Inhibitory effect of *Psidium guajava* Linn. stem bark extracts on community acquired methicillin-resistant *Staphylococcus aureus*

Chibuike Ibe [1], Reginald Azu Onyeagba[1], Solomon Charles Ugochukwu[2], Venatius Chiamaka Ubah[3] and Chinenyenwa Joy Nduka[4]

[1]Microbiology Department, Abia State University, PMB 2000 Uturu, Abia State, Nigeria
[2]Plant Science and Biotechnology Department, Abia State University, PMB 2000 Uturu, Abia State, Nigeria
[3]Animal and Environmental Department, Abia State University, PMB 2000 Uturu, Abia State, Nigeria
[4]Environmental Health Technology Department, Abia State College of Health Technology, PMB 7016 Aba, Abia State, Nigeria
chibuike_ibe@yahoo.co.uk

Abstract: This study was conducted to investigate the inhibitory effect of *Psidium guajava* stem bark extract on methicillin-resistant *Staphylococcus aureus*. The plant materials were extracted and phytochemicals quantified using standard techniques. The agar well diffusion procedure and agar dilution method were used for the assessment of the antibacterial activity of the extracts, and quantitative determination of the bacteriostatic and bacteriocidal activities of the plant extracts respectively. The mean inhibition zone diameter (IZD) of the extracts ranged from 5-22 mm with ethanolic extracts exhibiting higher activities. The minimum concentrations range at which 50% and 90% of the isolates were inhibited for water and ethanolic extracts and their specific concentrations are: 1250->5000 µg/ml (MIC_{50}, 2500 µg/ml; MIC_{90}, 5000 µg/ml), and 625-5000 µg/ml (MIC_{50}, 1250 µg/ml; MIC_{90}, 2500 µg/ml) respectively. The phytochemicals estimated in mg/g dry body weight included: total phenol (111.82±0.47), tannin (141.98±0.51), flavonoids (3.31±0.04), vitamin C (1.59±0.03), saponin (285.79±0.81), and alkaloid (111.066±0.38). The methanolic extracts of the plant showed appreciable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free-radical-scavenging ability of 0.056 mg/ml. Similarly, the plant extract had high trolox equivalent antioxidant capacity (TEAC) of 11.95±0.04 mM/gdw. The result of this study supports the use of *Psidium guajava* stem bark in folk medicine.

[Chibuike Ibe , Reginald Azu Onyeagba, Solomon Charles Ugochukwu, Venatius Chiamaka Ubah and Chinenyenwa Joy Nduka. **Inhibitory effect of *Psidium guajava* Linn. stem bark extracts on community acquired methicillin-resistant *Staphylococcus aureus***. *Nat Sci* 2013;11(10):64-72]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 11

Keywords: *Psidium guajava*; inhibitory effect; phytochemical analysis; community acquired methillin-resistant *Staphylococcus aureus*.

1. Introduction

Staphylococcus aureus (*S. aureus*) is a vanguard for both nosocomial and community-acquired infections (Hossain et al., 2013). The antimicrobial resistance ability and extraordinary virulence of community acquired methicillin resistant *S. aureus* which allow it infect healthy persons are major medical issues worldwide. The term "methicillin-resistant *Staphylococcus aureus*" (MRSA) refers to those strains of *S. aureus* that have acquired resistance, whether in the community or in the hospital, to the antibiotics methicillin, oxacillin, nafcillin, cephalosporins, Imipenem, and/or other beta-lactam antibiotics (Hena and Sudha, 2011; Al-Anazi, 2009). *S. aureus* especially methicillin-resistance *S. aureus* (MRSA) is relatively ubiquitous and is the cause of many, community infections (Peters et al., 2013; McDougal et al., 2010; Okwu et al., 2012; Olowe et al., 2007; Yamamoto et al., 2006), endemic and epidemic nosocomial colonization (Hena and Sudha, 2011; Moussa et al., 2011) and infections (Al-Baidani et al., 2011; Olowe et al., 2007).

The two major strains of MRSA are known to be hospital-acquired (HA) MRSA and community-acquired (CA) MRSA (Milyani and Ashy, 2012). Community acquired MRSA (CA-MRSA) infections which were first described in small series of adult and paediatric patients presenting skin and soft tissue infections (SSTIs), pneumonia, or bacteremia have become a significant public health threat (Peters et al., 2013).

Since 1930, the epidemiology of *S aureus* has changed dramatically, and methicillin-resistant *S. aureus* (MRSA) has reached epidemic levels in both hospitals and community settings (Stenehjem and Rimland, 2013). Community acquired MRSA (CA-MRSA) has been increasingly reported from India (INSAR, 2013). These CA-MRSA strains cause serious skin and soft tissue infections, necrotizing pneumonia, and sepsis in healthy children (Saravanan et al., 2013). The epidemiological success of CA-

MRSA strain is believed to stem from combination of antibiotic resistance fitness at low cost with extraordinary virulence, allowing these strains infect otherwise healthy individuals and spread sustainably in the population (Perveen et al., 2013). The resistance to antibiotics in MRSA is due to the presence of *mecA gene* on the mobile genetic element, termed the staphylococcus cassette chromosome (SCC) (Corvaglia et al., 2010), which expresses a novel cell wall synthesizing enzyme, penicillin-binding protein 2A (PBP2A) with low affinity for all β-lactams (Al-baidani et al., 2011; Perwiaz et al., 2007; Brown et al., 2005).

