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Antibacterial Activity of *Psidium guajava* Leaf and Stem Bark Extracts on Selected Bacteria in Ugbokolo, Benue State, Nigeria

Peter Adikwu[1*], Emmanuel Godwin Oyiwona[2,3], Adejor Johnson[2], Yuana Awua[1], Amina Ojochide Hassan[4], Terkaa Oluwakemisola Adenugba[2], Ene Theresa Ebiega[2]

[1]Biological Science Department, Benue State University, Makurdi, Nigeria
[2]Department of Science Laboratory Technology, Benue State Polytechnic, Ugbokolo, Nigeria
[3]Benue State Ministry of Energy, Science and Technology, Makurdi, Nigeria
[4]Department of Microbiology, Federal University of Agriculture, Makurdi, Nigeria
Email: *adikwupeter@gmail.com

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Abstract

Aim: In recent years, there has been a growing interest in researching and developing new antimicrobial agents from various sources to combat microbial resistance. The study was aimed at determining the phytochemical constituents and *in vitro* antibacterial activity of methanol and aqueous extracts of *Psidium guajava* leaves and stem bark on *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Proteus* sp. in Ugbokolo, Nigeria. **Materials and Methods:** The phytochemical screening of the plant materials for various bioactive components was conducted between July and December, 2019 using standard laboratory techniques. The extracts were purified using column chromatography. The identity of the test isolates were confirmed using morphological characteristics, gram stain, motility and appropriate biochemical tests such as indole, catalase, coagulase, triple sugar iron agar. The susceptibility of the isolates to each bioactive component was determined using the agar well diffusion method. The broth dilution method was employed for the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts. **Results:** The result of the study showed the presence of phenol, tannins, flavonoids and saponins as bioactive compounds. The antibacterial susceptibility of the isolates to aqueous and methanol extracts of leaf and stem bark of *Psidium guajava* varied significantly ($P < 0.05$). *Staphylococcus aureus* was the most susceptible isolate at 200 mg/ml concentration with average zone of inhibition of 13.05 mm for leaf extract and 15.34 mm for stem bark extract. *Proteus* sp. is the least susceptible with average zone of inhibition of 8.88 mm for the leaf extract and 12.36 mm for the stem bark extract respectively. Minimum Inhibitory Concentration

and Minimum Bactericidal Concentration of aqueous and methanol extract of *P. guajava* leaf and stem bark showed that dilutions of various concentrations of aqueous and methanol extracts can inhibit and/or kill the isolates. Lower MIC (3.125 mg/ml) was shown by methanol extract than aqueous extract. MBC of methanol extract ranges between 6.25 - 25.0 mg/ml. Statistical analysis of the result showed methanol extract is more effective than aqueous extract while the stem bark of the plant showed higher efficacy than the leaf. **Conclusion:** The findings of the study imply that the extract of *Psidium guajava* has shown promising properties against tested microorganisms. Further study of the extract is therefore recommended.

Keywords

Antibacterial, *Psidium guajava*, Leaf Extract, Stem Bark Extract

1. Introduction

Plants have been a source of medicinal agents for treating illnesses since the beginning of human civilization [1]. Recently, there has been a lot of attention focused on producing medicines and products that are natural [2]. Several leaves, stems and their extracts have been found to exhibit antimicrobial and antioxidant activities [1]. Researches into traditional plants and herbs have received a major boost due to increasing resistance of microorganisms to orthodox medicine [3]. Muhammad *et al.* [3] suggested that there is a need to search for new organic molecules of plants with antimicrobial properties.

The presence of bioactive components in medicinal plants makes them effective in treating human infections. These components are naturally occurring compounds in the medicinal plants such as tannins, saponins, flavonoids and they exhibit various important pharmacological activities against microorganisms, inflammation and cancer [4]. The World Health Organization (WHO) has categorized more than 20,000 plant species with medicinal properties and their use is gaining attention due to their availability, cost effectiveness, proven nature of specificity, biodegradability, low toxicity and minimum residual toxicity in the environment [5] [6] [7].

Guava (*Psidium guajava*) belongs to the family Myrtaceae and it is believed to have originated from South America. Guava tree grow in tropical and sub tropical area of the world like Asia and Africa. Guava has been reported by several researchers to be rich in tannins, phenols, flavonoids, essential oils, saponins. Birdi *et al.* [8] opined that *P. guajava* leaves have a broad spectrum of antimicrobial action against a wide range of pathogens. They further reported that guava leaf extract has analgesic, anti-inflammatory, antimicrobial, hepato protective and antioxidant activities.

