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Inhibition of lipid peroxidation and free radical scavenging activities of methanolic leaf extract of *Psidium guajava*

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Abstract

Objectives: Studies have linked the generation of free radicals with the incidence of degenerative diseases. Antioxidants from plant origin have been proved to play a major role in mitigating against free radicals-induced oxidative damage. This study aimed at assessing the *in-vitro* and *in-vivo* antioxidant capability of *Psidium guajava* leaf

Methods: The leaves were collected and extracted with 70% methanol. Total phenolic, and flavonoids contents, 2,2-diphenyl-1-picrylhydrazyl and Hydroxyl radicals scavenging activities, and inhibition of lipid peroxidation potential of the extract were assessed. Furthermore, rats (n=21) randomized into three groups were exposed to 50, 150, and 250 mg/kg body weight of the extract for 30 days. Control animals (n=7) received corn oil, after which blood and liver were excised for antioxidant assay.

Results: The extract is rich in phenolic and flavonoid compounds. It scavenged DPPH and hydroxyl radicals and inhibits lipid peroxidation *in-vitro*. *In-vivo*, it increased the activities of hepatic superoxide dismutase, catalase, glutathione peroxidase, and plasma paraoxonase, and the concentration of hepatic reduced glutathione and MDA.

Conclusion: *Psidium guajava* leaf extract is a potential source of natural antioxidant compounds, capable of supplementing the body's antioxidant defense system.

Keywords: *Psidium guajava*, antioxidant, phenol, flavonoids, paraoxonase

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Inhibition des activités de peroxydation lipidique et de piégeage des radicaux libres de l'extrait méthanolique de feuilles de *Psidium guajava*

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Résumé

Objectif de l'étude: Des études ont établi un lien entre la génération de radicaux libres et l'incidence de maladies dégénératives. Il a été prouvé que les antioxydants d'origine végétale jouent un rôle majeur dans l'atténuation des dommages oxydatifs induits par les radicaux libres. Cette étude visait à évaluer la capacité antioxydante *in-vitro* et *in-vivo de* la feuille de *Psidium guajava*

Méthode de l'étude: Les feuilles ont été recueillies et extraites avec du méthanol à 70 %. Les teneurs totales en composés phénoliques et en flavonoïdes, les activités de piégeage des radicaux 2,2-diphényl-1-picrylhydrazyle et hydroxyle et l'inhibition du potentiel de peroxydation lipidique de l'extrait ont été évaluées. De plus, des rats (n = 21) randomisés en trois groupes ont été exposés à 50, 150 et 250 mg/kg de poids corporel de l'extrait pendant 30 jours. Les animaux témoins (n = 7) ont reçu de l'huile de maïs, après quoi le sang et le foie ont été excisés pour un dosage antioxydant.

Résultat de l'étude: L'extrait est riche en composés phénoliques et flavonoïdes. Il a piégé les radicaux DPPH et hydroxyle et inhibe la peroxydation des lipides *in vitro. In vivo,* il a augmenté les activités du superoxyde dismutase hépatique, de la catalase, de la glutathion peroxydase et de la paronomase plasmatique, ainsi que la concentration de glutathion hépatique réduit et de MDA.

Conclusion: L'extrait de feuille de *psidium guajava* est une source potentielle de composés antioxydants naturels, capables de compléter le système de défense antioxydant de l'organisme.

Mots-clés : *Psidium guajava,* antioxydant, phénol, flavonoïdes, paraoxonase

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INTRODUCTION

Plants remain one of the major sources of several naturally occurring bioactive compounds (1). In traditional medicine, plant preparations have been used to cure a wide array of diseases (2). At present, phytochemicals and their products are increasingly attracting the interest of manufacturing industries such as the pharmaceutical, nutraceutical, and cosmetics industries. Although they are usually considered as a waste of no agricultural importance, leaves accumulate a large quantity of plant secondary metabolites and are therefore very rich in phytochemicals of medicinal importance (1, 3).

