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In-vitro Antibacterial Activity and Phytochemical Screening of *Psidium guajava* on Some Enteric Bacterial Isolates of Public Health Importance

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Authors' contributions

This work was carried out in collaboration between all the authors. Authors MA and AY designed the research. Conduct of the experiment and writing of the script was done by all the authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The study was conducted to investigate the antibacterial activity of *Psidium guajava* and its major antibacterial constituents (Phytochemicals).
Materials and Methods: The aqueous and ethanol extracts from the leaves and stem bark of the plant was tested using well diffusion method for their antibacterial activity against some members Enterobacteriaceae family isolated from diarrheic stool sample (*Escherichia coli, Shigella* spp*, Salmonella typhi* and *Pseudomonas aeruginosa*).
Results: The result shows that the extracts were active against the microorganisms. The ethanolic extract of stem bark showed the highest zones of inhibition against tested organisms compared to aqueous extract. Statistical analysis of the result shows that the extracts demonstrated higher antibacterial activity against the isolates tested with the average zone of inhibition of 15.44 mm,

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14.78 mm, 12.92 mm and 11.31 mm for *E. coli*, *Shigella* spp *S. typhi* and *P. aeruginosa,* respectively. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts ranges between 6.25 – 100 mg/ml. Preliminary phytochemical analyses showed that both stem bark and leaf extracts contain alkaloids, tannins, terpenoid, Anthraquinone, reducing sugar, amino acid, flavonoid, saponins, glycosides and phenols.

Conclusion: The extracts of the plant demonstrated antibacterial activity due to presence of phytochemical constituents hence, the application of the decoction of leaf and stem bark of the plants in ethno medicine is justified.

Keywords: Psidium guajava; enteric bacteria; phytochemical; well diffusion; antibacterial activity; extracts.

1. INTRODUCTION

There has been a lot of attention focused on producing medicines and products that are natural. Several leaves and leaves extracts have been found to have antibacterial activity against microorganisms [1]. There is no plant that does not have medicinal value [2]. The active components are normally extracted from all plant structure, but the concentration of these compounds varies from structure to structure. However, plants parts known to contain the highest concentration of these phytochemical constituents for therapeutic purpose can either be leaves, stem bark barks, root, bulks, corms, rhizomes, wood, flowers, fruits or the seeds [3].

The presence of phytochemical constituents in medicinal plants made them useful for healing as well as for curing of human diseases [4]. Phytochemical are naturally occurring compounds in the medicinal plants, [5]. Large populations of the world, especially in developing countries depend on the traditional system bark of medicine to treat variety of diseases [6]. Several hundred genera of plants were utilized traditionally for medicinal purposes. The world health organization [7] reported that 80% of the world population relies chiefly on traditional medicine and a major part of the traditional therapies which involve the use of plant extract and their constituents [8].

Guava, *P. guajava,* belongs to the family Myrtaceae which is considered to have originated from tropical South America, Guava tree grown in tropical and sub-tropical area of the world like Asia, Africa and Hawaii etc [9]. *Psidium guajava* common names are Guava (English), Gwaiba (Hausa), Goifa (Yoruba), Ugova (Igbo), Gutiba (Spanish), Goyave (French) and Goejaaba (Dutch) [10]. Many parts of the plant have been used in traditional medicine to manage conditions like malaria, Gastroenteritis, vomiting, diarrhea, dysentery, wound, ulcer, toothache, cough, sore throat and a number of other conditions [11]. The leaves are particularly rich in flavonoids and distinctly in quercetin [12]. The leaf methanolic extract also showed in vitro antibacterial activity against *E. coli*, *S. typhi*, *Staphylococcus aureus* and a strong antibacterial action have been reported on Gram positive and gram negative organism [11]. Therefore, the basic phytochemical investigation of its extract for major phytoconstituents is also vital. The phytochemical analyses of Guava plant shows that its extracts contain over twenty compounds [13].

The objective of the study was to determine the antibacterial activity of leaves and stem bark of *P. guajava* extracts (Both aqueous and ethanolic) against some members of Enterobacteriaceae namely *E. coli, Shigella* spp*, P. aeruginosa* and *S. typhi*, to screen for the phytochemical constituents of the extracts and to evaluate the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts against the test isolates.

