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Research Article

Total Phenolic Content, *In vitro* Antioxidant and Anti-inflammatory Activities of Ethanol Extract of *Psidium guajava* Linn. Leaves

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Abstract
Oxidative stress is an imbalance between pro-oxidants and antioxidants in an organism, which is a significant factor in the pathogenesis of neurodegenerative and inflammatory diseases. The qualitative phytochemical constituents and the total phenolic concentration of ethanol extract of leaves of *Psidium guajava* (EEPG) were evaluated using standard methods, while its antioxidant activities were assessed via the phosphomolybdenum, 1,1-diphenyl-2-2-picrylhydrazyl (DPPH), and the ferric ion reducing (FRAP) methods. The qualitative phytochemical constituents and the total phenolic concentration of ethanol extract of *Psidium guajava* (EEPG) were evaluated using standard methods, while its antioxidant activities were assessed via the phosphomolybdenum, 1,1-diphenyl-2-2-picrylhydrazyl (DPPH), and the ferric ion reducing (FRAP) methods. The carrageenan-induced rat paw oedema was used to investigate the anti-inflammatory activity of EEPG (100, 200, and 400 mg/kg). Secondary metabolites such as saponins, anthraquinones, terpenoids, phenpls, flavonoids, cardiac glycosides, and alkaloids were present in EEPG. The total phenolic content of EEPG was found to be 1.292±0.093 µg/mL of garlic acid equivalent, while the total antioxidant content and ferric reducing antioxidantpower were 0.982 ± 0.01 and 2.076 ± 0.24 µg/mL of ascorbic acid equivalent, respectively. The EEPG demonstrated antioxidant activity in scavenging DPPH radicals with a maximum inhibition of 63.86% at 1000 µg/mL, while ascorbic acid had percentage inhibition of 95.6 at the lowest dose (200 µg/mL). However, the 50% inhibitory concentration (IC_{50}) of EEPG and ascorbic acid were obtained as18.68 and 580.84 µg/mL respectively. EEPG at the tested doses showed significant anti-inflammatory activity in carrageenan-induced rat paw oedema at 4 hr and 5 hr. The study revealed that EEPG exhibited both antioxidant and anti-inflammatory activities that can be further explored for drug discovery.

Key Words: *Psidium guajava*, Phytochemical constituents, *In vitro* antioxidant, Anti-inflammatory, Carrageenan

INTRODUCTION

Oxidants are produced by our body cells under healthy or pathological circumstances. These oxidants are helpful to our body in eliminating microorganisms (Beatrice *et al*., 2020). Reactive oxygen species and other free radicals originating from oxygen are occasionally produced in an uncontrolled manner (Moriasi *et al*., 2020). Reactive oxygen species (ROS) are created during regular cellular metabolism, but when they are present in large concentrations, they become toxic and have negative effects like cancer and oxidative stress (Beatrice *et al*., 2020). To prevent the creation of free radicals, the human body has a natural protective defense system (Beatrice *et al*., 2020). Antioxidant supplements are also needed by the body to counteract the generation of free radicals whose defensive mechanisms are always disrupted under various disease conditions (Vijayakumar *et al*., 2015).
In recent years, there is a serious increase in search for natural antioxidants that are both safe and effective, particularly those derived from plants. The therapeutic value of plants is ascribed to specific chemical constituents that have a clear physiological effect on the human body. Alkaloids, flavonoids, tannins, and phenolic compounds,

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nitrogen-containing compounds, carotenoids, tocopherols, or ascorbic acid and its derivatives, have the strongest antioxidant activity. As a result, researchers are eager to find antioxidant remedies from natural sources without any negative side effects (Nićiforović *et al*., 2010).

Inflammation is the body's response to irritants, invading, physical trauma organisms, also a protective effort by the body to get rid of the injurious stimuli. (Johnny *et al*., 2011). For the prevention of diseases with an underlying inflammatory process such as cancer, rheumatoid arthritis, cardiovascular disorders, or neurological diseases, plant extracts with antioxidant activities are thought to be particularly crucial (Oyinloye *et al*., 2020).

