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Original Research Article

In-vitro Antioxidant and Antidiabetic Activities and *Ex-vivo* Hepatic Effects of *Psidium guajava* and *Ocimum gratissimum* Methanol Leaf Extracts

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ABSTRACT

Diabetes mellitus is a metabolic disorder characterized by impaired glucose regulation and associated oxidative stress, creating a need to explore plant-derived compounds with potential antioxidant and antidiabetic properties. This study evaluated the in-vitro antioxidant and antidiabetic activities of methanol leaf extracts of *Psidium guajava* and *Ocimum gratissimum* and their ex-vivo hepatic effects. Antioxidant activity was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, total antioxidant capacity (TAC), reducing power, and hydroxyl radical scavenging assays. α-Amylase and α-glucosidase inhibition were also determined for the invitro antidiabetic activities. Ex-vivo hepatic effects were assessed at 200, 400, and 600 µg/mL in a high-glucose Krebs–Henseleit buffer model, using metformin as a reference treatment. Calculated IC_{50} values for *O. gratissimum* and *P. guajava*, respectively, were 152.94 and 200.00 µg/mL for DPPH scavenging, 686.96 and 847.62 µg/mL for TAC, and 428.57 and 930.20 µg/mL for hydroxyl radical scavenging. For α-amylase inhibition, the corresponding values were 843.34 and 411.32 µg/mL, while those for α-glucosidase inhibition were 553.85 and 486.79 µg/mL. DPPH scavenging reached 86% and 81%, respectively, while reducing power reached 75% and 74%. Maximum α-amylase inhibition was 50% for *O. gratissimum* and 89% for *P. guajava*, whereas α-glucosidase inhibition reached 79% and 84%, respectively. Both extracts reduced hepatic glucose and malondialdehyde concentrations and significantly increase superoxide dismutase, catalase, and reduced glutathione activities. At 600 µg/mL, the activities of these enzymes and concentration of reduced glutathione were compared favourably with metformin. The findings demonstrate antioxidant and carbohydrate-digestive enzyme inhibitory activities, supporting further investigation of both extracts.

Keywords: Psidium guajava, Ocimum gratissimum, Antioxidant activity, α-glucosidase inhibition, Oxidative stress.

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Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycaemia resulting from inadequate insulin secretion, impaired insulin action, or a combination of both. Insulin is essential for the regulation of blood glucose by facilitating the uptake and utilization of glucose by tissues [1]. When insulin production or action is impaired, glucose accumulates in the circulation, leading to prolonged hyperglycaemia and progressive dysfunction of several organs and tissues [2]. The major forms of diabetes mellitus include type 1 diabetes mellitus, type 2 diabetes mellitus and gestational diabetes mellitus, with type 2 diabetes mellitus accounting for the majority of cases worldwide [3]. Diabetes has become a major global health concern because persistent hyperglycaemia is associated with complications affecting the cardiovascular system, kidneys, eyes, nervous system and other organs.

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The increasing burden of diabetes, particularly in low- and middle-income countries, has consequently intensified the search for effective strategies for its prevention and management.

Oxidative stress is an important biochemical feature associated with diabetes mellitus [4]. It occurs when the generation of reactive oxygen species and other reactive molecules exceeds the capacity of endogenous antioxidant defense systems to neutralize them. Persistent hyperglycaemia can promote oxidative stress through several mechanisms, including increased glucose oxidation, enhanced formation of reactive oxygen species and non-enzymatic glycation of proteins[5]. Excessive reactive oxygen species can subsequently interact with cellular lipids, proteins and nucleic acids, thereby disrupting normal cellular structure and function. Oxidative stress has therefore been implicated in the development and progression of metabolic disturbances and diabetic complications [4].

The antioxidant defense system consists of enzymatic and non-enzymatic components that work together to maintain cellular redox balance. Important enzymatic antioxidants include superoxide dismutase (SOD) and catalase, while glutathione constitutes an important component of the non-enzymatic antioxidant system [6]. Superoxide dismutase converts superoxide radicals into hydrogen peroxide, which is subsequently detoxified by catalase and other antioxidant enzymes. Glutathione also participates in the removal of reactive oxygen species and helps maintain the intracellular reducing environment [7]. When oxidative stress becomes excessive, membrane lipids may undergo peroxidation, resulting in the formation of products such as malondialdehyde (MDA)[8]. Assessment of antioxidant and oxidative stress markers such as SOD, catalase, glutathione and MDA can therefore provide useful information on alterations in cellular redox status associated with metabolic disorders [9].

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The liver is particularly important in glucose and lipid homeostasis and consequently plays a central role in the metabolic disturbances associated with diabetes [10]. Hepatic glucose uptake, storage, production and utilization are regulated by insulin and other metabolic signals. Persistent hyperglycaemia can expose hepatic tissues to oxidative and metabolic stress, which may interfere with normal carbohydrate and lipid metabolism and contribute to hepatic dysfunction [11]. Oxidative damage associated with hyperglycaemia can involve cellular lipids, proteins, DNA and mitochondrial structures [5]. Consequently, preservation of hepatic antioxidant capacity and normal glucose metabolism represents an important consideration in the management of diabetes and its associated complications.

Although conventional antidiabetic drugs remain central to diabetes management, the continuing burden of the disease and the need for complementary therapeutic approaches have encouraged increased interest in medicinal plants and plant-derived bioactive compounds. Plants contain diverse secondary metabolites, which include flavonoids, phenolic compounds, terpenoids, steroids and other constituents with biological activities of potential therapeutic relevance. Several plant-derived compounds have been investigated for their antioxidant, antihyperglycaemic and hepatoprotective properties. In particular, compounds capable of scavenging reactive species or modulating pathways involved in glucose metabolism may have potential value in addressing oxidative stress associated with diabetes [12].

Psidium guajava L., commonly known as guava, is a member of the family *Myrtaceae* and is widely distributed in tropical and subtropical regions. Different parts of the plant, particularly the leaves, have been use in traditional medicine for the management of several ailments. Scientific investigations have subsequently documented a broad range of biological activities associated with *P. guajava*, which include antioxidant, antimicrobial, anti-inflammatory, antidiabetic and hepatoprotective activities [13–15]

The biological properties of *P. guajava* leaves have been associated with their diverse phytochemical composition. Previous phytochemical investigations have reported the presence of flavonoids, phenolic acids, tannins, terpenoids, essential oils and other bioactive constituents [16,17]. Compounds such as quercetin, guaijaverin, kaempferol, myricetin, catechins, gallic acid, chlorogenic acid and caffeic acid have been reported in guava leaves and have been associated with various biological activities, particularly antioxidant and metabolic effects [17].

Ocimum gratissimum L., commonly known as African basil or clove basil, belongs to the family Lamiaceae and is widely used as a medicinal plant in several African communities. Traditionally, the plant has been used in the management of conditions including diabetes, gastrointestinal disorders, inflammation, pain and microbial infections [18]. Phytochemical investigations have identified diverse constituents in *O. gratissimum*, including phenolic acids, flavonoids, terpenoids and essential-oil components. Reported compounds include caffeic acid, ellagic acid, rosmarinic acid, chlorogenic acid, luteolin, apigenin, gallic acid, catechin, quercetin and rutin, while its volatile fraction contains compounds such as eugenol, thymol, linalool and carvacrol [19]. These constituents have been associated with antioxidant, anti-inflammatory, hepatoprotective and antidiabetic activities [19,20].

The antioxidant activity of *O. gratissimum* has been demonstrated using different experimental models, with its activity attributed partly to its phenolic and flavonoid constituents and essential-oil components. The plant has also demonstrated antidiabetic activity in experimental studies, suggesting that its constituents may influence glucose metabolism and oxidative balance [19].

Materials and Methods

Plant Material and Authentication

Fresh leaves of *Psidium guajava* L. and *O. gratissimum* were collected were collected from Faculty of Agriculture, Prince Abubakar Audu University in March, 2026 and authenticated at the Department of Plant Science and Biotechnology, Prince Abubakar Audu University, Anyigba with the voucher numbers; PAAU/PSB/HVN/982 and PAAU/PSB/HVN/578 respectively deposited at the herbarium unit. The leaves were carefully sorted to remove extraneous materials and washed with clean water to remove adhering dirt and other contaminants. The leaves were then air-dried at 27°C under shade until a constant weight was obtained. The dried leaves were pulverized into a fine powder using a clean laboratory blender and stored in an airtight container until extraction.

Preparation of Methanol Leaf Extract

The powdered leaves of *P. guajava* and *O. gratissimum* each were extracted using the cold maceration method. Exactly 150g of the powdered plant material was soaked in 600 mL of methanol and allowed to stand for 48 h at 27°C, with intermittent agitation to enhance extraction of the soluble constituents. At the end of the extraction period, the mixture was filtered through Whatman No. 1 filter paper. The filtrate was concentrated by evaporation of the solvent at 50 °C using a rotary evaporator [21]. The resulting concentrated extract was further allowed to dry to obtain the methanol leaf extract, which was weighed and stored in an airtight container at 27°C until required for the analyses.

In-vitro Antioxidant Assays

The antioxidant activity of the methanol leaf extract of *P. guajava* and *O. gratissimum* were evaluated using DPPH radical scavenging, total antioxidant capacity (TAC), ferric reducing antioxidant power (FRAP), and hydroxyl radical scavenging assays. The extract was evaluated at concentrations of 200, 400, 600, 800, 900 and 1000 µg/mL.

