1002 strain)	Okai et al. (1994)

TABLE 8.1 *(Continued)*

Bioactive Property	Sample	Testing Method	In Vitro or In Vivo Testing?	References
Antioxidant Antimutagenic [against aflatoxin B_1 (AFB_1)] Antiproliferative	7 marine macroalgae (rhodophyta, chlorophyta and ochrophyta species) methanolic, acetone and hexane extracts.	DPPH• and ABTS•+ radical scavenging assays. Ames test MTT assay	*In vitro* *In vitro* (*Salmonella typhimurium* TA98 and TA100 strains) *In vitro* (M12.C3.F6 cell line)	Osuna-Ruiz et al. (2016)
Antioxidant	Whole *Ulva* spp. and *Gracilaria* spp. (isolated and mixed with *Fucus* spp.)	GPx activity	*In vivo* (sea bass)	Peixoto et al. (2016)
Antioxidant Antigenotoxic (against CP)	Whole *U. rigida*, *F. vesiculosus* and *G. gracilis* (mix).	Catalase (CAT) and glutathione-S-transferase (GST) activities, and total glutathione (GSHt) content ENA and comet assays	*In vivo* (gilthead seabream)	Pereira et al. (2019)
Antioxidant	27 marine macroalgae (rhodophyta, chlorophyta and ochrophyta species) dichloromethane and methanolic extracts.	DPPH• radical scavenging and ORAC assays.	*In vitro*	Pinteus et al. (2017)
Antioxidant Antigenotoxic [against benzo[α]pyrene (BP)]	*U. fasciata* hydroethanolic extract.	DPPH• and ABTS•+ radical scavenging and lipid peroxidation assays. Micronucleus test.	*In vitro* *In vivo* (mice)	Rodeiro et al. (2015)
Antigenotoxic	Whole *P. umbilicalis*	Comet assay and micronucleus test.	*In vivo* [human papillomavirus (HPV)-transgenic mice]	Santos et al. (2019)
Antioxidant Antigenotoxic (against H_2O_2)	*G. tenuistipitata* aqueous extract.	DPPH• radical scavenging assay. Comet assay.	*In vitro* *In vitro* (H1299 cell line)	Yang et al. (2012)

TABLE 8.1 *(Continued)*

Bioactive Property	Sample	Testing Method	In Vitro or In Vivo Testing?	References
Antiproliferative	*G. tenuistipitata* methanolic extract.	WST-1 assay.	*In vitro* (Ca9-22 cell line)	Yeh et al. (2012)
Antioxidant Antiproliferative	*Palmaria palmata*, *L. setchellii*, *Macrocystis integrifolia* and *Nereocystis leutkeana* methanolic extracts.	Reducing activity assay. MTT assay	*In vitro* *In vitro* (HeLa cell line)	Yuan & Walsh (2006)
Antioxidant Antiproliferative	*P. palmata* methanolic extracts.	ORAC assay MTT assay	*In vitro* *In vitro* (B16-F1 cell line)	Yuan et al. (2009)
Antioxidant	*F. vesiculosus* hydroethanolic extracts.	Reducing power, DPPH•, ABTS•+ and •O_2^- radical scavenging and total radical antioxidant parameter (TRAP) assays. •O_2^- and NO• generation assays. Reducing power, O_2^- radical scavenging and ORAC assays, thiobarbituric acid-reactive substances (TBARS) and NO• contents, and SOD and paraoxonase-1 (PON-1) activities.	*In vitro* *In vitro* (RAW 264.7 cell line) *In vivo* (rats)	Zaragozá et al. (2008)
Antioxidant Antiproliferative	24 Rhodophyta species extracts (dichloromethane:methanol; 1:1).	DPPH• radical scavenging, reducing activity and BCB assays. WST-1 assay	*In vitro* *In vitro* (Daudi, Jurkat and K562 cell lines).	Zubia et al. (2009a)
Antioxidant Antiproliferative	10 Ochrophyta species extracts (different solvents).	DPPH• radical scavenging, reducing activity and BCB assays. WST-1 assay.	*In vitro* *In vitro* (Daudi, Jurkat and K562 cell lines).	Zubia et al. (2009b)

along the Danish coast globally revealed antioxidant activity on several *in vitro* antioxidant assays (Farvin and Jacobsen, 2013). Furthermore, extracts of *Sargassum dentifolium* showed protective effects against cyclophosphamide-induced genotoxicity (chromosome aberrations, micronuclei, and DNA fragmentation) induced in mice (Gamal-Eldeen et al., 2013). In turn, an aqueous-ethanolic extract of *U. fasciata* showed antioxidant and antigenotoxic effects against benzo[α]pyrene-induced micronuclei in mice (Rodeiro et al., 2015). Also, aqueous extracts of the edible *G. tenuistipitata* revealed *in vitro* protective capacity against H_2O_2-induced oxidative DNA damage (Yang et al., 2012). Yuan and Walsh (2006) determined the antioxidant and antiproliferative properties of *P. palmata*, *Laminaria setchellii*, *Macrocystis integrifolia* and *Nereocystis leutkeana* methanolic extracts on HeLa cells. Crude extracts of some Ochrophyta collected in the Brittany coasts also revealed antioxidant and antiproliferative effects on three different cancer cell lines (Zubia et al., 2009b).

Despite some *in vivo* studies, the majority addresses *in vitro* effects and only testing isolated fractions or extracts (Table 8.1). In this context, Holdt and Kraan (2011) echoed the recommendations from European Advisory Services stating that functional foods should not be pills or capsules, but must remain foods, demonstrating their effects in amounts that can normally be expected to be consumed in the diet (European Advisory Services, 2008). Based on this assumption, Marques et al. (2018, 2021) revealed the antigenotoxic potential of *U. rigida*, *F. vesiculosus* and two species of *Gracilaria*, *G. vermiculophylla* and *G. gracilis*, individually incorporated on the fruit fly (*Drosophila melanogaster*) diet, especially against streptonigrin-induced DNA damage. Likewise, Ferreira et al. (2019) showed that red macroalgae *P. umbilicalis* and *Grateloupia turuturu* displayed antigenotoxic and longevity-promoting potential in *D. melanogaster* after being incorporated in its diet. In tune, upon also adding *P. umbilicalis* to mice diet, antigenotoxic properties against human papillomavirus (HPV)-induced DNA damage were shown (Santos et al., 2019).

In the context of animal production, the commercial factor depends on the health, fitness, and survival of the organisms. In fact, in aquaculture, genetic damage can have a negative impact on fish fitness, with subsequent repercussion on the productivity (Silva et al., 2011), mainly due to a higher energy expenditure related with DNA repair processes (Olson and Mitchell, 2006) and homeostasis threatening (Pacheco and Santos, 2002). In line, marine macroalgae have demonstrated antioxidant and antigenotoxic properties, when incorporated on farmed fish diets. Particularly, a mixture of algae species representative of the genera *Ulva*, *Fucus* and *Gracilaria* enhanced the antioxidant response of *D. labrax* when incorporated in its feed, through the increase of glutathione peroxidase activity (GPx) (Peixoto et al., 2016). Moreover, a recent study highlighted the genoprotection enhancement of the same macroalgae mixture on *S. aurata*, specifically against erythrocytic nuclear abnormalities and DNA fragmentation induced by cyclophosphamide (Pereira et al., 2019). In line, a similar study demonstrated the potential of the same macroalgae mixture against erythrocyte population instability and chromosomal damage induced by two aqua-medicines frequently applied in aquaculture practices: oxytetracycline and formalin (Marques et al., 2019).

8.4 LINKING MARINE MACROALGAE PROPERTIES TO PHYTOCHEMICAL COMPOSITION

The current attention devoted to marine macroalgae's beneficial properties, especially genoprotective ones, ought to be directly linked to their unique phytochemical composition. In fact, those organisms must face highly diverse and harsh environments, coping with rapid environmental changes, which contributes significantly to the variety and quantity of the compounds and, even, the potential of their bioactivities (Tiwari and Troy, 2015) (Figure 8.1). For instance, the most abundant terrestrial phenolic compounds (flavonoids) have three interconnected rings. In turn, algae phenolic compounds, phlorotannins, may have up to eight interconnected rings, making them between 10 and 100 times more powerful and more stable as free radical scavengers than other polyphenols (for example, green tea catechins, have only four rings) (Mohamed et al., 2012). Furthermore, macroalgae phytochemical profile may vary due to the alga species, the harvesting period, the geographic habitat, and environmental factors (e.g. water temperature, light intensity and salinity), as well as nutrients and minerals availability (Mabeau and Fleurence, 1993; Marinho-Soriano et al., 2006; Marsham et al., 2007; Abreu et al., 2009; Tiwari and Troy, 2015). These factors may influence the phytochemical profile of macroalgae with eventual consequences on their bioactive properties as well. In this context, Marques et al. (2018) evaluated the impact of the growing conditions on the antigenotoxic potential of *U. rigida*, *F. vesiculosus* and *Gracilaria* sp. and concluded that *U. rigida* grown under aquaculture conditions showed higher antigenotoxic potential against exogenously-induced DNA damage, while wild-harvested *U. rigida* revealed to be more protective against endogenous damage. On the other

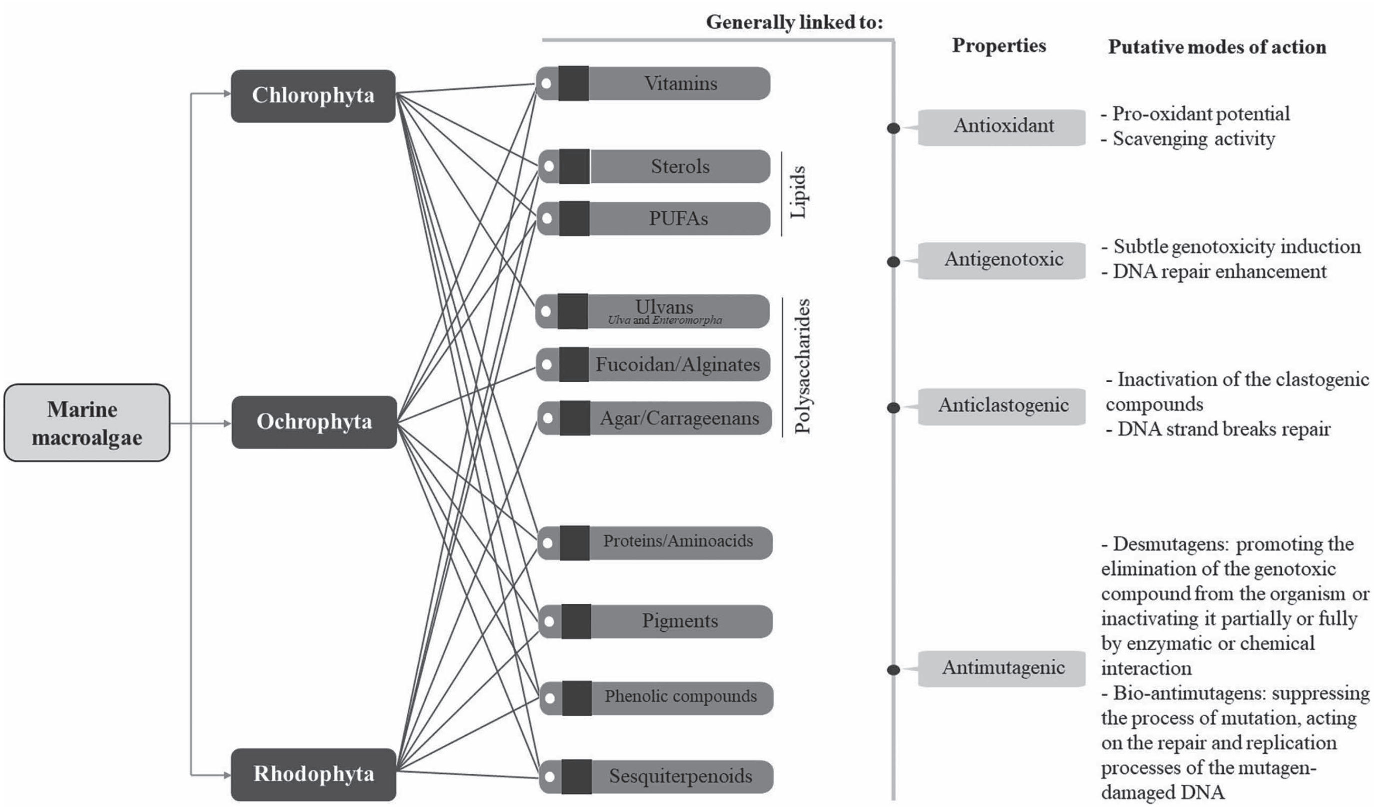

FIGURE 8.1 Schematic representation of the different classes of phytochemical compounds of Chlorophyta, Ochrophyta and Rhodophyta, generally linked to the macroalgae genoprotective properties and putative modes of action.

hand, the antigenotoxic potential of *F. vesiculosus* was not influenced by the growing conditions, while *Gracilaria* sp. showed ambivalent actions. Moreover, a recent study from the same research group confirmed the higher antigenotoxic potential of aquacultured *U. rigida* in comparison to the wild-harvested specimen, which was linked to the distinctive phytochemical profiles, namely the higher relative amounts of fatty alcohols, sterols, sesquiterpenoids and glycerol esters (Marques et al., 2021).

Keeping in mind the great taxonomic diversity of marine macroalgae, as well as their vast habitats, their phytochemical profile and possible applications may be considered an almost unlimited field regarding the exploration of functional ingredients (Tiwari and Troy, 2015). From a basic nutritional point of view, marine macroalgae contain a high concentration of proteins, carbohydrates (mainly dietary fibers), lipids, minerals, vitamins, pigments, and polyphenols (Holdt and Kraan, 2011). In this context, the phytocompounds must be available to exert their bioactivity following the algae ingestion, *i.e.*, they must be susceptible to the digestion process to be bioavailable to consumers (Wells et al., 2017). Most marine macroalgae have a protein content as high as soybean, leguminous plants, or eggs (Fleurence and Levine, 2016). In addition, marine algae have proved their value on human health because of their high content of essential amino acids (approximately 40% of total amino acids) (Fleurence and Levine, 2016). Carbohydrates are a source of energy to humans and other animals, but some more complex carbohydrates are not digestible by the gut. In specific, sulfated polysaccharides represent the major fraction of soluble dietary fiber in marine macroalgae, with some being greatly used in the food and pharmaceutical industries as thickeners, emulsifiers, and stabilizers (*viz.* agars and carrageenans from red macroalgae) (Gomez-Zavaglia et al., 2019). This brings rather beneficial effects, since these polysaccharides help slow down digestion, decrease blood cholesterol levels and maintain stable glucose levels, contributing to lower risk of obesity, diabetes, metabolic syndrome, and cardiovascular diseases, as well as lower risk of developing colon cancer (Patarra et al. 2011; Mohamed et al., 2012). Moreover, marine algae are rich in polyunsaturated fatty acids (PUFAs) of the *n*-3 and *n*-6 series (in higher amounts than land vegetables), which are considered essential fatty acids, since they are not synthesized by mammals and must be taken via food chains (Fleurence and Levine, 2016). The benefits of PUFAs, particularly eicosapentaenoic, arachidonic and docosahexaenoic acids (EPA, ARA, and DHA, respectively) are vast and well-studied, including cardiovascular effects, decrease of blood pressure and improvement of heart and liver function (Fleurence and

Levine, 2016). In line, they have also shown anti-inflammatory, antithrombotic, immunomodulatory and antioxidant effects, besides their role in the prevention of several types of cancer, pre and postnatal development of the brain and the retina, regulation of membrane fluidity, electron, and oxygen transport (Fleurence and Levine, 2016). In addition, they also are precursors of the biosynthesis of several bioregulators, exerting important functions on many cellular processes, mitochondrial function, inflammation processes, immune reactions and skin growth and protection (Fleurence and Levine, 2016). Sterols are a different class of lipids and, specifically, phytosterols are present in marine algae. Phytosterols have shown to decrease blood cholesterol levels and to hold antifungal, antibacterial, anti-inflammatory, anti-ulcerative antitumor, antioxidant, and antigenotoxic properties (Alarif et al., 2012; Tiwari and Troy, 2015). Marine algae are also rich in pigments, namely chlorophylls, fucoxanthin and phycoerythrin, which may vary according with the phylum, but generally display antioxidant, antiproliferative and cancer-preventive capacities (Holdt and Kraan, 2011; Tiwari and Troy, 2015; Fleurence and Levine 2016). Macroalgae have also a high content of minerals, some presenting larger amounts than terrestrial vegetables. For instance, marine algae are a natural and safe source of iodine, an important nutrient in metabolic and hormonal regulation (MacArtain et al., 2007). In turn, vitamins, essential micronutrients that an organism cannot synthesize, and which must be obtained through the diet, are also present in favorable amounts in marine algae (MacArtain et al., 2007; Wells et al., 2017). These have crucial cellular functions, serve as precursors of essential enzyme cofactors, and are needed for essential metabolic pathways. Deficiencies of vitamins have been connected to several human diseases/disorders (e.g. scurvy and anemia) and their inclusion have direct beneficial effects, such as antioxidant potential and reduction of cardiovascular diseases, cancer incidence and ophthalmologic disorders (Mohamed et al., 2012; Wells et al., 2017). Phenolic compounds, as phlorotannins, present a strong reducing power, showing a significant radical scavenging activity (Holdt and Kraan, 2011). They can work as preventive medicines for cardiovascular diseases, cancer, arthritis, and autoimmune disorders by helping to protect tissues against oxidative stress (Holdt and Kraan, 2011). In addition, polyphenols were found to be anti-inflammatory and have anti-allergic effect and antibacterial activity (Holdt and Kraan, 2011). The secondary metabolites mycosporine-like amino acids (MAAs) represent important constituents as well, mainly in red marine macroalgae (Chrapusta et al., 2017). Considering their photoprotective, immunostimulatory, antiproliferative, antioxidant, antigenotoxic, anti-inflammatory and antiaging properties, they have been used in the pharmaceutical/cosmetic industry as ingredients of creams and sunscreens (Becker et al., 2016; Chrapusta et al., 2017; Tarasuntisuk et al., 2019). MAAs can also be transported through the food chain, from algae to animals, since the latter do not have the capability of producing them (Chrapusta et al., 2017).