Psidum guajava is a well-known tropical tree that is widely grown for fruit, which originated in tropical South America. *Psidum guajava* is also referred to as guava, goiava, or guave (Killion, 2000). It is a member of the family Myrtaceae, the phylum Magnoliophyta, and theclass Magnoliopsida (Dakappa *et al*., 2013). Because of its edible fruit, it is now widely farmed in many tropical and subtropical nations. The therapy of numerous diseases may benefit from the usage of guava, a phytotherapeutic plant with bioactive components that is employed in folk medicine (Beatrice *et al*., 2020). Different components of the plant have historically been used to treat ailments like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothaches, cough, sore throat, swollen gums, and other disorders (Abdelrahim *et al*., 2002).

According to biological studies on *Psidium guajava* (*P. guajava*), previous studies have demonstrated that various extracts and isolated compounds from *Psidium guajava* exhibit antimicrobial, antihyperglycemic, anti-inflammatory, analgesic, antipyretic, spasmolytic, central nervous system (CNS)-depressant, analgesic, antipyretic, anticancer, antioxidant, antiallergic, anticancer, cardiovascular, hypotensive, antinociceptive, and wound healing properties (Wan Nur Zahidah *et al*., 2013).Additionally, phytochemical research conducted by earlier researchers revealed that this specie is capable of producing essential oils, tannins, phenolics, carotenoids, flavonoids, and triterpenoids (Sarah *et al*., 2012; Gayathri, and Kiruba, 2014). However, there is scanty information on the *in vitro* anti-oxidant models and in vivo anti-inflammatory activities of ethanol extract of *Psidium guajava* leaves grown in Ibadan, Oyo state, Nigeria. Therefore, in the present study we investigated total phenolic content, *in vitro* antioxidant and anti-inflammatory activities of ethanol extract of *Psidium guajava* leaves.

MATERIALS AND METHODS

Plant Material: The fresh leaves of *Psidium guajava* were collected from Zoological Garden, University of Ibadan, Ibadan, Oyo State, Nigeria. Authentication of the plant was established by Mr. Donatus A.O. of the Department of Botany, University of Ibadan, Ibadan.

Preparation of Plant Extract: The collected leaves were rinsed thoroughly and air-dried for two weeks after which it was grounded into powder. The powdered dried leaves (500 g) were soaked in mixture of 1.5 liters of 80% ethanol and 20% water at room temperature for 72 hours with occasional shaking and stirring. Fresh cotton beddings and filter paper (Whatman No. 1) were used to filter the extract in succession. The filtrate was allowed to evaporate using a rotary evaporator while under reduced pressure. The extract was then kept in a freezer at 4°C for storage.

Phytochemical screening: According to the method described by Trease and Evans (2002), the phytochemical constituents of ethanol extract of *P. guajava* leaves was tested for the presence of cardiac glycosides, alkaloids, anthraquinones, flavonoids, phenol, terpenoids, steroids, and saponins.

Total phenolic contents (TPC): The total phenolic contents of EEPG using Folin-Ciocalteu reagent were evaluated spectrophtometrically following the techniques described by Ebrahimzadeh *et al*. (2008). This is a colorimetric oxidation/reduction method for phenolic compounds. The ethanol extract of *Psidium guajava* was diluted to various concentrations (200, 400, 600, 800, and 1000 µg/mL) and 0.5 ml of each dilution was separately mixed with 5 mL of Folin-Ciocalteu reagent (to get a ratio of 1:10 dilution), and then left to stand for 5 min before being neutralized with 4 mL of 1M aqueous sodium carbonate The phenols were then measured using a colorimetric technique at 765 nm after the solution had been allowed to stand for an additional 15 min. The concentrations of phenolic compounds in the extract were expressed as mg of Gallic Acid Equivalents (GAEs) per gram of the dry extract.

