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Antimicrobial Effects and Phytochemical Analysis of Guava Leaf Extracts on Selected Microorganisms

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Abstract

The misuse of antimicrobial agents can lead to emergence of resistance to microbes which in turn can lead to the search for alternative therapy. The use of phytopharmaceuticals and herbal medicines have a positive impact and represents a promising alternative. Guava extract has been reported to have antimicrobial properties. However, the aim of this study was to determine the antimicrobial activities of methanol and ethanol extract of guava leave against some certain bacteria (Staphylococus aureus and Escherichia coli) and a fungus (Candida albicans). Qualitative phytochemical screening showed that phenols, tannins, saponins, terpenoids, flavonoids and glycosides are all present in the leaf extract;

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phenols and tannins are moderately present while others are present in minute quantities. Out of the different concentrations use (50, 100, 200 and 250), The highest concentration which is 250mg/ml showed the highest zone of inhibition for both methanolic and ethanolic extracts against the isolates. Minimum inhibitory concentration and Minimum Bactericidal Concentrations value for both S. aureus and E. coli are 200mg/ml and 250mg/ml respectively; while 50mg/ml and 100mg/ml are for Candida albicans.

Keywords: Concentration, Phytochemical, Susceptibility, Therapy.

INTRODUCTION

Antimicrobial resistance can be described as the ability of a microorganism to resist the action of antimicrobials, which regularly occurs through continuous exposure to them. The level of resistance of a mutational strain varies widely depending on the mechanism of resistance resulting in its evolution (Hughes and Andersson, 2017).

Plants produce a diverse array of secondary metabolite, many of which have antimicrobial activities against some pathogenic microbes that are implicated in an infection. Some of these compounds have constituents existing in healthy plants in their active form biologically and they elicit chemoprophylactic and chemotherapeutic properties against wide range of infectious agents (Oncho *et al.*, 2021). Phytochemicals are chemical compounds that occur naturally in plants (phyto means "plant" in Greek). Some are responsible for colour and other organoleptic properties, such as the deep purple of blueberries and the smell of garlic. Phytochemicals may have biological significance, for example carotenoids or flavonoids, but are not established as essential nutrients. The improper use of antimicrobials stimulated the emergence of genetic modifications that contributed to circumventing the mechanism of action of drugs. Therefore, the expansion of resistant strains results to public halt as it leads to infectious conditions that require difficult treatment (Hughes and Anderson, 2017; Pereira *et al.,* 2023). The continous evolution of multidrug resistant pathogens is a global clinical concern. This has led to the increase in search and research for new antimicrobial substances from various sources (Kenneth *et al.,* 2017). The use of phytopharmaceuticals and herbal medicines have positive impacts on therapy, representing a promising alternative since many microorganisms have developed resistance to synthetic drugs (Adamczak *et al.,* 2019; Pereira *et al.,* 2023).

MATERIALS AND METHODS

Study area

Collection of samples

The fresh leaves of *Psidium guajava* were gotten from compound Shao, Kwara state Nigeria. The leaves were taken to University of Ilorin Herbarium, Plant Biology Department for identification and a voucher number. The leaves were rinsed with water and air dried to crispiness at room temperature on the lab bench for one week. The dried leaves were grinded to powdered form using an electric blender and stored in an enclosed container.

Identification and maintenance of Test Organisms

Microbial isolates were collected and identified at Al-Hikmah University Ilorin, Kwara State, Nigeria. The organisms were*Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Nutrient agar for bacteria isolates at 37^{o}C and potato dextrose agar for fungi isolate at 27^{o}c.

These organisms were sub-cultured to ensure viable growth after incubation for 24hours and 48hours.

Preparation of Culture Media
Certain culture media were used to carry out this project; Nutrient agar (NA) was used for the culturing of the bacteria isolates; Potato dextrose agar (PDA) was used for fungus culture; Mueller Hinton agar (MHA) was used to carry out the antimicrobial susceptibility test. All media were prepared in accordance to the manufacturer's specification.

Extraction and Sterility Test of Plant Extracts
For the extraction, two solvents were used, which are the methanol and ethanol. forty grams (40g) of the leaf powder was soaked into 160ml of sterile distilled water for 72hours at room temperature with intermittent soaking using the rotary shaker at 200rpm. It was then filtered using muslin clothes; the filtrate was evaporated using water bath at 40^0C and until when needed was stored in the refrigerator.

Phytochemical Screening of the Leaf Extracts of *P. guajava*
Chemical tests for the screening and identification of bioactive chemical constituents in the guava were carried out with the extracts using the standard procedure as described by ; (Biswas *et al.,* 2013). For each test, 1 mL of each solvent extract was used for analysis, in exception for the saponin test in which 3 mL solvent extract was used.

Test for saponins; 0.5g of each extracts was placed in a test tube and shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for phenols and tannins; Extract was mixed with 2 mL of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

Test for Terpenoids (Salkowski's Test); Extract was mixed with 2 mL of chloroform. Then 2 mL of concentrated sulfuric acid was added carefully and shaken gently. A reddish brown coloration of the interphase was formed to show positive results for the presence of terpenoids.

