

Full Length Research Paper

Evaluation of antibacterial activity of *Pisidium guajava* and *Gongronema Latifolium*

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Pisidium guajava and *Gongronema latifolium* are local plants used traditionally in south-eastern Nigeria to treat ailments such as cough, loss of appetite, malaria and stomach disorders. In this study, aqueous and ethanolic leaf extracts of *P. guajava* and *G. latifolium* were screened for antibacterial activity against two clinically isolated organisms of the gastrointestinal tract, *Escherichia coli* and *Staphylococcus aureus*. Results obtained show that leaf extracts of both plants possess significant antibacterial activities against the two isolates. Ethanolic extracts showed more inhibitory effect compared to the aqueous extracts. Extracts of *P. guajava* exhibited higher inhibitory effect than that of *G. latifolium*. The diameter of zones of inhibition by the leaf extracts of *P. guajava* was 8 - 16 mm and 14 - 21 mm respectively for the aqueous and ethanolic extracts. The minimum inhibitory concentrations (MICs) were 5.0 and 0.625 mg ml⁻¹ respectively for the aqueous and ethanolic extracts of *P. guajava*. For the extracts of *G. latifolium*, the diameter of zones of inhibition was between 6 and 10 mm while MICs were 10.0 and 2.5 mg ml⁻¹ respectively for the aqueous and ethanolic extracts.

Key words: *Pisidium guajava*, *Gongronema latifolium*, antibacterial activity, minimum inhibitory concentration (MIC), *Escherichia coli*, *Staphylococcus aureus*.

INTRODUCTION

The use of medicinal plants in curing illnesses is as old as man (Grabley and Thierickee, 1999; Abinn et al., 2007). Large populations of people, especially in the developing world, rely on folk medicines for the treatment of common infections as well as persistent diseases. The plants are usually ingested as decoctions and teas or used as spices in the preparation of local delicacies (Okafor, 1975). There is a growing interest in plants with antimicrobial activity. Scientists are increasingly becoming involved in the screening of such plants with the aim of establishing their potential antimicrobial effects and identifying the compounds responsible for the antimicrobial properties (Aibinu et al., 2007; Ndukwe et al., 2007).

Pisidium guajava (guava) is one of the plants acclaimed to exhibit antibacterial activity (Gnan and Demello, 1999; Iwu, 1993). It is a common shade tree or shrub in the tropics which belongs to the family *Myrtaceae*. It is

believed to be a native of Brazil. The plant has been extensively used in the treatment of cough, sore throat and inflamed gums. Its medicinal effects have spurred many researchers into studying the properties of its extracts (Jordon et al., 2003). *Gongronema latifolium*, known as 'utazi' in the south-eastern and 'arokeke' in the south-western part of Nigeria, is a tropical rainforest plant which belongs to the family *Asclepiadaceae* (Ugochukwu and Baddy, 2002; Ugochukwu et al., 2003). It is a climber with a tuberous base found in deciduous forests from Guinea-Bissau to Western Cameroons. Various parts of these plants, particularly the stems and leaves, are used as chew-sticks or liquor in places such as Sierra Leone. The liquor, usually obtained after the plant is sliced and boiled with lime juice or infused in water for over three days, is frequently taken as a purge for colic and stomach pains as well as to treat symptoms connected with worm infection (Okafor, 1975).

Escherichia coli and *Staphylococcus aureus* are intestinal bacteria often implicated in several gastrointestinal disorders. Gastrointestinal diseases caused by *E. coli* are the most frequent causes of death in developing coun-

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countries (Caceres et al., 1993). The presence of Enterobacteria in food and water is a common cause of diarrhea and dysentery particularly in developing countries with short supply of social amenities and political instability. *S. aureus* constitutes a major public health threat, being one of the most common causes of hospital and community acquired infections (Aires-de-Sousa et al., 2006). The organism is frequently resistant to a wide variety of antibiotics. Infections caused by methicillin resistant *S. aureus* (MRSA) and Vancomycin resistant *S. aureus* are associated with high morbidity and mortality, high treatment cost and long stays in hospitals.

This study was designed to investigate the scientific basis for the traditional practice among the local people in south-eastern Nigeria of treating ailments related to microbial infections with extracts from these plants. It was also aimed at formulating better prophylaxis for bacterial infections. We report that the ethanolic and aqueous extract of *P. guajava* and *G. latifolium* exhibited significant antibacterial effects against clinical isolates of *E. coli* and *S. aureus* obtained from medical laboratory and culture collection centre. The ethanolic extracts yielded greater antibacterial effects. Extracts of *P. guajava* showed higher inhibitory effect on the growth of the isolates compared to that of *G. latifolium*.