2. MATERIALS AND METHODS

2.1 Plant Materials

The plant materials used in this research consisted of the leaves and stem bark of *P. guajava* which were collected from botanical garden of Government Secondary school Gundutse, Kura Local Government Area of Kano State at about 08:30 a.m. Identification and Authentification of the plant materials has been done at Herbarium unit in the department of Biological science Ahmadu Bello University, Zaria with the following voucher numbers 3253. Voucher specimen has been deposited there for future reference. The samples were washed with water to remove dust and rinsed with distilled water. Samples were air dried for two-weeks and

pulverized into powder form using sterile mortar and pestle in the laboratory as described by Mukhtar and Tukur [14]. The powdered samples were bagged in a black polythene bag and store in air tight container for further work.

2.2 Test Organisms

Clinical isolates of *E. coli, Shigella* spp*, P. aerugenosa and S. typhi* were obtained from Department of Microbiology of Murtala Muhammad Specialist hospital for further experiment. Identification and characterization of the isolates was conducted there by using three procedures namely Gram staining, cultural characterization using selective or indicative media and biochemical characterization with reference to Cheesbrough [15]. The pure isolates of each of the test organism were inoculated in sterile slants containing Nutrient agar and transported to the department of Microbiology KUST and refrigerated at 4°C before use.

2.3 Preparation of Plant Extracts

The ethanol and aqueous extract of both plant samples was carried out according to Bengum [13]. Twenty five gram (25 g) each of fine powder of the plant materials was dissolved in 250 ml of distilled water and ethanol in a conical flask and kept for 7 days in a cabinet and frequently shaken to dissolve the powder properly. After 7 days the solution of the plant materials was filtered using Whatman filter paper. The filtrate was kept in rotary evaporator for complete evaporation of the solvent. After running this procedure, a gummy extraction was obtained which was preserved in refrigerator before use.

2.4 Antibacterial Assay of Extracts

The mixture was filtered using Whatman No.1 filter paper and the extracts were evaporated to dryness using rotary evaporator and water bath. One gram solid residues obtained were reconstituted in 5 ml of 5% DMSO to form stock concentration of 200 mg/ml, stored in the refrigerator at 4℃ until used. The agar well method was used to determine the antibacterial activity of the plant extracts. 0.1 ml of the different standardized organisms were introduced separately and thoroughly mixed with Mueller Hilton Agar in a sterile Petri dish and allowed to set then labeled. A sterile cork borer 6mm was then used to punch holes (i.e. 5 wells) in the inoculated agar and the agar was then removed. Four wells that were formed were filled with different concentrations of the extract which were labeled accordingly; 200 mg/ml, 150 mg/ml, 100 mg/ml and 50 mg/ml while the 5th well contained the solution used for the research to serve as control, tetracycline (Chi pharmaceutical limited, Lagos Nigeria) 125 mg/ml, was used as control in this research. These were then left on the bench for 1 hour for adequate diffusion of the extracts and incubated at 37℃ for 24 hours. After incubation, the diameter of the zones of inhibition around each well were measured to the nearest millimeters along straight line and the mean of the readings were then calculated [2].

2.5 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Overnight Nutrient broth cultures of *E. coli, Shigella* spp*, S. typhi* and *P. aeruginosa* at 37°C were prepared. The culture was adjusted to obtain turbidity comparable to that of the turbidity of McFarland 0.5 standard, and then further dilute in Nutrient broth. The inoculums thus prepared expected to obtain 10^5 cfu/ml. The MIC and MBC were determined using procedure described by Kowser and Fatima [16].

2.6 Phytochemical Screening

This was done on different extract to ascertain the presence of bioactive component present in the leaves and stem bark of *P. guajava.* The presence of Alkaloid, saponin, Glycoside, Tannin, flavonoids, steroid, terpenoid, Anthraquinones, Protein and amino acid were determined using procedure described by Sofowora [17].

3. RESULTS AND DISCUSSION

3.1 Antibacterial Activity

The antibacterial activity of aqueous and ethanolic leaf extract *P. guajava* was represented in the Table 1 showing the mean diameter of zone of inhibition of extract on the test isolate with *Shigella* spp being the most susceptible isolate at 200 mg/ml concentration (23.3 mm) and *P. aeruginosa* the least susceptible (6 mm).

The antibacterial activity of aqueous and ethanolic stem bark extract *P. guajava* was represented in the Table 2 showing the mean diameter of zone of inhibition of extract on the test isolate with *Shigella* spp being the most susceptible isolate at 200 mg/ml concentration

(23.6mm) the least susceptible is *P. aeruginosa,* especially for ALE (11.0 at 100 mg/ml and 10.3 at 150, with no linear increase, and 12.6 at 200 mg/ml).

