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Anti-Inflammatory and Antioxidant Activities of *Psidium Guajava Linn* Aqueous Extract

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Abstract

Based on the local availability we have chosen the plant Psidium guajava Linn for our study, which are rich in polyphenols (bioactive compounds/molecules). The objective of our study is to evaluate the antioxidant and anti-inflammatory activities of aqueous extract of P.guajava Linn leaves. In this context, the in vitro antioxidant activity was done by 2,2-diphenyl1-1-picrylhydrazyl, hydroxyl radical and H2O2 radical scavenging, ferrous ion chelating, ferric reducing power, total antioxidant capacity and by the protection against peroxidation of β-carotene-linoleic acid in emulsion. The anti-inflammatory activity was evaluated by studying membrane of human red blood cells against different hypotonic concentrations of NaCl and against heat inhibiting the denaturation of albumin. P.guajava Linn showed in vitro anti-inflammatory activity by inhibiting the heat induced albumin denaturation and red blood cells membrane stabilization. Our results showed that aqueous leaf extract of P.guajava Linn has good antioxidant activity and anti-inflammatory properties. P.guajava Linn aqueous extract can be used to prevent oxidative damage and inflammatory processes.

Keywords: Psidium guajava Linn; Antioxidant and anti-inflammatory activity.

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1. Introduction

1.1. Antioxidants

Antioxidants are thought to protect the body against the destructive effects of free radicals. Antioxidants neutralize free radicals by donating one of their own electrons, ending the electron- gain reaction. Body produces several enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSHPX), that neutralize many types of free radicals. Supplements of these enzymes are available for oral administration. However, their absorption is probably minimal at best. Supplementing with the "building blocks" the body requires to make SOD, catalase, and glutathione peroxidase may be more effective. These building block nutrients include the minerals manganese, zinc, and copper for SOD and selenium for GSHPX. In addition to enzymes, many vitamins, minerals and hormones act as antioxidants in their own right, such as vitamin C, vitamin E, β-carotene, lutein, lycopene, vitamin B_2, coenzyme Q_{10}, and cysteine (an amino acid). Herbs, such as bilberry, turmeric (curcumin), grape seed or pine bark extracts, and ginkgo can also provide powerful antioxidant protection for the body. Melatonin is a hormone secreted by pineal gland and proves to be powerful antioxidant and free radical scavenger.

2. Materials and Methods

2.1. Chemicals

Psidium guajava Linn (Guava) leaves were collected from in and around our college campus. All other chemicals were purchased from Sigma-Aldrich, Chemicals Pvt. Ltd, India. All other chemicals used were of good quality and analytical grade.

2.2. Preparation of Extract

Psidium guajava Linn leaves were collected in and around from the college campus, washed well cleaned with distilled water, and air dried at room temperature for 12/12 light and dark conditions. The air dried plant material was ground in an electric grinder and sieved by 0.22 mm mesh size. 20 grams of *Psidium guajava Linn* leaves powder were dissolved in 200 ml of double distilled water. After 24 hours of Maceration at room temperature the filtrate was lyophilized.

2.3. Phytochemical Screening

The phytochemical investigation of the different extracts of *Psidium guavaja linn* was carried out with standard protocol. The phytochemical tests were carried out with Chloroform, Methanol, Ethanol & Water. The results are presented in Table 1.

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Table-1. Phytochemical Profile of *Psidium guavaja linn*

Chemical Test	Aqueous Extract	Chloroform Extract	Methanol Extract	Ethanol Extract
Test for Alkaloids:				
a) Dragendorff's test	+	+	+	+
b) Mayer's test	-	-	-	-
c) Hager's test	-	-	-	-
Test for Tannins:				
a) Ferric chloride test	+	+	+	+
b) Lead acetate test	+	-	+	+
c) Sodium hydroxide test	+	-	+	+
d) Shinoda test	+	-	+	+
Test for Steroids:				
a) Salkowski test	+	+	+	+
b) Libermann-Burchard reaction	+	+	+	+
Saponification test:				
Foam test	+	-	+	+
Test for Cardiac glycosides:				
a) Keller-killiani test	-	-	+	+
b) Legal's test	+	+	+	-
Test for Anthraquinone glycosides:				
Borntrager's test	-	-	+	+
Test for Saponin glycosides:				
a) Foam test	+	-	+	+
b) Heamolytic test	+	-	+	+
Test for Carbohydrates:				
a) Molisch's test	+	-	+	+
b) Fehling's test	+	+	+	+
c) Benedict test	-	-	+	+
Test for Proteins:				
a) Biuret test	-	-	+	-
b) Millions test	+	-	-	-
Test for Amino acids:				
Ninhydrin test	-	-	-	-

2.4. Determination of Total Phenolic Content

The phenolic compounds in plant extract was studied by using the Folin-Ciocalteu spectrophotometric method [1]. The reaction mixture consists of 200 µl of extract (40 µg/ml) with 1 ml of Folin-Ciocalteu reagent, diluted 10 times and 800 µl of sodium carbonate (75 mg/ml) added. The mixture incubated for 45 min at room temperature and the absorbance was measured against a blank at 760 nm. Gallic acid was used as the standard and the calibration curve was constructed. The results are expressed in mg of Gallic acid equivalent per gram of extract.

2.5. Determination of Total Flavonoid Content

Total flavonoid content in leaves extract was determined using the aluminium chloride method spectrophotometric assay [2, 3]. 1 ml of ethanolic extract containing 2% of $AlCl_3$ was incubation for 10 min the absorbance was measured at 430 nm against a methanol as blank. Quercetin was used as standard and standard calibration curve was constructed. Results were expressed as mg of Quercetin equivalent per gram of extract.

2.6. Determination of 2, 2-Diphenly-1-Picrylhydrazyl (DPPH) Radicals Scavenging Activity

Free radical scavenging activity of the extract was measured using the stable free radical DPPH test according to the method described before [4, 5]. A total of 250 µl of 0.8 mm/L DPPH in ethanol was mixed with 3.75 ml of extract kept for incubation for 30 min in dark, experiment was done in triplicate and the absorbance was measured at 517 nm. L-Ascorbic acid was used as reference standard. The percent of scavenging activity was calculated using the following equation.

$$\text{Scavenging Activity (\%)} = \left[\frac{(\text{Ac} - \text{As})}{\text{Ac}}\right] \times 100$$

Where, Ac stands for the absorbance of the control and As stands for absorbance of the sample.