DPPH Radical Scavenging Assay

The DPPH radical scavenging activity was determined according to the method of [22]. Different concentrations of the methanol leaf extract were prepared, and 1 mL of each concentration was mixed with 1mL of freshly prepared DPPH solution. The reaction mixtures were thoroughly mixed and incubated in the dark for 30 minutes. The absorbance was measured spectrophotometrically at 517nm against methanol as blank. Ascorbic acid was used as the standard antioxidant. The percentage inhibition of DPPH radicals was calculated.

$$\%\, Inhibition = \frac{A_c - A_s}{A_c} \times 100$$

where $A_c$is the absorbance of the control and $A_s$is the absorbance of the sample.

The concentration of extract required to inhibit 50% of the DPPH radicals (IC_{50}) was determined from the concentration-response relationship.

Total Antioxidant Capacity Assay

Total antioxidant capacity was determined using the phosphomolybdenum method described by [23]. Different concentrations of the extract were mixed with the phosphomolybdate reagent and incubated at 95°C for 90 minutes. It was allowed to cool in ice cold water to room temperature and the absorbance was measured spectrophotometrically at 765nm. The antioxidant activity was expressed as percentage inhibition and the IC_{50} value was determined from the concentration-response relationship.

$$\%\, Inhibition = \frac{A_s - A_c}{A_s} \times 100$$

where $A_c$is the absorbance of the control and $A_s$is the absorbance of the sample

Reducing Power assay

The reducing power of the methanol leaf extracts were determined according to the method described by [24]. Different concentrations of the methanol leaf extract (200, 400, 600, 800 and 1000 µg/mL) were prepared. For each concentration, 1.0 mL of the extract solution was mixed with 1.0 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 1.0 mL of 1% (w/v) potassium ferricyanide [$K_3Fe(CN)_6$]. The reaction mixtures were incubated at 50°C for 20 min in a water bath. After incubation, 1.0 mL of 10% (w/v) trichloroacetic acid (TCA) was

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added to each reaction mixture. The mixtures were centrifuged at 3,000 rpm for 10 min to obtain clear supernatants. From each supernatant, 2.5 mL was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% (w/v) ferric chloride ($FeCl_3$). The mixtures were allowed to stand at room temperature for 10 min, after which the absorbance was measured at 700 nm using a spectrophotometer.
Ascorbic acid was used as the reference antioxidant and was treated under the same experimental conditions. A reagent blank containing the respective reagents without the extract was prepared and used for baseline correction.

Hydroxyl Radical Scavenging Assay
The hydroxyl radical (•OH) scavenging activity of the methanol leaf extract of *Psidium guajava* and *O. gratissimum* were determined using the Fenton reaction-based 1,10-phenanthroline method described by [25]. The assay was based on the generation of hydroxyl radicals through the Fenton reaction and their interaction with the Fe^{2+}–1,10-phenanthroline complex. The formation of the Fe^{2+}–1,10-phenanthroline complex was monitored spectrophotometrically at 536 nm.
Briefly, 1.0 mL of 1,10-phenanthroline solution (1.0 mM) was mixed with 2.0 mL of the methanol leaf extract at concentrations of 200, 400, 600, 800, 900 and 1000 µg/mL. Subsequently, 1.0 mL of $FeSO_4 \cdot 7H_2O$ solution (1.0 mM) was added, and the reaction was initiated by the addition of 1.0 mL of H_2O_2 (0.03%, v/v). The reaction mixture was incubated at 37 °C for 60 min in a water bath. After incubation, the absorbance was measured at 536 nm against the appropriate reagent blank. Ascorbic acid was used as the reference antioxidant and was evaluated under the same experimental conditions.
A reaction mixture containing all reagents except the test extract or reference antioxidant served as the negative control, while a reaction mixture prepared without H_2O_2 served as the reagent blank. Hydroxyl radical scavenging activity was calculated using the absorbance values obtained for the sample, negative control and reagent blank. The percentage hydroxyl radical scavenging activity was expressed as:

$$\text{Hydroxyl radical scavenging activity (\%)} = \frac{A_s - A_n}{A_b - A_n} \times 100$$

where $A_s$represents the absorbance of the sample, $A_n$represents the absorbance of the negative control, and $A_b$represents the absorbance of the reagent blank. The concentration of extract required to scavenge 50% of the hydroxyl radicals (IC_{50}) was determined from the concentration-response curve. The IC_{50} value was obtained by plotting percentage hydroxyl radical scavenging activity against extract concentration and determining the concentration corresponding to 50% scavenging activity.

In-vitro antidiabetic enzyme inhibition assay
The in-vitro antidiabetic activity of the methanol leaf extracts was evaluated by determining its inhibitory effects on α-glucosidase and α-amylase activities.

α-Glucosidase inhibition assay
The α-glucosidase inhibitory activity of the methanol leaf extract was determined according to the method described by [26]. Different concentrations of the extract (200, 400, 600, 800 and 1000 µg/mL) were prepared. The extract solution was mixed with α-glucosidase enzyme solution prepared in phosphate buffer and pre-incubated. Following pre-incubation, p-nitrophenyl-α-D-glucopyranoside (pNPG) was added as the substrate and the reaction mixture was incubated under the specified assay conditions. The enzymatic reaction resulted in the release of p-nitrophenol, which was measured spectrophotometrically at 405 nm.
Acarbose was assayed at corresponding concentrations and served as the reference inhibitor, while a reaction mixture containing all assay components except the extract served as the control. Appropriate sample blanks were also prepared to correct for background absorbance. The assay was performed in triplicate.
The percentage inhibition of α-glucosidase was calculated using:

$$\%\,\text{Inhibition} = \left[1 - \frac{A_s - A_b}{A_c}\right] \times 100$$

where $A_s$is the absorbance of the sample reaction, $A_b$is the absorbance of the sample blank, and $A_c$is the absorbance of the control reaction.

α-Amylase inhibition assay
The α-amylase inhibitory activity of the methanol leaf extracts were determined according to the method of [27]. Different concentrations of the extract (200, 400, 600, 800 and 1000 µg/mL) were prepared and mixed with α-amylase enzyme solution in sodium phosphate buffer. The mixture was pre-incubated to allow interaction between the extract constituents and the enzyme. A starch solution was subsequently added as the substrate and the reaction mixture was incubated under the specified assay conditions.
The enzymatic reaction was terminated by addition of dinitrosalicylic acid (DNS) colour reagent, followed by heating in a boiling water bath to develop the colour. After cooling, the absorbance was measured spectrophotometrically at the wavelength specified for the assay. Acarbose was used as the standard inhibitor, while the enzyme reaction without extract served as the control. Appropriate reagent and sample blanks were included, and all determinations were performed in triplicate.
The percentage inhibition of α-amylase activity was calculated as:

$$\%\,\text{Inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

where $A_c$represents the absorbance of the control reaction and $A_s$represents the absorbance of the extract-treated reaction.

Experimental Animals and Liver Tissue Collection
Albino rats were used as the source of liver tissue for the *Ex-vivo* experiment. The animals were maintained under standard laboratory conditions with free access to feed and water. The experimental procedure was conducted in accordance with Kogi State University guidelines for the care and use of laboratory animals.
Following the approved experimental procedure, the animals were humanely sacrificed and the liver was rapidly excised. The liver tissue was immediately placed in ice-cold physiological medium and carefully freed from adhering connective tissue and blood [28].

Ex-vivo Induction of a Hyperglycemic Condition and Treatment in Liver Tissue
The *Ex-vivo* glucose uptake assay was carried out to evaluate the effect of the methanol leaf extract on glucose utilization by isolated rat liver tissue under normoglycaemic and hyperglycaemic conditions. The method was based on the ability of viable liver tissue to remove glucose from the incubation medium according to the methods described by [29,30].
Following an overnight fast (18 h), the experimental rats were sacrificed by cervical dislocation in accordance with the approved institutional guidelines for the care and use of laboratory animals. The abdominal cavity was immediately opened, and the liver was carefully excised, rinsed in ice-cold physiological saline to remove adhering blood, and gently blotted dry using filter paper. The liver was then cut into small pieces weighing approximately 200–250 mg each.
Each liver tissue piece was transferred into a 100 mL Erlenmeyer flask containing 20 mL of pre-warmed Krebs–Henseleit bicarbonate buffer (KRB). The buffer consisted of 118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4 and 25 mM $NaHCO_3$ and was prepared in distilled water and maintained at pH 7.4. The buffer was equilibrated with a 95% O_2/5% CO_2 gas mixture and maintained at 37 °C during tissue incubation for 10 min. Following equilibration, the tissues were transferred into fresh KRB buffer containing the appropriate glucose concentration and treatment.
The experimental groups consisted of:

Negative control: Liver tissue incubated in KRB buffer containing 25 mM (0.451 g glucose/100 mL), without any treatment (untreated hyperglycaemic control).

Positive control: Liver tissue incubated in KRB buffer containing 5 mM, 0.090 g glucose/100 mL (physiological glucose control).

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Standard group: Liver tissue incubated in KRB buffer containing 25 mM glucose and metformin (100 µg/mL).
Extract-treated groups: Liver tissue incubated in KRB buffer containing 25 mM glucose and the methanol leaf extract at concentrations of 200, 400, and 600 µg/mL.

The incubation media were aerated for approximately 5 min before incubation to ensure adequate oxygenation and maintain tissue viability. The Erlenmeyer flasks were then incubated at 37°C in a shaking incubator operating at 96 rpm for 3 h. Aliquots (0.5mL) of the incubation medium were collected at 0, 30, 60, 120 and 180 min for glucose determination. Following each sampling, an equal volume of fresh KRB buffer of the corresponding treatment was added to maintain a constant incubation volume.

Determination of Glucose Concentration in the liver homogenate

The glucose concentration of the incubation medium was determined using the glucose oxidase–peroxidase (GOD–POD) enzymatic method as described by [31].