Although, scientists have identified several phytochemicals in guava, only

small fractions have been studied closely and each one works differently [9]. Begum *et al.* [10] reported the extraction of triterpenoids guavanoic acid and guavacouremic acid from the leaves of guava. Four flavonoids were isolated and identified by Arima and Danno [11] to inhibit the growth of *Salmonella enteritidis* and *Bacillus cereus*. The study was aimed at determining the phytochemical constituents and *in vitro* antibacterial activity of methanol and aqueous extracts from *Psidium guajava* leaves on *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Proteus* spp in Ugbokolo, Nigeria.

2. Materials and Methods

Test Isolates

The study was conducted between July and December, 2019. The test organisms for the study; pure isolates of *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli and Proteus* spp were obtained from stool and blood samples of patients attending health centres in Ugbokolo, Benue State, Nigeria. The isolates were confirmed at the Microbiology Laboratory, Benue State Polytechnic, Ugbokolo using morphological and appropriate biochemical tests such as Indole, motility, Triple sugar iron, catalase and coagulase and stored in refrigerator at 4˚C.

Collection of Plant Materials

Leaf and Stem bark of *Psidium guajava* were collected from residential areas in Ugbokolo, Benue State. Identification and authentication of the plant materials was done at the Department of Botany, University of Agriculture, Makurdi, by a botanist, Dr Okoh. S.

Preparation of Extracts

Aqueous and methanol extracts of *Psidium guajava* leaves and stem barks were prepared separately. The leaves and stem barks were washed in water and air dried on the desk in the laboratory for two weeks. They were frequently turned to achieve uniform dryness. The dried leaves were then crushed and pulverized by grinding using local mortar and pestle. 50 g powder of the leaves and stem barks was soaked in 500 ml each of distilled water and methanol respectively. The flasks were kept at room temperature for 3 days with intermittent shaking. Filtration was done using Whatman filter paper. The extracts were evaporated at 70˚C in water bath with shaker until dried extract samples were obtained. The extracts were purified and separated based on polarity into discrete fractions of bioactive components by column chromatography by a method described by [12].

Phytochemical Screening

The screening of the plant materials for bioactive components was done by a method decribed by Sofowora [13] and Trease and Evans [12]. The extract was tested for the presence of the following bioactive compounds:

Test for Phenol:

2 ml of the extract was measured and dispensed into a test tube, subsequently

followed by 3 drops of ferric chloride. Dark blue or dark green colouration indicated the presence of phenol.

Test for Saponins

Frothing method was used to determine the presence of saponins in the extract. 3 ml of distilled water was measured and added to 1ml of the extract in a test tube and agitated vigorously for stable persistent froth. Formation of froth that is stable for up to 10 min is an indicative of the presence of saponins.

Test for Flavonoids

The ethanol and water extracts (5 ml) each was added differently to a concentrated sulphuric acid (1 ml) and 0.5 g of Mg. A pink or red coloration that disappear on standing (3 min) indicates the presence of flavonoids.

Test for Tannins

1 ml of the ethanol extract was added in 2 ml of water in a test tube. 2 to 3 drops of diluted ferric chloride solution was added and observed for green to blue-green (catechic tannins) or blue-black (garlic tannins) coloration.

Test for Alkaloids

20 ml of ethanol extract was evapourated and the dry residue dissolved in 5 ml of HCl (2N) and filtered. A few drops of Mayer's reagent and Wagner were added, the presence of precipitate indicates the alkaloids.

Antibacterial Susceptibility test

The sensitivity of each extract was determined by using the agar well diffusion method as described by Ahmed and Beg [14] and Arora and Arora [15]. The concentration of the test organisms was adjusted to 0.5 McFarland standard using Phosphate buffered saline (PBS). The inoculums were spread over the entire sterile surface of sterile Mueller-Hinton agar plate, rotating the plate to ensure an even distribution using a sterile cotton swab dipped into the suspension. A sterile 6 mm diameter cork borer was used to bore 6 wells in the agar medium. 0.1 ml of the extract were dispensed into the wells at different concentrations of 50, 100, 150 and 200 mg/ml with utmost care to prevent the spillage onto the surface of the agar. DMSO was dispensed into the fifth hole and Amoxicillin into the sixth hole as negative and positive control respectively. The plates were allowed to stand on the laboratory bench for 1 hour for proper diffusion of the extract into the medium. The plates were then incubated at 37˚C for 24 hours. The zones of inhibition were observed and measured at the end of the incubation period around the agar well to the nearest millimeter using a ruler.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the extracts was determined using broth dilution technique as described by Ali *et al.* [16]. Two fold serial dilutions of the extracts were prepared by adding 5 ml of 100 mg/ml of the extract into a test tube containing 5 ml of Nutrient broth and mixed vigorously, thus producing a solution containing 50 mg/ml of the extract. The process continued serially up to the fifth test tube and the last 5 ml was discarded leaving equal volume in the tubes, hence producing the following concentrations; 50, 25, 12.5,

6.25 and 3.125 mg/ml. The sixth test tube does not contain extracts and therefore serves as the negative control. 50 µl of 0.5 McFarland standards of test organisms were introduced into the test tubes and incubated at 37˚C for 24 hours. The test tubes were observed for turbidity. The least concentration with no turbidity was taken as the MIC.

