

*Full Length Research Paper*

## **Bacteriostatic and bactericidal effects of ethyl acetate root bark extract of *Terminalia avicennioides* on methicillin-resistant *Staphylococcus aureus***

**Adim Ekene Ugochukwu<sup>1</sup>, Dingwoke Emeka John<sup>1\*</sup>, Adamude Fatima Amin<sup>2</sup>, Edenta Chidi<sup>3</sup>, Nwobodo Ndubuisi Nwobodo<sup>4</sup>, Offiah Raymond Ogbonna<sup>5</sup>, Onyeka Ifeanyi Peter<sup>6</sup> and Ezeaku Ikenna Nnamdi<sup>1</sup>**

<sup>1</sup>Department of Biochemistry, Ahmadu Bello University, Main Campus, P. M. B. 1045, Samaru, Zaria, Kaduna State, Nigeria.

<sup>2</sup>Department of Biochemistry, Federal University, Lafia, Nasarawa State, Nigeria.

<sup>3</sup>Department of Biochemistry, Renaissance University, Ugbawka, Enugu State, Nigeria.

<sup>4</sup>Department of Pharmacology and Therapeutics, Division of Clinical Pharmacology and Translational Medicine, College of Medicine, Enugu State University of Science and Technology, Enugu State, Nigeria.

<sup>5</sup>Department of Pharmacology and Therapeutics, College of Medicine, Enugu State University of Science and Technology, Enugu State, Nigeria.

<sup>6</sup>Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

Received 9 December, 2017; Accepted 19 February, 2018

Root bark extract of *Terminalia avicennioides* was obtained by cool maceration with 750 ml n-hexane, chloroform, ethyl acetate and methanol, independently for 48 h using soxhlet extractor. ATCC 33591 standard strains of methicillin-resistant *Staphylococcus aureus* (MRSA) was used. Strain resistance to methicillin/oxacillin antibiotic was verified by a retest of its sensitivity to oxacillin antibiotic. The antimicrobial test and zone of inhibition were determined using the agar well diffusion method. The isolated bioactive fractions of the extract were subjected to FTIR and GC-MS analysis. Results revealed both fractions (TLb4 and TLb17) were both bacteriostatic and bactericidal. The growth of MRSA was inhibited at extract concentrations of 60, 120, 180 and 240 µg/ml, within the susceptible range of ≥ 14 mm, with a mean inhibitory zone sensitivity of 14 mm at 60 µg/ml, 15.76 mm at 120 µg/ml and 15.33 mm at 180 µg/ml for fraction TLb4 and 15.33 mm at 60 µg/ml, 17.33 mm at 120 µg/ml and 20 mm at 180 µg/ml for TLb17 (≥ 14 mm). GC-MS detected oleic acid and analogs of palmitic acid as pharmacological active compounds of both fractions. FTIR showed the presence of alkyl halides. These bioactive agents revealed could be effective therapeutic agents for the treatment of MRSA.

**Key words:** Methicillin-resistant, *Staphylococcus aureus*, inhibition, bacteriostatic, bactericidal, *Terminalia avicennioides*.

### **INTRODUCTION**

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are prevalent bacterial pathogen that causes both health care and community-associated infections, hence

the name, community-associated strains of methicillin-resistant *Staphylococcus aureus* (CA-MRSA). Over the years, CA-MRSA have emerged and spread rapidly, both



**Plate 1.** *T. avicennioides* plant in its natural habitat in Samaru, Sabon-Gari L.G.A., Kaduna State, Nigeria.

in the community and in the hospital (Chambers and DeLeo, 2009). Increasing resistance to the commonly prescribed antibiotics such as methicillin, resulted in antibiotic-resistant strains of *S. aureus* which is prevalent in Nigeria (Ajibade and Akinruli, 2016; Ajoke et al., 2012). Initially, MRSA strains have been suffered by patients with chronic illnesses, until the emergence of CA-MRSA strains in the 1990s. The CA-MRSA strains became epidemic, causing skin and soft tissue infections (SSTIs), including some with severe consequences, such as community-acquired pneumonia (Francis et al., 2005), pyomyositis (Burdette et al., 2012), sepsis, osteomyelitis (Kechrid et al., 2011) and necrotizing fasciitis (Changchien et al., 2011). The methicillin-resistance is mediated by a penicillin binding protein (PBP-2a) encoded by *mecA* gene which aids the successful life cycle of the organism, and renders methicillin and other beta-lactam antibiotics ineffective. Plant-based system continues to play an essential role in healthcare, especially in Africa (Tagboto and Townson, 2001). A large variety of secondary metabolites are produced by medicinal plants; they are either used as precursors or lead compounds in the pharmaceutical industry. *Terminalia avicennioides* (Plate 1) is a yellowish brown, hard and durable plant. It belongs to the family *combrataceae*, genus *Terminalia*, and species *avicennioides*. In Nigeria, it is called Baúsheè in Hausa, Idi in Yoruba and Edo in Igbo. The roots, used as chewing sticks have been shown to cure dental caries and skin infections (Gill and Akinwunmi, 1986). Previous study showed that the bark extract of *T. avicennioides* exhibited both vibriocidal and typhoidal activities

(Akinyemi et al., 2005). Traditionally, in different localities in the northern part of Nigeria, the root bark extract of *T. avicennioides* is drunk and bathed with, and in some cases, are mixed with local creams and used for the treatment of skin infections. Pharmacologically, crude methanolic extract of the *T. avicennioides* root bark exhibited broad growth inhibition against microbes causing infectious diseases (Mann et al., 2007, 2009). Based on the foregoing, there is need to explore alternative means of inhibiting MRSA strain. Therefore, this research presents novel inhibition model against MRSA using root bark extracts of indigenous *T. avicennioides*.