2.7. B-Carotene Bleaching Assay

Antioxidant activity of *P.guajava Linn* extract and the levels of butylated hydroxytoluene (BHT) were measured according to the published method [6, 7]. The emulsion mixture was prepared in 50 ml of round-bottom flask containing 1 ml of chloroform (HPLC grade), 0.5 mg β-carotene, 25 µl linoleic acid and 200 mg of Tween 40. Chloroform was completely evaporated using a vaccum evaporator at 40°C for 10 min. After evaporation, the mixture was diluted in 100 ml of distilled water. The ethanolic stock solution of the extract (350 µl, concentrations

were 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml) was repeated with BHT as positive control. Absorbance of the mixtures was measured at 470 nm immediately after their preparation (t = 0 min) and at incubation time t = 120 min against the blank. The percentage of inhibition was calculated with the following equation.

$$\text{Inhibition (\%)} = \left[\frac{(Aa_{120} - Ac_{120})}{Ac_0 - Ac_{120}}\right] \times 100$$

Where, Aa_{120} is the absorbance of the antioxidant at t = 120 min, Ac_{120} is the absorbance of the control at t = 120 min and Ac_0 is the absorbance of the control at t = 0 min.

2.8. Hydroxyl Radical Scavenging Assay

Scavenging activity of the hydroxyl radical of the extract was measured according to method [7], 500 µl of various concentrations of the *P.guajava Linn* leaves extract with a 3 ml final reaction solution, 1 ml of $FeSO_4$ (1.5 mmol/L), 0.7 ml hydrogen peroxide (6 mmol/L) and 0.3 ml sodium salicylate (20 mmol/L) were added and the reaction mixture was incubated for 1 hr at 37°C. L-Ascorbic acid was used as the standard. The colour developed was measured at 560 nm against a blank.

2.9. Hydrogen peroxide radical scavenging activity

The scavenging ability of the *P.guajava Linn* aqueous leaves extract on hydrogen peroxide was determined according to the method [8], A solution of hydrogen peroxide (40 mmol/L) was prepared in phosphate buffer (pH 7.4). 3.4 ml of aqueous extract was added to a hydrogen peroxide solution (2.80.6 ml, 40 mmol/L). Absorbance of hydrogen peroxide at 230 nm was measured 10 min later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extract and standard (L-Ascorbic acid) was calculated using the following equation.

$$\text{Scavenged } H_2O_2 \text{ (\%)} = (1 - \frac{A_s}{A_c}) \times 100$$

Where, A_c is the absorbance of the control (without the extract) and A_s is the absorbance in the presence of the extract. The experiment was repeated in triplicate.

3. Ferrous Ion Chelating Activity

Ferrous ion chelating activity was determined by inhibition of the formation of iron (II)-ferrozine complex, following the previous published method [9, 10]. 100 µl of 0.6 mmol/L $FeCl_2$ was added to 500 µl of different concentrations of the extract of the ethylenediamine tetra acetic acid (EDTA) (positive control). The reaction mixture was adjusted to a final volume of 1.5 ml with methanol, and then 100 µl of 5 mmol/L ferrozine were added. The mixture was shaken vigorously and left to stand at room temperature for 5 min. absorbance measured at 562 nm and percent of chelation was calculated using the following equation.

$$\text{Chelation (\%)} = \left(\frac{A_{sample}}{A_{control}}\right) \times 100$$

Where, A_{sample} is the absorbance of the sample and $A_{control}$ is the absorbance of the control.

3.1. Ferric Reducing Power Assay

Reducing power assay was determined by the method [11, 12], different concentration of the extract was mixed with 1.25 ml of 0.2 mol/L, pH 6.6 sodium phosphate buffer and 1.25 ml of potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min. After incubation, the reaction mixture was acidified with 1.25 ml of trichloroacetic acid (10%) and centrifuged at 3000 rpm per min for 10 min. finally, 0.5 ml of freshly prepared $FeCl_3$ (0.1%) was added to this solution, and the absorbance was measured at 700 nm. Ascorbic acid at various concentrations was used as standard.

3.2. Total antioxidant capacity

Total antioxidant capacity was estimated by phosphomolybdenum assay [13, 14]. The tubes containing the extract and reagent solution (0.6 mol/L sulphuric acid, 28 mmol/L sodium phosphate and 4 mmol/L ammonium molybdate) were incubated at 95°C for 90 min the solution was cooled to room temperature and the absorbance was read at 695 nm. Ascorbic acid was used as standard.

3.3. Antihemolytic Activity

3.4 Red Blood Cell Suspension

Blood was collected in heparinized tubes by venipuncture from our colleagues and centrifuged at 2000 rpm per min for 10 min at 4°C. After removing the plasma, red blood cells (RBCs) was washed for three successive times using phosphate buffer saline (PBS) (0.9% NaCl).

3.5. Hypotonic Solution Induced Hemolysis

Membrane stabilizing activity of the *P.guajava Linn* leaves extract was assessed using hypotonic solution induced hemolysis and the method [15], in hypotonic solution, the test sample consisted of washed stock erythrocyte (RBC) suspension (40 µl) with 1 ml of hypotonic solution (0.1%, 0.3%, 0.5%, 0.7%, 0.9% NaCl) in sodium PBS (pH 7.4) containing either of the different concentration of *P.guajava Linn* leaves aqueous extract. The mixture was

incubated for 30 min at 37°C under gentle stirring, centrifuged for 10 min at 2000 rpm for 10 min and the absorbance of supernatant was measured at 540 nm.

$$\text{Inhibition of Hemolysis (\%)} = \left[\frac{(OD_1 - OD_2)}{OD_1}\right] \times 100$$

Where, OD_1 is the optical density of hypotonic-buffered saline solution alone control and OD_2 is the optical density of test sample in hypotonic solution.

