International Journal of
Medicinal Chemistry & Analysis
www.ijmca.com
e ISSN 2249 - 7587
Print ISSN 2249 - 7595

ANTI-BACTERIA ACTIVITIES OF AQUEOUS AND ETHANOLIC EXTRACTS OF LEAVES OF GUAVA (*PSIDIUM GUAJAVA*)

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ABSTRACT

The antibacterial activities of aqueous and ethanolic extracts of *Psidium guajava* (guava) leaves were evaluated against two clinical isolates of staphylococcus aureus and Escherichia coli using disc diffusion and broth dilution methods to determine the diameter of zone of inhibition (DZI) and the minimum inhibitory concentration (mic) respectively. The aqueous and ethanolic extracts of the plants exhibited strong inhibitory activities against the two bacteria at a concentration of 10,000ug/ml (10mg/ml). At a potency of 1000mg /disc, the aqueous extracts produced a diameter of zone of inhibition of 12mm against *E.coli*. and S .aureus respectively while the ethanolic produced diameter zone of inhibition of 12mm and 10mm against the bacteria, respectively .These results suggest that the leaves of *Psidium guajava* contain potent antibacterial substances thus validating the medicinal uses of the leaves of the plant for treatment of bacterial infection.

Keywords: Aqueous and ethanolic extracts, *Psidium guajava*, *Staphylococcus aureus*, *Escherichia coli*.

INTRODUCTION

Psidium guajava (guava) is a perennial tree plant whose leafy part is used as a medicinal plant by indigenes of south-eastern Nigeria as antibacterial remedies in treatment of tropical diseases. A medicinal plant is one in which one or more of its organs like leaves, stems, roots and barks contain chemical substance that can be used for therapeutic purposes [1]. A medicinal plant can be in form of vegetable drug which may be organized by possessing cellular structure into: leaves, stems, petals, and roots. And or unorganized by not possessing cellular structure but contain medicinal agents such as gums, latex, and resins [2]. The medicinal plant that forms the subject matter of this investigation is guava, botanically called *Psidium guajava* is a rich source of antibacterial agents. Several parts of this cellular medicinal plant are used as therapeutic agents namely; roots, stems, barks Leaves, flowers, fruits. Medicinal plant extracts may be in form of decoction, which may be in cold water or prepared by boiling and then allowed to cool. The medicinal plant extract may be tisane which is a tea made either by decoction or infusion. Medicinal plants whether cellular or non-cellular contains secondary metabolites called phytochemical. Phytochemical is made of several classes of organic compounds with different functional groups. These organic compounds include; alkaloids, flavonoids, coumarines, quercetins, glycosides, gums, latex, polysaccharides, phenols, tannins, terpenes, and terpenoids [3-5].

Generally, plants produce bioactive compounds making them rich sources of medicaments. A lot of tropical disease experts and researchers have reported the antibacterial activities of some medicinal plants [6-9]. This is subject matter of this report; the antibacterial activities of aqueous and ethanolic extracts of leaves of guava (*Psidium guajava*), an indigenous medicinal plant of south-eastern Nigeria.

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MATERIALS AND METHODS

Sample collection

The leaves of guava (*Psidium guajava*) were harvested from the plant of guava grown within the Michael Okpara University of Agriculture, Umudike, Ikwuano L.G.A., Umuahia, Abia State, Nigeria. The harvested leaves were identified and authenticated by a botanist, Dr. Omosun Garuba of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike. A voucher specimen of the guava leaves was deposited at the University herbarium. All chemical reagents used in this work are analytical grades from May and Baker Chemicals, Ltd., USA.

Sample preparation

The harvested guava leaves were air-dried at the laboratory floor for two weeks. The dried leaves of guava were ground into a coarse powder using electric blender (binatone model), and sieved into a fine powder using 2 mm mesh-sieve. The powders were stored in a clean labeled air-tight container properly sealed until required for analysis.

