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(RESEARCH ARTICLE)

Phytochemical analysis and antibacterial activity of *Psidium guajava* L. leaf extracts

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Abstract

The increasing menace of antimicrobial resistance in many pathogenic microbes has led to the search for long lasting remedy. The aim of this study was to examine the phytochemical and antimicrobial properties of extracts of *Psidium guajava* leaves against some clinical bacterial isolates. The plant leaves were extracted in three solvents namely; water, ethanol and methanol. The pathogenic bacterial isolates were *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae* and *Klebsiella pneumonia*e all isolated from urine samples. The phytochemical analysis showed the presence tannins, flavonoids, alkaloids, saponins, glycosides and terpenoids in different proportions. The mean antibacterial activity of the extracts *in vitro* showed that the ethanolic extract was most efficacious at 25 mg concentration, inhibiting *P. aeruginosa* (9.50 mm), *E. coli* (9.00 mm), *S. pneumoniae* (10.50 mm) and *K. pneumoniae* (9.50 mm). The aqueous extract at 100 mg concentration inhibited *E. coli* (12.50 mm), *S. aureus* (14.50 mm) and *S. pneumoniae* (9.00 mm). This study has revealed that the leaves extract of *P. guajava* contains antibacterial and phytochemical substances which can be harnessed in satiation of human quest for better and healthier living.

Keywords: *Psidium guajava*; Plant extracts; Phytochemical; Antibacterial activity

1. Introduction

The continuous evolution of multidrug resistant pathogens is a global clinical concern [1-3]. In recent years there has been an increasing incidence of multiple drug resistance in human pathogenic microorganisms due to the indiscriminate uses of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases [4-5]. This has led to the increase in search and research for new antimicrobial substances from various sources like medicinal plants. The search for new antibacterial agents by the screening of many plant families is encouraged [6-7]. Additionally, using antibiotics is sometime associated with adverse effects [8-9]. Therefore, phytomedicine could be an alternative treatment method for bacterial infections which may decrease such problems [9].

Psidium guajava is a fruit bearing plant commonly known as guava, which belongs to the family Myrtaceae [4]. Guava grows nearly throughout Nigeria up to 1500 m height and is cultivated commercially in some regions. The plant is described by its particular thin, smooth, copper hued bark that fragments off, demonstrating a greenish layer underneath. Guava trees have spread generally all through the tropics since they flourish in a variety of soils, easily propagating and bearing fruits rapidly. The fruits are appreciated by fowls and monkeys, which scatter the seeds and makes natural dumps of guava saplings to cultivate all over the rainforest [10].

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The leaves and bark of guava tree have a long history of medicinal uses. Decoction of the leaves and bark of guava is used to cure diarrhoea, dysentery, vomiting, sore throats and also to regulate menstrual cycles. 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 labour. They are also an excellent source of fibre, potassium and retinoic acid [11].

Psidium guajava is a phytotherapic plant used in folk medicine and is believed to have active components that helps in treatment and management of various diseases [12]. Guava has exhibited remarkable antimicrobial activity against microorganisms such as *Bacillus*, *E. coli*, *Staphylococcus, Pseudomonas, Clostridium*, *Shigella*, *Salmonella* and yeast such as *Candida* species [13]. This study investigates the phytochemical and antibacterial properties of *Psidium guajava* L. leaf extracts.

2. Material and methods

2.1. Collection of Samples and Preparation

Fresh and healthy leaves of *P. guajava* L. were obtained at locations within Nasarawa state, North-Central Nigeria and identified at the Plant Science and Biotechnology herbarium in Nasarawa State University, Keffi. The leaves were washed in distilled water and allowed to dry at room temperature for 7 days. The leaves were then pulverized into coarse powder using blending machine.

2.2. Isolation and Identification of Bacterial Species

Bacterial species were isolated from urine samples of urinary tract infected (UTI)patients referred to the Microbiology Laboratory Unit of Federal Medical Centre, Keffi, Nigeria.The urine samples were cultured by streaking on Eosin Methylene Blue (EMB), MacConkey Agar (MA) and Mannitol Salt Agar (MSA), and incubated at 37 °C for 24 hrs.

Identification of the bacterial isolates was based on the cultural and morphological and biochemical characteristics such as, Indole, Methyl Red/Vorges-Proskauer, Citrate utilization, Catalase, Coagulase, Nitrate reduction, Urease and Sugar fermentation tests following standard microbiological procedures as described by Cheesebrough [14] .

2.3. Extraction of *Psidium guajava* leaves

Extracts were prepared following the method described by S´anchez et al. [15]. Briefly, 100 g of dried pulverized guava leaves were soaked in 500 mL of water, methanol and ethanol for 24 hrs at room temperature, under occasional shaking. Extraction was repeated three times, and the extracts obtained were filtered using Whatman filter paper number 1. After that, the extracts were concentrated to dryness under reduced pressure using a rotary evaporator at 45 °C.

