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Antimicrobial Activity and Phytochemical Analysis of Leaves and Stem Bark Extracts of *Psidium guajava* (guava) Against Selected Clinical Bacteria

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ABSTRACT

The use of crude extracts of plant parts and phytochemicals of known antimicrobial properties can be of great significance in the therapeutic of microbial infections. This study was conducted to investigate antibacterial activity and phytochemical screening of leaves and stem bark extracts of *Psidium guajava* against some selected clinical isolates. The leaf and stem bark of *P. guajava* were extracted by maceration using ethanol and water. The crude extracts obtained were screened for phytochemicals using qualitative methods. The yield of the extracts ranged from 16.10 to 22.50 g. The extracts contained flavonoids, alkaloids, phenols, tannins, saponins, glycosides and terpernoids. The clinical isolates used were *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*. The antibacterial activity of leaf and stem bark extracts was done using agar well diffusion method and the ethanol and water extracts showed significant antibacterial activity against the test isolates. The ethanolic leaf extract of *P. guajava* have the highest antibacterial activity against *Salmonella typhi* with zone of inhibition of 30 mm; *S. aureus,* 25 mm and *E. coli,* 16.50 mm. Similarly, the aqueous leaf extracts have the highest antibacterial activity against *Salmonella typhi* with zone of inhibition of 15 mm; *E. coli,* 10.50 mm and *S. aureus*, 9.50 mm. The ethanolic extract of stem bark had the highest antibacterial activity against *S. aureus* with zone of inhibition of 25 mm, *S. typhi* (21 mm) and *E. coli* (20 mm). The aqueous stem bark extracts have the highest antibacterial activity against *S. typhi* with

zone of inhibition of 15 mm. *Salmonella typhi* was more susceptible to the positive control, ampicillin, with zone of inhibition of 33 mm. The minimum inhibitory concentration and minimum bactericidal concentration of extracts of *P. guajava* ranged from 6.25 to 50.00 mg/mL. The antibacterial activity of extracts of *P. guajava* could be used to treat microbial infections.

Key words: Antibacterial, phytochemical, *Psidium guajava*, leaf and stem bark extracts

INTRODUCTION

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the 20th and 21st centuries. In recent times, the secondary bioactive constituents in plants have been of great application for pharmaceutical purposes and its use have gradually increased in many countries [1]. The use of herbal plants that have known antimicrobial potentials can be of great importance in the treatment of infectious diseases [2]. A number of researches have been carried out in different countries of the world to ascertain the efficiency of these plants. Different plants have been used because of their bioactive attributes, which are due to the presence of secondary bioactive metabolites produce by the plants [1, 3-5].

Psidium guajava is an example of the medicinal plants. *Psidium guajava* commonly referred as guava is a tree that belongs to the family, Myrtaceae [6]. It is grown in the tropical countries and it is therefore cultivated in countries having abundant rainfall and subtropical climate [7]. The plant have large amount of bioactive compounds such as, tannins, alkaloids, flavonoids, steriods, carbohydrates, cellulose, chlorophyll, mineral salts and other substances that possess antimicrobial properties [8]. Phytochemicals are substances produced by plants for their own protection but have been found to be effective in the treatment of humans against infectious diseases [9]. The plant is known for its therapeutic activities such as anti–inflammatory, hypoglycemic, anti-diarrheal and anti-oxidant properties which can be attributed to the presence of these bioactive substances [10].

The leaves and bark of medicinal plants including guava tree have been used in ancient times. Boiling of the plant parts in hot water can be used to treat stomach disorders such as diarrhea, dysentery, vomiting, and also to treat menstrual disorders [6]. The tribes of the Africa uses leaf decoction for mouth sores, bleeding gums, as douche for vaginal discharges and to

tighten and tone up vaginal walls after child delivery. They are also a good source of fibre, potassium and retinoic acid [11].

