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PHYTOCHEMICAL COMPOSITION, ANTIMICROBIAL ACTIVITY, ANTIOXIDANT POTENTIAL, AND ACUTE TOXICITY STUDIES OF *PSIDIUM GUAJAVA* (GUAVA) LEAF EXTRACT

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ABSTRACT

Plant species have been used in treatment of microbial infections as well as other health conditions due to their medicinal properties. The aim of this study was to investigate antimicrobial activity, antioxidant potential, and acute toxicity studies on *Psidium guajava* leaf extract. Qualitative phytochemical composition was determined in accordance with reference experimental protocols. The antimicrobial assays were done using eleven bacterial strains and a fungal strain. The determination of minimum inhibition concentrations (MICs) and minimum bactericidal concentrations (MBCs) were carried out by broth dilution method using 96 well plates. The extract was also evaluated for its antioxidant property by employing the DPPH radical scavenging method and the total antioxidant capacity test. The toxicity study of *Psidium guajava* extract was carried out in Sprague-Dawley rats (n= 6). The phytochemical screening revealed the presence of cardiac glycosides, saponins, tannins, flavonoids, reducing sugars, alkaloids, and terpenoids. The results of the antimicrobial study showed appreciable antimicrobial activity against the bacteria strains used, with the mean MIC values from 1.563 mg/mL to 12.50 mg/mL and MBC values ranged from 12.50 mg/mL to 50.00 mg/mL. For antioxidant potential of the extract, scavenging activity increases with increasing concentration. The antioxidant activity of the extract became comparable to ascorbic acid at the highest concentration tested (500 µg/mL), demonstrating concentration-dependent scavenging effectiveness. Acute toxicity study indicated no noticeable adverse effects on experimental animals. This suggests that the LD_{50} of the extract could exceed 5000 mg/kg. *Psidium guajava* leaf is a potential source of a novel antimicrobial and antioxidant agents.

KEYWORDS: *Psidium guajava*, antimicrobial activity, antioxidant potential, acute toxicity, medicinal plants, plant extract, rats.

I. INTRODUCTION

Medicinal plants remain a reliable source of active drugs due to the presence of secondary metabolites. These metabolites are the main sources of biological activity of plant extracts.[1,2,3] Currently, there has been increasing resistance pattern of pathogenic microbes to commonly used antimicrobial drugs in most parts of the world and this has made most scientists to investigate more into plant secondary metabolites in order to discover new therapeutic agents.[4] It is now obvious that, if care is not taken, pathogenic microbes will endanger our health care system.

According to World Health Organization (WHO), about 80 % of the global population, especially those in developing countries, largely depend on medicinal plants for treatment of so many infections.[5] Studies have shown that secondary metabolites in medicinal plants are responsible pharmacological properties such antimicrobial property.[3], anticancer activity, anti-inflammatory property and antioxidant activity.[6]

There are so many medicinal plants, which are used to treat many health conditions and one of these plants is *Psidium guajava*.[7] *Psidium guajava* leaves are used traditionally to treat diarrhea, dysentery, gastroenteritis, fever, diabetes, pain, hypertension, wounds and caries.[7] *Psidium guajava* leaves and seeds are used in Mexico, Africa, Asia and Central America for treating many health conditions.[7]

In Ghana, aqueous extract of *Psidium guajava* leaves are used traditionally to treat all kind of infections including diarrhea in both children and adults. In addition, the decoction of *Psidium guajava* leaves is used to treat malaria and bacterial infections.

Even though, *Psidium guajava* leaves are used extensively in Ghana, there is little scientific studies on it. Therefore, this study has been designed to investigate antimicrobial and antioxidant properties of *Psidium guajava* leaf extract and also to evaluate its acute toxicity profile in rats.