## MATERIALS AND METHODS

### Plant material and preparation of extracts

*T. avicennioides* root bark was collected from its natural habitat in Samaru, Sabon-Gari Local Government Area, Kaduna State, Nigeria. They were identified and authenticated at the Herbarium (voucher number 901452), Department of Biological Science, Ahmadu Bello University, Zaria. It was washed and dried at room temperature for 14 day, then pulverized using pestle and mortar, sieved and stored in polythene bag until needed. Three hundred grams (300 g) of the powdered *T. avicennioides* root bark were extracted by cool maceration in triplicates with 750 ml n-hexane, chloroform, ethyl acetate and methanol, independently for 48 h using soxhlet extractor. The extracts were filtered using Whatman filter paper No.1 and left at open air to dry at room temperature. The percentage yield of each extract was determined by the formula:

$$\text{Percentage yield} = \frac{\text{Mass yield of extract}}{\text{Mass of plant material}} \times \frac{100}{1}$$

### Chemicals and reagents

All chemicals and reagents were of analytical grade. All determinations were done in triplicates.

### Bacterial isolate

ATCC 33591 standard strain of MRSA was purchased in USA. Resistance of this strain to methicillin/oxacillin antibiotic was verified by a retest of its sensitivity to oxacillin antibiotic (Oxoid, UK) and interpreted, following the performance standard for antimicrobial susceptibility testing as described in the Clinical and Laboratory Standard Institute.

### Antimicrobial activity

The antimicrobial test was performed using the agar well diffusion

\*Corresponding author. E-mail: dinhimself@yahoo.com. Tel: +2347030665559.

method (Nair et al., 2005). A 0.1 ml standardized inoculum ( $1.5 \times 10^8$  cfu/ml 0.5 McFarland standard) of a 24 h culture was inoculated using a sterile cotton swab on the surface of sterile Muller Hinton agar which was earlier prepared and allowed to solidify. The plates were left on the bench for 1 h for the inocula to diffuse into the agar. Wells of 5 mm were made on the agar using a sterile cork borer. The cut agar disks were carefully removed by the use of forceps sterilized in flame. The extracts were dissolved in dimethylsulfoxide (DMSO), and a 0.5 ml of each extract was introduced into each well, in the following concentrations: 60, 120, 180 and 240  $\mu\text{g/ml}$ . Wells containing DMSO were negative controls while standard antibacterial drug (Augmentin) was used as positive control. The plates were inoculated at  $37^\circ\text{C}$  for 24 h. The zones of inhibition (clearance) were measured in (mm) from the edge of the plate to the point where the growth of the organism started after incubation and compared with Kirby Bauer standard chart.

Ethyl acetate extract revealed activity and was further used for this study. To narrow down the search for the particular active agent(s) in the ethyl acetate extract, it was subjected to thin layer chromatography (TLC).

#### Purification of the ethyl acetate extract

The ethyl acetate extract was purified by preparative thin layer chromatography (PTLC) using pre-coated TLC plates. A capillary tube was dipped into the extract and lightly applied on the thin layer plate ( $1'' \times 3''$ ) to form a spot of about 1 mm, then continuously applied, forming a streak on the plate. Ascending TLC was done by placing the plate vertically inside the chromatographic tank containing chloroform, ethyl acetate, methanol and water as the solvent system (15:8:4:1). Afterward, the TLC plates were viewed under UV light for detection of the separated compounds which appeared as bands. The bands were scraped off from the TLC plate and suspended in ethyl acetate and centrifuged at 1500 g for 10 min. The supernatants were concentrated to dryness at room temperature and reconstituted with DMSO. Each fraction obtained was tested for inhibitory activity against the bacterial isolate as described by Nair et al. (2005). The active fraction was subjected to GC-MS analysis and FTIR for functional group determination.

#### Qualitative phytochemical screening

Phytochemical screening of the distinct bioactive fraction obtained from TLC was carried out (Harborne, 1998; Trease and Evans, 1989).

#### Determination of minimum inhibitory concentration

The broth dilution method was used to determine the MIC (Leboffe and Pierce, 1999). Three times serial dilution was done as follows: 9 ml of Muller Hinton broth was dispensed into each of the test tubes, and then 1 ml of 60  $\mu\text{g/ml}$  of ethyl acetate fraction was added and mixed properly. From this first test tube, 1 ml of the mixture was taken and dispensed into the second test tube containing only 9 ml of the broth and mixed properly, then 1 ml was as well taken from this second tube into the third tube also having 9 ml of broth, then 1 ml was finally taken out of this third tube and discarded. Finally, 0.01 ml of standardized test organism of bacterial cells which has been streaked overnight on a Petri dish was added to each of the three test tubes. Controls were equally set up using broth and test organisms only and the next control was the broth only. The test tubes were incubated aerobically for 24 h at  $37^\circ\text{C}$ . The plates were then observed for the presence or absence of growth. The concentration that completely inhibits macroscopic growth was regarded as the MIC of the extract.

#### Determination of minimum bacterial concentration

A sample of the tubes used in MIC determination that did not show any visible growth after the incubation was streaked on Muller Hinton agar plates. The lowest concentration of the extract indicating a bactericidal effect after 24 h of aerobic incubation at  $37^\circ\text{C}$  was regarded as the minimum bactericidal concentration (MBC).

#### Fourier transformed infra-red (FTIR) spectroscopy analysis

The FTIR analysis of the TLC fraction with the highest MRSA inhibitory property was carried out to determine functional group(s) present using FTIR-8400S (Shimadzu model spectrophotometer) at the National Research Institute for Chemical Technology (NARICT) Laboratory, Zaria.

#### Identification of bioactive fraction by gas chromatography-mass spectroscopy

The active TLC bands of the ethyl acetate fraction of *T. avicennioides* root-bark were dissolved in DMSO and used for GC-MS analysis. Aliquots of each sample were injected into the sample inlet. The machine was programmed with syringe injection positioned at  $250^\circ\text{C}$  and 108.0 and at 80% column oven.

#### Statistical analysis

The results are presented as mean  $\pm$  standard deviation. Differences between the mean values of the zone of inhibition were compared with the standard using one way ANOVA. A post hoc multiple test was used to compare the level of significance. P value  $> 0.05$  was considered statistically not significant while P value  $< 0.05$  was considered statistically significant.

## RESULTS

#### Yield of successive extraction of *T. avicennioides* root bark

The yield of successive extraction of *T. avicennioides* root bark extract is presented in Table 1. Methanol gave the highest yield at 13.01% value, followed by chloroform 1.43%, n-hexane gave 0.73% and ethyl acetate 0.37%.