Extraction of bioactive ingredients

One hundred grammes of the fine powdered leaves of the guava (*Psidium guajava*) were weighed out and transferred into a clean labeled conical flask containing measured 250 ml of distilled deionised water and allowed to percolate for 24 hours. The resulting extract was filtered using what-man filter paper No1 into clean flask. The filter ate called aqueous extract of guava leaves was evaporated to dryness using thematically controlled water bath at 50-60^{o}C. The resulting dark brown gummy material was solution was filtered using What-man No 40 filter paper into a clean flask. The filtrate known as aqueous extracts of leaves of guava was evaporated to dryness using temperature regulated electrical water bath. The concentrated extract after evaporation, dark brown, gummy in nature was stored in an air-tight container and placed in a desiccators filled with grains of silica-gels until required for analysis. The residues for the aqueous extracts were thereafter; transferred into a clean labeled conical flask containing 250 ml of 95% ethanol, shake gently for thorough soaking and allowed percolate for 24 hours. The ethanolic extract was filtered using What-man No1 filter paper into a clean flask. The filtrate known as the ethanolic extract of the guava leaves was evaporated to dryness in water bath. The thick gummy dark brown compound obtained after evaporation was stored in an air-tight container and placed in desiccators spiked with a silica-gel grains until required for analysis.

Qualitative chemical analysis

A standard qualitative chemical analysis of the extract was carried out using 0.2g amount of the extracts. The extracts were of flavonoids, alkaloids, tannins, phenol and other Phyto chemicals according to the methods described by Edeoga et al [10, 11].

Screening for antibacterial activity

A clinical isolates of *Staphylococcus aureus* and *Escherichia coli*, isolated from urine sample obtain from patients at the University medical centre of Michael Okpara University of Agriculture, Umudike, were used for antibacterial activity of the extracts.

Preparation of paper Disc

A paper discs 6mm in diameter were cut from filter paper No1 using an office a paper perforator. The paper discs were placed in a clean glass Petri dish and satirized at 160^{o}C for 1h in a hot-air oven. Stock solution (1000mg /ml) of the extracts were made by dissolving the ethanolic extract in 50%v/v ethanol in water and the aqueous extract was dissolved in water .Appropriate dilutions of the stock solutions were made and 20ml amounts were dispersed onto each paper disc to obtain 2mg, 5mg, and 10mg of extract per disc . The discs, were dried at 37-40^{o}C in an incubator overnight. The discs of Ampicillin (10mg) and erythromycin (15ug) were similarly prepared.

Agar disc diffusion assay

Mueller –Hinton agar plates were prepared by dispersing 25ml of autoclaved molten agar medium into sterile 90mm plastic petri dishes .The agar was left to solidify and then dried at 45^{o}C in the incubator. An overnight broth culture of each of the test organisms was adjusted to McFarland standard tube no.5 with sterile distilled water. A sterile cotton swab was dipped into standardized inoculum .The swab was used to streak the surface of the Muller –hintor agar plate previously dried in the incubator. The discs of the plant extracts and the standard antibiotics were aseptically placed on the surface of inoculated muller-hintor plate using a pair of flame –sterilized forceps. The plates were placed in the incubator for 20-30 minutes, and then turned upside down for further incubation at 37^{o}C under aerobic conditions for 16-18h. The diameter of zone of inhibition around the discs was measured using transparent plastic ruler.

Minimum inhibitory concentration (mic) : A serial 2- fold dilutions of the extracts was made in sterile peptone water to give concentration ranging from 200ug/ml to 1.95ug/ml .Each tube was inoculated with 0.1ml(100ul) of 5x10^{6}cfu/ml of the test organisms. The tube with lowest concentration of extracts showing visible growth as compared with the control was taken as the minimum inhibitory concentration for the plant extract. The standard antibiotics were prepared in concentration ranging from 128ug/ml to 0.5ug/ml. The treatments given to the extracts as described above were also given to the standard antibiotics.

RESULTS AND DISCUSSION

The antimicrobial activity and the phytochemical screening of the aqueous and ethanolic extracts of the leaves of *P. gujava* (guava) have been carried out. The results of the phytochemical screening of the aqueous leaf extract are shown in Table 1. Tannins, flavonoids, saponins, phenols, and alkaloids were detected in the extracts .The presence of these Phytochemicals in the leaf extracts have been reported in the previous studies (reviewed by Gutiernez, et al [10]. Although, Begum et al [11] reported the isolation of two triterpenoids from the fresh leaves of guava .We did not detect terpenoids in our screening system. This could be due to several factors including whether the plant material is fresh or dried, the stage of growth or maturity or season of collection of the plant materials, the solvents used in the extraction and the screening method used [12].

