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Research Article

Phytochemical Screening and Antimicrobial Activity of Guava Leaf Fractions of *Psidium guajava* (Guava) Cultivated in Idah, Nigeria

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Abstract:
This study aimed to determine the phytochemicals and antimicrobial activities of *Psidium guajava* leaves. *Psidium guajava* (guava) belonging to the Myrtaceae family is an evergreen tree cultivated for its precious fruits and high biological properties. This research work was done to screen the phytochemical present and determine the antimicrobial properties of *Psidium guajava* leaf fractions. Ethanol solvent was used to extract the sample (using the cold maceration method), and the crude extract was further fractionated using n-hexane, acetone, and methanol solvents respectively. The screening of the phytochemicals (to test for alkaloids, tannins, steroids, flavonoids, polyphenols, terpenoids, and saponins) was done using the standard procedure, and evaluation of the antimicrobial activity of the plant was done using agar well diffusion method. The results showed that *Psidium guajava* leaf extracts contain all the phytochemicals tested, with an n-Hexane fraction containing Alkaloids, flavonoids, tannins polyphenols, and steroids. The acetone fraction reveals Alkaloids, tannins, terpenoids, polyphenols, and steroids, while the methanol fraction contains Alkaloids, flavonoids, terpenoids, tannins, and saponins. The results also showed *Psidium guajava's* promising antimicrobial properties, with the n-hexane fraction showing the greatest antimicrobial activity. This research has confirmed the widespread traditional medicinal applications, which is most prevalent among people living in northern Nigeria.

Keywords: Antimicrobial, Extracts, Phytochemical Screening, *Psidium Guajava* Leaf

1. INTRODUCTION

Research into the biological and chemical properties of natural products over the past 200 years has led to a rapid development in the medicinal chemistry, which is an important field for the development of new and potent therapeutic agents as well as the manufacturing of drugs to treat a variety of diseases (Carbonell-Capella et al., 2014). The range of chemical compounds that nature has produced as bioactive secondary metabolites in plants is astounding, and therefore it is crucial to carry out more and more bioactivity studies of plants and assess them both quantitatively and qualitatively in the search for new biomolecules (Sunday et al., 2020).

Psidium guajava belongs to the Myrtaceae family and it is an important herb in Nigeria, India, Indonesia, Pakistan, Bangladesh, and South America (Sharma et al., 2017). The leaves of the guava plant have been studied for their medicinal applications benefits which are attributable to their plethora of phytochemicals, such as quercetin, avicularin, apigenin, guaijaverin, kaempferol, hyperin, myricetin, gallic acid, catechin, epicatechin, chlorogenic acid, epigallocatechin gallate, and caffeic acid (Ashraf et al., 2016). Research has shown that preparations of the leaves of *Psidium guajava* have been used in medicine in several countries, mainly as anti-diarrheal remedy (Jiang et al., 2020). It has revealed that txtracts of Guava has been used to treat gastrointestinal diseases such as vomiting and simple diarrhea, and also in the treatment of wounds, caries, and are also widely used for their antispasmodic, cough sedative, anti-inflammatory, antidiarrheic, antihypertension, antiobesity, and antidiabetic properties (Mazumdar et al., 2015). Studies on animal models have also established the role of guava leaves isolates as potent antitumor, anticancer, and cytotoxic agents (Ashraf et al., 2016; Jiang et al., 2020). The presence of phenolic compounds, such as gallic acid, pyrocatechol, taxifolin, ellagic acid, ferulic acid, and several others, is responsible for the antioxidant roles

of guava leaves (Farag et al., 2020). High-performance liquid chromatography analysis of guava extracts revealed the presence of seven major flavonoids: quercetin, hesperetin, kaempferol, quercitrin, rutin catching, and apigenin, while other bioactive compounds, such as kaempfertin, isoquinoline, and corilaginoline alkaloids, were also identified (Taha et al., 2019).

Based on the description above, research was carried out with the aim of determining the phytochemical and antimicrobial activity of *Psidium guava* leaves.

2. MATERIAL AND METHOD

Sample Collection and Identification

Fresh leaf samples of *psidium guajava* were collected within the premises of Ajaka, Igalamela Local Government, Kogi State, Nigeria. This was done in the month of January, 2024. The leaf samples were identified by Botany unit within the department of Science Laboratory Technology, Federal Polytechnic Idah, Kogi State, Nigeria.

Sample Preparation

After identification, the leaf samples were thoroughly washed with distilled water to remove debris and contaminants, air-dried at room temperature and grounded into powder using mortar and pestle and stored in an air-tight and away from moisture until used (Deshpande et al., 2014).

Extraction procedure

Two hundred grams (200 g) of the powdered sample was soaked in 600 mL of ethanol (1/3 w/v) in a reagent bottle for seven days with constant shaking stirring. The sample mixture was then filtered using a whatman filter paper. The crude extract was allowed to evaporate to dryness (Deshpande et al., 2014).

