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PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL SCREENING OF THE METHANOLIC LEAVES EXTRACT OF GUAVA (*PSIDIUM GUAJAVA LINN)*

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ABSTRACT

Psidium guajava linn commonly known as guava and belonging to the family Myrtaceae is widely used in folk medicine. The phytochemical analysis of the methanolic extract of the leaves showed the presence of tannins, saponins, flavonoids, steroids, proteins and triterpenoids but alkaloids and glycosides were absent. The presence of these phytochemicals indicates the pharmacological property and nutritive value of the plant. The sensitivity of five test organisms grown on MacConkey and blood agar were tested on the extract of different dilutions. The result showed that *Escherichia coli* indicated sensitivity with a zone of inhibition (ZOI) of 1.50cm only at full strength, *Staphylococcus aureus* with a (ZOI) of 2.5cm each at 50% and 100% strength and *Proteus vulgaris* with (ZOI) 0.60cm, 0.80cm and 1.00cm at 10%, 50% and 100% strength respectively. *Streptococcus pneumonia* and *Pseudomonas spp*. did not show sensitivity towards the extract.

Key words: Guava (*Psidium guajava linn),* phytochemical, antimicrobial, zone of inhibition (ZOI).

INTRODUCTION

The significant roles played by plants in metabolic activities such as photosynthesis and respiration cannot be overemphasized (Jack and Orubite, 2008). The search for and use of drugs and dietary supplements derived from plants have accelerated recently due to the world population explosion and food depletion. This choice of plants as food and drugs rests on the

presence of chemical substances called phytochmicals that are present in plants (Nsi and Dyegh, 2004).

Though non nutritive, these phytochemicals have protective or disease preventive properties (Ezeghara, 2014). Recent research has demonstrated that indeed phytochemicals can be harnessed in the treatment and control of diseases (Iwu *et al.,* 1999; Ayandele and Adebiyi, 2007) while others are known to inhibit the growth of micro-organisms (Farnsworth, 1996, Ogbeche *et al.,* 1997; Okerelu and Ani, 2001; Craig, 2005 and Alinor, 2006).

The plant *Psidium guajava linn* commonly known as guava and belonging to the family Myrtaceae is a phytotherapeutic plant that is used widely in folk medicine. Though guava originated from Mexico, Central America and Northern part of South America, it is now cultivated and grows wild in a number of countries including India, Thailand, Brazil, West Indies and Nigeria. African folk medicine uses guava leaves to treat many diseases such as diabetes, diarrhea, cough, painful menses and hypertension (Sharon, 2011). It is the success recorded in the effective use of guava leaves for the treatment of diarrhea, gastroenteritis and other ailments that has prompted this work on the phytochemical analysis and the antimicrobial potentiality of the methanolic extracts of guava (*Psidium guajava L.*) leaves. Such knowledge will guarantee the safe and accurate usage of this plant leaves.

MATERIALS AND METHODS

All reagents and solvents used were purified before use. The fresh leaves of guava were collected from the Rivers State University of Science and Technology farm at Nkpolu, thoroughly washed with water and sun dried for about 18 hours within three days and ground into a powder. 40g of the powder was put into a 2 litre volumetric flask and 1 litre of methanol was put into it. The flask was covered and allowed to stand at room temperature for about 36 hours with occasional shaking. The methanol extract was filtered through 110mm Whatman filter paper and evaporated to give 16.4g of the extract used for the various tests below.

Phytochemical Analysis

The phytochemical analysis of the extract was carried out by the standard methods provided by Odebiyi and Ramstard, 1978 and Waterman, 1993.

Test for Tannins

(a) 1cm^3 of freshly prepared 10% KOH was added to 1cm^3 of the extract in MeOH and observed for dirty white precipitate.

(b) 2 drops of 5% $FeCl_3$ was added to 1cm^3 of the methanolic extract and observed for green precipitate.

Test for Saponins (frothing test)

2cm^3 of the extract was put into a test tube and vigorously shaken for two minutes and observed for persistent foaming.

Test for Flavonoids

To 3cm^3 of the extract was added 1cm^3 of 10% NaOH and observed for yellow colouration.

Salkwoski Test for Steroids

5cm^3 drops of conc. H_2SO_4 was added to 1cm^3 of the extract and observed for red colouration.

Test for Proteins

To 5cm^3 of the extract, 2cm^3 of 4% NaOH was added with few drops of 5% $CuS0_4$ solution and observed for pink colouration.

Test for Glycosides

10cm^3 of 50% H_2S0_4 was added to 1cm^3 of the extract in a test tube. The mixture was heated in a boiling water bath for 15 minutes and 10cm^3 of Fehling's solution was added to it while still boiling and observed for brick red colour.

Test for Alkaloid

1cm^3 of 1% HCl was added to 3cm^3 of the extract in a test tube. The mixture was heated for 20 minutes, cooled and filtered. 2 drops of Wagner's reagent was added to 1cm^3 of the filtrate and observed for reddish-brown precipitate.