II. MATERIALS AND METHODS

2.1 Plant Collection and Identification

The *Psidium guajava* leaves were collected at Ejisu in the Ashanti Region, in November 2023. The plant sample was identified and authenticated by a horticulturist, Mr. Clifford Osafo Asare at the Department of Herbal Medicine, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. The specimen was deposited in the herbarium of the department with a voucher number KNUST/HMI/L019/2023.

2.2 Sample Preparation and Extraction

The fresh *Psidium guajava* leaves were air dried for two weeks were pulverized by an electronic grinding mill and the powdered sample was then extracted with methanol. The powered sample (500 g) was extracted with 1.8 L of methanol for three days by cold maceration. The filtrate was decanted and filtered using Whatman filter paper 1. After filtration, the filtrate was concentrated to dryness by using Buchi rotavapour R-210 with vacuum pump V-710V and water bath B-491 at 40 ^{o}C.

2.3 Phytochemical Screening of the Extract

The methanolic extract of *Psidium guajava* leaf was screened for some major secondary metabolites such as cardiac glycosides, saponins, tannins, flavonoids, cyanogenic glycosides, reducing sugars, alkaloids, terpenoids, and coumarins using standard procedures.[8]

2.3.1Test for Cardiac Glycosides

Five (5) mL of distilled water added to 0.3 g of the extract. Four drops of glacial acetic were added to the content, followed by three drops of iron (III) chloride solution. Finally, few drops of concentrated H_2SO_4 were added. The formation of a brown colour at the interface indicated the presence of cardiac glycosides.

2.3.2 Test for Saponins (Frothing Test)

About 8 mL of distilled water was added to 0.2 g of the extract in a test tube. The content was then shaken continuously for 30 seconds. The formation of persistent foam or froth column of about 2 cm in height showed the presence of saponins.

2.3.3 Test for Tannins

Three (3.0) mL of 5 % $FeCl_3$ was added to 0.3 g of the extract in a test tube. The development of a bluish black colour signifies the presence of tannins.

2.3.4 Test for Terpenoids/Steroids (Salkowski's Test)

About 3.0 mL of chloroform was added to 0.3 g of the extract in a conical flask. The content was then shaken and the mixture was filtered. Four (4) drops of Salkowski's reagent were added to the filtrate. The appearance of a reddish colour at the interface indicated the presence of terpenoids.

2.3.5 Test for Coumarins

A 0.5 g of the extract was placed in a test tube and 10 mL of chloroform was added to it and the mixture filtered. Hot distilled water was used to treat the residue and allowed it to cool. About 1 mL of 10 % NH_3 was added to the resulting solution and observed under UV light at 365 nm. The development of intense blue green colour under UV light shows the presence of coumarins.

2.3.6 Test for Alkaloids

Two milligrams (2 mg) of the extract was dissolved in 4 mL of 1% hydrochloric acid with vigorous stirring in a water bath. The mixture was filtered and 4 drops of Dragendrof's reagent were added. The formation of a reddish precipitate indicated the presence of alkaloids.

2.3.7 Test for Reducing Sugars

About 1.0 mL of Fehling's solution was put into a test tube and 1.0 mL of the extract was added to the solution and heated in a water bath. The formation of a brick red precipitate indicates the presence of reducing sugars.

2.3.8 Test for Cyanogenic Glycosides

A strip of sodium picrate paper was suspended by means cork in the neck of a test tube containing 0.4 g of the sample was warmed gently on a water bath. The release of hydrocyanic acid made the paper to change colour indicating the presence of cyanogenic glycosides.

2.3.8 Test for Flavonoids

One hundred milligram of the extract was dissolved in 4 mL of methanol and pieces of magnesium chips were added, followed by addition of 4 drops of concentrated hydrochloric acid. Pink or red colouration of the solution.

2.4 Antimicrobial Assay

2.4.1 Microbial Strains

The standard reference microbial strains were obtained from Microbiology Laboratory of the Department of Pharmaceutics, School of Pharmacy and Pharmaceutical Sciences, University for Development Studies.