Briefly, 10 µL of each incubation medium sample was mixed with 1.0 mL of the GOD–POD working reagent and incubated at 37°C for 10 min. The absorbance was then measured spectrophotometrically at 500 nm against the reagent blank. Glucose concentration was calculated according to the manufacturer's instructions using a glucose standard of known concentration.

The amount of glucose utilized by the liver tissue was determined by calculating the difference between the initial glucose concentration and the residual glucose concentration remaining in the incubation medium after incubation.

The glucose concentration was calculated using the equation:

$$\textit{Glucose Concentration (mg/dL)} = \frac{\textit{Absorbance of Sample}}{\textit{Absorbance of Standard}} \times \textit{Concentration of Standard}$$

Glucose uptake by the isolated liver tissue was determined using the following equation:

Glucose Uptake (mg/dL) =

Initial Glucose Concentration − Residual Glucose Concentration

The percentage glucose uptake was calculated as:

$$\textit{Percentage Glucose Uptake} = \frac{\textit{Initial Glucose Concentration} - \textit{Residual Glucose Concentration}}{\textit{initial Glucose Concentration}} \times 100$$

To account for differences in tissue size, glucose uptake was expressed as milligrams of glucose utilized per gram of liver tissue using the equation:

$$\textit{Glucose Uptake (mg/g protein)} = \frac{\text{Glucose Utilized (mg)}}{\text{Weight of Liver Tissue (g)}}$$

The antihyperglycaemic activity of the methanol leaf extract was assessed by comparing glucose uptake in the extract-treated groups with that of the untreated hyperglycaemic control (25 mM glucose), the physiological glucose control (5 mM glucose), and the metformin-treated group. Enhanced glucose uptake by the liver tissue in the extract-treated groups compared with the untreated hyperglycaemic control was considered indicative of improved hepatic glucose utilization and potential antihyperglycaemic activity.

Determination of Total Protein Concentration of the Homogenate

The total protein concentration of the liver tissue homogenate was determined using the Biuret method described by [32].

In an alkaline medium, cupric ions react with the peptide bonds of proteins to form a purple-coloured copper–protein complex. The intensity of the colour produced is proportional to the protein content of the sample.

Exactly 0.10 mL of appropriately diluted liver tissue homogenate (×10) was pipetted into a clean test tube, followed by the addition of 0.9 mL of distilled water and 4.0 mL of Biuret reagent. The mixture was vortexed thoroughly to ensure uniform mixing and allowed to stand undisturbed for 30 minutes at 27°C for colour development. A reagent blank was prepared by mixing 4.0 mL of Biuret reagent with 1.0 mL of distilled water. The absorbance of the coloured solutions was measured at 540 nm using a UV-Vis spectrophotometer (Model UV752, Shanghai, China) against the reagent blank.

Protein standards were prepared from bovine serum albumin (BSA) at concentrations ranging from 1 to 10 mg/mL and treated in the same manner as the liver tissue samples. A calibration curve was generated by plotting absorbance against BSA concentration. The protein concentration of the liver tissue homogenate was obtained from the calibration curve.

Calculation

The protein concentration of the liver tissue sample was calculated as:

Protein concentration (g/dL) = $0.1 \times C \times 10$

Where:

C = protein concentration obtained from the BSA calibration curve (mg/mL)

10 = dilution factor of the liver tissue homogenate.

Determination of Malondialdehyde (MDA)

The extent of lipid peroxidation in the isolated liver tissue was determined by measuring malondialdehyde (MDA) concentration in the liver supernatant following the method described by [33].

The assay was based on the reaction of MDA with 2-thiobarbituric acid (TBA) under acidic conditions and elevated temperature to form a pink-coloured MDA–TBA_2 complex. The intensity of the colour formed was measured spectrophotometrically at 532 nm and was proportional to the concentration of MDA in the liver supernatant.

Briefly, 0.2 mL of appropriately diluted liver supernatant (×10) was mixed with 0.8 mL of Tris-KCl buffer (pH 7.4). Thereafter, 0.25 mL of 20% trichloroacetic acid (TCA) was added, followed by 0.25 mL of 0.67% thiobarbituric acid (TBA). The reaction mixture was incubated in a water bath at 80°C for 45 minutes to facilitate the formation of the MDA–TBA_2 complex. The mixture was subsequently cooled on ice and centrifuged at 4000 × *g* for 15 minutes. The absorbance of the resulting pink-coloured supernatant was measured at 532 nm using a UV-visible spectrophotometer against distilled water as the blank.

$$\text{MDA (units/mg protein)} = \frac{\text{Absorbance} \times \text{Total Volume of Reaction Mixture}}{\varepsilon 532 \times \text{Volume of Sample} \times \text{mg Protein}}$$

Where:

ε_{532}= Molar absorptivity of MDA–TBA complex at 532 nm = $1.56 \times 10^5 M^{-1}cm^{-1}$

Total volume of reaction mixture = 1.5 mL

Volume of sample = 0.2 mL

mg protein = Protein concentration of the liver tissue

Determination of Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) activity in the isolated liver tissue was determined using the method described by [34]. The assay was based on the ability of SOD to inhibit the auto-oxidation of epinephrine to adrenochrome under alkaline conditions. At pH 10.2, superoxide radicals generated during the auto-oxidation of epinephrine promoted the formation of adrenochrome, which exhibited maximum absorbance at 480 nm. The degree of inhibition of adrenochrome formation was used as an indication of SOD activity in the liver supernatant.

For the assay, 100 µL of appropriately diluted liver supernatant (×10) was mixed with 1,000 µL of 0.05 M carbonate buffer (pH 10.2) in a clean cuvette. The reaction mixture was equilibrated for 5 minutes at 27°C. The reaction was initiated by adding 150 µL of freshly prepared 0.3 mM epinephrine, and the contents were gently mixed. A reference cuvette containing 1,000 µL of carbonate buffer, 150 µL of epinephrine and 100 µL of distilled water was prepared simultaneously. The change in absorbance was measured at 480 nm at 30-second intervals for 2.5 minutes using a UV-visible spectrophotometer.

$$\Delta A/\text{min} = \frac{A_3 - A_0}{2.5}$$

Where A_0 = initial absorbance

A_3 = absorbance after 2.5 minutes

ΔA/min = Change in the absorbance per minute

$$\text{Inhibition (\%)} = \frac{\Delta A_{\text{blank}} - \Delta A_{\text{sample}}}{\Delta A_{\text{blank}}} \times 100$$

Where

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$\Delta A_{blank=}$ change in absorbance of blank
ΔA_{sample}= change in absorbance of sample
1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adenochrome per minute

$$\text{SOD Units} = \frac{\%\text{Inhibition}}{50} \times \text{Dilution Factor}$$

50 = 1 unit of SOD is the amount of enzyme that inhibit the reaction by 50%

$$\text{SOD Activity (U/mg protein)} = \frac{\text{SOD Units}}{\text{mg protein in assay volume}}$$

Determination of Catalase Activity

Catalase activity in the isolated liver tissue was determined using the procedure described by [34]. The method was based on the reaction of hydrogen peroxide with dichromate in acetic acid at high temperature to form a coloured chromic acetate complex. The intensity of the colour formed was proportional to the concentration of hydrogen peroxide and was used to determine catalase activity.

Briefly, 1 mL of appropriately diluted liver supernatant (×10) was mixed with 1 mL of 800 µmol/mL hydrogen peroxide solution and 1.25 mL of phosphate buffer (pH 7.0). At 60-second intervals, 1 mL aliquots of the reaction mixture were withdrawn and added to 2 mL of dichromate-acetic acid reagent containing 5.0 g potassium dichromate ($K_2Cr_2O_7$) in 100 mL of glacial acetic acid. The tubes were incubated in a water bath at 95°C for 10 minutes. Following incubation, the tubes were cooled to 27°C, and the absorbance was measured at 570 nm using a UV-visible spectrophotometer. A blank and hydrogen peroxide standards were treated under similar conditions.

Catalase activity was calculated based on the absorbance of the sample in relation to the blank and hydrogen peroxide standard, taking into consideration the total assay volume, concentration of the hydrogen peroxide standard, volume of liver enzyme extract and protein concentration of the liver tissue. The result was expressed as U/mg protein.

$$\text{Catalase (U/mg protein)} = \frac{(\text{Ablank} - \text{Asample}) \times \text{Conc of standard} \times \text{Volume of assay}}{\text{Ablank} \times \text{Volume of enzyme} \times \text{mgprotein}}$$

Where:
A_{blank}= Absorbance of the blank
A_{sample}= Absorbance of the test sample
Volume of assay = Total volume of reaction mixture
Conc. of standard = Concentration of standard H_2O_2 = 800 µmol/mL
Volume of enzyme = Volume of enzyme extract used
mg protein = Protein concentration of liver tissue

Determination of Reduced Glutathione (GSH)

The concentration of reduced glutathione (GSH) in the isolated liver tissue was determined using the procedure of [35]. The assay was based on the formation of a stable yellow-coloured product following the reaction of sulfhydryl compounds with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), also known as Ellman's reagent. The coloured product exhibited maximum absorbance at 412 nm, with the intensity of the colour being proportional to the concentration of reduced glutathione in the liver supernatant.

For the assay, 1 mL of liver supernatant was mixed with 1.5 mL of 4% sulfosalicylic acid and allowed to stand at 27°C for 10 minutes. The mixture was subsequently centrifuged at 1800 × *g* for 10 minutes. Thereafter, 0.25 mL of the clear supernatant was added to 2 mL of 0.1 M phosphate buffer (pH 7.4), followed by the addition of 0.25 mL of Ellman's reagent. The absorbance was measured at 412 nm using a UV-visible spectrophotometer. The GSH concentration was determined from a GSH calibration curve.

