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Fourier Transform Infrared Spectroscopic Analysis, Phytochemical Constituents and Anti-staphylococcal Efficacies of Aqueous Leaf Extracts of *Baphia nitida* and *Annona muricata*

*Akinjogunla, O.J., Asamudo, N.U., Okon, M.U. and Nya, P. U.
Department of Microbiology, Faculty of Science, University of Uyo, P.M.B. 1017, Uyo, Akwa Ibom State.

ABSTRACT

Phytochemical constituents, Fourier Transform Infrared Spectroscopic (FT-IR) analysis and anti-staphylococcal efficacies of aqueous leaf extracts of *Baphia nitida* (ALEBN) and *Annona muricata* (ALEAM) were determined using chemical method, Fourier Transform Infrared Spectrophotometer and disc diffusion technique, respectively. The total percentage occurrences of *Staphylococcus* spp obtained from the samples in decreasing order were as follows: 100 % (water) > 87.5 % (RTE-food) > 75.0 % (soil) > 62.5 % (urine). A total of ten (10) methicillin resistant (MR) *Staphylococcus* spp, comprising eight (8) MR- *S. aureus* (MRSA) and two (2) MR-coagulase negative *Staphylococcus* spp (MRCoNS) were obtained. The results showed between 75.0 % and 100 % MRSA were sensitive to different concentrations of ALEBN, while ≤ 90 % MR-*Staphylococcus* spp tested were sensitive to ALEAM with the lowest and highest inhibitory zone of 7.8 ± 0.2 mm and 17.7 ± 1.5 mm, respectively. The MIC ($mgml^{-1}$) of ALEBN on MRSA ranged from 6.25 to 50.0, while MIC of ALEBN on MRCoNS ranged from 6.25 to 12.5. The ALEAM showed both bacteriostatic (MBC/MIC > 4) and bacteriocidal (MBC/MIC ≤ 4) effects on the strains of MR-*Staphylococcus* spp. The secondary metabolites detected in varied concentrations in both ALEBN and ALEAM were flavonoids, saponins, alkaloids, tannins, anthraquinones, deoxy sugar, cardiac glycosides and phlobatanins. The FT-IR analysis of ALEBN and ALEAM revealed multiple biologically active functional groups of carboxylic acids, amines, esters, alkenes, ketones, alkanes, aromatics and alcohols with absorption bands ranging 534.3 to 3410.26 cm^{-1}. This study consequently supports the utilization of these plants for therapeutic purposes and equally validates their anti-staphylococcal activities especially on the strains of MR-*Staphylococcus* spp

Key words: Susceptibility, *Baphia nitida*, *Annona muricata*, *Staphylococcus*, FT-IR.

INTRODUCTION

The *Staphylococci* are facultative anaerobes, gram positive bacteria, belonging to the family *Staphylococcaceae* (Akinjogunla *et al.*, 2014). *Staphylococcus* spp are usually classified as pathogenic and non - pathogenic strains based on their capability to synthesis coagulase (Prescott *et al*., 2008). *S. aureus* has been vastly studied owing to its potential pathogenicity against humans and infections caused by methicillin resistant *S. aureus* (MRSA) are of global concern (Wendlandt *et al*., 2013). *Staphylococcus* spp has a remarkable ability of evolving different mechanisms of resistance to antibiotics (Montefiore *et al*., 1989). The emergence of multi-drug resistant microorganisms such as *S. aureus* all over the world and predominantly in developing countries may be attributed to unselective usage of antibiotics for treatment of infectious diseases caused by the organisms (Akinjogunla *et al*., 2014). Most developed countries have reported an increase in infections caused by methicillin resistant coagulase negative *Staphylococcus* spp (MRCoNS) such as *S. epidermidis*, *S. saprophyticus* and S. *lugdunensis* (Longauerova, 2006), whereas there are scanty data on infections caused by MRCoNS in developing countries (Akinjogunla and Enabulele, 2010). Utilization of plants, owing to its availability and affordability, as traditional remedies has occupied a fundamental place in the world's population (Planta and Gundersen, 2000). Scientists and researchers are progressively searching for new, efficient antimicrobial substances, turning their attention to medicinal plants (Akinjogunla *et al*., 2012). Plants contain a diverse range of bio-active secondary metabolites such as alkaloids, flavonoids, cardiac glycoside, tannins and phenolics, thus, purportedly provide excellent leads for new drug developments (Newman *et al*., 2000; Akinjogunla *et al*.., 2012). *Annona muricata* (Soursop), belonging to the family *Annonaceae*, is a naturally occurring plant, widely distributed in India, Central America and some parts of Africa (Chauhan and Mittu, 2015). The leaves, bark and stems of *A. muricata* have been reported to have anti-spasmodic, anti-diarrhoeal, anti-dysenteric, anti-inflammatory, anti-diabetic, anti-tumour, anti-malarial and anti-oxidant activities (Sousa *et al.*, 2004).