2.4.2 Inoculum Preparation of Test Microorganisms

The Gram-positive and Gram-negative bacteria were streaked on 20 mL Sterile Nutrient Agar plates and the fungus strain was streaked on 20 mL Sterile Sabouraud Dextrose Agar (SSDA) plate. They were incubated at a temperature of 37 ^{o}C and their colonies were fished and suspended in sterile water of 10 mL in test tubes and each turbidity was compared to the 0.5 McFarland standard and read with the eye.

2.4.3 Preparation of the Extract and Standard Drugs for Minimum Inhibitory Concentration

Two-fold serial dilutions of the guava leaf extract were prepared in single sterile Mueller Hinton broth (100 - 0.781 mg/mL). The standard drugs (ciprofloxacin and fluconazole) used in the assay were also prepared in two-fold serial dilutions with concentration range of 25 µg/mL to 0.195 µg/mL. A 180 µL of the prepared concentrations of the guava leaf extract and the standard drugs were transferred into separate 96 well bottom flat microtiter plates. After 24 h of sub-culturing the organisms, 20 µL of diluted form of each organism was taken and the diluted form was compared to 0.5 McFarland turbidity standard before adding 180 µL of the prepared concentrations of the extract or the standard drugs. All the plates were incubated at 37 ℃ for 24 h. After incubation, minimum inhibitory concentrations against test microbes were determined by addition of 20 µL (0.125 %) tetrazolium bromide solution. The wells were observed for inhibition or killing of microbial growth after 30 minutes of addition of the dye. All wells that stained purple colour indicate microbial growth and wells which remained yellow are indicative of inhibition.[9]

2.4.4 Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) of the Extract

The minimum bactericidal and fungicidal concentrations were determined by sub-culturing 100 µL of all negative or yellow cultures contained in wells from the minimum inhibitory concentration determinations against test microbes into another fresh100 µL sterile broth in 96 well plate. The plate was incubated at 37 ℃ for another 24 h and the results were observed for specific concentrations that kill 99.9 % growth of the test microorganisms and recorded.[9]. All the assays were performed in triplicate.

2.5 Determination of Total Phenolic Content in the Extract

The total phenolic content of the extract was determined by using Folin–Ciocalteu method.[10] To about 2 mL of prepared concentrations (500, 250, 125, 62.5, 31.25, 15.625, 7.8125 and 3.90625 µg/mL) of the guava extract, 4.5 mL of Folin–Ciocalteu reagent (diluted 1:10 in methanol) was added and allowed to stand for 5 minutes at room temperature and then 7.5 mL of 20 % Na_2CO_3 was added. The mixtures were shaken to form homogenous solutions and then incubated in the dark for 2 h. After which 250 uL each of the mixtures was pipetted into a 96-bottom flat plastic micro-well plate followed by the absorbance measurement at 760 nm against a blank using a spectrophotometer (microplate reader), Infinite M200, Tecan, Austria. The results were expressed in milligrams equivalents of gallic acid per milligram of dry weight. The calibration curve was established by using 500, 250, 125, 62.5, and 0.00 µg/mL of gallic acid as a standard under comparable conditions.[11] The experiment was repeated in triplicates.

2.6 Screening for DPPH Antioxidant Activity of the Extract

The antioxidant capacity of the extract was evaluated using a standard DPPH radical scavenging activity assay.[12] Test tubes were serially made with different concentrations of the extract and ascorbic acid (500 µg/mL, 250 µg/mL, 125 µg/mL, 61.25 µg/mL, 31.25 µg/mL, 15.625 µg/mL, 7.8125 µg/mL, and 3.90525 µg/mL). A 9.0 mL of 0.3 mM DPPH was freshly prepared in methanol and then combined with 1.0 mL each of the prepared concentrations in newly cleaned test tubes. The resulting solutions were incubated for 30 minutes at room temperature in the dark. A 200 µL of each resulting solution was pipetted into a round bottom flat 96-well plate, and the absorbance was measured using a microplate reader, Infinite M200, Tecan, Austria at 517 nm. The experiment was repeated in triplicates. The following formula was used to determine the DPPH scavenging activity:

$$Percentage\ Scanvenging\ ability = \frac{(A_{control} - A_{test\ sample})}{A_{control}} x100$$

$A_{control}$ denotes mean absorbance of the negative control.

