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***In Vitro* Pro-Inflammatory Enzyme Inhibition, Anti-Oxidant Potential and Biomolecule Oxidation Protective Activity Using Different Extracts of Soursop (*Annona muricata* L.) Leaves and Fruits**

Malarkodi R.^{1,2}, Gokulakrishnan A.^{1*}, Sudha D.^{1,3} and Liyakath Ali A.¹

¹Department of Biochemistry, Islamiah College, Vaniyambadi, Tamil Nadu, India

²Department of Biochemistry, Marudhar Kesari Jain College for Women, Vaniyambadi, Tamil Nadu, India

³Department of Biochemistry, Islamiah Women's Arts and Science College, Vaniyambadi, Tamil Nadu, India

*Corresponding Author

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Abstract: *Annona muricata* L. plant extracts have been studied for anti-inflammatory, anti-oxidant and anti-arthritic properties which provides an excellent supplement for the pharmacological industry. The aim of this study was to investigate *in vitro* antioxidant activity, free radical scavenging activity, inhibition of pro-inflammatory enzymes and biomolecule oxidation of different solvent extract of soursop (*Annona muricata* L.) leaves and fruits. *In vitro* radical scavenging activities of leaves and fruit extracts were assessed using DPPH, H₂O₂, hydroxyl radical scavenging activity and total antioxidant capacity. *In vitro* pro-inflammatory enzymes inhibitory activity was assessed using inhibition of hyaluronidase, lipoxygenase and xanthine oxidase enzymes. Anti-arthritic activity of the plants was analyzed using protein degradation method. In DPPH assay, the ethanol solvent extract showed greater potential in leaves and fruit amongst all other solvent extracts. Ethanol leaves extract showed the highest value of H₂O₂, hydroxyl radical scavenging activity and total antioxidant capacity. In *In vitro* pro-inflammatory enzymes inhibitory activity, the ethanol extract of leaves showed greater activity than fruit extracts. Inhibition of protein oxidation and lipid peroxidation were found higher in leaves ethanol extracts compared to other solvent extracts. Leaves showed the highest free radical scavenging potential and anti-inflammatory activity. These findings suggest that the ethanol leaves extract could be used as a preventive therapy against inflammatory diseases and arthritis associated with protein denaturation.

Keywords: Antioxidants, *Annona muricata* L., Soursop, Pro-Inflammatory enzymes, Lipid peroxidation, Protein denaturation

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Introduction

Inflammation is a complex disease associated with various symptoms like redness, fever, pain and

swelling with changes in the skin covering at the site (Yogesh *et al.*, 2013) which needs immediate

pharmacological treatment. Excessive productions of inflammatory mediators result in chronic hepatitis, arthritis and inflammatory disorders (Gupta *et al.*, 2104). Free radicals are either beneficial or harmful to the body depending on its level. The small amount, it plays an important role in biological functions like-- act as signal transducers, growth factor regulator and secondary messengers. However, the excessive generation of free radicals lead to damage to predominant biomolecules such as lipids, proteins and nucleic acids (Lobo *et al.*, 2010) leading to a condition termed oxidative stress which is a key factor in the pathogenesis of many inflammatory diseases. This pathological condition stimulates cytokines and activation of pro-inflammatory enzymes like lipoxygenase, xanthine oxidase and hyaluronidase (Dobrian *et al.*, 2011).

Antioxidant can reduce and prevent the generation of free radicals in the human body and inhibit the activity of oxidative enzymes such as lipoxygenase. Phytochemicals such as phenols, flavonoids, anthocyanins, terpenoids and alkaloids from fruits and vegetables have been shown to possess significant antioxidant potential that may be associated with lower incidence of many degenerative diseases in human (Chinnici *et al.*, 2004). Thus, antioxidants have gained numerous attentions in the last few years especially in pharmacological fields. Different biological properties, antioxidant activities and free radical scavenging activities of various medicinal plants extracts have been widely demonstrated by many researchers.