#### Mean zone of inhibition of different concentrations of the root bark extracts of *T. avicennioides* on methicillin-resistant *S. aureus*

The mean zone of inhibition is shown in Table 2. The values down the column bearing the same alphabetical superscript were not significantly different ( $P > 0.05$ ). Thus, the inhibitory activities observed in the extracts at all concentrations were significantly lower ( $P < 0.05$ ) as compared to the standard drug. Similarly, at 180 and 240  $\mu\text{g/ml}$ , activities were equally significantly lower ( $P < 0.05$ ) in the extracts than it was in the standard drug. However, at 180  $\mu\text{g/ml}$ , activity in chloroform was significantly

**Table 1.** The yield of successive extraction of *T. avicennioides* root bark.

Solvent	Yield (g)	Yield (%)
n-Hexane	2.18	0.73
Chloroform	4.29	1.43
Ethyl acetate	1.10	0.37
Methanol	39.01	13.01

**Table 2.** Zone of inhibition of different concentrations of root bark extracts of *T. avicennioides* on MRSA.

Solvent extracts	60 µg/ml (Mean ± SD)	120 µg/ml (Mean ± SD)	180 µg/ml (Mean ± SD)	240 µg/ml (Mean ± SD)
Methanol	18.50 ± 0.71 <sup>x</sup>	19.00 ± 0.00 <sup>xy</sup>	19.50 ± 2.12 <sup>x</sup>	15.50 ± 0.71 <sup>z</sup>
Chloroform	14.33 ± 1.15 <sup>y</sup>	17.00 ± 0.00 <sup>y</sup>	15.00 ± 1.00 <sup>y</sup>	14.50 ± 0.71 <sup>z</sup>
Ethyl acetate	19.50 ± 0.71 <sup>x</sup>	19.33 ± 1.15 <sup>x</sup>	18.00 ± 2.00 <sup>xy</sup>	19.67 ± 0.58 <sup>x</sup>
n-Hexane	14.50 ± 0.71 <sup>y</sup>	14.50 ± 0.71 <sup>z</sup>	17.33 ± 1.53 <sup>xy</sup>	17.50 ± 0.71 <sup>y</sup>
Std. drug (Augmentin)	26.67 ± 1.15 <sup>w</sup>	26.67 ± 1.15 <sup>w</sup>	26.67 ± 1.15 <sup>w</sup>	26.67 ± 1.15 <sup>w</sup>

Values are represented as mean zone of inhibition ± standard deviation (SD) and in millimetre (mm) unit zone of inhibition. Values bearing the same superscript down column are not significantly different in millimetre (mm) unit zone of inhibition. "w" is values with significant difference from values with x. "x" is values with significant difference from values with y. "y" is values with significant difference from values with z. Superscript "xy" is values not significantly different from other values with xy, "z" is the least significant difference of all values with superscripts.

higher ( $P < 0.05$ ) as compared to what was observed in ethyl acetate extract but not significantly different ( $P > 0.05$ ) from the inhibitory activities recorded in other extracts of the same concentration. Activities of chloroform extract at 240 µg/ml were significantly higher ( $P < 0.05$ ) than that of other extracts of the same concentration. At 60 µg/ml of the extracts, the inhibitory activities did not differ significantly ( $P > 0.05$ ) from chloroform and n-hexane extracts which both showed lower activities as compared to what was observed in methanol and ethyl acetate extracts which also did not show a significant difference ( $P > 0.05$ ) in activities. At 120 µg/ml, activity was significantly lower ( $P < 0.05$ ) in n-hexane extract than what was observed in other extracts, where it appeared activity was higher in ethyl acetate extract as compared to the activities in both methanol and chloroform extracts but only differ significantly ( $P < 0.05$ ) from the latter. At 180 µg/ml, the highest inhibitory activity was observed in methanol extract as compared to others among which only chloroform extract showed a significant difference ( $P < 0.05$ ). At 240 µg/ml, while there was no significant difference ( $P > 0.05$ ) observed between the inhibitory activity in methanol and chloroform extract, they both showed a significantly lower activity than what was observed in n-hexane and ethyl acetate extracts in which the former showed a significantly lower activity. Mean values in Roman numeral across the row at different concentrations describes a mean zone of inhibition of different concentrations of the same extract. Roman numeral i is significantly higher than ii ( $i > ii$ ). The

values for each extract at different concentrations bearing the same superscript across the row are not significantly different ( $P > 0.05$ ).

#### Phytochemicals present in the most bioactive extract of *T. avicennioides*

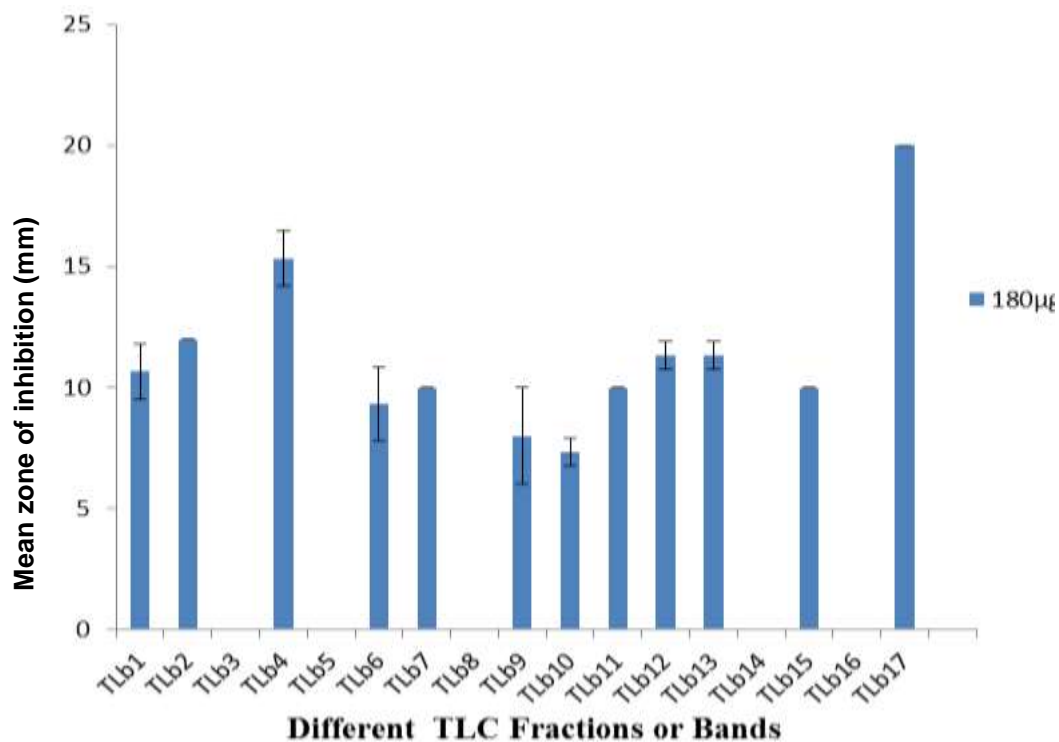
The phytochemical revealed the presence of triterpens, alkaloids, tannin, cardiac glycoside, terpenoid and anthraquinon. Quantitatively, 42 µg of alkaloids, 184.25 µg of tannins and 19.80 µg of anthraquinone were estimated in 1 mg of the extract.