8.5 CONCLUSION

Regardless of the apparent plethora of beneficial properties afforded by marine macroalgae, it is important to consider, as with any food or feed, some aspects concerning the safety about their consumption. In particular, the origin and/or growing conditions of the macroalgae should be contemplated. Indeed, an important issue regarding wild harvested macroalgae or from unknown origin relies on the possible absorption and contamination with toxic elements, namely metals, dioxins, pesticides, ammonia, radioactive isotopes or microplastics (Spiegel et al., 2013; Tiwari and Troy, 2015; Desideri et al., 2016; Gutow et al., 2016). Additionally, some macroalgae hold a great iodine content and, if consumed in excess, this compound can be deleterious and contribute to thyroid-related disorders (Fleurence and Levine, 2016). Besides that, some species may also possess antinutritional factors. There are reports suggesting some health complications came from direct consumption of species of the genera *Caulerpa*, *Gracilaria* and *Acanthophora*, which were associated to intrinsic compounds, as caulerpin and caulerpinic acid (genus *Caulerpa*) or prostaglandins and polycavernosides (genus *Gracilaria*) (Fleurence and Levine, 2016). Moreover, carrageenan, which is possibly the most used macroalgae isolated product, has been in the center of some controversy. Some studies have suggested it may contribute to harmful gastrointestinal disorders (Tobacman, 2001), while recent review articles have been defended this food additive to be safe (McKim, 2014; Weiner, 2014).

Nonetheless, the number of harmful effects reports is small and occurring sporadically and, in most cases, the source of toxins involved is questionable and may not even be produced by the macroalgae, but instead produced by epiphytic cyanobacteria contaminants (Fleurence and Levine, 2016), which toxicity is well documented (Carmichael, 2001; Navarro et al., 2015). Besides, the treatment given to macroalgae before their consumption (e.g.

washing, cooking method) should be also taken into consideration on these singular events (Fleurence and Levine, 2016).

KEYWORDS

- **antigenotoxic**
- **antimutagenic**
- **antioxidant**
- **antiproliferative**
- **functional feeds**
- **functional foods**
- ***in vitro* trials**
- ***in vivo* trials**
- **seaweeds**

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CHAPTER 9

ALGAE-BASED PHARMACEUTICALS FOR CANCER TREATMENT: PRESENT STATUS AND FUTURE APPLICATION

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ABSTRACT

Cyanobacteria anticancer metabolites are observed as a better alternative for cancer therapies. They have various applications in pharmacological field and their efficiency as a potent therapeutic compound in the past two decades have been studied. Around 50% of the blue-green algal species are grown commercially to extract the bioactive agents that are effective in causing cytotoxicity to a wide variety of cancerous cells through apoptosis or by inactivation of the cell signaling enzymes. They contain huge amount of several types of vitamins, proteins, minerals and dietary fibers but polysaccharides extracted from them have a major role as an anti-cancerous agent commercially. These algae-based pharmaceuticals have a number of advantages like better drug efficacy because of enhanced solubility for drug delivery, increment in half-life and better accumulation of drug in target cancerous cells. But there are challenges that must be taken into account when developing plans for the production and commercialization of algae-based pharmaceuticals for cancer treatment. This chapter discusses about the bioactive compounds obtained by cyanobacteria having anti-cancerous properties and their present status in commercialized platform, the challenges

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that have arisen and the strategies that can be used for overcoming them and the promise that it holds for the future.

9.1 INTRODUCTION

Cancer is a collective phrase for a number of diseases that are caused when cells that are not normal start dividing quickly, and further proliferate and spread to other parts of tissues and organs (https://www.healthline.com/health/cancer). Such abnormal cells are called as cancer cells, tumor cells, or malignant cells. Cancer is one of the major reasons for death in the world, with an approximate 57% (8 million) of the new cancer patients, 65% (5.3 million) of the deaths caused because of cancer and 48% (15.6 million) of the five years prevailing cases of cancer arising in the regions that are less developed (Johnson et al., 2011; Siegel and Jemal 2013). The uncontrolled cell division i.e. Malignant tumors are associated to considerable pathologic variations (https://www.medicinenet.com/cancer/article.htm, El-Hack et al., 2019). More than 200 several kinds of malignant tumors are introduced along with various kinds of cancers that can be transformed into different tissues resulting in deadly metastatic cancers. Due to this high level of impact, significant focus has been given to combat cancer (Jayaprakasam et al., 2013).

Cancer consists of a huge batch of pathologies linked to the uncontrolled division of cells of the body (https://www.cancer.gov/about-cancer/understanding/what-is-cancer.). Around 14 million new cancer patients came up worldwide in the year 2012 that further led to around 8 million deaths (McGuire 2016). According to a report on cancer presented by the World Health Organization (WHO), cancer will remain the major cause of death worldwide and this will continue to rise with an approximate 13.1 million approximate deaths by the year 2030 (around an increment of 70%) (https://www.medicinenet.com/cancer/article.htm). According to the European Cancer Observatory, approximation of the four most usual kinds of cancer in the European Union in 2012 were as follows: around 82075 patients of skin melanoma, 358967 patients of breast cancer, 309589 patients of lung cancer (along with trachea and bronchus cancer) and 342,137 patients of colon cancer (Martínez et al., 2018). It is one of the serious health issues in the United States and is estimated to exceed heart diseases as the major reason for death in the coming years (Siegel et al., 2015). Selection of most suitable treatment for cancer depends on the type and stage of the disease. Most of the treatments are considered so as to fit the individual patient's illness. Although, most of the medication consist of at least one of the following or can consist of all: radiation therapy, surgery and chemotherapy (https://www.medicinenet.com/cancer/article.htm). Chemotherapy is generally the first line medication to treat cancers; these drugs are capable enough to damage or at the minimum stop the growth of cancer cells. These medicines are related through malignancy that varies from a moderate reaction to rigorous life-threatening disease. Presently several therapies for cancer treatment are available, but most of them are associated with severe limitations, risks, adverse effects and ethical issues. Chemotherapy, gene therapy, radiation therapy, cryosurgery, transplantations, photodynamic therapy, biological therapy, angiogenesis inhibitors, lasers, hyperthermia and targeted cancer therapies are some of them. Baldness and loss of appetite are some of the side effects of using chemotherapeutic drugs (El-Hack et al., 2019). The condition needs efficacious treatment approach to fight the increment in the incidence rate, efficient in long term, immune-enhancer, without adverse side-effects along with being economical (Kiruba et al., 2018). Research for the cure of cancer is being conducted by a lot of scientists so as to enhance the results of patients suffering from the disease. These attempts comprise the evolvement of more efficient and lesser toxic therapies like cancer vaccines, targeted therapies and immunotherapies as well as the advancement of treatments that are present for decades. Some of the studies describe better administration of a therapy's noxious effect, hence improve an individual's capacity to sustain effective treatment of cancer. Decades of studies on the cancer biology have disclosed the understanding of the mechanisms that causes the disease. Information from the molecular and several other researches specify that even in a particular cancer, there are few variations in how the cancer acts and how it responds to therapy. Along with the recognition of genetic, epigenetic, and other molecular variations that can encourage the evolvement and spread of tumors, scientists have known about the processes that helps tumors survive and expand in the body. For instance, cancerous cells have the capacity to generate blood supplies of their own, make changes in the immune system to choke responses generated by the immune, and employ non-cancerous cells to help them in their growth. Cancerous cells can neglect signals that generally inform old or injured cells to die. This new insight has given opportunities to invent targeted therapies i.e. medications that focus on the particular variations, most commonly in proteins, that is fundamental for the evolvement and spread of cancer (https://www.cancer.gov/about-cancer/understanding/what-is-cancer). Another main opportunity arises after the realization of the fact that molecular variations that are alike are also same among different cancers that occurs at various parts in the body. For instance, recently The Cancer Genome Atlas (TCGA) research network recognized similarities within genome between endometrial and various other kinds of cancer, counting colorectal, breast and ovarian cancers. Hence, treatment which focus on particular molecular variations may be effective not only for the cancer for which it was designed, but also for cancer from different sites which seems to have the similar variations. Despite various developments have been achieved in cancer therapy in recent time but still there are a number of limitations present to attain the aim of giving the best possible results for all patients detected with cancer (https://www.cancer.gov/about-cancer/understanding/what-is-cancer). The characterization of genome of tumors has given both chance for cancer therapy and challenges. The finding that every patient's cancer has a novel constellation of mutations in gene and other variations increases the difficulty in identification of therapies that are suitable to work appropriate for a specific individual's cancer. Sometimes, also within a single individual, different metastatic tumor nodules or different constituents of a single tumor in the same individual, may not be similar in terms of the molecular variations present. This leads to the probability that a medicine may be efficient for one part of an individual's tumor but not in the other. Despite the huge advancement made in the field of immunotherapy recently, this perspective to treat cancer is still in its initial stage. A lot of limitations are still left, counting how to improve the immune response to eliminate tumor while ignoring runaway results that leads to autoimmune injury to non-cancerous tissues. An added limitation is to determine why the recent immunotherapies are effective in some individuals but not in others. Cyanobacteria have been an important source of traditional, clinically important drugs for the cure of various types of tumors and even if the real compounds obtained from algae did not function as drugs, they offer leads for the evolution of potent new agents (Somasekharan et al., 2016). As such, finding algae for efficient anticancer compounds with lower side effects seems to offer an attractive approach even for the evolution of the anticancer therapy in the modern era.

9.2 CYANOBACTERIA

Marine plants have acted as an important and a variety of sources of natural products from a long time. Marine algae (phytoplankton) among the marine plants, comprises a major component of the ocean flora as the largest source for production of oxygen and forms the foundation of the marine food chain (Pulz and Gross, 2004). Microalgae are microscopic organisms that are photosynthetic and present an important component of phytoplankton of marine and fresh water. They are capable enough to grow either alone or synchronously with other organisms and it can also withstand a wide scale of unfavorable environmental circumstances (Cha et al., 2008). Algae can be categorized into green algae (*Chlorophyta*), brown algae (*Phaeophyta*) and red algae (*Rhodophyta*). They can also be categorized according to their size as macroalgae and microalgae. Macroalgae are the large-size multicellular organisms that can be seen by the naked eye, whereas microalgae are the microscopic single cell organisms that can be either prokaryotic or eukaryotic. About 40% of the global productivity is contributed by microalgae (Moreno et al., 2008). They are present at the bottom of aquatic food webs and they generally have shorter generation times and have colonized themselves in almost all the biotopes present i.e. from temperate to adverse conditions such as cold conditions and hydrothermal vents. Generally, microalgae comprise of various proportions of nucleic acids, proteins, carbohydrates and lipids and this proportion can vary depending on the species and growth environment (Das, 2011). Moreover, they also consist of pigments, polysaccharides, minerals and vitamins (Brennan and Owende, 2010; Martínez et al., 2018). For example, the study conducted on marine microalgae (*Nannochloropsis*) cells exposed its components of pigment such as β-carotene, violaxanthin, vaucheraxanthin, anteraxanthin and zeaxanthin; amino acids such as hydroxyproline, glutamate, proline, tryptophan, aspartate, cysteine, methionine and histidine; and ω-3 fatty acids such as eicosapentaenoic acid. They are a rich source of essential and trace minerals (Ibrahim et al., 2015). They can be easily cultured in photo-bioreactors (e.g., in 100000 L bioreactors) to get a huge biomass and present a renewable and still poorly-explored agents that can be used in drug discovery. They utilize solar energy and fix CO_2 at efficiencies greater than terrestrial crops and contribute to the reduction of the effects of greenhouse gas and the removal of derivatives of nitrogen and phosphorous that can be pollutants on the basis of their concentration (de Morais et al., 2015). According to some studies, the bioactivity of microalgae can vary for various clones and can be different depending on the culturing environment such as availability of nutrient, intensity of light, growth phase and temperature. Green algae are a promising source of renewable fuels and several other bioproducts. Their potential to produce biomass with minimal inputs makes them a promising option for the generation of economical proteins as well. The extensive application of microalgae has built a promising future for humans in several daily life areas like nutraceutical, cosmetics, pharmaceuticals and aquaculture applications. Now, the researches related to therapeutics using algae have been evolved because of their ability of their bioactive components against various pathogens (Ribalet et al., 2008; Ingebrigtsen et al., 2016; Lauritano et al., 2016).

They are a valuable source of nutrition in the food and animal feeding sectors; for example, various species of microalgae are produced commercially as a supplement of unsaturated fatty acids (Spolaore et al., 2006) and single cell proteins (Ravindra, 2000). Moreover, marine algae are generally utilized as food additives, coloring and flavoring agents (Noel and Kim 2002; Sunda et al., 2002). Currently, various kinds of biologically active compounds have been utilized in marine algae and have various applications in medicinal field. For example, it is found that the cell wall of marine brown algae contains huge amount of sulfated polysaccharides (SPs) (Cumashi et al., 2007; Ngo and Kim 2013), that have various potentially therapeutic applications including anticoagulatory, antiallergic, antiviral, hypoglycemic, hypolipidemic, immunomodulatory, antioxidant and anti-inflammatory properties (Itoh et al., 1993; Huang et al., 2005; Gademann and Portmann, 2008; Wase and Wright, 2008; Mayer et al., 2009; de Andrade et al., 2011; Amaro et al., 2013; Fan et al., 2014). The potential of microalgae to adapt in the highly adverse environment like temperature and hydrothermal vents lead them to be a good option for discovery of drug; this can attribute to their novel agents that are produced for survival and defense (Landsberg 2002; Caldwell 2009). Metabolic plasticity acts as a benefit in marine drug discovery that can induce the generation of various compounds with possible usage in several biotechnology fields such as health, energy, food, biomaterials and environment. The pharmaceutical industries in global market is on a great rise. Pharmaceutical industries dominate around 70% to 80% of the market in India and this market size is evolving each year. Algae always had the ability to be useful to mankind; especially the utilization of cyanobacteria (blue-green algae), for antibiotics and pharmacologically active agents have gained ever increasing interest (Ariyanti and Handayani, 2012; El-Sheekh et al., 2012; Parab and Tomar, 2012; Silva et al., 2013; Sulaymon et al., 2013).

9.3 CANCER AND ALGAE

The unique therapeutic and pharmaceutical characteristics of microalgae has gained great interest worldwide. A lot of research is being done to identify the potential applications of a number of metabolites so as to utilize these in several diseases of humans. The ever-growing field of production of compounds derived from algae have gained attention in drug industry (Dias et al., 2012; Shanab et al., 2012; Martins et al., 2014; David et al., 2015; de Morais et al., 2015).

Various compounds that are derived from microalgae have undergone a number of tests for the analysis of their bio medicinal and therapeutic potential for cancer (Campos et al., 2012; Farooqi et al., 2012). The scientific research conducted on natural compounds obtained from microalgae has revealed about their anti-cancerous properties (Talero et al., 2015). There are a lot of difficulties regarding the collection of specimen but still these have succeeded to gain to the approval for their application in the pharmaceutical industry as supplements that are beneficial for health (Pangestuti and Kim, 2011). The progressive evolvement of compounds derived from marine algae as better medicinal molecule compared to the counterparts that are terrestrial is still going on despite a number of difficulties like lack of evidences that are related to ethnomedical along with some technical problems related to the strategies for collection of marine flora. A lot of efforts have been put up by the research and pharmaceutical organizations in order to isolate and identify bioactive compounds from microalgal species that are unique (Pyne et al., 2017). Marine flora is still not explored fully and the work done till now has ignited the spark for promoting the research in this area further. Various species of cyanobacteria like *Oscillatoria*, *Microcystis*, *Nostoc*, *Anabaena*, etc. provide a number of secondary metabolites that are active biologically and diverse chemically. These compounds usually belong to lipopeptides, alkaloids, fatty acids amides, cyclic peptides and saccharides. It was reported that around 50% of the marine cyanobacteria have bioactive compounds that are capable enough to kill the cancerous cells by activating the mechanism of apoptosis (Farooqi et al., 2012; Ahmed et al., 2017) (Table 11.1).

Some compounds obtained from different microalgae and their mechanism of action. It has been seen that the cancer cells are becoming resistant to presently available drugs such as taxanes and vinca alkaloids which is regarded as one of the major reasons for the failure of the chemotherapeutic treatment of cancer. Therefore, there is an acute requirement of novel drugs for cancer. Moreover, the development of new type of cancer like glioblastoma is increasing quickly. In the 1990s, the Laboratory of Moore (Oregon State University) and Gerwick (University of Hawaii) had first initiated the screening of extracts of cyanobacteria for developing novel drugs for cancer (El-Hack et al., 2019). These compounds with anti-cancerous properties have been reported to bring variation in various mechanisms of cells like

TABLE 9.1 Some Compounds Obtained from Different Microalgae and Their Mechanism of Action

Compound	Organism	Mode of Action	References
Borophycin	*N. linckia* and *N. spongiaeforme* var. *tenu*	Cytotoxicity against human colorectal adenocarcinoma and human epidermoid carcinoma (LoVo) activity.	Vijayakumar & Menakha (2015)
Apratoxin-A	*Lyngbya majuscule*, *Lyngbya bouillonii*	Inhibit several cancer cell lines like U2OS osteosarcoma, HT29 colon adenocarcinoma, and HeLa cervical carcinoma.	Vijayakumar & Menakha (2015); Bajpai et al. (2018); El-Hack et al. (2019)
Calothrixin-A	*Calothrix*, *Lyngbya majuscule*	Cytotoxicity to adenocarcinoma.	Vijayakumar & Menakha (2015); El-Hack et al. (2019)
Cryptophycin-1	*N. linckia*, *Nostoc* sp. GSV 224	Cytotoxicity against human solid tumors and human tumor cell lines.	Vijayakumar & Menakha (2015); Bajpai et al. (2018)
Cryptophycin-8	*N. spongiaeforme*	Greater therapeutic efficiency and lower toxicity than cryptophycin-14 in vivo.	Vijayakumar & Menakha (2015)
Largazole	*Symploca* sp.	Antiproliferative activity against cancer cell lines MDA-MB-23I breast cancer and U2OS osteosarcoma A549 lung cancer and HCT-116 colorectal carcinoma.	Vijayakumar & Menakha (2015); El-Hack et al. (2019)
Coibamide	*Leptolyngbya* sp.	Cytotoxicity against NCIeH460 lung, mouse neuro-2a cells and human NCIH460.	Vijayakumar & Menakha (2015); El-Hack et al. (2019)
Curacin-A	*L. majuscule*, *Lyngbya aestuarii*	Antitumoral activity by inhibiting microtubule assembly.	Vijayakumar & Menakha (2015); El-Hack et al. (2019)
Dolastatin-10	*Symploca* sp.	Binds to tubulin on rhizoxin-binding site, and inhibits microtubule assembly.	Vijayakumar & Menakha (2015)
Dolastatin-15	*Lyngbya* sp.	Breast cancers (binds directly to vinca alkaloid site of tubulin).	Vijayakumar & Menakha (2015)
Fucoxanthin	*Chaetoceros* sp., *Cylindrotheca closterium*, *Odontella aurita*, *Phaeodactylum tricornutum*, *Haematococcus pluvialis*, *Chlorella zofingiensis*, *Chlorococcum* sp. and *Cladosiphon okamuranus*.	Inhibits Akt and activator protein-1 pathways that influenced the suppression of cell growth, migration and invasion and the induction of apoptosis on osteosarcoma cells; used for oral cancer, bladder cancer, colon cancer, leukemia cancer; hepatocellular carcinoma.	Alves et al. (2018); Martínez et al. (2018); El-Hack et al. (2019)

TABLE 9.1 *(Continued)*

Compound	Organism	Mode of Action	References
Methanol extract	Eleven strains of benthic diatoms *Ostreopsis ovata*, *Amphidinium operculatum*, *Chlorella stigmatophora*, *Phaeodactylum tricornutum*, *Spirulina fusiformis*.	Anti-inflammatory, immunomodulatory and analgesic activities.	Martínez et al. (2018); El-Hack et al. (2019)
Aqueous extract	Canadian marine microalgal pool, *Chlorella sorokiniana*, *Chlorella*, *Stigmatophora*, *Phaeodactylum tricornutum* and *Spirulina fusiformis*.	Anti-inflammatory, immunomodulatory and analgesic activities.	Martínez et al. (2018); El-Hack et al. (2019)
Scytonemin	*Stigonema* sp.	Antiproliferative and anti-inflammatory activities.	El-Hack et al. (2019)
C-Phycocyanin	*Chlorella* and *Arthrospira*, *Spirulina platensis*, *Porphyridium* sp., cyanobacteria such as *Lyngbya* sp. and *Symploca* sp.	Activities of microalgae against cancer (breast, ovary, skin, lung, colon, kidney, stomach).	El-Hack et al. (2019)

downregulation of invasion of cancer cells, cytotoxication and activation of apoptosis for cancer cells (Farooqi et al., 2012; Lee et al., 2013). The natural dynamic anti-malignant activity of compounds derived from algae is suggested by various cellular and molecular explorations (Kumar et al., 2013; Talero et al., 2015). Fucoxanthin which is a carotenoid found in microalgae, diatom and brown seaweeds have offered potential anticancerous characteristics by preventing the growth of malignant cells, stimulating the suppressor genes of cancer and arresting cell cycle (Peng et al., 2011; Takahashi et al., 2015) (Figure 11.1).