Soursop (*Annona muricata* L.) is an important medicinal plant that demonstrate the antioxidant properties. The leaves of this plant are reported to be used in the treatment of diabetes, cystitis, headaches, fever, diarrhea, malaria, skin rashes and heart and liver diseases (Moghadamtousi *et al.*, 2015). Soursop leaves have been reported to be a rich source of alkaloids (Coria-Téllez *et al.*, 2016), flavonoids, phenolic acids and acetogenins. Many studies have reported that its leaves contain anti-inflammatory (Laksmitawati *et al.*, 2016),

anticancer (Adewole *et al.*, 2009), antiulcer (Bento *et al.*, 2016) , wound healing (Moghadamtousi *et al.*, 2015), and antioxidant activity (Gavamukulya *et al.*, 2014). However, the literature survey did not show any reference to previous work on comparing the antioxidant activity of this plant, inhibition of pro-inflammatory enzymes such as hyaluronidase, lipoxygenase, xanthine oxidase and reduction of biomolecule oxidative damage. Therefore, the present study was aimed to evaluate the potent antihyaluronidase activity and the potential role of different extracts of soursop to prevent lipid and protein against oxidative damage.

Materials and Methods

Chemicals:

2, 2-diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide [$K_3 Fe (CN)_6$], gallic acid (GA), ascorbic acid (AS), and $FeCl_3$, were purchased from Sigma-Aldrich Chemical Co., (St. Louis, MO, USA), and ammonium molybdate, Folin-Ciocalteu's phenol reagent, and sodium carbonate were from Merck Chemical Supplies (Darmstadt, Germany). All the other chemicals used, including the solvents, were of analytical grade.

Preparation of plant extracts:

The soursop (*Annona muricata* L.) leaves and ripe fruits were harvested and washed thoroughly under running tap water, oven dried for a week at 40-60°C. The dried leaves and fruit pulp were uniformly ground using an electric grinder. The powdered material (100 g) was extracted for 3 days in 500 ml of distilled water, ethanol and methanol. The separated extracts were then filtered through Whatman No.1 filter paper and the filtrates were evaporated to dryness using rotary evaporator at 40°C. The semisolid extract was then dried at room temperature and stored at -20°C until further use.

Total antioxidant capacity:

The total antioxidant activity of extracts and standard were evaluated based on reduction of molybdate[VI] to molybdate[V] at acid pH and

formation of a green phosphate complex according to the method described by Prieto *et al.* (1999). The absorbance of the solution was read at 695 nm against a blank. Ascorbic acid was used as standard.

2, 2- diphenyl – 1 - picrylhydrazyl (DPPH) radical scavenging activity:

The DPPH radical scavenging activity of leaves and fruit extracts were determined by the method of Brand-Williams *et al.* (1999) and the absorbance of the reaction mixture was read at 517 nm using UV-Vis spectrophotometer. Ascorbic acid was used as standard and the percentage of inhibition was calculated and IC₅₀ value was determined.

Hydroxyl radical scavenging activity:

In vitro hydroxyl radical scavenging activity was determined by the method of Klein *et al.* (1991), with some modifications, using ascorbic acid as reference compound. The absorbance of the sample was read at 510 nm and the percentage of inhibition was calculated.

Hydrogen peroxide scavenging activity:

The hydrogen peroxide scavenging activity of extracts was determined by the method of Ruch *et al.* (1989) with slight modifications. The absorbance of the solution was measured at 230 nm using UV-Vis spectrophotometer against blank solution without H₂O₂. All the experiment performed was in triplicated at each concentration and ascorbic acid was used as the experimental control. The percentage of inhibition of H₂O₂ was calculated and the IC₅₀ value was determined.

Lipid peroxidation inhibition assay:

The lipid peroxidation inhibition was determined by thiobarbituric acid (TBA) method described by Choi *et al.* (2007) with slight modifications. The linoleic acid-20 was used as lipid rich media and formation of malondialdehyde (MDA) was measured using UV-Vis spectrophotometer at 532 nm. BHT was used as standard and percentage inhibition was calculated.

Protein oxidation inhibition assay:

The protein oxidation inhibition was measured by the protein carbonyl formation as described by Zakrys *et al.* (2008). The absorbance was read at 370 nm and carbonyl concentration was calculated based on the extinction coefficient of DNPH. The percentage inhibition was calculated and IC₅₀ value was determined.