The global emergence and increase of MRSA, also known as multidrug resistant or oxacillin resistant *S. aureus* have caused a shortage of effective beta-lactam antibiotics to MRSA based infections (Esimone et al., 2012). MRSA infections are very difficult to cure because MRSA strains are resistant to almost all clinically available antibiotics (Okwu et al., 2012).

Plants are the largest biochemical and pharmaceutical stores ever known on our planet. These living stores are able to generate endless biochemical compounds. In their living, human and animals are using only a small portion (1 to10%) of plants available on Earth (250,000 to 500,000 species) (Abdallah, 2011).

Psidium guajava (*P. guajava*) Linn. commonly known as guava is a plant of the family Myrtaceace. *P. guajava* is a low ever green tree or shrub 5 to 8 m high (Noudogbessi et al., 2013), with wide spreading branches and square downy twigs. It is a native of Tropical America (Esimone et al., 2012), but knows a good development in the tropical regions of Africa (Noudogbessi et al., 2013). *P. guajava* is one of the plants in folk medicine that has been used for the management of various disease conditions and is believed to be active. Various parts of the plant have been used in traditional medicine to manage conditions (Buvaneswari et al., 2011), extracts of root bark and leaves are used to treat gastroenteritis, vomiting, diarrhoea, dysentery, wound, ulcers, toothache, cough, sore throat, inflamed gums and a number of other conditions (Esimone et al., 2012). The aim of the present study was to determine the inhibitory effect of *P. guajava* stem bark extracts on the growth of 10 community acquired methicillin-resistant *S. aureus* strains isolated from healthy individuals in Uturu rural communities, Abia State, Nigeria.

2. Materials and Methods

2.1. Cell cultures

Stock cultures of 10 methicillin-resistant *S. aureus* isolated from healthy individuals maintained on nutrient agar slants at 4°C at the Department of Plant Science and Biotechnology Laboratory, Abia State University, Nigeria were used in this study. These stock cultures were sub cultured on mannitol salt agar and incubated at 37°C for 24 hours to check for their purity and reidentification. The colonial morphology of the different bacterial isolates were observed and identified accordingly. After 24 hours of incubation of the organisms in a mannitol salt agar plates, a single colony of each bacterial isolate was picked and streaked on a fresh mannitol agar plate and incubated at 37°C for 24 hours. A pure colony of the pure culture was Gram stained and examined for Gram positive cocci in cluster. This was followed by testing the ability for DNase production on agar plates as well as the ability to produce catalase, and coagulase enzymes. Once the identity of each isolate was confirmed by Bergey's (Breed et al., 1957) manual according to Al-Jumaily et al. (2012), the isolate was inoculated onto nutrient agar slant, incubated overnight and then stored at 4°C until needed for further studies.

2.1.2. Oxacillin susceptibility testing

The oxacillin discs used was procured from Oxoid, Germany. The antimicrobial susceptibility testing of the isolates was determined using the disc diffusion technique assay as described by Orji et al. (2012). The discs were prepared by MAYO diagnostic laboratory, Nigeria according to the recommendation by Clinical and Laboratory Standard Institute (CLSI, 2007).

With a sterile wire loop, few colonies of each of the isolates were emulsified in 5 ml of sterile peptone water to a turbidity corresponding to 0.5 McFarland standards (corresponding to approximately 10^8 cfu/ml). Then 0.5 ml of each inoculum was dispensed unto the surface of dried Mueller Hinton agar plate using sterile Pasteur pipette. These were spread evenly on the agar surface with sterile swab sticks (one for each inoculum). The excess inocula were discarded into a disinfectant jar. The inoculated plates were kept on the bench for 3 minutes to dry. The oxacillin disc (1 μg) was then placed centrally on the inoculated plates aseptically using a sterile forceps.

The preparations were incubated aerobically for 24 hours at 35°C. The diameter of zone of inhibition produced by each of the disc was measured, recorded and the isolates were classified as resistant (≤ 10 mm) or sensitive (≥13 mm) based on the standard interpretative chart as described by the Clinical and Laboratory Standard Institute (CLSI, 2010; CLSI, 2007).

2.1.2. Plant collection and preparation

Fresh stem bark of *P. guajava* (guava) was collected from Uturu, Abia State, Nigeria. The plant

materials were identified and authenticated in the taxonomy unit of Department of Plant Science and Biotechnology, Abia State University, Nigeria. The plant materials were chopped into small pieces, air-dried at room temperature and ground into powder using a manual blender. Exposure to direct sunlight was avoided to prevent the loss of active components. The dried powder was stored at 4°C until further analysis.

Analytical grade ethanol, BDH and distilled water were used for extraction. Reagents for phytochemical screening of extracts were freshly prepared using standard methods.