#### Zone of inhibition of different TLC fractions of root bark bioactive ethyl acetate extract of *T. avicennioides*

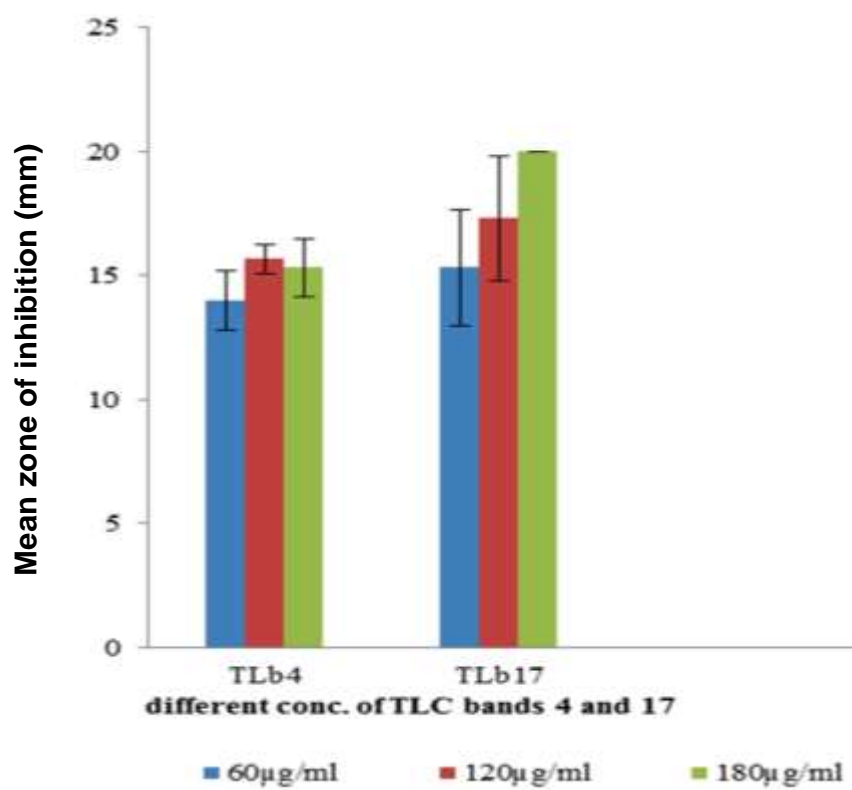
The mean zone of inhibition in millimeter units at concentration of 180 µg/ml is shown in Figure 1. TLb4 and TLb17 being  $\geq 14$  mm indicate sensitivity of MRSA within the Kirby-Bauer standard for (methicillin) antibiotic susceptibility to the compounds in the bands.

#### Mean zone of inhibition of graded concentrations of TLC bands 4 and 17 of root bark bioactive ethyl acetate extract of *T. avicennioides*

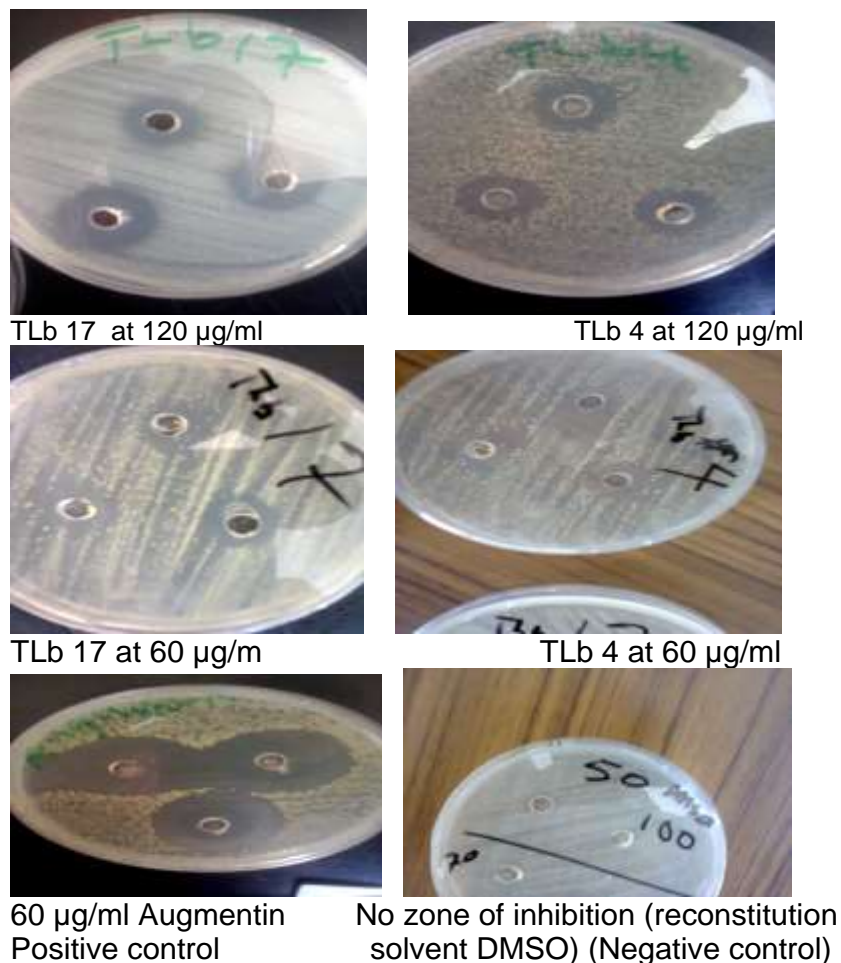
The mean zone of inhibition in millimeter is shown in