Hyaluronidase inhibition activity:

Hyaluronidase inhibitory activity of leaves and fruit extracts were determined by spectrophotometric method as described by Sahasrabudhe *et al.* (2010) with slight modifications. The absorbance of the reaction mixture was measured using UV-Vis spectrophotometer at 585 nm. Tannic acid was used as the reference compound. Per cent enzyme inhibition was calculated and IC₅₀ value was determined.

Xanthine oxidase inhibition assay:

Xanthine oxidase inhibitory activity of the sample extracts were determined by the conversion of xanthine to form uric acid and hydrogen peroxide as described by Lee *et al.* (1998). The absorbance was read at 295 nm against blank solution and allopurinol was used as the reference standard. Percentage inhibition of xanthine oxidase was calculated and the IC₅₀ value was determined.

Lipoxygenase inhibition assay:

Lipoxygenase inhibitory activity of soursop leaves and fruit extracts was determined by a spectrophotometric method as described by Perera *et al.* (2018). The formation of hydroperoxyoctadecadienoic acid was measured at 234 nm using UV-Vis spectrophotometer against blank and standard solution. Baicalein was used as the reference standard and the percentage inhibition was calculated.

Inhibition of protein denaturation:

Inhibition of protein denaturation was determined by the method of Padmanabhan *et al.* (2018) with some modifications. Bovine serum albumin was

used as protein source and diclofenac sodium was used as the reference standard. The absorbance of the reaction mixture was measured at 660 nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated and IC₅₀ value was determined.

Statistical analysis:

The experimental results were expressed as mean \pm SE of three replicates. The data were subjected to One-way Analysis of Variance (ANOVA). Student's *t*-test was used to calculate the statistical significant differences between the control groups and the test samples. The IC₅₀ value was calculated by interpolation from linear regression analysis using XLSTAT software. A value of $P \leq 0.05$ was considered to be significant.

Results and Discussion

Free radical scavenging activity:

Three solvents namely water, ethanol and methanol were used for the extraction of phenolic compounds from soursop leaves and fruit. The mean antioxidant activity of the sample extracts increased in the following order: water < methanol < ethanol. Antioxidants have been studied for their effectiveness in preventing the deleterious effects of inflammation and related diseases. DPPH is a stable free radical and gives purple colour in methanol, which reduced to yellow after receiving a proton from phenolic compounds. In the present study ethanol extract of leaves recorded maximum DPPH radical scavenging activity (68.41%) with IC₅₀ value of 317.58 μ g/ml (Table 1) while fruit pulp showed lowest percentage inhibition (47.14%). The aqueous extract of test samples showed least inhibition activity as compared to ethanol and methanol extracts (Fig. 1 A).

Hydrogen peroxide is an unstable radical which can be associated to form hydroxyl and singlet oxygen radicals causing lipid peroxidation and cell damage. Soursop (*Annona muricata* L.) extract was an effective scavenger of hydrogen peroxide radical (Fig. 1 B) and this activity was

compared to that of ascorbic acid. The IC₅₀ value of the test sample was 192.12 μ g/ml whereas that of ascorbic acid was 73.84 μ g/ml (Table 1). The ethanolic extract of leaves and fruit showed significantly higher scavenging activity as compared to aqueous and methanol extracts. The percentage inhibition of leaves ethanol extract was 78.21% whereas the fruit recorded 44.18% scavenging activity. No significant activity was found between ethanol and methanol fruit extracts but leaves extract showed significant activity. The water extract of soursop leaves and fruit showed the lowest hydrogen peroxide radical scavenging activity.

The current investigation demonstrated that the scavenging action of hydroxyl radicals was concentration-dependent, with greater concentrations increasing scavenging activity. The radical scavenging activity of the extract was calculated from the standard ascorbic acid to inhibit hydroxyl radical-mediated deoxyribose degradation in a Fe³⁺-EDTA-ascorbic acid and H₂O₂ reaction mixture. The maximum activity was observed in the ethanol extract of leaves (72.42%) samples (Fig. 1 C). The IC₅₀ value of the extract and the standard ascorbic acid in this assay were 301.19 μ g/ml and 77.83 μ g/ml, respectively (Table 1). The sample extract's IC₅₀ value was higher than the standard, indicating reduced activity. Significant scavenging activities were found among samples and different solvent extracts. The aqueous extract of fruit and leaves samples showed lowest inhibitory activity as compared to ethanol and methanol extracts.