$A_{test\ sample}$ denotes mean absorbance of the extract or ascorbic acid (standard).

2.7 Acute Toxicity Studies

2.7.1 Animals Used

The animals used were Sprague-Dawley Rats (SDR) of weights ranging from 190-250 g of both sexes. These animals were obtained from the animal house of Center for Plant Medicine Research, Mampong-Akwapim, Ghana. The entire experiment was conducted at the same animal house with the help of the experts in the animal house. All the animals used in this study were humanely handled during the entire experimental period, by following the Animal Welfare Regulations and the Public Health Service Policy on Humane Care and Use of Laboratory Animals. Moreover, the studies on the animals were conducted with the approval of the Research and Ethics Committee of University for Development Studies, Tamale, Ghana (UDS/RB/176/24).

2.7.2 Acute toxicity Study

The Organization for Economic Cooperation and Development (OECD) 425 guideline for testing chemicals was used to assess the acute toxicity profile of the extract.[13] The Sprague-Dawley Rats (SDR) of both sexes were used in the study. The rats were selected randomly and put into a group of six. The experimental rats were allowed to acclimatize to the laboratory environment for one week and fasted for one day with access to water. The rats were weighed before oral administration of the extract in doses of 500 mg/kg (Group II), 1000 mg/kg (Group III), 2000 mg/kg (Group IV), 3000 mg/kg (Group V), and 5000 mg/kg (Group VI). The positive control group (Group I) received 10 mL/kg of distilled water. The experimental rats were observed closely for weight loss, diarrhea, unusual locomotion, skin and fur position, lethargy, salivation, reactivity to touch, coma, eye colour, sleep, urination and death at 0 min, 30 min, 60 min, 120 min and 200 min, 24 h and 14 days after administration of the extract.

2.8 Statistical Analysis

Data analysis was done by using SPSS software package, SPSS IBM version 23 for windows and the results expressed as mean ± standard deviation.

III. RESULTS AND DISCUSSION

3.1 Phytochemical Composition

The phytochemical screening of *Psidium guajava* leaf extract revealed the presence of secondary metabolites such as cardiac glycosides, saponins, tannins, flavonoids, reducing sugars, alkaloids, and terpenoids. However, coumarins and cyanogenic glycosides were absent. These phytochemicals have pharmacological properties. The presence of these phytochemical constituents in guava leaf may be attributed to the claim that guava leaves are used traditionally to treat diarrhea, gastrointestinal issues, fever, cough, menstrual disorders, fertility problems, respiratory issues, and wound healing.[7]

The summary of the results from the phytochemical screening carried out on the methanolic extract from guava leaves are presented in Table 1.

Table 1: Phytochemical constituents in *Psidium guajava* leaf.

Phytochemicals	Results
Cardiac glycosides	+
Saponins	+
Tannins	+
Flavonoids	+
Reducing sugars	+
Coumarins	-
Cyanogenic glycosides	-
Alkaloids	+
Terpenoids	+

Key: (+) present and (-) absent.

