Antibacterial Activity and Phytochemical Screening of *Psidium guajava L.* (GUAVA) Leaf Against *Escherichia coli* Isolated from Stool Samples of Children Attending Specialist Hospital, Gusau

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Abstract

Guava (Psidium guajava) leaf is commonly used as a medicine against gastroenteritis and child diarrhea by those who cannot afford or do not have access to antibiotics. This study was aimed to determine the antibacterial activity of Guava leaf (Psidium guajava) extracts on E. coli using agar well diffusion method. Two different extracts were obtained from the guava leaf (aqueous-soluble and ethyl acetate soluble extracts). Psidium guajava leaves were extracted with water and ethyl acetate using maceration extraction method. Phytochemical screening of the Psidium guajava leaf extracts was carried out using standard methods. Agar well diffusion and agar dilution methods were employed to determine the zone of inhibition, minimum inhibitory concentration, and minimum bactericidal concentration. The antibacterial test results showed that the Psidium guajava ethyl acetate and aqueous extracts have potential antimicrobial activity against Gram-negative bacteria. The Ethyl acetate extract showed the maximum zone of inhibition on test isolates (17mm at 100mg/ml) While the Aqueous extract showed the least inhibitory effect on test isolates (8mm at a concentration of 12.5mg/ml). The minimum inhibitory concentration (MIC) showed that both E. coli isolates was sensitive to both Ethyl acetate and aqueous extract (at the lowest concentration of 12.5mg/ml). The minimum bactericidal concentration (MBC) of aqueous extract was 100mg/ml for both the isolates and ethyl acetate extract for 50mg/ml for both the isolates, this result indicate that Guava leaf extracts have antibacterial activities against the test isolates, and the Ethyl acetate extract is more effective compared to the aqueous extract.

Keywords: *Antibacterial activity, E coli, MIC, MBC*

Introduction

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Almodaifer *et al*., 2017). They protect plants from disease and damage and contribute to the plants color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Abdullahi, and Hamza 2020).

The Guava plant (*Psidium guajava*) is a tropical plant easily found in Nigeria (Eze and Maxwell, 2021). Many parts of this plant are utilized by humans, especially its fruits and leaves. Notably, its fruit is commonly consumed as fresh fruit or processed food. Guava fruit contains tryptophan lysine, pectin, calcium, phosphor, minerals and vitamin (Li *et al.*, 2024). Its fruit is currently used to treat diabetes mellitus patients and people who have high-level blood cholesterol (Oluwajuyiita *et al.*, 2021). This study was aimed to determine the antibacterial activity of ethyl acetate and aqueous extracts of *Psidium guajava* leaves against *Escherichia coli recovered* from stool samples of diarrhoeal children.

MATERIALS AND METHODS

Sample collection

Fresh and healthy leaf of *P. guajava* was collected in Damba area of Gusau Metropolis and then transported to the Herbarium section in the Department of Biological Science, Federal University Gusau where identification and authentication of the plant take place with the following voucher number 113. After authentication, a voucher plant specimen was deposited in the herbarium of the University for future reference.

Preparation of plants material

The fresh Guava leaves were washed under running tap water and air dried in shade so as to prevent the decomposition of chemical constituents for 14 days. The dried leaf material was grinded into fine powder using sterile pestle and mortar under laboratory condition. The powder was kept in dark and air tight container before use.

Preparation of Extract

Water and ethyl acetate were used as solvents in the extraction process. Forty grams (40g) of the powdered leaves was weighed out and mixed with 400ml of distilled water and ethyl acetate separately. Both aqueous and ethyl acetate extracts were extracted by maceration method (Subeno and Makkiyah, 2024). The extracts were evaporated and dried at 40 °C using water bath. The extract yields were weighed, stored in dark air tight container at 4°C (Singla *et al*., 2020).

Phytochemical tests

Phytochemical screening of the plant materials was conducted using the method adopted by (Tiwari *et al*., 2011) as follows;

Test for alkaloids

Extract was mixed with 2ml of 1% HCl and heated gently. Mayer's and Wagner's reagent were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Test for steroids

Extract was mixed with chloroform and concentrated H_2SO_4 was added sidewise. A red color produced at the lower chloroform layer indicates presence of steroids.

Test for terpenoid

Extract was dissolved in 2 ml of chloroform and evaporated to dryness. To this, 2 ml of concentrated H_2SO_4 was added; a reddish-brown coloration formed at the interface indicated the presence of terpenoids.

Test for Tannins

0.2 g sample of *psidium guajava* was stirred with water and filtered. A dirty-green precipitate, or blur-black, or blue-green precipitates, on addition of few drops of 5 % ferric chloride to the test extract would be taken as an indication of the presence of tannins.