Determination of Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MBC) of the extracts was determined by a process described by Ali *et al.* [16].

Minimum bactericidal concentration (MBC) was performed to determine if the test microorganisms in the tubes without turbidity were killed or inhibited. 10 µl was aseptically transferred from tubes with different concentrations that showed no turbidity onto the solidified Mueller-Hinton agar medium and incubated at 37˚C for 24 hours. The MBC was recorded as the lowest concentration of the extract that had less than 99% growth on the agar plates.

Statistical Analysis

The data of average zone of inhibition produced by the isolates against the extracts used was analyzed using One-way ANOVA on the Statistical Package for Social Sciences (SPSS 20). Significance level for the differences was set as $P < 0.05$.

3. Results

Phyto-chemical screening

The phytochemical constituents of *P. guajava* leaf and stem bark are as presented in Table 1. The result showed the presence of saponin, flavonoid, tannin and phenol.

4. Antibacterial Activity of the Extracts

Leaf Extracts

The antibacterial activity of aqueous and methanol leaf extracts of *Psidium guajava* on the isolates is as shown in Table 2. The result showed that *Staphylococcus aureus* is the most susceptible isolate at 200 mg/ml concentration with zone of inhibition of 11.9 mm (ALE) and 19.0 mm (MLE) and *Proteus* sp. is the least susceptible with zone of inhibition of 8.5 mm (ALE) and 12.4 mm (MLE).

Stem Bark Extract

The antibacterial activity of aqueous and methanol stem bark extract of *Psidium guajava* on the isolates is as presented in Table 3. The result showed that *Staphylococcus aureus* is the most susceptible isolate at 200 mg/ml concentration with zone of inhibition of 14.1 mm (ASE) and 22.3 mm (MSE). *Proteus* sp. is the least susceptible with zone of inhibition of 13.6 mm (ASE) and 17.4 (MSE).

MIC and MBC

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of aqueous and methanol extract of *P. guajava* leaf and stem bark is represented in Table 4. The result showed dilutions of various concentrations of aqueous

Table 1. Phytochemical constituents of *P. guajava* leaf and stem bark extracts.

S/N	Phytochemicals	Test	Leaf extract	Stem bark extract
1	Saponin	Foam test	+	+
2	Flavonoid	Lead acetate test	+	+
3	Tannin	Gelatin test	+	+
4	Phenol	Ferric chloride test	+	+

Table 2. Antibacterial activity of aqueous and methanol leaf extracts of *Psidium guajava*.

Isolates/zone of inhibition (mm)					
Leaf extracts	Conc. (mg/ml)	*E. coli*	*S. typhi*	*S. aureus*	*Proteus* sp.
ALE	50	9.5	8.6	10	7.0
	100	9.9	8.8	10.8	7.2
	150	10.0	9.0	11.5	7.9
	200	11.1	10.4	11.9	8.5
MLE	50	9.6	8.8	10.9	7.6
	100	12.6	10.8	12.8	8.8
	150	15.1	12.5	17.5	11.6
	200	16.8	13.1	19.0	12.4
Control (Amox.)	100	17.4	15.0	21.7	13.1

χ = 10.731, P < 0.05. Key: ALE: Aqueous Leaf extract, MLE: Methanol Leaf Extract.

Table 3. Antibacterial activity of aqueous and methanol stem bark extracts of *Psidium guajava on* Isolates.

Isolates/Zone of inhibition (mm)					
Stem bark extracts	Conc. (mg/ml)	*E. coli*	*S. typhi*	*S. aureus*	*Proteus* sp
ASE	50	11.6	10.2	12.0	9.0
	100	12.0	11.8	12.8	9.8
	150	12.8	12.0	13.6	10.4
	200	13.8	13.7	14.1	13.6
MSE	50	12.8	12.0	12.9	9.9
	100	13.4	14.9	15.2	12.7
	150	18.9	17.4	19.8	16.0
	200	21.6	19.0	22.3	17.4
	100	17.4	15.0	21.7	13.1

χ = 26.036, P < 0.05. Key: ASE: Aqueous Stem Bark Extract. MSE: Methanol Stem Bark Extract.

and methanol extracts can inhibit and/or kill the isolates. Lower MIC (3.125 mg/ml) was shown by methanol extract than aqueous extract. MBC of methanol extract ranges between 6.25 - 25.0 mg/ml.

Table 4. MIC and MBC of *Psidium guajava* extracts.

