INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES
(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com
Coden:IJCROO(USA) Volume 3 Issue 3 – 2016

Research Article

SOI: http://s-o-i.org/1.15/ijcrcps-2016-3-3-1

BIOACTIVE COMPONENTS AND ANTIBACTERIAL ACTIVITIES OF N-HEXANE EXTRACT OF *MORINGA OLEIFERA* ROOT BARK ON CLINICAL ISOLATES OF METHICILIN RESISTANT *STAPHYLOCOCCUS AUREUS.*

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Abstract

Bioactive components and antibacterial activities of n-hexane extract of *Moringa oleifera* root bark on clinical isolates of methicilin resistant *Staphylococcus aureus* was evaluated using agar dilution method. The identification and confirmation of the *S. aureus* were done using selective and differential medium (Mannitol salt agar) for *S. aureus* and by coagulase/staphylase test using Oxoid® reagents kits (DR0595A). Antibiotics susceptibility pattern was done on the characterised *S. aureus* isolates using standard antibiotic discs diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI). MRSA confirmation was done using Oxoid® DR0900 penicillin binding protein (pbp2") latex agglutination test kits. Pulverised *Moringa oleifera* root bark was defatted by cold maceration over night with n-hexane solvent to yield hexane extract fraction (HEF). Qualitative phytochemical analyses of the extracts were carried out using standard procedures. The antibacterial activities of HEF were evaluated on the MRSA, the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were recorded and compared with the standard disc antimicrobial test results. The extract fraction was analysed using gas chromatographic-mass spectrometry (GC-MS) for their bioactive compounds. The results showed characterized clinical isolates to be 58 *S. aureus* strains. Antibiotic susceptibility tests indicated varied percentages of MRSA that were resistant to various antibiotics thus: oxacillin (62.1 ± 3.2%), vancomycin (60.4 ± 3.8%), cephalexin (55.2 ± 1.2%), levofloxacin (56.9 ± 2.2%), ciprofloxacin (56.9 ± 0.9%), tetracycline (65.5 ± 2.3%), cotrimoxazole (68.9 ± 0.8%), gentamicin (67.2 ± 1.3%), clindamycin (62.1 ± 3.3%) and rifampicin (62.1 ± 4.1%). Latex agglutination test confirmed 39 strains of the clinical isolates to be MRSA. The *S. aureus* isolates resistant to all the antibiotics including vancomycin at 30 µg/ml were sensitive to the extract as follows, HEF: MIC (7.0 ± 0.5 to 8 ± 1.1 mg/ml) and MBC (7.0 ± 0.5 to 9 ± 0.5 mg/ml). Phytochemical analysis of the extract showed the presence of alkaloids, glycosides, steroids, terpenoids, flavonoids, saponins, tannins, resins, reducing sugars, proteins, fats and oil and carbohydrates. GC-MS analysis revealed 45 distinct compounds with the following compounds dominating the fraction 9, 12-Octadecadienoic acid (41.08 %), 2-Chloroethyl linoleate (41.08 %), n-Hexadecanoicacid (17.35 %), 12-Octadecadienoic acid (8.55 %), Stigmasterol (4.44 %), Ergost-22-en-3-one (4.44 %) and Campesterol (3.46 %).

Keywords: Bioactive, components, antimicrobial, activities, *S. aureus*, Methicillin, Resistance, n-hexane, *Moringa oleifera*

Introduction

Moringa oleifera root extract possesses secondary metabolites with antimicrobial activities. Presently, numerous scientific investigations have confirmed the effectiveness of these traditional remedies [1] Also based on research the plant is very nutritious, earning it the WHO (World Health Organization) candidate in the fight against malnutrition [2].

It is a native to India, occurring wild in the sub-Himalayan regions of Northern India and cultivated throughout the country. It is commonly known as Sajina, sajna (Bengali); Horseradish tree, drumstick tree (English); Sahinjan, mungna(Hindi); Murinna, muringa, tishnagandha (Malyalam); Sevaga, segata (Marathi); Sohanjana (Punjabi); Sobhanjana, sigru, murungi,

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dvishiguru (Sanskrit) and Sehjan(Urdu) in varied Indian languages and regions [3-5]. It also thrives well in Pakistan, Bangladesh, Sri Lanka, tropical Africa, Arabia, Philippines, Cambodia and Central, North and South America [6-10]. Described as "one of the most amazing trees God has created", almost every part of drumstick viz. bark, root, fruit, flowers, leaves, seed and gum is a rich repository of proteins, vitamins and minerals including potassium, calcium, phosphorus, iron, folic acid as well as carotene. Leaves can be eaten fresh, cooked or stored as dry powder for many months without refrigeration, without loss of nutritional value. Almost all the parts of this plant have been used for various ailments in the indigenous medicine of South Asia [11, 12].