A. muricata possess acetogenins and other secondary metabolites, hence, are traditionally used for treatment of arthritis, asthma, hypertension, rheumatism and skin diseases (Adeyemi *et al*., 2008). *Baphia nitida* (camwood) is widely

*Corresponding Author's E-mail / Phone No: **papajyde2000@yahoo.com /** 08064069404

distributed in the tropical Africa, extremely abundant in under wood in the African dense forests and belongs to the family *Fabaceae*, suborder *Caesalpinieae* (Okon *et al*., 2013).

The leaves of *B. nitida* possess a dose dependent analgesic activity, diverse pharmacological properties such as acting against sprain, nosebleed, arthritis, rheumatism and asthma (Ouattara, 2006) and are also effective in the treatment of venereal diseases, dysentery, skin disorders and skin wounds. Fourier Transform Infrared (FT-IR) Spectrophotometer is a rapid, valuable, reliable and sensitive tool for the characterization and identification of chemical functional groups present in compounds or plant samples based on the measurement of vibration of a molecule excited by infrared radiation in the wavelength range of 400 to 4000 cm^{-1} (Maobe and Nyarango, 2013; Ashokkumar and Ramaswamy, 2014). The aim of this study was to determine the anti-staphylococcal activities, phytochemical constituents and chemical functional groups of aqueous leaf extracts of *A. muricata* and *B. nitida.*

MATERIALS AND METHODS

Collection of Samples

A total of thirty two (32) samples comprising mid-stream urine (n=8); soil (n=8), water (n=8) and Ready–to-eat (RTE) food (n=8) were aseptically collected using sterile wide mouth containers. The samples were appropriately labelled after collection and immediately transported to the Microbiology laboratory for bacteriological analysis.

Bacteriological Analysis of Samples

One (1) gram of each food / soil sample was separately added into 9 ml of peptone water and then shaken vigorously to dislodge adhered organisms. One (1) ml of each mid-stream urine (MSU) / water sample was separately added into 9 ml of peptone water. Serial dilutions were made to obtain 10^{-1} to 10^{-5} dilutions. Zero point one (0.1) ml of each serially diluted sample was streaked onto each plate of Mannitol Salt Agar and incubated overnight at 37 °C. After incubation, the yellow colonies (indication of fermentation of mannitol) on the plates were subcultured onto nutrient agar plates and incubated overnight at 37 °C. Pure cultures of isolates were streaked onto nutrient agar slants, incubated overnight at 37 °C and stored in the refrigerator at 4 °C. The isolates were Gram stained, subjected to various biochemical and sugar fermentation tests using standard methods.

Phenotypic Detection of Methicillin Resistant *Staphylococcus* spp

Methicillin resistant (MR) *Staphylococcus* spp isolated from the samples were detected phenotypically using disc diffusion test as described by Clinical and Laboratory Standards Institute (CLSI, 2005). Zero point one (0.1) ml of *Staphylococcus* spp, prepared directly from an overnight agar plate, adjusted to 0.5 McFarland Turbidity Standard, was inoculated onto plate containing Mueller-Hinton Agar (MHA). Commercially available 1 µg Oxacillin and 30 µg Cefoxitin discs (Oxoid, UK) were placed on the plate of MHA and incubated aerobically at 37 ^{o}C for I8 hrs. After incubation, the inhibitory zones (IZ) were measured in millimetres (mm) and interpreted as follows: Cefoxitin (methicillin resistant: IZ $\leq$ 21 mm; methicillin sensitive: IZ $\geq$ 22 mm). Oxacillin (methicillin resistant: IZ $\leq$ 12 mm; methicillin sensitive: IZ $\geq$ 13 mm).