The *Psidium guajava* commonly known as guava is one such plant that is used traditionally for curative purposes as a result of its various medicinal properties. Data obtained from traditional medical practitioners revealed that different parts of *Psidium guajava L* have been used globally in the treatment of several diseased conditions such as diabetes, stomachache, diarrhea among other conditions. *Psidium guajava* leaves are dark green, oval, elliptical, with a characteristic obtuse-type apex. Furthermore, data from the previous investigation showed that the leaf can be used in the management of gastrointestinal and respiratory abnormalities. It had also been shown to be efficient in the treatment of dengue fever (4). Various authors have also reported anti-inflammatory, antispasmodic, anti-diarrheic, cough sedative, anti-obesity, anti-diabetic, anti-hypertension (5), antitumor, anticancer, and cytotoxic (6) properties of *Psidium guajava* leaf.

These multiple health benefits of *Psidium guajava* leaves had been attributed to the presence of a plethora of phytochemicals in the leaves extract. These phytochemicals include avicularin, quercetin, guaijaverin, apigenin, kaempferol, myricetin, hyperin, gallic acid, epicatechin catechin, chlorogenic acid, caffeic acid, ferulic acid, and epigallocatechin gallate (7). All these secondary metabolites have been proposed to possess potent immunostimulant and antioxidant potentials (8).

Oxygen plays an important role in aerobic respiration as it is the final acceptor of electrons during the process of electron transport chain (oxidative phosphorylation) in the inner mitochondria membrane, a key source of cellular ATP production. Meanwhile, during this process of oxidative phosphorylation, free radicals are produced as a result of partial reduction of the molecular oxygen. This can lead to the incidence of oxidative stress when there is an imbalance between the rate at which free radicals are produced and the rate at which antioxidants are converting them to less deleterious compounds. Meanwhile, oxidative stress had been linked to the pathogenesis of a plethora of diseases such as ischemic diseases, inflammatory diseases, neurological disorders, emphysema, hemochromatosis, acquired immunodeficiency syndrome, among others (1, 8). This study was carried out to investigate the antioxidant activity of *Psidium guajava* leaf methanolic extract.

MATERIALS AND METHOD

Chemicals and Reagents

Ferrous sulfate, acetic acid, thiobarbituric acid (TBA), sodium dodecyl sulfate (SDS), 1,1- diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide, potassium ferricyanide, trichloroacetic acid (TCA), ferrous chloride, sodium acetate, Folinn-Ciocalteu reagent, sodium carbonate, gallic acid, ascorbic acid and all other chemicals used for the other assays were of pure grade and were gotten from Sigma-Aldrich, Missouri, U. S. A. and British Drug House (BDH) Chemicals Limited, Poole, England. The diagnostic kit for the determination of superoxide dismutase activity is the product of Fortress Diagnostic Laboratory, Unit 2C, Antrim Technology Park, Belfast Road, Antrim, Northern Ireland, United Kingdom.

Plants Material and Plant Extract Preparation

The *Psidium guajava* leaves were harvested around the premises of the Old Biochemistry Department, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria, and were authenticated by Prof A. T. J. Ogunkunle of the Department of Pure and Applied Biology LAUTECH, Ogbomoso, Nigeria. Fresh healthy leaves of *psidium guajava* were air-dried at room temperature for two weeks and powdered. Thereafter, 10 grams of the powdered leaves were exhaustively extracted in 100 ml of methanol at room temperature (about 27°C) for 72 hours. The methanolic filtrate was then concentrated with the aid of a rotary evaporator at 60°C to obtain the methanolic extract of *Psidium guajava* leaves

In-vitro study

Total phenols: The total phenolic content of the leaf extract was determined using the method of Mc-Donald *et al*. (9), which measures the ability of the phenolic compounds to reduce the Folin Ciocalteu. Gallic acid was used as standard and the absorbance was read at 760 nm.

Total flavonoids: The total flavonoids contents of the extract were determined spectrophotometrically at 415 nm based on the ability of Aluminium chloride to form complexes with flavonoids as described by Chang *et al*. (10) using quercetin as the standard

Antioxidants activity

Percentage 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities: Percentage DPPH free radical scavenging activity was evaluated following the method of Mensor *et al*. (11) using ascorbic acid as the standard. In this method, when DPPH reacts with an antioxidant compound that is capable of donating hydrogen, the DPPH is reduced with a concomitant change in color from deep violet to golden/light yellow the absorbance of which can be measured at 518 nm.