The total antioxidant activity is based on reduction of molybdate[VI] to molybdate[V] at acid pH and formation of a green phosphate complex, which can be quantified spectrophotometrically at 695 nm. This assay shows the abilities of the conversion of molybdate [VI] to molybdate[V] by the leaves and fruit extracts and standard ascorbic acid (Fig. 1 D). The leaves ethanol extract showed highest percentage of scavenging activity (83.36%) than other test samples. The IC₅₀ value of the extract and

Table 1: IC₅₀ values of different extracts of soursoap (*Annona Muricata* L.) leaves and fruit for different inhibitory activities compared with the suitable reference compound. Each Values represent the mean \pm SE for each group, (n=3). The values are statistically significance at P<0.05 by hypothesis testing method which includes ANOVA followed by post hoc analysis

Parameters ($\mu\text{g/ml}$)	Leaves			Fruits			Reference compound
	Aqueous	Methanol	Ethanol	Aqueous	Methanol	Ethanol	
DPPH scavenging activity	556.6 \pm 2.35	374.5 \pm 1.74	317.6 \pm 0.93	810.8 \pm 3.47	516.1 \pm 2.94	518.9 \pm 1.37	105.8 \pm 0.47
H ₂ O ₂ scavenging activity	510.9 \pm 2.05	322.9 \pm 1.65	192.1 \pm 0.87	1111.5 \pm 5.22	629.3 \pm 2.89	616.1 \pm 2.31	73.8 \pm 0.59
Hydroxyl radical scavenging activity	585.6 \pm 1.43	438.4 \pm 1.01	301.2 \pm 0.79	882.1 \pm 2.25	696.5 \pm 3.58	439.6 \pm 1.66	77.8 \pm 0.86
Total antioxidant capacity	550.9 \pm 2.01	248.0 \pm 2.87	168.1 \pm 1.14	925.4 \pm 1.45	535.7 \pm 1.81	397.2 \pm 0.98	57.6 \pm 0.63
Protein oxidation inhibition	1938.3 \pm 3.45	612.3 \pm 2.84	615.5 \pm 2.33	2726.4 \pm 4.69	2269.9 \pm 3.21	1149.5 \pm 2.78	83.4 \pm 0.87
Lipid peroxidation inhibition	1181.7 \pm 1.36	619.3 \pm 1.89	576.0 \pm 0.75	2185.2 \pm 2.22	1917.0 \pm 2.67	1689.7 \pm 2.95	72.3 \pm 0.92
Hyaluronidase inhibition	940.0 \pm 1.57	772.4 \pm 1.21	766.5 \pm 1.97	1454.4 \pm 2.31	1244.6 \pm 2.04	1646.2 \pm 2.58	103.6 \pm 1.02
Lipoxygenase inhibition	1895.2 \pm 2.41	519.5 \pm 2.06	466.6 \pm 2.67	2228.3 \pm 3.45	1428.7 \pm 2.74	1285.1 \pm 3.01	166.7 \pm 1.15
Xanthine oxidase inhibition	1250.7 \pm 1.46	527.8 \pm 1.67	520.2 \pm 1.1	1648.3 \pm 1.97	1062.9 \pm 1.63	1064.7 \pm 1.12	140.3 \pm 0.96
Inhibition of protein denaturation	815.2 \pm 1.25	314.4 \pm 0.97	294.8 \pm 0.81	1919.1 \pm 1.87	942.7 \pm 1.36	974.3 \pm 1.64	178.5 \pm 1.11