Test for Saponins

0.2 g sample *Psidium guajava* was dissolved in 5 ml of distilled water. 2 ml of the resulted solution was taken into a test tube and will be shaken vigorously for a few minutes. Frothing which persists on warming was taken as evidence of the presence of saponins.

Test for Flavonoids

A small quantity of the extracts was dissolved in dilute 2% NaOH. A yellow solution that turns colorless on addition of 1% HCl acid indicates the presence of flavonoids.

Test for Phenols

Test extracts was dissolved in ferric chloride solution. Blue-black or brown coloration indicates the presence of phenols.

Test for Triterpenes

Equal volume acetic anhydride was added to the extract. One millimeter of concentrated H_2SO_4 was added downside the tube and the color charge was observed immediately and later. Red, pink and purple indicated the presence of triterpenes.

Test for Glycosides

The 2.5 ml of 50 % H_2SO_4 was added to 5 ml of each extracts in test tubes. The mixture was heated to boiling for 15 minutes. Cooled and neutralized with 10 % NaOH 5ml of Fehling's solution was added and the mixture was boil again. A brick-red precipitate was observed, which indicated presence of glycoside.

Collection of clinical samples

The 10 stool samples of diarrhoea children with gastrointestinal tract infection were collected from specialist Hospital Gusau, Zamfara state, Nigeria.

Isolation and Identification of Bacterial Species

Bacterial species were isolated from stool samples of gastrointestinal tract infected (GIT) Patients referred to Microbiology laboratory federal University Gusau. The stool samples were cultured by streaking on EMB agar and then subcultured on nutrient agar and incubated at 37 °C for 24 hrs.

Identification of *Escherichia coli* isolate was based on cultural, morphological and biochemical characteristics such as, Indole, Methyl Red, Vorges-Proskauer, Citrate utilization, following standard microbiological procedures as described by (Kennith *et al.,* 2017). The isolates were monitored throughout the duration of the study on agar slant.

Preparation of both Aqueous and Ethyl Acetate Extract Concentration for antibacterial assay

0.2 g of *Psidium guajava* extract was dissolved in 2 ml of Dimethyl sulfoxide (DMSO) to make 100 mg/ml. it was then serially diluted to obtain 50, 25 and 12.5 mg/ml concentrations.

Antibacterial Activity of the Extracts

The agar well diffusion method was used to determine the antibacterial activity of the plant extracts as described by (Ali *et al*., 2017). 0.1 ml volume of the different standardized organisms (0.5 McFarland) was introduced onto the surface of freshly prepared Mueller Hinton agar in a sterile Petri dish and allowed to set and then labeled. Four wells was filled with different concentrations of the extract which were labeled accordingly; 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml, while the 5th well will contain the control, 5 µg of Ciprofloxacin (Oxoid UK) as positive control and water as negative control in this research.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the extracts was determined using broth dilution technique (Sule *et al*., 2011).

Determination of Minimum Bactericidal Concentration (MBC)

From each tube that did not show visible growth in the MIC, 0.1ml was aseptically transferred onto extract free Mueller Hilton agar plates. The plates were 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 (Adikwu *et al.,* 2022)

RESULTS

The qualitative phytochemical screening of *P. guajava* leaf aqueous and ethyl acetate extract was presented in Table 1. The result indicated the presence of alkaloid, saponin, phenol, flavonoid,

tannin and terpenoid in both extracts while steroid was present only in ethyl acetate leaf extract. Resin, glycoside and triterpenes were absent in both the extracts.

The result of identification of isolates is presented in Table 2. The result showed that *E. coli* is *Gram* negative bacteria, positive for MR and indole tests but negative for VP and citrate utilization test. The isolates produced green metallic sheen on Eosin methylene blue agar plate.

The antibacterial activity of aqueous and ethyl acetate extracts of *P. guajava* leaf were indicated in Table 3. The result showed that the mean diameter of zone of inhibition of extracts on the test isolate and highest zone of inhibition recorded was 17mm from 100 mg/ml ethyl acetate extract while the lowest zone of inhibition was 8 mm from 12.5mg/ml aqueous extract. The zones of inhibition recorded by the control (10 µg ciprofloxacin) were 24, 26 and 25 mm for *E. c1, E.c2 and E.c3* respectively.

The MIC of the extracts against the isolates were indicated in Table 4. The MIC result showed that the extracts inhibit the growth of the isolates at the concentration of 12.5 mg/ml.