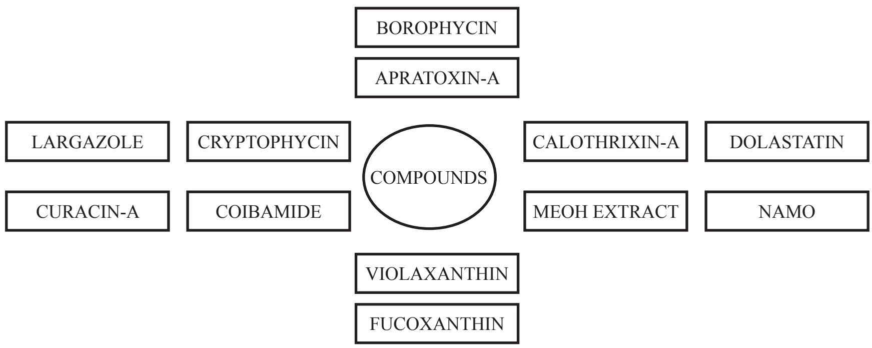

FIGURE 9.1 Different compounds obtained from cyanobacteria.

Similarly, in the late 1980s, the seaweeds came into limelight because of the anti-tumor properties that held a promising future for the development of cancer drugs. Huge amount of literature is present that offers evidences that seaweeds, their extraction or compounds that are obtained from them have anti-tumor and anti-oxidant properties. Their therapeutic importance has been proved by various in-vitro studies. According to the pilot studies and present research, the compounds from cyanobacteria has a potent application in the area of drug discovery and development for cancer. It has also been reported that lycopene along with vitamin E considerably reduces the MCF-7 cell line growth by 55.83% and 48.3% respectively (Kiruba et al., 2018). A case control study was conducted among the Korean women related to consumption of seaweed and the incidence of breast cancer and it was seen that the *Porphyra* spp. uptake decreases the risk of breast cancer (Yang et al., 2010). According to a clinical trial conducted among the menopausal women in America, dietary seaweed was reported to lower the case of breast cancer post menopause by lowering uPAR (Kiruba et al., 2018). A study conducted by the Japan Public Health Center also identified a positive relation between the consumption of seaweed and the risk of papillary cancer in post-menopausal women (Teas et al., 2011). Seaweed and soy consumption lowers IGF-1 serum concentrations relating it with the less cases of breast cancer in Asian countries (Teas et al., 2013). The presence of photosynthetic organelle i.e. chloroplast makes cyanobacteria a better option for making complex but targeted drugs for cancer (Aditya et al., 2016). Various researches are being conducted and scientists are working hard to develop an alga which is genetically engineered. These will be helpful for killing the cancerous cells. This is regarded as the major step in the evolvement of the drugs for cancer. Algae have a potent capacity of folding proteins into three dimensional complex structures. Human antibodies were produced by algae successfully in San Diego. Human therapeutic drugs like Human vascular endothelial growth factor were used on patients that suffered from pulmonary emphysema (Silva et al., 2013; Singh et al., 2014; Suryanarayanan and Johnson, 2014; Visconti et al., 2015; Basystiuk and Kostiv, 2016; Montoya et al., 2016). These microalgae have played a major role in the evolvement of anti-cancer drugs like the compound called cryptophycin which is obtained from blue green algae and is a good option for the development of anti-cancer drugs. Alkaloid neurotoxins like saxitoxin and polyketide are also produced by them and they have both anti-cancerous and anti-inflammatory characteristics (Raja 2013; Vinale 2014). According to Humis and his colleagues, cyanobacteria possess anti-leukemic agents as various strains that are studied well can activate apoptosis for AML (Acute myeloid leukemia) cells but the non-malignant cells such as hepatocytes remain unaffected (Ahmed et al., 2017). All the productive research conducted for finding novel clinical anti-cancer leads from the diversified marine life in the past few decades will translate into a variety of novel options for the cancer treatment in the coming years (Vijayakumar and Menakha, 2015).

9.4 BIOACTIVE COMPOUNDS FROM ALGAE

9.4.1 BOROPHYCIN

Borophycin which is an acetate derived polyketide cytotoxin and is basically extracted from marine strains of *Nostoc spongiaeforme* var. *tenue* and *N. linckia* (Vijayakumar and Menakha, 2015; El-Hack et al., 2019). This metabolite contains boron and is found to be very effective as drugs for cancer in human epidermoid carcinoma and colorectal adenocarcinoma activity (Davidson 1995; Vijayakumar and Menakha, 2015). Its gross structure was determined by some spectral methods and its stereochemistry was established by X-ray crystallography. It has shown strong anticancerous activity against KB (KERATIN-forming tumor cell line HeLa) and LoVo cell lines in human (Hemscheidt et al., 1994). Borophycin 8 is extracted from *Nostoc linckia*. It consists of two identical halves with the overall structure which is similar to other antibiotics that contain boron (Arai et al., 2004).

9.4.2 APRATOXIN-A

It is a secondary metabolite obtained from cyanobacteria and is powerful cytotoxin. It is derived from the apratoxin family. The mixed peptide-polykitide natural compound is obtained from a polyketide synthase/nonribosomal peptide synthase mechanism. It has been reported to induce G1-phase cell cycle and also cause apoptosis. Its anti-cancerous activity has gained a lot of interest and is now the most preferred target for the development of cancer drugs. They are very useful in cancer treatment. It has been seen to be prominently cytotoxic in both the in vivo and in vitro studies. Although a lot of studies have been done to know the mechanism of action of apratoxin-A but the understanding of how apratoxin-A is effective as anti-cancer drugs in humans is still unknown (Vijayakumar and Menakha, 2015).

9.4.3 CRYPTOPHYCIN

It performs its action by attacking the tubulin microfilaments present in the eukaryotic cells and eventually it prevents cell division and reproduction. According to a hypothesis, cyanobacteria produce this energetically expensive compound named cryptophycin because it is antifungal in nature and hence it helps to prevent fungi and other different types of algae that can compete among themselves for food and sunlight. The amount of cryptophycin is produced only by any one alga at any specific time on the basis of present environmental scenario. It has an excellent recognizing characteristic and this helps it to identify tumor cells, even the solid tumors like breast cancer, pancreas, ovarian, brain, lung, prostrate and colon and also cause destruction in the tumors that are multidrug-resistant. Such cancers are very difficult to be treated by even chemotherapy and around 85% of all deaths due to such cancers occurs in United States (Back and Liang, 2005). Cryptophycin 1 is extracted from *Nostoc* species GSV 224 and it also possesses the anti-cancerous activity against both human cancer cell lines and solid tumors (Shin 2001; El-Hack et al., 2019). Cryptophycin 5 is a chemical analog of cryptophycin-1 (Liang et al., 2005). Cryptophycin 249 and 309 are two other analogs that have better stability and water solubility and are being regarded as second-generation clinical agents (Vijayakumar and Menakha, 2015). It was reported that cryptophycin-8 which is a semi synthetic form of cryptophycin (Carmichael, 1992; Vijayakumar and Menakha, 2015) was assessed for its preclinical activity against subcutaneous tumors of human and mouse origins both. Cryptophycin-8 was four times less powerful than cryptophycin-1; however, it was more soluble in water and had greater therapeutic efficiency. It has shown more efficient in vivo antiproliferative action (Medina et al., 2008).

9.4.4 STYPOLDIONE

It constitutes an anti-quinone functional group that halts a number of biological activities like cell division. According to Medina et al. (2008), it binds covalently to sulfhydryl groups of thiol-containing component by the addition of sulfur to the C-4' position of the quinone ring. They had assessed the potential of stypoldione to add to sulfhydryl groups a lot of substances containing thiol, along with beta-mercaptoethanol, gluthione, thiophenol, and the protein tubulin.

9.4.5 LARGAZOLE

It is a cyclic depsipeptide found in a cyanobacterium that belongs to the genus *Symploca* and is a marine natural compound with a new chemical frame and is capable enough to inhibit Class 1 histone deacetylases (HDACs). It constitutes highly distinctive growth inhibiting activities and hence targeting only cells that are transformed rather than the non-transformed cells. Largazole has gained a lot of interest of the synthetic chemistry industry because of its fascinating structure and biological activity to define synthetic paths and to identify its ability as a therapeutic for cancer. Therefore, the current advances pay attention on the discovery, production, identification of target, structure-activity relationships (Luesch et al., 2001).

9.4.6 MICROCOLIN A

It is isolated from *L. majusculata*. It is a linear peptide that is an immunosuppressive which suppresses the lymphocyte reaction even at nanomolar concentration (Feldman et al., 1999).

9.4.7 CURACIN A

It is obtained from the organic extract of *L. majusculta*. It contains thiozoline (Carmichael, 1992). It is seen to have considerable anti-cancerous activity against breast cancer (Fukui et al., 1987).

9.4.8 SYMPLOCIN-A

It is a N,N-dimethyl-terminated peptide obtained from the Bahamian cyanobacterium *Symploca* sp. Chiral-phase high-performance liquid chromatography of the corresponding 2-naphthacyl esters was performed to assign the complete configuration of symplocin-A, along with the unexpected D configurations of the terminal N,N-dimethylisoleucine and valic acid residues. High performance liquid chromatography is a sensitive and effective method for assignment of N-blocked peptide residues as compared to Marfey's method which is not effectual. Symplocin-A possesses a considerable activity as it acts as an inhibitor of cathepsin E (Fukui et al.,1987).

9.4.9 CALOTHRIXIN-A

It is a pentacyclic metabolite that is extracted from microalgae that shows growth inhibiting activities like *Calothrix* species. The cell extract obtained were reported to show potent activity in a dose dependent manner against the growth of HeLa (cervix epithelial carcinoma cancer) cells even in nanomolar concentration (El-Hack et al., 2019). It was also seen to inhibit the growth of malarial parasite *Plasmodium falciparum* in vitro that became resistant to chloroquine. More than 50% of the marine cyanobacteria are capable enough to produce calothrixin. This compound is capable enough to kill tumor cells by either inducing apoptosis or by hampering cell signaling by activating the members of protein family kinase-c. These can exert their activity in nanomolar concentrations (Cardellina et al., 1979; Khalifa et al., 2019).

9.4.10 ORGANIC FRACTIONS FROM AMPHIDINIUM CARTERAE

Samarakoon et al. (2013) reported the anti-cancerous property of several extracts obtained from the dinoflagellate *Amphidinium carterae* on carious cancer cell lines like A549 (adenocarcinomic human alveolar basal epithelial cells), B16F10 (mouse melanoma tumor cells) and HL-60 (Human promyelocytic leukemia cells). The mouse monocyte macrophage cell line was also used for the cytotoxicity assays. The biomass obtained from the cultured marine microalgae was firstly freeze-dried and then it was converted into a fine powder. It was extracted later by using 80% methanol and then was homogenized at 25°C for 90 min using sonication. In order to concentrate the crude methanol extract, it was evaporated by using a solvent using a rotary evaporator under reduced pressure and was further partitioned. In order to obtain the fractions for testing, solvent-solvent partition chromatography was done using analytical grade n-hexane, ethyl acetate, chloroform and water. Then MTT (3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide) assay was used to measure the inhibition in cell growth. *A. carterae* chloroform fraction showed the maximum activity and reduced the viability of HL-60 cell by 50% after exposing it for 24 hours at 50 µg/mL concentration.

9.4.11 CHRYSOLAMINARAN POLYSACCHARIDE

Kusaikin et al. (2010) reported a polysaccharide that belonged to the family chrysolaminaran from the diatom *Synedra acus.* These storage polysaccharides are commonly known as water soluble biopolymers. The anti-cancerous property of the chrysolaminaran obtained from *S. acus* was studied on human colon cancer cell lines HTC-116 (human colorectal carcinoma cell line) and DLD-1 (colorectal adenocarcinoma cell line). The viability of cells was monitored by the MTS method. Tumor cells were then treated with 25, 50 and 100 µg/mL of concentration of chrysolaminaran for about 72 hours. The inhibition pattern in the various experiments was irregular, but the IC50 values for each cell line was determined: 54.5 µg/mL for HCT-116 and 47.7 µg/mL for DLD-1. Also, the authors did not observe any toxicity on the respective cell lines at a concentration above 200mg/mL and since most of the anti-cancerous drugs show toxicity at this level so this is a very potent characteristic (Hildebrand et al., 2017).

9.4.12 DOLASTATIN 10

This metabolite is obtained from sea hare *Dolabella auricularia* in lesser quantity but was originally referred to as a cyanobacterial metabolite after it was directly obtained from *Symploca* species (Kobayashi et al., 1997). It is basically a pentapeptide and contains four unique amino acids dolaisoleucine, dolavaline, dolaphenine and dolaproline. It has antiproliferative property. It attaches itself to tubulin on the rhizoxin-binding site and hampers the assembly of microtubule and causes cell arrest in G2/M phase. However, it failed as single agent in phase II of clinical tests because of peripheral neuropathy development in around 40% of the patients and there was lack of potent activity in patients suffering from hormone refractory metastatic adenocarcinoma (Natsume et al., 2003) and platinum-sensitive adenocarcinoma (Vijayakumar and Menakha, 2015). TZT-1027 (auristatin PE or soblidotin) is an analog of dolastatin 10. It is different from dolastatin 10 as there is no thiazoline ring from the dolaphenine residue. It was reported that it had shown its activity in two xenograft human models, LX-1 (lung carcinoma) in mice and MX-1 (breast carcinoma) (Cunningham et al., 2005). It shows efficacy against both mutant and p53 normal cell lines (Rickards et al., 1999; Martins et al., 2014).

9.4.13 DOLASTATIN 15

It is another member of the family dolastatin. It is a linear peptide that has anti-cancerous activity against cancer cell lines. There is a vinca alkaloid site on tubulin where it goes and binds and halts the transition into M phase. However, this metabolite has still not entered the clinical trial phase because of certain limitations such as complexity in structure, poor solubility in water and lower synthetic yield. Cematodin (LU103793) which is an analog of dolastatin 15, is soluble in water. It has a terminal benzylamine moiety in place of dolapyrolidone. It has higher cytotoxicity in vitro. BASF Pharma (Varanasi, India) had conducted its clinical trial and it had shown potent activity in Phase I for breast and other cancers but Phase II trial could not take place because of unfavorable results. Presently, ILX-651 (synthadotin) which is a third-generation analog has a terminal tert-butyl moiety in place of dolapyrolidone and has passed Phase I clinical trial successfully, and a Phase II trial has been suggested (Dias et al., 2012).

9.4.14 CAROTENOIDS

Chlorella are regarded as a good source of carotenoids like astaxanthin, lutein, zeaxanthin and β-carotene. Kwang et al. (2008) extracted carotenoids from the green algae *C. vulgaris* and *C. ellipsoidea* and reported the anti-proliferative property after it was tested on human colon carcinoma cell line (HCT116). Firstly, ethanol was used to extract freeze-dried Chlorella powder and then saponification was done by treating it with KOH and further partitioned was done using hexane. The hexane phase obtained was rich in carotenoids and it was further analyzed by utilizing HPLC-ESI-MS to identify the composition of ajorcarotenoid. The carotenoid extract obtained from *C. ellipsoidea* was composed of violaxanthin and two types of xanthophylls – zeaxanthin and antheraxanthin in lesser ratio. Lutein was the main component of the extract obtained from *C. vulgaris*. After the exposure of 24 hours with the microalgal extract, MTT assay was performed for measuring the anti-cancer activity. Kwang et al. (2008) had also performed annexin V-fluorescein assay to check the translocation of phosphatidylserine to find if apoptosis was the reason of the anti-proliferative activity. After the treatment with the extracts obtained from both *C. vulgaris* and *C. ellipsoidea*, the apoptotic activity was confirmed. In fact, the extract obtained from *C. ellipsoidea* had apoptosis activity 2.5 times higher (Martínez et al., 2018).

9.4.15 METHANOLIC EXTRACTS FROM AMPHIDINIUM CARTERAE, PROROCENTRUM RHATHYMUM, SYMBIODINIUM SP., COOLIA MALAYENSIS, OSTREOPSIS OVATA, AMPHIDINIUM OPERCULATUM, AND HETEROCAPSA PSAMMOPHILA

Shah et al. (2014) cultured around eleven different strains of benthic dinoflagellates (*Amphidinium carterae*, *Prorocentrum rhathymum*, *Symbiodinium* sp., *Coolia malayensis* strain 1, *Ostreopsis ovata* strain 1, *Ostreopsis ovata* strain 2, *Coolia malayensis* strain 2, *Amphidinium operculatum* strain 1, *Heterocapsa psammophila*, *Coolia malayensis* strain 3 and *Amphidinium operculatum* strain 2). In 2011, these were isolated from the coast of Jeju Island (Korea) and were used against HL-60 (human promyelocyticleukemia cell line) and RAW 264.7 (murine macrophage cell line) cells. They had described the particular location of sampling and the microbial growth phase tested. The freeze-dried biomass obtained from the cultured marine microalgae was converted into a fine powder, then 80% methanol was used for extraction and then sonication was done for homogenizing at 25°C for 90 min to obtain methanolic extracts. After the exposure of extract for 24 h at 37°C, the cell viability was tested using the MTT assay.