3.6. Heat Induced Hemolysis

Different concentrations of the *P.guajava Linn* leaves extract (25-500 µg/ml) and aspirin dissolved in isotonic PBS (pH 7.4) was mixed with 1 ml of 2% RBCs suspension. The reaction mixture was incubated in a water bath at 56°C for 30 min, tubes were cooled under running tap water, centrifuged at 2000 rpm for 10 min and the absorbance of the supernatant was measured at 560 nm [16]. The percentage of protection against heat induced hemolysis was calculated by using the following equation.

$$\text{Production (\%)} = (1 - \frac{OD_{sample}}{OD_{control}}) \times 100$$

3.7. Oxidant Induced Hemolysis

1 ml of RBC suspension (5%) in PBS (pH 7.4) was incubated for 15 min at 37°C with 1 ml of the *P.guajava Linn* leaves extract of various concentrations. After pre-incubation, the mixture was centrifuged at 2000 rpm for 10 min at 4°C, the supernatant was removed and packed RBCs were resuspended and incubated with 0.5 mmol/L HOCl in PBS, absorbance was measured at 540 nm [17, 18].

$$\text{Production (\%)} = (1 - \frac{OD_{sample}}{OD_{control}}) \times 100$$

3.8. Inhibition of Albumin Denaturation

0.2% (w/v) of egg albumin was prepared in a PBS (pH 6.4), 50 µL of the *P.guajava Linn* leaves extract with different concentration was added to 5 ml of this stock solution. The test tubes were heated at 72°C for 5 min and cooled. The absorbance was measured at 660 nm [19].

4. Result and Discussions

4.1. Phenolic Content

Many studies report that phenolic compounds play an important role in human health due to their antioxidant activity. The total phenols of *P.guajava Linn* aqueous extract [208.85±16.04 mg Gallic acid equivalent/g of extract] was calculated according to the equation y = 0.006x+0.027 (R^2 = 0.990).

4.2. Flavonoid Content

Construction of the calibration curve [y = 0.022x+0.182 (R^2 = 0.004)] collected data clearly showed a good amount of flavonoid content in the *P.guajava Linn* aqueous extract [(14.070±0.097) mg Quercetin equivalent/g of extract].

4.3. DPPH Scavenging Activity

DPPH radical scavenging activity determined in terms of inhibition percent as shown in Graph 1. The parameter used to compare the radical scavenging activity of the *P.guajava Linn* extract and standard ascorbic acid. IC_{50} value determined and is defined as the concentration of antioxidant required for 50% scavenging of DPPH radicals. The IC_{50} value for Ascorbic acid was 25 µg/ml which was comparatively lower than the IC_{50} [8.957±0.279)µg/ml] of *P.guajava Linn* aqueous extract.

4.4. B-Carotene Bleaching

Antioxidant activity was evaluated by β-carotene bleaching method was used to measure the ability of the extract to inhibit lipid peroxidation. The antioxidant activity was expressed as percent inhibition. Graph 2 shows that the antioxidant activity increases with the increasing concentrations of the extract, similar results were obtained for BHT [% inhibition = 97.89±0.35 (positive control)] and *P.guajava Linn* extract (% inhibition = 88.05±2.22) at 1000 µg/ml, which indicates a high potential antioxidant activity of the *P.guajava Linn* extract.

4.5. Hydroxyl Radical Scavenging

Hydroxyl scavenging activity of the aqueous extract was evaluated by its ability to compete with salicyclic acid for hydroxyl radicals, as shown in Graph 3, hydroxyl radical scavenging increased with increase in concentrations. The ascorbic acid [IC_{50} = (759.84±8.41) µg/ml] showed more effective scavenging ability when compared to that of *P.guajava Linn* aqueous extract [IC_{50} = (1016.75±47.36) µg/ml]. The maximum scavenging activity was found to be (98.81±0.19) % for ascorbic acid and (80.24±2.15) % for *P.guajava Linn* aqueous extract at 2 mg/ml.

4.6. Hydrogen Peroxide Radical Scavenging Activity

Scavenging activity of the *P.guajava Linn* extract and ascorbic acid as standard against hydrogen peroxide in terms of effective concentrations was remarkably different and were shown to be 84.15 % (600 µg) and 97.4 % (90 µg), respectively Graph 4. According to the results, *P.guajava Linn* showed an activity dependent on the concentration and the H_2O_2 scavenging IC_{50} was (115.78±17.87) µg/ml, which indicates a less effective scavenging potential referring to ascorbic acid [IC_{50} = (50.20±3.71) µg/ml].

4.7. Ferrous Ion Chelating Activity

The most important mechanisms is the chelating of pro-oxidant metals such as iron, Ferrozine forms a complex with Fe^{2+} with a characteristic red color but in the presence of chelating agent, the complex formation is disrupted and the red colour is decreased. Measurement of colour reduction, therefore, allows the estimation of the chelating activity of the *P.guajava Linn* extract. The metal chelating effect of investigated extract and EDTA were dependent on concentrations Graph 5. EDTA [IC_{50} = (58.22±0.45) µg/ml] in this assay demonstrated relatively high activity and comparison to the *P.guajava Linn* extract [IC_{50} = (1015.31±37.22) µg/ml].

4.8. Ferric Reducing Power

P.guajava Linn extract showed concentration dependent reducing power. However, it's reducing power [IC_{50} = (157.56±18.41) µg/ml] was lower than that of ascorbic acid [IC_{50} = (57.73±3.80) µg/ml].

4.9. Total Antioxidant Capacity

Test was based on the reduction of Mo(VI) to Mo(V) by the *P.guajava Linn* extract and formation at acid pH of green phosphate/Mo(V) complex. Results showed the antioxidant activity of the extract and ascorbic acid in a dose dependent manner at concentrations 100-500 µg/ml the IC_{50} value of antioxidant capacity for the ascorbic acid [(293±8.55) µg/ml] was greater than the *P.guajava Linn* extract IC_{50} [(462.68±5.17) µg/ml) (Graph 7).

