Special Conference Edition, November, 2017
http://dx.doi.org/10.4314/bajopas.v10i1.23S

Bayero Journal of Pure and Applied Sciences: 10(1): 112 - 115
ISSN 2006 – 6996

SYNERGISTIC ANTIBACTERIAL EFFECT OF STEM BARK EXTRACTS OF *Faidherbia albida* AND *Psidium guajava* AGAINST METHICILLIN RESISTANT *Staphylococcus aureus*

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ABSTRACT
The study was aimed at screening the stem bark extracts of Faidherbia albida and Psidium guajava for synergistic antibacterial effect against methicillin resistant Staphylococcus aureus (MRSA). The powdered plant materials were extracted with methanol using cold maceration technique and the extracts were screened for alkaloids, flavonoids, tannins, saponins, and steroids using standard methods. The test organism was isolated from subjects with boils in Gombe State and confirmed using Gram staining and standard biochemical procedures as well as cefoxitin susceptibility test. Confirmed MRSA were subjected to susceptibility test of the plant extracts using agar well diffusion and broth dilution techniques. The results of the study showed percentage extraction yields of 15.7% and 7.5% for F. albida and P. guajava stem barks respectively. Sensitivity test of MRSA isolates to the extracts using agar well diffusion method revealed zone diameters of 8-10mm while combination of extracts showed activity of 21mm .The minimum inhibitory concentration (MIC) of the extracts combination was found to be 0.25mg/ml while the minimum bactericidal concentration (MBC) was determined to be 0.5mg/ml. The study revealed that stem bark methanol extracts combination of the two plants possess synergistic antibacterial activity against MRSA and so can be exploited as a viable option in production of safer plant-based drugs against the bacterium.
Keywords: Synergistic, Faidherbia albida, Psidium guajava, Methicillin Resistant, Staphylococcus aureus.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been emerging worldwide as one of the most important hospital and community acquired pathogens, and it is a topic of increasing concern in the realm of healthcare (Aliyu *et al.*, 2008; Esimone *et al.*, 2012). It causes local purulent infections like furuncles, carbuncles, wound infections, sinusitis, post-influenza pneumonia, and sepsis. It is also responsible for toxin-caused illnesses like food poisoning, and toxic shock syndrome (Kayser *et al.*, 2005).

The problem of microbial resistance to antibiotics is fast growing particularly in the hospitals, where methicillin-resistant *Staphylococcus aureus* has become a global threat to antimicrobial chemotherapy. As a result, the search for new ways to treat MRSA infections stimulates research into natural products from medicinal plants (Aliyu *et al.*, 2008).

In Northern Nigerian traditional medicine, many indigenous plants are widely used in the treatment of various infectious diseases; one of such plants is *Faidherbia albida*. This plant has records of widespread claims of therapeutic effectiveness against skin infections across Northern Nigeria, West Africa and beyond (Aliyu *et al.*, 2008).

In tropical America folk medicine, extracts of root, bark, and leaves of *Psidium guajava* are used to treat gastroenteritis, vomiting, diarrheoa, dysentery, wound, ulcers, toothache, cough, sore throat, inflamed gums and a number of other conditions (Esimone *et al.*, 2012). Therefore this work was set up with the aim of determining the synergistic antibacterial activity of *F. albida* and *P. guajava* stem barks against MRSA.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Stem barks of *Faidherbia albida* and *Psidium guajava* were collected using a knife at Malamsidi and Kalshingi towns of Gombe State respectively. The plant parts were authenticated at the Department of Plant Biology, Bayero University Kano, Nigeria, with *F. albida* having BUK Herbarium Accession number 0346 and *P. guajava* having BUK Herbarium Accession number 0336. Samples were air-dried under a shade for two weeks and pulverized to powder using aluminium mortar and pestle as described previously by Aliyu *et al*. (2008) and Esimone *et al*. (2012).

Extraction and Phytochemical Screening

One hundred grams of each of the powdered plant materials were put in separate conical flasks containing 500mls each of methanol, kept for two weeks with shaking at regular intervals, then filtered and evaporated at 40^{o}C (Sarker *et al.,* 2006).

Phytochemical screening was carried out for the detection of Alkaloids, Saponins (Frothing test), Tannins, Steroids (Salkowski's test), Flavonoids, Anthraquinones (Borntrager's test), Cardiac glycosides (Keller Killiani's test) and Reducing sugars (Aiyelaagbe and Osamudiamen, 2009).

Bioassay Studies

Test Isolate

The test organism was isolated in the Gombe State University Campus and identified using Gram Staining, Microscopy, Biochemical Tests, and Cefoxitin susceptibility test (Cheesbrough, 2006; Reynolds *et al.,* 2010; Clinical Laboratory Science Institute, 2014; Okwu *et al.,* 2015).

Preparation of Stock Solution of Extract

This was prepared by dissolving 0.2g of the extract in 2mls of 20% DMSO (Esimone *et al.,* 2012) to yield a concentration of 100mg/ml, 5-fold serial dilutions were then made.

Inoculum Standardisation

Direct colony suspension (CLSI, 2014) was employed, 24h old colonies of the isolate were picked using sterile wire loop and dissolved in 2 mls of sterile normal saline. The turbidity was then adjusted with sterile normal saline to match the turbidity of 0.5 McFarland Standard.