The MBC of the extracts against the isolates were indicated in Table 5. The MBC result showed that the ethyl acetate extract against the isolates was lower than that of the aqueous extract. This showed that both the extracts exhibit bactericidal activity against the isolates

Table 1: Phytochemical screening of *P. guajava* leaf Extracts

S/N	Phytochemicals	Extract type	
		Aqueous	Ethyl acetatic
1	Alkaloid	+	+
2	Saponin	+	+
3	Phenol	+	+
4	Flavonoid	+	+
5	Tannin	+	+
6	Terpenoid	+	+
7	Resin	-	-
8	Glycoside	-	-
9	Steroid	-	+
10	Triterpenes	-	-

Key: + = Presence of phytochemical, - = Absence of phytochemical.

Table 2: Morphological and characteristics of the test Isolates

S/N	Test	Sample 1	Sample 2	Sample 3
1	Gram staining	-	-	-
2	Indole	+	+	+
3	Methyl-red	+	+	+
4	Voges Proskauer	-	-	-
5	Citrate utilization	-	-	-
6	EMB agar growth	Green metallic sheen	Green metallic sheen	Green metallic sheen

Table 3: Antibacterial activity of *P. guajava* leaf Extracts against the clinical isolates

Isolates	Extracts	12.5	25	50	100	Control (Ciprofloxacin)	*P*-value
E. coli$_1$		10	11	11	13	24	0.00001
E. coli$_2$	Aqueous	09	08	11	14	26	
E. coli$_3$		10	11	12	13	25	
E. coli$_1$		11	12	13	16	24	
E. coli$_2$	Ethyl acetate	10	13	15	17	26	
E. coli$_3$		10	12	13	16	25	
***P*-value**	**0.00013**						

Key: The result is significant at $p < 0.05$

Table 4: Minimum inhibitory concentration MIC of the extracts of *Psidium guajava* leaves

Isolates	Aqeous Extract	Ethyl acetate Extract
E. coli$_1$	12.5	12.5
E. coli$_2$	12.5	12.5
E. coli$_3$	12.5	12.5

Table 5: Minimum bactericidal concentration MBC of the extracts of *Psedium guajava* leaves

Isolates	Aqeous Extract	Ethhyl acetatic Extract
E. coli$_1$	100	50
E. coli$_2$	100	50
E. coli$_3$	100	50

Discussion

The result of the preliminary phytochemical screening of the leaf extract (ethyl acetate and water) of *P. guajava* revealed the presence of the following constituents; alkaloid, saponin, phenol, flavonoid, tannin and terpenoid, which disrupt bacterial cell membranes and inhibit growth. The presence of these phytochemical agents reported in this study is similar to the reports of (Abdullah *et al*., 2019), in a study he conducted in Bayero University Kano, Nigeria using *Psidium guajava* leaf and stem extracts. The findings of this study is also similar with the findings of Raj *et al.*, (2020), in a study he conducted using the leaf of *Psidium guajava* extract.

The result of this study showed that *Psidium* guajava extract exhibited varied antibacterial activity against *E. coli* associated with gastrointestinal infection. The result of antibacterial activity of the extracts against isolates showed that the ethyl acetate extract demonstrated higher activity against the isolates tested than aqueous extract. This might be attributed to better solubility of the active components by the ethyl acetate than water. There are several reports that showed that the activity of extracts was largely depend on the types of extraction solvent used as observed in this work and is in conformity with the report of (Ilesanmi *et al*., 2020). Activity index of the extracts showed that the extracts can be a good source of drugs.

MIC and MBC of the extracts showed it has both bacteriostatic and bactericidal activity against the isolates. Antibacterial activity of the extracts against the isolated might be attributed to the presence of phytochemicals such as alkaloid, tannins and saponin. *P. guajava* contain several phytochemicals which is responsible for its medicinal value (Raj *et al*., 2020).

Conclusion

The finding of this study showed that the leaf of *P. guajava* contain several phytochemicals such as alkaloid, saponin, phenol, flavonoid, tannin, steroid and terpenoid, which disrupt bacterial cell membranes and inhibit growth. The plant extracts demonstrated activity against the *E. coli* isolated from stool samples of diarrhea patients. However, ethyl acetate extract showed considerable higher activity than aqueous extract. The antibacterial activity of the plant may be attributed to the presence of phytochemicals. The activity index of the plant leaf extract indicated that the plant leaf is a good candidate as a source of drugs. MIC and MBC of the extracts showed it has both bacteriostatic and bactericidal activity against the isolates.

Recommendation

Government, non-governmental organizations (NGOs) and philanthropists should encourage researchers in this field so that the spread of antibacterial resistant pathogens can be restrained.

CONFLICT OF INTEREST

The authors declare no conflict of interest exist

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