2.1.2.1. Preparation of plant materials aqueous extract

This was done according to Soniya et al. (2013) with minor modification. Fifty grams of dried powdered plant (stem bark) material was macerated with 500 ml of distilled water and allowed to stand for 24 hours while agitating at regular time intervals. The extract was filtered through Whatman No. 1 filter paper, and evaporated to dryness at 40° C.

2.1.2.1. Preparation of plant materials solvent extract

Fifty gram of dried powdered plant (stem bark) material was soaked separately for 24 hours in 500 ml 50% (v/v) ethanol. The soaked material was agitated at regular time intervals. After 24 hours the soaked material was filtered using Whatman filter paper No. 1 on separate filtration setups. The final filtrates was collected in wide mouthed evaporating bowls and evaporated to dryness at 40° C.

The dried extracts were weighed to calculate the extractability percentage. The extracts were stored at 4°C until further use.

2.1.3. Sensitivity test: agar well diffusion assay

The assay was conducted using agar-well diffusion method described by Esimone et al. (2012). An 80 mg/ml concentration of both ethanol and water extracts of *P. guajava* was constituted by dissolving 0.16 g of the dry extract in 2 ml each of 20% v/v dimethyl sulfoxide (DMSO) and 2-fold dilutions made to obtain 40 mg/ml and 20 mg/ml. A single colony of the isolates each was suspended in 2 ml of sterile peptone water. The suspension of each isolate was standardized as stated previously and used to inoculate the surface of the Mueller Hinton agar and the excess fluid drained into disinfectant jar. The inoculated agar surface was allowed to dry and the plates appropriately labeled. Using a cork borer, two wells of 5 mm in diameter was bored in the inoculated Mueller Hinton agar. With a micropipette, 50 µl of each concentration of the test extracts was delivered into each well. The plates were left on the bench for 30 minutes to allow the extracts to diffuse into the agar. Thereafter, the plates were incubated at 37°C for 24 hours. After incubation, the plates were observed for inhibition zones around the wells. The diameter of the zones was measured with metre ruler to the nearest whole millimetre. Each test was carried out thrice and the mean inhibition zone diameter (IZD) recorded to the nearest whole millimetre.

2.1.4. Minimum inhibitory concentration (MIC) of plant extracts

This was carried out using agar dilution method following the procedure outlined by Esimone et al. (2012) and CLSI (2007). 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 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, and 0.3125 mg/ml.

One millilitre each of the extracts 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 appropriately. 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 plates with wire loop and inoculated into 2 ml sterile peptone water to make a suspension of each test isolate. Each suspension was standardized as stated previously. Using a micropipette, a 10 µl of the standardized broth cultures was placed on the surface of the plates containing various concentrations of the extracts. Plain Mueller Hinton agar (that is, without the extract) was also streaked and served as negative controls. Inoculated plates were then incubated at 37°C for 24 hours 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.

2.1.5. Minimum bactericidal concentration (MBC) of plant extracts

After completion of the MIC procedure, the agar plates showing no growth in the MIC test 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 hours 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.

2.1.6. Phytochemical Analyses

These analyses determine the biologically active non-nutritive compounds that contribute to the flavour, colour, and other characteristics of plant parts. Quantitative analyses were done at the International Institute of Tropical Agriculture (IITA), Ibadan.

2.1.6.1. Preparation of methanolic extract

Methanolic extract of the guava powder (stem bark) was prepared following the method of Chan et al. (2007), by adding 25 ml of methanol to 0.5g of sample contained in a covered 50 ml centrifuge tube, and shaking continuously for 1 hour at room temperature. The mixture was centrifuged at 3,000 rpm for 10 minutes, and then the supernatant was collected and store at -4°C for further analysis.

2.1.6.2. Determination of total phenol content (TPC)

The total phenol content of sample methanolic extract was determined according to the Folin–Ciocalteu method reported by Chan et al. (2007). Briefly, 300 µL of extract was dispensed into test tube (in duplicates). To this was added 1.5 ml of Folin–Ciocalteu reagent (diluted 10 times with distilled water), followed by 1.2 ml of Na_2CO_3 solution (7.5% w/v). The reaction mixture was mixed, allowed to stand for 30 minutes at room temperature before the absorbance was measured at 765 nm against a blank prepared by dispensing 300 µL of distilled water instead of sample extract. TPC was expressed as gallic acid equivalent (GAE) in mg/g material.

2.1.6.3. Determination of tannin content

Tannin content of sample was determined according to the method of Padmaja (1989) as follows. Sample (0.1 g) was extracted with 5 mL of acidified methanol (1% HCl in methanol) at room temperature for 15 minutes. The mixture was centrifuged at 3,000 rpm for 20 minutes. About 0.1 mL of the supernatant was added with 7.5 ml of distilled water, 0.5 ml of Folin-Denis reagent, 1 ml of 35% sodium carbonate solution and diluted to 10 ml with distilled water. The mixture was shaken well, kept at room temperature for 30 minutes and absorbance was measured at 760 nm. Blank was prepared with water instead of the sample. Tannin content was expressed as tannic acid equivalent (TAE) in mg/g material.