Not only moringa is glorified as a traditional mother care plant", for the leaves are highly nutritious for pregnant women [13]. Until now, only a very few attempts have been made to compile the myriad of potential uses of this "miracle tree". In view of a number of recent findings of ethno pharmacological importance, an updated appraisal was much needed.

The present research is an attempt to explore the claims so far and prepare the ground for development of effective novel herbal formulations of *M. oleifera*. in the treatment of infections caused by much dreaded Methicilin resistant *Staphylococcus aureus* [14].

Biological resistance refers to changes that result in the organism being less susceptible to a particular antimicrobial agent than has been previously observed. When antimicrobial susceptibility has been lost to such an extent that the drug is no longer effective for clinical use, the organism is then said to have achieved clinical resistance [15]. It is important to note that often, biologic resistance and clinical resistance do not necessarily coincide. From a clinical laboratory and public health perspective it is important to realize that biologic development of antimicrobial resistance is an ongoing process, while clinical resistance is dependent on current laboratory methods and established cut-offs. Our inability to reliably detect all these processes with current laboratory procedures and criteria should not be perceived as evidence that they are not occurring [15-16].

Many plasmid-encoded determinants have recently become inserted into the chromosome at a site associated with the methicillin resistance determinant [16]. There may be an advantage to the organism having resistance determinants in the chromosome because they will be more stable. There are essentially four mechanisms of resistance to antibiotics in bacteria: (1) enzymatic inactivation of the drug, (2) alterations to the drug target to prevent binding, (3) accelerated drug efflux to prevent toxic concentrations accumulating in the cell, and (4) a by-pass mechanism whereby an alternative drug-resistant version of the target is expressed [16-17].

Hospital strains of *S. aureus* are often resistant to many different antibiotics. Indeed strains resistant to all clinically useful drugs, apart from the glycopeptides vancomycin and teicoplanin, have been described [17]. The term MRSA refers to methicillin resistant *Staphylococcus aureus*. Plasmid-associated vancomycin resistance has been detected in some enterococci and the resistance determinant has been transferred from enterococci to *S. aureus* in the laboratory and may occur naturally [18].

Materials and Methodology

Collection, authentication and processing of plant materials

The root of *Moringa oleifera* was collected from Nsukka Local Government Area, Enugu State, Nigeria. The plant materials were identified and authenticated by a Botanist at the Biological Science Department, University of Nigeria, Nsuka. Confirmation of taxonomic identity of the plants was achieved by Mrs. Immanuela Udoma by comparison with voucher specimens kept at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Uyo, Uyo and use of documented literature [19]. The plant materials were air-dried in the laboratory for four weeks. The dried samples were grinded to coarse powder with a mechanical grinder and stored for future use.

Extraction of root extract

The pulverized root of *Moringa oleifera* (3 kg) was defatted with 10 litres of n-hexane by cold merceration over night to yield hexane extract fraction (HEF). The hexane extract fraction (HEF) was concentrated *in-vacuo* using rotary evaporator and yielded percentage was calculated. The dried extracts were stored in amber coloured bottles and kept in the refrigerator until use.

Qualitative phytochemical analysis of the extract

Qualitative phytochemical analyses of the extracts were carried out using standard procedures (Trease and Evans (1996), Sofowora (1984) [20].

Media preparation

All the media used for culturing and sub culturing of the Clinical isolates used were prepared according to manufacturer"s Protocols.

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Standardization of inoculum

The inocula were prepared from the stock cultures, which were maintained on nutrient agar slant at 4 ^{0}C and subcultured onto nutrient broth using a sterilized wire loop. The density of suspension inoculated onto the media for susceptibility test was determined by comparison with 0.5 McFarland standard of barium sulphate solution [21].

Characterization of the clinical isolates

The clinical isolates were characterized by cultural characteristics, microscopy and biochemical tests (Catalase and Mannitol fermentaion). *S. aureus* isolates were confirmed by coagulase/staphylase test [21].