2.4. Phytochemical Analysis

2.4.1. Test for Alkaloids

The extracts (20 μL) was applied on TLC plate (Silica Gel 60G, 5 × 10 cm) and eluted using toluene-ethyl acetate-diethylamine (70: 20: 10) as solvent system. Alkaloid was detected after spraying Dragendorff's reagent as orange-brown spots on the TLC plate [16].

2.4.2. Test for Quinones

Extracts suspended in ethanol (1 mL) were treated with 1mL of concentrated sulfuric acid. Formation of red colour showed the presence of quinones [17].

2.4.3. Test for Glycosides

One ml of glacial acetic acid, 3 drops 5% W/V ferric chloride and concentrated sulphuric acid were added to test tubes containing 2 ml of extracts and observed. The disappearance of reddish brown colour at the junction of two layers and bluish green in upper layer indicates the presence of glycosides [18].

2.4.4. Test for Tannins

Extracts were treated with 1mL of 5% ferric chloride. The presence of tannin was indicated by the formation of bluish black or greenish black precipitate [19].

2.4.5. Test for flavonoids

Few fragments of magnesium metal ribbon (3-4 pieces) was added to 1 mL of the extracts, followed by drop wise addition of concentrated hydrochloric acid. Formation of pink or red colour indicated the presence of flavonoids [20].

2.4.6. Test for Saponin

The 2 mL of distilled water was added to extracts suspended in ethanol and was shaken vigorously. The formation of profuse foam layer indicated the presence of saponins [20].

2.4.7. Test for Terpenoids

One mL of acetic anhydride and 5 drops of concentrated sulfuric acid (H_2SO_4) was added to the extracts.A colour change from violet to blue confirms the presence of steroids [21] and formation of blue-green ring indicates the presence of terpenoids [22].

2.5. Determination of Antibacterial Activity of *Psidium guajava* leaves

Antibacterial activity of crude extracts (aqueous, methanol and ethanol) of *P. guajava* leaves was carried out using cup-plate agar diffusion bioassay [23] as follows; 100 μL of fresh culture (Standardized to 0.5 McFarland) was spread uniformly on a sterile Mueller-Hinton agar (MHA) plates and allowed to air dry. After that, wells of 6 mm in diameter were made in the MHA plates using a sterilized cup-borer and the base was seeded with molten MHA and approximately 100 μL for each concentration (50 mg/L, 25 mg/L, 12.5 mg/L, 6.25 mg/L, 3.125 mg/L, 1.56 mg/L and 0.78 mg/L) of the extract was dispensed into the wells and the plates were allowed to stand for 1 hr at room temperature for pre-diffusion and then incubated at 37 °C for 24 hrs and the diameter zone of inhibition against the test strain is measured and recorded. Ciprofloxacin (5µg) was used as control.

3. Results and discussion

The qualitative screening of phytochemical properties of *P. guajava* leaves extracts showed the presence of moderate quantities (+) and large quantities of tannins, alkaloids, saponins, glycoside, terpenoids, and flavonoids in the different solvents used (Table 1).

Table 1 Phytochemical test on solvent fractions of *P. guajava* leaves extracts

Fractions	Tannins	Flavonoids	Alkaloids	Saponins	Glycoside	Terpenoids
Ethanol	+	++	+	-	-	+
Methanol	++	++	-	+	+	+
Aqueous	++	+	+	+	+	+

- =Absent; + = Present in moderate quantity; ++ = Present in large quantity

The antibacterial activity of crude ethanol extracts of *P. guajava* against clinical bacterial isolates (Table 2) showed that the ethanolic extracts has antibacterial activity at concentrations 25 mg above against all tested bacteria with the exception of *S. aureus* which was inhibited only at 50 mg concentration. Table 3 showed the antibacterial activity of crude methanolic extract of leaves of *P. guajava* against clinical bacterial isolates. In the results, the highest inhibitory activity was observed against *K. pneumoniae* where 16.5mm mean zone of inhibition was observed at 50mg concentration. *E. coli* was mostly resistant to this extract and only showed 1.0 mm mean zone of inhibition at 50mg concentration. The antibacterial activity of crude aqueous leaves extract of *P. guajava* against clinical bacterial isolates (Table 3) showed significant level of activity against all test bacterial isolates at 100 mg concentration with decreased activity at lower concentrations.