2.1.6.4. Determination of total flavonoid content (TFC)

Total flavonoid content was determined using aluminum chloride method as reported by Kale et al. (2010). About 0.5 ml of methanolic extract was dispensed into test tube, followed by 1.5 ml of methanol, 0.1 ml of aluminum chloride (10%), 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The reaction mixture was mixed, allowed to stand at room temperature for 30 minutes, before absorbance was read at 514 nm. TFC was expressed as quercetin equivalent (QE) in mg/g material.

2.1.6.5. Determination of vitamin C content

The vitamin C content of the aqueous extract was determined using the method reported by Benderitter et al. (1998). Briefly, 75 µL DNPH (2 g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg $CuSO_4.5H_2O$ in 100 ml of 5 M H_2SO_4) was added to 500 µL reaction mixture (300 µL appropriate dilution of hydrophilic extract with 100 µL of 13.3% trichloroacetic acid and distilled water). The reaction mixture was subsequently incubated for 3 hours at 37°C, then 0.5 ml of 65% H_2SO_4 (v/v) was added to the medium, and the absorbance was measured at 520 nm, and the vitamin C content of the sample was subsequently calculated from the calibration curve prepared with ascorbic acid standard.

2.1.6.6. Determination of total saponin content

Total saponin was determined by the method described by Makkar et al. (2007). About 0.5 g of sample was extracted with 25 ml of 80% aqueous methanol by shaking on a mechanical shaker for 2 hours, after which contents of the tubes were centrifuged for 10 minutes at 3,000 rpm. In a test tube an aliquot (0.25 ml) of the supernatant was taken to which 0.25 ml vanillin reagent (8% vanillin in ethanol) and 2.5 ml of 72% aqueous H_2SO_4 were added. The reaction mixtures in the tubes were heated in a water bath at 60°C for 10 minutes. Then tubes were cooled in ice for 4 minutes and then allowed to acclimatize to room temperature. Subsequently, the absorbance was measured in a Uv/Visible spectrophotometer at 544 nm. Diosgenin was used as a standard and the results obtained were expressed as mg diosgenin equivalent per g of sample dry matter.

2.1.6.7. Determination total alkaloid content

The total alkaloid content of the sample was measured using 1,10-phenanthroline method described by Singh et al. (2004) with slight modifications. About 100mg sample powder was extracted in 10 ml 80% ethanol. This was centrifuged at 5000rpm for 10 minutes. Supernatant obtained was used for the further estimation total alkaloids. The reaction mixture contained 1 ml plant extract, 1 ml of 0.025 M $FeCl_3$ in 0.5 M HCl and 1ml of 0.05 M of 1,10-phenanthroline in ethanol. The mixture was incubated for 30 minutes in hot water bath with maintained temperature of 70 ± 2°C. The absorbance of red coloured complex was measured at 510 nm against reagent blank. Alkaloid contents were estimated and it was calculated with the help of standard curve of quinine (0.1 mg/ml, 10 mg dissolved in 10 ml ethanol and diluted to 100 ml with

distilled water). The values were expressed as mg/g of dry weight.

2.1.7. Antioxidant Activity Determination

2.1.7.1. Estimation of DPPH free-radical-scavenging ability

The free-radical-scavenging ability of the methanolic extract against 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was evaluated as described by Cervato *et al.* (2000) with slight modification. Briefly, appropriate dilution of the extracts (1 ml) was mixed with 3 ml of 60 µM methanolic solution of DPPH radicals; the mixture was left in the dark for 30 minutes before the absorbance was taken at 517 nm. The decrease in absorbance of DPPH· on addition of test samples in relation to the control was used to calculate the percentage inhibition (%Inh.) following the equation: $\%Inh. = [(A517_{control} - A517_{sample}) \div A517_{control}] \times 100$. The IC_{50}, which stands for the concentration of extract required for 50% scavenging activity, was calculated from the dose-inhibition linear regression curve of each extract.

2.1.7.2. Estimation of ABTS* radical-scavenging ability

The ABTS* radical-scavenging ability of extract was determined according to the method described by Sellappan and Akoh (2002). The ABTS* radical was generated by incubating equal volume of a 7 mM ABTS aqueous solution with $K_2S_2O_8$ (2.45 mM) in the dark for 16 hours at room temperature and adjusting the absorbance at 734 nm to 0.7 ± 0.02 with 95% ethanol. Then 0.2 ml appropriate dilution of the extract was added to 2.0 ml ABTS* solution and the absorbance was measured at 734 nm after 15 minutes The trolox equivalent antioxidant capacity (TEAC) was subsequently calculated. The calibration equation for TEAC was $Y = -0.0505x + 0.1954$ ($R^2 = 0.9902$).

