

*Full Length Research Paper*

# Antimicrobial activity of *Psidium guajava* Linn. stem extracts against methicillin-resistant *Staphylococcus aureus*

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The antimicrobial activities of the water and methanolic extracts of *Psidium guajava* Linn. stem bark were evaluated against eight methicillin-resistant *Staphylococcus aureus* (MRSA) isolates. The plant material was extracted and phytochemical analyses were performed by standard procedures. The agar diffusion method was employed for the assessment of the sensitivity of the extracts, while the agar dilution technique was employed for the quantitative determination of the bacteriostatic and bacteriocidal activities of the plant extracts. The phytochemical studies of *P. guajava* revealed the presence of carbohydrates, glycosides, tannins, and proteins as its major constituents. Results show that the methanolic and water extracts of *P. guajava* stem bark exhibited antibacterial activity against methicillin resistant *S. aureus* bacteria. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the stem bark water extracts ranged from 125 to 500 µg/ml while that of the stem bark methanol extract ranged from 62.5 to 250 µg/ml.

**Key words:** *Psidium guajava*, antimicrobial activity, phytochemical screening, methicillin-resistant *staphylococcus aureus*.

## INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a topic of increasing concern in the realm of healthcare. Before the advent of antibiotics, *S. aureus*-associated bacteraemia was linked to an 82% mortality rate mostly amongst young adults (Cui and Hiramatsu, 2003). The discovery of penicillin and the susceptibility of *S. aureus* were seen as a welcome development. However, the emergence of resistant strains of *S. aureus* to penicillin created a need for newer drugs and a new wave of beta-lactam antibiotics were developed including methicillin

and oxacillin (Alan, 2007). The global emergence and increase of MRSA, also known as multi-drug resistant or oxacillin resistant *S. aureus* (Klein et al., 2007) have caused a shortage of effective beta-lactam antibiotics to MRSA based infections (Cui and Hiramatsu, 2003).

Historically, plants have provided sources of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being (Iwu et al., 1999). *Psidium guajava* Linn. commonly known as guava is a plant of the family Myrtaceae. *P. guajava* is a low ever green tree or shrub 6 to 25 feet high with wide spreading branches and square downy twigs. It is a native of Tropical America. In folk medicine, extracts of root bark and leaves are used to treat

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gastroenteritis, vomiting, diarrhea, dysentery, wound, ulcers, toothache, cough, sore throat, inflamed gums and a number of other conditions (Morton, 1987).

Antimicrobial and gastrointestinal activities have been reported from the plant (Guan and Demello, 1999; Geidam et al., 2007; Esimone et al., 2003; Egharevba et al., 2010; Buvaneswari et al., 2011). However, the use of *P. guajava* stem bark extracts against MRSA has not been previously reported. The activities of *P. guajava* stem bark extracts against eight MRSA isolates were evaluated and described herein.

## MATERIALS AND METHODS

### General experimental procedures

An analytical grade of methanol (Sigma-Aldrich) and distilled water were used for extraction. Reagents for phytochemical screening of extracts were freshly prepared using standard methods. Nutrient agar and nutrient broth (Oxoid, UK) were used for the bioassay studies.

### Plant collection and preparation

Fresh stem bark of *P. guajava* was collected from Nsukka, in Enugu State, Nigeria. They were identified by Mr. Alfred Ozioko of the Herbarium Unit Bio resources Development and Conservation Program (BDCP), Nsukka, Nigeria. The plant materials were chopped into small pieces, air-dried at room temperature and ground into powder.

### Extraction of plant material

Fifty grams of the ground plant material were extracted in 200 ml of methanol for 48 h and another 50 g was also boiled in 200 ml of water for 30 min. The extracts were filtered using Whatman filter paper and the filtrate evaporated to dryness at 40°C. After evaporation, the extracts were recovered and stored in air tight containers at 4°C in the refrigerator until ready for use.

### Phytochemical analysis of plant extracts

The qualitative phytochemical composition of the methanolic and water extracts of the stem bark of *P. guajava* was evaluated using standard procedures (Evans, 2002; Sofowora, 2008).

### Cell cultures

Stock cultures of eight methicillin-resistant *S. aureus* isolated from man and animals and maintained on nutrient agar slants at 4°C in the Microbiology Diagnostic, Unit Department of Veterinary Pathology and Microbiology, University of Nigeria Nsukka were used in this study. These stock cultures were sub cultured on nutrient agar and incubated at 37°C for 24 h to check for their purity and re-identification. The colonial morphology of the different bacterial species were observed and identified accordingly. After 24 h incubation of the organisms in a nutrient agar plate, a single colony of the bacteria isolate was picked and streaked on a fresh nutrient agar plate and incubated at 37°C for 24 h. A pure colony of the pure culture was gram stained and examined for gram positive cocci in cluster. Once the identity of each isolate was confirmed, the isolate

was inoculated onto nutrient agar slants, incubated overnight and then stored at 4°C until needed for further studies.