**Figure 1.** Mean zone of inhibition in (mm) of different TLC fractions of bioactive root bark ethyl acetate extract of *T. avicennioides* at 180 µg/ml on MRSA.



**Figure 2.** Mean zone of inhibition of TLC bands 4 and 17 of ethyl acetate root bark extract of *T. avicennioides* at different concentrations.



**Figure 3.** Zone of inhibition of TLb 4 and TLb 17 at different concentrations: 60 µg/ml Augmentin (Negative control) and DMSO (positive control) on MRSA.

Figure 2. The sensitivity plates are shown in Figure 3.

**Minimum inhibitory concentration and minimum bactericidal concentration of the partially purified compound: TLC bands 4 and 17 against MRSA**

The minimum concentration of the partially purified compound at which it was inhibitory to MRSA is 0.6 µg/ml for both TLC bands 4 and 17. It was as well bactericidal at the same concentration.

**Gas chromatography-mass spectroscopy analysis of TLb4 and TLb17 fractions of root bark bioactive ethyl acetate extract of *T. avicennioides***

The gas chromatography-mass spectroscopy analysis is presented in Tables 3 and 4, respectively while the chromatograph is presented in Figure 4. Seven compounds were present in TLb4 while six compounds




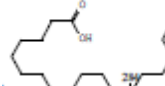
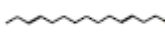
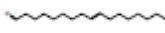
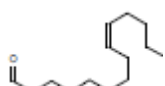
were present in TLb17. These compounds are analogs of palmitic acid (n-hexadecanoic with molecular weight of 256, percentage abundance of 3.95 and 1.52), methyl ester (11-octadecenoic acid with molecular weight of 296, percentage abundance of 2.90 and 1.82), stearic acid (octadecanoic acid with molecular weight of 284, percentage abundance of 4.07 and 2.97) and oleic acid with molecular weight of 282, percentage abundance of 22.10 and 13.79, for both TLb4 and TLb17 fractions respectively.

**FTIR analysis for TLb4 and TLb17 fraction of ethyl acetate extract of root barks of *T. avicennioides***

FTIR analysis is presented in Figures 4 and 5. The functional groups detected includes alkyl halides: R-Br, R-F, methyne: (CH-), nitro compounds: R-NO<sub>2</sub>, amides: R-C(O)-NH<sub>2</sub>, R-C(O)-NH-R., isothiocyanate: (-NCS), nitril: C≡N, carboxylic acids, saturated aliphatic (alkyl): methylene C-H, alkanes, in both TLb4 and TLb17, while

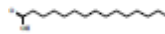


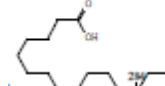
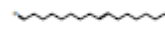
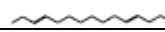


**Table 3.** Gas chromatography mass spectroscopy analysis of fraction TLb4 of bioactive ethyl acetate root barks extract of *T. avicennioides*.

PN	SI	PN	Structure	MW	MF	RT	PA	A (%)
1	93	n-Hexadecanoic acid		256	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	18.546	9986682	3.95
2	92	11-Octadecenoic acid		295	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	20.205	7339337	2.90
3	94	Oleic Acid		282	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	21.350	55865451	22.10
4	90	Octadecanoic acid		284	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	21.537	10294647	4.07
5	91	3,11-Tetradecadien-1-ol		210	C <sub>14</sub> H <sub>26</sub> O	24.153	7787536	3.08
6	91	9-Octadecenal		266	C <sub>18</sub> H <sub>34</sub> O	24.589	11094234	4.39
7	90	(9Z)-9-Teradecenal		210	C <sub>14</sub> H <sub>26</sub> O	26.550	3722523	1.47

PN= Peak number, SI = Stable ion, PN = Peak name, MW = Molecular weight, MF = Molecular formula, RT = Retention time, PA = Peak area, A = Abundance.

**Table 4.** Gas Chromatography mass spectroscopy analysis of fraction TLb17 of bioactive ethyl acetate root barks extract of *T. avicennioides*.

PN	SI	PN	Structure	MW	MF	RT	PA	A (%)
1	85	n-Hexadecanoic acid		256	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	8.523	2746442	1.52
2	92	11-Octadecenoic acid		296	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	20.207	3282366	1.82
3	94	Oleic Acid		282	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	21.296	24859094	13.79
4	90	Octadecanoic acid		284	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	21.501	5347465	2.97
5	91	9-Octadecenal		266	C <sub>18</sub> H <sub>34</sub> O	24.587	6723795	3.73
6	92	3,11-Tetradecadien-1-l		210	C <sub>14</sub> H <sub>26</sub> O	26.550	1457472	0.81