Sensitivity Testing

A sterile swab stick was used to swab the surface of Mueller-Hinton Agar plates with the standardized inoculum. Sterile cork borer of 8mm was used to bore two wells in each of the plates. One hundred microlitres from each of the dilutions were put in separate wells of the petri dishes, Gentamicin 30µg was used as control. The plates were incubated at 34^{o}C for 24h and then zones of inhibition were measured to the nearest millimitre. The procedure was repeated for the extract combination.

Minimum Inhibitory Concentration (MIC)

This was carried out for the extract combination using Agar dilution technique (CLSI, 2014). One millilitre (1 ml) of the extract dilutions were added to separate 19 mls of sterile molten Mueller hinton agar, mixed thoroughly and poured into separate sterile Petri dishes. Ten microlitres (10µl) of a standardized culture was placed on the surface of each of the plates containing various concentrations of the extract combination. Plain Mueller hinton agar (that is, without the extract) was also inoculated and served as negative control. Inoculated plates were incubated at 37°C for 24 h and observed for any visible bacteria 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)

This was determined using broth dilution method (Esimone *et al*. 2012), 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 corresponding test tubes of fresh nutrient broth that acted as the recovery medium. One test tube containing nutrient broth was left blank to serve as control, and then the tubes incubated for 24 h at 34°C. At the end of incubation, microbial growth was ascertained by checking the turbidity of the media in comparison with the blank nutrient broth test tube. The absence of change in turbidity in the recovery media was used as evidence of total cell death, and vice-versa.

RESULTS AND DISCUSSION

The result of extraction showed a percentage extract yield of 7.5% and 15.7% for *F. albida* and *P. guajava* stem barks respectively, this may be as a result of the high polarity of the extraction solvent. Both of the extracts had dark brown colours and smooth textures. The results of phytochemical analysis showed that the *P. guajava* stem bark extract tested positive for tannins, flavonoids, anthraquinones, alkaloids, reducing sugars, cardiac glycosides, and saponins, but tested negative for steroids, while the *F. albida* stem bark tested positive for flavonoids, anthraquinones, alkaloids, reducing sugars, cardiac glycosides and saponins, but tested negative for tannins and steroids. These seconadary metabolites are known to have good antimicrobial properties (Doughari, 2012).

The presumed MRSA isolate on nutrient agar, mannitol salt agar, and MRSA select agar produced spherical colonies that were cream coloured, yellow coloured, and deep pink coloured respectively. Results of Gram staining, microscopy, and biochemical tests showed that the presumed isolate was Gram positive, its colonies appeared purple in colour, spherical shaped, arranged in clusters, and was catalase positive, coagulase positive, deoxyribonuclease (DNAse) positive, and positive for mannitol fermentation. Cefoxitin susceptibility test confirmed the presumed isolate to be MRSA (Cheesbrough, 2006; Reynolds *et al.,* 2010; CLSI, 2014; Okwu *et al.,* 2015).

Sensitivity of the test isolate to the stem bark extracts of *F. albida* (Table 1) and *P. guajava* (Table 2) both singly and in combination (Table 3) using well diffusion method was established by measurement of zones of inhibition formed around the wells containing various concentrations of the extracts. Absence of visible growth on agar plates indicated the inhibitory activity of the extract combination and was taken as the lowest concentration of the extract that resulted in this activity (0.25mg). Absence of turbidity in the recovery medium used in MBC test showed the bactericidal activity of the extract combination and was taken as the lowest concentration (0.5mg) from the MIC range which resulted in no growth in the recovery medium of the MBC test. The activity of this combination of plant extracts can be attributed to the high amounts of tannins and alkaloids indicated from the initial phytochemical analysis, because tannins and alkaloids have been shown to have good antibacterial properties since tannins bind to and precipitate proteins while alkaloids act by targeting and affecting a wide range of cellular molecular targets such as biomembranes and nucleic acids (Roberts and Wink, 1998; Akiyama *et al.,* 2001).

Table 1: Extraction of plant materials with methanol

Plant Part	Percentage yield	Color	Texture	Consistency
F. albida stem bark	7.5	Dark brown	Smooth	Broken
P. guajava stem bark	15.7	Dark brown	Smooth	Intermediate

Table 2: Phytochemical constituents of *F. albida* and *P. guajava* stem barks extracts

Plant Part	Alkaloids	Flavonoids	Tannins	Steroids	Saponins
F. albida stem bark	++	+	+++	-	++
P. guajava stem bark	++	+	++	-	+++

Key: - = absent (no response), + = present in small amounts (low response), ++ = present in moderate amounts (medium response), +++ = present in high amounts (high response)

Table 3: Sensitivity of the test isolate to the stem bark extracts of *F. albida* and *P. guajava* both singly and in combination

Concentration (per well)	1mg	0.5mg	0.25mg	0.125mg	Gentamicin (control)
P. guajava stem bark	8	8	8	8	32
F. albida stem bark	10	8	8	8	32
P. guajava stem bark + *F. albida* stem bark	21	18	8	8	32

Table 4: MIC and MBC of *F. albida* and *P. guajava* stem barks extracts combination

Minimum Inhibitory Concentration (MIC)	0.25mg/ml
Minimum Bactericidal Concentration (MBC)	0.5mg/ml

CONCLUSION

It can be concluded that the stem barks of *Faidherbia albida* and *Psidium guajava* contain considerably high amounts of bioactive compounds which when combined posses synergistic antibacterial activity against MRSA and so can be exploited as viable options in production of safer plant-based drugs against infections caused by this bacterium and thus reducing or even eliminating some of the problems caused by antibiotic resistance.

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