### Test for methicillin resistance

The *Staphylococcus* isolates were evaluated for methicillin resistance using the agar diffusion technique (CLSI, 2002). Antibiotic discs of oxacillin (30 µg) (Oxoid Limited, Basingstoke Hampshire, England) were used. A single colony of each test isolate was picked with wire loop and inoculated into nutrient broth and incubated for 3 h. The turbidity of each broth culture was adjusted to correspond to 0.5 McFarland turbidity standards (corresponding to approximately  $10^8$  cfu/ml). Each standardized broth culture was used to inoculate the surface of the nutrient agar plate. The excess broth was drained into disinfectant jar and the surface of each inoculated plate was allowed to dry. Using a disc dispenser, the antibiotic discs were aseptically placed on the surface of the inoculated agar plates, one disc for each plate and the plates were then incubated at 37°C for 24 h. After incubation, the plates were examined for inhibition zone around the disc. The diameters of the zones were measured with a meter ruler and recorded. Each test was conducted three times and the mean inhibition zone diameter (IZD) recorded to the nearest whole millimeter. Each test isolate was classified as resistant to oxacillin in accordance with the guidelines given by the CLSI (2002).

### Antimicrobial test methods against methicillin resistant *S. aureus*

#### Sensitivity test: Agar well diffusion assay

The assay was conducted using agar-well diffusion method (Perez et al., 1990). An 80 mg/ml concentration of both methanol and water extracts of *P. guajava* was constituted by dissolving 0.08 g in 2 ml each of 20% v/v dimethyl sulfoxide (DMSO) and 2-fold serial dilutions made. A single colony of each test isolate was suspended in 2 ml of sterile nutrient broth. The suspension of each isolate was standardized as stated previously and used to inoculate the surface of the nutrient agar and the excess fluid drained into disinfectant jar. The inoculated agar surface was allowed to dry and the plates were appropriately labeled. Using a cork borer, four wells of 8 mm in diameter were bored in the inoculated nutrient agar. With a micropipette, 50 µl of each concentration of the test extract was delivered into each well. The plates were left on the bench for 30 min to allow the extract to diffuse into the agar. Thereafter, the plates were incubated at 37°C for 24 h. After incubation, the plates were observed for inhibition zones around the wells. The diameters of the zones were measured with meter ruler to the nearest whole millimeter. Each test was carried out thrice and the mean IZD recorded to the nearest whole millimeter.

#### Minimum inhibitory concentration (MIC) of plant extracts

This was carried out using agar dilution method following the procedure outlined by CLSI (2002). For each extract, 80 mg was weighed and dissolved in 2 ml of 20% v/v DMSO to get a stock solution with concentration of 40 mg/ml. Sterile test tubes were arranged on a test tube rack and 1 ml of sterile distilled water was dispensed into them. From the stock solution, 1 ml was transferred into the first test tube and serial dilution of the extract was carried out and the resultant concentrations in the test tubes were 40, 20, 10, 5, 2.5, 1.25, 0.625, 0.3125 mg/ml.

One ml of the extract dilution was added to 19 ml of sterile molten nutrient agar, mixed thoroughly and poured into sterile Petri dishes. The plates were allowed to solidify and then labeled appro-

**Table 1.** Phytochemical constituents of *P. guajava* stem bark.

Plant constituent	Concentration
Carbohydrates	+++
Reducing sugar	++
Alkaloids	++
Cardiac Glycoside	+++
Saponins	++
Tannins	+++
Flavonoids	-
Resin	-
Proteins	+++
Oil	++
Steroids	+
Terpenoids	+

-, Absent, +, low in concentration; ++, moderate concentration; +++, high concentration.

priately. The plates were kept overnight in the incubator to check for their sterility.

A single colony of each test isolate was picked from the sub cultured plate with wire loop and inoculated into 2 ml sterile nutrient broth to make a suspension of each test isolate. Each suspension was standardized as stated previously. Using a micropipette, a 10  $\mu$ l of the standardized broth cultures were placed on the surface of the plates containing various concentrations of the extracts. Plain nutrient agar (that is, without the extract) was also streaked and served as negative controls. Inoculated plates were then incubated at 37°C for 24 h and observed for any visible bacterial growth. MIC was taken as the lowest concentration of extract that resulted in no visible growth on the surface of the agar.

#### Minimum bactericidal concentration (MBC) of plant extracts

After completion of the MIC procedure, the agar plates showing no growth in the MIC tests were used for the determination of the MBC. Blocks were cut out from the plates that showed no growth in the MIC test and transferred to a corresponding test tube of fresh nutrient broth, acting as the recovery medium. The newly inoculated broth medium was incubated for 24 h at 32°C. At the end of incubation, microbial growth was ascertained by checking the turbidity of the medium. The absence of turbidity in the recovery medium was evidence of total cell death.