Psidium guajava is a plant used in traditional medicine and is believed to have active components that helps in treatment and management of various diseases [12]. Guava has shown to have considerable therapeutic effects against a wide range of microorganisms including *Bacillus*, *E. coli, Staphylococcus*, *Pseudomonas*, *Clostridium*, *Shigella*, *Salmonella* and yeast such as *Candida* species [13]. The antimicrobial activity of methanol and ethanol extracts of guava showed inhibitory activity against *S. typhi*, *S. aureus, B. cereus* and *E. coli* [14]. In addition, Ekeleme et al [15] conducted study on the phytochemical analysis and antibacterial activity of *Psidium guajava.* Leaf extracts had inhibitory activity against *Pseudomonas aeroginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Klebsiella pneumonia.* Furthermore, Growther and Sukirtha [16] conducted a phytochemical analysis and antimicrobial properties of *Psidium guajava* leaves and bark extracts. The results showed significant antibacterial and antifungal activity against *Staphylococcus aureu*s, *Streptococcus pyogenes*, *E. coli*, *Pseudomonas aeroginosa* and *Candida albicans.* The antimicrobial properties of guava extracts were attributed to the presence of different phytochemical constituents.

However, variation in plant bioactive compounds and antimicrobial activities exist, among other factors, due to biochemical reaction within species, geographical locations, methods of extraction and solvents used for extraction. Due to the fact that phytochemical constituents vary as a result of geographical locations and literature about guava within Benue State, Nigeria, with respect to phytochemical composition and antimicrobial activities are hardly available, there is a need to investigate phytochemical and antimicrobial properties of guava from Benue State for treatment of *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* infections.

This study aimed to investigate antimicrobial activity and phytochemical composition of ethanolic and aqueous extracts of leaf and stem bark of *Psidium guajava* (guava). The objectives of the study were: to identify compounds responsible for antimicrobial activity; to assess the antimicrobial properties of extracts of *Psidium guajava* and to determine minimum inhibitory and bactericidal concentrations of the plant extracts against the clinical isolates.

MATERIALS AND METHODS

Study area

The study was carried out in the Microbiology Laboratory Joseph Sarwuan Tarka University Makurdi, Benue State, Nigeria. The duration of the study was from December, 2023 to February, 2024.

Collection of plant material

The parts of *Psidium guajava* were collected in Joseph Sarwuan Tarka University Makurdi Benue State, Nigeria. The plant was identified and authenticated by a taxonomist in the Department of Botany, Joseph Sarwuan Tarka University Makurdi, Benue State, Nigeria. They were given herbarium voucher specimen number (JOSTUMH 7018)

Processing of plant material

The parts of the plant collected were washed with tap water, after it was dried in the shade for four weeks and then the plant was ground into powder using mortar and pestle. The powder was stored in air tight bottles at 4 °C for future use [17].

Extraction of plant material (Maceration)

About 100 grams of powdered parts of *P. guajava* plant were soaked in 200 ml of ethanol and water contained in 500 ml conical flasks and covered with cotton wool separately. Thereafter, it was shaken occasionally for one week. The extracts were filtered by Whatman No. 1 filter paper to get a good filtrate. The filtrate was evaporated using an oven at 40 °C to obtain a dry extract. The dried extract was stored at 4°C for further use [18, 19].

The percentage yield of the plant extract obtained was calculated as

$$\text{Percentage Yield (\%)} = \frac{\text{weight of plant sample after extraction}}{\text{weight of dried powder extract}} \times 100$$

Preparation of culture media

The media used were prepared according to manufacturer's guide. Mueller Hinton agar was prepared by weighing 39 g of the powdered agar dispensed into 1000 ml of distilled water in a conical flask. It was shaken until it became a mixture. It was then covered with a foil and was autoclaved at 121 °C, 115 atmospheric pressure for 15 minutes. The medium was allowed to cool

at 47 ºC. Thereafter, 20 ml of the molten medium was dispensed into petri dish and allowed to solidify [20].

Collection of test isolates

The isolates used in the study include *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi.* They were collected from the Microbiology Laboratory, Benue State University Teaching Hospital, Makurdi, Benue State, Nigeria. The identity of the test isolates were confirmed by standard microbiological procedures [20]. The test isolates were preserved at 37 ºC on nutrient agar slant and sub-cultured before use.