The concentration of GSH was calculated using the absorbance at 412 nm, total assay volume, molar extinction coefficient of the TNB product (ε = 0.0136 µmol^{-1} mL^{-1} cm^{-1}), path length of the cuvette, sample volume and protein concentration of the liver tissue. The result was expressed as µmol/mg protein.

$$\text{GSH (µmol/mg protein)} = \frac{A \times Vt}{\varepsilon \times l \times V_s \times \text{mgProtein}}$$

Where:
A = Absorbance at 412 nm
V_t = Total volume of assay mixture (mL)
ε = Molar extinction coefficient of TNB = 0.0136 µmol^{-1} mL^{-1} cm^{-1}
l = Path length of cuvette (typically 1 cm)
V_s = Volume of sample used in the assay (mL)
mgProtein = Concentration of protein in the liver tissue (mg/mL)

Statistical Analysis

All experimental data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA). Differences among treatment groups were analysed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Differences were considered statistically significant at $p < 0.05$.

Result and Discussion

Invitro antioxidant activity

The DPPH radical scavenging activity of *Psidium guajava* and *Ocimum gratissimum* methanol leaf extracts was evaluated to determine their free-radical scavenging capacities (figure 1). The activity of *O. gratissimum* increased from 52% at 200 µg/mL to 86% at 1000 µg/mL, representing a 65.38% increase, whereas *P. guajava* increased from 50% to 81%, representing a 62.00% increase over the same concentration range. At each tested concentration, the DPPH scavenging activities of both extracts were significantly lower than that of ascorbic acid ($p < 0.05$). The activities of *O. gratissimum* and *P. guajava* were not significantly different from each other at the tested concentrations ($p > 0.05$).

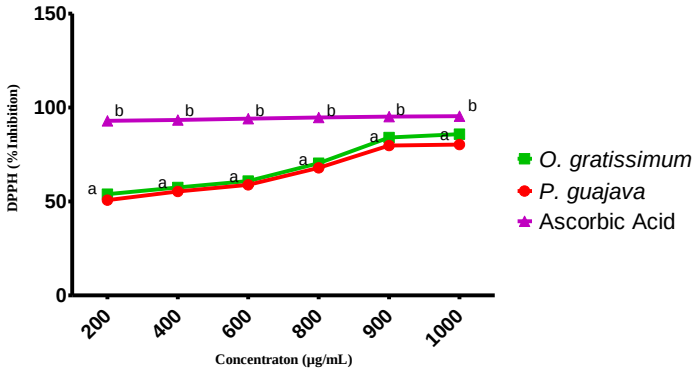

Figure 1: DPPH Radical Scavenging Assay**.** Values are expressed as Mean ± SEM (n = 3).

Total antioxidant capacity was determined to assess the overall antioxidant activity of the methanol leaf extracts. The total antioxidant capacity of *O. gratissimum* increased from 36% at 200 µg/mL to 59% at 1000 µg/mL, representing a 63.89% increase, while that of *P. guajava* increased from 33% to 54%, representing a 63.64% increase. Ascorbic acid produced significantly higher antioxidant capacity than both extracts at the tested concentrations ($p < 0.05$). The total antioxidant capacities of *O. gratissimum* and *P. guajava* did not differ significantly at the tested concentrations ($p > 0.05$) (Figure 2).

The reducing power activity of the extracts was determined to assess their ability to reduce the assay system. *O. gratissimum* showed an increase in reducing power from 47% at 200 µg/mL to 75% at 1000 µg/mL, representing a 59.57% increase, whereas *P. guajava* increased from 41% to 74%, representing an 80.49% increase. The reducing power activities of the two extracts were not significantly different at the tested concentrations where they shared the same statistical grouping ($p > 0.05$). Ascorbic acid differed significantly from the extract groups at concentrations where different statistical groupings were observed ($p < 0.05$) (Figure 3).

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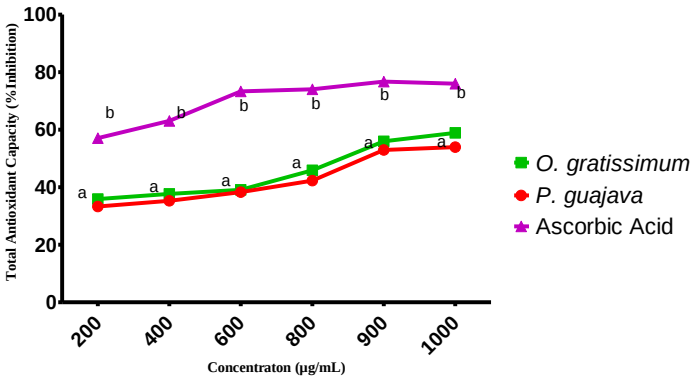

Figure 2: Total Antioxidant Capacity. Values are expressed as Mean ± SEM (n = 3).

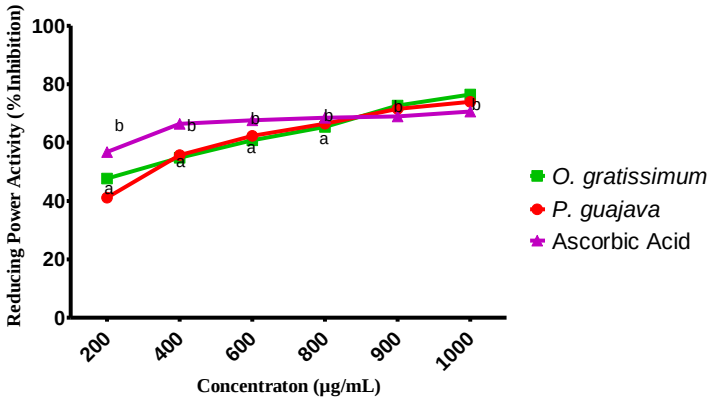

Table 3: Reducing power activity. Values are expressed as Mean ± SEM (n = 3

Hydroxyl radical scavenging activity was evaluated to determine the ability of the extracts to scavenge hydroxyl radicals. The activity of *O. gratissimum* increased from 46% at 200 µg/mL to 60% at 1000 µg/mL, representing a 30.43% increase, whereas *P. guajava* increased from 47% to 50%, representing a 6.38% increase. The activities of the two extracts were not significantly different from each other from 200 to 800 µg/mL ($p > 0.05$). At 900 and 1000 µg/mL, the hydroxyl radical scavenging activity of *P. guajava* differed significantly from that of *O. gratissimum* ($p < 0.05$). Ascorbic acid produced significantly higher hydroxyl radical scavenging activity than the extracts at the tested concentrations ($p < 0.05$) (figure 4).

The antioxidant activities of *Psidium guajava* and *Ocimum gratissimum* methanol leaf extracts were evaluated using DPPH radical scavenging, total antioxidant capacity, reducing power, and hydroxyl radical scavenging assays. The extracts demonstrated concentration-dependent antioxidant activities across the evaluated assays, although the magnitude of activity varied according to the antioxidant system assessed. The observed antioxidant effects may be attributed to the presence of phenolic acids, flavonoids, tannins, and other redox-active phytoconstituents capable of donating electrons or hydrogen atoms and thereby neutralizing reactive oxygen species and free radicals. Previous investigations have established that both *P. guajava* and *O. gratissimum* contain diverse phenolic and flavonoid constituents associated with substantial antioxidant and pharmacological activities [20,36].

The DPPH radical scavenging assay demonstrated a concentration-dependent increase in radical scavenging activity in both plant extracts. *O. gratissimum* exhibited significantly greater DPPH radical scavenging activity at the higher extract concentrations, whereas ascorbic acid retained the strongest radical scavenging capacity across the experimental range. The progressive increase in DPPH radical neutralization with increasing extract concentration indicates the presence of concentration-dependent electron- or hydrogen-donating constituents in the extracts. Phenolic compounds are particularly relevant to this activity because their hydroxyl groups can participate in hydrogen atom donation and stabilization of the resulting radicals through resonance. The antioxidant activity of *P. guajava* leaves has previously been associated with their high phenolic and flavonoid content, while *O. gratissimum* has been reported to contain several bioactive phenolic compounds, including caffeic acid, rosmarinic acid, chlorogenic acid, gallic acid, catechin, quercetin, and kaempferol [20,36,37].

A similar concentration-dependent response was observed in the total antioxidant capacity assay. Both extracts exhibited significantly increased total antioxidant capacity with increasing extract concentration, with *O. gratissimum* demonstrating greater antioxidant capacity than *P. guajava* at the higher concentrations. The total antioxidant capacity assay reflects the cumulative reducing capacity of the constituents present in the extracts and therefore provides complementary information to radical-specific assays. The observed activity may be related to the cumulative contribution of phenolic and flavonoid constituents capable of reducing oxidized intermediates. The comparatively greater activity of *O. gratissimum* in this assay may indicate a higher contribution of redox-active phytochemicals within the tested extract concentration range. The antioxidant properties of *O. gratissimum* have been extensively associated with its phenolic constituents and have been implicated in its reported antioxidant and hepatoprotective activities [20].

The reducing power assay further demonstrated a concentration-dependent increase in the reducing capacity of both extracts. At higher concentrations, the reducing activities of the plant extracts were comparable with that of the reference antioxidant. This finding indicates that the methanol extracts contain constituents with electron-donating capacity capable of reducing oxidized intermediates. Electron donation represents an important mechanism through which phytochemicals can terminate oxidative reactions and prevent the propagation of free-radical-mediated processes. The reducing activity observed in the present study is consistent with previous reports demonstrating substantial antioxidant capacity in *P. guajava* leaves, which has been associated with their abundance of phenolic and flavonoid compounds [36].