Antimicrobial susceptibility testing

The characterised *S. aureus* isolates were further identified as MRSA by disc diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI)., using ten Standard antibiotic discs [22].

Penicilin-binding protein (PBP2) latex agglutination test

The PBP2 test was performed only on *Staphylococcus* species (Gram + positive *cocci*). A coagulase test confirming the isolates used for this test to be *S. aureus* was done prior to the PBP2 test. A pure clinical isolates of *S. aureus* were used for this test according to the manufacturer's procedures Oxoid U.K. MRSA strain.

ATCC® 43300 (Oxoid Culti – Loops C9022) was used as positive control and ATCC® 25923 (Oxoid Culti – Loops® CL7010) was used as negative control [23].

Determination of MIC of methanol extract and fractions

The minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) were carried out using Agar (MHA) dilution method (solid phase testing) according to the Clinical Laboratory Standards Institute (CLSI).

Control dish with only solvent without the extract was also inoculated to confirm non interference of the DMSO: water in the activity of the extract. Experiments were repeated three times and the average was recorded.

Table 1: Preparation of Hexane extract fractions concentrations for agar dilution MIC and MBC Tests.

S/N	C1 (mg/ml)	V1 (ml)	C2 (gm/ml)	Volume of MHA (ml)	V2 (ml) Volume of reaction mixture
1	50	4.00	10	16.00	20
2	50	3.60	9	16.40	20
3	50	3.20	8	16.80	20
4	50	2.80	7	17.20	20
5	50	2.40	6	17.60	20
6	50	2.00	5	18.00	20
7	50	1.60	4	18.40	20
8	50	1.20	3	18.80	20
9	50	0.80	2	19.20	20
10	50	0.40	1	19.60	20

Determination of MBC of HEF

The value of MBC is an extension of MIC. The agar plates showing no growth in the MIC tests were used for the determination of the MBC. Discs were cut from the agar plate of the MIC concentration and two preceding concentrations and transferred into the corresponding containers of the fresh Muller Hinton broth (recovery medium). The media were also incubated at 35 ^{0}C for 48hrs. At the end of incubation the media were observed for any visible growth or turbidity. The absence of growth in the recovery medium is evidence of total cell death. The minimal concentration of the antimicrobial agent that produces total cell death is taken as the MBC [21].

GC-MS determination of bioactive components of N-hexane extract fraction

Analyses were carried out in A GC-MS equipment with Agilent technologies 7890B for GC systems and Agilent technologies 5975 series for MS system. The investigations were carried out for each fraction at oven equilibration Time of 1 min, maximum temperature of 450°C, Oven Program On 50°C for 5 min then 5°C/min to 280°C for 9 min, total Run Time 60 min [24-25].

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Statistical analysis

Results were expressed as mean ± SD and differences between sets obtained were determined using ANOVA followed by Duncan post Hoc Test with the use of SPSS v 17 software. Differences were considered significant at $p < 0.05$.

Results

Table 2: Results of phytochemical analysis of the N-Hexane fraction

Chemical Constituents	Test	N-hexane fraction (HEF)
Alkaloids	Dragendorff's reagent Mayer's reagent Wagner's reagent	-
Glycosides	Fehling's solution I and II	-
Steroids	General Test	+ + + +
Terpenoids	General Test	+ + +
Flavonoids	Ammonium Test 1 % Aluminium Chloride solution Test.	-
Saponins	Frothing Test Emulsion Test Fehling's Test	-
Tannins	Ferric chloride Test Lead Acetate Test	-
Resins	Precipitation Test Colour Test	+ +
Reducing Sugar	Fehling's solution I and II	-
Proteins	Millon's Test Xanthoproteic Reaction Test Picric Acid Test Biuret Test	-
Fats and Oil	General filter paper Test	++++
Carbohydrate	Molisch's	-

Key:
(-): Not present.
(+): Present in small concentration.
(++): Present in moderately high concentration.
(+++): Present in very high concentration.
(++++): Abundantly present.