9.4.16 MONOGALACTOSYL GLYCEROLS

Andrianasolo et al. (2008) reported two different varieties of monogalactosyl glycerols obtained from *Phaeodactylum tricornutum* and were used for testing against immortal mouse epithelial cells (W2 and D3). In the D3 cell line, the function of apoptosis is disabled by the mechanism of gene deletion and the W2 cell line is a wild type. In this test, the minimum values needed for induction of apoptosis were a growth rate of atleast 10% on the D3 cell line and at a death rate of 20% on the W2 cell line.

9.4.17 NAMO (NONYL 8-ACETOXY-6-METHYLOCTANOATE)

Samarakoon et al. (2014) had extracted 8-acetoxy-6-methyloctanoate from *Phaeodactylum tricornutum* and had reported its anti-cancerous activity after testing it on three different cell lines: mouse melanoma cell line (B16F10), human lung carcinoma cell line (A549) and human promyelocytic leukemia cell line (HL-60). It was then screened at 25 and 50 µg/mL for 48 hours. It was seen to be active only against HL-60 cells at both the concentrations specified. At a concentration of 50 µg/mL of NAMO, the highest growth inhibitory activity of around 70% was observed on HL-60 cells. NAMO performs its action by inducing DNA damage and increment in the formation of apoptotic body. It was observed that proportional to the concentration of NAMO, there was cell cycle arrest and there is accumulation of cells in the sub-G1 phase. The increment in the expression of both p53 proteins and caspase-3, suppression of the anti-apoptotic protein Bcl-x and pro-apoptotic protein Bax's activation were observed by the author.

9.4.18 STIGMASTEROL

Kim et al. (2014) extracted Stigmasterol obtained from *Navicula incerta* using chromatography methods like preparative thin layer chromatography (PTLC) and silica gel open column chromatography. The isolated stigmasterol was used to screen the anti-proliferative properties on HepG2 (human liver hepatocellular carcinoma cell line) at a concentration of 5, 10 and 20 μM. A dose-dependent trend was indicated by the values of cytotoxicity of 40%, 43% and 54% respectively. Stigmasterol which has a structure similar to phytosterol with double bonds in the C-5 and C-22 positions are observed to induce apoptosis. Here flow cytometric measurements of cell cycle arrest, apoptosis pathways analysis, fluorescence-activated cell sorting, controlling morphological changes and gene expression levels were used to study apoptosis (Ryu et al., 2009).

9.4.19 VIOLAXANTHIN

Pasquet et al. (2011) used the extracts obtained from the green algae *Dunaliella tertiolecta* on four different cell lines: LNCaP (Lymph Node Carcinoma of the Prostate cell line), MDA-MB-231, MCF-7 and A549 for assessing the anti-cancerous activity. A wide range of solvents like ultrapure water, dichloromethane and ethanol that varied in terms of polarity were used for preparing different extracts. Cell viability was measured using MTT assay. Considerable activity was shown by dichloromethane extract against MCF-7 cancer cell line. Subfraction of dichloromethane extract was obtained using RP-HPLC (Reversed-phase high performance liquid chromatography) analysis and fractionation and was screened at concentrations between 0.1 μg/mL and 40 μg/mL for 72 hours. The subfraction at the rate of 95% was recognized as violaxanthin. Also, the DNA obtained from both the treated and non-treated cells were extracted and analysis was done using gel electrophoresis.

9.4.20 HYDROPHOBIC FRACTION FROM SKELETONEMA MARINOI

Lauritano et al. (2016) had identified and studied 32 species of microalgae by 18S sequencing and microscopy. With a ratio of acetone:water (1:1), the microalgal biomass was extracted from the cultured marine microalgae and further AmberliteRXAD16N resin with acetone was used to fractionate so as to obtain a fraction that is hydrophobic. The hydrophobic fractions obtained from *Alexandrium andersonii*, *Alexandrium tamutum*, *Alexandrium minutum* and *Skeletonema marinoi* were tested at a concentration of 100 μg/mL on melanoma cancer cell line (A2058). Further tests performed on normal lung fibroblast (MRC-5) cell line proved that *Alexandrium* species were toxic.

To check the cytotoxicity in both normal and cancer cell lines, the colorimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, atetrazolium dye were utilized for the quantification of viable cells.

9.4.21 FUCOXANTHIN

It is found in both micro and macro algae and is one of the most studied metabolites. This pigment belongs to the xanthophylls family and is an important carotenoid present in brown algae (Martínez et al., 2018). Ishikawa et al. (2008) performed an experiment to evaluate the toxicity of fucoxanthin and fucoxanthinol in mice. They had also used a higher dose of 200mg/kg for 28 days but no toxicity was developed. Another experiment was performed to assess toxicity of fucoxanthin by using a dose of about 10 mg/kg and 50 mg/kg for 28 days. It was considered as a safe pharmaceutical agent because it did not cause any toxicity. Hosokawa et al. (1999) had also reported the considerable anti-proliferative property of fucoxanthin against HL-60 cells and they induced apoptosis. The cells were treated with fucoxanthin in concentration of 11.3 and 45.2 μM and after 24 hours had shown viabilities of 46.0% and 17.3%, respectively. Tryphan blue was used for dye exclusion test for measuring viability of cells. He had also tested the toxicity of fucoxanthin against three human colon cancer cell lines (HT-29 (human colon adenocarcinoma), DLD-1 and Caco-2 (colorectal adenocarcinoma cells)). After the analysis by WST-1 assay, it was observed that the Caco-2 cell line was affected more as compared to the other two cell lines. Peng et al. (2011) reported that microalgae species *Phaeodactylum tricornutum*, *Chaetoceros*, *Odontella aurita* and *Cylinrotheca closterium* produce fucoxanthin and they had also summarized studies related to their role as bioactive compound like as an anti-inflammatory, anti-diabetic, anti-cancerous, antioxidant, bone protective agent and skin protective agent. Kotake-Nara et al. (2001) had studied 15 different types of carotenoids on three different types of prostate cancer cell lines: LNCaP, DU145 (prostate cancer cell lines) and PC-3 (human prostate carcinoma) and it was observed that fucoxanthin is the most active compound with anti-cancerous property. 20 μM of fucoxanthin was added and after 72 hours, MTT assay was used to determine cell viability in percentage as 9.8% for LNCaP, 5.0% for DU145 and 14.9% for PC-3.

9.4.22 AQUEOUS EXTRACT FROM A CANADIAN MARINE MICROALGAL POOL

Somasekharan et al. (2016) extracted the marine microalgal compound from Canada (dried powder) to study the anti-proliferative property on 8 different varieties of cell lines, along with the anti-colony forming activity. The microalgal powder obtained was suspended at a concentration of 30mg/ml in distilled water and then was sonicated. In order to release the contents of cytosol, it was then passed through 25-gauge needle after sonication after syringe filtration by 0.2 μm filters. This aqueous extract was tested against A549, MNNG (bone cancer cell line), DU145, BxPC-3 (pancreas cancer cell line), N87 (stomach cancer cell line), PC-3, MCF7 (Breast cancer cell line) and H460 (lung adenocarcinoma cell line). Then the cells were incubated with the extract at 0 (control), 1, 2 and 5 mg/mL for 72 hours. Then MTT assay was performed to check cell viability. No significant activity was shown by the extract at 1–2mg/mL except for on the MNNG cell line as the cell viability reduced to 50% at 2 mg/mL. But at a concentration of 5 mg/mL, all the cell lines were considerably inhibited. A crystal violet test was also performed by the authors at 0.5–5mg/mL to check the anti-colonial activity of the extract. This test proved that even at a low concentration of 0.5 mg/ml, the extract was successful to inhibit the colony forming ability of all the cancer cell lines.

9.4.23 EICOSAPENTAENOIC ACID (EPA)

Nappo et al. (2012) performed the analysis of the anti-cancerous activity of the extract obtained from the marine diatom *Cocconeis scutellum* on BRG-M (Burkitt's lymphoma cells), COR (Epstein-Barr Virus-transformed B cells isolated from human tonsils), JVM2 (lymphoblast immortalized with Epstein-Barr virus), MB-MDA468 (human breast cancer), BT20 (breast tumor cell line) and LNCaP (human prostate adenocarcinoma cells). It was determined that the extract obtained from that *C. scutellum* showed more effectivity on the BT20 cell line. Diethyl ether extract which is the most active form obtained from *C. scutellum* after fractionation could produce further three fractions with different activities. No significant reduction in cell viability was observed from fraction 1 and 2 but fraction 3 was successful in reducing it by 56.2%. Annexin V/propidium iodide staining methods were used for the evaluation of DNA fragmentation. Further the analysis of composition of all three fractions were carried out and it was observed that fraction 1 constituted 2.4% fatty acids and 77.2% of glycerides, fraction 2 constituted 3.2% sterols, 66.7% fatty acids and 11% monoglycerides and fraction 3 constituted 2.3% 4-methylcholesterol and 81.7% fatty acids. By evaluating these outcomes, it was concluded that the anti-proliferative activity was governed because of the presence of fatty acids, particularly eicosapentaenoic acid because it is the only product present in the fraction that induced apoptosis. Further analysis done by Western blot also confirmed the activation of caspases 3 and 8. But it was still not concluded that whether EPA is the only compound which is alone responsible for causing apoptosis or it is a result of any association which is synergic (Chajès et al., 1995).

9.4.24 POLYUNSATURATED ALDEHYDES (PUAs)

Miralto et al. (1999) reported three polyunsaturated aldehydes obtained from the marine diatoms *P. delicatissima*, *S. costatum* and *Thalassiosira rotula*. They found that 2-trans-4-trans-decadienal, 2-trans-4-trans-7-cis-decatrienal and 2-trans-4-cis-7-cisdecatrienal had anti-proliferative activity on the human colon adenocarcinoma cell line (Caco-2). They had utilized various concentrations of PUAs between 0 and 20 μg/mL after incubation of 48 h to determine the anti-proliferative activity. It was further concluded that concentration of 11–17 μg/mL was effective in reducing cell viability to about 0%. Also, to determine the occurrence of apoptosis to check the DNA fragmentation, a TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) assay was performed. Sansone et al. (2014) used colon COLO 205, lung A549 and adenocarcinoma cell lines for testing the commercially available PUAs-2-trans-4-trans-heptadienal (HD), 2-trans-4-trans-octadienal (OD) and 2-trans-4-trans-decadienal (DD). These polyunsaturated aldehydes were tested at different concentrations (i.e., 2, 5 and 10 μM) and at different time of exposure (i.e., 48 and 72 h). Cell viability of 70%, 50% and 18% was induced when A549 cells were exposed to DD at a concentration of 2, 5 and 10 μM respectively after 24 hours. Cell viability of 80%, 44% and 26% was induced when COLO 205 cell lines were exposed to DD at a concentration of 2, 5 and 10 μM respectively. Cell viability decreased to 35% when A549 cells were exposed to OD at a concentration of 10 μM for 72 hours. Cell viability decreased to 60%, 60% and 41% when COLO205 cells were exposed to OD at a concentration of 2, 5 and 10 μM for 72 hours. Cell viability reduced to 10% and 0% when A549 cells were exposed to HD at a concentration of

10 μM for 48 and 72 hours respectively. Cell viability reduced to 40% and 28% when COLO205 cells were exposed to HD at a concentration of 10 μM for 48 and 72 hours respectively. They had also concluded that PUAs are non-toxic to normal cells by testing it on epithelial BEAS-2B cell lines.

9.4.25 AQUEOUS EXTRACT FROM *CHLORELLA SOROKINIANA*

Chlorella species is usually produced in Asia and is commonly used as a dietary supplement in most of the countries. There are various patents regarding its application as dietary supplement like for example US2005/0196389A1 (Lin et al. 2017).

Lin et al. (2017) used lung adenocarcinoma cell lines to study the activity of hot water extracts obtained from the diatom *Chlorella sorokiniana*. Reflux extraction was done to obtain extracts from the dried biomass using distilled water and further filtration done using N 0.5 filter paper. MTT assay was performed at 15.625 to 1000 mg/mL concentration to determine cytotoxicity and the outcomes proved that there was a reduction in the dose-dependent in cell viability in both the cancer cell lines. Annexin V/propidium iodide staining was performed to study the mechanism of action of the extract obtained from *C. sorokiniana* so as to confirm possible apoptotic process. Even after the exposure of 24 hours, cell cycle was not observed but in sub-G1 phase, there was an increase in the cell number which indicates apoptosis. After the exposure of extract after 24 hours, Western blot analysis was performed and it was observed that activated forms of Poly (ADP-ribose) polymerase (PARP), caspase 3 and caspase 9 increased in both the cell lines. It was reported that the main pathway involved in apoptosis was because of mitochondrial pathway in case of activation of caspase 3 and caspase 9. Increment in the ratio of Bax/Bcl-2 (pro/anti-apoptotic proteins) after 24 hours which is another indication of apoptosis.

9.4.26 ETHANOL AND ETHYL ACETATE EXTRACTS FROM *CHAETOCEROS CALCITRANS*

Ebrahimi et al. (2013) produced an ethanolic extract obtained from the planktonic diatom *Chaetoceros calcitrans* on peripheral blood mononuclear cells (PMBC), breast epithelial (MCF-10A) and breast adenocarcinoma (MCF-7). Homogenization of biomass from microalgae was done to obtain ethanolic extract and a 0.2 μm filtration unit and filter cotton were used for filtration.

The results obtained were compared to the already present drug for breast cancer named as tamoxifen. MTT assay was done to check the viability of cells in PMBC, MCF-10 A and MCF-7 cells. No cytotoxicity was seen on PMBC cells after using the extract from *C. calcitrans* even at concentrations higher than the normal and its activity is specific for only cancerous cells. Goh et al. (2014) studied the activities of the extract obtained from *C. calcitrans* on a number of cancer cells. They had used Caov3 (human ovarian adenocarcinoma), MDA-MB-231, HepG2, HT-29, A549, MCF-7, HeLa, PC-3 and 4T1 (mouse breast carcinoma). There were four crude solvents named methanol, hexane, ethyl acetate and dichloromethane whose cytotoxicity was measured.

9.4.27 SCYTONEMIN

This is obtained from *Stigonema* species. Its anti-proliferative properties have been reported against many forms of human fibroblast and endothelial cells. It plays major role in regulating mitotic division and cell cycle (Rickards et al., 1999). Also, *Leptolyngbya* species is used for extracting a new compound named n-methyl-depsipeptide coibamide. It was reported to induce the formation of autophagosome and cause individual cell death in human SF295 and U87-SG glioblastoma cells and mouse embryonic fibroblasts (MEF) cells. Scytonemin has anti-inflammatory and anti-proliferative property (Stevenson et al., 2002).

9.4.28 COIBAMIDE

This compound possesses strong cytotoxic impact against NCI-H460 lung cancer cell even in nanomolar concentration. It is also responsible for causing upregulation of G1 cells in dose dependent manner. It was also tested for over 60 cell lines for ovarian, breast and colon malignant cancers in humans. It was also reported to be active against MDA-MB-231 cell line (human breast adenocarcinoma) (Hau et al., 2013).

9.4.29 C-PHYCOCYANIN

It is obtained from microalgae *Spirulina platensis*. It has the capability to induce DNA fragmentation and pathogenic alteration. It is responsible for both upregulating ICAM (Intercellular Adhesion Molecule) and FAS (Fatty Acid Synthase Gene) expression and downregulate Bcl2 (B-cell lymphoma 2) expression. It is also responsible for activation of caspases 2, 3, 4, 6, 8, 9, 10 in MCF7 and HeLa cell line (Medina et al., 2008). Inhibitory effect was shown by ethanolic extracts obtained from *Aphanizomenon flos-aquae* at G0–G1 stage against AML cell lines (Li et al., 2006). Potent anti-cancerous activity was shown by *Haematococcus pluvialis* and *Aphanizomenon flos-aquae* against HL-60 cell lines and MV-4-11 (Bechelli et al., 2011). The treatment using either extracts or the growth media obtained from *Phormidium* mole on adherent cell lines such as FL (Fogh and Lund), Jurkat, 3T3, HeLa, RD (Rhabdomyosarcoma), A2058 and U-937, it was observed in the clinical trials that it induces alterations and causes destruction in dose dependent manner (Dzhambazov et al., 2006). Another compound obtained from *Symploca* species is known as LU 103793 (N, N dimethyl-I-valyl-L-valyl-W-methyl-L-valyl-L-prolyl-L-prolinebenzylamide). This compound possesses anti-tumor properties that interfere with the microtubule synthesis (Kerbrat et al., 2003).

9.5 BENEFITS OF USING ALGAE-BASED PHARMACEUTICALS FOR CANCER

The ocean has been identified as a rich source of natural products and new chemical compounds that possess unique activities that can be utilized for finding drugs that have better efficacy and increased specificity for disease treatment in humans (Haefner, 2003). Micro flora have existence of around 3.5 billion years old and are still the best source of chemicals in nature. Cyanobacteria produce a number of chemical compounds to survive in harsh conditions like variation in temperature, salinity and pressure present in their surroundings (Guedes et al., 2011). The chemical compounds produced are novel in terms of their either structure or function (Ibrahim et al., 2015). The cyanobacterial population provides a great chance in new drug discovery. They possess antibacterial, anti-cancerous, anti-viral, antioxidant and antifungal properties (Alves et al., 2018). All these characteristics can be used to treat a number of deadly diseases. The compounds extracted from cyanobacteria have shown promising results specially in cancer treatment. Oral cancers can be cured by the use of β-carotenes that have antioxidant properties (Aditya et al., 2016). The present method used for cancer treatment i.e. chemotherapy has a number of side-effects that are life threatening. Resistance among cancer cells have also been reported. A lot of drugs currently available for cancer have efficient efficacy but have very serious side-effects. Their supply is also limited and are expensive (Feng and Chein, 2003). The antioxidant property of compound present in cyanobacteria inhibits the growth of cancer cells by causing regression of premalignant regressions (Bisen 2016; Brandão and Longhi, 2016; Ichihara et al., 2016; Liu et al., 2016; Pastorino et al., 2016; Shukla et al., 2019). It has been reported that cyanobacteria help in cancer prevention by stopping oxidative damage by scavenging free radicals and active oxygen. The sulfate polysaccharides that are obtained from cyanobacteria have radical scavenging properties (Abass et al., 2016; Ariza and Alberto, 2016; Fortela et al., 2016; Osman et al., 2016; Saleh, 2016). There are a number of methods for extraction designed by the researchers for obtaining compounds with beneficial properties. The drug discovery is a time taking and an expensive process. It can take around 15 to 20 years and billions of dollars. Therefore, identification of a less expensive method for drug production is the call of the hour. Scientists are putting great efforts to find drugs that are not only effective but also impart lesser side effects. Research is going on to develop novel methods of administration and new devices for drug delivery for better efficacy. The application of metabolites present in cyanobacteria having anti-cancerous properties may improve the cancer treatment. Their potential as an excellent source of novel therapeutic lead compounds have been identified in the last two decades as they possess a broad spectrum of activities. These natural metabolites when used as drug offers no or less cytotoxicity as compared to the synthetic drugs and hence no compromise with the quality of patient's life. Another benefit of using cyanobacteria as a source for drug discovery is present in the economical cultivation in comparison to other microorganisms as cyanobacteria require only simple organic nutrients for growth. Secondary metabolites from cyanobacteria have a source of novel entities because of a higher microbial diversity that leads to new pharmaceutical development (Shi et al., 1999). The cyanobacteria possess an additional benefit because of presence of metabolic plasticity. They can be exposed to a variety of external stress for enhancing the production of secondary metabolites (Olaizola, 2003). Cyanobacteria holds a promising future in the development of drugs for cancer with lower cost but higher benefits. They have high availability (Tonon et al., 2002; Somasekharan et al., 2016) (Figure. 11.2).