Test for Triterpenoids

To 5cm^3 of the extract was added 2cm^3 of $CHCl_3$. This was followed by 3cm^3 H_2S0_4 and observed for reddish-brown interface.

Antimicrobial Screening

Pure cultures of the test organisms namely *Escherichia coli*, *Staphylococcus aureus, Proteus vulgaris, Streptococcus pneumonia* and *Pseudomonas spp* were obtained from the Braithwaite Memorial Specialist Hospital (BMSH).

MacConkey Agar

1.05g of (MacConkey agar) powder was weighed and dispersed in $20cm^3$ of distilled water. It was allowed to soak for 10 minutes and gently mixed with a stirring rod. It was then sterilized by autoclaving for 15 minutes at 121^0C, cooled to 45^0C and then turned into a petridish where it solidified.

Blood Agar

0.56g of nutrient agar powder was weighed and dispersed in $20cm^3$ of distilled water, allowed to soak for 10 minutes and gently mixed with a stirring rod. It was then sterilized by autoclaving for 15 minutes at 121^0C and allowed to cool to 45^0C. $5cm^3$ sheep blood was added, then shaken and emptied into a petri dish where it solidified.

Inoculation and Sensitivity Test

Inoculation of viable colonies of the different organisms was done with the help of a wire loop which was sterilized by flaming it and allowed to cool before using it to pick, transfer and spread the organisms in different agar plates. By intermittently sterilizing the wire loop, *E. coli, S. aureus and P. valgaris* were inoculated on MacConkey agar plate while *S pneumonia* and *Pseudomonas* spp were inoculated on blood agar. Sterilized filter papers which were soaked in different dilutions of the methanolic extract were taken out with sterilized forceps and placed on the inoculated petri dishes containing the media and the test organisms.

The plates were incubated at 37^0C for 24 hours and observed for zones of inhibition (ZOI) measured in centimeters diameter using a transparent ruler.

RESULTS

Table 1: Phytochemical Analysis of the Methanolic Leaves Extract

S/N	Active Principle	Test	Inference
1.	Tannins	$FeCl_3$	+
2.	Saponins	Frothing	+
3.	Flavonoids	NaOH	+
4.	Steroids	Salkwoski	+
5.	Glycosides	Fehling's	-
6.	Proteins	$NaOH/CuSO_4$	+
7.	Alkaloids	Wagner's	-
8.	Triterpenes	$CHCl_3$	+

Key

\+ = Present

\- = Absent

Table 2: Zone of Inhibition of Test Organisms at different dilutions of the methanolic extract

Dilutions	*E. coli* (ZOI) cm	*Staph. aureus cm*	*S. pneumonia cm*	*P. vulgaris cm*	*Pseudomonas cm*
10%	0.00	0.00	0.00	0.60	0.00
50%	0.00	2.50	0.00	0.80	0.00
100%	1.50	2.50	0.00	1.00	0.00

DISCUSSIONS

The phytochemical analysis of the methanolic extract of guava leaves reveals that tannins, saponins, flavonids, steroids, proteins and triterpenoids are present. The presence of these active principles corroborates evidence that the leaves have medicinal properties since these compounds are used in most cases as starting materials for some synthetic drugs. The absence of glycosides and alkaloids is instructive as the leaves are not toxic but used as diabetic agent.

In the antimicrobial screening of the extract as shown in table 2, it is obvious that the test organisms *Streptococcus pneumonia* and *Pseudomonas* did not show any sensitivity towards the extract in all dilutions as there was no zone of inhibition (ZOI) on the agar plates. However, when the disc paper was impregnated with the extract at different dilutions, *E. coli, S. aureus* and *P. vulgaris* showed sensitivity towards the extract at varying degrees. For instance, *E. coli* showed inhibitory effect of 1.5cm diameter at full strength of 100% extract but not in any other dilutions. *S. aureus* showed zone of inhibition of 2.5cm each when 50% and 100% extracts were impregnated on the disc paper but no inhibition with 10% extract. Though the inhibitory effect of *P. vulgaries* was the least i.e. 0.60cm, 0.80cm and 1.00cm

compared to 1.50 and 2.50cm, it is very clear that this organism is the most sensitive to the methanolic extract in all dilutions of 10%, 50% and 100%.

CONCLUSION

Considering the sensitivity of the three organisms *Escherichia coli, Staphylococcus aureus and Proteus vulgaris* shown toward the methanolic guava leaves extract out of five test organisms, the broad spectrum activity of guava leaves on micro-organisms can no longer be in doubt. Thus, it can be used as an antibacterial agent for the treatment and control of bacterial infections. The absence of alkaloids and the presence of many phytochemicals are indicators that the plant is environmental friendly, safe for food and can provide cheap antibacterial drug.

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