3.2 Antimicrobial Activity

The results of the antimicrobial study revealed that the methanolic extract of guava leaf demonstrated appreciable antimicrobial activity against the reference strains of bacteria used, with the mean MIC range of 1.563 mg/mL to 12.50 mg/mL. Also, the MBC values obtained against the same strains of bacteria ranged from 12.50 mg/mL to 50.00 mg/mL. Moreover, it was observed that, the standard drug for antibacterial assay showed MIC and MBC values from 1.563 µg/mL to 6.25 µg/mL, except *E. coli ESLB* which recorded MBC >25.00 µg/mL. For the *C. albicans*, fluconazole recorded the mean MIC value of 12.50 µg/mL and MBC >25.0 µg/mL. Among the bacteria screened, the most susceptible strain to the extract was *B. cereus* with MIC of 1.563 mg/mL. However, the less susceptible strains were *S. aureus* and *P. aeruginosa* with MIC values of 12.50 mg/mL. It was observed that, *E. coli ESLB* was more susceptible to the test drug at 6.25 µg/mL but quite resistant the ciprofloxacin. For the fungal strain, the guava leaf extract at 6.25 mg/mL was more active against *C. albicans* than the reference drug fluconazole for the MIC (Table 2). A similar result was also recorded for MFC against the fungal strain, *C. albicans*. Moreover, it was observed that, *N. gonorrhoeae* was highly susceptible to the extract at 3.125 mg/mL but quite resistant to the reference drug. This means that isolating phytochemicals from the extract may lead to the discovery of potent antimicrobial agents for treating *N. gonorrhoeae*, *E. coli ESLB* and *C. albicans* infections. This is in line with the reported literature which indicates that tannins and saponins have antimicrobial activities and these secondary metabolites can be found in guava leaf.[14,15] Additionally, terpenoids have both antibacterial and antifungal activities and are used to treat microbial infections [16]. It therefore means that the presence of these secondary metabolites could be responsible for excellent antibacterial and antifungal activities exhibited by the plant extract. Table 2, presents the summary of the results.

Table 2: MIC and MBC values of guava leaf extract and standard drug recorded against test organisms

Test organisms	Extract/Standard drugs						
	Extract (mg/mL)			Ciprofloxacin (µg/mL)		Fluconazole (µg/mL)	
	MIC	MBC	MFC	MIC	MBC	MIC	MFC
S. aureus (ATCC 25923)	12.5	25.0	NA	0.195	3.125	NA	NA
MRSA	6.25	25.0	NA	0.195	3.125	NA	NA
E. faecalis (ATCC 29212)	6.25	25.0	NA	0.195	6.25	NA	NA
P. aeruginosa (ATCC 4853)	12.5	50.0	NA	3.125	6.25	NA	NA
E. coli ESLB (NCTC 13351)	6.25	50.0	NA	12.5	>25.0	NA	NA
S. typhi (ATCC334538)	6.25	50.0	NA	0.39	1.563	NA	NA
S. typhimurium (ATCC13311)	6.25	25.0	NA	0.39	1.563	NA	NA
N. gonorrhoeae (NCTC12700)	3.125	25.0	NA	3.125	12.5	NA	NA
E. coli (ATCC 25922)	3. 125	25.0	NA	0.39	1.563	NA	NA
K. pneumomiae (ATCC7004603)	6.25	50.0	NA	0.39	1.563	NA	NA
B. cereus (NCTC10320)	1.563	12.5	NA	0.39	1.563	NA	NA
C. albicans (ATCC10031)	6.25	NA	25.00	NA	NA	12.50	>25.0

NA means not applicable.

3.3 DPPH Radical Scavenging Activity of *Psidium guajava*

The ability of an antioxidant to add an electron or a hydrogen atom to the DPPH radical, a stable, deep violet free radical is the basis for the DPPH assay. The solution's deep violet colour changes to a bright yellow hue when an odd electron partners with an antioxidant agent that scavenges free radicals, reducing the DPPH radicals to the corresponding hydrazine, or DPPH-H.[17]

The potential of *Psidium guajava* leaf extract to absorb free radicals that may be present in both humans and animals was determined and studied using the extract's DPPH scavenging activity. Methanolic extract of *Psidium guajava* and ascorbic acid (standard drug) of concentrations ranging between 3.90625 to 500.00 µg/mL.