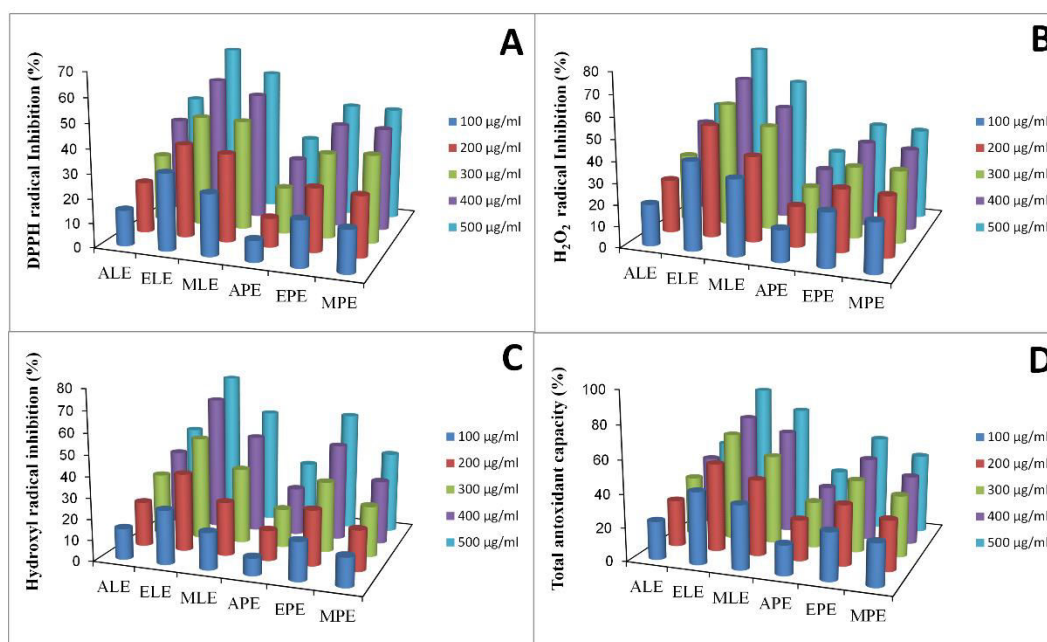


Fig. 1: Free radical scavenging activity of different extracts soursoap (*Annona muricata* L.) leaves and fruit. ALE: aqueous leaf extract, ELE: ethanol leaf extract, MLE: methanol leaf extract, APE: aqueous fruit pulp extract, EPE: ethanol fruit pulp extract and MPE: methanol fruit pulp extract. Each Values represent the mean \pm SE for each group, (n=3). The values are statistically significance at P<0.05 by hypothesis testing method which includes ANOVA followed by post hoc analysis.

standard were 168.07 µg/ml and 57.62 µg/ml, respectively (Table 1). The higher phenolic content in ethanol leaves extracts might be related to its higher antioxidant activity. The low free radical scavenging activity observed in soursop fruit ethanol extract may be due to a low level of phenolic compound, as evidenced by the high IC₅₀ value when compared to ascorbic acid. This result is very closely related to the studies of Lee *et al.* (2016) and Kuhnert *et al.* (2014). Based on this finding further it is validated that the leaves of soursop possess higher antioxidant potential than fruit.

Inhibition of biomolecule oxidation:

The formations of carbonyl groups have been widely selected as a general index of protein oxidation which involves irreversible modifications of essential amino acids. Figure 2 A shows the results of the inhibition of carbonyl group formation by the plant extract and this activity was comparable to that of silymarine as reference compound. The effect of silymarine and different solvent extracts of soursop leaves and fruits on oxidative damage of bovine serum albumin were analyzed.

Ethanol extracts of soursop leaves showed highest percentage (43.11%) inhibitory activity on protein carbonyl formation induced by H₂O₂, Fe³⁺ and ascorbic acid system as compared to methanol and aqueous extracts. The calculated IC₅₀ of the sample extracts were more than the standard, indicating that the standard is more potent inhibitor on carbonyl group formation than the plant extract. The IC₅₀ value of the extract and the standard were 612.26 µg/ml, and 83.35 µg/ml, respectively (Table 1). The aqueous extract of leaves and fruit samples showed significantly lower inhibitory activity than other solvent extract while, the IC₅₀ value was greater than the standard and other extracts, 1938.34 µg/ml (leaves) and 2726.35 µg/ml (fruit). Control samples had significantly higher amounts of protein carbonyls than the sample extract and standard treated samples. These results suggest that samples treated with ethanol extract of soursop leaves had

significant effect in controlling final carbonyl concentrations.