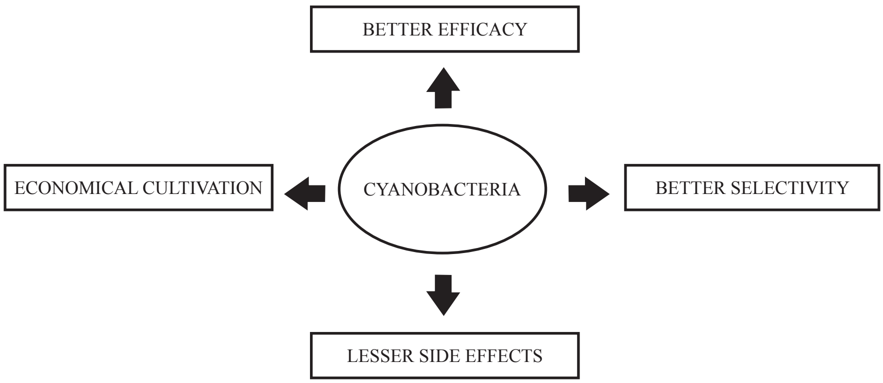

FIGURE 9.2 Benefits of using the compounds obtained from cyanobacteria.

The compounds obtained from Cyanobacteria have more novelty and structural diversity as compared to the synthetic compounds. These natural metabolites can easily interact with proteins and other biological molecules inside in body. They have more complexity in structure that allows them to bind selectively to the targets (https://www.cancerquest.org/patients/discovery-and-development-drugs). These natural metabolites are capable of being absorbed and eventually metabolized in the body (Calixto, 2019). Other advantages of using pharmaceuticals obtained from cyanobacteria are low risk to induce multi-drug resistance, various routes of administration, and ease of absorption. Cyanobacteria are a promising area for the development of cancer drugs for next generation (Kiruba et al., 2018).

9.6 STATUS OF ANTI-CANCEROUS DRUGS FROM CYANOBACTERIA IN COMMERCIALIZED PLATFORM (PRESENT SCENARIO)

Out of the total drugs that were approved between the years 1983 to 1994, around 80% of the drugs for bacterial infection and cancer treatment were obtained from natural source. Pharmaceutical producers have kept their focus on conventional microbial drug producers such as Hyphomycetes and Actinomycetes for decades. These natural therapeutic agents obtained from marine sources have a considerable contribution in the anti-cancer drug's development after they gained public approval for clinical trials in Europe and USA specially. These natural products obtained from cyanobacteria have paved path for new phase of research (Bajpai et al., 2018). *Spirulina*, which is a cyanobacterial species, is consumed directly without undergoing any pre-processing steps because of its nutritional value. These have been reported to boost the immune system and have anti-cancerous properties and hence these provide protection to cells against a number of disorders like cancers and inflammation (Deo et al., 2014; Mishra et al., 2014). Over the past few years, both pharmaceutical organizations and research institutes have made considerable efforts in order to isolate and identify the novel products present in cyanobacteria. A lot of these natural products have gained significant importance after their successful clinical trials and a lot of them are still under clinical or pre-clinical trials and are under further investigations (Table 11.2). Some compounds obtained from different microalgae and their status.

Although a number of cyanobacterial compounds have failed as they were not approved in phase-1 or phase-2 of clinical trials because severe neuromuscular toxicity and anaphylaxis but still the search for a better anti-cancer drug from cyanobacteria has not stopped as they were more potent than the traditional anti-cancer drugs (Mayer et al., 2010).

Presently a number of natural products have been isolated after a lot of research has been published that proves their anti-cancer properties (Lee et al., 2013). Still only a few of them have been commercialized and reached the markets. The identification of commercial and medical applications of cyanobacteria have arisen huge interest among the scientists to study these organisms more deeply. These have also acted as lead compounds for synthetic analog's development with better bioactivity. Main emphasis is given for finding the drugs to cure fatal human diseases like AIDS and cancer. Scientists have discovered various drugs for these diseases in past few years but the diversity possessed by the marine population in terms of chemical or biological is immeasurable and is therefore an extraordinary source for the discovery of novel drugs for cancer. These drugs after successful clinical trials will be translated into a lot of new treatments for cancer in the future. Some techniques are developed and standardized for steps such as extraction, isolation, detections and further purification of the bioactive compounds from cyanobacteria. Two antibody drug conjugates named as tisotumab vedotin and pinatuzumab vedotin that have been obtained from cyanobacteria and mollusks, are reported to be effective against chronic lymphocytic leukemia and non-Hodgkin lymphoma (Advani et al., 2017) because they have the ability to inhibit the growth of tumor (de Goeij and Lambert, 2016) and are approved for phase-1 of clinical trials. The cyanobacterial species have a bright future in research and holds many opportunities for the discovery of drugs for cancer. On contrast, many clinical trials are terminated of some of the compounds. Around 33% of these drugs were not approved because they were toxic and 43% of them were rejected because of lack of efficiency. Many of them were not approved because of their non-specificity

TABLE 9.2 Some Compounds Obtained from Different Microalgae and Their Status.

Compound	Organism	Status	References
Apratoxin-A	*Lyngbya majuscule*, *Lyngbya bouillonii*	Preclinical; Effective against a colon tumor and ineffective against a mammary tumor.	Vijayakumar & Menakha (2015); Bajpai et al. (2018); El-Hack et al. (2019)
Calothrixin-A	*Calothrix*, *Lyngbya majuscule*	Cytotoxicity against mouse neuro-2a cells and NCIH460 lung.	Vijayakumar & Menakha (2015); El-Hack et al. (2019)
Cryptophycin-1	*N. linckia*, *Nostoc* sp. GSV 224	In market	Vijayakumar & Menakha (2015); Bajpai et al. (2018)
Largazole	*Symploca* sp.	Preclinical; Inhibition of the growth of highly invasive transformed MDA-MB-231.	Vijayakumar & Menakha (2015); El-Hack et al. (2019)
Coibamide	*Leptolyngbya* sp.	Cytotoxicity against NCIH460 lung and mouse neuro-2a cells.	Vijayakumar & Menakha (2015); El-Hack et al. (2019)
Curacin-A	*L. majuscule*, *Lyngbya aestuarii*	Hepatocellular carcinoma cell line HEPG2, colon adenocarcinoma cell line HT-29.	Vijayakumar & Menakha (2015); El-Hack et al. (2019)
Fucoxanthin	*Chaetoceros* sp., *Cylinrotheca closterium*, *Odontella aurita*, *Phaeodactylum tricornutum*, *Haematococcus pluvialis*, *Chlorella zofingiensis*, *Chlorococcum* sp. and *Cladosiphon okamuranus*	The viability of HL-60 cells was decreased.	Martínez et al. (2018); Alves et al. (2018); El-Hack et al. (2019)
Scytonemin	*Stigonema* sp.	Inhibition of Jurkat T cell proliferation.	El-Hack et al. (2019)
C-Phycocyanin	*Chlorella* and *Arthrospira*, *Spirulina platensis*, *Porphyridium* sp. cyanobacteria such as *Lyngbya* sp. and *Symploca* sp.	Inhibition of cell proliferation and reducing colony formation ability of MDA-MB-231 cells.	El-Hack et al. (2019)

as they destroyed considerable number of normal cells along with cancer cells (Bajpai et al., 2018). Although the idea of utilizing drugs obtained from natural source for the prevention and treatment of cancer is widely accepted but still the research is limited in this field (Somasekharan et al., 2016). More research is being done to identify strains that have yield, for optimizing cultivation and also utilize the approach of genetic engineering for modification of the strain to produce high-value added products (Markou et al., 2015). These compounds have the capability to induce or modify cell metabolism (Yu et al., 2015). Cyanobacteria is still a resource that is unexplored and therefore some strategies are required to design an ideal and optimal environment to obtain high quantity of products from cyanobacteria.

9.7 CHALLENGES

Although the compound isolated from cyanobacteria have been identified to possess potential applications in bioindustries but there are still many challenges that need to be met to realize scalable productions. The first challenge in commercialization is achieving production yields (Kiruba et al., 2018). A lot of them are not able to reach clinical trials because they are even difficult to be synthesized in laboratory. Cryptophycin is one of them. It is expensive to be produced synthetically in laboratory because of complexity in its structure although it is produced naturally as well. Back and Liang (2005) had reported the high-level production of cryptophycin. So, few of them are only produced at industrial level (Clarens et al., 2010; Norsker et al., 2011). There is not enough information regarding structure. Also, their production either in laboratory or at industrial level is time consuming. There are also chances that these natural compounds function differently than the compounds isolated from their source (https://www.cancerquest.org/patients/discovery-and-development-drugs-). There are also challenges related to the safe accessibility to marine sources with proper identification and efficient screening of samples and compounds. Technical category and supply deals with challenges related to knowing the mechanism of action for any specific target. Now as discussed, the synthesis and production are a costly process so this will be an important factor in determining its price in the market in future. If the drug developed is costly, then not all the sections of society will be able to afford such medicines. So, making the cyanobacterial drugs cost-effective is another challenge. The discovery of drug and eventual commercialization would create pressure on the sustainable resource and will lead to harmful environment issues. Now synthesis of these active molecules is also an option but not all molecules are accountable for the complete synthesis and therefore the dependence on the lead resources will continue (Martins et al., 2014). Other challenges associated with commercialization of the pharmaceutical agents obtained from cyanobacteria are accessibility and enough supply, the complications in the chemistry of natural product related to slowness of functionality with natural products and intellectual property right concerns (Baker et al., 2007; McChesney et al., 2007; Rishton, 2008). Some of the species like *Microcystis* is reported to be toxic to human and animals. So, before a drug is commercialized, its toxicity should be determined thoroughly in clinical trials (Sharma et al., 2011). So, these challenges should be considered when designing strategies for development of cyanobacteria based anti-cancer drugs and their commercialization (Bajpai et al., 2018).

9.8 STRATEGIES

To exploit the benefits available, we need to design novel methodologies for isolation and culturing of microorganisms that produce natural products. It is necessary to identify and produce only the active parts of compounds to minimize the risks of several activities of toxins. More research and clinical trials are needed to overcome the problems of delivering these compounds to the right cells at the right time. Previously the scientists used the "one target one drug" model for systematically modulating various targets. The experiments of interaction between the drug and the target is both costly and labor intensive so strategies should be planned to bring more advancement in the *in silico* prediction processes which is an efficient way of providing information. Identification of drug-target protein interaction helps to discover genomic drug (Alves et al., 2018) (Table 11.3).

Some compounds obtained from different microalgae and their target cells. Proper clinical trials should be conducted to determine the safety and efficacy of a number of anti-cancer compounds at the biomedical platform (Bajpai et al., 2018). Their therapeutic index should also be monitored to determine the efficacy in cancer treatments. The other reason for the low number of compounds in clinical trial or in market in comparison to the total number of anti-cancer compounds isolated is also because of insufficient funding by pharmaceutical organizations. We need to develop high-throughput screening technologies that will help to attain accurate and faster results. There are various reports that provide evidence of the cytostatic and cytotoxic characteristics of compounds derived from cyanobacteria and some studies have also described the intracellular signaling pathways. *Sphaerococcus coronopifolius* is one

TABLE 9.3 Some Compounds Obtained from Different Microalgae and Their Target Cells

Compound	Organism	Target Cells	References
Polysaturated aldehydes	*Thalassiosira rotula*, *Skeletonema costatum* and *Pseudonitzschia delicatissima*	Colon adenocarcinoma (Caco-2), Lung adenocarcinoma (A549).	Martínez et al. (2018)
Carotenoid extract	*Chlorella ellipsoidea*	Colon carcinoma (HCT-116).	Martínez et al. (2018)
Violaxanthin	*Dunaliella tertiolecta*	Breast adenocarcinoma (MCF-7).	Martínez et al. (2018)
Eicosapentaenoic acid (EPA)	*Cocconeis scutellum*	Breast carcinoma (BT20)	Martínez et al. (2018)
Fucoxanthin	*Chaetoceros* sp., *Cylinrotheca closterium*, *Odontella aurita*, *Phaeodactylum tricornutum*, *Haematococcus pluvialis*, *Chlorella zofingiensis*, *Chlorococcum* sp. and *Cladosiphon okamuranus*, *Tokida*	Promyelocytic leukemia (HL-60), Caco-2, colon adenocarcinoma (HT-29), DLD-1 and prostate cancer (PC-3, DU145 and LNCaP).	Martínez et al. (2018); El-Hack et al. (2019); Alves et al. (2018)
EtOH extract	*Chaetoceros calcitrans*	MCF-7 Breast adenocarcinoma (MDA-MB-231).	Martínez et al. (2018)
CH_3Cl	*Amphidinium carterae*	HL-60 HL60, Skin melanoma (B16F10), A549.	Martínez et al. (2018)
MeOH extract	Eleven strains of benthic diatoms *Ostreopsis ovata*, *Amphidinium operculatum*, *Chlorella*, *Stigmatophora*, *Phaeodactylum tricornutum*, *Spirulina fusiformis*	HL-60	Martínez et al. (2018); El-Hack et al. (2019)
Stigmasterol	*Navicula incerta*	Liver hepatocellular carcinoma (HepG2).	Martínez et al. (2018)
NAMO	*Phaeodactylum tricornutum*	HL-60 Mouse epithelial cell lines (W2, D3).	Martínez et al. (2018)
Aqueous extract	Canadian marine microalgal pool, *Chlorella sorokiniana*, *Chlorella*, *Stigmatophora*, *Phaeodactylum tricornutum* and *Spirulina fusiformis*	A549, lung carcinoma (H460), prostate carcinoma (PC-3, DU145), stomach carcinoma (N87), MCF-7, pancreas adenocarcinoma (BxPC-3) and osteosarcoma (MNNG).	Martínez et al. (2018); El-Hack et al. (2019)

of the evidences as its some of the bioactive agents have cytotoxic activities on various cell lines but the intracellular signaling pathways involved in its activities is still not characterized deeply. Hence there is a need to design and conduct studies to access these types of molecules in a biological system that is more complex along with in vivo models. 3D chemical structural modeling technique is another significant tool that remains unexplored and can be used to find novel biochemical targets for the compounds derived from cyanobacteria. Evaluation of the compounds obtained from cyanobacteria as co-adjuvants in therapies should also be done (Alves et al., 2018). Another challenge is the sustainable production of these anti-cancer compounds to provide the adequate supply for pre-clinical, clinical and commercialization in future because slow growth, seasonality and low extraction yields of cyanobacteria are some of the limitations. This will not only fulfill the sustainable and continuous production but also enhance or improve the functional zones of the bioactive molecules. To fulfill the continuous demand, aquaculture is another interesting approach. However more studies need to be done to see if cyanobacteria can produce the specific compounds under desired conditions. Another potent technique for overcoming limitations and improving the potentials of these compounds is by the application of nanotechnology by the development of nanoformulations. But further some new technologies are needed to improve the anti-cancer property in nanoformulations. The establishment of inter-disciplinary teams comprising biologists, pharmaceuticals, biotechnologists and chemists will help to design a faster bioprocessing method including isolation, identification to full validations of their anti-cancer properties (Lau et al., 2015). A centralized database of these phytochemical compounds can also be constructed for conducting the molecular and genetic level studies. All these strategies should be applied to remove the hurdles present in the way of developing anti-cancer drugs of cyanobacterial origin that have maximum efficacy and minimum side effects (Kiruba et al., 2018).

9.9 FUTURE

The natural compounds obtained from algal and cyanobacterial community that are biologically active are utilized in a number of biomedical and industrial sectors from the beginning of the civilization (Bajpai et al., 2018). Cyanobacteria that are found all over the globe, are loaded with a variety of novel characteristics that helps them to survive in all the severe situations that these encounters in the ecosystem. The novel properties are present because of the variations in the macro and micro-molecular constituents in the cells that are formed under the adverse conditions. These metabolites have unique properties. There are around thousands of cyanobacterial species but only around 25–35% of them have been recognized and collected. Hence, there is a huge resource available that is unexplored and needs to be commercialized in the pharmaceutical industry (Bhattacharjee, 2016). Cyanobacteria produce a variety of therapeutic agents that are either obtained from biomass or are extracellularly released into the medium. They contain many bioactive compounds like sterols, vitamins, polysaccharides, enzymes, proteins, lipids, polykitides, lectins, antioxidants, MAAs, steroids and halogenated compounds with nutritional and pharmaceutical importance (Priyadarshani and Rath, 2012). Our understanding in the field of metabolites obtained from cyanobacteria has improved a lot and this is going to get more better in future after all the challenges are met. The complex organic compounds obtained from cyanobacteria have unique structure and stereochemistry. These compounds either are difficult to synthesize artificially or are expensive. So, for utilizing cyanobacteria at the large scale, it has to be set as "cell factories" for producing compounds of interest and addressed through post-genomic technologies. Besides, the proteomic evaluation has been considered vital. Conjugation of genomic revolution with the natural product obtained from cyanobacteria will change the scenario of research in future. A revolution has been brought in the discovery of natural products by the genetic characterization of the secondary metabolites found in cyanobacteria like dolastatin, cryptophycin and the metabolites that belong to the class of non-ribosomal peptide synthase (NRPS) gene family and polyketide synthase (PKS). Barrios-Llerena et al. (2007) reported that of all the 21 strains of cyanobacteria, 19 strains have the PKS genes and NRPS genes are present in the 18 strains. More information regarding the biosynthesis of metabolites that are biologically active can be gathered after understanding the design of these gene clusters. The traditional methods of identification and characterization of these bioactive molecules are labor intensive, expensive and time-taking. The recent advancement in the number of whole genome sequencing of cyanobacteria have accelerated the process of utilizing bioinformatics tools. Novel therapeutic molecules can be generated in the field of cyanobacterial drug discovery by the evolution of the field of system biology with a cross-talk between the areas of proteomics, transcriptomics and genomics. Moreover, detailed understanding of fundamental concepts of biosynthesis of natural product would stabilize the research on cyanobacteria and its applications in therapies (Raja et al., 2016). Further technologies such as nanoformulations are required to realize the effectivity of these anti-cancer metabolites. Green technology and nanofusion technology can be fused to exploit the cyanobacterial anti-cancer drug nano-formulations. To achieve all the benefits at the commercialized platform, further research and innovation has to be done continuously.