The scavenging percentage of the extract increases from 14.26±0.73 % at 3.90625 µg/mL to 82.2984±0.6843 % at 500µg/mL whereas the scavenging activity of ascorbic acid, the reference standard used in this study increases from 14.47±0.20 % at 3.90625 µg/mL to 95.94±0.18 % at 500 µg/mL indicating that both the extract and ascorbic acid exhibit a concentration-dependent increase in scavenging activity. This is because as scavenging activity increases with increasing concentration for both substances as shown in Table 3.

The extract has limited antioxidant activity compared to ascorbic acid at lower concentrations, as the scavenging activity of ascorbic acid increases from 14.47±0.20 % to 18.38±3.09 % that of the extract also increases just slightly from 13.34±0.33 % to 14.84±0.26 % (3.90625 to 15.625 µg/mL). However, as the concentration increases, both the extract and ascorbic acids show an increasing scavenging activity with ascorbic acid demonstrating much stronger scavenging ability than the extract.

The extract's antioxidant activity becomes comparable to ascorbic acid only at the highest concentration tested (500 µg/mL), demonstrating concentration-dependent scavenging effectiveness. These findings suggest that the methanolic extract of *Psidium guajava* leaf possesses antioxidant properties, but with lower potency than ascorbic acid, particularly at lower concentrations.

According to previous studies, essential oils extracted from guava leaves were found to function as moderate antioxidant with an IC_{50} value of 460.37 ± 1.33 µg/mL.[18] This value is higher than the obtained in this study (263.74±2.16 µg/mL). This difference could be explained on the basis that an isolated compound was used in the reported literature. This result however, conforms to a finding in literature which suggests that, extracts of guava in water and organic solvents have a large quantity of antioxidants which can stop the oxidation reaction and the concentration of the compounds in the extracts become high with increase in concentration [19]. Guava fruits and the leaves are a great source of antioxidants, which can help lower the prevalence of degenerative illnesses like arthritis, cancer, heart disease, inflammation, and brain dysfunction.[19]

The extract from guava leaves has antioxidant property and this study has opened up a new therapeutic avenue for treating a variety of diseases and complications. However, further investigations are required in this regard to find the actual mechanism involved in antioxidant and other pharmacological activities of guava leaf extract. Table 3 presents the summary of the radical scavenging percentage values of the extract of *Psidium guajava* and ascorbic acid.

Table 3: Radical scavenging percentage (%) of the extract of *Psidium guajava* and ascorbic acid across a range of concentrations

Concentration (µg/mL)	Scavenging Percentage (%)	
	Extract	Ascorbic acid
3.90625	13.34±0.33	14.47±0.20
7.8125	14.26±0.73	15.94±0.19
15.625	14.84±0.26	18.38±3.09
31.25	14.90±0.23	28.03±0.28
62.5	18.37±0.08	50.88±0.30
125	30.28±0.19	82.50±0.43
250	51.96±0.13	88.25±0.27
500	82.29±0.68	95.94±0.18

3.4 Total Phenolic Content of Methanolic Extract of *Psidium guajava* Leaf

Gallic acid was used as the standard in order to calculate the total phenolic content of the extract using the Folin-Ciocalteu (F-C) method. The absorbance values obtained at different gallic acid concentrations (500, 250, 125, 62.5, and 0.00µg/mL) were used to produce the calibration curve. Phenolic chemicals transport electrons to phosphomolybdic phosphotungstic acid complexes in alkaline environments, generating blue-colored complexes known as PMoW11O40)-4, which are detected spectrophotometrically at 765 nm. This is the basis for the F-C method.[17]

Total phenolic content of the extract was calculated from the regression equation of calibration curve, A = 0.003467*C + 0.327963 and R^2 = 0.953127, where A is absorbance and C is the concentration. Table 4 shows the results of total phenolic content of the extract and expressed as mg gallic acid equivalents (GE) per gram of sample in dry weight (mg GAE/g).