Sources of Medicinal Plants

The leaves of *Baphia nitida* and *Annona muricata* were obtained in Uyo, Akwa Ibom State, and confirmed by a taxonomist in Department of Botany and Ecological Studies. The leaves of *B. nitida* and *A. muricata* were subsequently transferred to Pharmacognosy and Natural Medicine Laboratory, Faculty of Pharmacy, University of Uyo for processing. The leaves of *B. nitida* and *A. muricata* were separately washed with water so as to remove extraneous substances, dried for three weeks, and pulverized using mortar and pestle into fine powder. The powdered leaves of *B. nitida* (3 kg) were soaked in water (1 litre) for 72 hrs with constant shaking at room temperature, filtered using Whatman No 1 filter paper, the filtrate evaporated to dryness using water bath, the dried extract weighed and stored in a refrigerator in screw capped bottle until required for use. The same procedure was also repeated for powdered *A. muricata*. The graded concentrations (12.5, 25 and 50) $mgml^{-1}$ of the extracts were aseptically prepared using 100 ml of Dimethyl sulphoxide (DMSO) and were shaken vigorously to obtain a homogenous mixture.

Phytochemical Screening Of Extracts

The phytochemical constituents of the aqueous leaf extracts *B. nitida* (ALEBN) and *A. muricata* (ALEAM) were analyzed as described by Trease and Evans (1996). Fourier Transform - Infrared Spectroscopic (FT-IR) analysis. Each dried extract powder (10 mg) was mixed with 100 mg potassium bromide and then compressed to prepare translucent sample disc (3 mm diameter)

Plate I: Leaves of *Annona muricata*

Plate II: Leaves of *Baphia nitida*

The disc was kept in the sample holder, the FT-IR analysis was performed using Fourier Transform Infrared Spectrophotometer (Shimadzu FT-IR–8400s, Japan) with a scan range from 400 to 4000 cm^{-1} at the resolution of 4 cm^{-1}. The FT-IR spectral peak values, their functional groups separated based on the peaks ratio were observed and recorded (Rajiv *et al*., 2016).

Antibacterial Activities of ALEBN and ALEAM

The antibacterial activities of ALEBN and ALEAM on MR-*Staphylococcus* spp were determined by disc diffusion method (Akinjogunla and Fatunla, 2017). MR-*Staphylococcus* spp (0.1 mL), prepared directly from an overnight agar plate, adjusted to 0.5 McFarland Turbidity Standard, was inoculated onto plate containing MHA. Each sterile filter paper disc (6 mm) impregnated with graded concentration (12.5, 25 and 50) $mgml^{-1}$ of ALEBN was placed onto MHA plates inoculated with MR-*Staphylococcus* spp and incubated at 37 ^{o}C for 18 hr. The same procedure was repeated for ALEAM as described above. The susceptibilities of the MR-*Staphylococcus* spp to Clindamycin, Erythromycin and DMSO were also determined and used as controls. The experiments were performed in triplicates and the mean inhibitory zone diameter was determined. Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentrations (MBC) of ALEBN and ALEAM on MR- *Staphylococcus* spp ALEBN (10 g) were weighed and dissolved into distilled water (100 mL) to obtain 100 $mgml^{-1}$. The 100 $mgml^{-1}$ of ALEBN was serially diluted to obtain concentrations of 50, 25, 12.5 and 6.25 ($mgml^{-1}$). To 0.1 ml of varying concentrations of ALEBN in test tubes, nutrient broth (9 ml) and a loopful of the MR-*Staphylococcus* spp were added. A tube of nutrient broth inoculated with only test organism serve as control. The same procedure was repeated for ALEAM as described above. All the culture tubes were incubated at 37 ^{o}C for 24 hr and examined for MR- *Staphylococcus* spp growth (turbidity). The MIC was the least concentration that inhibited the growth of the test isolate (Akinjogunla and Fatunla, 2017).