Test for antibacterial activity

The antibacterial activity of the plant extracts (ethanol and water) against some selected bacterial isolates were determined using agar well diffusion assay (AWDA) method [17, 21]. The diameters of the zone of inhibition were measured in millimeters (mm). About 5 ml of the medium was dispensed into petri dish, after which, a well measuring 6 mm in diameter was created using a cork borer in cooled nutrient agar plate, streaked with a target isolate (~10^6 cfu/ml). Thereafter, 100 µl of the plant extracts were dispensed into the well and the plates were incubated at 37 ºC for 24 hours. For each bacterial isolate, ampicillin and dimethyl sulfoxide were used as positive and negative controls respectively.

Test for minimum inhibitory concentration (MIC)

The MIC tests of the extracts were performed for each of the isolates at different concentrations of 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml [1, 22]. To obtain this, 1 ml of the plant extract was placed in test tubes. One milliliter (1 mL) was added and then a loopful of the test organism previously diluted to 0.5 McFarland turbidity stand was placed into the tubes. All the test tubes were incubated at 37 ºC for 24 hours and were evaluated for MIC.

Test for minimum bactericidal concentration (MBC)

A loopful of test isolate from the broth was collected from the test tubes, which did not show any visible of growth and inoculated on nutrient agar plates by streaking. The test organisms were streaked on Nutrient agar plates which served as control. The plates were then incubated at

37º C for 24 hours. After incubation the concentration at which no visible growth occurred was considered as the minimum bactericidal concentration [23].

Preliminary phytochemical analysis

Test for tannins

The plant extracts were mixed with 15% ferric chloride solution. The change in color was observed. The appearance of blue color showed the presence of tannin [24, 25].

Test for alkaloids

The plant extracts were dispensed in 10 ml methanol in a test tube containing chloroform and the solution was removed with dilute H_2SO_4 and acid layer taken and tested for presence of alkaloids:

Dragendroff's test

About 5 ml of acid layer of test solution was mixed with 5 mL of Dragendroff's reagent (potassium bismuth iodide solution) in a test tube and shaken vigorously to obtain a stable solution. Thereafter, 5 mL of dilute HCl were added. The appearance of orange-red precipitate showed the presence of alkaloids [2].

Test for cardiac glycosides (Keller-Kilian's test)

About 0.5 mL of glacial acetic acid was dissolved in conical flask containing 50 ml of test solution and one drop of ferric chloride solution was added in the conical flask. Then 0.5 mL of concentrated sulphuric acid was added to the test solution. The appearance of a brown ring at the interface indicated the presence of cardiac glycosides [26].

Test for saponins:

About 1 mL of the plant extract was dispensed in test tube containing water and then shaken occasionally. The sample was warmed and the appearance of frothing, which persist on warming showed preliminary evidence for the presence of saponins. Thereafter, few drops of olive oil was added to 1ml of the extracts and vigorously shaken. Formation of soluble emulsion in the extract indicated the presence of saponins [2, 15].

Test for flavonoids (Shinoda reduction test).

About 5 mL of distilled water was added to 0.5 g of each plant extracts in the test tube and mixtures were shaken to obtain the extract solution. The extracts were mixed with magnesium

ribbon fragments and drops of concentrated hydrochloric acid were added. The formation of an orange, red, pink or purple coloration indicates the presence of flavonoids [27].

Test of phenol

About 3 ml of aqueous or alcoholic extract were added with 5% FeCl3 solution. The appearance of deep blue-black color shows the presence of phenol [3, 26].

Test for terpenoids

About 5 mL of plant extracts were mixed with 2 mL of CHCl3 in a test tube. About 3 mL of concentrated H_2SO_4 was carefully added to the mixture to form a layer. The appearance of an interface with a reddish brown coloration indicated the presence of terpenoids [3].

RESULTS AND DISCUSSION

The ethanol and aqueous extracts of *P. guajava* were screened for phytochemicals composition and antibaterial activity against *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*. Table 1 shows results of the percentage yield of the crude leaf and stem bark extracts of *Psidium guajava*. The ethanol stem bark extracts of *P. guajava* had the highest yield (22.50%) while aqueous leaf extract of *P. guajava* produced the lowest (16.10/ %).