Table 3: Antimicrobial susceptibility test

S/N	Antimicrobial	MSSA	% MSSA	MRSA	% MRSA
1	Oxacilin 5 µg/ml	22.3 ± 0.5	37.93	36.0 ± 0.5	62.07
2	Vancomycin 30 µg/ml	23.0 ± 1.0	39.65	35.6 ± 1.1	60.35
3	Cephalexin 30 µg/ml	26.0 ± 1.0	44.82	32.3 ± 0.5	55.18
4	Levofloxacin 5 µg/ml	25.3 ± 0.5	43.10	32.6 ± 0.5	56.90
5	Ciprofloxacin 5 µg/ml	21.0 ± 0.5	34.48	37.6 ± 0.5	65.52
6	Tetracycline 30 µg/ml	18.3 ± 0.5	31.03	39.6 ± 0.5	68.97
7	Cotrimoxazole 25µg/ml	20.0 ± 0.5	32.75	39.3 ± 0.5	67.25
8	Gentamycin 30µg/ml	21.3 ± 1.0	37.93	36.3 ± 0.5	62.07
9	Clindamycin 2 µg/ml	21.3 ± 0.5	36.20	37.3 ± 0.5	63.79
10	Rifampicin 5µg/ml	21.3 ± 0.5	37.93	36.3 ± 0.5	62.07

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Table 4: Penicillin binding protein (PBP2[1]) latex agglutination test

S/N	Sputum	L T	Inf.	Skin swab	L T	Inf.	Absces s	LT	Inf.	Open wound	LT	Inf.	Ear/ Nasal	LT	Inf.
1	SP4	+	MRSA	SS8	+	MRSA	AB5	-	MSSA	OW12	-	MSSA	EN4	-	MSSA
2	SP22	+	MRSA	SS10	-	MSSA	AB20	+	MRSA	OW30	+	MRSA	EN8	-	MSSA
3	SP35	-	MSSA	SS19	-	MSSA	AB31	-	MSSA	OW36	+	MRSA	EN35	+	MRSA
4	SP46	-	MSSA	SS27	-	MSSA	AB53	-	MSSA	OW53	+	MRSA	EN38	+	MRSA
5	SP651	+	VISA	SS33	+	MRSA	AB61	+	MRSA	OW66	-	MRSA	EN62	+	MRSA
6	SP720	-	MSSA	SS42	+	MRSA	AB187	+	MRSA	OW101	-	MSSA	EN127	+	VISA
7	SP1172	+	MRSA	SS46	-	MSSA	AB570	+	MRSA	OW123	+	VISA	EN208	+	VISA
8				SS57	+	MRSA	AB600	+	VISA	OW154	+	VISA	EN390	+	VISA
9				SS235	+	MRSA	AB841	+	MRSA	OW238	-	MSSA	EN504	-	MSSA
10				SS310	+	MRSA	AB1009	+	MRSA	OW270	-	MSSA	EN551	-	MSSA
11							AB1956	+	MRSA	OW417	+	MRSA	EN831	+	MRSA
12										OW578	+	MRSA			
13										OW620	+	MRSA			
14										OW819	+	MRSA			
15										OW940	+	MRSA			
16										OW947	+	MRSA			
17										OW1104	+	MRSA			
18										OW1420	+	MRSA			
19										OW1827	+	VISA			
T T	7 (58)			10 (58)			11 (58)			19 (58)			11 (58)		

Key:

LT: Latex Test

Inf.: Inference

+ : Agglutination

- : No agglutination

T T: Total

Positive Control
A known MRSA strain was used following the method given in the test procedure, agglutination occurs within 3 minutes.

Negative Control
A know Methicillin – Sensitive *Staphyloccocus aureus* was used following the method given in the test procedure, there was no agglutination within 3 minutes.

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Table 5: MIC and MBC of n-hexane fraction in mg/ml