5. Membrane Stabilizing Activity

The Graph 8 and 9 shows the *P.guajava Linn* extract prevented the erythrocyte membrane against lysis induced either by hypotonic solution and heat. For hypotonic solution induced hemolysis, at concentration range of 0.250-1.500 mg/ml, the *P.guajava Linn* extract showed significant inhibitory effect against RBCs hemolysis [(47.16±0.71)%, (80.54±0.44) %, (71.79±2.39) %, (72.96±3.27) % and 0.9 % of NaCl when the concentration of the *P.guajava Linn* extract was 1.500 mg/ml. In heat induced hemolysis. *P.guajava Linn* extract inhibited lysis of erythrocyte membrane in the range of (46.71±2.31) % (80.67±2.93) % at concentration range of 0.025-0.500 mg/ml. Aspirin demonstrated the protection in the range of (26.66±2.58) % - (59.50±2.24) %.

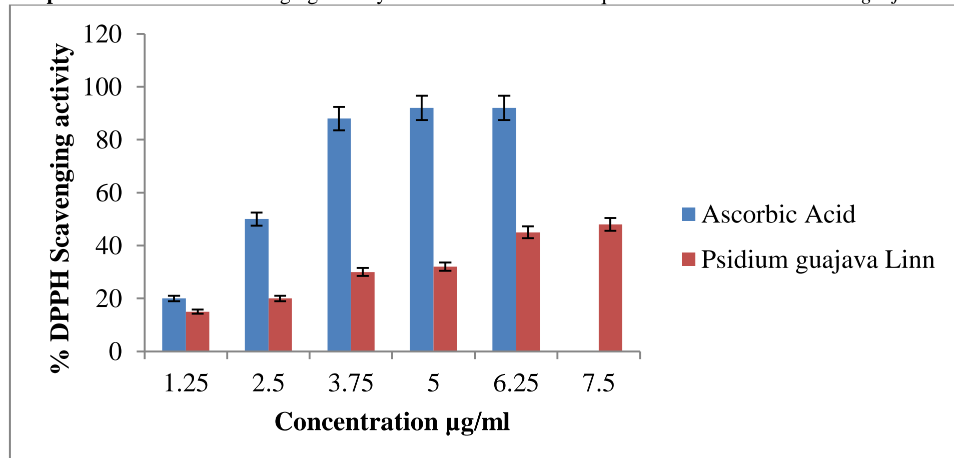
5.1. Oxidant Induced Hemolysis

From the Graph 10, protective effect of the *P.guajava Linn* extract against HOCl induced hemolysis was dose dependent. In fact the hemolysis ratio gradually decreased with the increasing dose of the *P.guajava Linn* extract. Protection was already evident at 1 mg/ml of the *P.guajava Linn* extract with (74.91±3.09) % protection.

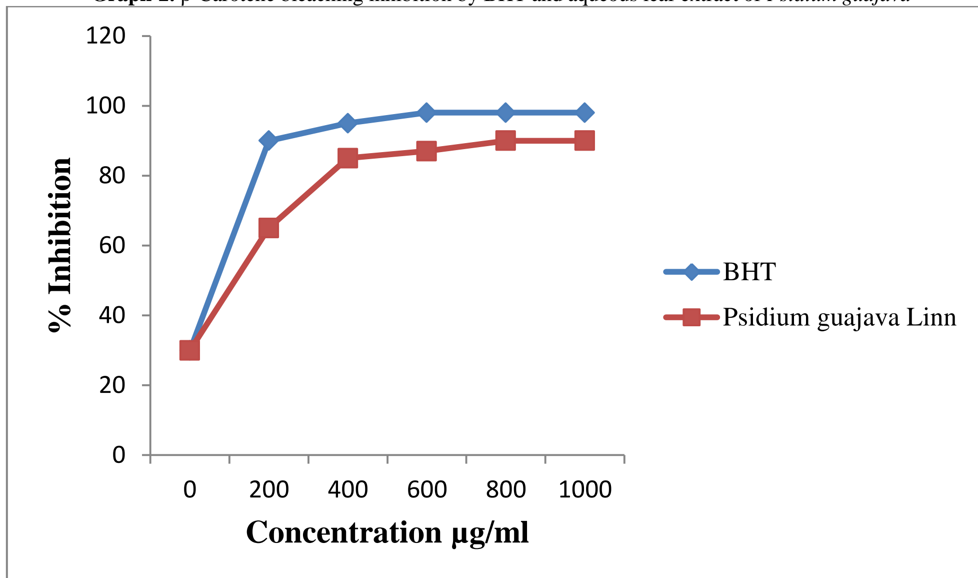
5.2. Inhibition of Albumin Denaturation

Protein denaturation is involved in inflammation and *P.guajava Linn* extracts showing inhibition of denaturation are often tested for anti-inflammatory activity. For inhibiting thermally induced denaturation of albumin, the extract showed an astonishingly effect at different concentration as shown in Graph 11. A maximum inhibition of (75.29±0.87) % was observed at 500 µg/ml for the *P.guajava Linn* extract and (93.24±0.33) % at 500 µg/ml for aspirin the anti-inflammatory standard.

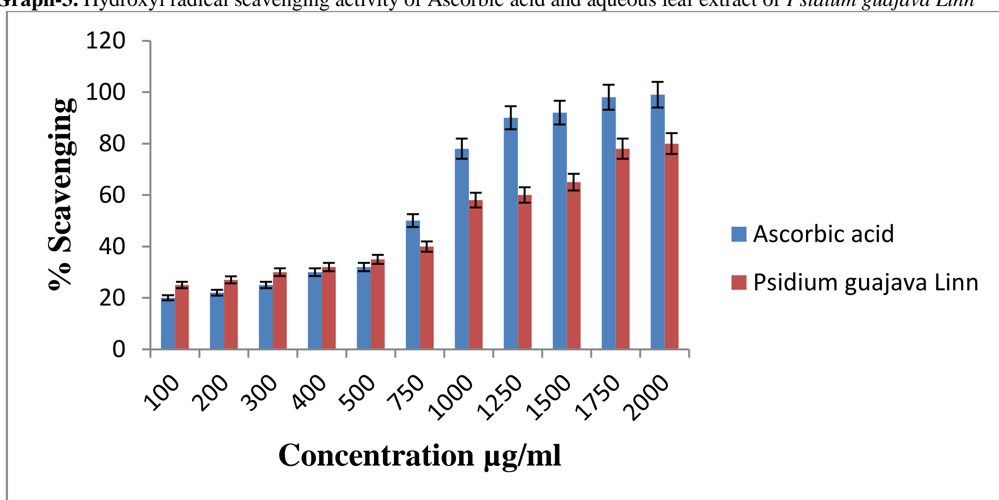
Graph-1. DPPH radical scavenging activity of Ascorbic Acid and Aqueous leaf extract of *Psidium guajava Linn*