Test for Flavonoids (Shinoda Test); Extract was mixed with magnesium ribbon fragments, and concentrated hydrochloric acid was added drop wise. Orange, red, pink, or purple coloration indicates the presence of flavonoids.

Test for Glycoside; Extract was mixed with 2 mL of glacial acetic acid containing 2 drops of 2% $FeCl_3$. The mixture was poured into another tube containing 2 mL of concentrated sulfuric acid. A brown ring at the interphase indicates the presence of glycosides. (Lali and Sukirtha, 2018).

Antimicrobial and Antibiotics Sensitivity Testing
For the antimicrobial sensitivity testing, agar well diffusion method of (CLSI, 2021) was used. Mueller Hinton agar and Potato Dextrose agar were used for this test and was prepared as instructed by the manufacturer. A loop of the standardized bacteria and fungus cell were evenly inoculated into 2ml of normal saline already dispensed into sterilized test tubes, compared with the Mc Farland solution and incubated for 4hours. Using a swab stick, each organism was streaked on molten agar plates. Using a sterile cork borer, five wells each were made on the agar plates and two drops of each plant extract was discharged into each well (7mm in diameter). The plates were allowed to stand on the work bench for one hour for the

pre-diffusion of the extracts to occur before incubating for 24hours at 37^0C for bacteria and 48hours for fungi. The plates were then observed for growth and zone of inhibition of microorganisms. A zone of clearance around each well signifies the presence of antimicrobial property of each extract. Each of the isolates was also tested on broad spectrum antibiotics such as, Gentamycin and Streptomycin for bacteria and Nystatin for the fungus. The antibiotics discs for bacteria were placed on the Mueller Hinton agar plate that had been inoculated on with bacteria using sterile pair of forceps while the liquid Nystatin was used to fill the holes on the plates containing fungus culture and inoculated at 27^0C for 24 and 48 hours respectively for microbial growth and inhibition (Bale and Mukhtar, 2021.

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

The agar dilution method described by Pereira *et al.,* 2023 was used. The minimum inhibitory concentration (MIC) and minimum cidal concentration (MCC) were evaluated using four (4) final concentrations namely 50, 100, 200 and 250mg/ml respectively in 20ml of nutrient agar each. In the first set of assay, *Staphylococcus aureus* was used and each concentration of ethanolic and methanolic extract was in duplicate plates of 20ml of nutrient agar each. Each plate was streaked with over- night nutrient broth culture of *Staphylococcus aureus*.
In the second set of assay, *Escherichia coli* was used and each concentration of ethanolic and methanolic extract was in duplicate plates of 20ml of nutrient agar each. Each plate was streaked with over- night nutrient broth culture of *Escherichia coli*.

In the third set of assays, *Candida albicans* was used and each concentration of ethanolic and methanolic extract was in duplicate plates of 20ml of nutrient agar each. Each plate was streaked with over- night nutrient broth culture of *Candida albicans*. The inoculated plates were incubated at 37^0C and 27^0C for 24, 48 and 72 hours respectively and were examined for growth.

RESULTS

Table 1 showed the qualitative phytochemical screening of *Psidium guajava* methanolic and ethanolic extracts. This was done to extract and identify the active components present in the leaf extracts. The results showed that phenols, tannins, saponins, terpenoids, flavonoids and glycosides are all present in the leaves extracts but phenols and tannins are moderately present while the others are present in small quantities. The antimicrobial activity of ethanolic and methanolic leaf extracts of *P. guajava* against the isolated organism showing the zones of inhibition in millimeter (mm) (Table 2). The result shows that there were four (4) different concentrations in Milligram per mill (mg/ml) (50, 100, 200 and 250). The highest concentration which is 250mg/ml shows the highest zones of inhibition for both methanolic and ethanolic extracts for the isolated organisms.

Table 3 showed the zone of inhibition in millimeter (mm) by guava extracts and those of already existing antibiotics (Gentamycin, Streptomycin and Nystatin). The result showed that the methanolic extracts and the antibiotics have similar zone of inhibition. The Minimum cidal and Minimum Inhibitory Concentrations of the ethanol extracts (Table 4). The result shows that MIC value is 200mg/ml and MBC value was 250mg/ml for *Staphylococcus aureus* and *Escherichia coli* while MIC value is 50mg/ml and MBC value is 100mg/ml for *Candida albicans*. The Minimum cidal and Minimum Inhibitory Concentrations of the methanolic extracts (Table 5). The result showed that MIC value is 200mg/ml and MBC value is 250mg/ml for *Staphylococcus aureus* and *Escherichia coli* while MIC value is 50mg/ml and MBC value is 100mg/ml for *Candida albicans*.

Table 1: Phytochemical screening of *Psidium guajava* leaves

Extracts	Phenols & Tannins	Saponins	Terpenoids	Flavonoids	Glycosides
Methanol	++	+	+	+	+
Ethanol	++	+	+	+	+

Key: ++ = moderately present, + = present in small quantity

Table 2: Antimicrobial activity of ethanolic and methanolic leaf extracts of *P. guajava* against the selected organism (mm).