Percentage hydroxyl radical scavenging activities: The percentage hydroxyl radicals scavenging activity of the plant extract was determined using iron/EDTA/H_2O_2 complex following the procedure described by Aruoma and Halliwell (12). In this method, hydroxyl radical generated by the reaction of an iron-EDTA complex with H_2O_2 in the presence of ascorbic acid, attack the deoxyribose to form products that upon heating with Thiobarbituric acid at low pH will produce a pink chromogen. Added hydroxy radicals 'scavengers compete for the hydroxyl radicals leading to diminished chromogen formation.

Percentage inhibition of lipid peroxidation: The percentage inhibition of ferrous sulfate-induced lipid peroxidation of the plant extract was evaluated by a modified thiobarbituric acid reactive substances (TBARS) assay to determine the amount of lipid peroxide formed using egg yolk as a lipid-rich medium as described by Ruberto *et al. (*13).

Experimental Animals: Twenty-eight (28) adult male Wistar rats (130–150 g), aged about 10 weeks were gotten from The Animal House, Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria, and were kept in compartmentalized cages and grouped into four, comprised of seven animals each in a well-ventilated room under controlled laboratory settings of temperature (25 ± 2°C) and standard light-dark cycle (12 h light/dark). The rats had unrestricted access to rat pelletized feed and water *ad libitum*. Experimental plan and animal handling were done according to the guiding principle permitted by the Experimental Animals Ethics Committee of Biochemistry Department, LAUTECH, Ogbomoso, Nigeria (LAU/BCH/20/0028), which is in total agreement with the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health. The rats were allowed to acclimatize for two weeks, during which they were properly observed for any changes in their behavior.

At the expiration of the two weeks acclimation period, the group I animals (control) received 1 ml/kg body weight (bwt) of corn oil orally. Group II animals were orally administered 50 mg/kg bwt of the plant extract dissolved in corn oil daily for 30 days. Animals in groups III and IV orally received 150 and 250 mg/kg bwt of the extract in corn oil daily for 30 days respectively. The administrations were done through oral intubation, between 7 and 8 am each day.

Twenty-four (24) hours after the last treatment, the rats were anesthetized using diethyl ether after an overnight fast, and the blood was collected into heparinized tubes via cardiac puncture. The blood samples were centrifuged at 5000 rpm for 10 minutes to obtain the plasma. The livers were excised from the animals and processed for biochemical analyses.

In-vivo study

Preparation of liver homogenate: A 10% liver homogenate was prepared by homogenizing 1 g of the liver in 4 ml of 10 mM potassium phosphate buffer (pH 7.4) with 6 up and down strokes at 1100 rpm using a power-driven, Teflon pestle in a glass homogenizing cup maintained at 4°C. The homogenate was then centrifuged at 10 rpm, for 15 minutes in a cold centrifuge at 4°C, and the resultant supernatant was used for the biochemical assays.

Determination of hepatic superoxide dismutase (SOD) activity: SOD was estimated using Fortress diagnostic kit which employed the Arthur method (14). Briefly, xanthine and xanthine oxidase are used to generate free radicals which in turn react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyl tetrazolium chloride (I.N.T) to form a red formazan dye. One unit of SOD is that which causes a 50% inhibition of the rate of reduction of I.N.T under the conditions of the assay.

Determination of hepatic catalase (CAT) activity*:* The activity of catalase (CAT) was measured by the method of Aebi (15). In brief, the assay mixture maintained at 20°C consisted of 0.1 ml of supernatant tissue homogenate, 1.9 ml of 50 mM phosphate buffer, and 1 ml of 30 mM H_2O_2. The decomposition of H_2O_2 was

continuously monitored spectrophotometrically at 240 nm for 60 s. The change in absorbance was the measure of CAT activity and was expressed as unit/mg protein.

Glutathione peroxidase (GPx) activity: GPx activity was determined using H_2O_2 as a substrate in the presence of reduced GSH, according to the method of Rotruck *et al*. (16). The reaction medium contained 0.2 mL of phosphate buffer (0.4 M), 0.1 mL of sodium azide (10 mM), 0.2ml of tissue homogenate and 0.2 mL of GSH. The reaction was initiated by adding 0.2 mM H_2O_2 to the mixture. The GSH content was quantified using Ellman's reagent. The activity was expressed as unit per milligram protein where a unit is mmol of GSH consumed per minute.