Scale-up commercialization with technology development should also be an important work for economical marine microalgae nano formulations. The unique functions of microalgae secondary metabolites should be explored in future (Bajpai et al., 2018).

9.10 CONCLUSION

It is evident now that cyanobacteria hold a promising and bright future in the development of pharmaceuticals in cancer treatment (El-Hack et al., 2019). A number of strains are commercially useful in the last several decades. Cyanobacteria are recognized as an important source of both pre-existing compounds and unexplored compounds that have the ability to provide both sustainable economy and health benefits (Aditya et al., 2016). Their advantage as a good source of novel therapeutic lead compounds has been recognized in the past two decades (Singh et al., 2011). The compounds extracted from cyanobacteria have been reported to display anti-proliferative and anti-tumorigenic property and the current pipeline has proved it. Out of the 9 drugs, six of them are used in treatment of cancer and several other compounds that are obtained from cyanobacteria and have applications in oncologic therapeutics are undergoing clinical trials (El-Hack et al., 2019). The sustainability of cyanobacterial products has increased in the field of drug development after it gained confirmation by the scientists (Aditya et al., 2016). There is an increase in the incidences of cancer because of increase in malnutrition, global warming and several other environmental concerns. Natural derivatives play a major part to decrease the cancer incidences because synthetic drug formulations impart several life-threatening side effects to human beings (Sithranga and Kathiresan, 2010). Moreover, maximum reports suggest that necrosis, apoptosis and lysis of the cancer cells is the major reason behind the mechanism of action of inhibition of tumor growth both in in vivo and in vitro models (Khalifa et al., 2019). The drugs for cancer therapy which is produced from cyanobacteria exist on both carbon dioxide present in the air and sunlight and can be manufactured at one thousandth of today's expense, that makes it economical. Marine algae contribute 65.63% of the total natural anti-cancerous compounds obtained so far. The marine flora is a source of several anti-cancer drugs that are potent, cheaper and safer because of diverse chemical ecology and it further needs an extensive investigation (Sithranga and Kathiresan, 2010). Economical cultivation of cyanobacteria as compared to other microorganisms is another reason why scientists are putting so much efforts on them for drug discovery. They need only simple inorganic nutrients for growth (Singh et al., 2011). But their commercialization is still hampered because of several concerns related to handling (Vijayakumar and Menakha, 2015). The scientific advancement and technological innovation have offered a baseline for exploring biologically active, chemically unique and taxonomically diverse marine flora (Khalifa et al., 2019). Various advancement in molecular and biological investigations discussing the role and the efficiency of such cyanobacterial derived-anti-cancerous metabolites along with its description are still required. Also, thorough investigations, in-vivo or in-vitro, should be performed on the bioactive metabolites of cyanobacteria to assess their potent use against a number of cancers. The unexplored section of cyanobacteria is expected to be discovered in the coming years and their translation to clinical applications can be a reality (El-Hack et al., 2019). Bioreactors are being used for the production and are widely promoted. After seeing the above studies, the ongoing researches and the improvement in drugs are possible in future (Sithranga and Kathiresan, 2010).

KEYWORDS

- **bioactive compounds**
- **breast cancer**
- **cancer**
- **carotenoids**
- ***Chlorella***
- **drug discovery**
- **polyunsaturated aldehydes**
- **seaweed**
- **secondary metabolites**
- ***Spirulina***
- **therapeutic agents**
- **tumor**

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CHAPTER 10

ALGAE-BASED COSMECEUTICAL PRODUCTS IN THE MARKET – PRESENT SCENARIO AND FUTURE PERSPECTIVES

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ABSTRACT

In the world cosmetics are staggeringly in use by citizens to defend the skin from external stimuli. Marine algae have increment enormous attention in cosmeceuticals. As the synthetic cosmetic products caused unfavorable side effects and consequence in low absorption rate due to the chemicals' bigger molecular size the consumer predilection towards natural cosmetic commodity has enhanced. Algae an oxygenic photosynthetic organism that are mainly recovered in marine milieus and marshes. In the tract of cosmeceuticals there is an accelerative affinity in the consumption of photosynthetic microorganisms involving macro-algae and micro-algae by integrated the bulk commodity infusion from its biomass into aesthetic preparations. There is numerous technological corroboration that proves the ability of algae but of course, it depends on extract, how it is prepared and its utilization. Due to the flourishing economic prospect of the cosmetic commercial enterprise, the need for nontoxic, harmless, and economic natural raw constituent has become an intense necessity. Reported to certain research reports, algal products utilized in cosmeceuticals is appropriate alternate with creative effect

Algal Farming Systems: From Production to Application for a Sustainable Future.
Jeyabalan Sangeetha, Devarajan Thangadurai (Eds.)

even after protracted usage. Various algal variety are now being used broadly for the treatment of diverse skin related difficulties by acting as a moisturizer or texture heighten, sunblock, anti-wrinkling, anti-aging, anticancer, antioxidant, anti-inflammation, and antimicrobial agents etc. This chapter aims in describing on the commercialized worth of diverse algal product in the biotechnology and cosmetic industry, extracted from both macro-algal and micro-algal genera for its application.

10.1 INTRODUCTION

The term "cosmeceuticals" is an alliance of cosmetics and pharmaceuticals, encircling the naturally active compounds retaining beneficial value. These are assortments of numerous chemical combinations, some of which are obtained from natural sources like animals, plants, algae, minerals, while others are artificial like polyvinyl pyrrolidone (PVP), sodium lauryl sulfate, ethyl paraben. Algal extracts is used in the cosmetic industry as antioxidants, sunscreen, thickening agents, skin sensitizers, nourishing agents to improve the capability of skin against scratches (scratches, tanning (browning), etc. (Wang et al., 2015). They are also used in hair products having additional benefits (Figure 10.1). Algae are multicellular eukaryotes or primitive unicellular, which are photosynthetic, i.e., they are primary manufacturers connecting energy from sunlight and converting it into biochemical energy for the biosynthesis of organic compounds such as sugars. Algal species comprise a green pigment known as chlorophyll, an imperative component in the process of photosynthesis. These pigments aid in absorption of energy from the light source and transferring it to the reaction center of photosystem II and I. These pigments can be discriminated into two types, chlorophyll a and chlorophyll b. Thus, carbon dioxide, water, and sunlight are utilized, to transform oxygen into sugars like glucose/starch and biomass (Croce and Amerongen, 2014). Algal species can withstand tremendous environment conditions of temperature, pH, osmotic pressure, salinity, and exposure to ultraviolet rays, an aerobiosis and are able to thrive efficiently under these dissimilar conditions. They are able to defend its cellular constituents by the counter manufacture of primary metabolites such as oleic acids, vitamin E, lutein, vitamin B_{12} and zeaxanthin (de Morais et al., 2015). Secondary metabolites are also produced under harsh circumstances in which they might be present. These metabolites retain antibiotic and antimicrobial outcome against pathogenic fungi and viruses (Wang et al., 2014). Algae are further distributed into two chief classifications, micro algae and macro algae.

FIGURE 10.1 Diverse applications of algae in commercial cosmeceutical products.

10.2 MACROALGAE

Macro-algae are considered as eukaryotic, macroscopic multicellular algae, extensively recognized as seaweeds. The home of macro-algal genera is marine water or seawater with the optimal obtainability of light (Milledge et al., 2014). They are benthic plants, therefore, their feasibility changes on how narrowly enclosed they are with the seabed or a solid primary layer of rock. Macro-algal genera retain basic structure comprising of thallus, lamina, holdfast, kelp, and fern sorus, thus morphologically discriminating it from the distinctive terrestrial plant, containing of compound tissue and organ organization. Macroalgae can be distributed into three major collections based on their coloration (Milledge et al., 2014): Phaeophyceae (brown algae), Chlorophyceae (green algae), and Rhodophyceae (red algae).

10.3 MICROALGAE

Cyanobacteria (microalgae) blue green algae are prokaryotic, miniscule unicellular algae having an approximate diameter of 1-50µm. They are phototrophic, but some might also grow heterotrophically. They carryout oxygenic photosynthesis that is quite alike to that establish in terrestrial plants, utilizing carbon and light (radiant) energy for their metabolism (Wolkers et al., 2011). They exist exclusively, in chains or cluster. Micro algae comprise phosphorous, iron, calcium, vitamin A, B, C, E, folic acid, biotin, beta-carotene, pantothenic acid, and vitamin B_{12} (Fabregas and Herrero, 1989). There are some micro algal genera that might adapt to variations when phosphorus is exhausted in the atmosphere; some genera of microalgae retain the ability to replacement non-phosphorus membrane lipids in place of phospholipids (Bonachela et al., 2011).

10.4 SOLICITATIONS OF ALGAE IN COSMETICS

10.4.1 SKIN WHITENING AND ANTI-WRINKLING

When direct contact between skin and UV rays is recognized for a long period of time, the radioactivity is engrossed by melanin, a complex polymer pigment which imparts colour to human skin and acts as a defensive obstruction for human skin cells (Thomas and Kim, 2013). So continual exposure to sunlight upsurges melanin in the skin subsequent in tanning. Radioactivity from sunlight helps synthesize tyrosinase, which aids to catalyze reactions for the creation of melanosomes, which then mature into melanin and is additional distinguished into keratinocytes to supplement the disrepair of skin. Therefore, the hydroxylation of L-tyrosine to L-DOPA and 3,4-dihydroxy-L-phenylalanine takes place anywhere the later endures oxidation subsequent in the development of dopaquinone. Melanin is then transformed from the dopaquinone designed. The huge quantity of melanin produced causes skin pigmentation and wants to be constrained. Therefore, tyranose inhibitors are used to catalyze rate-limiting step in the procedure of pigmentation (Wang et al., 2011). Pigments from algae i.e. fucoxanthin from brown algae *Alaria*, *Chorda*, *Laminaria japonica*, and *Macrocystis* assist to decrease the activity of melanogenesis and tyrosinase (Shimoda et al., 2010).

10.4.2 ALGAE AGAINST SKIN AGING

Skin aging is a complicated biological activity that indicates to the damage of elasticity of skin, ridges, appearance of fine lines, creases and staining of the skin with growing age (Ganceviciene et al., 2012). Our skin is exposed to extreme severities of insensitive environmental factors and thus, skin complications like thinning, skin laxity, fragility, dryness, enlarged pores, and drooping of skin leads to premature wrinkles as the elastin fibers slowly undergo deterioration (Peytavi et al., 2016). The natural procedure of crumpling of skin is amplified if there is a constant exposure of nutrient deficiency, heavy metals, and lack of moistness on the epidermis. The utmost common cause of skin maturing is reactive oxygen species (ROS), such as superoxide, peroxides, hydroxyl radical, and singlet oxygen. The protein kinase is inspired by ROS that phosphorylates transcription factor, activator protein 1, whose task is to generally regulate gene expression in reaction to cytokines. This transcription factor activates an increased regulation of matrix metalloproteinase foremost to the decrepitude of collagen from the skin (Thomas and Kim, 2013). Modern scientific reports have led to favorable assumptions about how algal commodities, for example vitamin E is a fat-soluble antioxidant and pigments for example carotene might revitalize and aid the skin to be resistant towards skin aging, and similarly reduces the hazard of skin cancer midst the users (Keen and Hassan et al., 2016). The antioxidant properties of β-carotene discovered in red and green algae assist against skin aging (Schagen et al., 2012). Algal genera such as *Ahnfeltiopsis*, *Colpomenia*, *Gracilaria*, *Halymenia*, *Laurencia*, *Hydroclathrus*, *Padina*, *Polysiphonia*, *Turbinaria ornata*, are used as anti-aging vehicles (Kelman et al., 2012). Mycosporine like amino acid (MAAs) defends against UV-A, which might cause skin impairment and early skin aging. Mycosporine like amino acid MAAs are originated in *Porphyra umbilicalis* (Daniel et al., 2004).

10.4.3 ALGAE AS MOISTURIZING VEHICLE

A moisturizer (conditioner) is prepared of a complex combination of chemical compounds, which construct the epidermis of skin smooth. If the skin is not appropriately conditioned, it is disposed to to acne provocation and can even lead to begin eczema. Thus, moisturizers aids in retaining the moistness of the skin avoiding bruising, drying, and wrinkling. Water and acids such as hyaluronic aids in moisturizing the human skin (Bonte, 2011). Polysaccharides such as agar, alginate, carrageenan, and fucoidans (Table 10.1) from certain algal genera help to normalize the circulation of water in the skin. These polysaccharides are economical, non-toxic plentiful in the algal biomass, which might be used as an alternative for lightweight oils, such as silicone-derived, or acetyl alcohol ingredients (Wang et al., 2014). Reports have revealed how polysaccharides from evident algal species like *Chondrus crispus*, *S. japonica*, and *Codium tomentosum* benefits in the absorption of moisture or water, delivering comforting effect, which assists in appropriate water transmission. This maintains the skin nourished in tremendously warm and dry atmospheres (Wang et al., 2013).

10.4.4 ALGAE AS THICKENING AGENT AND SKIN SENSITIZER

Congealing agents are used in lotions or other cosmetic commodity if the water content is high in the formulation is to stop discrepancy. Thickening agents used in cosmetics include vegetable gum and polyethylene glycol (Kadajji et al., 2011). In the cell wall of red algal species Agar acts as a binder, which is found *Gelidium* and *Gracilaria*. Carrageenan acquired by *Chondrus crispus*, is additional sort of thickening and soothing agent. Some algal species might also be trained in cosmetics as skin sensitizers as they include pigments such as vitamin A, proteins, phycocyanin, and carrageenan, sugars that are valuable and beneficial for skin (Couteau and Coiffard, 2016).

TABLE 10.1 Macroalgal Species Used in Cosmetics

Algal Species	Kind	Pigment	Fatty Acids/Metabolites	Applications/Products
Irish moss	Red algae	Phycoerythrin	Consist of omega-3 fatty acids, omega-6 fatty acids.	Emollient, moisturizing, sheaths injured or dry hair, skin calming, nutritive, anti-inflammatory (http://seaboost.ca/products/irsh-moss.html)
Sea lettuce (*Ulva lactuca*)	Green algae	Chlorophyll a, chlorophyll b, β-carotene.	Oleic acid, linoleic, and linolenic acid.	Antioxidant, anti-inflammatory, collagen synthesis, skin elasticity, anti-wrinkle, emollient, moisturizing (http://www.irishseaweeds.com/sea-lettuce-ulva-lactuca)
Sea palm (*Postelsia palmaeformis*)	Brown algae	Chlorophyll c, fucoxanthin	–	Skin softening, anti-wrinkle, nutritious, conditioning or moisturizing, anti-inflammatory (https://www.truthinaging.com/review/marine-ingredients-for-anti-aging-skin-care)
Fucus vesiculosus	Brown algae	Chlorophyll-c, fucoxanthin.	–	Tightening effect and enhances metabolism (https://www.ewg.org/skindeep/ingredient/719135/FUCUS_VESICULOSUS_EXTRACT)
Porphyra umbilicalis	Red algae	Phycoerythrin	α-Linolenic acid	Used in skin-conditioning agent (http://cosmetics.specialchem.com/inci/porphyra-umbilicalis-extract.)
Ascophyllum nodosum	Brown algae	Chlorophyll-c	Fucoxanthin, alginates	Anti-aging agent, smoothing agent anti-wrinkle agent (http://cosmetics.specialchem.com/inci/ascophyllum-nodosum-extract)

10.4.5 ALGAE AS ANTIOXIDANTS

Antioxidants are compounds that transmit electrons to an oxidizing agent delivering blooming skin by averting skin impairment. An antioxidant aids in skin constriction, decreases inflammation and reduction of wrinkles. Retinoic acid is a kind of vitamin A, which lessens dark circles dark spots, and wrinkles; it also leads to improvements in skin elasticity (Kelman et al., 2012). It has been discovered that cyanobacteria blooms yield retinoic acid (Wu et al., 2012). Carotenoids are fat-soluble auxiliary pigments, which aid algae to produce light in combination with chlorophyll to transmit on the procedure of photosynthesis. Vitamin A and Vitamin C aids as biological antioxidants. Algae like *Chlorella vulgaris* and *Spirulina maxima* comprises vitamins aids in skin toning, healing of dark circles, cleansing skin, boosting hair growth by curing dandruff.

10.5 ALGAL SPECIES USED IN COSMETICS

In certain cosmetic commodities with a varied range of purposes, algal species establish in pools, and seabeds are collected and through dissimilar approaches the biomolecules/pigments are removed and assimilated. The metabolites assist as agents for the cure of skin, like anti-wrinkle or moistening agents. Polysaccharides such as carrageenan, alginates, and agar originated from Rhodophyceae and Phaeophyceae act as gelling agents in numerous lotions, shampoos, etc. Apart from this, the constituents of microalgae acquire preserving, stabilizing, and organoleptic (ingredients that can be apparent through senses including smell, touch and sight) properties (Table 10.2). Algae extracts from microalgae *Thalassiosira* and *Nannochloropsis* species have shown efficacy towards skin hydration and restoration of trans-epidermal loss of water. This occurs due to the presence of diversity of biomolecules with moisturizing stuffs i.e. polysaccharides, fatty acids (sophorolipids, rhamnolipids and mannosylerythritol) and proteins (Fitton et al., 2015). Reports on two microalgae, *Undaria pinnatifida* extract (85% fucoidan) and *Fucus vesiculosus* co-extract (60% fucoidan) revealed that fucoidan helped in skin defense, soothing and protection (Tomita et al., 2019). *Euglena gracilis* is a unicellular eukaryotic alga; synthesize amino acids such as arginine, alanine, leucine, lysine, and valine when incubated for three days in the presence of oxygen. These amino acids have been commercially used in cosmetics (Mourelle et al., 2017) (Table 10.2). Many radioprotective phytochemicals have been reported in marine microalga,

TABLE 10.2 Microalgae Species Used in Cosmetics

Algal Species	Kind	Pigment	Fatty Acids/Metabolites	Applications/Products
Spirulina sp.	Blue green algae	Phycocyanin	Gamma-linolenic acid, phycoerythrobilin, phycocyanobilin.	Anti-aging, anti-wrinkle, anti-inflammatory, collagen synthesis, nurturing, antioxidant (Hosseini et al., 2013)
Isochrysis sp.	Brown algae	Fucoxanthin, cantaxanthin	Oleic acid, mistiric acid	Antioxidant, suncare, soothing agent, anti-irritant (http://www.oilgae.com/blog/2012/01/cosmetics-from-algae.html)
Dunaliella salina	Green algae	Chlorophyll-a, chlorophyll-b, β-carotene	Palmitic acid, linolenic acid, β-cryptoxanthin.	Antioxidants, smoothing agent, anti-inflammatory (Widowati et al., 2017)
Chlorella vulgaris	Green algae	β-carotene, chlorophyll-a, chlorophyll-b	Palmitic acid palmitoleic acid, polysaccharides.	Anti-aging, de-pigmentation, moisturizing and stiffening agent (http://www.osmosisskincare.com/research/files/chlorella-extract-on-skin)
Tetraselmis suecica	Green algae	β-carotene, chlorophyll-a, chlorophyll-b	Palmitic acid, stearic acid, and vitamin E (α-tocopherol).	Anti-oxidant, defensive activity (Sansone et al., 2017)
Botryococcus braunii	Green algae	Chlorophyll-a, chlorophyll-b, β-carotene	Palmitic acid, stearic acid.	Antioxidant (Rao et al., 2016)

phlorotannins, polysaccharides, and carotenoids to name a few. Besides these some other compounds have been explored in recent researches that might be used as constituents in cosmetic formulations for providing protection against ultraviolet radiation (Leska et al., 2018).