Extracts	Organism	Extract Concentration (mg/ml)			
		250	**200**	**100**	**50**
	Staphylococcus aureus	12	15	11	8
	Escherichia coli	18	15	15	10
Ethanol	*Candida albicans*	20	17	12	8
	Staphylococcus aureus	17	14	10	9
	Escherichia coli	20	16	13	14
Methanol	*Candida albicans*	25	16	15	14

Table 3: Antimicrobial activities of *P. guajava* leaf extract and some selected antibiotics against the selected organisms (mm)

Organisms	Extract		Antibiotics		
	Ethanol	**Methanol**	**Gen**	**Strep**	**Nys**
Escherichia coli	18	20	20	18	_
Staphylococcus aureus	12	17	16	16	_
Candida albicans	20	25	_	_	25

Key: Gen= Gentamycin; Strep= Streptomycin; Nys= Nystatin; - = Not present

Table 4: Minimum Cidal and Minimum Inhibitory Concentrations of ethanolic leaf extracts of *P. guajava* against the selected organisms.

Test Organisms	Inspection Duration	ExtractConcentrations (Mg/ml)			
		250	**200**	**100**	**50**
	24hrs	-	+	+++	+++
Staphylococcus aureus	48hrs	-	-	++	++
	72hrs	-	-	+	++
	24hrs	+	+	++	+++
Escherichia coli	48hrs	-	+	++	+++
	72hsr	-	-	++	++
Candida albicans	24hrs	-	-	-	+
	48hrs	-	-	-	-
	72hrs	-	-	-	-

Key: + =scanty growth, ++ = moderate growth, +++ = heavy growth, - = No growth

Table 5: Minimum Cidal and Minimum Inhibitory Concentrations of methanolic leaf extracts of *P. guajava* against selected organisms.

Test Organisms	Inspection Duration	ExtractConcentrations (Mg/ml)			
		250	200	100	50
Staphylococcus aureus	24hrs	-	-	++	++
	48hrs	-	-	+	++
	72hrs	-	-	+	++
Escherichia coli	24hrs	+	+	+	++
	48hrs	-	-	+	+
	72hrs	-	-	+	+
Candida albicans	24hrs	-	-	-	+
	48hrs	-	-	-	-
	72hrs	-	-	-	-

Key: + =scanty growth, ++ = moderate growth, - = No growth

DISCUSSION

The results of this work indicated that the extract of guava leaves have antibacterial properties. All the species of microbes were inhibited by the extracts. The most susceptible of the three organisms was *Candida albicans* which showed the highest zone of inhibition of 25mm (methanolic extracts) and *Escherichia coli* which showed zone of inhibition of 20mm (methanolic extracts) while the least susceptible was *Staphylococcus aureus* with 16mm (methanolic extracts) (Table 2). This showed that methanolic extracts is more potent than ethanolic extracts as reported by Biswa *et al.* (2013); Dhiman *et al.* (2011) and Pereira *et al.* (2023). The factors responsible for this high susceptibility of *Escherichia coli* and *Candida albicans* to the extracts could be attributed to the presence of plant secondary metabolites. Meanwhile, according to Pereira *et al. (*2023) findings, the aqueous extract of leaves, roots and stem bark of *P. guajava* showed different potencies against *S. aureus* (MIC= 500, 125 and 250ug/ml) and practically inactivity against Gram-negative bacteria such as *E. coli* and *P. aeruginosa* (MIC > 1000ug/ml). This showed that there is a potent active substance in guava leaf with the potential for use as antibiotics for curing infections. The qualitative phytochemical screening was done on the extract which showed the presence of some important active ingredient in the extract which could be the reason for its inhibition ability (Table 1). This result could be linked to Lin *et al*. (2002) report on the inhibitory activity of guava extract on *Salmonella* and *Escherichia coli* This study is in contrast with the findinds of Biswas *et al.*, (2013) where *E. coli* has no zone of inhibition in both N-hexane, Ethanol and methanol extract. In Table 2. *E. coli* showed highest zone of inhibitions against both ethanol and methanol extracts; this is slightly similar to the findings of Kenneth *et al*. (2017) where *E. coli* showed highest zone of inhibition in only ethanol extract while *S. aureus* showed highest in methanol extract of *P. guajava*.

The diameter of the inhibition zone obtained against commonly used antibiotics is presented in Table 4, which showed that the extracts and the antibiotics have the same zone of inhibition. The guava extract proved to have a good inhibitory and lethal effect at the highest concentration most especially on the bacteria while the lowest concentration had inhibitory and lethal effect on *Candida albicans*.

CONCLUSION

From this study, it is clear that guava leaf extract is a good antibacterial and antifungal agent. The results indicated that the extracts are more effective against *Candida albicans* than the two bacteria. The use of guava as an edible fruit indicated that it has little or no negative effects on human. This study provides insight to further determine antimicrobial principles and

investigate other pharmacological properties of guava. *Psidium guajava* leaves possess the capabilities of being a good candidate in the search for natural antimicrobial agent against infections or diseases caused by *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli.*

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