The hydroxyl radical scavenging assay demonstrated concentration-dependent radical scavenging activity in both extracts. *O. gratissimum* exhibited a significantly increased hydroxyl radical scavenging response at the higher concentrations, whereas *P. guajava* demonstrated comparatively lower activity within the same concentration range. Hydroxyl radicals are highly reactive oxygen species capable of initiating oxidative modification of lipids, proteins, and nucleic acids [38]. Consequently, the ability of the extracts to neutralize hydroxyl radicals may have important implications for their capacity to limit oxidative cellular damage. The antioxidant effects observed across the different assays collectively indicate that both extracts possess multiple antioxidant mechanisms, although the relative activity of each extract is dependent on the specific radical or redox system examined.

The antioxidant activities observed in the present study are consistent with previous reports on the phytochemical composition and antioxidant properties of *P. guajava* and *O. gratissimum*. Researchers reported substantial antioxidant activity associated with polyphenolic constituents of *P. guajava* leaves and the antioxidant potential of *O. gratissimum* was found to be associated with its diverse phenolic and flavonoid constituents [20,36]. The differences in antioxidant responses among the assays may reflect differences in assay principles, radical reactivity, and the capacity of individual phytochemicals to participate in electron transfer or hydrogen atom donation.

Carbohydrate-digesting Enzymes Inhibition

The inhibitory activities of the methanol leaf extracts against α-amylase were evaluated to determine their effects on carbohydrate-digesting enzyme activity. The α-amylase inhibitory activity of *O. gratissimum* increased from 17% at 200 µg/mL to 50% at 1000 µg/mL, representing a 194.12% increase, whereas *P. guajava*

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increased from 36% to 89%, representing a 147.22% increase. The α-amylase inhibitory activity of *O. gratissimum* was significantly lower than those of *P. guajava* and acarbose at all tested concentrations ($p < 0.05$). The inhibitory activity of *P. guajava* did not differ significantly from that of acarbose at the tested concentrations ($p > 0.05$) (Figure 5). The α-glucosidase inhibitory activities of the extracts were determined to assess their effects on another key carbohydrate-digesting enzyme. The inhibitory activity of *O. gratissimum* increased from 27% at 200 µg/mL to 79% at 1000 µg/mL, representing a 192.59% increase, whereas *P. guajava* increased from 31% to 84%, representing a 170.97% increase. The α-glucosidase inhibitory activity of *O. gratissimum* was significantly lower than that of *P. guajava* at all tested concentrations ($p < 0.05$). The activity of *P. guajava* did not differ significantly from that of acarbose at 200, 600, 800 and 1000 µg/mL ($p > 0.05$), whereas a significant difference was observed at 400 µg/mL ($p < 0.05$) (Figure 6).

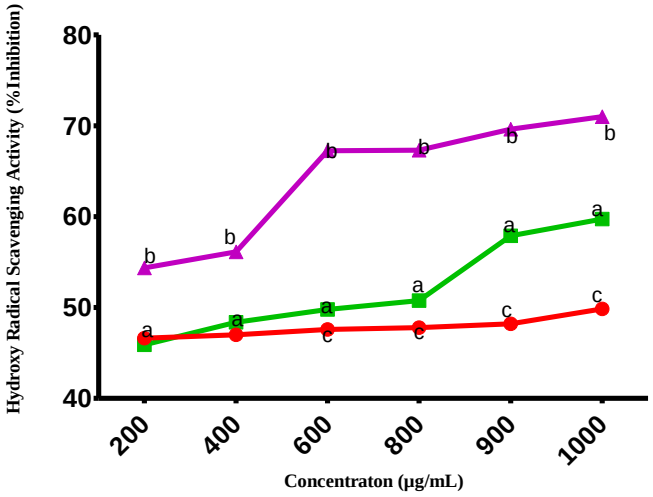
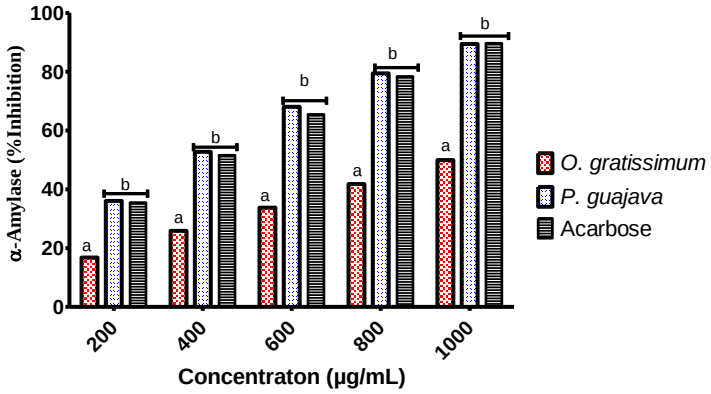
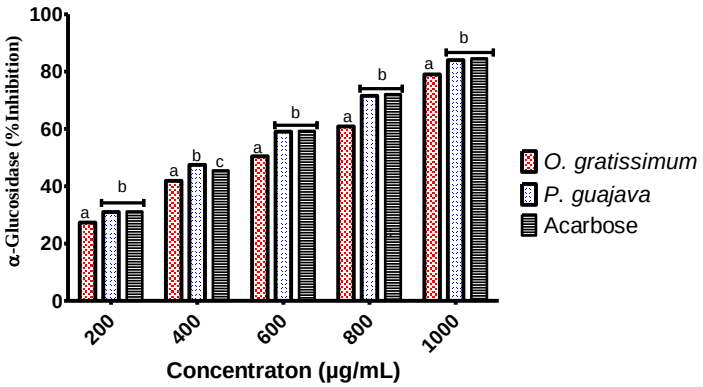


Figure 4: Hydroxyl Radical Scavenging Assay. Values are expressed as Mean ± SEM (n = 3)



Values are expressed as Mean ± SEM (n = 3). Bars with different superscripts within the same parameter are significantly different at p < 0.05.

Figure 5: Effects of *Psidium guajava* and *O. gratissimum* Methanol Leaf Extract on percentage inhibition of α-amylase activity.



Values are expressed as Mean ± SEM (n = 3). Bars with different superscripts within the same parameter are significantly different at p < 0.05.

Figure 6: Effects of *Psidium guajava* and *O. gratissimum* Methanol Leaf Extract on percentage inhibition of α-glucosidase activity.

The two methanol leaf extracts demonstrated concentration-dependent inhibition of α-amylase activity. *P. guajava* exhibited significantly greater α-amylase inhibitory activity than *O. gratissimum* across the evaluated concentration range, with its inhibitory response approaching that of acarbose at the higher concentrations. This finding indicates that *P. guajava* contains constituents capable of interfering with the catalytic activity of α-amylase and consequently may limit the enzymatic hydrolysis of complex carbohydrates.

α-Amylase catalyzes the hydrolysis of α-1,4-glycosidic linkages in starch and other polysaccharides, producing smaller carbohydrate molecules that can subsequently undergo further digestion and absorption [39]. Inhibition of this enzyme therefore represents an important mechanism for reducing the rate of carbohydrate digestion. The substantial inhibitory activity demonstrated by *P. guajava* suggests that its phytochemical constituents may interact with the enzyme and interfere with substrate hydrolysis. Phenolic compounds and flavonoids have been reported to inhibit digestive carbohydrate-hydrolyzing enzymes through interactions with catalytic or substrate-binding regions of the enzymes [40,41].

The present finding agrees with the study of Ahmed and other [40], who demonstrated α-amylase inhibitory activity in phenolic fractions obtained from *P. guajava* leaves. The authors associated the inhibitory activity with phenolic constituents capable of interacting with metabolism-related enzymes. Findings from research reported inhibitory effects of *P. guajava* leaf extract against digestive enzymes and attributed the activity, in part, to the phenolic constituents present in the leaves [41]. Although extraction solvent and experimental conditions can influence enzyme inhibitory activity, these findings provide evidence supporting the potential of *P. guajava* leaf phytochemicals as inhibitors of carbohydrate-digesting enzymes.

O. gratissimum also demonstrated concentration-dependent inhibition of α-amylase activity, although its inhibitory response was significantly lower than that observed with *P. guajava*. The observed inhibition demonstrates that *O. gratissimum* contains constituents capable of modulating carbohydrate-hydrolyzing enzyme activity. The reported presence of flavonoids, phenolic acids, tannins, and other secondary metabolites in *O. gratissimum* may contribute to this activity [20]. Differences in the qualitative and quantitative composition of phytochemicals between the two plant species may account for the differential inhibitory responses observed in the present investigation.

Inhibition of α-glucosidase activity

The two methanol leaf extracts demonstrated concentration-dependent inhibition of α-glucosidase activity. *P. guajava* exhibited significantly greater inhibitory activity than *O. gratissimum* at the higher concentrations, and its inhibitory response was comparable with that of acarbose. This observation indicates that *P. guajava* may interfere

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with the terminal stage of carbohydrate digestion mediated by α-glucosidase.

α-Glucosidase catalyzes the hydrolysis of terminal α-glycosidic bonds in oligosaccharides and disaccharides, facilitating the release of absorbable glucose. Inhibition of this enzyme can consequently attenuate the enzymatic release of glucose from dietary carbohydrates [42]. The concentration-dependent inhibition observed in the present study therefore provides evidence of the potential of both extracts to modulate carbohydrate digestion. The comparatively stronger activity of *P. guajava* suggests that its phytochemical constituents may possess greater affinity or inhibitory activity toward α-glucosidase under the experimental conditions employed.