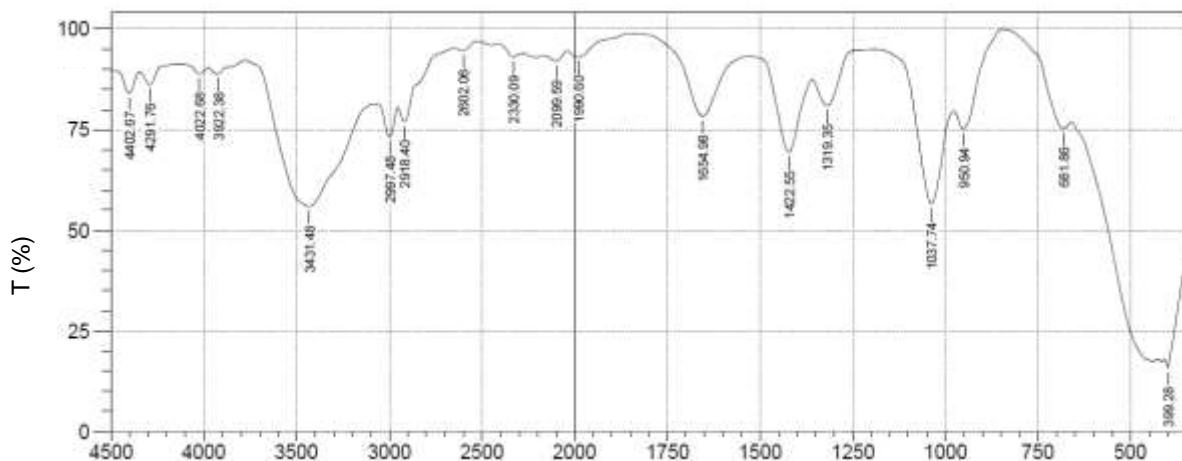
PN= Peak number, SI = Stable ion, PN = Peak name, MW = Molecular weight, MF = Molecular formula, RT = Retention time, PA = Peak area, A = Abundance.

alkyl halide: R-I, allenes and amines: R-NH<sub>2</sub> were found in addition to TLb17 only.

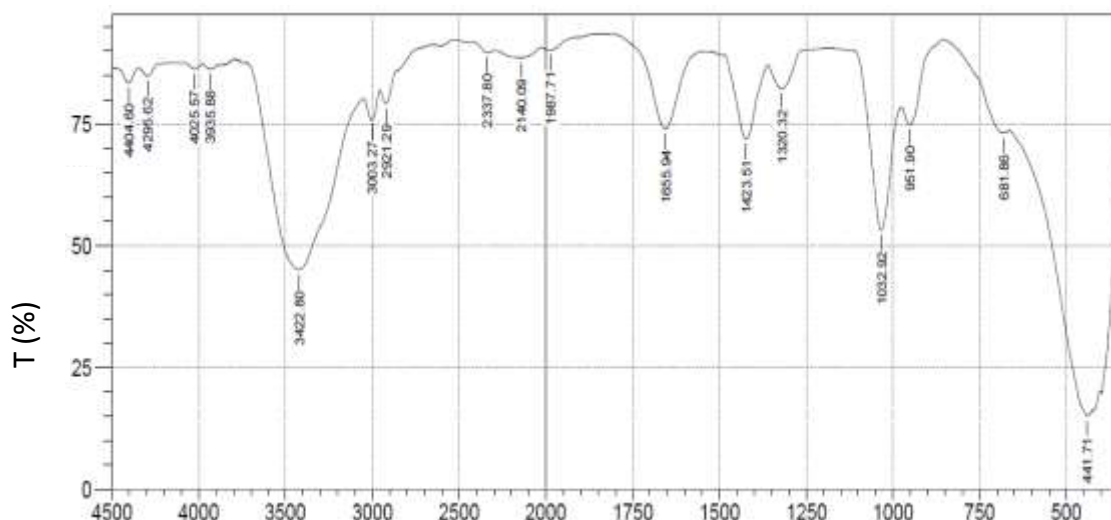
## DISCUSSION

It has been established that plants produce bioactive secondary metabolites, thereby attracting researcher's interest for the scientific investigations on herbs. The ethyl acetate root bark extract of *T. avicennioides* presents a novel model for antimicrobial agent. To the best of our knowledge, this is the first time 3,11-Tetradecadien-1-ol is found among compounds with anti-

methicillin-resistant MRSA potential, and also, the first time the quantitative phytochemical estimation of the root bark of *T. avicennioides* is reported. The result obtained revealed that ethyl acetate is the solvent of choice for the extraction of bioactive agent in the root bark of *T. avicennioides*, (Table 2). This is probably due to its intermittently polar (polarity index of 4.4), thus having the ability of extracting series of saturated and unsaturated fatty acids with antimicrobial potentials such as oleic acid. It is interesting that the extract at all concentrations were inhibitory to the test organism within the susceptible range ( $\geq 14$  mm) as defined by Kirby-Bauer standard chart for methicillin antibiotic susceptibility. The inhibitory



**Figure 4.** The FTIR spectrum of fraction TLb4 of root barks bioactive ethyl acetate extract of *T. avicennioides* showing spectra peaks.



**Figure 5.** The FTIR spectrum of fraction TLb17 of root barks bioactive ethyl acetate extract of *T. avicennioides* showing spectra peaks.

activity exhibited by the extract could be most probably linked to the presence of the secondary metabolites. This finding is in conformity with previous study in which the presence of the secondary metabolites in *T. avicennioides* was concluded to be responsible for its potential use as drugs (Mann et al., 2008).

A similar finding was reported on the anti-MRSA of *T. avicennioides*; though, the leaf part of the plant was used, but was found to be potent against MRSA isolated from Primary School Pupils in Ekiti State, Nigeria (Ajibade and Akinruli, 2016). This is an indication that harnessing *T. avicennioides* is advancement of the search for novel and viable anti-MRSA agent. The possible mechanism of action of the bioactive fraction of the extract against the test organism, MRSA, may be

deduced from the presence of the functional groups. Previous studies (Hollosoy et al., 2002; Litvin et al., 2004) indicated that halogen substituents on aryl rings resulted in compound with improved biological activity, hence, incorporation of halogen atoms into drug candidates have generally been correlated with increased lipophilicity; this enhances the penetration of agents into cells through the lipid membrane. The alkyl halides in the bioactive fractions may have explained the bactericidal mechanism of action of the agent against the MRSA. In other related studies, it was shown that functional groups on the nitrogen-containing heterocyclic compounds, and amides were the active species that exhibited anti-MRSA, by inhibiting dihydrofolate reductase activity (Wyatt et al., 2008; Pauk et al., 2013; Zadrzilova et al., 2014). The



biphenyl ether, was found to possess broad spectrum antibiotics activity, targeting the fatty acid biosynthetic pathway by inhibiting enoyl-acyl carrier protein reductase (Tipparaju et al., 2008), likewise the vancomycin mechanism (Rybak et al., 2009). Also, isothiocyanates from cruciferous plants showed bactericidal activity against MRSA of about 87% with MIC values between 2.9 and 110 µg/ml (Dias et al., 2014).