The sample extract and standard inhibited lipid peroxide formation in a concentration-dependent manner resulting from the oxidative degradation of lipids (Fig. 2 B). BHT was used as the standard and showed a protective effect against the lipid peroxidation. The extracts also showed inhibition of peroxidation effect in all concentrations. The ethanol and methanol extracts were more efficient than aqueous extracts of soursop leaves and fruit. The IC₅₀ value of the standard was 72.26 µg/ml whereas, the sample extracts recorded higher than the standard value, 575.98 µg/ml (ethanol), 619.26 µg/ml (methanol) and 1181.65 µg/ml (aqueous) of soursop leaves and 1689.73 µg/ml (ethanol), 1916.96 µg/ml (methanol) and 2185.18 µg/ml (aqueous) for fruit samples (Table 1). From this results we observe that the aqueous extract exhibits very poor inhibitory activity than other solvent extracts.

Anti-arthritic activity:

Figure 2C illustrates the *in vitro* anti-inflammatory effect of soursop leaves and fruit extracts. The results observed that the sample extract exhibited a dose-dependent inhibition protein denaturation from 100 to 500 µg/ml. The higher percentage of inhibition obtained with leaves ethanol extract was 79.12%, while the fruit ethanol extracts recorded as 30.65%. The statistical analysis showed significant difference (P<0.0001) between leaves and fruit samples while there was no difference between the ethanol and methanol extracts of fruit and leaves samples. Aqueous extracts recorded significantly lower inhibitory activity than solvent extracts. However, the solvent extract was more active than aqueous extract. This was further confirmed by comparing their IC₅₀ values. The ethanol extracts showed the highest inhibition of protein denaturation with the lowest concentration of IC₅₀ values (294.78 µg/ml) as compared to that of aqueous extract (815.22 µg/ml) (Table 1). Diclofenac was used as the reference compound in this assay which also exhibited the percentage inhibition of protein

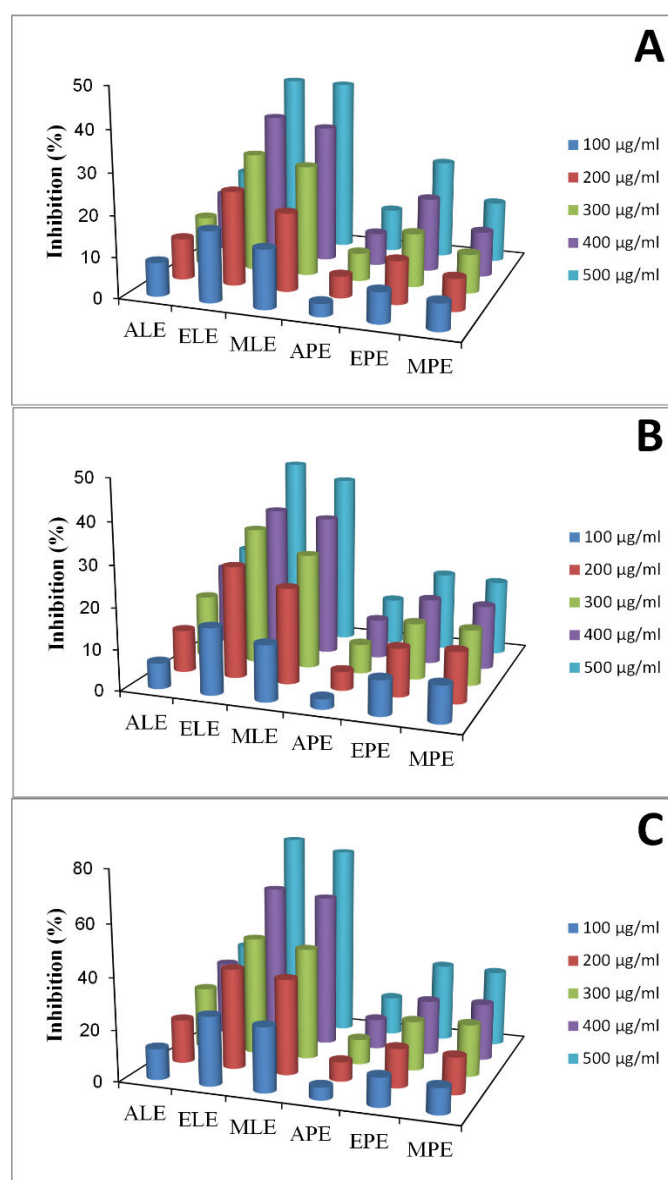


Fig. 2: Inhibition of protein and lipid peroxidation of different extracts soursop (*Annona muricata* L.) leaves and fruit (A: protein oxidation, B: lipid peroxidation and C: protein denaturation). ALE: aqueous leave extract, ELE: ethanol leaves extract, MLE: methanol leaves extract, APE: aqueous fruit pulp extract, EPE: ethanol fruit pulp extract and MPE: methanol fruit pulp extract. Each Values represent the mean \pm SE for each group, (n=3). The values are statistically significance at $P < 0.05$ by hypothesis testing method which includes ANOVA followed by post hoc analysis.