The results of the initial antimicrobial screening test using the agar dilution streaking method are shown in Table2. .At a concentration of 10,000ug/ml (10mg/ml), the aqueous extract exhibited strong inhibition against *S.aureus* and *E .coli*. At lower concentrations, the extracts produced reduced growth (partial inhibition) or no inhibition as compared with the control culture plate. The results of the disc agar diffusion test measured by the diameter of zone of inhibition (DZI) are presented in Table 3. At 2000ug/ml (equivalent to a concentration of 2mg/ml) ,the aqueous extract produce DZI of 12mm against S. aureus and 10mm against *E.coli*. The ethanol extract produce DZI of 14mm against S. aureus and 17mm against *E.coli*. In comparison with standard commercial antibiotics, 10ug Ampillicin produce no inhibitory activity against the strain of *E.coli*, tested in this study while 15ug of erythromycin produce DZI, of 10mm and 19mm against S. aureus and *E.coli* respectively. The results of this are consistent with those previously published which showed that the various leaf extracts of guava possessed broad spectrum of antimicrobial activity against Gram-positive and Gram-negative bacteria Dhumian et al reported that the methanolic leaf extract of *P.gujava* from India possessed broad spectrum of antibacterial and antifungal activity. The antibacterial activity of the baqueous and alcoholic extracts of roots and leaves of guava *S,aureus, streptococcusmutans, pseudomonas aeruginosa, schmonella enteritidis bacillus cereus, proteus spp, shigella spp* and *E.Coli*. A major of mechanism of action of Ampicillin an (amino penicillin antibiotics) is the inhibition of the transpeptidation in the synthesis of peptidoglygcan component of the cell wall. The possibility of finding bioactive compounds with novel mechanism of antimicrobial activity is one of the strongest motivations for scientific exploration of the huge untapped ethno medical knowledge of the use of the medicinal plants in traditional medical practice.

The antimicrobial activities of the leaves of *P.gujava*, have been shown to be due to the flavonoidal compounds principally the quercetins. According to cowan et al, flavonoids and tannins are among the important Phytochemicals with antimicrobial activities. These groups of Phytochemicals were detected in the aqueous and ethanolic extracts of the leaves of *P.gujava* in this study. It is noteworthy that the extracts were active against a strain of *E.coli*; against which ampicillin at a standard concentration of 10ug/ml disc had no activity. This may suggest that the bioactive substances in the extracts have different mechanism of action, different from that of Ampicillin.

Table 1. Detection of Phytochemicals in the aqueous extract of leaves of *Psidium gujava*

Phytochemical compounds	Result of phytochemical tests
Tannins	+
Flavonoids	+
Saponins	+
Terpenoids	-
Phenols	+
Alkaloids	+

Key: + = detected, – = not detected

Table 2. Antimicrobial effects of the aqueous and ethanol extracts of leaves of *P. gujava* on the growth of test organisms at different concentrations

Concentration mg/ml	*S.aureus*		*E.coli*	
	Aqueous	Ethanol	Aqueous	Ethanol
100	*S*1	SI	S1	SI
50	P1	P1	N1	N1
25	P1	N1	N1	N1

Key: S1=Strong inhibition, P1=Partial inhibition, N1= No inhibition

Table 3. Diameter of zone of inhibition (mm) of leaf extract of guava against the test organisms

Extract type/Antibiotics	Concentration(ug/disc)	Diameter of zone of inhibition(mm) *S.aureus*	Against test organisms *E.coli*
Aqueous	2000	14	17
	1000	12	12
	500	8	12
Ethanol	2000	10	12
	1000	10	11
	500	9	8
Ampicillin	10	9	0
Erythromycin	15	10	19

CONCLUSION AND RECOMMENDATION

The results of this study validate the folkloric use of this plant as a medicinal plant in the treatment of ailments associated with microbial infections. The results serve as scientific basis for further research and development of therapeutic potential of this plant as a source of broad spectrum antimicrobial agents .We recommend that a comprehensive structural elucidation of the antimicrobial substances in the leaf extracts should be undertaken. The mechanisms of action of the active substances as well as toxicology of the extracts also need to be done.

ACKNOWLEDGEMENTS

We appreciate the supervisory role of the Directorate of Research and Development, Michael Okpara University of Agriculture, Umudike, and Tertiary Education Trust Fund (TET-FUND), Nigeria for funding the project for 2009 – 2010 intervention years.

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