The α-glucosidase inhibitory activity of *P. guajava* is supported by previous investigations demonstrating inhibitory effects of guava leaf constituents against carbohydrate-digesting enzymes. Phenolic compounds and flavonoids identified in *P. guajava* leaves, including quercetin derivatives and other polyphenolic constituents, have been associated with inhibition of α-glucosidase activity [41,43]. These compounds may inhibit the enzyme through hydrogen bonding, hydrophobic interactions, and interactions with catalytic residues within the enzyme structure. The present observation therefore provides further evidence that the biological activities of *P. guajava* leaves extend beyond antioxidant effects to include modulation of carbohydrate-digestive enzymes.

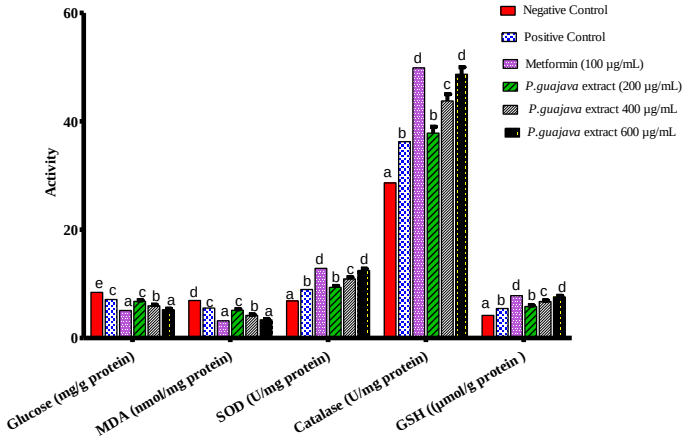
The α-glucosidase inhibitory activity of *O. gratissimum* also increased progressively with extract concentration. This activity is consistent with the established phytochemical profile of the species, which includes several phenolic acids and flavonoids with reported enzyme-modulating properties [20,44]. However, the significantly greater inhibitory activity observed for *P. guajava* indicates that the two extracts do not possess equivalent inhibitory capacities against α-glucosidase. The differential activity may arise from differences in phytochemical composition, concentration of active constituents, extract polarity, and interactions among individual compounds.

The simultaneous inhibition of α-amylase and α-glucosidase observed with the plant extracts is of particular biological relevance. Inhibition of these enzymes at different stages of carbohydrate digestion may reduce the rate at which dietary carbohydrates are hydrolyzed and glucose becomes available for absorption. The stronger inhibitory activity demonstrated by *P. guajava* against both enzymes suggests that the plant may possess considerable potential for the modulation of carbohydrate metabolism.

Ex-vivo Effects of Psidium guajava Methanol Leaf Extract on Glucose Concentration and Oxidative Stress Markers in Rat Liver

Ex-vivo exposure of the liver tissue to high-glucose Krebs-Henseleit buffer (negative control) significantly increased the glucose concentration by 14.29% when compared with tissue incubated in low-glucose Krebs-Henseleit buffer (positive control) ($p < 0.05$). Addition of *P. guajava* methanol leaf extract at 200, 400 and 600 µg/mL significantly decreased the glucose concentration by 12.50%, 25.00% and 37.50%, respectively, when compared with the negative control ($p < 0.05$). At 200 µg/mL, the glucose concentration was not significantly different from the positive control ($p > 0.05$), whereas treatment at 400 and 600 µg/mL produced significantly lower glucose concentrations by 14.29% and 28.57%, respectively ($p < 0.05$). When compared with metformin, the glucose concentrations at 200 and 400 µg/mL were significantly higher by 40.00% and 20.00%, respectively ($p < 0.05$), while the value at 600 µg/mL was not significantly different from metformin ($p > 0.05$) (Figure 7).

High-glucose *ex-vivo* exposure also significantly increased the MDA concentration by 16.67% when compared with the positive control ($p < 0.05$), indicating increased lipid peroxidation under the high-glucose condition. Treatment with *P. guajava* methanol leaf extract at 200, 400 and 600 µg/mL significantly decreased MDA concentration by 42.86%, 28.57% and 57.14%, respectively, when compared with the negative control ($p < 0.05$). The MDA concentration at 200 µg/mL was not significantly different from the positive control ($p > 0.05$), whereas treatment at 400 and 600 µg/mL resulted in significantly decrease values by 16.67% and 50.00%, respectively ($p < 0.05$). When compared with metformin, MDA concentration was significantly increase by 33.33% and 66.67% at 200 and 400 µg/mL, respectively ($p < 0.05$), while the value at 600 µg/mL was not significantly different from metformin ($p > 0.05$) (Figure 7).



Values are expressed as Mean ± SEM (n = 3). Bars with different superscripts within the same parameter are significantly different at $p < 0.05$

Figure 7: *Ex-vivo* Effects of *Psidium guajava* Methanol Leaf Extract Glucose Concentration and Oxidative Stress Markers in Rat Liver

The antioxidant defense system was also affected by high-glucose exposure, as SOD activity was significantly decrease by 22.22% when compared with the positive control ($p < 0.05$). Administration of *P. guajava* methanol leaf extract at 200, 400 and 600 µg/mL significantly increased SOD activity by 28.57%, 57.14% and 85.71%, respectively, when compared with the negative control ($p < 0.05$). At 200 µg/mL, SOD activity was not significantly different from the positive control ($p > 0.05$), whereas the activities at 400 and 600 µg/mL were significantly higher by 22.22% and 44.44%, respectively ($p < 0.05$). In comparison with metformin, SOD activity was significantly lower by 30.77% and 15.38% at 200 and 400 µg/mL, respectively ($p < 0.05$), while the activity at 600 µg/mL was not significantly different from metformin ($p > 0.05$) (Figure 7).

A similar pattern was observed for catalase activity. Liver tissue maintained under the high-glucose condition showed a significant decrease in catalase activity by 22.22% when compared with the positive control ($p < 0.05$). Treatment with *P. guajava* methanol leaf extract at 200, 400 and 600 µg/mL significantly increased catalase activity by 35.71%, 57.14% and 75.00%, respectively, when compared with the negative control ($p < 0.05$). Catalase activity at 200 µg/mL was not significantly different from the positive control ($p > 0.05$), whereas activities at 400 and 600 µg/mL were significantly higher by 22.22% and 36.11%, respectively ($p < 0.05$). When compared with metformin, catalase activity was significantly lower by 24.00% and 12.00% at 200 and 400 µg/mL, respectively ($p < 0.05$), while the activity at 600 µg/mL was not significantly different from metformin ($p > 0.05$) (Figure 7).

The reduced glutathione status further demonstrated the effect of the extract on the hepatic antioxidant system. High-glucose exposure significantly decreased GSH concentration by 20.00% when compared with the positive control ($p < 0.05$). Treatment with *P. guajava* methanol leaf extract at 200, 400 and 600 µg/mL significantly increased GSH concentration by 50.00%, 75.00% and 100.00%, respectively, when compared with the negative control ($p < 0.05$). The GSH concentration at 200 µg/mL was not significantly different from the positive control ($p > 0.05$), whereas concentrations at 400 and 600 µg/mL were significantly higher by 40.00% and 60.00%, respectively ($p < 0.05$). When compared with metformin, GSH concentration was significantly decrease by 25.00% and 12.50% at 200 and 400 µg/mL, respectively ($p < 0.05$), whereas the concentration at 600 µg/mL was not significantly different from metformin ($p > 0.05$) (Figure 7).

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The *Ex-vivo* exposure of rat liver tissue to the high-glucose medium produced a significant alteration in hepatic glucose concentration and antioxidant status, as evidenced by the significantly increase glucose and malondialdehyde (MDA) concentrations and the comparatively decrease activities of superoxide dismutase (SOD), catalase and concentration of reduced glutathione (GSH) observed in the negative control. This coordinated alteration indicates that excessive glucose availability was associated with oxidative imbalance in the liver tissue. Hyperglycaemia is known to enhance the generation of reactive oxygen species and to increase oxidative damage through several interrelated metabolic pathways. Consequently, excessive glucose exposure can overwhelm endogenous antioxidant defense mechanisms and promote lipid peroxidation[11]. The present findings therefore suggest that the high-glucose incubation condition successfully produced a hyperglycaemia-associated oxidative stress environment in the isolated liver tissue.

The glucose result showed that the negative control had the highest hepatic glucose concentration and was significantly different from all the other treatment groups. The positive control had a significantly decrease glucose concentration than the negative control, while the metformin-treated tissue showed a similar glucose concentration to the positive control. Treatment with *P. guajava* methanol leaf extract further reduced hepatic glucose concentration, with the 200 and 400 µg/mL concentrations producing significantly decrease values than the negative control. The 600 µg/mL extract produced the lowest glucose concentration compare to all the groups and was significantly lower than both the positive control and metformin-treated groups. This finding demonstrates a concentration-related glucose-lowering effect of *P. guajava* methanol leaf extract under the experimental conditions. The observation is consistent with previous reports demonstrating the antihyperglycaemic activity of *P. guajava* leaf preparations. There was a reported significant hypoglycaemic activity of *P. guajava* leaf extract [46], and a reviewed evidence supporting the ability of *P. guajava* leaf constituents to influence glucose metabolism was documented[47]. The result of research has shown that *P. guajava* leaf extract reduced blood glucose and improved antioxidant parameters in streptozotocin-induced diabetic rats [48].