The result of the GC-MS analysis conforms to previous findings. The antimicrobial activity and therapeutic efficacy of oleic acid against methicillin-resistant *S. aureus* have been proven (Huang et al., 2011). Fatty acids, particularly methyl ester possess antibacterial activities (Chandrasekaran et al., 2008). Compounds possessing anti-microbial effects against MRSA have been reported in previous studies, they include palmitic acid, stearic acid, (9Z)-9-Tetradecenal among fatty acids (Gheda et al., 2013; Ololade et al., 2014). Interestingly, this is the first time 3,11-Tetradecadien-1-ol is found among compounds with anti MRSA effect. These potential antimicrobial activities is said to be attributed to different compounds belonging to a diverse range of chemical classes (Ozdemir et al., 2004). In the current findings, oleic acid was among the compounds, it was shown that oleic acid effectively eliminates *S. aureus* through cell wall disruption and can inhibit the growth of Gram-positive bacteria, including hospital and community-associated MRSA (Chen et al., 2011); therefore, it is inferred that the observed anti-MRSA effect of the TLb4 and TLb17 fractions of the root bark ethyl acetate extract of *T. avicennioides* could be due to the presence of oleic acid.

Fatty acids are known to interfere with the bacterial cell structure, which in turn obstructs cellular energy production, causing disruption of the electron transport chain and oxidative phosphorylation (Carpo et al., 2007; Parfene et al., 2013). Fatty acids also inhibit enzyme activity, impair nutrient uptake, generate cellular degradation products, and/or cause direct lysis of infectious cells (Carpo et al., 2007; Parfene et al., 2013). In this regard, the organic compounds found in this medicinal plant would play important role as agents for the treatment of infections associated with MRSA.

## Conclusion

This study explored novel inhibition model for MRSA. It has scientifically validated the ethno-medical use of root bark extracts of *T. avicennioides* traditionally in the treatment of skin infections, particularly caused by the test organism. Result of the GCMS analysis identified analogs of palmitic acids as the bioactive agent, which possibly could have synergized with other compounds to have elicited the observed inhibition against MRSA. The minimum concentration of the partially purified bioactive agent at which it became inhibitory, bacteriostatic and

bactericidal to MRSA was 0.6 µg/ml for both TLC bands 4 and 17. FTIR revealed functional groups present in the bioactive agent, which include alkyl halides: R-Br, R-F, methyne: (CH-), nitro compounds: R-NO<sub>2</sub>, amides: R-C(O)-NH<sub>2</sub>, R-C(O)-NH-R, isothiocyanate: (-NCS), Nitril: C≡N, carboxylic acids, saturated aliphatic (Alkyl): methylene C-H and alkanes.

## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

## ACKNOWLEDGEMENTS

Authors thank the staff of the Department of Biochemistry, Ahmadu Bello University, Zaria, for their respective efforts during this research. They acknowledge Mallam Yauza of Veterinary Medicine Department, Ahmadu Bello University, Zaria, for his technical assistance during this research. They also thank Oluwadamilare Stephen Ojo for helping with the statistical analysis.

## REFERENCES

- Ajibade VA, Akinruli FT (2016). Antibacterial Effects of *Phyllanthus discoideus* and *Terminalia avicennioides* on Methicillin Resistant *Staphylococcus aureus* Isolated from Primary School Pupils in Ekiti State. *Global Journal of Science Frontier Research* 16(3):456-461.
- Ajoke OI, Ikenna OO, Olumide AO, Okwori AEJ (2012). Prevalence of methicillin-resistant staphylococcus aureus from healthy community individuals volunteers in Jos South, Nigeria. *The Journal of Microbiology, Biotechnology and Food Sciences* 1(6):1389-1405.
- Akinyemi KO, Olukayode O, Okwara CE, Ibe CC, Fasura KA (2005). Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. *BMC Complementary Alternate Medicine* 5(1):6.
- Burdette SD, Watkins RR, Wong KK, Mathew SD, Martin DJ, Markert RJ (2012). *Staphylococcus aureus* pyomyositis compared with non-*Staphylococcus aureus* pyomyositis. *Journal of Infection* 64(5):507-512.
- Carpo BG, Verallo-Rowell VM, Kabara J (2007). Novel antibacterial activity of monolaurin compared with conventional antibiotics against organisms from skin infections: an in vitro study. *Journal of Drugs in Dermatology* 6(10):991-998.
- Chambers HF, Deleo FR (2009). Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nature Reviews Microbiology* 7(9):629-641.
- Chandrasekaran M, Kannathasan K, Venkatesalu V (2008). Antimicrobial activity of fatty acid methyl esters of some members of chenopodiaceae. *Zeitschrift für Naturforschung C* 63(5-6):331-336.
- Changchien CH, Chen YY, Chen SW, Chen WL, Tsay JG, Chu C (2011). Retrospective study of necrotizing fasciitis and characterization of its associated methicillin-resistant *Staphylococcus aureus* in Taiwan. *BMC Infectious Diseases* 11(1):297-301.
- Chen CH, Wang Y, Nakatsuji T, Liu YT, Zouboulis C, Gallo R, Zhang L, Hsieh MF, Huang CM (2011). An innate bactericidal oleic acid effective against skin infection of methicillin-resistant *Staphylococcus aureus*: a therapy concordant with evolutionary medicine. *Journal of Microbiology Biotechnology* 21(4):391-399.
- Dias C, Aires A, Saavedra MJ (2014). Antimicrobial Activity of