S/N	Clinical isolates	MIC	MBC	S/N	Clinical isolates	MIC	MBC
1	SP4	6.3 ± 0.5	7.5 ± 0.5	21	EN390	7.5 ± 0.5	8.6 ± 1.1
2	SS8	5.5 ± 1.1	6.5 ± 1.1	22	SS310	6.3 ± 0.5	7.5 ± 0.5
3	AB20	5.3 ± 1.5	6.6 ± 0.5	23	OW417	5.6 ± 0.5	6.6 ± 0.5
4	SP22	6.5 ± 0.5	7.3 ± 0.5	24	AB570	6.5 ± 0.5	7.6 ± 0.5
5	OW30	7.3 ± 1.5	8.5 ± 0.5	25	OW578	6.7 ± 0.5	8.5 ± 1.0
6	SS33	5.6 ± 0.5	6.3 ± 1.0	26	AB600	7.5 ± 1.1	8.7 ± 1.0
7	EN35	7.3 ± 1.1	8.3 ± 0.5	27	OW620	6.8 ± 0.5	8.5 ± 0.5
8	OW36	6.5 ± 1.1	7.6 ± 0.5	28	SP651	7.6 ± 0.5	8.8 ± 0.5
9	EN38	6.7 ± 0.5	8.3 ± 0.5	29	OW819	5.8 ± 0.5	6.6 ± 0.5
10	SS42	7.3 ± 1.0	8.5 ± 0.5	30	EN831	7.5 ± 0.5	7.5 ± 1.1
11	OW53	6.8 ± 0.5	7.5 ± 0.5	31	AB841	6.8 ± 0.5	8.5 ± 0.5
12	SS57	7.3 ± 0.5	8.3 ± 1.0	32	OW940	7.5 ± 0.5	8.7 ± 0.5
13	AB61	5.6 ± 0.5	6.6 ± 0.5	33	OW947	6.9 ± 0.5	8.5 ± 0.3
14	EN62	6.5 ± 0.5	7.3 ± 0.5	34	AB1009	7.5 ± 0.5	7.6 ± 1.1
15	OW123	5.6 ± 0.5	5.6 ± 1.0	35	OW1104	6.3 ± 0.5	7.5 ± 0.5
16	EN127	6.7 ± 0.5	7,5 ± 0.5	36	SP1172	7.5 ± 0.5	8.6 ± 1.0
17	OW154	7.3 ± 0.5	7.3 ± 0.5	37	OW1420	6.8 ± 0.5	7.8 ± 0.5
18	AB187	6.8 ± 0.5	8.5 ± 1.1	38	OW1827	7.5 ± 0.35	7.5 ± 0.5
19	EN208	5.6 ± 0.5	6.5 ± 0.5	39	AB1956	6.5 ± 0.5	7.6 ± 1.1
20	SS235	6.6 ± 0.5	7.3 ± 0.5				

Values were expressed as Mean ± SD, N = 3

MSSA – Methicilin sensitive *S. aureus*
MRSA – Methicilin resistant *S. aureus*
Key:
SP: Sputum
SS: Skin swab
AB: Abscess
OW: Open wound
EN: Ear/Nasal

N-hexane extract fraction (HEF)

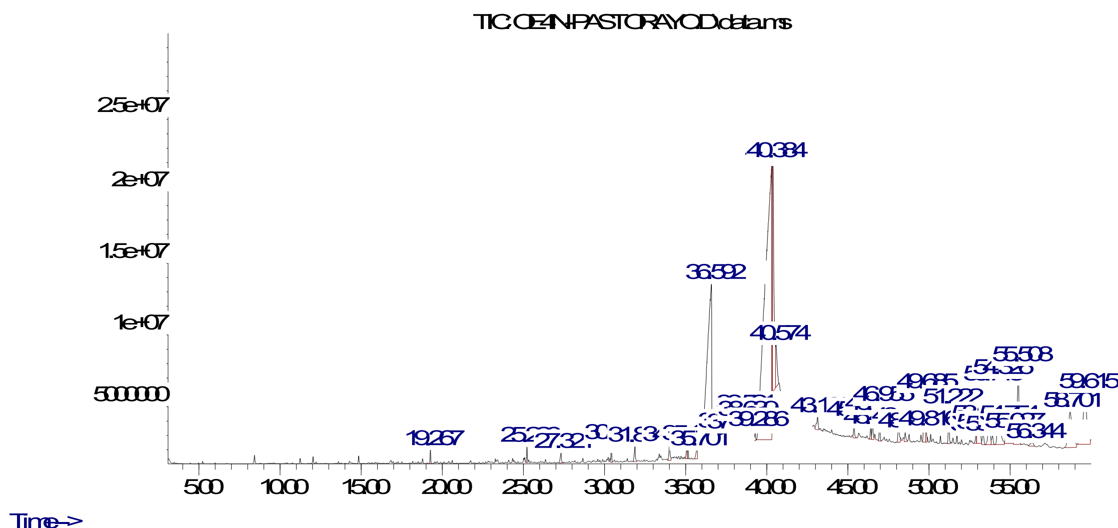

Fig. 1: MS fragment of n-hexane fraction composition

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Table 6: A GC-MS report of n-hexane fraction