Antioxidant Activities

Total antioxidant contents (TAC): According to the process previously reported by Phatak and Hendre (2004), the total antioxidant capacity of the ethanol extract of *Psidium guajava* was assessed using the phosphomolybdenum method. The assay is based on the extract's reduction of Mo (VI) to Mo (V), which is followed by the development of a green phosphate/Mo(V) complex at an acidic pH. The molybdate reagent solution was prepared by mixing 20 mL of distilled water with 1 mL each of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate and made up to 50 mL with distilled water. After being serially diluted to various concentrations (200, 400, 600, 800, and 1000 µg/mL), the ethanol extract of *Psidium guajava* was added in an equal volume to each of the five test tubes, each of which contained 3 mL of molybdate reagent solution. The test tubes with reaction solution were incubated at 95^0C for 90 min, allowed to stand at room temperature for 20-30 min and then absorbance of the reaction solution was measured at 695 nm. The antioxidant activity results were expressed as mg Ascorbic acid Equivalent (ASCE) per gram of dry extract.

Ferric ion reducing antioxidant power assay (FRAP): The FRAP technique was used to determine the total antioxidant activity of the extract. This experiment evaluates the conversion of ferric ion to ferrous form when antioxidant chemicals are present. The method described by Trease and Evans (2000) was used to determine ferric ion reducing activity of the extract. The fresh FRAP reagent was made up of 50 mL of 2, 4, 6-Tri (2- pyridyl)-s-triazin (TPTZ) (10 mM), 50 mL of FeCl3.6H2O (50 mM), and 500 mL of acetate buffer (300 Mm, pH 3. 6).For the assay, 2 mL of FRAP reagent and 75 mL of various concentrations of

ethanol extract of *Psidium guajava* (200, 400, 600, 800, and 1000 g/mL) were mixed with 2 ml of FRAP reagent. After two minutes, the optical density was measured at 593 nm against a blank.

DPPH free radical-scavenging activity evaluation

Preparation of DPPH solution: The crystalline solid of about 0.1 mM DPPH was placed in a test tube, and an organic solvent (methanol) was used to slowly dissolve it in order to create the DPPH solution. The ethanol extract, standard (ascorbic acid), and control (without the test compound but with an equivalent amount of methanol) at various concentrations (200, 400, 600, 800, and 1000 g/mL) of 3 mL each were taken in test tube (Amarawiez *et al*., 2008). To the ethanol extract,1 mL of the DPPH solution was added gradually. The concentrate solution was shaken and kept in the dark at room temperature for about 30 min and the absorbance was measured at 517 nm using a spectrophotometer. The decrease in absorbance of the substance is a measure of the free radical scavenging activity (Deguchi and Miyazaki, 2010).The percentage inhibition of DPPH radical was calculated using the formula below

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{\text{Absorbance of Control}} \times 100$$

The antioxidant activity was expressed as $IC_{50.}$ The IC_{50} value is the measure of concentration in µg/mL of extract that inhibits 50% of DPPH radicals. The value was obtained by a simple calculation from linear regression analysis.

Experimental animals: Adult Wistar rats of both sexes weighing between 200 to 250 g were procured from Animal house of the Institute for Advance Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Ibadan. They were maintained under standard laboratory conditions, and allowed free access to standard dry pellet diet and water ad libitum. The rats were acclimatized to laboratory condition for 10 days before commencement of the experiment. All experimental procedures in the present study were carried out in accordance with National Institutes of Health guidelines