Reduced glutathione determination (GSH)*:* Reduced glutathione (GSH) in the liver was determined using the method of Moron *et al*. (17). The supernatant (1 mL) was treated with 0.5 mL Ellman's reagent in 0.1% sodium citrate. After that, phosphate buffer (2 mL) and 0.5 mL of DTNB were added before the absorbance was read at 412 nm.

Determination of hepatic malonaldehyde (MDA) concentration*.:* The level of lipid peroxidation was quantified by MDA content in the liver. Hepatic MDA was determined using the thiobarbituric acid reactive substance assay, as described by Buege and Aust (18) with slight modifications. Briefly, 10% of testis homogenate was prepared in 0.15-M KCl. One volume of homogenate was mixed thoroughly with two volumes of a stock solution of 15% w/v trichloroacetic acid, 0.375% w/v thiobarbituric acid, and 0.25 M hydrochloric acid. The mixture was heated for 15 min in a boiling water bath. After cooling, the mixture was centrifuged at 1000 x g for 10 min to remove the precipitate. The absorbance of the clear supernatant was determined at 535 nm and MDA concentration was calculated using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}, The MDA concentration was expressed as nmol per gram protein.

Determination of plasma paraoxonase activity: Paraoxonase (PON1) enzyme activity was measured against paraoxon (substrate). PON1 activity towards paraoxon (PONase) was evaluated using the method of Furlong and co-workers (19). Briefly, paraoxon (1.2 mM) freshly prepared in 50 mM Tris-HCl buffer pH 8.5 containing 1.32 mM $CaCl_2$ was incubated at 37°C with appropriate volumes of plasma. The detection of released p-nitrophenol following enzymatic hydrolysis of paraoxon was measured spectrophotometrically at 405 nm. A molar extinction coefficient of 18,050 $M^{-1}cm^{-1}$ was used to estimate enzyme activity.

Statistical analysis

Results are expressed as mean ± SD. The level of homogeneity among the groups was assessed using analysis of variance (ANOVA). Where heterogeneity occurred, the groups were separated using Tukey's test. All analyses were done using GraphPad Prism® version 5.

RESULTS

Total phenolic contents of methanol extract of *Psidium guajava* leaf

The result for the total phenolic contents of methanol extract of *Psidium guajava* leaf is as presented in Table 1. The result showed that the concentration of total phenol in the extract increased in a concentration-dependent manner. For instance, the total phenolic content of the extract ranged from 0.286 ± 0.083 µg/mg GAE at 50 µg/mL of the extract, to 0.417 ± 0.089 (100 µg/mL), 0.627 ± 0.032 (150 µg/mL), and 0.748 ± 0.063 (200 µg/mL).

Total flavonoid contents of methanol extract of *Psidium guajava* leaf

Table 2 depicts the flavonoid content of the methanol extract of *Psidium guajava* leaf expressed as mg/g QE. As depicted, the flavonoids contents of the extract increase as the concentration of the extract increases. The extract when tested at 50, 100, 150, and 200 µg/mL revealed that the extract contained 0.174 ± 0.014, 0.215 ± 0.011, 0.296 ± 0.004, and 0.420 ± 0.019 mg/g QE total flavonoids respectively.

Percentage DPPH radical scavenging activities of methanol extract of *Psidium guajava* leaf

The percentage DPPH radical scavenging activity of methanol leaf extract of *Psidium guajava* is presented in Table 3. As shown, the methanol extract of the leaf scavenged DPPH radical in a concentration-dependent fashion. For instance, at 50, 100, 150, and 200 µg/mL, the extract scavenged 17.79%, 30.17%, 56.8%, and 61.44% of the total DPPH free radical.

Percentage hydroxyl radical scavenging activities of methanol extract of *Psidium guajava* leaf

The percentage hydroxyl radical scavenging activity of the methanol extract of *Psidium guajava* leaf is shown in Table 4. At the

various concentration of the extract tested, the plant extract scavenged the hydroxyl radical in a concentration-dependent manner. Specifically, the extract scavenged 16.41%, 28.69%, 46.29%, and 59.67% of the hydroxyl radical at 50, 100, 150, and 200 µg/mL respectively.