The reduction in glucose cannot be attributed solely to systemic mechanisms such as reduced intestinal glucose absorption or increased pancreatic insulin secretion because the administration of the extract was done in an isolated liver tissue. The finding suggests that constituents of *P. guajava* may influence hepatic glucose handling directly or may modify intracellular metabolic processes involved in glucose utilization. Previous in-vivo investigations have demonstrated that guava leaf extract can improve glucose homeostasis and alter enzymes associated with glucose metabolism. In a streptozotocin-induced diabetic rat model, *P. guajava* leaf extract significantly reduced blood glucose and improved several biochemical abnormalities associated with diabetes[49]. Similarly, experimental work has reported antidiabetic effects of *P. guajava* leaf preparations, including effects on enzymes involved in glycogen metabolism [50].

The reduction in hepatic glucose concentration was accompanied by a significant decrease in MDA concentration. The negative control had the highest MDA concentration, indicating increased lipid peroxidation under the high-glucose condition. The positive control showed a significant decrease in MDA, whereas metformin produced a further decrease. Treatment with *P. guajava* extract produced a concentration-dependent decrease overall, with 600 µg/mL concentrations producing the lowest MDA values and does not significantly differ from that of metformin. The reduction in MDA suggests that *P. guajava* attenuated lipid peroxidation induced by the high-glucose environment. This result agrees with previous findings that the *P. guajava* leaf extract decrease hyperglycaemia and oxidative stress in streptozotocin-induced diabetic rats and improved antioxidant enzyme status [51].

The increased MDA concentration in the negative control may resulted from enhanced production of reactive oxygen species during excessive glucose exposure. MDA is a commonly used indicator of lipid peroxidation and therefore provides an indication of oxidative damage to cellular membranes. When reactive oxygen species exceed the capacity of endogenous antioxidant systems, membrane polyunsaturated fatty acids become susceptible to oxidative attack, resulting in the formation of lipid peroxidation products [52]. Thus, the decrease MDA concentrations in the *P. guajava*-treated groups suggest that the extract either reduced reactive oxygen species generation, enhanced their removal, protected membrane lipids from oxidation, or exerted a combination of these effects.

The SOD result provides additional evidence of improvement in hepatic antioxidant defense following *P. guajava* treatment. The negative control exhibited the lowest SOD activity and was significantly different from the positive control, metformin and all extract-treated groups. The metformin and 600 µg/mL extract groups had the highest SOD activities and they were not significantly different from each other. The 400 µg/mL extract also increase SOD activity, although it remained statistically different from the groups with the highest values. These findings may be attributed to the ability of the *P. guajava* extract to enhanced the enzymatic capacity of the liver tissue to deal with superoxide radicals. This is consistent with the report of Akhter and other, in which *P. guajava* leaf extract restored antioxidant enzymes in diabetic rats [48]. Jayachandran [51]also reported that diabetic rats treated with *P. guajava* leaf extract showed improvement in tissue antioxidant enzyme status.

Catalase activity showed a similar pattern. The negative control had the lowest catalase activity, while the positive control showed a significant increase. Metformin produced the highest catalase activity among the treatment groups, and the 600 µg/mL *P. guajava* extract produced a catalase activity that was statistically comparable with metformin. The 400 µg/mL extract also produced a significant increase in the activity of this enzyme and the 200 µg/mL extract produced a lowest activity compare to all the treated groups. The improvement in catalase activity is important because catalase is responsible for the decomposition of hydrogen peroxide into water and oxygen. Increased catalase activity therefore provides an important means of limiting the accumulation of hydrogen peroxide and subsequent formation of more reactive oxygen species. The present finding agrees with previous research that *P. guajava* leaf extract can restore hepatic antioxidant enzymes under diabetic conditions[48,51]

The GSH result further supports the antioxidant activity of the extract. The negative control had the lowest GSH concentration, whereas the positive control showed a significantly higher concentration. Metformin and the 600 µg/mL *P. guajava* extract produced the highest GSH concentrations and were statistically comparable. The 200 and 400 µg/mL extract treatments also increased GSH compared with the negative control, although their responses were lower than those of the metformin and 600 µg/mL groups. GSH is a major intracellular non-enzymatic antioxidant that participates in the maintenance of cellular redox balance and provides reducing equivalents for the detoxification of reactive oxygen species. Its depletion under high-glucose conditions may reflect increased utilization during oxidative stress, whereas its restoration following *P. guajava* treatment suggests improved capacity to maintain hepatic redox homeostasis.

The concurrent significant decrease in MDA and decrease in SOD, catalase and GSH are particularly important because they demonstrate that the effect of *P. guajava* was not limited to glucose regulation. Rather, the extract improves several interconnected components of the antioxidant defense system. This agrees with the research findings which showed that *P. guajava* leaf extracts possess antioxidant properties and glucose-modulating effects [53]. Extracts of *P. guajava* leaves exhibited strong antioxidant activity in DPPH, ferric-reducing and phosphomolybdenum assays, with the methanol fraction showing particularly strong activity [37].

The antioxidant effect of the methanol extract may be associated with the presence of phenolic and flavonoid constituents in *P. guajava* leaves. The reported substantial antioxidant activity of methanolic *P. guajava* leaf fractions was attributed the activity to their phenolic constituents [37]. Phytochemical investigations have also identified polyphenolic compounds such as quercetin, kaempferol and avicularin in *P. guajava* leaf extracts [54]. These compounds may contribute to antioxidant protection through radical scavenging and other mechanisms capable of limiting oxidative damage.

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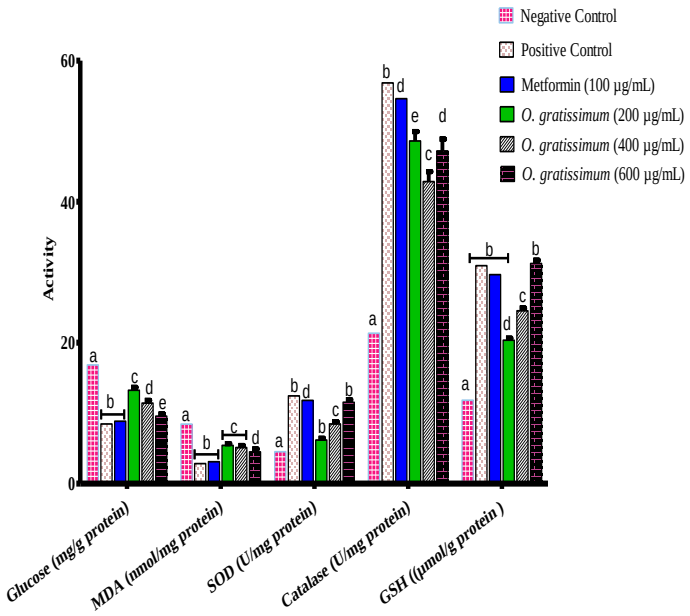
Effects of Ocimum gratissimum Methanol Leaf Extract on Glucose Concentration and Oxidative Stress Markers in Rat Liver

Ex-vivo exposure of the liver tissue to high-glucose Krebs-Henseleit buffer (negative control) significantly increased the glucose concentration by 88.89% when compared with tissue incubated in low-glucose Krebs-Henseleit buffer (positive control) ($p < 0.05$). Addition of *O. gratissimum* methanol leaf extract at 200, 400 and 600 µg/mL significantly decreased the glucose concentration by 17.65%, 35.29% and 41.18%, respectively, when compared with the negative control ($p < 0.05$). When compared with the positive control, the glucose concentrations at 200, 400 and 600 µg/mL were significantly higher by 55.56%, 33.33% and 11.11%, respectively ($p < 0.05$). The glucose concentration at 600 µg/mL was not significantly different from metformin ($p > 0.05$), whereas the values at 200 and 400 µg/mL were significantly higher than metformin by 55.56% and 22.22%, respectively ($p < 0.05$) (Figure 8).



Values are expressed as Mean ± SEM (n = 3). Bars with different superscripts within the same parameter are significantly different at $p < 0.05$

Figure 8: *Ex-vivo* Effects of *Ocimum gratissimum* Methanol Leaf Extract on Glucose Concentration and Oxidative Stress Markers in Rat Liver**.**

High-glucose *Ex-vivo* exposure also significantly increased the MDA concentration when compared with the positive control ($p < 0.05$), indicating increased lipid peroxidation under the high-glucose condition. Treatment with *O. gratissimum* methanol leaf extract at 200, 400 and 600 µg/mL decreased MDA concentration by 27.78%, 22.22% and 38.89%, respectively, when compared with the negative control. The MDA concentration at 200 µg/mL was not significantly different from the positive control ($p > 0.05$), whereas the concentrations at 400 and 600 µg/mL were significantly different from the positive control ($p < 0.05$). When compared with metformin, the MDA concentrations at 200 and 400 µg/mL were significantly higher, whereas the value at 600 µg/mL was not significantly different from metformin ($p > 0.05$) (Figure 8).

The antioxidant defence system was also affected by high-glucose exposure, as SOD activity was significantly decreased when compared with the positive control ($p < 0.05$). Treatment with *O. gratissimum* methanol leaf extract at 200, 400 and 600 µg/mL significantly increased SOD activity by 61.54%, 7.69% and 7.69%, respectively, when compared with the negative control ($p < 0.05$). The SOD activity at 200 µg/mL was significantly lower than the positive control, whereas the activities at 400 and 600 µg/mL were not significantly different from the positive control ($p > 0.05$). When compared with metformin, SOD activity at 200 µg/mL was significantly higher, while the activities at 400 and 600 µg/mL were not significantly different from metformin ($p > 0.05$) (Figure 8).