10.6 COMMERCIAL PRODUCTS HAVING ALGAL METABOLITES

In the modern scenario, a huge number of minor and large-scale commercial entities have motivated amply towards using algal metabolites in their cosmetic products. For example, the algal products of these companies variety from sea-weed dust and manures from Red algae, astaxanthin in cosmetics, algal proteins for hair therapy, food and beverages, etc. In anti-ageing cream Swiss snow algae varied with sea almond oil are used. These firms frequently assure the commodities to be absolutely health and organic centric. Organisms such as *Dunaliella* spp., *Spirulina*, *Chlamydomonas* spp., etc. enhance value to these commodities by producing metabolites for example asthaxanthin, certain MAA, proteins, etc. that assists in the nourishment of hair and skin. Red algal species obtains cleansing properties, which recover the skin supporting in the complete health of skin cells (Table 10.3).

10.7 SOME LATEST COSMETIC PRODUCTS

10.7.1 STEMLAN

Japanese beauty company has developed Shiseido (patent No. WO2018074606A1) having new anti-aging active ingredient Stemlan-173. This compound has the capacity to prevent the degradation of laminin-511. Laminin-511 improves skin texture, rejuvenates skin, and protects the epidermal stem cells. Shiseido contains the extracts from green algae, red algae and brown algae.

10.7.2 PURE CLAY MASK

L'Oreal Paris' skin experts have developed a pure exfoliating clay mask enriched with red algae extract to exfoliate skin.

TABLE 10.3 Commercialized Algal Products

Company	Product Name	Algae	Applications/Products	References
Aquarev industries	Kappa carrageenan	Red algae	Carrageenan and *Kappaphycus alvarezii* cultivation, sargassum seaweed powder, seaweed, liquid fertilizer.	www.aquarev.in
Algatech	Astapure astaxanthin	Green algae	Supplies astaxanthin, acquired from *Haematococcus pluvialis*, which is used as nutritional supplements, in cosmetics, food and beverages.	https://www.algatech.com
Nykaa	Iraya algae body serum	Green algae and *Spirulina*	It moisturizes and conditions the skin, making it soft.	http://www.nykaa.com
L'Oreal Paris	Pure face mask	Red algae	Made from clay and red algae. It exfoliates, improves the skin and possesses cleansing properties of red algae.	https://www.lorealparis.co.in
La Prairie	Cellular Swiss ice crystal dry oil	Snow algae (*Chlamydomonas nivalis*)	Anti-aging cream, which contains Swiss snow algae, mixed with sea almond oil.	http://www.laprairieswitzerland.com
Aubrey Organics	Balancing protein shampoo release conditioning mask.	Blue-green algae	Algal protein present aids in firming of hair and prevents breaking and split ends.	http://aubreyorganics.com
Algenist	Reveal color correcting eye serum brightener.	Green algae (*Dunaliella salina*, *Haematococcus pluvialis*)	A concealer that diminishes rough skin around the eye and covers dark circles.	https://algenist.borderfree.com
Dove	Dove regenerative repair shampoo	Red algae	It provides nourishment to damaged hair and repairs it, restoring hair strength.	https://www.purplle.com
Jenelt	Ultra UV defense brightening cream with SPF 30 sunscreen.	Red algae (*Porphyra umbilicalis*)	A sunblock with antioxidants, which avoids the skin from UV rays and early skin ageing.	http://www.jenelt.com
Osea	Osea eyes and lips	Red algae (*Chondrus crispus*)	It hydrates the delicate skin around the eyes and lips.	https://oseamalibu.com

10.7.3 GALLINEE'S FACE VINEGAR

It is a toner, containing a cocktail of hibiscus vinegar, and algae prebiotics which help to tranquil jittery skin.

10.7.4 ALGALLURE ALGARICHE

AlgEternal Technologies has developed microalgae-based skin care products AlgAllure AlgaRiche. This product used a marine red microalgae extract. AlgEternal reduces the appearance of wrinkles, hydrates the skin, and guards against evident signs of aging.

10.7.5 ALGENIST

Algenist is a first color-changing, transformative mask which is developed from a prebiotic extract from algae containing alguronic acid. This product maintains, detoxifies and increases skin radiance. Algenist also launched the Genius Sleeping Collagen, which is an overnight treatment for plump, dewy skin. The components in the formula include the brand's patented alguronic acid and oil extracted from microalgae, which is naturally rich in omegas 3, 6 and 9 and vitamin E.

10.7.6 ALGAKTIV® LIGHTSKN™

It is a product by Greenaltech which is a whitening agent. It is developed from microalgae fractions aimed to decrease melanin production and remove spots from skin.

10.7.7 INNISFREE

Lip peeling booster containing algae extract has been developed by Innisfree (Amorepacific) which exfoliate lips giving smooth lips.

10.7.8 ACQUASEAL® ALGAE

Active concepts has developed AcquaSeal® Algae to promote youthful skin, by decreasing inflammation and enhancing cellular proliferation. It is obtained from green algae *Chlamydomonas reinhardtii*.

10.7.9 EKSPERIENCE™

Eksperience™ is the thalassotherapy hair range by Revlon Professionals that prevents hair loss, sensitive scalp, oily scalp and other hair problems. Revlon Thalassotherapy marine algae scalp treatment is an amalgamation of marine algae and vital extracts, creating active mud with *Spirulina* and Vitamin B.

10.8 FUTURE PERSPECTIVES

Algal species are currently vigorously being utilized capably in the varied cosmetic products as a reliable organic component and also to add value to these products. The main components of the algal product are the pigments created by these photosynthetic organisms. The algal metabolites such as proteins, polysaccharides, MAAs, etc. have varied purposes. They recover the health of the skin by acting as antioxidant, anti-aging, anti-wrinkling, anti-inflammatory, and collagen enhancing agent. Algal genera are also used in numerous other biotechnological businesses such as biofuels, dietary supplements, bio-fertilizers etc. This chapter typically emphasizes on the growing purposes of algae in the cosmetic industry. The encroachments in algae biotechnology has momentous role for novel and viable renewables wherein the manipulation and directing of bioactive components between these organisms has to fit the increasingly niche product stipulations. Our knowledge on the algae-derived oligosaccharides has been augmented substantially and many investigation progressions are available in this decade on their chemical structure diversity and their biological applications. Accepting the basic ecology of these derivatives consuming omics technology, biochemistry and practical biotechnology through characterization and screening of bioactive molecules, developing omics tools to rebuild metabolic paths, and low-cost production of algae-derived oligosaccharides will emphasis upon driving bio product optimization and discovery in algal systems. Additional analytical reports of oligosaccharides, composed with molecular investigations will improve our understanding on the mechanisms of their biological action. Hereafter, these insights on cellular and molecular mechanisms of algae-derived oligosaccharides will eventually result in innovative green cell-factories. Currently, there is a growing interest in seaweeds due to the credit of abundant new bioactive compounds. Antimicrobials, antioxidants, anti-inflammatory, anticancer, anti-aging, are just certain of its astonishing possessions to use as cosmeceuticals, pharmaceuticals nutraceuticals, and in agriculture or feeding. There is additional and additional awareness of sustainable usage of natural resources, somewhat than artificial and treated commodities with subsequent detrimental side effects to the customer. All the increasing interest in these potentialities led to the fostering of macroalgae invention, as well as, to do study on them. Seaweeds are a resource to uphold and preserve with unique properties.

KEYWORDS

- **algae**
- **anti-ageing**
- **antioxidants**
- **anti-tanning**
- **cosmeceuticals**
- **moisturizers**
- **skin sensitizers**
- **soothing agents**
- **texture enhancer**
- **thickening agent**

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CHAPTER 11

NANOPARTICLES FROM ALGAE: A GREEN APPROACH FOR SYNTHESIS AND THEIR BIOLOGICAL ACTIVITY

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ABSTRACT

There is an incredible interest in the homegrown medications from both the creating just as created countries were given their adequacy, security, and lesser symptoms when contrasted with manufactured atoms. These medications likewise offer therapeutics for age-related disarranges like memory misfortune, osteoporosis, safety issue, and so forth for which no advanced medication is accessible. It shows the promising contribution of natural medications in both economic and wellbeing concerns, in this way, it is ground enough to contribute to their development. As the viability and adequacy of the homegrown items significantly rely on their quality. The absence of normalization is the key factor because of that India despite its rich conventional information, a legacy of homegrown drugs, and huge biodiversity has a bleak portion of the fare to the world market. Subsequently, the quality affirmation of homegrown medication/items is the need of the hour. Consequently, normalization has become a fundamental segment of homegrown medication/items that guarantee the advantages of home grown medications. Along these lines, by normalization of natural medications, the Indian homegrown market and even the fare of homegrown meds will expand, making "India the Pharma Power House."

Algal Farming Systems: From Production to Application for a Sustainable Future.
Jeyabalan Sangeetha, Devarajan Thangadurai (Eds.)

11.1 INTRODUCTION

"Wellbeing for all" is a fantasy and an objective which humankind everywhere offers and takes a stab at, lamentably, it has been demonstrated without question that cutting edge pharmaceuticals are and will stay far off for an enormous extent of the human populace for the not so distant future. This has made thankfulness and a requirement for the utilization of different wellsprings of human information to give normal medical advantages. Elective and customary medications, to a great extent homegrown in nature, are presently viewed as significant yet underutilized apparatuses against infection.

Nature consistently remains as a brilliant imprint to epitomize the exceptional marvels of advantageous interaction. Normal items from plants, creatures, and minerals have been the premise of the treatment of human illness. The utilization of plant-based medicines and different botanicals in the West has expanded complex as of late. Around two centuries prior, our therapeutic practices were to a great extent ruled by plant-based medicines (Bukay et al., 2020). However, the restorative utilization of herbs went into a fast decrease when more predictable synthetic drugs were made generally accessible. Interestingly, many creating countries proceeded to benefit from the rich information on clinical herbalism. For instance, Ayurvedic medication in India, Kampo medication in Japan, conventional Chinese medication (TCM), and Unani medication in the Middle East and South Asia are as yet utilized by a lion's share of people. In the ongoing time of natural renaissance, the interest in homegrown drugs and different botanicals by Western people groups has been expanding consistently, especially in the course of recent decades. Alongside homegrown meds, other homegrown items, for example, makeup, scents, teas, wellbeing nourishments, and nutraceuticals are similarly famous and comprise an enormous extent of the worldwide homegrown business (Dhas et al., 2014).

In former times, in India vaidyas used to treat patients on a singular premise, and get ready medication as indicated by the necessity of the patient. However, the situation has changed now; natural meds are being made for the huge scope in Pharmaceutical units, where produces go over numerous issues, for example, the accessibility of good quality crude material, confirmation of crude material, accessibility of norms, legitimate normalization strategy of single medications and detailing quality control parameters. The utilization of homegrown medication because of the poisonousness and symptoms of allopathic prescriptions has prompted an unexpected increment in the number of natural medication makers.

India has a rich legacy of conventional medication comprising its various segments like Ayurveda, Siddha, Unani, Homeopathy, and naturopathy. Customary social insurance has been thriving in this nation for a long time. The developing utilization of botanicals by the general population is driving moves to assess the wellbeing cases of these operators and to create norms of value and assembling. Most prescriptions before being offered to consumers experience thorough proof-based clinical testing; this isn't valid for herbs, due to their long chronicled clinical utilize and solid restorative adequacy. Different conventional medication frameworks, particularly the Indian arrangement of medication pulled in the worldwide consideration, and numerous large pharmaceutical organizations are utilizing the customary medication as a phenomenal pool for finding characteristic bioactive mixes. With the developing requirement for more secure medications, consideration has been attracted to their quality, viability, and norms of conventional Indian medication. Each conventional arrangement of drugs has its strategy for normalization for guaranteeing quality most in human semantic terms. This technique for assessment must be mulled over in the normalization of homegrown medication/drugs (Rajasulochana et al., 2012).

Nanotechnology is currently known to be creating the field of science, designing, and innovation-based fair and square of nanoscale. Nanotechnology items are nanoparticles or nanomaterials (NP), going from 10^{-9} m and sizes from 1 to 100 nm. Fundamentally three kinds of NPs are there: characteristic nanoparticles built nanoparticles and coincidental nanoparticles. The huge surface zone of the nanoparticles, the potential to associate without breaking a sweat, and other related variables make them an alluring field. NPs are known to have progressed biotechnological applications. Nanotechnology is a magnificent region of center nowadays as a result of its multidimensional physicochemical properties (Debasish et al., 2020). Wet strategy is referred to by the customers just as a most adequate technique with the end goal of nanoparticles combination. To the extent concoction techniques for nanoparticles, amalgamation is concerned, a fluid containing different reagents, similar to sodium borohydride or hydrazine and so forth could be utilized. Stabilizers, for example, sodium dodecyl benzyl sulfate are applied to the response blend to forestall amassing just as oxidations of metal nanoparticles (Prasad et al., 2013). Compound strategies that are most normally utilized are substance decrease and so on whereas in the event of physical techniques typically pyrolysis just as ball processing is utilized. Grinding includes particles' steady loss through any component which brings about size decrease. Pyrolysis mostly includes consuming the forerunner by going it through a high-weight port. Substance techniques are very less expensive for huge volumes, yet they additionally have a significant disservice of including concoction antecedent tainting, harmful solvents, and union of different side-effects which are dangerous. The downsides that are related to physical techniques are moderate creation speed, cost, high, and prerequisite of high vitality. It is important to supplant poisonous strategies with an earth cordial technique for NP combination. To manage this circumstance, specialists are progressively engaged in organic techniques for nanoparticle amalgamation. Natural techniques are helpful, non-harmful, and great from a biological perspective. There are different reports which show the utilization of plant extricates, microbial species, catalysts just as green growth for NP blend. Specialists these days are increasingly engaged towards a rising method for NP combination, for example, green growth blend. Undoubtedly, green growths are viewed as a significant gathering of photosynthetic living beings. Green growth might be unicellular or multicellular, that live in changed ecological conditions, for example, ocean water or wet stone surfaces. Microalgae and macroalgae are two fundamental arrangements of green growth. They are known to have numerous applications in different fields like clinical, pharmaceutical, and so on. Different business items like regular colors are gotten from green growth. Up to this point, various gatherings of green growth have been utilized for the biosynthesis of metallic NPs, for example, chlorophytes, pheophytes, cyanophile and so on. The green growth's metal aggregation capacity and metal particle's diminishing ability make them the best open door for nanoparticle biosynthesis. Furthermore, green growth is good to be utilized, additionally, they have numerous different focal points, for example, union at low temperature with more noteworthy vitality effectiveness, less harmfulness and natural dangers.

In various physical-concoction techniques, different economically accessible surfactants have been utilized as models and obstructing operators in the amalgamation of NP with various morphologies. Removal of remaining parts turns into a significant point. As this utilization of normally perfect techniques has been created, this infers the union of NP utilizing different bio-helped courses that could normally change the shape or size of a more excellent gem. Among the organic materials, bio-nano factories are the elective name given to green growth, since dead and dry biomass was utilized for the amalgamation of metal nanoparticles (Rajasulochana et al., 2010). Green growth like *Spirulina platensis* and *Chlorella vulgaris* has been utilized as a modest technique for the amalgamation of silver nanoparticles. *Ulva fasciata* extricate has been utilized as a diminishing specialist for the blend of silver nanoparticles which hinders the development of *Xanthomonas campestris*. Notwithstanding green growth, diatoms are accounted for to have the capacity to blend gold nanoparticles (Parial et al., 2012).

Contrasted with different living beings, green growth is additionally a significant life form in NPs union; hence, the investigation of green growth intervened biosynthesis can be brought to another branch and has been characterized as phytonano innovation (Pingarron et al., 2008). Accordingly, this clarifies the utilization of green growth blended nanoparticles for present and future points of view.

11.2 TYPES OF NANOPARTICLES

11.2.1 POLYMERIC MICELLES

Polymeric micelles are nanostructures made with amphiphilic block copolymers that accumulate themselves to form a central shell structure. The hydrophobic drugs are present in the hydrophobic core, while the hydrophilic coating makes the entire system water-soluble and performs core stabilization. Polymeric micelles are less than 100 nm in size and normally have a reduced distribution, allowing them to accumulate in tumor tissue due to the EPR effect. These nanostructures have great potential to deliver hydrophobic drugs, as their internal structure allows the accumulation of this type of drug resulting in improved stability and bioavailability. There are two approaches for polymeric micelles synthesis. Such as easy direct dissolution of the solvent-based polymer followed by the dialysis and precipitation. Factors such as the size of the hydrophobic chain in the amphiphilic molecule, the solvent system, and the temperature influence the formation of micelles. The creation of the micellar assembly begins when the amphiphilic molecules reach the minimum concentration known as the critical micellar concentration (CMC) (Bao & Lan, 2018).

11.2.2 DENDRIMERS

Dendrimers are monodispersed highly forked and three-dimensional structures. They are spherical in shape and their surface is easily functionalized in a controlled manner, making these structures excellent as agents for drug delivery. Dendrimers can be manufactured in two ways: the first is the different path in which the dendrimer begins to form from its core and then spreads outward, and the second is convergent starting from outside the dendrimer. The dendrimers are categorized into different types depending on their functionalization fractions, among which PAMAM, is the most studied for oral drug administration because it is soluble in water and maybe epithelial tissue, increasing transmission through the paracellular pathway (Singaravelu et al., 2007).

Because of the presence of amino groups, dendrimers have limited clinical applications. They are positively charged, making them toxic, so dendrimers are generally adapted to reduce or eliminate this toxicity problem. The drug is loaded into the dendrimers by the following mechanisms like encapsulation, interaction, and conjugation.