3.2 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Conc. (MBC)

Table 3 shows minimum inhibitory concentration (MIC) of the plant extracts. The result shows that both aqueous and ethanolic leaf extract of the plant can inhibit the growth of the test isolates at concentration of 6.25 – 25 mg/ml. The aqueous stem bark extract has higher MIC of 50mg/ml in *E. coli and Shigella spp* than in *S. typhi* and *P. aeruginosa* but MIC of 12.5-50 mg/ml for ethanolic extract. This shows that ethanolic extract is more effective against the test isolates.

3.3 Phytochemical Screening

The Table 4 showed that the phytochemicals were present in both leaf and stem bark of *P. guajava.* Both leaf and stem bark contain Alkaloid, saponin, phenol, flavonoids, protein and amino acid, Anthraquinones, terpenoid and tannin except Steroid.

Table 1. Mean diameter zones of inhibition of aqueous and ethanolic leaf extract *P. guajava*

Conc. (mg/ml)		Microorganisms/Zone of inhibition (mm)			
		E. coli	***Shigella spp***	***P. aeruginosa***	***S. typhi***
ALE	50	0.96	10.6	06.0	07.3
	100	11.3	11.6	11.0	10.6
	150	11.6	12.3	10.3	18.0
	200	13.6	16.3	12.6	18.3
Control		18.0	18.6	17.3	17.6
ELE	50	11.6	11.3	0.76	0.83
	100	15.3	18.3	14.3	10.6
	150	17.3	20.3	15.6	13.3
	200	20.0	23.3	17.0	18.6

*ALE = Aqueous Leaf Extract, ELE = Ethanolic Leaf Extract, * = No Inhibition*
E. coli = Escherichia coli, P. aerugenosa = Pseudomonas aerugenosa, S. typhi = Salmonella typhi

Table 2. Mean diameter zones of inhibition of aqueous and ethanolic stem bark extract *P. guajava*

Conc. (mg/ml)		Microorganisms/Zone of inhibition (mm)			
		E. coli	***Shigella spp***	***P. aeruginosa***	***S. typhi***
ALE	50	12.3	13.3	11.6	11.3
	100	14.0	14.6	12.3	13.3
	150	18.3	20.0	14.6	17.3
	200	22.3	23.3	18.3	20.3
Control		18.6	18.3	17.3	17.6
ELE	50	14.3	14.6	12.0	13.3
	100	15.3	17.3	13.3	16.0
	150	20.3	21.3	17.3	18.3
	200	23.3	23.6	20.3	21.3

*ASE = Aqueous Stem bark Extract, ESE = Ethanolic Stem bark Extract, * = No Inhibition*
E. coli = Escherichia coli, P. aerugenosa = Pseudomonas aerugenosa, S. typhi = Salmonella typhi

Table 3. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of the *P. guajava* extracts against test isolates

Extracts	ORGANISMS/ MIC/MBC (mg/ml)			
	E. coli	***Shigella spp***	***P. aeruginosa***	***S. typhi***
ALE	25/25	25/25	50/50	50/50
ELE	12.5/25	12.5/25	25/50	25/25
ASE	25/50	12.5/50	25/ND	12.5/ND
ESE	6.25/25	6.25/25	12.5/50	6.25/25

ALE = Aqueous leaf extract, ELE = Ethanolic Leaf Extract, ASE = Aqueous Stem Bark Extract, ESE = Ethanolic Stem Bark Extract, ND = Not Detected.
E. coli = Escherichia coli, P. aerugenosa = Pseudomonas aerugenosa, S. typhi = Salmonella typhi

Table 4. Phytochemical constituents of leaves and stem bark of *P. guajava*

S/N	Phytochemical	Leaf extract	Stem bark extract
1	Alkaloid	+	+
2	Saponin	+	+
3	Phenol	+	+
4	Flavonoid	+	+
5	Protein and amino acid	+	+
6	Tannin	+	+
7	Reducing sugar	+	+
8	Anthraquinone	+	+
9	Steroid	-	-
10	Terpenoid	+	+