The aliquot (1 ml) from each of MIC broth tubes without visible growth was inoculated onto each of the sterile nutrient agar plates using sterile pipette and streaked. The inoculated plates were incubated at 37 °C for 24 hr. After incubation, the least concentration of the ALEBN that visibly killed the MR-*Staphylococcus* spp was observed. The same procedure was repeated for ALEAM as described above. The MBC was the least concentration that killed the growth of the test bacterial isolate. The ratio of MBC/MIC was used to determine the anti-staphylococcal activites of ALEBN and ALEAM as either bacteriostatic (MBC/MIC > 4) or bacteriocidal (MBC/MIC ≤ 4) (Gatsing *et al*., 2009).

RESULTS

The morphological and biochemical characteristics of *Staphylococcus aureus* and CoN-*Staphylococcus* spp isolated from the samples are presented in Table 1.

A total of twenty six *Staphylococcus* spp comprising twenty *S. aureus* and six CoN-*Staphylococcus* spp were obtained from RTE-food (n=8), water (n=8), urine (n=8) and soil (n=8). The total percentage occurrences of *S. aureus* and CoN-*Staphylococcus* spp in decreasing order were as follows: 100 % (water) > 87.5 % (RTE-food) > 75.0 % (soil) > 62.5 % (urine) (Table 2). Of the 20 *S. aureus* isolates, 8 (40.0%) were methicillin resistant and 12 (60.0%) were methicillin sensitive. The no (%) of methicillin resistant *S. aureus* (MRSA) in the samples were: RTE-food (n=2, 40.0 %), water (n=3, 42.9 %), urine (n=1, 33.3 %) and soil (n=2, 40.0 %). Of the six CoN *Staphylococcus* spp isolated, 2 (33.3 %) were methicillin resistant and 4 (66.7 %) were methicillin sensitive (Table 2). Table 3 shows the phytochemical constituents of ALEBN and ALEAM. The results showed that ALEBN contained flavonoids, alkaloids, saponins, tannins, steroids, cardiac glycosides, anthraquinones, deoxy sugar and phlobatanins, while the eight secondary metabolites detected in ALEAM were flavonoids, saponins, alkaloids, tannins, cardiac glycosides, anthraquinones, deoxy sugar and phlobatanins. Figs I and II illustrate the FT-IR analysis of the ALEBN and ALEAM, absorption bands and wave numbers of the prominent peaks obtained. Eighteen (18)

compounds were found in ALEAM with the absorbance bands ranging from 557.45 to 3392.90 cm^{-1}, while ALEBN had 16 compounds with the absorbance bands ranging from 534.30 to 3410.26 cm^{-1}. The FT-IR analysis revealed multiple biologically active functional groups of carboxylic acids, amines, esters, alkenes, ketones, alkanes, aromatics and alcohols (Figs I and II).