denaturation by concentration dependent manner. The result showed that the highest percentage of inhibition activity (95.24%) of diclofenac was recorded at 500 µg/ml (Fig. 2 C). Denaturation of proteins is well-documented causes of inflammatory and arthritic disease. Agent that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development. The present study showed that the

soursop leaves and fruit extracts are the good inhibitor of protein denaturation. The presence of polyphenols could be the basis of the better activity showed by these extracts (Raja *et al.*, 2012).

In vitro pro-inflammatory enzymes inhibition:

The result represented in Figure 3 A indicates that the hyaluronidase inhibitory activity of soursop

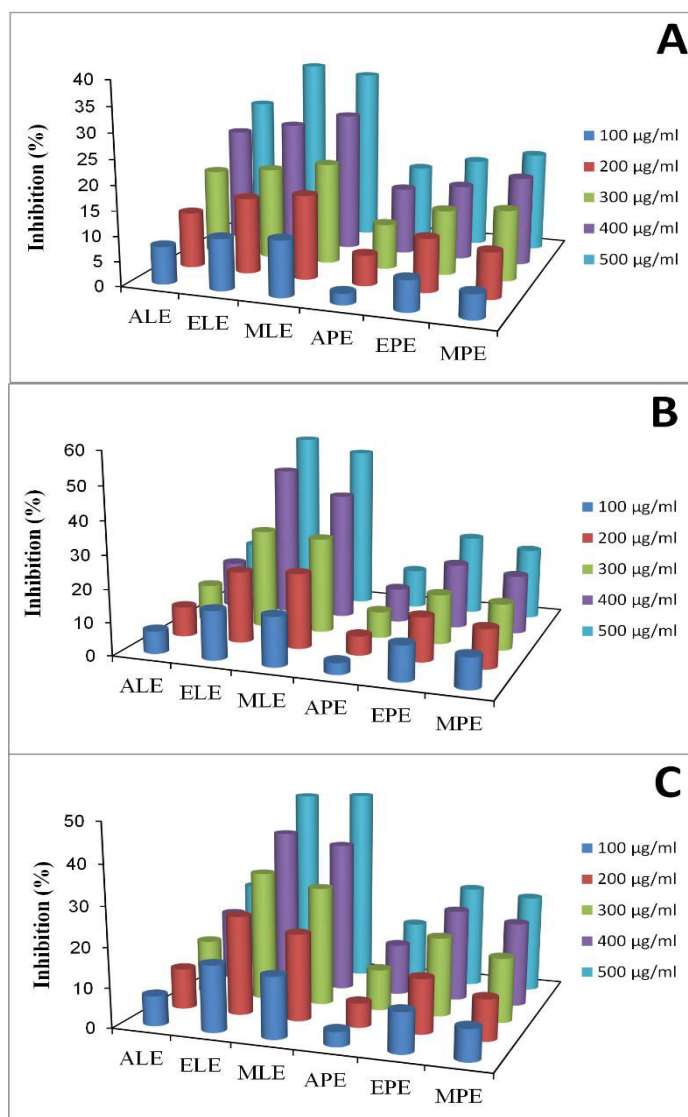


Fig. 3: Inhibition of pro-inflammatory enzymes of different extracts soursop (*Annona muricata* L.) leaves and fruit (A: hyaluronidase, B: lipoxygenase and C: xanthine oxidase). ALE: aqueous leave extract, ELE: ethanol leaves extract, MLE: methanol leaves extract, APE: aqueous fruit pulp extract, EPE: ethanol fruit pulp extract and MPE: methanol fruit pulp extract. Each Values represent the mean \pm SE for each group, (n=3). The values are statistically significance at $P < 0.05$ by hypothesis testing method which includes ANOVA followed by post hoc analysis.