Percentage inhibition of lipid peroxidation of methanol extract of *Psidium guajava* leaf

The ability of the methanol leaf extract of *Psidium guajava* to inhibit LPO is presented in Table 5. As shown in the table, the potentials at which the extract can inhibit LPO increase with the increasing concentration of the *Psidium guajava* leaf extract. More specifically, the extract at 50, 100, 150, and 200 µg/mL showed 37.01%, 51.86%, 63.15% and 69.94% inhibition of LPO capability respectively.

Hepatic superoxide dismutase activity of animals exposed to methanol extract of *Psidium guajava* leaf

The hepatic SOD activity of the animal exposed to various concentrations of *Psidium guajava* methanolic leaf extract is presented in Fig. 1. The administration of the extract led to a significant ($p < 0.05$) enhancement of the activity of the SOD in the liver of the treated animals compared with the control animals. At 50 mg/kg bwt, the extract caused about a 2-fold increase in the activity of the enzyme. A subsequent increase in the concentration of the extract to 150 and 250 mg/kg bwt, led to about 1.5, and a 3-fold increase in the activity of the enzyme respectively, when compared with the control group.

Hepatic catalase activity of animals exposed to methanol extract of *Psidium guajava* leaf

As illustrated in Fig. 2, methanol extract of *Psidium guajava* leaf up-regulated the activity of catalase significantly ($p < 0.05$) in the hepatocytes of the animals that were exposed to the extract. As shown (Fig. 2), the administration of the extract elicited a hormetic response in the activity of the antioxidant enzyme compare with the control group. The lowest dose (50 mg/kg bwt) increased the activity of the enzyme from 8.72 ± 1.10 unit/mg protein (control) to 12.23 ± 0.62 unit/mg protein. Furthermore, the other two doses (150 and 250 mg/kg bwt) increased the activity to 11.17 ± 0.32 and 10.41 ± 0.25 unit/mg protein respectively.

Hepatic glutathione peroxidase activity of animals exposed to methanol extract of *Psidium guajava* leaf

As illustrated in Fig. 3, the administration of methanol extract of *Psidium guajava* leaf caused a dose-dependent significant ($p < 0.05$) increase in the activity of GPx in the liver of the treated rats. As shown, the administration of 50, 150, and 250 mg/kg bwt of the extract resulted in about 54%, 80%, and 100% increase in the activity of the enzyme respectively compared with the animals in the control group.

Hepatic reduced glutathione concentration of animals exposed to methanol extract of *Psidium guajava* leaf

The effects of methanol extract of *Psidium guajava* leaf on the concentration of hepatic GSH is represented by Fig. 4. As presented, the administration of the extract resulted in a steady but significant ($p < 0.05$) increase in the concentration of GSH in the hepatocytes in a dose-dependent fashion. For instance, the extract increased the hepatic GSH content from 1.96 ±0.15 (control) to 2.43 ± 0.29 (50 mg/kg bwt), 2.52 ± 0.25 (150 mg/kg bwt) and 2.71 ± 0.20 (250 mg/kg bwt).

Hepatic MDA concentration of animals exposed to methanol extract of *Psidium guajava* leaf

The concentration of MDA in the liver of the Wistar rats treated with methanol extract of *Psidium guajava* leaf is shown in Fig. 5. As illustrated, the extract significantly ($p < 0.05$) reduced the MDA concentration in the hepatocytes of the animals that received various doses of the extract. When compared with the control, the extract reduced the hepatic MDA content by 11% (50 mg/kg bwt), 14% (150 mg/kg bwt) and 23% (250 mg/kg bwt).

Plasma paraoxonase 1 activity of animals exposed to methanol extract of *Psidium guajava* leaf

The activity of PON 1 in the plasma of the animals that received 50, 150, and 250 mg/kg bwt of methanol extract of *Psidium guajava* leaf significantly ($p < 0.05$) increased with increasing doses of the extract (Fig. 6). As illustrated, the extract significantly ($p < 0.05$) increased the activity of the enzyme from 138.8 ± 9.99 in the control group to 183.0 ± 3.54 (50 mg/kg bwt), 198.8 ± 6.50 (150 mg/kg bwt), and 218.8 ± 7.19 (250 mg/kg bwt).

DISCUSSION

This study investigated the antioxidant activity of *Psidium guajava* leaf methanolic extract. Data from the present study revealed that *Psidium guajava* leaf extract is rich in phenolic, and flavonoid compounds. It also effectively scavenged free radicals and inhibits lipid peroxidation. Furthermore, it increased the activities of SOD, catalase, GPx, and PON1. It also increases the concentration of GSH as well as reduced the concentration of MDA in the liver.