The results of antibacterial activities of ALEBN on MR - *Staphylococcus* spp showed that 6 (75.0 %), 7 (87.5 %) and 8 (100 %) of the MRSA were sensitive to growth inhibition of the ALEBN at 12.5, 25 and 50 ($mgml^{-1}$) concentrations, respectively (Table 4). Among MRSA, the ALEBN had the lowest activity with inhibitory zone (mm ± SD) of 7.7±0.2 as obtained in the plate containing MRSA-09, while the highest activity with inhibitory zone (mm ± SD) of 18.1±1.2 was obtained in the plate containing MRSA-13. All the MRCoNS were sensitive to growth inhibition of the ALEBN at 12.5, 25 and 50 ($mgml^{-1}$) with inhibitory zones ranging from 8.0 ± 0.2 mm as obtained in MRCoNS-01 to 14.5 ± 1.0 mm as obtained in MRCoNS-03 (Table 4). The disc containing 50 $mgml^{-1}$ of ALEAM showed the highest inhibitory zone against MR-*Staphylococcus* spp isolated from the samples, while the disc containing 12.5 $mgml^{-1}$ showed the lowest inhibitory zone, consequently, exhibiting concentration dependent activity. Of the 10 MR-*Staphylococcus* spp tested, between 60 % and 90 % isolates were sensitive to growth inhibition of different concentrations of ALEAM with the lowest and highest inhibitory zone of 7.8 ± 0.2 and 17.7± 1.5 mm, respectively (Table 5). The MIC ($mgml^{-1}$) of ALEBN on MRSA ranged from 6.25 to 50.0, while MIC ($mgml^{-1}$) of ALEBN on MRCoNS ranged from 6.25 to 12.5. The MBC of ALEBN on MRSA-04, MRSA-16 and MRCoNS-01 was 100 $mgml^{-1}$ (Table 6). The MIC and MBC of ALEAM on MRCoNS-01 were the same, indicating its bacteriocidal activity on this isolate, while the MIC and MBC of ALEAM on MRSA-07 and MRSA-16 was 12.5 and 100 ($mgml^{-1}$), respectively (Table 6). The results showed that ALEBN had bacteriostatic effect on MRSA-08 and MRCoNS-01; the ALEAM had bacteriostatic effect on MRSA-07 and MRSA-16, while their effects on other MR-*Staphylococcus* spp were bacteriocidal (MBC/MIC $\leq$ 4).

Table 1: Morphological and Biochemical Characteristics of *Staphylococcus* spp Isolatedfrom the Samples

	Results	
Tests	*Staphylococcus aureus*	CoN- *Staphylococcus* spp
Gram Staining	+ cocci	+ cocci
Catalase	+	+
Citrate	+	+
Oxidase	-	-
Coagulase	+	-
Indole	-	-
Urease	+	+
Glucose	+	+
Lactose	+	+
Sucrose	+	+
Mannitol	+	+
Motility	-	-
Methyl red	+	+
Vogues Proskauer	-	-

Keys: +: Positive; -: Negative; CoN: Coagulase negative

Table 2: Occurrence of Methicillin Resistant and Methicillin Sensitive *Staphylococcus* spp in the Samples

Food Samples	No (%) of	No (%) of Occurrence		No (%). of CoN	No (%) of Occurrence	
	S. aureus	MRSA	MSSA	*Staphylococcus* spp	MRCoNS	MSCoNS
RTE-Food (n=8)	5 (62.5)	2 (40.0)	3 (60.0)	2 (25.0)	1 (50.0)	1 (50.0)
water (n=8)	7 (87.5)	3 (42.9)	4 (57.1)	1 (12.5)	0 (0.0)	1 (100)
Urine (n=8)	3 (37.5)	1 (33.3)	2 (66.7)	2 (25.0)	1 (50.0)	1 (50.0)
Soil (n=8)	5 (62.5)	2 (40.0)	3 (60.0)	1 (12.5)	0 (0.0)	1 (100)
Total (32)	20 (62.5)	8 (40.0)	12 (60.0)	6 (18.8)	2 (33.3)	4 (66.7)

Keys: CoN: Coagulase negative; MRSA: Methicillin Resistant *S. aureus*; MSSA: Methicillin Sensitive *S. aureus*; MRCoNS: Methicillin Resistant coagulase negative *Staphylococcus* spp; MSCoNS: Methicillin Sensitive coagulase negative *Staphylococcus* spp; Values in parenthesis indicated percentages

Table 3: Phytochemical Constituents of Aqueous Leaf Extracts of *Baphia nitida* and *Annona muricata*

Plant Extracts	Bio-Active Constituents	Occcurence
B. nitida	Flavonoids	+
	Saponins	+
	Alkaloids	+
	Tannins	+
	Cardiac glycosides	+
	Steroids	+
	Anthraquinones	+
	Deoxy sugar	+
	Phlobatanins	+
A. muricata	Flavonoids	+
	Saponins	+
	Alkaloids	+
	Tannins	+
	Cardiac glycosides	+
	Steroids	-
	Anthraquinones	+
	Deoxy sugar	+
	Phlobatanins	+