11.2.3 INORGANIC NANOPARTICLES

Inorganic nanoparticles include silver, iron oxide nanoparticles, etc. The studies focused on it are not as numerous as exist on other types of nanoparticles discussed, they have several potential applications. Most of the nanoparticles are still in the clinical testing phase. Silver and gold nanoparticles have special properties such as SPR that liposomes, dendrimers, and micelles do not have (Sicard et al., 2010). They have demonstrated numerous advantages, such as biocompatibility and versatility in terms of surface functionalization. Drug administration activity studies have not been able to determine whether the ionized form is associated with toxicity, still, there is insufficient information on the transport and absorption mechanism in vivo. Drugs can be adhered to the surfaces of gold nanoparticles by ionic or covalent bonds and can be administered by biological stimuli or light activation. Silver nanoparticles have shown antimicrobial activity, but very few studies have been conducted on drug delivery (Silver et al., 2006).

11.2.4 NANOCRYSTALS

Nanocrystals are drugs without carrier molecules and are generally stabilized with polymeric stabilizers or surfactants. A suspension of nanocrystals in a marginal liquid medium is usually illuminated by the addition of a surfactant. In this case, the dispersion medium is mainly aqueous or non-aqueous medium including liquid polyethylene glycol and oils. Nanocrystals have specific characteristics that allow them to overcome problems such as increasing the solubility of saturation, a higher dissolution rate, and a higher viscosity on the surface/cell membranes. The top-down approach includes crystallization, precipitation, high gravity controlled gravity technology, and low impact liquid jet precipitation technique. The bottom-up approach involves the rectification of procedures and higher pressure homogenization. Of all methods, grinding and homogenization are the most commonly used methods for nanocrystal production. The mechanisms by which nanocrystals support the incorporation of a drug into the system include improving the solubility, the rate of suspension, and the ability to firmly support the gut wall.

11.2.5 QUANTUM DOTS

Quantum dots are semiconductor nanocrystals in a size range between 2 and 10 nm, absorption and photoluminescence properties of QDs are dependent on size. QDs have received interest in the nanomedicine field because, unlike conventional organic dyes, QDs have emissions in the near IR region, a highly desirable feature in the biomedical imaging field, due to reduction in tissue absorption and light scattering. In addition, the same light source could be used for manufacturing QDs of different sizes, compositions, which results in the separation of colors over a wide spectrum range. QDs are very interesting for multi images (Singh et al., 2013).

11.2.6 PROTEIN AND POLYSACCHARIDES NANOPARTICLES

Polysaccharides and proteins both are referred to as natural biopolymers and biological sources such as plants, animals, etc., are their source of extraction. They are generally degradable, and easily functionalized due to their binding to specific drugs (Tian et al., 2007). They are produced from soluble proteins such as bovine and human serum albumin and insoluble proteins such as zein and gliadin. Common methods of synthesizing them are co-protection, solvent extraction, complex co-protection, and electrospray. They are chemically modified to combine target ligands that define exact cells to support and enhance targeting mechanisms (Sudha et al., 2013). The composition and biological sources of these monomers can give these polysaccharides a range of specific physicochemical properties.

11.3 SYNTHESIS OF NPs USING ALGAE

The abundance and ease of availability of algae make them a worthwhile source for the synthesis of nanoparticles (Sharma et al., 2009). Algae have been proved as a boon with indefinite applications in numerous fields that have been employed as a substitute to chemical reductants for the tailoring of

Au-NPs. Au-NPs have been synthesized from four different classes of algae, such as Cyanophyceae (Blue-green algae), Chlorophyceae (Green algae), Phaeophyceae (Brown algae), and Rhodophyceae (Red algae) (Sharma et al., 2007).

11.3.1 SYNTHESIS OF GOLD NANOPARTICLES FROM GREEN ALGAE

The intracellular algae-based synthesis of nano-gold was reported in unicellular green alga *Chlorella vulgaris* (Tkachenko et al., 2003).

11.3.2 BIOSYNTHESIS OF NANOPARTICLES USING CELL-FREE SUPERNATANT

Cell-free supernatant generally refers to the method where algal cells are removed by centrifugation and the supernatant is used for NP biosynthesis. However, in some instances, the cells can be removed by filtration as well. For example, cell-free supernatants of various species of cyanobacteria and Chlorophyta can promote the synthesis of AgNPs (Sanguansri et al., 2006; Shakibaie et al., 2010; Senapati et al., 2012).

Various literatures reported the synthesis of Ag nanoparticles using the marine red alga *G. corneum*. The dried algae powder (5 g) was mixed with 100 ml of distilled water. The extract was collected and then it was diluted with water at different extract rates in water to synthesize silver nanoparticles (AgNPs). For the synthesis of AgNPs, the optimized concentration of a filter-sterilized metal salt solution ($AgNO_3$) was used under optimum conditions (Shiny et al., 2013). The synthesis was first monitored by the visual color change in the reaction mixture (from slightly reddish to dark brown) followed by spectral analysis (UV-Vis). NPs were purified by centrifugation at 5000 rpm for 10 minutes at first; this allowed the removal of large particles, followed by second centrifugation at 18,000 rpm to collect the nanoparticles (Shukla et al., 2012). The obtained nanoparticles were thoroughly washed with distilled water to remove unconverted metal ions or any other reaction component. Subsequently, the samples were first dried at –80°C and then freeze-dried to powder NPs and used for characterization.

Biosynthesis of AgNPs was also reported using an aqueous extract of *Neochloris oleoabundans*. As per the report, the chlorophyll content and illumination play an essential role in biosynthesis, results highlight that agitation of the extract during the experiment positively influences nanoparticles' size and shape. Other noteworthy research in recent years on the biogenesis of nanoparticles from microalgae includes the synthesis of CuNPs using *Chlorella kessleri*, *Dunaliella tertiolecta*, and *Tetraselmis suecica*, AgNPs using *Gelidium amansii* (Pugazhendhi et al., 2018; Singh et al., 2019).

11.4 APPLICATION OF ALGAL-SYNTHESIZED NPs

The algal-synthesized NPs are becoming more important as far as biomedical applications are concerned with various essential therapeutic properties (Figure 11.1).

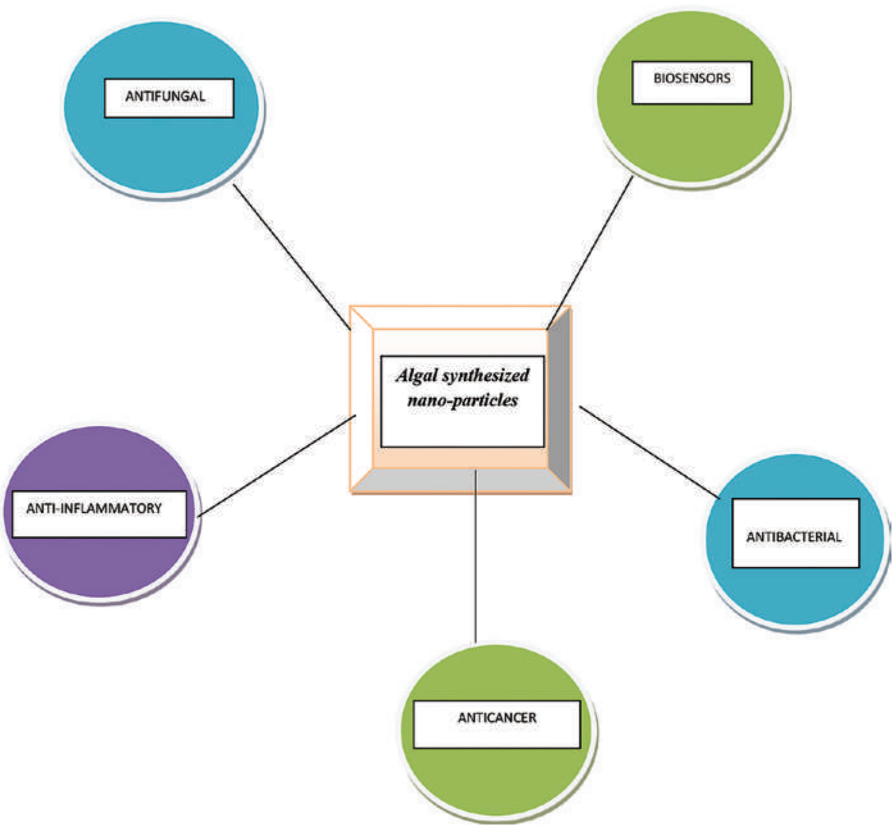

FIGURE 11.1 Applications of algal synthesized nanoparticles.

11.4.1 ANTIBACTERIAL ACTIVITY

NPs synthesized with algae are known to have effective antibacterial activity. *Bifurcaria bifurcata* which is brown algae has been reported for copper oxide nanoparticles synthesis of approximately 5.5 nm, which shows antibacterial efficacy against *Enterobacter aerogenes* and *S. aureus* (Rahman et al., 2009). Gold nanoparticles synthesized with *Galaxaura elongata* were evaluated to have potential antibacterial activity against *E. coli*, *K. pneumoniae* etc. In another study, silver chloride NPs synthesized by *Sargassum plagiophyllum*, which were analyzed by various microscopic techniques showed potential bactericidal activity against *E. coli*. Synthesis of AgNP using fresh extract and *Chlorococcum humicola* inhibited the growth of Gram-negative *E. coli* bacteria. Recently, fuxanthine, a lymphocyte pigment, an aqueous extract of an *Amphora*-46 diatom was used for the light-bound biosynthesis of polycrystalline AgNP, for which it was responsible for the reduction of the Ag ion. Synthesized AgNPs (Sahayaraj et al., 2012; Sangeetha et al., 2012) were also tested for Gram-positive and Gram-negative bacteria for their antibacterial activity. AgNPs synthesized with seaweed, *Caulerpa racemosa*, showed antibacterial activity against *S. aureus* and *Proteus mirabilis*. *Microcoleus* sp. is used to synthesize AgNP and improved the antibacterial activity of antibiotics against *Proteus vulgaris* and *Salmonella typhi*, etc. Various marine microalgae like *C. calcitrans*, *C. salina* are used for AgNPs synthesis (Focsan et al., 2011).

High growth inhibition of *E. aerogenes* and *P. vulgaris*, exhibited by AgNP synthesized with extracts of *Sargassum cinereum* algae were tested. Moreover, nanoparticles synthesized with algae extracts have cotton fabrics stabilizing potential. The *Gracilaria corticata*, which is red algae, the aqueous extract of it acts as a reducing agent has been investigated for its antibacterial effect against gram-positive and gram-negative bacteria. Another study shows AuNP synthesized with *Turbinaria conoides*, which acts as a potential antibacterial agent against *Streptococcus* sp. The Ag-Au nanoparticles were synthesized from *Gracilaria* sp., which showed potential antibacterial activity against *S. aureus* and *K. pneumoniae*. *Padina pavonica* mediated extracellular synthesis of AgNP is reported to inhibit the growth of *Fusarium oxysporum* and *Xanthomonas campestris.* The synthesized AgNP and nanocomposite material showed bactericidal activity *B. pumilus*. In a work performed by Suganya et al. protein-mediated synthesis of AuNP was performed using *S. platensis* was performed; In addition, it showed effective antibacterial activity against *B. subtilis* and *S. aureus* (Rajathi et al., 2012).

11.4.2 ANTIFUNGAL ACTIVITY

NP synthesized with algae has been used as effective antifungals. In this respect, only an accounting number of works has been performed. This includes AgNPs synthesis by using the aqueous extract of *Gelidiella acerosa*, the synthesized nanoparticles thus exhibit antifungal properties against *Humicola insolens*. In a study, the effect of AgNP synthesized from *Sargassum longifolium* against *Aspergillus fumigatus* has been determined (Perez-de-luque et al., 2012).

11.4.3 ANTICANCER ACTIVITY

In a work performed by chitosan-coated silver nano of triangle shape were synthesized which have been used as photothermic agents against a human lung cancer cell line. In another article, *Sargassum vulgare* mediated synthesis of AgNPs was done and has been tested for its ability to destroy myeloblastic leukemia cancer cells and cervical cancer cells.

11.4.4 OTHER APPLICATIONS

Algae synthesized NPs are being investigated in other application areas, including the synthesis of a palladium nanocrystals with an aqueous solution using photosynthetic reaction in *C. vulgaris*. The antioxidant capabilities of *G. corticata* synthesized AgNP have also been estimated. In another study, *Lemanea fluviatilis* mediated synthesis of AuNPs was done (Tkachenko et al., 2003), where the dry biomass acts as both a reducing agent and a stabilizer. In addition, the antioxidant property was determined using the DPPH assay.

11.5 FUTURE APPLICATION OF ALGAL-SYNTHESIZED NPs

11.5.1 ANTIBIOFILM AGENTS

Nanoparticles as antibiofilm agents are an emerging research area, to prevent widespread use and abuse of antibiotics, as many pathogens have become resistant to multiple drugs. Since bacteria are inefficient in developing resistance to nanoparticles, thus they act as an efficient therapeutic agent against biofilms (Zinicovscaia et al., 2012). Nanoparticles can penetrate the EPS and cell membranes (Husain et al., 2019). Silver nanoparticles are more effective than the others and exhibit antibiofilm activity against several pathogens. In a work performed, chemically synthesized CuNP by a one-pot synthesized method is used to inhibit biofilm against *P. aeruginosa* and weakening of the extracellular polymeric substance. *Stenotrophomonas maltophilia* and *Ochrobacterium* sp., have been used to synthesize zero-valent selenium and tellurium NPs which were found to be effective against *E. coli* biofilm (Tsibakhashvili et al., 2011). Likewise, *E. faecalis* mediated synthesis of AgNP was used in the form of nanocoloids, which plays an important function in the inhibition of MDR biofilms. *S. aureus* biofilms are reported to get inhibited by green synthesized AgNP.

11.5.2 LIPID NANOPARTICLES

Lipid-rich marine organisms can be used because of their potential to produce lipid nanoparticles using algae, fungi, and bacteria (Uma et al., 2015). Synthesis of lipid nanoparticles can be done by using organisms by heating them to liquefy fatty acids; the addition of active substances of pharmacological and cosmetic interest was done. The addition of hot surfactant along with homogenization under high pressure can be done by ultrasound. They could be used in diverse applications like the production of food, cosmetics, and medicines (Vaidyanathan et al., 2009).

11.5.3 BIOSENSING

Algae synthesized NPs could be investigated in biosensor applications. AuNPs are an efficient tool for detecting hormones in the urine sample in pregnant women. Platinum functions as a new highly sensitive biosensor for the determination of adrenaline to treat allergies. The synthesis of Au-Ag nanoscale alloy synthesized with chloroplasts showed high electrocatalytic activity which could be used as an early-stage cancer detection tool (Doria et al., 2012).

11.6 CONCLUSION

Due to their unique properties and diverse range of applications, the production of engineered NPs has continuously shown an increasing trend in the past two decades. It is estimated that the production of NPs doubles every three years. Nature act like a large "bio laboratory" comprising of plants, algae, fungi, yeast, etc., which are composed of biomolecules. The developing era of nanoscience is a renowned gift for the development of science all over the world. Despite numerous studies conducted over the last decade, there are still considerable gaps in our knowledge about the biotechnological potential of green-synthesized nanoparticles. Furthermore, the precise basis of their antibiotic and antibiofilm activity has yet to be defined. However, the toxicity of nanoparticles to eukaryotic cells is a legitimate concern and remains uncharacterized. One way of avoiding this potential drawback might be to target green-synthesized nanoparticles to the specific site of an infection so that toxic nanoparticles concentrations are localized. In addition, improvements in the way that green-synthesized nanoparticles are incorporated into medical devices could increase their efficacy and diminish any side effects, but considerable research effort is still required to perfect this technology.

The use of different types of algae in the synthesis of NPs has encouraged the designing of simple, green, cost and time-effective approaches thereby, minimizing the use of chemicals, and solvents. But the limitation with the use of algae is that not all the species can be exploited for the synthesis as some of them contain toxic compounds and the mechanism for synthesis has not been fully explored yet.

The pharmacopeial guidelines in Ayurvedic Pharmacopeia of India are not sufficient enough to guarantee the nature of plant materials. Homegrown medications/items normalization is hugely wide and profound. The medications are fundamentally apportioned as water decoction or ethanolic separate or as new plant parts, juice, or rough powder. Consequently, therapeutic plant parts ought to be bona fide and liberated from destructive materials like pesticides, overwhelming metals, microbial or radioactive pollution, and so on. The concentrates should then be checked for the demonstrated organic movement in a trial creature model(s) as polyherbal definitions contain multiple herbs so their consolidated impact ought to be contemplated. It ought to be then normalized based on dynamic standard or major compound(s) alongside fingerprints utilizing chromatographic and spectroscopic techniques and adjustment with a base time of usability of longer than a year, experience administrative or restricted security concentrates in creatures. Assurance of the plausible method of activity will clarify the helpful profile. The homegrown medications created in this mode ought to be apportioned as doctor-prescribed medications or even OTC items relying on infection thought and by no means as wellbeing nourishments or nutraceuticals.

Homegrown medication is making an emotional rebound and expanding number of patients are visiting elective medication centers as the reactions of engineered medication are disturbing. Under the overall conditions, further examinations concerning the idea of polyherbal details ought to be attempted. It tends to be inferred that polyherbal plans ought not to be excused uniquely on the premise that they don't withstand current research. Ayurveda and homegrown medication has establishes in restorative herbs and they have been polished for centuries. There is the requirement for the advancement of strategies that incorporates both customary techniques for assessment and current strategies for assessment. This will improve the nature of the medication and propels the experts to get increasingly associated with the normalization procedure.

KEYWORDS

- **algae**
- **antibacterial**
- **anti-biofilm**
- **anticancer**
- **antifungal**
- **bio-sensing**
- **dendrimer**
- **green synthesis**
- **herbal drugs**
- **micelle**
- **nanoparticles**
- **nanotechnology**
- **pharma**

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Algal Farming Systems

From Production to Application for a Sustainable Future

The farming and cultivation of algae can provide sustainable solutions for issues such as food security-related problems, costly health-related products, sustainable fuels, and more. However, the use of algae is currently restricted to high-value, low-volume markets, mainly due to the high investment and production costs involved. In recent years, algaculture for food and fuel purposes has begun a transition from R&D and pilot-scale operations to commercial-scale systems. This new book presents the latest technological innovations in algae production, market status, and prospects for algal applications.

The book provides an informative overview of different perspectives on the commercial production of algae-based food, health, and high-value cosmeceutical products, providing an institutional framework to support and promote the development and commercialization of algal farming. The book discusses phycotechnology and highlights the current trends and future scope of algal technology. It also presents new information on algal culture conditions and cultivation strategies, including a look at geographic positions and local climate as key factors in the implementation of microalgae-based processes. Algal production, marketing strategies, and their commercialization are discussed as the industrial applications of algae, focusing mainly on nutraceutical, pharmaceutical, and cosmeceutical applications of microalgae and macroalgae.

ABOUT THE EDITORS

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