3. Results and Discussion

Methicillin-resistant *S. aureus* (MRSA) is primarily a nosocomial pathogen that emerged as a major cause of infection and colonization in hospitalized patients, whereas, community-associated MRSA infections are increasing in incidence and are said to be severe enough to cause fatality (Mandell et al., 2005). Strains of MRSA that cause infections have also developed resistance to antibiotics commonly used for therapeutic purposes (Milyani and Ashy, 2012). Plant products, particularly spices and extracts of various plant parts have been used extensively as natural antimicrobials and antioxidants (Milyani and Ashy, 2012). From the result of this study, the extractability percentage of *P. guajava* stem bark is 16.6% for water and 19.04% for ethanol. This indicates that the chemical components (antibacterial principles) of *P. guajava* were more soluble in ethanol than in water. The water and ethanolic extracts of *P. guajava* stem bark were active against all the 10 isolates of MRSA tested with a mean inhibition zone diameter (IZD) which ranged from 5-22 mm as shown in table 1. The ethanolic extracts had more activity than water extracts with a maximum IZD of 22 mm on 1 tested MRSA isolate. This result is in agreement with the work of Joseph and Priya (2011), who in related study reported a maximum inhibition zone diameter of 21 mm against *S. aureus* using methanolic extract of *Psidium guajava* stem bark. Water extract only recorded a maximum inhibition zone diameter of 18 mm on 2 MRSA isolates. The activity observed with ethanolic extract may be used to improve the medicinal value (enhance application) of the plant in common practice in traditional medicine to use the plant extracts prepared in form of infusion or decoctions.

The MIC results showed that the 10 isolates were inhibited by ethanolic extract with activities that ranged from 625-5000 µg/ml while only 9 isolates were inhibited by water extract with activities that ranged from 1250-5000 µg/ml (table 2). The MBC results showed 8 isolates to be susceptible to ethanolic extract within the range of 625-5000 µg/ml whereas 6 isolates were susceptible to the water extract with activity range of 1250-5000 µg/m (table 3). The results of well diffusion assay, MIC and MBC on the MRSA isolates agreed with the results of Anas et al. (2008) who in a related study reported higher activities in the methanolic extract of *P. guajava* leaves than in aqueous extracts. The results of this study is in contrast to the reports of Esimone et al. (2012) who in a similar study reported maximum activities with water extracts of *P. guajava* stem bark than methanolic extracts on MRSA isolates and also with that of Buvaneswari et al. (2011) who in a related study reported higher antibacterial activity with water extracts of *P. guajava* leaves than with 75% methanol extracts.

This study indicated that ethanolic extracts had lower and more active MIC_{50} and MIC_{90} concentrations than water extracts. The ethanolic extracts had MIC_{50} and MIC_{90} at 1250 µg/ml and 2500 µg/ml respectively, whereas, water extracts were at 2500 µg/ml and 5000 µg/ml (table 4).

Table 1: Susceptibility of MRSA to *P. guajava* stem bark extract

Extract (Conc. in (mg/ml)	Mean IZD of MRSA isolates (mm)									
	1	2	3	4	5	6	7	8	9	10
Water (40)	15	13	14	17	6	18	11	15	18	15
(20)	8	9	10	11	5	13	7	10	15	7
Ethanol (40)	17	13	18	20	6	20	12	16	22	16
(20)	9	10	10	13	6	14	8	10	15	9

Table 2: Minimum inhibition concentration (MIC) of *P. guajava* stem bark extracts on MRSA isolates

Extract	MRSA isolates (MIC (µg/ml))									
	1	2	3	4	5	6	7	8	9	10
Water	2500	5000	5000	2500	>5000	1250	5000	1250	1250	2500
Ethanol	1250	2500	1250	2500	5000	625	2500	1250	625	625

Table 3: Minimum bacteriocidal concentration (MBC) of *P. guajava* stem bark extract on MRSA isolates

Extract	MRSA isolates (MBC (µg/ml))									
	1	2	3	4	5	6	7	8	9	10
Water	5000	>5000	>5000	2500	>5000	2500	>5000	5000	1250	2500
Ethanol	2500	2500	2500	2500	>5000	1250	>5000	1250	625	2500

Table 4: MIC_{50} and MIC_{90} (µg/ml) of *P. guajava* extract for MRSA isolates

n = 10

Extract	MIC (µg/ml) MIC range	MIC_{50}	MIC_{90}
Water	1250–>5000	2500	5000
Ethanol	625–5000	1250	2500

The result of this study supports the findings of other workers on the antimicrobial studies of *P. guajava* extracts. The antibacterial activity of organic extracts and essential oils of *P. guajava* leaves was investigated by Goncalves et al. (2008), and the methanolic extract showed the highest inhibition against shrimp isolates and type strains of *S. aureus*, *E. coli* and *Salmonella* species.

The phytochemical contents of the stem bark are presented in table 5.

Table 5: Total phenol, tannin, flavonoids, vitamin C, saponin, and alkaloid expressed in mg/g dry body weight

Phytochemical	*P. guajava* (stem bark)
Total phenol	111.82±0.47
Tannin	141.98±0.51
Flavonoids	3.31±0.04
Vitamin C	1.59±0.03
Saponin	285.79±0.81
Alkaloid	111.066±0.38

Data represent the mean ± standard deviation of duplicate readings.