Keys: +: Present ; - : Not detected

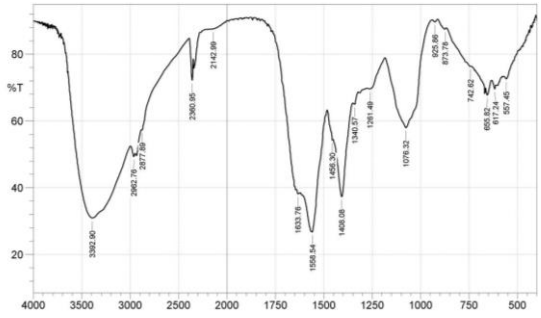

Fig 1: FT-IR Spectra of Aqueous Leaf Extracts of A. muricata

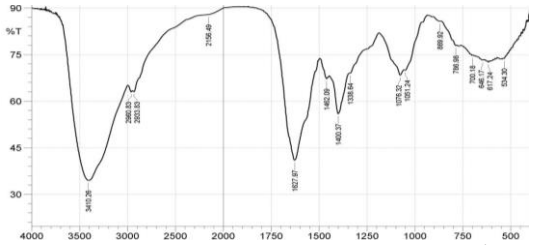

Fig I1: FT-IR Spectra of Aqueous Leaf Extracts of *B. nitida*

Table 4: Antibacterial Activity of Aqueous Leaf Extracts of *Baphia nitida* on Methilicent Resistan *Staphylococcus* spp

Bacterial Isolates	Isolate Code	Zone of Inhibition (mm ± S.D) 12.5 mgml^{-1}	25 mgml^{-1}	50 mgml^{-1}	Erythromycin mm ± S.D	Clindamycin mm ± S.D	DMSO mm ± S.D
S. aureus	MRSA-01	11.1 ± 0.4^{a}	13.9 ± 0.5^{b}	16.2 ± 0.5^{c}	16.7 ± 1.2^{c}	17.0 ± 0.5^{c}	NZ
S. aureus	MRSA-04	NZ	8.4 ± 0.2^{a}	11.3 ± 0.3^{a}	NZ	NZ	NZ
S. aureus	MRSA-07	9.5 ± 0.1^{a}	11.1 ± 0.5^{a}	14.4± 1.0^{b}	17.1 ± 1.1^{c}	19.5 ± 1.0^{c}	NZ
S. aureus	MRSA-09	7.7 ± 0.2^{a}	9.4 ± 0.1^{a}	12.6 ± 0.5^{b}	13.5 ± 0.5^{b}	15.1 ± 0.2^{b}	NZ
S. aureus	MRSA-13	11.0 ± 0.2^{a}	15.9 ± 0.5^{b}	18.1± 1.2^{c}	NZ	20.5 ± 1.5^{c}	NZ
S. aureus	MRSA-16	NZ	NZ	9.6 ± 0.3^{a}	15.5 ± 0.5^{b}	16.9 ± 1.1^{c}	NZ
S. aureus	MRSA-17	10.5 ± 0.5^{a}	14.4 ± 0.5^{b}	17.7 ± 1.1^{c}	14.7 ± 1.0^{b}	18.7 ± 0.5^{c}	NZ
S. aureus	MRSA-21	9.9 ± 0.1^{a}	13.1 ± 0.7^{b}	16.5 ± 1.0^{c}	13.3 ± 0.3^{b}	14.1 ± 0.7^{b}	NZ
CoN *Staphylococcus* spp	MRCoNS-01	8.0 ± 0.2^{a}	8.7 ± 0.1^{a}	9.4 ± 0.2^{a}	NZ	11.8 ± 0.5^{a}	NZ
CoN *Staphylococcus* spp	MRCoNS-03	11.3 ± 0.5^{a}	12.9 ± 0.5^{b}	14.5 ± 1.0^{b}	15.9 ± 1.0^{b}	17.8 ± 0.5^{c}	NZ

Keys: CoN: Coagulase negative; NZ: No Inhibitory Zone; mm: mean; S.D: Standard Deviation; DMSO: Dimethyl Sulphoxide; Mean within the column followed by the different superscript letters are significant as determined by Duncan's multiple range test ($P < 0.05$).