Fractionation of the crude

The crude extract was further fractionated using three solvents of increasing order of polarity (n- hexane, acetone and methanol, respectively). The fractions were dried and named n-hexane fraction, acetone fraction, and methanol fraction (Deshpande et al., 2014).

Phytochemical Screening

The dried fractions were separately diluted with their respective fractionating solvents for the purpose of conducting phytochemical screening to test the presence of alkaloids, flavonoids, terpenoids, polyphenols, tannins, saponins, and steroids using the procedure explained below:

Test for Alkaloids (Mayer's test)

To test the presence of alkaloids, 2 drops of Mayer's reagent were added to 2 ml of each fraction. Creamy precipitate indicated the presence of alkaloids (Indhumathi et al., 2018).

Test of Flavonoids (Salkowski's test)

For flavonoids, 2 ml of sulfuric acid was added to 4 ml of each fraction. Formation of orange color confirmed the presence of flavonoids (Singh & Kumar, 2017).

Test of Terpenoids (Ferric Chloride test)

For terpenoids detection, 2 ml of chloroform and 3 ml of concentrated sulfuric acid were added to 4 ml of each fraction forming a layer. A reddish brown color confirmed the presence of terpenoids (Singh & Kumar, 2017).

Test of Tannins (ferric chloride test)

To test the presence of tannins, 3 drops of 1% ferric chloride were added to 1 ml of each fraction. Green precipitate confirmed the presence of tannins (Pandey & Tripathi, 2014).

Test of Saponins (Froth test)

For saponins, 1 ml of distilled water was added to 0.5 ml of each fraction and shaken vigorously. Formation of frothing confirmed the presence of saponins (De Silva et al., 2017).

Test of Polyphenols (Salkowskis's test)

In the case of polyphenols detection, 3 drops of 5% solutions of lead acetate was added to 1 ml of each fraction. Yellow precipitate indicated the presence of polyphenols (Pandey & Tripathi, 2014).

Test of Steroids (Salkowski's Test)

To test steroids, 2 ml of chloroform and 2 ml of concentrated sulfuric acid was added to 2 ml each fraction. The appearance of yellowish-green fluorescence indicated the presence of steroids (De Silva et al., 2017).

Test of glycosides (keller-Killian test)

To detect glycosides, 2 ml of chloroform and 2 ml of ammonia solution were mixed with 2 ml of each fraction. Pinkish color indicated the presence of glycosides (Ezeonu & Ejikeme, 2016).

Antimicrobial Procedure

Preparation of the Media

The media used were Nutrient Agar (NA) and Potato Dextrose Agar (PDA) for antibacterial and antifungal

screening, and were prepared according to manufacturer's instructions. The media were prepared by weighing 28 g of NA and 39 g of PDA (using digital weighing balance) and dispersed in a separate 1500 ml conical flasks that contained 1000 ml distilled water each, the conical flasks were immediately plugged with cotton wools and wrapped with aluminium foil and heated for complete dissolution. The dissolved media were sterilized by autoclaving at 121^0C for 15 minutes and then cooled at room temperature for 45 minutes and poured into sterilized petri-dishes, solidified for 24 hours before inoculation of the sample (Kebede et al., 2021).

Collection of pathogenic organism

The test Organisms consisting of two bacteria (*Staphylococus* aureus and *Actinomycetes*) and two fungi (*Aspergillus niger* and *Epicoccins*) were obtained from Microbiology unit of the Federal Polytechnic, Idah.

Standardization of the Inoculum

A suspension of the test bacteria was made by emulsifying loop full of colony into test tube containing normal saline solution. Inoculum density of the bacterial suspension was adjusted to that of 0.5 ml McFarland standard (Kebede et al., 2021).

Preparation of the Stock Solution

Stock solution was prepared by mixing 16 mg of each extract in 2 ml of dimethyl sulfoxide (DMSO) to give 8 mg/ml. Concentrations of 15 %, 30%, and 60 % were prepared using serial dilution method. Gentamycin (20 µg/ml) and Nystatin (50 µg/ml) were used as standards for bacterial and fungi respectively. Negative control was a solvent of dilution, Dimethyl Sulfoxide (Kebede et al., 2021).

Bioassay Procedure

After sterilization, the media were allowed to cool at 40 to 450C and poured into petri-dishes. The petri-dishes were labelled with culture name and the fractions to be used. A sterile cotton wool swab (swab stick) was dipped into the standardized bacterial and fungal suspension to seed the entire surface of the agar media. By the use of sterile Cork borer, 5 wells (6 mm in diameter) were stroke at the centre of each agar medium of each petri-dish. The wells created were then filled with the prepared fraction and incubated at 370C for 24 hours. The zone of inhibition, (in millimetres) was measured using a ruler (Kebede et al., 2021).