Plant-derived phenolic compounds are well known to exhibit antioxidant activity through a variety of mechanisms, including free-radical scavenging, lipid peroxidation and chelating of metal ions. They were reported to eliminate radicals due to their hydroxyl groups. In addition to these antioxidant effects, phenols were reported to inhibit alpha-amylase, sucrase, as well as the action of sodium glucose-transporter 1 (SGLUT-1) of the intestinal brush border, hence their antidiabetic action (Balakrishnan et al., 2010). They have also been reported to exert anti-inflammatory and anti-carcinogenic effects.

Tannin, the dietary anti-nutrients that are responsible for the astringent taste of foods and drinks, are known to cause browning or other pigmentation problems in both fresh foods and processed products. The presence of tannin in *P. guajava* stem bark suggests that it may have astringent properties and in addition, could quicken the healing of wounds and burns. The antibacterial action of tannins against *S. aureus*, is attributed to the antimicrobial mechanisms of their (I) astringent property (II) toxicity, and (III) complexation of metal ions (Esimone et al., 2012).

Flavonoids have antioxidant activity and could therefore lower cellular oxidative stress, which has been implicated in the pathogenesis of various neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. The high flavonoid content of the stem bark may have contributed to the medicinal properties (Irondi et al., 2012).

The total saponin content of the plant was found to be quite high. The biological activities of saponins have been reviewed and include haemolytic, hypoglycaemic, antioxidant and hypolipidaemic activities, lowering of cancer risks, virucidal activity,

reduction of protein digestibility, antimicrobial activity, among others.

Alkaloids are a group of basic organic substances of plant and microbial origin, containing at least one nitrogen atom in a ring structure in the molecule. They were reported to be the most efficient therapeutically significant plant substances. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents due to their analgesic, antispasmodic and antibacterial properties (Oboh and Irondi, 2012).

As a water-soluble antioxidant, vitamin C is in a unique position to 'scavenge' aqueous peroxyl radicals before these destructive substances damage the lipids. These phytochemicals observed in the *P. guajava* stem bark may either individually or in combination be responsible for the antibacterial activity exhibited.

DPPH, a stable free radical with a characteristic absorption at 517 nm, was used to study the radical scavenging effects of extracts of the stem bark of *P. guajava*. As antioxidants donate protons to this radical, the absorption decreases. The decrease in absorption is taken as a measure of the extent of radical scavenging (Irondi et al., 2012; Oboh and Irondi, 2012). The IC_{50} value for the extract, defined as the concentration of extract causing 50 per cent inhibition of DPPH absorbance, is shown in table 6.

Table 6: DPPH IC_{50}, and Trolox equivalent antioxidant capacity (TEAC) of *P. guajava* stem bark methanolic extracts.

Antioxidant Activity	*P. guajava* stem bark
DPPH IC50 (mg/ml)	0.056
TEAC (mM/gdw)	11.95±0.04

Data for TEAC represent the mean ± standard deviation of duplicate readings.

P. guajava had IC_{50} of 0.056 mg/ml. Since IC_{50} is a measure of inhibitory concentration, a lower IC_{50} value is a reflection of greater antioxidant activity of the sample. Hence *P. guajava* displayed a high free radical scavenging ability.

ABTS* scavenging ability reported as the Trolox equivalent antioxidant capacity (TEAC) is presented in table 6. The results revealed that the ABTS* scavenging ability of *P. guajava* stem bark is high (11.95±0.04 mM/gdw). The ABTS assay is based on the inhibition of the absorbance of the radical cation ABTS* which has a characteristic long wavelength absorption spectrum.

ABTS radicals are more reactive than DPPH radicals and unlike the reactions with DPPH radical which involve H atom transfer the reactions with ABTS radicals involve electron transfer process. From our results, there is a linear correlation between total phenol content and the antioxidant activity of *P. guajava* stem bark extract. The high reducing power of *P. guajava* extract is attributable to its high total phenol, tannin, total flavonoid and saponin contents, which are also reflected in its high DPPH free-radical-scavenging ability and TEAC.

In conclusion, the results of this study showed that *P. guajava* has promising medicinal properties due to the high content of phytochemicals which have been shown to exhibit antimicrobial characteristics and have validated the folkloric use of *P. guajava* in the treatment of bacterial infections especially those associated with *S. aureus*. Natural products still represent an important source of interesting leads for drug development. The plant could be exploited greatly in the development of phytomedicines for the control or management of resistant bacteria like CA-MRSA.

Corresponding author:
Chibuike Ibe
Microbiology Department
Abia State University
PMB 2000 Uturu
Abia State, Nigeria
Tel: +234(0) 805 9322 893
Email: chibuike_ibe@yahoo.co.uk