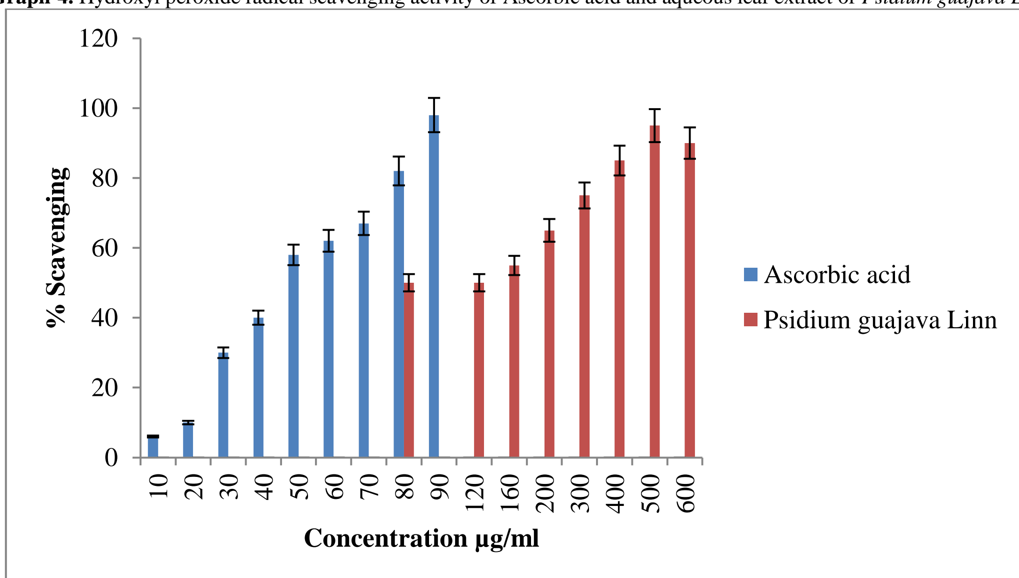
Graph-2. β-Carotene bleaching inhibition by BHT and aqueous leaf extract of *Psidium guajava*



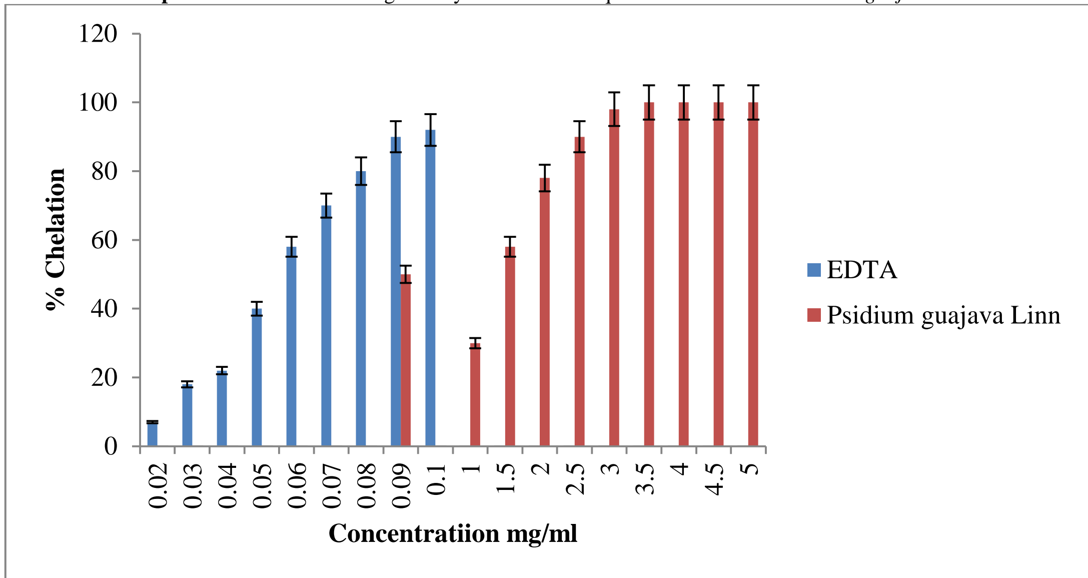
Graph-3. Hydroxyl radical scavenging activity of Ascorbic acid and aqueous leaf extract of *Psidium guajava Linn*



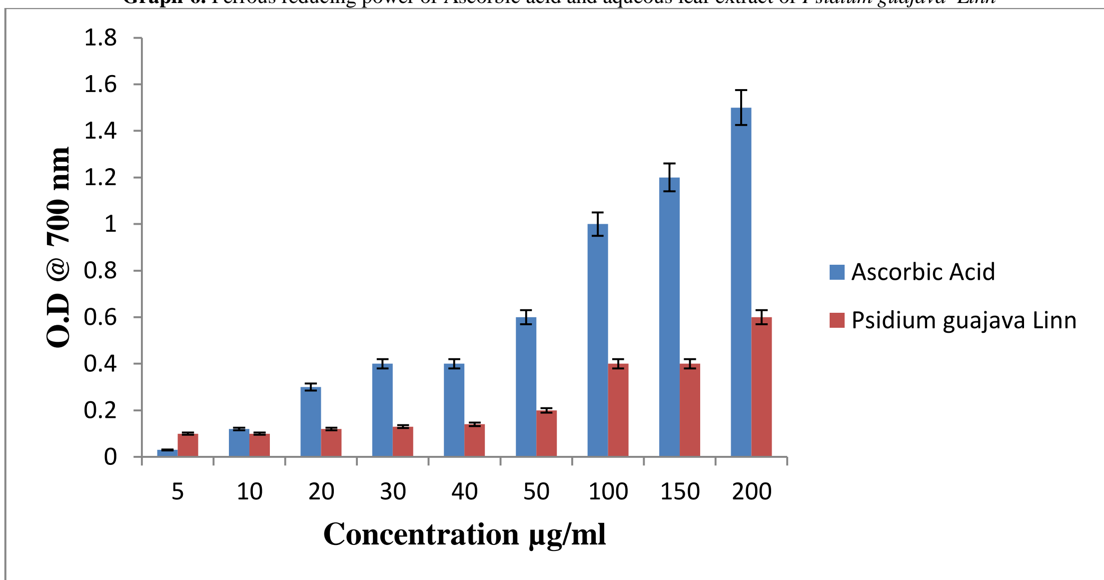
Graph-4. Hydroxyl peroxide radical scavenging activity of Ascorbic acid and aqueous leaf extract of *Psidium guajava Linn*