Table 1: Yield (%) of the extracts of *Psidium guajava*

Plant Part	Type of Extract	Weight of Powdered Sample(g)	Weight of Extract(g)	Yield of Extract (%)
Leaf of *Psidium guajava*	Ethanol	30	5.30	17.66
	Aqueous	30	4.42	16.10
Stem bark of *Psidium guajava*	Ethanol	25	4.5	22.50
	Aqueous	20	3.8	19.00

Table 2 shows the results qualitative phytochemical screening of of *Psidium guajava*. In the ethanolic leaf extract, phenols, flavonoids, saponinns, alkaloids, tannins, steroids and glycosides were present. In aqueous leaf extract of *Psidium guajava*, phenols, flavonoids, saponins, alkaloids, tannins, steroids and glycosides were also present. Furthermore, the ethanolic stem

bark extracts of *Psidium guajava* showed the presence of phenols, flavonoids, saponins, alkaloids, tannins, steroids and glycosides while the aqueous stem bark of *Psidium guajava* showed the presence of phenol, flavonoids, saponins, tannins, steroids and glycosides.

Table 2: Qualitative Phytochemical Analysis of *Psidium guajava*

Plant part	Extracts	Phenols	Flavonoids	Saponins	Alkaloids	Tannins	Terpenoids	Glycosides
Leaf	Ethanol	++	++	++	+	++	+	++
	Aqueous	++	++	+	+	++	+	+
Stem bark	Ethanol	++	++	++	++	++	+	+
	Aqueos	++	++	+	+	++	+	+

KEY:+= Positive; -=Negative; ++=Highly present

Table 3 shows zone of inhibition (mm) extracts of *P. guajava* against selected bacteria. The ethanolic leaf extracts of *Psidium guajava* have the highest zone (30 mm) on *Salmonella typhi*, followed by 25 mm on *S. aureus* and 16.50 mm against *E. coli* while the positive control Ampicillin shows the highest inhibition in diameter on *Salmonella typhi* (33 mm). The aqueous leaf extracts of *P guajava* had the highest zone of inhibition of 15 mm against *S. typhi*, followed by 10 mm against *E. coli* while the least zone of inhibition was shown against *S. aureus* (9.50 mm). Similarly the ethanolic stem bark extracts of *Psidium guajava* had the highest mean zone of inhibition on *S. aureus* (25 mm) followed by 21 mm against *S. typhi* and the least zone of inhibition was 20 mm against *E. coli* while the positive control, ampicillin, showed the highest inhibition on E. coli (29 mm). The aqueous stem bark extracts of *P.guajava* had the highest zone of inhibition of 15 mm on *S. typhi* followed by 12.50 mm against *S. aureus* while the lowest zone of inhibition was 10 mm on *E. coli*.

Table 3: Zones of Inhibitions of Various Extracts and Controls (mm) on various Bacteria

Plant part	Bacteria	Ethanol	Aqueous	Ampicilin	DMSO
Leaf of *Psidium guajava*	*E. coli*	16.50	10.50	29.50	0
	Salmonella typhi	30.00	15.00	33.00	0
	S. aureus	25.00	9.50	29.50	0
Stem bark of *Psidium guajava*	*E. coli*	20.00	10.00	29.50	0
	Salmonella typhi	21.00	15.00	33.00	0
	S. aureus	25.00	12.50	29.00	0

Table 4 shows the MIC of extracts of *Psidium guajava* on selected bacteria. The results showed that ethanolic extracts of *P. guajava* have lower values than aqueous extracts

Table 4: Minimum Inhibitory Concentration of Various Extracts (mg/ml) on Bacteria

Plant part	Bacteria	Ethanol	Aqueous
Leaf of *Psidium guajava*	*E. coli*	9.37	25.00
	S. typhi	50.00	18.75
	S. aureus	25.00	9.37
Stem bark of *Psidium guajava*	*E. coli*	12.50	25.00
	S. typhi	12.50	9.37
	S. aureus	18.75	25.00

Table 5 shows MBC of ethanolic and aqueous leaf and stem bark extracts of *Psidium guajava* on selected bacteria. The ethanolic extracts have lower MBC values than the aqueous extracts.