References

1. Abdallah EM. Plants: An alternative source for antimicrobials. Journal of Applied Pharmaceutical Science, 2011;1(06):16-20.
2. Al-Anazi AR. Prevalence of *Methicillin-Resistant Staphylococcus aureus* in a teaching hospital in Riyadh, Saudi Arabia. Biomedical Research, 2009; 20(1):7-14.
3. Al-baidani AR, El-shouny WA, Shawa TM. Antibiotics Susceptibility Pattern of Methicillin-Resistant *Staphylococcus aureus* in Three Hospitals at Hodeidah City, Yemen. Global Journal of Pharmacology, 2011;5(2):106-111.
4. Al-Jumaily EF, Mohamed DA, Khanaka HH. Molecular epidemiology and Antibiotic susceptibility patterns of clinical strains of methicillin resistant *Staphylococcus aureus* (MRSA) in Sulaimani city-Iraq. Global Advanced Research Journal of Microbiology, 2012;1(6): 081-089.
5. Anas K, Jayasree PR, Vijayakumar T, Manish KPR. *In vitro* antibacterial activity of *Psidium guajava* Linn. leaf extract on clinical isolates of multidrug resistant *Staphylococcus aureus*. Indian Journal of Experimental Biology, 2008; 46:41-46.
6. Balakrishnan N, Balasubramaniam A, Nandi P, Dandotiya R, Begum S. Antibacterial and Free

Radical Scavenging Activities of stem bark of *Psidium guajava* Linn. International Journal of Drug Development & Research, 2010;3(4):255-260.
7. Benderitter M, Maupoil V, Vergely C. Studies by electron paramagnetic resonance of the importance of iron in the hydroxyl scavenging properties of ascorbic acid in plasma: Effects of iron chelators. Fundamental and Clinical Pharmacology, 1998;12:510-16.
8. Breed RS, Murray EGD, Smith NR, editors. Bergey's Manual of Determinative Bacteriology. 7th ed. The Williams & Wilkins Company, Baltimore, 1957; 464-466.
9. Brown DFJ, Edwards DI, Hawkey PM, Morrison D, Ridgway GL, Towner KJ, Wren MWD. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant Staphylococcus aureus (MRSA). Journal of Antimicrobial Chemotherapy, 2005;56:1000–1018.
10. Buvaneswari S, Raadha CK, Krishnaveni N, Jayashree S. *In-vitro* Antimicrobial activity of *Psidium guajava* against clinically important strains. E-Journal of Life Sciences, 2011;1(1):14-22.
11. Chan EWC, Lim YY, Chew YL. Antioxidant activity of Camellia sinensis leaves and tea from a lowland plantation in Malaysia. Journal of Agriculture and Food Chemistry, 2007;102:1214-1222.
12. Clinical and Laboratory Standard Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing, Seventeenth International Supplement, CLSI document M100-S17. Vol. 27-1 [ISBN 1-56238-625-5], PA, USA, 2007.
13. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Approved standard M100-S20. Vol. 30, No. 1. National Committee for Clinical Laboratory Standards, Wayne, PA. USA, 2010.
14. Corvaglia AR, François P, Hernandez D, Perrona K, Lindera P, Schrenzel J. A type III-like restriction endonuclease functions as a major barrier to horizontal gene transfer in clinical Staphylococcus aureus strains. Proceedings of the National Academy of Sciences, 2010;107(27):11954–11958.
15. Esimone CO, Attama AA, Mundi KW, Ibekwe NN, Chah KF. Antimicrobial activity of *Psidium guajava* Linn. stem extracts against methicillin-resistant *Staphylococcus aureus. African Journal of Biotechnology*, 2012;11(89):15556-15559.
16. Goncalves FA, Andrade-Neto M, Bezerra JNS, Macrae A, Sousa OV, Fonteles-Filho AA, Vieira RHSF. Antibacterial activity of guava, *Psidium guajava* Linnaeus, leaf extracts on diarrhea-causing enteric bacteria isolated from seabob shrimp, *Xiphopenaeus kroyeri* (Heller). Revista do Instituto de Medicina Tropical de São Paulo, 2008;50(1):11-15.
17. Hena, JV, Sudha SS. RFLP analysis of clinical MRSA isolates. International Journal of Pharmacy and Biological Sciences, 2011;2(1):637-645.
18. Hossain M, Chowdhury DUS, Farhana J, Akbar MT, Chakraborty A, Islam S, Mannan A. Identification of potential targets in *Staphylococcus aureus* N315 using computer aided protein data analysis. Bioinformation, 2013;9(4):187-192.
19. Indian Network for Surveillance of Antimicrobial Resistance (INSAR). Methicillin resistant *Staphylococcus aureus* (MRSA) in India: Prevalence & susceptibility pattern. Indian Journal of Medical Research, 2013;137:363-369.
20. Irondi AE, Oboh G, Akintunde JK. Comparative and Synergistic Antioxidant Properties of *Carica papaya* and *Azadarichta indica* Leaves. International Journal of Pharmaceutical Sciences and Research, 2012;3(12):4773-4779.
21. Kale A, Gaikwad S, Mundhe K. Quantification of Phenolics and Flavonoids by Spectrophotometer From -*Juglans regia.* International Journal of Pharmacy and Biological Sciences, 2010;1:1-4.
22. Makkar HPS, Siddhuraju P, Becker K. Plant secondary metabolites. Humana Press Inc., Totowa, NJ, USA, 2007.
23. Mandell GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases. 6th ed. Churchill Livingstone, London, 2005.
24. McDougal LK, Fosheim GE, Nicholson A, Bulens SN, Limbago BM, Shearer JES, Summers AO, Patel JB. Emergence of Resistance among USA300 Methicillin-Resistant *Staphylococcus aureus* Isolates Causing Invasive Disease in the United States. *Antimicrobial Agents and Chemotherapy,* 2010;54(9):3804–3811.
25. Milyani R, Ashy N. Inhibitory effect of some plant extracts on clinical isolates of *Staphylococcus aureus.* African Journal of Microbiology Research, 2012;6(40):6822-6829.
26. Moussa IM, Al-Qahtani AA, Gassem MA, Ashgan MH, Ismail DK, Ghazy AI, Shibl AM. Pulsed-field gel electrophoresis (PFGE) as an epidemiological marker for typing of methicillin-resistant *Staphylococcus aureus* recovered from KSA. *African Journal of Microbiology Research*, 2011; 5(12):1492-1499.
27. Noudogbessi, JP, Chalard P, Figueredo GA, Alitonou G, Agbangnan P, Osseni A, Avlessi F,