+ = Presence of phytochemical, - = Absence of phytochemical

The antibacterial activity is higher at a concentration of 200mg/ml against *Shigella* spp and *Escherichia coli* as compared to *Pseudomonas aeruginosa* and *Salmonella typhi*. *Psidium guajava* ethanolic extract showed higher antibacterial activity compared to aqueous extract, this is attributed to better solubility of the active component by the ethanol than water. This shows similarities to the findings of Nwanneka et al. [18] which investigated the antibacterial activity of *Psidium guajava* leaf extract, the results showed that both aqueous and ethanolic extracts of guava leaf inhibited the growth of the bacteria and fungi tested but the ethanolic extract showed stronger inhibition than the aqueous extract against the organisms. This result was also in conformity with that of Pandey and Shweta [19] where the results of antibacterial activity of *Psidium guajava* leaf and stem bark reveals that ethanolic extract showed stronger anti-bacterial activity than aqueous extract. However, this was contrary to the findings of Emmanuel, [20] who investigate the antibacterial effects of some Ghanian aromatic plants including *Psidium guajava*, he stated that the antibacterial activity of aqueous extract of *Psidium guajava* is higher than that of ethanolic extract. This is due to differences in geographical location of the plant he used compared to the one of the present study. The result of antibacterial effects of *Psidium guajava* leaf extract by Elekwa et al. [21] showed that aqueous extract of guava leaf had higher inhibitory effects on some organisms than ethanolic extract. The results of the present research on the antibacterial activity of stem bark and leaf of *Psidium guajava* on the tested isolates revealed that stem bark extract posses higher antibacterial activity than corresponding leaf extract. This hold true with the results of Elekwa et al. [21] in which the antibacterial study on the effect of *Psidium guajava* showed that the stem bark extract is more effective than leaf extract. The higher antibacterial activity shown by stem bark extract is as result of having higher quantity of the phytochemicals in stem bark extract compared to leaves extract.

From the results of MIC determination (Table 3), the minimum inhibitory concentration showed that a very low concentration of 6.25 - 12.5 mg/ml the ethanolic stem bark extract of *Psidium guajava* inhibit the growth of all tested isolates.

Preliminary Phytochemical Analysis of *P. guajava* In the present study was carried out to identify the active constituents such as alkaloids, flavonoids, sterols, terpenoid, Anthraquinones, protein and amino acid, phenol, carbohydrate and cellulose present in the leaves and stem bark of guava plant. Preliminary phytochemical analysis of leaf and stem bark extract, (ethanol and water) of *P. guajava* showed the presence of the following chemical constituents; Alkaloid, saponin, phenol, flavonoids, protein and amino acid, Anthraquinones, terpenoid and tannin (Table 4). Earlier work have revealed the presence of alkaloids, flavonoids, glycosides, poly-phenols, reducing compounds, saponins and tannins in the aqueous extract of *P.guajava* leaf [22]. This finding can be attested to the work of Offo [23] who also reported similar finding on the phytochemical of guava leaf extract, which contain alkaloid, saponin, flavonoids, phenol, steroid, tannin, protein and glycoside. Pandey and Shweta, [18] reported the phytochemicals mainly present in *Psidium guajava* were reducing sugar, tannin, saponin, phlobatannin, terpenoid, alkaloid and phenols. The finding is also similar to that of Joseph and Priya, [24] where the preliminary phytochemical analysis of leaf, stem bark and root extracts of *Psidium guajava* showed the presence of carbohydrate, glycoside, saponin, Anthraquinone, Flavonoid, tannin and alkaloids at high concentration.

4. CONCLUSION

The antibacterial activity of the plant showed that the plant part extracts demonstrated antimicrobial effect against the test isolates with higher activity in stem bark extract compared to leaves extract. Higher antibacterial activity was recorded in ethanol extract compared to aqueous extract. The antibacterial activity of the plant parts is due to the present of the Phytochemicals identified in this study. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts ranges from 6.25 – 50 mg/ml of the extracts. The Phytochemical screening of the plant parts revealed the present of Alkaloid, Tannin, Saponin, and Cardiac glycoside, protein and amino acid, Flavonoid, Terpenoid, Phenols and Anthraquinone. Findings from this work support the use of extracts from *P. guajava* stem bark and leaves as medicinal plant. It is recommended that Government, Non-governmental organizations (NGOs) and philanthropists should encourage researchers in this field so that the spread of antibacterial resistant pathogens can be restrained.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval (issue number HMB/GEN/488/ VOL. 1) was obtained from Kano State Hospital Management Board based on the consent of Murtala Muhammad Specialist Hospital ethical committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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