Structural analogue	Rt(min)	Area %	Peak
Dodecane, 2,6,11-trimethyl- Tridecane, 1-iodo- 1,3-Dimethylcyclopentanol	18.26	0.22	1
Heneicosane, Pentadecane, Dodecane	25.22	0.27	2
Dodecanoic acid	27.32	0.29	3
Heneicosane , 2-Bromo dodecane, Nonane,	30.41	0.32	4
Tetradecanoic acid	31.87	0.40	5
Pentadecanoic acid	34.01	0.43	6
Heptacosane Heneicosan, Hexadecane	35.06	0.33	7
Hexadecanoic acid, Pentadecanoic acid,	35.14	0.24	8
Palmitoleic acid, 9-Hexadecenoic acid , 4-Methyl-dodec-3-en-1-ol	35.70	0.31	9
n-Hexadecanoic acid	36.59	17.35	10
n-Hexadecanoic acid , Cyclic octaatomic sulfur7-Amino-7H-S-triazolo[5,1-c]-S-triazole-3-thiol	37.38	0.25	11
cis-Vaccenic acid, Cyclopentadecanone, 2-hydroxy- 9-Hexadecenoic acid	37.92	0.23	12
9,12-Octadecadienoic acid, methyl ester	38.52	0.56	13
9-Octadecenoic acid	38.63	0.30	14
Hexacosane , Octadecane, 2-methyltetracosane	39.28	0.22	15
9,12-Octadecadienoic acid, 2-Chloroethyl linoleate	40.30	41.08	16
9,12-Octadecadienoic acid (Z,Z)-2-Chloroethyl linoleate	40.33	1.73	17
40.384 8.55 C 9,12-Octadecadienoic acid	40.38	8.55	18
9,12-Octadecadienoic acid	40.57	1.35	19
9,12-Octadecadienoic acid	43.12	0.47	20
Oleic Acid, 2-Methyl-Z,Z-3,13-octadecadienol, 1-Naphthalenol, 1,2,3,4-tetrahydroacetate	45.37	0.38	21
Octadecane, 1-(ethenyloxy)- 9,17-Octadecadienal, (Z)-Z,E-3,13-Octadecadien-1-ol	46.40	0.25	22
Bis(2-ethylhexyl) phthalate	46.51	0.54	23
Dodecanoic acid, Phenyl methyl ester, Undecanoic acid, phenyl methyl ester, Octadecanoic acid, phenyl methyl ester	46.95	0.63	24
Difluoro(methylamino)phosphine sulfide, Cinnamylcinnamate	48.11	0.52	25
Difluoro(methylamino)phosphine sulfide, Cinnamyl cinnamate, Naphthalene, 1,2,3,4-tetrahydro-1	48.53	0.26	26
Morpholine, 4-[2-phenyl-1-(phenylethyl)ethenyl]- (N-(Benzyloxycarbonyl)glycyl)-l-rine hydrazide 1,3-Benzoxazin-2-one,	49.68	1.55	2;7
12-Methyl-E,E-2,13-octadecadien-1-Ol , Cyclohexane ethanol, 4-methyl-.beta-methylene-trans-4,9-Decadienoic acid, 2-nitro-ethyl ester	49.81	0.25	28
Dibut-3-enyl phthalate, Benzothiazole, 2-methyl-Phthalic acid, 3,4-dimethylphenyl isobutyl ester	51.22	0.82	29
1-Bromo-11-iodoundecane, C(14a)-Homo-27-nor-14.beta.-gammaceran-3.alpha-ol, Oxalic acid, pentadecyl propyl ester	52.91	0.23	30
9-(3-Fluorobenzyl)-9-hydroxy-3,6,10,10-tetramethyl-9,10-dihydrophena, Nthrene, 2,3-Dimethoxy-5-methyl-6-dekaisoprenyl-chinon, 7H-Pyrazolo[4,3-E][1,2,4]triazolo[1,5-c] pyrimidine.	53.01	1.13	31
Cyclopropane, carboxamide, 2-cyclopropyl-2-methyl-N-(1-cyclopropylethyl)-2,4,4-Trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexene beta. Carotene	53.32	0.41	32
Cholest-5-en-3-ol, (3.beta.)-carbonochloridate, Cholest-5-en-3-ol (3.beta.)-, propanoate1H-Isoindole,	53.71	2.24	33
Silane, [[(3.beta.)-lanosta-9(11), 24-dien-3-yl]oxy]trimethyl- 3.beta.-Hydroxy-5-cholen-24-oic acid, 9,19-Cyclolanost-23-ene-3,25-diol,	53.90	0.31	34
Cholesta-6,22,24-triene, 4,4-dimethyl, Stigmasteryl tosylate Stigmastan-6,22-dien	54.32	3.42	35
Stigmastan-3,5-diene, Hexacosanoic acid, A-Norcholestan-3-one	54.75	0.64	36
Stigmasteryltosylate Oxazole, 5-(2-furfurylamino)-2-(4- methyl phenyl)-4-phenylsulfonyl- Stigmasta-5,22-dien-3-ol.	55.02	0.39	37
Stigmastan-3,5-diene, .beta.-Sitosterol acetate, Ergosta-4,6,22-trien-3.beta.-ol	55.50	2.97	38
1-Nonadecene , 1,14-Dibromotetradecane, 2-Dodecen-1-yl(-)succinic anhydrid	56.34	0.26	39
Campesterol	58.70	3.46	40
Stigmasterol, Ergost-22-en-3-one,	59.61	4.44	41