Table 5: Antibacterial Activity of Aqueous Leaf Extracts of *Annona muricata* on Methicillin Resistant *Staphylococcus* spp

Bacterial Isolates	Isolate Code	Zone of Inhibition (mm ± S.D) 12.5 mgml^{-1}	25 mgml^{-1}	50 mgml^{-1}	Erythromycin mm ± S.D	Clindamycin mm ± S.D	DMSO mm ± S.D
S. aureus	MRSA-01	9.4 ± 0.2^{a}	12.2 ± 0.5^{b}	15.0 ± 1.0^{b}	16.7 ± 1.2^{c}	17.0 ± 0.5^{c}	NZ
S. aureus	MRSA-04	NZ	NZ	8.9 ± 0.1^{a}	NZ	NZ	NZ
S. aureus	MRSA-07	8.8 ± 0.1^{a}	9.7 ± 0.2^{a}	12.0± 0.5^{b}	17.1 ± 1.1^{c}	19.5 ± 1.0^{c}	NZ
S. aureus	MRSA-09	NZ	10.5 ± 0.3^{a}	14.7± 1.0^{b}	13.5 ± 0.5^{b}	15.1 ± 0.2^{b}	NZ
S. aureus	MRSA-13	11.5 ± 0.5^{a}	15.3 ± 0.8^{b}	17.7± 1.5^{c}	NZ	20.5 ± 1.5^{c}	NZ
S. aureus	MRSA-16	8.4 ± 0.1^{a}	10.5 ± 0.2^{a}	11.3 ± 0.2^{a}	15.5 ± 0.5^{b}	16.9 ± 1.1^{c}	NZ
S. aureus	MRSA-17	NZ	9.1 ± 0.1^{a}	9.8 ± 0.5^{a}	14.7 ± 1.0^{b}	18.7 ± 0.5^{c}	NZ
S. aureus	MRSA-21	7.8 ± 0.2^{a}	10.6 ± 0.2^{a}	14.2 ± 1.0^{b}	13.3 ± 0.3^{b}	14.1 ± 0.7^{b}	NZ
CoN *Staphylococcus* spp	MRCoNS-01	NZ	NZ	NZ	NZ	11.8 ± 0.5^{a}	NZ
CoN *Staphylococcus* spp	MRCoNS-03	10.8 ± 0.4^{a}	14.3 ± 0.5^{b}	17.2 ± 1.2^{c}	15.9 ± 1.0^{b}	17.8 ± 0.5^{c}	NZ

Keys: CoN: Coagulase negative; NZ: No Inhibitory Zone; mm: mean; S.D: Standard Deviation; DMSO: Dimethyl Sulphoxide; Meanwithin the column followed by the different superscript letters are significant as determined by Duncan's multiple range test ($P < 0.05$).

Table 6: Minimum Inhibitory Concentration and Minimum Bacteriocidal Concentration of *B. nitida* and *A. muricata* on Methicillin Resistant *Staphylococcus* spp

		ALEBN			ALEAM		
		MIC	MBC		MIC	MBC	
Bacterial Isolates	Isolate Codes	mgml^{-1}	mgml^{-1}	MBC / MIC	mgml^{-1}	mgml^{-1}	MBC / MIC
S. aureus	MRSA-01	6.25	50.0	8	12.5	50.0	4
S. aureus	MRSA-04	25.0	100	4	50.0	100	2
S. aureus	MRSA-07	12.5	50.0	4	12.5	100	8
S. aureus	MRSA-09	12.5	50.0	4	25.0	50.0	2
S. aureus	MRSA-13	6.25	12.5	2	6.25	25.0	4
S. aureus	MRSA-16	50.0	100	2	12.5	100	8
S. aureus	MRSA-17	6.25	25.0	4	25.0	100	4
S. aureus	MRSA-21	12.5	12.5	1	12.5	50.0	4
CoN *Staphylococcus* spp	MRCoNS-01	12.5	100	8	100	100	1
CoN *Staphylococcus* spp	MRCoNS-03	6.25	12.5	2	6.25	25.0	4

Keys: CoN: Coagulase negative; ALEBN: Aqueous Leaf Extracts of *B. nitida*; ALEAM: Aqueous Leaf Extracts of *A. muricata* IC: Minimum Inhibitory Concentration; MBC: Minimum Bacteriocidal Concentration.