Table 2 Antibacterial activity of crude ethanol extract of *P. guajava* leaves against clinical bacteria isolates

Bacterial isolates	Diameter zone of inhibition (mm)						
	50 mg	25 mg	12.5 mg	6.25 mg	3.125 mg	1.56 mg	0.75 mg
P. aeruginosa	12.5 ± 0.71	9.5 ± 0.78	0.00	0.00	0.00	0.00	0.00
E. coli	16.0 ± 1.41	9.0 ± 1.41	0.00	0.00	0.00	0.00	0.00
S. aureus	10.0 ± 0.74	0.00	0.00	0.00	0.00	0.00	0.00
S. pneumoniae	12.0 ± 1.43	10.5 ± 2.10	0.00	0.00	0.00	0.00	0.00
K. pneumoniae	11.5 ± 0.77	9.5 ± 0.72	0.00	0.00	0.00	0.00	0.00

Results expressed as mean ± SD

Table 3 Antibacterial activity of crude methanolic extract of *P. guajava* leaves against clinical bacteria isolate**s**

Bacterial isolates	Diameter zone of inhibition (mm)						
	50 mg	25 mg	12.5 mg	6.25 mg	3.125 mg	1.56 mg	0.75 mg
P. aeruginosa	14.0 ± 1.51	10.5 ± 1.55	7.5 ± 0.87	0.00	0.00	0.00	0.00
E. coli	1.0 ± 1.51	0.00	0.00	0.00	0.00	0.00	0.00
S. aureus	14.0 ± 1.51	10.0 ± 1.45	0.00	0.00	0.00	0.00	0.00
S. pneumonia	11.0 ± 1.61	0.00	0.00	0.00	0.00	0.00	0.00
K. pneumoniae	16.5 ± 1.71	9.5 ± 0.45	5.6±0.58	0.00	0.00	0.00	0.00

Results expressed as mean ± SD

Table 4 Antibacterial activity of crude aqueous extracts of *P. guajava* leaves against clinical bacteria isolates

Bacterial isolates	Diameter zone of inhibition (mm)						
	50 mg	25 mg	12.5 mg	6.25 mg	3.125 mg	1.56 mg	0.75 mg
P. aeruginosa	18.0 ± 1.51	10.5 ± 1.55	5.5 ± .87	0.00	0.00	0.00	0.00
E. coli	12.5 ±2.11	0.00	0.00	0.00	0.00	0.00	0.00
S. aureus	14.5 ± 0.69	14.5 ± 1.45	0.00	0.00	0.00	0.00	0.00
S. pneumonia	12.0 ± 1.45	9.0 ±1.42	0.00	0.00	0.00	0.00	0.00
K. pneumonia	12.5 ± 1.71	16±1.67	5.6±0.58	0.00	0.00	0.00	0.00

Results expressed as mean ± SD

This study on the phytochemical and antimicrobial properties of *P. guajava* leaves extracts on clinical bacterial isolates using alcoholic solvents (ethanol and methanol) and water revealed that the ethanolic extracts inhibits *P. aeruginosa*, *E. coli*, *S. pneumoniae*, and *K. pneumoniae* at 25 mg concentration whereas *S. aureus* was inhibited at 50 mg concentration of the ethanolic extracts. However, the methanolic extracts was potent at 12.5 mg concentration against *P. aeruginosa* and *K. pneumoniae*, while the aqueous extract was potent at 25 mg and above against tested bacterial isolates. Fugaban [13] has reported that the alcoholic extract of *P. guajava* leaves have antimicrobial activity against the fungus *Tricophyton metagrophytes*. Pandey et al. [12] has also demonstrated the antifungal properties of *P. guajava* leaves extracts. In line with this research report also, Biswas *et al*. [24] has reported that *P. guajava* has Gram-negative and Gram-positive antibacterial characteristics.

This research has also demonstrated the presence of alkaloids, flavonoids, tannins, saponins, glycosides and terpenoids in the leaves extracts of *P. guajava*. Also, these phytochemicals has been shown to have inhibitory activity against some clinical bacterial isolates *in vitro*. Reports have been made on the use of plants for therapeutic purposes dating back to centuries before the advent of antibiotics. The cinchona plant has been reportedly used to treat malaria [25-26]. Since microorganisms have become increasingly resistant to available antibiotics, scientists have been exploring various sources, including plants, to remediate the menace of antimicrobial resistance.

In recent years, reports on the antimicrobial and phytochemical properties of different plants have been published [12-13, 27-33]. This research result is believed to contribute in a way as humans continue to source for total cure for infectious diseases especially with the growing trends of antimicrobial resistivity.

4. Conclusion

The phytochemicals and antimicrobial studies of *P. guajava* leaf extracts provided scientific evidence for the rationale use of *P. guajava* leaves in prevention of disorders due to presence of some useful phytochemicals, and in treatment of diseases caused by some bacterial pathogens such as *P. aeruginosa, E. coli, S. aureus, S. pneumoniae* and *K. pneumonia*e. Further research is necessary to reveal its detailed molecular mechanism behind these phytochemical and antibacterial activities.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Authors have declared that no competing interests exist.

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