DPPH (2,2-diphenyl-1-picrylhydrazyl) is an organic chemical compound that can be used to monitor chemical reactions involving radicals, most notably it is a common antioxidant assay (20), Hydroxyl radicals are one of the most powerful oxidizing agents able to react unselectively and instantaneously with the surrounding chemicals including biological systems where they play an important role in immunity and metabolism (21), while Inhibition of lipid peroxidation is a mechanism through which antioxidants block the process of lipid peroxidation and prevent further degradation into more active oxidizing forms (22).

At different concentrations (50 mg/kg, 100 mg/kg, 150 mg/kg, 200 mg/kg), the *Psidium guajava* leaf methanolic extract was able to scavenge DPPH radical at 21.32%, 28.91%, 48.67% and 53.28% respectively. With an increase in the concentration of the *Psidium guajava* leaf methanolic extract, a significant ($p < 0.05$) increase in DPPH radical scavenging activity was observed. Similarly, hydroxyl radicals scavenging activity of *Psidium guajava* leaf methanolic extract increased with a progression of 2.38%, 21.86%, 37.61%, and 41.84% relative to the concentration used. Percentage inhibition of lipid peroxidation also increased with an increase in concentration. Inhibition of LPO by the *Psidium guajava* leaf methanolic extract increased from 52.96% at 50 mg/kg, 60.18% at 100 mg/kg, and 82.67% at 150 mg/kg to 89.28% at 200 mg/kg. Thus, *Psidium guajava* leaf methanolic extract has great antioxidant potential.

Superoxide dismutase (SOD) is an enzymatic biomarker of oxidative stress that alternately catalyzes the dismutation of the superoxide (O_2^-) radical into ordinary molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). Superoxide is produced as a by-product of oxygen metabolism and if not regulated, causes many types of cellular damage e.g. oxidative stress (**23**). A decrease in the activity of SOD makes the system prone to oxidative damage. However, hepatic SOD activity was upregulated at all concentrations of *Psidium guajava* leaf methanolic extract relative to control. SOD activity was at its peak at 250 mg/kg, 250 mg/kg > 50 mg/kg > 150 mg/kg.

Furthermore, catalase (CAT) is an enzymatic biomarker of oxidative stress that catalyzes the decomposition of hydrogen peroxide (H_2O_2) to water (H_2O) and oxygen (O_2) and through this reaction, protects the cell from oxidative damage by reactive oxygen species (ROS) (**24**). The upregulation of catalase activity was observed at all concentrations of *Psidium guajava* leaf methanolic extract when compared to control. Thus, this extract confers protection against oxidative damage.

Glutathione peroxidase (GPx) is an enzyme that catalyzes the decomposition of hydrogen peroxide or either peroxide with the simultaneous oxidation of GSH to glutathione disulfide (GSSG) (25). Hepatic GPx activity significantly increased at all concentrations of *Psidium guajava* leaf methanolic extract relative to control with the peak of the enzyme observed at 250 mg/kg.

Reduced glutathione (GSH) is a non-enzymatic biomarker of oxidative stress capable of preventing damage to important cellular components caused by reactive oxygen species (ROS). This prevention of oxidative stress is done by neutralizing the reactive oxygen species and free radicals (**26**). It was observed that at all concentrations of *Psidium guajava* leaf methanolic extract, the concentration of GSH was up-regulated.

Malondialdehyde (MDA), a by-product of lipid peroxidation is a biomarker of oxidative stress. Lipid peroxidation is a chain reaction of oxidative degradation of lipids. (27). Data from this study demonstrated that at a varying concentration of *Psidium guajava* leaf methanolic extract (50 mg/kg, 100 mg/kg, 150 mg/kg, 200 mg/kg), the extract can decrease the process of lipid peroxidation. Malondialdehyde levels decreased with an increase in the concentration of the extract.