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Discussion

MIC and MBC of N-hexane extract fraction (HEF) in mg/ml

The HEF shows good activity against the MRSA, possibly because of the presence of high concentration of fatty acids, terpenoids and phenols which was in agreement with previous studies to enhance antimicrobial property of a plant [26]. The fractions as shown in table 5 inhibited the MRSA isolates at the least MIC and MBC of 5.3 mg/ml and 5.6 mg/ml while the highest MIC and MBC are 7.6 mg/ml and 8.8 mg/ml. This confirms the antibacterial potency of the fractions compare to Vancomycin which was completely resisted by the MRSA. These activities may be due to the presence of some non-polar contents present in the n-hexane fraction as seen in the phytochemical analysis and the GC-MS report in Table 6.

Gas chromatography-mass spectrometry (GC-MS) of n-hexane fraction

The HF fraction activity is also encouraging against the MRSA isolates, 45 compounds identified with the following compounds dominating the fraction 9, 12-Octadecadienoic acid (41.08 %), 2-Chloroethyl linoleate (41.08 %), n-Hexadecanoicacid (17.35 %), 12-Octadecadienoic acid (8.55 %), Stigmasterol (4.44 %), Ergost-22-en-3-one (4.44 %), Campesterol (3.46 %). The fractions contain high content of Fatty acids, terpenoids and steroids their nature and activities of the compound agrees with references [27].

Conclusion

N-hexane fraction of *Moringa oleifera* contains bioactive substances which serve as promising sources of novel antibiotic prototypes. Moreover, these compounds could be of clinical importance to improve health care in the infection caused by MRSA.

Conflict of interests

Authors have declared that no competing interests exist.

Acknowledgments

Authors acknowledged the contributions of Mr. Fadare Olatomide of Central Science Lab., O. A. U. Ile-Ife for his assistance in the GC-MS analysis.