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Anti-inflammatory evaluation: Carrageenan-induced rat paw oedema: Overnight fasted rats were grouped into five (Group 1-5; n = 5). Group 1 (which served as control) received normal saline (10 mL/kg body wt., p.o.) while Group 2, 3, and 4 received oral administration of EEPG at the doses of 100, 200 and 400 mg/kg body wt., respectively. Group 5 (which served as reference) were treated with diclofenac (50 mg/kg body wt., p.o.). The groups of rats after various treatments were injected with carrageenan. The right hind paw of each rat received 0.1 mL of a freshly prepared suspension of the carrageenan diluted to 1% (w/v) to induce acute inflammation. A plethysmometer (Ugo Basile, Italy) was used to measure the paw volume at 0 hour before injection of carrageenan and at 1, 2, 3, 4 and 5 hour. The difference between the paw volume at time Vx and at zero hour V0 was taken as the volume of oedema, and was considered as inflammatory response. The percentage of inhibition at 5 hour was calculated with respect to control and percentage of inhibition was expressed using the following formula: (Bhattacharya and Haldar, 2012).

$$\text{Percentage Inhibition} = \frac{(\text{Change in volume in control group} - \text{Change in volume in treated group})}{(\text{Change in volume in control group})} \times 100$$

Statistical analysis: Data were presented as mean ± standard error of mean (SEM). The IC_{50} values were determined by linear regression analysis. Data was compared using the one-way Analysis of Variance (ANOVA) followed by the Duncan multiple range test. The P value < 0.05 was considered significant.

RESULTS

Phytochemical screening: The result of the phytochemical screening of EEPG leaves are given in Table 1. The phytochemical analysis revealed moderate presence of saponins, anthraquinones, terpenoids and phenol, while the presence of flavonoids, cardiac glycosides steroids, and alkaloids were abundant.

Table 1:
Preliminary phytochemical screening of the ethanol extract of *Psidium guajava* leaves

Phyto-constituents	Qualitative data
Saponins	+
Flavonoids	++
Cardiac glycosides	++
Anthraquinones	+
Terpenoids	+
Steroids	++
Alkaloids	++
Phenol	+

+ve : Moderately present; ++ve : Abundantly present

Total Phenolic Contents: The Total Phenolic Contents of EEPG leaves in this study were expressed as garlic acid equivalents mg (GAE) per gram of dry fraction. The Total Phenolic Contents (TPC) result was concentration dependent. The highest phenolic content was found at1000 µg/mL (1.385 mg of GAE/g). The mean TPC of EEPG at various concentrations was 1.292±0.093 mg of GAE/g (Table 2).

Total antioxidant content (TAC): At different concentrations, the results showed different degrees of antioxidant activity as shown in Table 2. All concentrations showed increasing antioxidant activity with increasing concentration. However, it was shown that 1000 µg/mL possessed the highest total antioxidant content equivalent of 0.990 mg/g ascorbic acid equivalent. The mean TAC of EEPG at different concentrations was estimated to be 0.982±0.005 mg of ASCE/g.

Ferric Reducing Antioxidant Power (FRAP): Generally, all the tested concentrations of EEPG leaves exhibited remarkable concentration-dependent increase in reducing power (Table 2). The EEPG at 1000 µg/mL showed higher reducing power (2.390±1.19 mg of ASCE/g) when compared to 800 µg/mL (2.222±1.45 mg of ASCE/g), 600 µg/mL (2.054±1.03 mg of ASCE/g), 400 µg/mL (1.954±0.51 mg of ASCE/g) and 200 µg/mL (1.759±0.51 mg of ASCE/g).

Table 2:
Total Phenolic Content (TPC), Total antioxidant content (TAC) and Ferric Reducing Antioxidant Power (FRAP) of ethanol extract of *Psidium guajava* leaves

EEPG Concentration (µg/mL)	200	400	600	800	1000	Mean ±SEM
TPC (GAE)	1.192	1.198	1.317	1.371	1.385	1.292±0.093
TAC (ASAE)	0.976	0.979	0.980	0.984	0.990	0.982±0.005
FRAP (ASAE)	1.759	1.954	2.054	2.222	2.390	2.076±0.24