Paraoxonase 1 (PON1) is a hydrolytic enzyme with a wide range of substrates, and the competence to guard against lipid oxidation. Originally, paraoxonase was discovered as an enzyme required for the hydrolysis of exogenous poisonous organophosphate compounds such as insecticide paraoxon. The three members of the paraoxonases family that are known are Paraoxonase 1 (PON1), Paraoxonase 2 (PON2), and Paraoxonase 3 (PON3) (28). PON1 is

regarded as a protein that is responsible for most of the antioxidant properties of High-density lipoprotein (HDL) (29). Purified PON1 protects HDL and low-density lipoprotein (LDL) from oxidation catalyzed by copper ions, inhibits copper-induced HDL oxidation by prolonging oxidation lag phase, ***and reduces peroxide and aldehyde content in oxidized HDL (30).*** The activity of Plasma paraoxonase 1 was up-regulated at all concentrations of *Psidium guajava* leaf methanolic extract. The trend of increased activity is given as, 50 mg/kg > 150 mg/kg > 250 mg/kg.

CONCLUSION

Our findings from this study revealed that the methanolic extract of *Psidium guajava* leaf has the potential to serve as an antioxidant. Isolation and characterization of the active compounds responsible for the observed antioxidant activities will further assist in the possible utilization of the plant in the prevention and management of free radicals-induced disorders.

Conflict of interest: The authors declare no conflict of interest.

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Table 1: Total phenolic contents of methanol extract of *Psidium guajava* leaf expressed as µg/mg gallic acid equivalent (GAE)

Concentration of the extract (µg/mL)	Total phenolic contents (µg/mg GAE)
50	0.286 ± 0.043
100	0.417 ± 0.089
150	0.627 ± 0.032
200	0.748 ± 0.063

Values are expressed as the mean of triplicate measurements ± standard error of the mean (SD)

Table 2: Total flavonoids contents of methanol extract of *Psidium guajava* leaf expressed as mg/g ± SD Quercetin equivalent (QE)

Concentration of the extract (µg/mL)	Total flavonoids contents (mg/g QE)
50	0.174 ± 0.014
100	0.215 ± 0.011
150	0.296 ± 0.004
200	0.420 ± 0.019

Values are expressed as the mean of triplicate measurements ± standard error of the mean (SD)

Table 3: Percentage DPPH radical scavenging activities of methanol extract of *Psidium guajava* leaf

Concentration of the extract (µg/mL)	Percentage DPPH radical scavenging activities
50	19.79%
100	30.17%
150	56.80%
200	61.44%

Values are expressed as the mean of triplicate measurements ± standard error of the mean (SD)

Table 4: Percentage hydroxyl radical scavenging activities of methanol extract of *Psidium guajava* leaf

Concentration of the extract (µg/mL)	Percentage hydroxyl radical scavenging activities
50	16.41%
100	28.69%
150	46.29%
200	59.67%

Values are expressed as the mean of triplicate measurements ± standard error of the mean (SD)

Table 5: Percentage inhibition of lipid peroxidation of methanol extract of *Psidium guajava* leaf

Concentration of the extract (µg/mL)	Percentage inhibition of lipid peroxidation
50	37.01%
100	51.86%
150	63.15%
200	69.94%

Values are expressed as the mean of triplicate measurements ± standard error of the mean (SD)

Figure 1: Hepatic superoxide dismutase activity of animals exposed to methanol extract of *Psidium guajava* leaf. Each value is mean ± SD of five animals in each group. Bars with different alphabets are significantly different at $p < 0.05$ across the groups.

Figure 2: Hepatic catalase activity of animals exposed to methanol extract of *Psidium guajava* leaf. Each value is mean ± SD of five animals in each group. Bars with different alphabets are significantly different at $p < 0.05$ across the groups.

Figure 3: Hepatic glutathione peroxidase activit exposed to methanol extract of *Psidium guajav* value is mean ± SD of five animals in each group Bars with different alphabets are significantly dif *0.05* across the groups

Figure 6: Plasma paraoxonase 1 activity of anin to methanol extract of *Psidium guajava* leaf. E mean ± SD of five animals in each group. Bars with different alphabets are significantly dif *0.05* across the group

Figure 5: Hepatic MDA concentration of animals exposed to methanol extract of *Psidium guajava* leaf k. Each value is mean ± SD of five animals in each group. Bars with different alphabets are significantly different at $p < 0.05$ across the groups

Figure 4: Hepatic reduced glutathione concentration of animals exposed to methanol extract of *Psidium guajava* leaf. Each value is mean ± SD of five animals in each group. Bars with different alphabets are significantly different at $p < 0.05$ across the groups