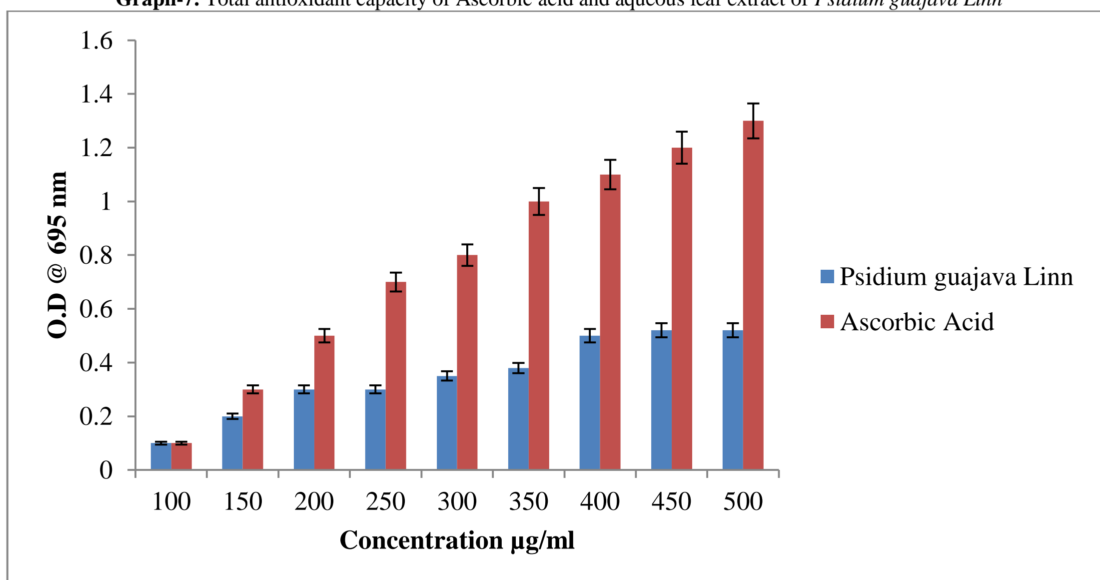
Graph-5. Ferrous ion chelating activity of EDTA and aqueous leaf extract of *Psidium guajava Linn*



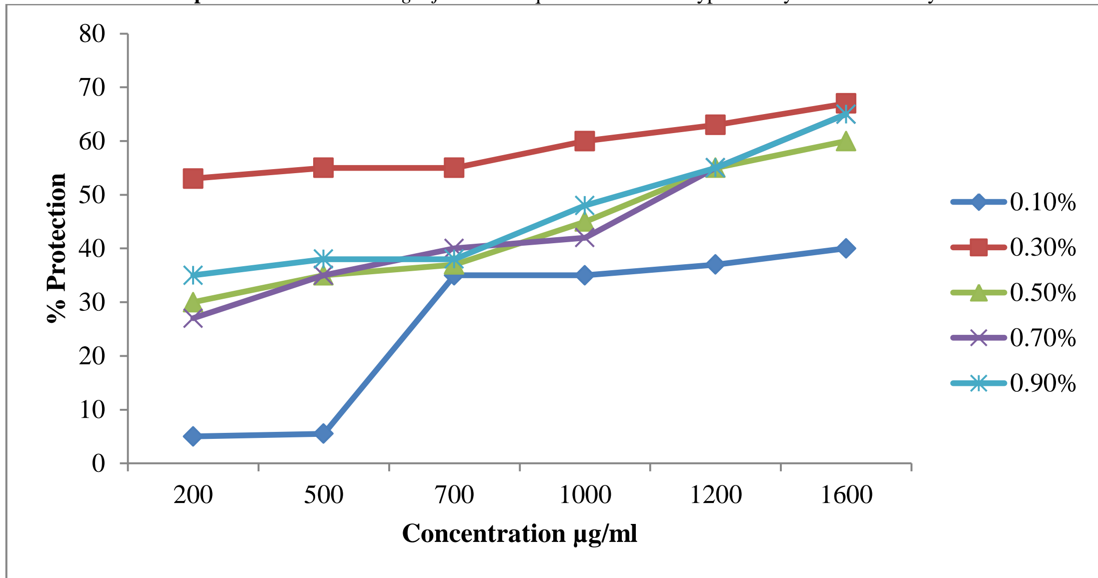
Graph-6. Ferrous reducing power of Ascorbic acid and aqueous leaf extract of *Psidium guajava Linn*