Table 3:
DPPH scavenging activity (% inhibition) by ascorbic acid and ethanol extract of *Psidium guajava* leaves

EEPG Concentration (µg/mL)	200	400	600	800	1000	Mean ±SEM	IC_{50}
% Inhibition of DPPH by Ascorbic acid	95.6	95.68	95.68	95.82	96.99	95.95±0.58	18.68 (µg/mL)
% Inhibition of DPPH by EEPG	44.87	46.69	59.39	63.51	63.86	55.66±9.21	580.84 (µg/mL)

Table 4:
Effect of ethanol extract of *Psidium guajava* against carrageenan-induced paw edema in rats

Treatments	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	% Inhibition at 5th hour
Normal Saline	0.23±0.02	0.30±0.06	0.36±0.01	0.96±0.09	1.72±0.02	1.95±0.07	-
EEPG 100 mg/kg	0.24±0.04	0.29±0.01	0.33±0.05	0.65±0.03	0.51±0.03*	0.48±0.01*	75.38
EEPG 200 mg/kg	0.22±0.01	0.28±0.07	0.31±0.02	0.55±0.02	0.34±0.02*	0.30±0.03*	84.62
EEPG 400 mg/kg	0.25±0.02	0.27±0.06	0.30±0.04	0.53±0.04	0.32±0.07*	0.29±0.02*	85.13
Diclofenac (50mg/kg)	0.24±0.06	0.28±0.03	0.31±0.02	0.51±0.01	0.28±0.03*	0.23±0.04*	88.21

Values are mean ± SEM (n=5) *p<0.05 compared with normal saline (control group)

Determination of free radical scavenging activity by DPPH method: The result of DPPH radical scavenging activity of different concentrations of ethanol extract of *Psidium guajava* leaves and the standard ascorbic acid is presented in Table 3. All the concentrations of EEPG leaves tested demonstrated *in vitro* DPPH radical scavenging activities in a dose-dependent manner. Likewise, the standard (ascorbic acid) exhibited increase in DPPH radical scavenging activities as the concentrations increased. The IC50 value for the EEPG leaves was 580.84 µg/mL, while that of the ascorbic acid was18.68 µg/mL (Table 3 and Figure 1).

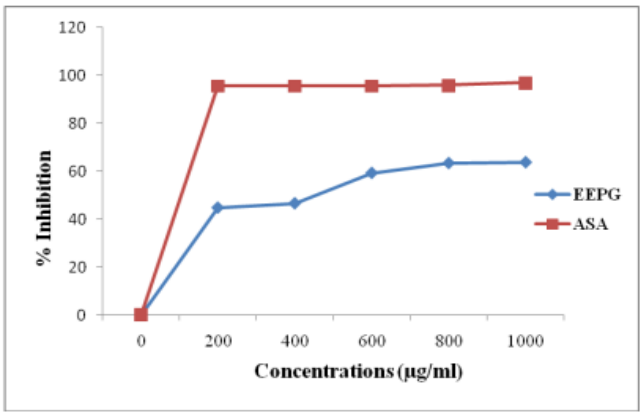

Figure 1:
Plot of DPPH radical scavenging percentage between ascorbic acid and ethanol extract of *Psidium guajava* leaves

Anti-inflammatory activity of EEPG in carrageenan-induced paw oedema produced a progressive increase in paw volume peaked at the third hour (Table 4). Groups pre-treated with EEPG (100, 200 and 400 mg/kg) showed decrease paw volume in a dose-dependent manner, with significant ($p<0.05$) reduction at the fourth and fifth hours. Likewise, diclofenac (50 mg/kg) showed significant ($p<0.05$) reduction of paw volume at the fourth and fifth hours. A dose-dependent inhibition of carrageenan-induced paw edema was observed in rats that received 400 mg/kg (85.13%), 200 mg/kg (84.62%), and 100 mg/kg (75.38%) of EEPG leaves, while that of diclofenac (50 mg/kg) gave 88.21% inhibition when compared with the control at 5 hour (Table 4).