## RESULTS AND DISCUSSION

The preliminary phytochemical constituents of methanolic and water extracts of *P. guajava* stem bark are presented in Table 1. The table indicates that methanolic and water extracts of *P. guajava* contained carbohydrates, cardiac glycosides, tannins and proteins at high concentration while reducing sugar, alkaloids, saponins and oil were present in moderate concentration, steroids and terpenoids were present at low concentrations. These results are consistent with findings in other phytochemical studies which have identified more than 20 compounds in guava extracts (Begum et al., 2002). This study indicates

that *P. guajava* is an important source of tannin, cardiac glycosides and saponins.

The agar well diffusion test was carried out on eight clinical isolates of MRSA and the results of the screening test of the stem bark of *P. guajava* against MRSA are shown in Table 2. The methanolic and water extracts of *P. guajava* stem bark were active against all of the eight MRSA tested with a mean inhibition zone diameter (IZD) ranging from 5 to 20 mm. The water extract had more activity, with an IZD of 20 mm against more isolates than the methanolic extract. The activity observed with the water extract may be associated with the common practice in traditional medicine to use the plant extracts prepared in the form of infusions and decoctions.

The MIC and MBC were determined on previously tested MRSA isolates. The agar dilution method was used. The MIC results reported in Table 3 show that five of the isolates were inhibited by the methanol extracts with activities ranging from 62.5 to 125  $\mu$ g/ml while seven were inhibited by the water extracts between 125 and 500  $\mu$ g/ml. On Table 4, the MBC results showed that five of the isolates were susceptible to the methanol extracts within the range 62.5 to 125  $\mu$ g/ml and five of the isolates were susceptible to the water extracts within the range 125 to 250  $\mu$ g/ml.

The results of the MIC and MBC on the MRSA isolates confirmed the antimicrobial potency of the plant extracts as previously observed by the disc diffusion assay. This gives credence to the findings of other workers on antimicrobial studies of *P. guajava*. Anas et al. (2008), in a related study, recorded higher activities in the methanolic extract of *P. guajava* leaves than in the aqueous extract, though both extracts were susceptible to MDR clinical isolates of *S. aureus*. The antibacterial activity of organic extracts and essential oils of *P. guajava* leaves was also investigated, and the methanolic extract showed the highest inhibition against shrimp isolates and type strains of *S. aureus*, *E. coli* and *Salmonella* spp (Goncalves et al., 2008). This highlights the interesting activity of *P. guajava* as an antimicrobial agent and to the best of our knowledge, this is the first report on the activity of *P. guajava* stem bark on MRSA.

The antimicrobial activity revealed by *P. guajava* extract against methicillin-resistant *S. aureus* (MRSA) indicated that the extract could be a better antimicrobial agent in cases of blind emergency treatment, where the cause of the infection is not known and in cases of resistance to conventional antibiotics. Tannins found in the phytochemical analysis may be responsible for the antibacterial effects. Akiyama et al (2001), in their study of the antibacterial action of tannins against *S. aureus*, attributed the antimicrobial mechanisms to their (I) astringent property (II) toxicity, and (III) complexation of metal ions.

Conclusively, the results of this study show that *P. guajava* Linn. has promising medicinal properties. The plant could be exploited in the development of phytome-

**Table 2.** Sensitivity of MRSA isolates to *P. guajava* stem bark extracts.

Extract (concentration in mg/ ml)	MRSA isolates diameters of the inhibitory zones (in mm)							
	1	2	3	4	5	6	7	8
Water (40 mg)	16	12	20	20	20	6	18	14
	(20 mg)	12	10	14	18	8	6	10
Methanol (40 mg)	16	12	20	20	10	6	16	12
	(20 mg)	10	10	12	15	10	5	10

**Table 3.** Minimum inhibition concentration (MIC) of stem bark extracts of *P. guajava* on MRSA isolates.

Extract	MRSA isolate (MIC (µg/ml))							
	1	2	3	4	5	6	7	8
Water	250	250	125	125	125	500	500	> 500
Methanol	125	125	62.5	62.5	> 500	> 500	> 500	> 500

**Table 4.** Minimum bacteriocidal concentration (MBC) of stem bark extracts of *P. guajava* on MRSA isolates.

Extract	MRSA isolate (MBC (µg/ml))							
	1	2	3	4	5	6	7	8
Water	250	250	125	250	250	> 250	> 250	> 250
Methanol	125	125	62.5	62.5	125	> 250	>250	>250

dicines for the control or management of resistant bacteria or as like the MSRAs. The results of this study are in agreement with previous reports and have validated the folkloric use of *P. guajava* in the treatment of diarrhea, gastrointestinal disorders, dysentery and wounds (Chah et al., 2006).

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