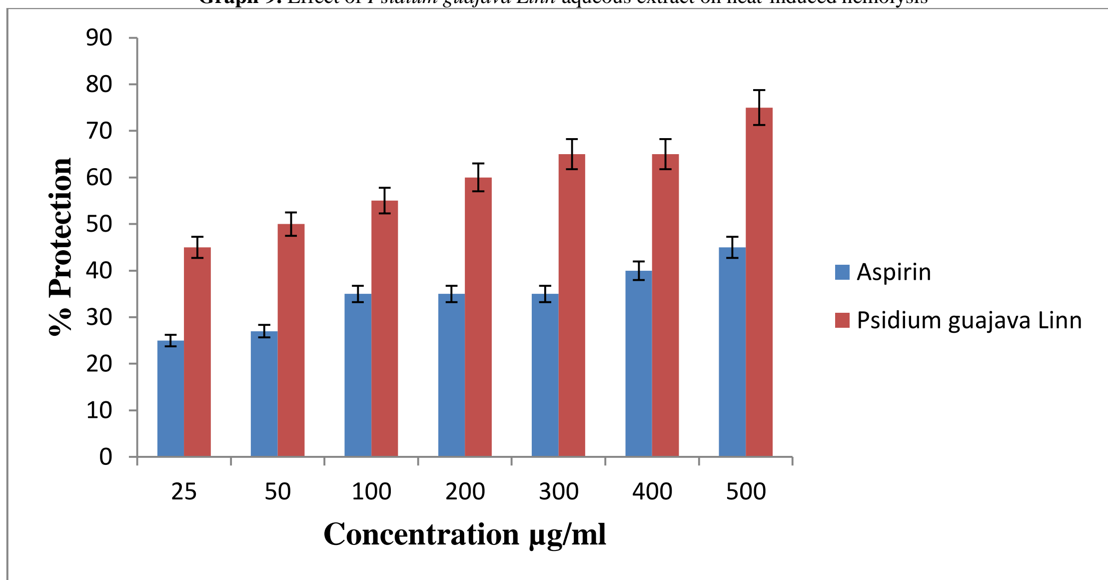
Graph-7. Total antioxidant capacity of Ascorbic acid and aqueous leaf extract of *Psidium guajava Linn*



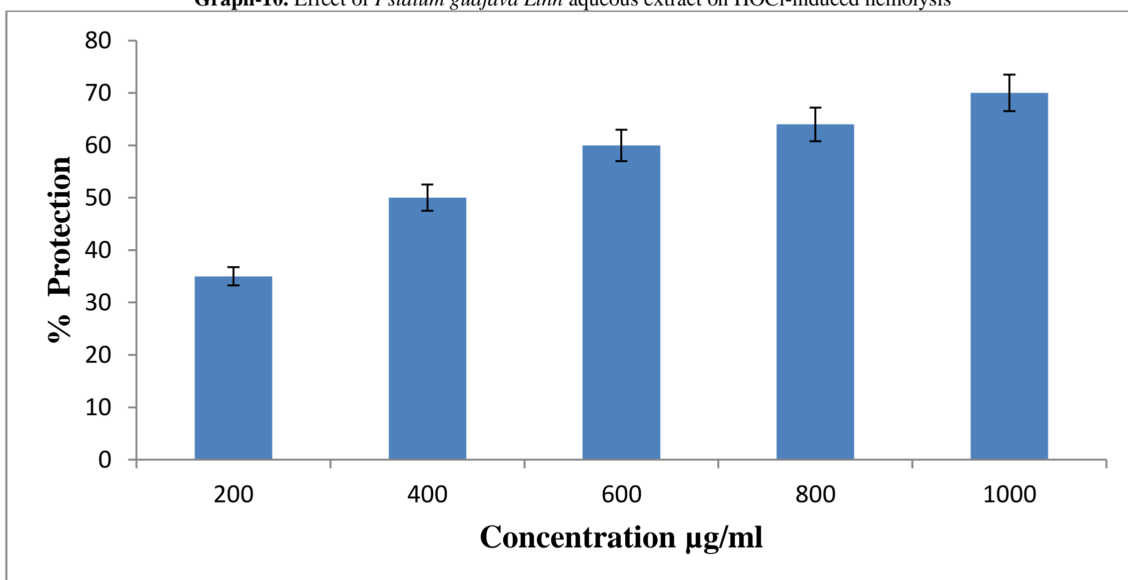
Graph-8. Effect of *Psidium guajava Linn* aqueous extract on hypotonicity-induced hemolysis



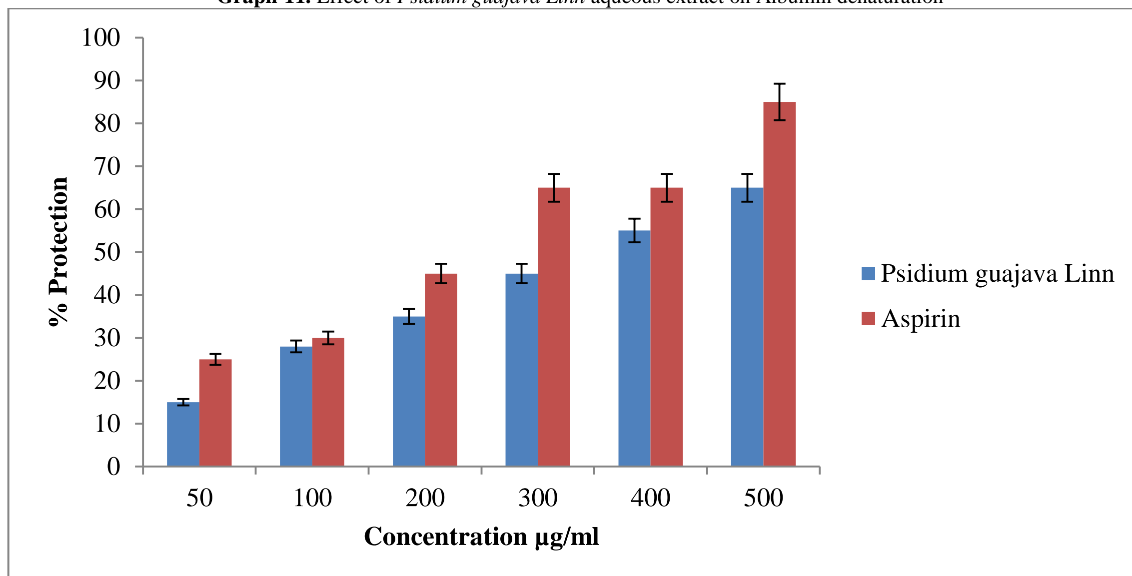
Graph-9. Effect of *Psidium guajava Linn* aqueous extract on heat-induced hemolysis



Graph-10. Effect of *Psidium guajava Linn* aqueous extract on HOCl-induced hemolysis



Graph-11. Effect of *Psidium guajava Linn* aqueous extract on Albumin denaturation



6. Summary and Conclusion

Antioxidants are substance that prevents various pathologic changes in living cells by protecting mechanism/oxidation of its major constituents (DNA, Proteins, Carbohydrates, Lipids [20]. In plant extract the antioxidant activity is performed by polyphenols and is correlated positively with their concentrations. From the obtained results, we can see a great antioxidant potential of the studied extract when compared to other species described in the literature.