3. RESULT AND DISCUSSION

Weights and Color of the Crude Extract and Fractions

Table 1. Weight and color of the crude and the fractions

S/N	Extract	Weight (g)	Colour
1	Crude	13.00	Green
2	N-Hexane	4.00	Green
3	Acetone	4.25	Green
4	Methanol	4.75	Brown

Table 1 contains the weights and color of the crude extract and fractions. Yields of fractions were relative with rude because they were obtained from the crude during fractionation. It was shown that methanol fraction has highest yields among the three fractions, followed by acetone fraction, and then n-hexane fraction respectively. The methanol fraction was brown in color, while the crude, acetone and n-hexane were found to be green. Crude extract dissolved completely by methanol during fractionation process.

Phytochemical screening of the extract

Table 2. Phytochemical screenings of *Psidium guajava* leaf fractions

Phytochemicals	N-hexane	Acetone	Methanol
Alkaloids	-	+	+
Flavonoids	+	-	+
Terpenoids	+	+	-
Steroids	-	-	+
Saponins	+	+	-
Polyphenols	-	+	+
Tannins	+	+	+

Key: + =Present (positive) - = Absent (negative)

The phytochemical screenings, according to table 2 above, shows that the leaf fractions of *Psidium guajava* contains alkaloids, flavonoids, saponins, tannins, steroids, terpenoids, and polyphenols.

Alkaloids, steroids, and polyphenols were absent in n-hexane fraction but all present in methanol fraction. Terpenoids and saponins were absent in methanol fraction but present in n-hexane fraction. The only two phytochemicals not found in acetone fraction were flavonoids and steroids.

Antimicrobial Activity of Psidium guajava

Antibacterial Activity Psidium guajava

Table 3. Inhibition Zones of *Actinomycetes*

Inhibition zones	**Concentrations**		
Extracts	**15 %**	**30 %**	**60 %**
N-hexane (mm)	10	10	11
Acetone (mm)	8	10	11
Methanol (mm)	10	11	12

Key: Control = 15 mm

Table 3 above presents the results of antibacterial activity of the leaf fractions of *psidium guajava* against *Actinomycetes* bacteria in comparison with control, with methanol fraction showing greatest activity, followed by n-hexane and acetone fractions, respectively. *Actinomycetes spp* was susceptible to the guava leaf extract, with a zone of inhibition of 11 mm at 60% concentration for n-hexane fraction and acetone fractions, 10 mm at 30% concentration for n-hexane and acetone fractions, and 8 mm and 10 mm for acetone and n-hexane/methanol fractions at 15% concentration.

Table 4. Inhibitions Zones of *Staphylococcus aureus*

Inhibition zones	**Concentrations**		
Extracts	**15 %**	**30 %**	**60 %**
N-hexane	6.00 mm	8.00 mm	10.00 mm
Acetone	0.00 mm	0.00 mm	0.00 mm
Methanol	0.00 mm	6.00 mm	6.00 mm

Key: Control = 12 mm

Table 4 above presents the results of antibacterial activity of the leaf fractions of *Psidium guajava* against *Staphylococcus aureus* bacteria in comparison with control, with n-hexane fraction showing greatest activity, followed by methanol fraction. It was observed that acetone fraction did not show any activity against *Staphylococcus aureus*.

Antifungal Activity of Psidium guajava

Table 5. Inhibition Zones of *Aspergillus niger*

Inhibition zones	**Concentrations**		
Extracts	**15 %**	**30 %**	**60 %**
N-hexane	15.00mm	19.00mm	20.00mm
Acetone	15.00mm	19.00mm	21.00mm
Methanol	13.00mm	17.00mm	20.00mm

Key: Control = 25 mm

The inhibition zone of *psidium guajava* leaf fractions was presented in table 5. The results show a remarkable antifungal activity against *Aspergillus niger,* with acetone fraction showing largest zone of inhibitions against *Aspergillus niger,* followed by n-hexane and methanol fractions, respectively.

Table 6. Inhibition Zones of *Epicoccins*

Inhibition zones	**Concentrations**		
	15 %	**30 %**	**60 %**
N-hexane	10.00mm	15.00mm	18.00mm
Acetone	12.00mm	15.00mm	17.00mm
Methanol	10.00mm	14.00mm	17.00mm

The inhibition zone of *psidium guajava* leaf fractions was presented in table 6. The results also show a remarkable antifungal activity against *Epicoccins,* with n-hexane fraction showing largest zone of inhibitions against *Epicoccins,* seconded by acetone fraction, and then methanol fraction.

4. CONCLUSION

The phytochemical screening confirmed the presence of various phytochemicals, which include; alkaloids, saponins, tannins, flavonoids, glycoside, steroids, Polyphenol and terpeniods which are reported to have medical properties. The results also show that *guava* leaf extracts have antimicrobial activities against the bacterial and fungal isolates tested. This confirms the wide traditional medicinal applications of the plant, which is more noticeable in northern Nigeria.

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