DISCUSSION

The increasing occurrence and difficulties encountered in treating infections caused by antibiotic resistant bacterial strains such as methicillin-resistant *Staphylococcus* spp have necessitated investigations on natural plant resources as alternative therapeutic agents.

In this study, MR-*Staphylococcus* spp obtained from the samples were sensitive to growth inhibition of different concentrations of ALEBN and ALEAM as evinced by the varied zones of inhibition obtained, consequently, ALEBN and ALEAM possess promising antibacterial properties especially against MR-*Staphylococcus* spp. The antibacterial activities of ALEAM on MR-*Staphylococcus* spp in this study corroborate the previous reports of Pathak *et al.* (2010) and Gajalakshmi *et al.* (2012) who in their respective investigations reported the growth inhibition of *Staphylococcus* spp by the aqueous leaf extracts of *A. muricata.* The anti-staphylococcal activity of aqueous leaf extracts of plants have been shown by many scientists and this study confirmed their previous reports as ALEBN tested against *Staphylococcus* spp in our study obviously possessed anti-staphylococcal capability. The result of the antibacterial activity of *B. nitida* is in conformity with the reports of Agwa *et al.* (2012) recorded that *S. aureus* were susceptible to aqueous leaf extracts of *B. nitida* leaf with inhibitory zone diameter of 14 mm and MIC of 75 mgml^{-1}. The disc containing 50 mgml^{-1} of ALEBN and ALEAM showed the widest inhibitory zones against the MR-*Staphylococcus* spp, while the disc containing 12.5 mgml^{-1} of ALEBN and ALEAM showed the lowest inhibitory zones; therefore, exhibiting concentration dependent activity.

The preliminary qualitative phytochemical analysis of the ALEBN and ALEAM in this study revealed the presence of tannins, phlobatannins, saponins, cardiac glycosides, alkaloids, deoxy-sugar, anthraquinones and flavonoids in varied amounts. The occurrence of alkaloids, tannins and flavonoids in ALEBN in this study is in agreement with the reports of Okon *et al.* (2013) who obtained these secondary metabolites in the ALEBN. The presence of flavonoids and alkaloids in ALEBN and ALEAM showed that these secondary metabolites may be responsible for the antibacterial activity especially on MR-*Staphylococcus* spp (Olowosulu and Ibrahim, 2006). The medicinal value of herbal remedies lies in those chemical substances such as alkaloids, flavonoids and tannins that produce definite physiological actions on the human body (Akande and Ajao, 2011). Flavonoids have the ability to complex with extracellular, soluble proteins and lipoflavonoids in disrupting the microbial cell membrane and exert antimicrobial property (Tsuchiya *et al.*, 1996). Tannins inactivate microbial adhesion, enzymes and cell envelope transport protein to form complex with polysaccharides (Oana, 2009). Studies have also shown that alkaloids have powerful effects on phagocytosis and it increases T- helper cells (Cruz and Jubilo, 2014). FT-IR is a reliable and sensitive technique, employed for detection of bio-molecular composition (Kumar and Prasad, 2011). The FT-IR analysis of ALEAM and ALEBN revealed multiple biological active functional group components of carboxylic acids, amines, esters, alkenes, ketones, alkanes, aromatics and alcohols with absorption bands ranging from 534.5 to 3410.26 cm^{-1}. The detection of functional group components of amines, esters, carboxylic acid and aromatics in the ALEAM and ALEBN using FTIR spectroscopic method in this study is similar to that of Gaurav *et al.* (2010) and Ragavendran *et al.* (2011).

CONCLUSION

The susceptibility of MR-*Staphylococcus* spp isolated from the samples to the ALEAM and ALEBN at low MIC and MBC indicates that the plants could be useful for the development of new

antimicrobial drugs. This equally supports the ethno-medicinal usage of these plants and validates their anti-staphylococcal activities especially on strains of MR-*Staphylococcus* spp.

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