So, the phenolic compounds/content of the studies extract exceed that reported [21, 22] with (170.3±1.4) mg GAE/g and with (172.21±6.29) mg GAE/g, that of various *Malva parviflora L* [23], with (1.49±0.063) mg/g, *Anacardium excelsum* with (1.49±0.03) mg/g and *Piper putumayoense* [24] with (10.20±0.03) mg/g. In the first part of our study we focused on the antioxidant activity of the aqueous extract of *P.guajava Linn*. The large amount of polyphenols, in this plant can explain the scavenging and antioxidant activity due to their loss of proton properties, chelate formation, dismutation of radicals and giving up hydrogen atoms from their hydroxyl groups with radicals to form stable phenoxyl radicals [25]. Monitored in our study by several tests, this potential was confirmed in comparison to other works too. So, DPPH scavenging activity, in comparison to other works on the aqueous extract of *P.guajava Linn* [21, 22, 26], [488.3 µg/ml, (74.8±7.4) µg/ml, and (88±8) µg/ml, our sample presented a lower IC_{50} which revealed a higher scavenging activity. The hydroxyl radical the representative reactive oxygen species generated in biological systems, can be formed from superoxide anion and hydrogen peroxide in the presence of metal ions. Considered as the most reactive free radical, hydroxyl radical is most often implicated in the pathology of free radical because of its ability to interact with intracellular targets such as DNA, thus causing significant damage. The *P.guajava Linn* extract was found to be less effective scavenger of hydroxyl radical compared to reported results [(180.160±0.015) % at 1 mg/ml for *Quisqualis indica* [27]. Hydrogen peroxide itself is not very reactive, but it can sometime be toxic to cell because it can give rise to hydroxyl radical in the cells [28, 29]. Thus, the removal of H_2O_2 is very important for antioxidant defense in cell systems or food [30]. Compared to the scavenging effect reported [31], with (26.02±1.91) % at 250 µg/ml, our study in our *P.guajava Linn* extract showed a better scavenging activity with (61.54±2.19) % at the same concentration. The ability of the extract to slow down the β-Carotene bleaching is used in the evaluation of its antioxidant activity and its ability to inhibit lipid peroxidation. The inhibition percentage for *P.guajava Linn* extract at 1 mg/ml, was significantly important compared to those reported [32], for *Labisia pumila var. alata* with (89.72±0.95) % and *Labisia pumila var. pumila* with (59.09±2.24) % at 40 mg/ml. These data lead us to believe the great biological activity of the *P.guajava Linn* extract such as anti-cancer activity, for which one of the causes of its occurrence is lipid peroxidation. *P.guajava Linn* showed a stronger chelating activity ferrous ion chelating activity (IC_{50}) compared to report data for aqueous extract of *Smilax excels* [33] with (2.56±0.07) mg/ml. This chelating potential indicated a significant protective activity of the extract against oxidative damage by sequestering iron (II) ions that may turn into catalyst for Fenton-type reactions or participate in metal-catalyzed hydroperoxide decomposition reactions [34]. Ferric reducing power is a simple test of antioxidant capacity and often used as indicator of antioxidant potential for a plant extract. In this test, electron donation leads to the neutralization of the free radical [35]. An increase in absorbance corresponds to an increase of the reducing power of the extract tested [36, 37]. *P.guajava Linn* aqueous extract was characterized by higher ferric reducing power than other data reported for the same aqueous extract of the plant [22], [IC_{50} = (288.8±16.7) µg/ml]. *P.guajava Linn* leaves extract showed greater total antioxidant capacity compared to *Pistacia lentiscus* [38], [IC_{50} = (500.0±22.3) µg/ml]. This result suggests an important electron donating ability of the extract and so a great antioxidant capacity.

We explored anti-inflammatory activity of *P.guajava Linn* extract through the study of its ability to stabilize RBCs membrane. For hypotonic solution induced hemolysis, compared to 24.5% produced by *Momordica charantia* aqueous extract [39] at 2 mg/ml, the studied extract presented stronger protection. In heat induced hemolysis, compared to *Murraya paniculata* [40], [(33.49±0.51) % at 2mg/ml], the plant extract showed greater

protection. While the protection percentage against HOCl induced hemolysis was lower than recorded for the aqueous extract of *Rhus typhina* [41], [(61.06±2.53) % at the concentration 20 µg/ml].

Our results provide evidence of anti-inflammatory activity of the *P.guajava Linn* extract which showed a good protective effect of RBCs against heat oxidant and hypotonic solution induced hemolysis. This feature can be explained by the ability of the extract to edit the calcium influx in erythrocytes [42]. Knowing that the erythrocyte membrane resembles to lysosomal membrane and as such, the effect of extracts on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane [43]. The anti-inflammatory activity can also be explained by the inhibition of release of lysosomal content at the site of inflammation [44]. During the investigation of the activity of the plant extract on albumin denaturation we observed that guava extract showed a greater protection comparatively to data observed by *Erythrina indica* [45], [(65.21±1.77) % at 800 µg/ml]. According to the fact that proteins denaturation is the cause of inflammation and rheumatoid arthritis, the protection of albumin denaturation confirms and contributes to anti-inflammatory activity of guava extract.

We demonstrated in vitro antioxidant and anti-inflammatory activities of *P.guajava Linn* aqueous leaves extract, through screening, chelating and reducing activities indicated in the performed tests, showed a good antioxidant activity. Furthermore, the protection of RBCs indicated a membrane stabilizing effect of the extract. Our results lead to the conclusion that *P.guajava Linn* aqueous extract has good antioxidant and anti-inflammatory potential.

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