DISCUSSION

The result of the phytochemical screening revealed the presence of saponins, anthraquinones, terpenoids, phenol, flavonoids, cardiac glycosides, steroids, and alkaloids. When compared to data from previous studies, the qualitative phytochemical analysis is in agreement with research conducted by Morais-Bragan *et al*. (2016). Many beneficial effects of the phytochemicals found in *P. guajava's* leaves have been reported previously. For instance, phenolics and flavonoids exhibit a wide range of biological activities, including wound healing, anti-inflammatory, anticancer, and antibacterial capabilities (Türkylmaz *et al*., 2013), antioxidant activity (da Gama *et al*., 2014) and antimicrobial activity (Bouaziz *et al*., 2015). On the other hand, steroids have been shown to have cardiotonic, insecticidal, and antibacterial characteristics (Iqbal and Lim, 2015), whereas saponins can be used to treat diabetes (Keller *et al*., 2011). Terpenoids have also been employed to treat human illnesses such as cancer, malaria, inflammation, and a number of infectious disorders (Mbaveng and Kuete, 2014).

Antioxidants are essential components that protect our bodies from the harms brought on by oxidative stress caused by free radicals. Numerous vital substances found in plants provide protection against oxidative stress by scavenging free radicals, inhibiting lipid peroxidation, and employing other ways (Sen *et al*., 2013). For evaluating antioxidant activity, a variety of *in vitro* evaluation approaches are taken into consideration (Mwihia, 2017; Akinmoladun *et al*., 2010). The hydroxyl scavenging activity, lipid peroxidation inhibition capacity (LPIC), oxygen radical absorbance capacity (ORAC) method, nitric oxide scavenging assay, ferric reducing antioxidant power (FRAP), and DPPH scavenging effects are some of the *in vitro*antioxidant assays employed (Akinmoladun *et al*., 2010).

The Folin-Ciocalteu technique was used to determine the amount of total phenolics in the extracts in terms of mg GAE/g. The results of this investigation showed that the ethanol extract of *P. guajava* contained significant total phenolic content. Our findings support those of Braga *et al*. (2014), who showed the antioxidant activityof *P. guajava* leaves. Free radical scavengers, hydrogen donors, and reducing agents are all functions of phenolic compounds (Beatrice *et al*., 2020). The presence of considerably good amount of phenolics in the ethanol leaf extract of *Psidium guajava* may contribute significantly to its antioxidant properties. These characteristics may be responsible for its utilization in a number of conventional herbal remedies.

The total antioxidant capabilities of plant extracts can be evaluated using a variety of assays. Phosphomolybdenum technique, which is based on the reduction of Mo (IV) to Mo (V) and the subsequent creation of green phosphate/Mo (V) complex with a maximum absorption at 695 nm, was used to assess the total antioxidant capacity of *Psidium guajava* extracts spectrophotometrically. A high absorbance value of the sample indicates its strong antioxidant capacity. This method is a quantitative one, since the antioxidant capacity is expressed as the number of equivalent of ascorbic acid (Prieto *et al*., 1999).The ethanol extract of leaves of *Psidium guajava* showed increasing antioxidant activity with increasing concentration. The findings of the study supported those of Beatrice *et al*. (2020), who claimed that the antioxidant activity of a *V. lasiopus* extract depends on the concentration of antioxidant compounds contained in the plant extract.