The total phenolic content (TPC) of *Psidium guajava* in mg GAE/g of dry extract is presented across a range of concentrations from 3.90625 to 500 µg/mL, with values increasing as concentration also increases. This trend indicates a concentration-dependent relationship, where higher concentrations yield higher phenolic content values.

Similar to the radical scavenging activity results, the TPC data for the extract also showed a concentration-dependent trend, with scavenging percentages rising with higher concentrations. This correlation between higher TPC values and increased radical scavenging activity suggests that the phenolic compounds in *Psidium guajava* leaf extract contribute significantly to its antioxidant capacity.

Table 4: Total phenolic content (TPC) of *Psidium guajava* in mg GAE/g of dry extract across a range of concentrations.

Concentration	Average TPC
3.90625	3.94±1.59
7.8125	38.89±1.64
15.625	54.65±2.29
31.25	56.18±0.15
62.5	58.21±1.17
125	58.64±3.43
250	63.49±2.59
500	67.91±2.11

3.5 Acute Toxicity Profile of *Psidium guajava* Leaf Extract

Preliminary acute toxicity study of methanolic extract of *Psidium guajava* leaves at doses of 500, 1000, 2000, 3000, and 5000 mg/kg showed no noticeable adverse effects. Also, no mortality of the experimental animals was recorded during the observational period of 14 days. This suggests that LD_{50} value of the extract could exceed 5000 mg/kg, meaning methanolic extract of guava leaves is not toxic. The other parameters like abnormal movement, sensitivity to touch, diarrhea, urination patterns, changes in eye colour, skin and fur condition, excessive salivation, and coma were either absent or normal. This implies the extract did not cause any significant adverse effects even as the dosage increased. Also, lack of diarrhea in the experimental rats further supports the fact that test drug did not cause gastrointestinal toxicity. Overall, the absence of observable toxic effects at any of the administered dose levels points to a favourable acute toxicity profile. These findings about the safety profile of *Psidium guajava* leaf extract in this study agrees with previous reports.[20]

Table 5 presents the summary of physical appearance and behavioural signs recorded during the observational period.

Table 5: General appearance and behavioural observations of the experimental rats for control and tested groups (mg/kg).

Toxicity signs	Control	500	1000	2000	3000	5000
Mortality	0	0	0	0	0	0
Eye colour	Nm	Nm	Nm	Nm	Nm	Nm
Coma	-	-	-	-	-	-
Salivation	Nm	Nm	Nm	Nm	Nm	Nm
Sleep	Nm	Nm	Nm	Nm	Nm	Nm
Unusual locomotion	-	-	-	-	-	-
Weight loss	-	-	-	-	-	-
Lethargy	Nm	Nm	Nm	Nm	Nm	Nm
Diarrhea	-	-	-	-	-	-
Skin & fur position	Nm	Nm	Nm	Nm	Nm	Nm
Urination	Nm	Nm	Nm	Nm	Nm	Nm
Reactivity to touch	Nm	Nm	Nm	Nm	Nm	Nm

The Table highlights physical appearance and behavioural observations of the experimental rats for acute toxicity study conducted. Number of rats per group, n = 6, 0 = No death, Nm = normal, - = absent

IV. CONCLUSION

The results of this study revealed that the extract of *Psidium guajava* leaf demonstrated appreciable antimicrobial activity against both Gram-positive and Gram-negative bacteria.

For antioxidant potential of the extract, scavenging activity increases with increasing concentration. The antioxidant activity of the extract becomes comparable to ascorbic acid at the highest concentration tested, demonstrating concentration-dependent scavenging effectiveness. Acute toxicity studies of the extract indicated no noticeable adverse effects on experimental animals. This suggests that the LD_{50} value of the extract could exceed 5000 mg/kg, meaning the methanolic extract of *Psidium guajava* leaf is not toxic. Hence, *Psidium guajava* leaf is a potential source of a novel antibacterial and antioxidant agents.

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