fruit and leaves was concentration dependent which ranged from 100 to 500 $\mu\text{g/ml}$. The ethanol leaf extracts showed the highest activity (36.21%) followed by the methanol extract (34.88%), while the lower activity (17.88%) was found in fruit ethanol extract as compared to methanol extract (20.11%). The aqueous extract showed lowest inhibitory activity against hyaluronidase enzyme (Fig. 3 A). Tannic acid was used as the standard in this assay. The calculated IC_{50} value of the

standard was 103.60 $\mu\text{g/ml}$ which was lower than the sample extract. The IC_{50} value of soursop leaves ethanol, methanol and aqueous extracts were 766.52 $\mu\text{g/ml}$, 772.35 $\mu\text{g/ml}$ and 940.02 $\mu\text{g/ml}$, respectively (Table 1). Whereas, the ethanol, methanol and aqueous extract of soursop fruit pulp were 1646.19 $\mu\text{g/ml}$, 122.63 $\mu\text{g/ml}$ and 1454.44 $\mu\text{g/ml}$, respectively. The results presumably recommended that the leaves extract may contribute an excellent anti-aging activity by

degradation of hyaluronates underneath the skin. The hyaluronidase inhibitory properties of soursop leaves and fruits using various solvent extracts are recorded for the first time to upgrade the medicinal value of the plant.

Based on the result, the per cent inhibition in the screening assays revealed that the ethanol leaves extract had the highest (53.31%) lipoxygenase inhibitory activity followed by the methanol extract (49.86%), while the leaf aqueous extract had the lowest activity (16.32%) (Fig. 3 B). The activities of the extracts were found to be significantly different from the standard. Baicalein was used as the reference compound, which showed a strong dose dependent activity against lipoxygenase enzyme activity. The IC_{50} value of the standard and the sample extract were 166.69 $\mu\text{g/ml}$ and 466.63 $\mu\text{g/ml}$, respectively (Table 1). The calculated IC_{50} value of all the sample extracts were more than the positive control which indicates that the standard had strong inhibitory activity than sample extracts.

The results revealed that, all extracts showed the inhibitory activity against xanthine oxidase enzyme. The activities were ranged from 3.71 to 48.31%. The methanol leaf extract had the highest inhibitory activity (49.14%) than ethanol (48.31%) and aqueous (22.41%) extract (Fig. 3 C). Whereas the fruit sample recorded lower activity of ethanol (25.94%), methanol (24.68%) and aqueous (15.19%) extracts against xanthine oxidase enzyme with respect to the standard allopurinol. The IC_{50} value of the leaves ethanol extract was 520.19 $\mu\text{g/ml}$ (Table 1) whereas that of allopurinol was 140.34 $\mu\text{g/ml}$.

This study demonstrated that anti-xanthine oxidase capacity of the extract of soursop may be used in the treatment of arthritis. Xanthine oxidase plays an important role in pathogenesis of hyperurisemia by imparting inflammation and catalyzing the formation of uric acid leading to arthritis. The high potential of soursop leaves extract to decrease uric acids probably related to the presence of high phenolic and vitamin C compounds which acts as xanthine oxidase

inhibitor and antioxidant agent (Chen *et al.*, 2015). Our screening results demonstrated that the strongest pro-inflammatory enzyme inhibitory activities were found in soursop leaves extracts. The results revealed that the effective pro-inflammatory enzyme inhibition of leaves extracts is probably due to its richness of phenolic and flavonoid compounds (Gupta *et al.*, 2019).

Conclusion

Soursop (*Annona muricata* L.) extract is the principal source of phenolic compounds and also contains vitamins, flavonoids and alkaloids. The extracts showed significantly highest antioxidant, anti-biomolecule oxidation and anti-inflammatory activity. Among the three solvent extract, the ethanol extract of leaves of soursop showed the highest free radical scavenging activity, anti-inflammatory activity and also inhibition of biomolecule oxidation. The fruit extracts showed the lowest antioxidant potential due to the presence of lower phenolic and flavonoid compounds. Therefore, the ethanol extract of soursop (*Annona muricata* L.) leaves is identified as a source of anti-arthritic agent, which can be further studied to isolate and characterize bioactive constituents.

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