MATERIALS AND METHODS

Collection of samples

Fresh samples of *P. guajava* leaves were harvested from a matured guava tree at Alor town in Anambra state while the fresh leaves of *G. latifolium* were purchased from traditional dealers at a local market in Awka, Anambra State. The plants were identified and authenticated at the Herbarium of the Department of Biological Sciences, Nnamdi Azikiwe University, Awka, Anambra State. A voucher specimen was deposited at the Herbarium for reference purposes.

Preparation of extracts

Fresh leaves of *P. guajava* and *G. latifolium* were dried for 5 hours in an oven at 60°C and then ground to powder. Two solvent were used for the preparation of the extracts, namely distilled water and 60% (v/v) ethanol.

Two hundred and fifty grams (250 g) of the dried powdered leaves of *P. guajava* and *G. latifolium* were respectively extracted in 200 ml of distilled water for 3 h using the reflux method. These were respectively filtered using Whatman No.1 filter paper and concentrated *in vacuo* to obtain the aqueous extracts of *P. guajava* (greenish-brown colour) and *G. latifolium* (dark-green colour) used for the subsequent studies. The prepared extracts were kept at 4°C in a refrigerator for at least 24 h before the subsequent tests. The ethanolic extracts were prepared by weighing out 250 g of the dried powdered leaves of *P. guajava* and *G. latifolium* respectively and extracting with 60% ethanol for 5 h using Soxhlet extractor. The extracts were concentrated by fractional distillation.

Test organisms

Clinical isolate of *S. aureus* was obtained from Glanson laboratories, Awka, Anambra State, Nigeria while the strain of *E. coli* was from Sammlung von Mikroorganismen (DSM) Germany. The isolates were propagated on nutrient agar plates and maintained on

the plate at 4°C. The isolates were sub-cultured in nutrient broth at 37°C for 8 h prior to antibacterial testing.

Antibacterial sensitivity testing

Agar well diffusion technique as described by Adeniyi et al. (1996) was used to determine the antibacterial activity of the extracts. Sensitivity test agar plates were seeded with 0.1 ml of an overnight culture of each bacterial isolate (equivalent to $10^7 - 10^8$ CFU mL⁻¹). The seeded plates were allowed to set and a standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of the agar. The wells were then filled with 0.3 ml of each extracts at a concentration of 10 mg mL⁻¹. Ethanol (60%) and distilled water which served as controls were also put in separate wells. All the plates were incubated at 37°C for 24 h. The assay was conducted at regular intervals of 24 h until marked decline in the potency of the extracts to inhibit the growth of the test organisms was noticed. Zones of clearance round each well means inhibition and the diameter of such zones were measured.

Determination of minimum inhibitory concentrations (MICs)

Agar well dilution method as described by Russell and Furr (1972) were used to determine the minimum inhibitory concentration (MIC) of the extracts. Different dilutions of the extracts were prepared to give final concentration in the range of 10.0, 5.0, 2.5, 1.25, 0.625, and 0.313 mg mL⁻¹. Two milliliter (2 ml) of each dilution was mixed with 18 ml of Mueller Hinton agar (MHA, Difco, France) and poured into Petri-dishes and allowed to set. The agar was streaked with an overnight broth culture of the bacterial isolates and incubated overnight. The plates were then examined for the presence or absence of growth. The minimum concentration that completely inhibited macroscopic growth was regarded as the minimum inhibitory concentration of the respective extracts.

RESULTS

Antibacterial activity of the extracts

The results of the antibacterial activity of the aqueous and ethanolic extracts against the test organisms, *E. coli* and *S. aureus*, are shown in Tables 1 and 2. The zone of inhibition of the growth of the isolates is a function of the relative antibacterial activity of the extracts. *P. guajava* showed more antibacterial activity against the organisms compared to *G. latifolium*. Higher growth inhibitory activity was obtained with the ethanolic extracts. Distilled water and ethanol (60%) used as respective controls were inactive against the test organisms.

Minimum inhibitory concentration (MIC) of the extracts

Results of the minimum inhibitory concentration (MIC) are summarized in Table 3. Generally, higher values were obtained for the aqueous extracts. Higher concentrations of the respective extracts were needed to inhibit *E. coli* compared to *S. aureus*. The MIC of the aqueous extract of *Pisidium guajava* was 5.0 and 2.5 mg mL⁻¹ for *E. coli* and *S. aureus* respectively whereas that of the ethanolic extract was 1.25 mg mL⁻¹ and 0.625 mg mL⁻¹ for *E. coli* and *S. aureus* respectively. The MIC of the aqueous extract of

Table 1. Antibacterial activity of the aqueous extracts of *Pisidium guajava* and *Gongronema latifolium* against *Escherichia coli* and *Staphylococcus aureus*.