- Isothiocyanates from Cruciferous Plants against Methicillin-Resistant *Staphylococcus aureus* (MRSA) International Journal of Molecular Sciences 15(11):19552-19561.
- Francis JS, Doherty MC, Lopatin U, Johnston CP, Sinha G, Ross T, Cai M, Hansel NN, Perl T (2005). Severe community-onset pneumonia in healthy adults caused by methicillin-resistant *Staphylococcus aureus* carrying the Panton-Valentine leukocidin genes. *Clinical Infectious Diseases* 40(1):100-107.
- Gheda SF, Khalil MA, Gheida SF (2013). *In vitro* and *in vivo* preliminary results on *Spirulina platensis* for treatment of impetigo. *African Journal of Biotechnology* 12(18):2498-2509.
- Gill LS, Akinwunmi C (1986). Nigerian Medicine Practice and Beliefs of the Ondo People. *Journal of Ethnopharmacology* 18(3):257-266.
- Harborne JB (1998). *Phytochemical Method, a Guide to Modern Technique of Plant Analysis*. 3<sup>rd</sup> Edn. Chapman and Hall, London. pp. 1-198.
- Hollósy F, Seprodi J, Orfi L, Eros D, Keri G, Idei M (2002). Evaluation of lipophilicity and antitumor activity of parallel carboxamide libraries. *Journal of Chromatography B* 780(2):355-363.
- Huang CM, Chen CH, Pornpattananangkul D, Zhang L, Chan M, Hsieh MF, Zhang L (2011). Eradication of drug resistant *Staphylococcus aureus* by liposomal oleic acids. *Biomaterials* 32:214-221.
- Kechrid A, Pe'rez-Va' ZM, Smaoui H, Hariga D, Rodri'guez-Ban' OS M, Vindel A, Baquero F, Canto' NR, Del Campo R (2011). Molecular analysis of community acquired methicillin-susceptible and resistant *Staphylococcus aureus* isolates recovered from bacteraemic and osteomyelitis infections in children from Tunisia. *Clinical Microbiology and Infection* 17(7):1020-1026.
- Leboffe JM, Pierce EB (1999). *Photographic Atlas for the Microbiology Laboratory*. 2<sup>nd</sup> Edition. pp. 85-90.
- Litvin AA, Kolyvanov GB, Zherdev VP, Arzamastsev AP (2004). Relationship between physicochemical characteristics and pharmacokinetic parameters of 1,4-benzodiazepine derivatives. *Pharmaceutical Chemistry Journal* 38(11):583-586.
- Mann A, Amupitan JO, Oyewale AO, Okogun JI, Ibrahim K (2007). Antimicrobial potentials of *Anogeissus leiocarpus* and *Terminalia avicennioides* in treating infectious diseases prevalent in Hospital Environments in Nigeria. *Journal of Women Technology Education Employment* 5(1):67-74.
- Mann A, Amupitan JO, Oyewale AO, Okogun JI, Ibrahim K (2009). Antibacterial activity of terpenoidal fractions from *Anogeissus leiocarpus* and *Terminalia avicennioides* against community acquired infections. *African Journal of Pharmacy and Pharmacology* 3(1):22-25.
- Mann A, Yahaya Y, Banso A, John F (2008). Phytochemical and antimicrobial activity of *Terminalia avicennioides* extracts against some bacteria pathogens associated with patients suffering from complicated respiratory tract diseases. *Journal of Medicinal Plants Research* 2(5):94-97.
- Nair R, Kalariya T, Chanda S (2005). Antibacterial activity of some selected Indian medicinal flora. Antibacterial activity of some selected Indian medicinal flora. *Turkish Journal of Biology* 29(1):41-47.
- Ololade ZS, Olatunde OZ, Oyelese OJ, Olaoye OO, Odewande RA (2014). Total Phenolic Content, Free Radical Inhibition, Antioxidant and Antibacterial Potentials of the Medicinal Organic Compounds in the Fruit of *Terminalia catappa* Linn. *National Science* 12(2):46-50.
- Ozdemir G, Karabay NU, Dalay MC, Pazarbasi B (2004). Antibacterial activity of volatile component and various extracts of *Spirulina platensis*. *Phytotherapy Research* 18(9):754-757.
- Parfene G, Hornicar V, Tyagi AK, Malik A, Bahrim G (2013). Production of medium chain saturated fatty acids with enhanced antimicrobial activity from crude coconut fat by solid state cultivation of *Yarrowia lipolytica*. *Food Chemistry* 136(3-4):1345-1349.
- Pauk K, Zadrazilova I, Imramovsky A (2013). New derivatives of salicylamides: preparation and antimicrobial activity against various bacterial species. *Bioorganic and Medicinal Chemistry* 21(21):6574-6581.
- Rybak M, Lomaestro B, Rotschafer JC, Moellering R, Craig W, Billeter M, Dalovisio JR, Levine DP (2009). Therapeutic monitoring of vancomycin in adult patients: A consensus review of the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious Diseases Pharmacists. *American Journal of Health-System Pharmacy* 66(1):82-98.
- Tagboto S, Townson S (2001). Antiparasitic properties of medicinal plants and other naturally occurring products. *Advanced Parasitology* 50:199-295.
- Tipparaju KS, Mulhearn CD, Klein MG, Chen Y, Tapadar S, Bishop HM, Yang S, Chen J, Ghassemi M, Santarsiero DB, Cook LJ, Johlf M, Mesecar DA, Johnson EM, Kozikowski PA (2008). Design and Synthesis of Aryl Ether Inhibitors of the *Bacillus Anthracis* Enoyl-ACP Reductase. *Medical Chemistry* 3(8):1250-1268.
- Trease GE, Evans WC (1989). *Pharmacognosy*. 13<sup>th</sup> Edn. ELBS/Baillier Tindall, London pp. 345-773.
- Wyatt EE, Galloway RJDW, Thomas LG, Welch M, Loiseleur O, Plowright TA, Spring DR (2008). Identification of an anti-MRSA dihydrofolate reductase inhibitor from a diversity-oriented synthesis. *Chemical Communications* (40):4962-4964.
- Zadrazilova I, Pospisilova S, Pauk K, Imramovsky A, Vinsova J, Cizek A, Jampilek J (2014). *In Vitro* Bactericidal Activity of 4- and 5-Chloro-2-hydroxy-N-[1-oxo-1-(phenylamino) alkan-2-yl]benzamides against MRSA. *BioMed Research International* 2015:287-295.