Chalchat JC, Sohounhloue DCK. Chemical Compositions and Physical Characteristics of Volatile Extracts of Leaves of *Psidium Guajava* Linn and *Lantana Camara* Linn of Benin. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2013;4(1):28-37.

28. Oboh G, Irondi EA. Comparative Phytochemical Composition and Antioxidant Activities of *Mangifera indica* and *Mucuna urens* Seeds. Research & Reviews: Journal of Herbal Science, 2012;1(3):8-17.
29. Okwu M, Bamgbala S, Aborisade W. Prevalence of Nasal Carriage of Community-associated Methicillin-resistant *Staphylococcus aureus* (CA-MRSA) among Healthy Primary School Children in Okada, Nigeria. Journal of Natural Sciences Research, 2012;12(4):61-65.
30. Olowe OA, Eniola KIT, Olowe RA, Olayemi AB. Antimicrobial Susceptibility and Beta-lactamase detection of MRSA in Osogbo, SW Nigeria. Nature and Science, 2007;5(3):44-48.
31. Orji I, Nworie A, Eze UA, Agberotimi IO, Okereke EC, Azi SO. The prevalence and antimicrobial susceptibility profile OF methicillin resistant *Staphylococcus aureus* isolates from clinical specimens in a tertiary hospital, south east Nigeria. Continental Journal of Pharmaceutical Sciences, 2012;6(1):23 – 29.
32. Padmaja G. Evaluation of techniques to reduce assayable tannin and cyanide in cassava leaves. Journal of Agricultural and Food Chemistry, 1989;37:712-716.
33. Perveen I, Majid A, Knawal S, Naz I, Sehar S, Ahmed S, Raza MA. Prevalence and Antimicrobial Susceptibility Pattern of Methicillin-Resistant *Staphylococcus aureus* and Coagulase-Negative Staphylococci in Rawalpindi, Pakistan. British Journal of Medicine & Medical Research, 2013;3(1):198-209.
34. Perwaiz S, Barakzi Q, Farooqi BJ, Khursheed N, Sabir N. Antimicrobial susceptibility pattern of clinical isolates of Methicillin Resistant *Staphylococcus aureus*. Journal of the Pakistan Medical Association, 2007;57(1):2-4.
35. Peters PJ, Brooks JT, McAllister SK, Limbago B, Lowery HK, Fosheim G, Guest JL, Gorwitz RJ, Bethea M, Hageman J, Mindley R, McDougal LK. Rimland D. Methicillin-Resistant Staphylococcus aureus Colonization of the Groin and Risk for Clinical Infection among HIV-infected Adults. *Emerging Infectious Disease,* 2013;19(4):623-629.
36. Saravanan M, Anima NA, Tesfaye T. Antibiotic Susceptibility Pattern of Methicillin Resistant *Staphylococcus aureus* from Septicemia Suspected Children in Tertiary Hospital in Hosur, South India. American Journal of Microbiological Research, 2013;1(2):21-24.
37. Singh DK, Srivastva B, Sahu A. Spectrophotometric determination of *Rauwolfia* alkaloids, estimation of reserpine in pharmaceuticals. Analytical Sciences, 2004; 20:571-573.
38. Soniya M, Kuberan M, Anitha S, Sankareswari P. *In vitro* antibacterial activity of plant extracts against Gram positive and Gram negative pathogenic bacteria. International Journal of Microbiology and Immunology Research, 2013; 2(1):001-005.
39. Stenehjem E, Rimland D. MRSA nasal colonization burden and risk of MRSA infection. American Journal of Infection Control, 2013;4:1405-1410.
40. Yamamoto T, Dohmae S, Saito K, Otsuka T, Takano T, Chiba M, Fujikawa K, Tanaka M. Molecular Characteristics and In Vitro Susceptibility to Antimicrobial Agents, Including the Des-Fluoro (6) Quinolone DX-619, of Panton-Valentine Leucocidin-Positive Methicillin-Resistant *Staphylococcus aureus* Isolates from the Community and Hospitals. Antimicrobial Agents and Chemotherapy, 2006;50(12):4077–4086.

8/21/2013