Plant	Test organism	Zone of Inhibition (mm)				
		Control (water)	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³
<i>Pisidium guajava</i>	<i>Escherichia coli</i>	-	16	9	-	-
	<i>Staphylococcus aureus</i>	-	11	8	-	-
<i>Gongronema latifolium</i>	<i>Escherichia coli</i>	-	8	-	-	-
	<i>Staphylococcus aureus</i>	-	6	-	-	-

(-) = No inhibition of growth; (10⁰, 10⁻¹, 10⁻², 10⁻³) = Different dilutions.

Table 2. Antibacterial activity of the ethanolic extracts of *Pisidium guajava* and *Gongronema latifolium* against *Escherichia coli* and *Staphylococcus aureus*.

Plant	Test organism	Zone of Inhibition (mm)				
		Control (water)	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³
<i>Pisidium guajava</i>	<i>Escherichia coli</i>	-	21	12	9	-
	<i>Staphylococcus aureus</i>	-	14	11	8	-
<i>Gongronema latifolium</i>	<i>Escherichia coli</i>	-	10	8	-	-
	<i>Staphylococcus aureus</i>	-	9	8	-	-

(-) = No inhibition of growth; (10⁰, 10⁻¹, 10⁻², 10⁻³) = Different dilutions.

Table 3. Minimum inhibitory concentration (MIC) of the aqueous and ethanolic extracts of *Gongronema latifolium* and *Pisidium guajava*.

Test organism	<i>Pisidium guajava</i>		<i>Gongronema latifolium</i>	
	Aqueous extract (mg ml ⁻¹)	Ethanolic extract (mg ml ⁻¹)	Aqueous extract (mg ml ⁻¹)	Ethanolic extract (mg ml ⁻¹)
<i>Escherichia coli</i>	5.00	1.25	10.00	2.50
<i>Staphylococcus aureus</i>	2.50	0.625	10.00	5.00

Gongronema latifolium was 10.0 mg ml⁻¹ for both *E. coli* and *S. aureus* while that of the ethanolic extract was 5.0 mg ml⁻¹ and 2.5 mg ml⁻¹ for *E. coli* and *S. aureus* respectively.

DISCUSSIONS AND CONCLUSIONS

The result of the study showed that aqueous and ethanolic extracts *P. guajava* and *G. latifolium* have concentration dependent inhibitory effect on the test organisms *E. coli* and *S. aureus*. Gnan and Demello (1999) obtained a concentration-dependent growth inhibition of *S. aureus* by aqueous extract of guava leaf. Antibacterial potential of the aqueous leaf extract of *P. guajava* against *E. coli* and *S. aureus* was reported by Iwu (1993). Aqueous and methanol leaf extracts of *G. latifolium* has been reported to exhibit antibacterial effects on a number of bacteria including *E. coli* and *S. aureus* (Eleyinmi, 2007; Oshodi et al., 2004). The findings of workers such as Heath (1977) and Vail et al., (1978) show that many plants used as spices in preparing local delicacies have significant anti-

bacterial activity. Most of the plants have been shown to contain aromatic oils from which they derive their main flavoring character. Phytochemical studies have also shown that the antibacterial properties of these plants depend on certain active ingredients, especially the oils such as saponins, tannins and flavonoids. *G. latifolium* contain saponins and these have been known to be responsible for its antioxidant and antimicrobial properties (Morebise and Fafunso, 1998; Morebise et al., 2002). Berdy et al. (1981) attributed the antibacterial effects of guajava extracts to the flavonoids, guajaverine and psydilic acids, present in the leaf. Flavonoids are known to be inhibitory to *S. aureus* and it has been used in treatment of inflamed tissues (Ali et al., 1996). It has been established that lectins found in guava leaf bind *E. coli*, thereby preventing its adhesion to the intestinal wall and the chance of infection (Berdy et al., 1981; Mota et al., 1985). This study has shown that ethanolic extracts of the plants exhibited greater inhibitory effects on the organisms. This is attributable to the ability of ethanol to extract essential oils inhibitory to the organisms. The anti-

antibacterial effect of *G. latifolium* and *P. guajava* which is evident from this study explains the long history of the use of these plants in traditional medicine for the treatment of different bacterial infections.

In conclusion, leaf extracts of *P. guajava* and *G. latifolium* showed antibacterial activity against *E. coli* and *S. aureus*. This study has provided the basis for the use of *P. guajava* and *G. latifolium* in the treatment of inflamed gum and stomach pains caused by *E. coli* and *S. aureus*. The potential antibacterial effects of the plants could be enhanced by extracting with ethanol instead of water as applied in the traditional practice.

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