The ferric reducing antioxidant power (FRAP) assay was used in the current investigation. This approach is based on the analyte's capacity to convert ferric ion (Fe3+) to ferrous ion (Fe2+) (Ulçin, 2010; MacDonald-Wicks *et al*., 2006). Hence, the Fe2+ formation can be examined by absorbance capacity at 700nm (MacDonald-Wicks *et al*., 2006). Increasing absorbance at this wavelength means that the reducing power is increasing (MacDonald-Wicks *et al*., 2006). The results of this investigation showed that the absorbance values of the ethanol extract of *Psidium guajava* increased in a concentration-dependent manner, indicating a significant ferric reducing antioxidant power. Based on the result, it showed that P. guajava have an ability of transferring the Fe3+ into Fe2+and minimizes the oxidative damage in the tissues. We discovered that the result of the present study was consistent with an *in vitro* study by Vijayakumar *et al*. (2015), who demonstrated the reducing power of ethanol extract of *P. guajava* leaves in a concentration-dependent manner when compared to ascorbic acid as the standard.

In order to guard against the damaging effects of free radicals in food and biological systems, antioxidant properties, in particular, radical scavenging activities are crucial. DPPH assay is considered as a valid method to evaluate scavenging activity of antioxidants, since the compound (DPPH) is a stable free radical, unlike other radical assays. Due to the spare electron's delocalization over the entire molecule, DPPH is known as a stable free radical, since it does not dimerize like most other free radicals do (Amal *et al*., 2016).The deep violet color which is shown by an absorption band in methanol solution with a peak at about 517 nm, is likewise a result of delocalization. When a DPPH solution is mixed with one of a substance that may donate a hydrogen atom, the reduced form results, with the loss of the violet color to yellow (Amal *et al*., 2016).

The amount of DPPH that vanishes from test samples reflects the antioxidant action. When an antioxidant is present in the medium, the violet color typically dries out or disappears. As previously shown by Subba *et al*., (2018), our study revealed concentration-dependent relationship in DPPH scavenging ability. Lower IC_{50} values are connected to higher DPPH radical-scavenging action. On the other hand, the IC_{50} value (580.84 µg/mL) in the present study is comparable with previous study by Vijayakum *et al*. (2015), who reported IC50value of 590 µg/mL with the same plant extract (ethanol extract of *Psidium guajava).* The importance of phenolic compounds as free radical scavengers has been highlighted in numerous reports (Sultana *et al*., 2020). The activity is

primarily attributed to their redox properties, which are crucial in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or breaking down peroxides (Zheng and Wang, 2001).

The current work demonstrates the acute anti-inflammatory activity of *P. guajava* leaves extract in experimentally-induced acute inflammation in Wistar rats. Using irritants or phlogistic chemicals, it is easy to create an inflammatory reaction in paw oedema. When such substances as carrageenan and formalin are injected into the dorsum of the foot of rats, they quickly cause acute paw oedema. Most frequently, rat paw oedema caused by carrageenan has been employed as an excellent experimental animal model for acute inflammation (Bhattacharya *et al*., 2012). Carrageenan-induced acute inflammatory oedema is generally believed to be a biphasic response. The early phase is attributed to be caused by the release of serotonin, histamine, bradykinin, and substance P (0–1 hr) (Bhattacharya *et al*., 2011). The late phase (after one hour) is primarily brought on by neutrophil infiltration into the inflammatory site and the production of significant amount of pro-inflammatory mediators like PGE2 and different cytokines like IL-1, IL-6, IL-10, and TNF-α (Bhattacharya *et al*., 2011). The results of our study showed that ethanol extract of *P. guajava* demonstrated significant anti-edematous activity at the fourth hour, with maximum activity occurring at the fifth hour. This suggests that the anti-inflammatory activity may be mediated by inhibition of prostaglandins in the late phase of inflammation, which is consistent with earlier research by Ismail *et al*. (2016), who reported maximum anti-inflammatory activity of Boswellia serrata on carrageenan-induced paw oedema in Wistar rats.

CONCLUSION

The ethanol extract of leaves of *Psidium guajava* possess antioxidant and anti-inflammatory activities. The pharmacological activities of this plant might be as a result of the phytochemicals present in the plant. Future studies include the isolation and characterization of the actual bioactive compounds, and determination of the possible mechanisms of action of compounds.

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