A similar pattern was observed for catalase activity. Liver tissue maintained under the high-glucose condition showed a significant decrease in catalase activity when compared with the positive control ($p < 0.05$). Treatment with *O. gratissimum* methanol leaf extract at 200, 400 and 600 µg/mL significantly increased catalase activity by 138.10%, 104.76% and 123.81%, respectively, when compared with the negative control ($p < 0.05$). Catalase activity at 200 µg/mL remained significantly lower than the positive control, whereas the activities at 400 and 600 µg/mL were also significantly lower than the positive control ($p < 0.05$). When compared with metformin, catalase activity at 200, 400 and 600 µg/mL was significantly decrease by 10.91%, 18.18% and 10.91%, respectively (Figure 8).

The reduced glutathione status further demonstrated the effect of the extract on the hepatic antioxidant system. High-glucose exposure significantly decreased GSH concentration by 61.29% when compared with the positive control ($p < 0.05$). Treatment with *O. gratissimum* methanol leaf extract at 200, 400 and 600 µg/mL significantly increased GSH concentration by 75.00%, 108.33% and 166.67%, respectively, when compared with the negative control ($p < 0.05$). The GSH concentration at 200 µg/mL was significantly lower than the positive control, whereas the concentration at 400 µg/mL was also significantly lower than the positive control. The value at 600 µg/mL was not significantly different from the positive control ($p > 0.05$). When compared with metformin, GSH concentration at 200 and 400 µg/mL was significantly lower, whereas the value at 600 µg/mL was not significantly different from metformin ($p > 0.05$) (Figure 8).

Ex-vivo exposure of rat liver tissue to the high-glucose Krebs-Henseleit buffer produced a coordinated disturbance in hepatic glucose homeostasis and oxidative status, characterised by increased glucose and malondialdehyde (MDA) concentrations and reduced activities or concentrations of the antioxidant markers, superoxide dismutase (SOD), catalase and reduced glutathione (GSH). This pattern is consistent with the established relationship between excessive glucose availability and oxidative stress. Hyperglycaemia can promote oxidative stress through several interconnected pathways, including glucose auto-oxidation, mitochondrial reactive oxygen species generation, activation of the polyol and protein kinase C pathways and increased formation of advanced glycation end products [11] In hepatic tissue, persistent oxidative stress impair cellular redox balance and promote lipid peroxidation, thereby increasing oxidative products such as MDA[55]. The present findings therefore suggest that incubation of the liver tissue in the high-glucose medium successfully produced a metabolically stressful condition characterised by impaired glucose regulation and antioxidant imbalance.

The increased glucose concentration observed in the negative control indicates that exposure of the liver tissue to the high-glucose medium resulted in greater glucose availability within the experimental system. In contrast, treatment with *Ocimum gratissimum* methanol leaf extract reduced hepatic glucose concentration, with the higher extract concentrations producing greater decrease. This finding suggests that the extract may enhance hepatic glucose handling under hyperglycaemic conditions and is consistent with previous reports of the hypoglycaemic activity of *O. gratissimum*. Research has reported that ethanolic leaf extract of *O. gratissimum* significantly reduced plasma glucose concentrations in both normal and alloxan-induced diabetic rats[56]. Researcher has also reported the significant antidiabetic activity of *O. gratissimum* leaf fractions in a rat model of type 2 diabetes, with the n-butanol fraction producing substantial improvement in fasting blood glucose and insulin sensitivity [57]. These findings revealed that constituents of *O. gratissimum* possess glucose-lowering properties.

The glucose-lowering effect observed in the present study may be associated with phenolic constituents of *O. gratissimum*. Several phenolic compounds, which include L-caftaric acid, L-chicoric acid, eugenyl-β-D-glucopyranoside and vicenin-2, in an *O. gratissimum* leaf extract and were identified and demonstrated hypoglycaemic activity of its fractions [58]. Chicoric acid was identified as one of the active constituents capable of significantly reducing glycaemia in diabetic mice. The authors also suggested that other constituents may

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contribute to the overall hypoglycaemic effect of the plant [59]. Therefore, the glucose-lowering activity observed in the present *Ex-vivo* experiment may reflect the combined action of several bioactive constituents rather than a single compound.

The reduction in glucose concentration was accompanied by an improvement in the oxidative stress profile of the liver tissue. MDA, a commonly used indicator of lipid peroxidation, was increased in the high-glucose negative control, whereas treatment with *O. gratissimum* extract reduced MDA concentration. Increased MDA under hyperglycaemic conditions reflects enhanced oxidative modification of membrane lipids and is consistent with the ability of excessive glucose metabolism to increase reactive oxygen species generation. Hyperglycaemia-induced oxidative stress may initiate lipid peroxidation, resulting in the accumulation of secondary products such as MDA and other aldehydes [11]. The reduction in MDA following *O. gratissimum* treatment therefore suggests that the extract attenuated lipid peroxidation in the liver tissue. Similar antioxidant effects have previously been reported for *O. gratissimum*. The leaf extract of *O. gratissimum* was reported to reduce MDA and increase glutathione in an experimental model of inflammation, supporting its ability to modulate oxidative stress [60].

The decrease in SOD activity observed in the hyperglycaemic negative control further supports the presence of oxidative stress in the liver tissue. Under conditions of excessive reactive oxygen species production, depletion or impairment of antioxidant enzymes may reduce the capacity of the tissue to neutralise oxidative intermediates. Diabetes-associated liver injury have been identified to reduce SOD and catalase activities as important consequences of oxidative stress in hepatic tissue [61]. In the present study, treatment with *O. gratissimum* methanol leaf extract improved SOD activity, suggesting restoration of an important component of the hepatic antioxidant defense system.

The improvement in catalase activity following treatment with *O. gratissimum* provides further evidence of enhanced antioxidant protection. An increase in catalase activity following extract treatment could facilitate more efficient removal of hydrogen peroxide generated during oxidative metabolism. This result agrees with the findings of [56], who reported significant dose-dependent increases in hepatic SOD and catalase activities following administration of *O. gratissimum* leaf extract to rats. This relationship between their findings and the present *Ex-vivo* observation strengthens the suggestion that *O. gratissimum* contains constituents capable of supporting hepatic antioxidant defense.

The increase in GSH concentration following treatment is also important because GSH represents a major non-enzymatic antioxidant involved in maintaining intracellular redox balance. GSH participates directly in the neutralisation of reactive oxygen species and serves as a substrate for glutathione-dependent antioxidant enzymes. Depletion of hepatic GSH has been associated with increased oxidative damage under hyperglycaemic conditions, whereas preservation of GSH contributes to cellular protection against oxidative injury [11]. Therefore, the increased GSH concentration observed following *O. gratissimum* treatment suggests that the extract may help maintain the reducing environment of the liver during high-glucose exposure. The simultaneous increase in SOD, catalase and GSH, together with the reduction in MDA, is important because it indicates that the effect of the extract was not restricted to a single antioxidant parameter but involved several components of the hepatic antioxidant defense system.

The antioxidant effects observed in the present study may be attributable to the polyphenolic composition of *O. gratissimum*. [58] reported that methanol extract of *O. gratissimum* leaves contained appreciable amounts of phenolics, flavonoids, flavonols and proanthocyanidins and demonstrated DPPH and ABTS radical-scavenging as well as ferric-reducing activities. The methanol extract had high phenolic and flavonoid contents among the extracts examined [58]. These compounds can contribute to antioxidant activity through radical scavenging and other mechanisms that help limit oxidative damage.

The phytochemical basis of the observed antioxidant effect was provided by [62], who identified caffeic acid, rutin, ferulic acid, apigenin and quercetin in the methanolic *O. gratissimum* leaf extract [58]. Several of these compounds possess recognised antioxidant properties and could contribute to the reduction in oxidative stress observed in the present experiment. Thus, the improvement in MDA, SOD, catalase and GSH following treatment may reflect the collective antioxidant activity of several phenolic and flavonoid constituents present in the methanol leaf extract rather than the action of a single compound.

The concentration-related improvement observed with *O. gratissimum* treatment further suggests that the biological response was influenced by the amount of extract present in the incubation medium. The higher extract concentrations generally produced greater decrease in glucose concentration and improve the antioxidant status than the lower concentration. A concentration-related response is biologically plausible because increasing extract concentration may increase the availability of bioactive constituents capable of interacting with hepatic metabolic and redox pathways. Previous experimental work has reported dose-dependent enhancement of hepatic SOD and catalase activities following *O. gratissimum* administration [60].

Conclusion

The findings of this study demonstrate that methanol leaf extracts of *Psidium guajava* and *Ocimum gratissimum* possess antioxidant and antidiabetic properties, as evidenced by their concentration-dependent activities in the in-vitro assays. Both extracts exhibited free-radical scavenging activity, total antioxidant capacity, reducing power and hydroxyl radical scavenging activity, indicating their potential to counter oxidative processes. In the carbohydrate-digesting enzyme assays, both extracts inhibited α-amylase and α-glucosidase activities, with *P. guajava* generally demonstrating stronger inhibitory effects than *O. gratissimum*. The α-amylase inhibitory activity of *P. guajava* was comparable to that of acarbose at the tested concentrations. The ex-vivo liver experiments showed that both extracts reduced glucose and malondialdehyde concentrations while improving superoxide dismutase, catalase and reduced glutathione responses under high-glucose conditions. At selected concentrations, particularly 600 µg/mL, the responses to the extracts were comparable with those of metformin for some measured parameters. These findings suggest that the two plant extracts possess promising antioxidant, carbohydrate-digesting enzyme inhibitory and hepatic protective potential under the experimental conditions. Further studies are required to identify the bioactive constituents, establish their mechanisms of action, evaluate their safety and efficacy in appropriate animal models, and determine their potential application in the management of hyperglycaemia-associated oxidative stress.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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