Table 5: Minimum Bactericidal Concentration of Various Extracts (mg/ml) on Bacteria

Plant part	Bacteria	Ethanol	Aqueous
Leaf of *Psidium guajava*	*E. coli*	18.75	37.50
	S. typhi	18.75	12.50
	S. aureus	25.00	37.50
Stem bark of *Psidium guajava*	*E.coli*	6.25	9.37
	S. typhi	6.25	50.00
	S. aureus	-	12.50

This study which evaluated the qualitative phytochemical screening of the extracts of guava showed that phenols, alkaloids, saponins, flavonoids, steroids, terpenoids, and tannins were present. This agrees with the findings of Oncho et al [28] who reported the presence of phytochemical constituents in the extracts including alkaloids, tannins, saponins, flavonoids, steroids and terpenes. In another study Ekeleme *et al* reported that guava plant contains similar phytochemical constituents [15]. Furthermore, Biwas *et al* reported that different extracts of guava were rich in secondary metabolites such as tannins, saponins, flavonoids, cardiac glycosides terpenoids, phenols, steroids and alkaloids [27]. The presence of the secondary bioactive compounds in the ethanolic and aqueous extracts of *P. guajava* is attributed for their antimicrobial properties.

The findings of the present study revealed that leaf and stem bark extracts of *P. guajava* exhibited antibacterial activity against the test bacterial isolates. This agrees with the findings of Biwas et al [27] who reported that leaves extracts of guava have therapeutic effect against a wide range of bacteria. Moreover, Anbulsevi and Jeyanthi [29] reported that the extracts (ethanol, methanol and aqueous) of *Psidum guajava* showed antimicrobial activity against wide range of

bacteria including *S. aureus*. In another study, Ifeanyichukwu *et al* [30] reported that extracts of *P.guajava* have great inhibitory effect against microbial pathogens.

According to this study, the plant parts extracted by ethanol possess greater antimicrobial action on test isolates compared to the aqueous extracts of guava. This agrees with the findings of Bansode and Chavan [31] that plant extracts of polar solvents (ethanol, methanol and acetone) provided more reliable antimicrobial activity compared to the ones extracted by water. It can be seen that some bioactive substances can dissolve more readily in polar solvent than non-polar solvents.

The MIC of this study showed that MIC values of the different extracts of guava varied at different concentrations against isolates. This agrees with the findings of some researchers who reported that different guava leaf solvent extracts have varied MIC against different test micro-organisms [16, 17]. This confirms the antimicrobial potency of the extracts.

The minimum bactericidal and fungicidal concentration of this study varied at different concentrations against microorganisms. According to the results of this study, the ethanol extracts of guava have lower values than aqueous extracts. This agrees with the findings of Ekeleme *et al* [15] who opined that aqueous extracts plant extracts have higher MBC values than the ethanolic extracts.

There were few limitations encountered in the course of carrying out this research: collection of plant materials were conducted during dry season and as a result, most of the plants were not actively in their growing stage. This could pose challenge as the plant may have lost some vital secondary bioactive substances that are responsible for its therapeutic properties. Furthermore, the samples of *E. coli*, *S. typhi* and *S. aureus* collected at the sampling points were not analyzed immediately due to unavailability of some reagents and equipment thereby posing a limitation to this study.

CONCLUSIONS

The investigation of antibacterial activity and phytochemical screening of leaf and stem bark extracts of *Psidium guajava* against some selected clinical isolates was carried out in this research work. From the result, *P. guajava* (Guava) extracts contain high bioactive substances or phytochemicals which at different concentrations have therapeutic effects on *E.coli*, *S. typhi* and *S. aureus.* The use of natural antimicrobials from *Psidium guajava* can help diversify the options

of combating antibiotic resistance, potentially slowing down the emergence of resistant strains. Exploring natural sources as potential antimicrobial agents may offer alternative solutions to combat drug-resistant pathogens. The results may have implications for the development of alternative antimicrobial therapies.

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