References

1. Oliveira JTA, Silveira SB, Vasconcelos IM, Cavada BS, Moreira RA. 1999. Compositional and nutritional attributes of seeds from the multipurpose tree *Moringa oleifera* Lamarck. J Sci Food Agric 79: 815–820.
2. Fahey, Sc.D., *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic and prophylactic properties, Part1.http://www.TFLJournal.org. 2005, 1(5): 1-15.
3. Mughal MH, Ali G, Srivastava PS, Iqbal M. 1999. Improvement of drumstick [*M. pterygosperma Gaertn.*] – a unique source of food and medicine through tissue culture. Hamdard Med 42: 37–42.
4. Dahot M. U. 1988. Vitamin contents of flowers and seeds of *Moringa oleifera*. Pak J. Biochem 21: 1–24.
5. Faizi S, Siddiqui B, Saleem R, Saddiqui S, Aftab K. 1994a. Isolation and structur elucidation of new nitrile and mustard oil glycosides from *M. oleifera* and their effect on blood pressure. J. Nat Prod 57: 1256–1261.
6. Gilani AH, Janbaz KH, Lateef A, Zaman M. 1994b. Ca channel blocking activity of *Artemisia scoparia* extract. Phytother Res 8: 161–165.
7. Bennett RN, FA Mellon, N Foidl, JH Pratt, MS DuPont, L Perkins and PA Kroon (2003) Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees *M. oleifera* L. (Horseradish tree) and *M. stenopetala* L. J. Agric . Food Chem. 51: 3546-3553.
8. Morton J. F. 1991. The horseradish tree, *Moringa pterigosperma [Moringaceae*]. A boon to arid lands. Econ Bot 45: 318–333.
9. Ghasi S, Nwobodo E, Ofili JO. 2000. Hypocholesterolemic effects of crude extract of leaf of *Moringa oleifera* Lam in high-fat diet fed Wistar rats. J. Ethnopharmacol. 69: 21–25.
10. Maravic G., Macrolide resistance based on the Ermmediated rRNA methylation, Curr. Drug Targets. Infect. Disord., 2004, 4, 193-202
11. Rezanka T., Spizek J., Sigler K., Medicinal use of lincosamides and microbial resistance to them, Anti-infect. Agents Med. Chem., 2007, 6, 133-144.
12. S, Nwobodo E, Ofili JO. 2000. Hypocholesterolemic effects of crude extract of leaf of *Moringa oleifera* Lam in high-fat diet fed Wistar rats. J. Ethnopharmacol. 69: 21–25.
13. Guevara AP, Vargas C, Sakurai H et al. 1999. An antitumor promoter from *Moringa oleifera* Lam. Mutat Res 440: 181–188.
14. Bharali R, Tabassum J, Azad MRH. 2003. Chemomodulatory effect of *Moringa oleifera,* Lam, on hepatic carcinogen metabolizing enzymes, anti-oxidant parameters and skin papillomagenesis in mice. Asia Pacific J Cancer Prev4: 131–139.

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15. Maravic G., Macrolide resistance based on the Ermmediated rRNA methylation, Curr. Drug Targets. Infect. Disord., 2004, 4, 193-202.
16. Weisblum B., Erythromycin resistance by ribosome modification, Antimicrob. Agents Chemother. 1995, 39, 577-585 .
17. Rezanka T., Spizek J., Sigler K., Medicinal use of lincosamides and microbial resistance to them, Anti-infect. Agents Med. Chem., 2007, 6, 133-144.
18. Bozdogan B., Appelbaum P.C., Oxazolidinones: activity, mode of action, and mechanismof resistance, Int. J. Antimicrob. Agents, 2004, 23,113-119.
19. Oliver, B. (1960). Medicinal Plants in Nigeria.,Nigerian College of Arts, Sciences and Technology, Ibadan, Nigeria, P. 49-50.
20. Trease, G.E and Evans, W.C, (1996). *Textbook of Pharmacognosy*. 14th Ed. W.B.National committee for Clinical Laboratory Standards. (2000 Approved Standard Fifth Edition. Example of preparing the dilutions of antimicrobial agents used in agar dilution. (NCCLS M100-S12 table 5).
21. Olowe O.A., Eniola K.I.T., Olowe R.A., Olayemi A.B, Antimicrobial Susceptibility and Beta-lactamase detection of MRSA in Osogbo. SW Nigeria, Nature and Science, 5(3), 2007,
22. Yamazumi T., Marshall S.A., Wilke W.W., et al (2001). *J. Clin. Microbiol.*, **39**: 53-56.
23. Ahmed S. John, Kumar P., GC-MS Study of the *Excoecaria agollocha* Leaf extract from Pitchavaram, Tamil nadu , India, *Researcher* 2012;4(6):10-14]. (ISSN: 1553-9865). http://www.sciencepub.net/researcher.
24. Adams 2007 r1, Release Guide, MSC.Software Corporation, 2 MacArthur Place, Santa Ana, CA 92707, USA. www.mscsoftware.com.
25. Nikkon F, Saud ZA, Rehman MH, Haque ME. 2003. In vitro antimicrobial activity of the compound isolated from chloroform extract of *Moringa oleifera* Lam. Pak J Biol Sci 22: 1888–1890.
26. Gassenschmidt U, Jany KD, Tauscher B, Niebergall H. 1995. Isolation and characterization of a flocculating protein from *Moringa oleifera* Lam. Biochim Biophys Acta 1243: 477– 481.

How to cite this article:
A.A. Agboke and A. A. Attama. (2016). Bioactive components and antibacterial activities of n-hexane extract of *Moringa oleifera* root bark on clinical isolates of methicilin resistant *Staphylococcus aureus*. Int. J. Curr. Res. Chem. Pharm. Sci. 3(3): 1-9.

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