	MIC/MBC (mg/ml)			
Extracts	*S. typhi*	*S. aureus*	*E. coli*	*Proteus* sp.
Aqueous Leaf Extract	12.5/25.0	6.25/12.5	12.5/25.0	25.0/50.0
Aqueous Stem bark Extract	6.25/12.5	6.25/6.25	12.5/25.0	12.5/25.0
Methanol Leaf Extract	6.25/12.5	3.125/6.25	6.25/12.25	12.5/25.0
Methanol Stem bark Extract	6.25/6.25	3.125/6.25	3.125/6.25	6.25/12.5

5. Discussion

The findings of this study showed the presence of flavonoids, saponins, alkaloids, Tannins and Phenol as bioactive compounds from the phytochemical screening of leaf and stem bark extracts of *P. guajava*. This agrees with the findings of Uboh *et al.* [17] and Offor [18] who reported the presence of alkaloids, tannins, steroids and flavonoids as bioactive compound in the *P. guajava* extracts. Ibe and Maduagwu [4] and Nwanneka *et al.* [19] also reported the efficacy of Alkaloids as anti-imalaria, anticancer and antiasthma. Ngene *et al.* [20] reported that tannins are effective in treating intestinal disorders such as diarrhea and dysentery.

Results of the study showed that the zone of inhibition of the extracts on the isolates varied significantly. The extracts exhibited the highest antimicrobial activity at a concentration of 200 mg/ml against *S. aureus* as compared to *E. coli*, *S. typhi* and *Proteus* sp. This could be as a result of the susceptibility of Gram positive bacteria to therapeutic agents than Gram negative bacteria which may be attributed to inhibition of cell wall development by the extracts. This result is supported by findings of Aliyu *et al.* [21], Joseph and Priva [22] and Muhammad *et al.* [3] who revealed that the leaf and stem extracts of *P. guajava* had more antibacterial activity on Gram positive bacterium (*Staphylococcus aureus*) than Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*).

One of the important findings of this work is that *Psidium guajava* methanol extracts showed higher antibacterial activity compared to aqueous extracts. This could be attributable to better solubility of the active component by the methanol than water. This corroborates the findings of Nwanneka *et al.* [19] who reported that ethanol extracts of *P. guajava* showed higher inhibition against bacteria and fungi than aqueous extract. However, this disagrees with the findings of Elekwa *et al.* [23] and Egharevba *et al.* [24] who reported that aqueous extracts of *P. guajava* had higher inhibitory effect than ethanol extract.

The results of the present research on the antibacterial activity of *P. guajava* extracts of leaf and stem on tested isolates revealed that stem bark extracts possessed higher antibacterial activity than the leaf extract. This agrees with/or holds true with the findings of Elekwa *et al.* [23] which showed that stem bark was more effective than leaf extract.

The minimum inhibitory concentrations (MICs) of the extract against the

isolates showed that aqueous leaf and stem bark extracts inhibited the growth of *S. aureus* and *S. typhi* at 6.25 mg/ml while methanol leaf and stem bark extracts inhibited the growth of *S. aureus* at 3.125 mg/ml. This corroborates the findings of Sushmita *et al.* [2]. Ngene *et al.* [20] equally reported that Gram positive organisms exhibited smaller MIC when exposed to extracts of *Psidium guajava* than those of Gram negative organisms. The Minimum Bactericidal Concentration showed that the leaves and stem extracts of the plant can kill the isolates at 12.5 - 50 mg/ml. Methanol leaf and Stem bark extracts however, exhibited MBC at 6.25 mg/ml. *S. aureus* had the least MBC. This is consistent with the findings of Biswal *et al.* [25], El-Ahmady *et al.* [26] and Muhammad *et al.* [3].

6. Conclusion

The phytochemical screening of leaf and stem bark extract of *P. guajava* showed the presence of flavonoids, saponins, Tannins and Phenol as bioactive compounds. The antibacterial activity of the extracts showed that methanol extracts had higher antibacterial activity as compared to aqueous extract. The result equally showed that the Gram positive bacterium was more susceptible to the extracts than the Gram negative bacteria. The findings of this study justify the antibacterial efficacy of leaf and stem bark extracts of *P. guajava* in the treatment of infections of bacterial origin.

Limitations of the Study

Paucity of funds did not allow for quantitative analysis of the phytochemicals.

Authors Contributions

A.P. and E.E.T. designed the study, did the experimental studies, Bench work and data acquisition for analysis. A.J. did the data analysis and interpretation of results. A.P. did the drafting of the manuscript. Revision of the manuscript for critical intellectual content was done by A.P., O.G.E., A.Y. and H.A.O. All authors gave final approval of the version to be published.

Conflicts of Interest

There is no conflict of interest between the authors. All authors of the manuscript have read and agreed to its content and are accountable for all aspects of the accuracy and integrity of the manuscript. The submitted article is our original work and it is not being considered or reviewed by any other publication, and has not been published elsewhere in the same or a similar form.

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