International Journal of Trend in Scientific Research and Development (IJTSRD)
International Open Access Journal
ISSN No: 2456 - 6470 | www.ijtsrd.com | Volume - 2 | Issue – 3

Evaluation of Bioactivecomponents and Antistaphylococcal Activitiesof Ethyl Acetate and Dicloromethanefractions of *Moringa Oleifera* Root Bark on Clinical Isolates of Methicillin Resistant *Staphylococcus Aureus*

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ABSTRACT

This study evaluated the phytochemical components, Bioactive compounds (GC-MS) and antistaphylococchal activities of ethyl acetate and dichloromethane fractions of *Moringa oleifera* root bark. MRSA (Methicillin resistance *staphylococcus aureus*) was used for the antistaphylococcal activities. The isolates from 3 different hospitals in south-east geopolitical region of Nigeria were confirmed by coagulase/staphylase test using Oxoid® reagents kits (DR0595A). MRSA confirmation was done using Oxoid® DR0900 penicillin binding protein (pbp2) latex agglutination test kits. Pulverised *Moringa oleifera* root bark was extracted with methanol using Soxhlet extractor to obtain methanol crude extract (ME). ME was adsorbed on Silical gel (60-200 mesh) and eluted with ethyl acetate solvent to get ethyl acetate fraction (EAF) and also with dichloromethane solvent to get dichloromethane fraction (DMF). Qualitative phytochemical analyses of the EAF and DMF were carried out using standard procedures. The antistaphylococcal activities of EAF and DMF 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 two extract fractions were analyzed using gas chromatographic-mass spectrometry (GC-MS) for their bioactive compounds. Statistical analysis was done with ANOVA followed by Duncan post Hoc test using SPSS v 17 software. The results obtained showed: DMF MIC (8.0 ± 1.1 to 10 ± 0.5 mg/ml) and MBC (8.0 ± 0.5 to 10 ± 0.5 mg/ml);EAF: MIC (5.0 ± 1.1 to 8.0 ± 0.5 mg/ml) and MBC (5.0 ± 0.5 to 8.0 ± 0.5 mg/ml. Phytochemical analysis of the extract fractions showed: DMCwith concentrations high in Resins followed by steroids, fats and oil with traces of alkaloids, terpenoids and flavonoids, while EAF has high concentrations of Flavonoids, with traces of glycosides, terpenoids, steroids and carbohydrates. The GC-MS analysis revealed the bioactive components from the two solvents. Ethyl acetate (EAF) fraction is more potent than dichloromethane fraction; this indicates that the most active compound against the MRSA can be isolated from the EAF.

Keywords: *Activities, Antimicrobial, Bioactive, Components, Compounds, Dichloromethane, Ethyl acetate, MRSA, Phytochemical*

INTRODUCTION

The evaluation of various plant products according to their traditional uses and medicinal value based on their therapeutic efficacy leads to the discovery of newer and recent drugs for treat ingvarious ailments [1]. This fact forms the basis for the development of new drugs from various plant sources. One of such plants of medicinal value is *Moringa olifera,* belonging to the family *Moringaceae*, commonly known as 'sahajan' in Hindi, Horse radish in English. It is a small, fast, growing, evergreen, or deciduous

tree that usually grows up to 10 or 12 m in height. It is distributed among Sub Himalayan Tracts, Assam, Bengal and Peninsular India [1]. Various properties are attributed to it like antispasmodic, diuretic, expectorant and abortifacient [2].

Moringa oleifera

The Horticultural College & Research Institute of Tamil Nadu Agricultural University has released two improved annual moringa varieties (PKM1, PKM2) within a span of 10 years, for commercial cultivation [3, 4]. The folklore claims and ancient literature report moringa to be an abortifacient antidote, antirheumatic, bactericide, diuretic, ecbolic, emetic, expectorant, purgative, rubefacient, stimulant, tonic, vermifuge and vesicant [5-7]. (Pharma Products Pvt Ltd, Thayavur, India) and Livospin (Herbals APS Pvt. Ltd., Patna, India), which are available for a variety of ailments [8]. Ayurvedic preparations include Ratnagiri Rasa, Sarasvata Ghrta, Sudarsana churna, Sarsapadi Pralepa, Visatimduka Taila etc [4, 5].

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 ethnopharmacological 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 resitant *Staphylococcus aureus*

Antibacterial and antifungal activities

Moringaroots have antibacterial activity [9] and are reported to be rich in antimicrobial agents. These are reported to contain an active antibiotic principle, pterygospermin, which has powerful antibacterial and fungicidal effects. A similar compound is found to be responsible for the antibacterial and fungicidal effects of its flowers [10]. The root extract also possesses antimicrobial activity attributed to the presence of 4-α-L-rhamnosyloxybenzyl isothiocyanate [11]. The aglycone of deoxy-niazimicine [N-benzyl, S-ethyl thioformate] isolated from the chloroform fraction of an ethanol extract of the root bark was found to be responsible for the antibacterial and antifungal activities [12]. The bark extract has been shown to possess antifungal activity [13], while the juice from the stem bark showed antibacterial effect against *Staphylococcus aureus* [14]. The fresh leaf juice was found to inhibit the growth of microorganisms [*Pseudomonas aeruginosa* and *Staphylococcus aureus*], pathogenic to man [15].

The seeds also possess antimicrobial properties [16, 17] reported that a recombinant protein in the seed is able to flocculate Gram-positive and Gram-negative bacterial cells. In this case, microorganisms can be removed by settling in the same manner as the removal of colloids in properly coagulated and flocculated water [17]. On the other hand, the seeds may also act directly upon microorganisms and result in growth inhibition. Antimicrobial peptides are thought to act by disrupting the cell membrane or by inhibiting essential enzymes.

It was reported that the seeds could inhibit the replication of bacteriophages [17, 18, 19]. The antimicrobial effects of the seeds are attributed to the compound 4[α-L-rhamnosyloxy] benzyl isothiocynate [19].

MATERIALS AND METHODS

MATERIALS

Clinical isolates: The clinical isolates used include *Staphylococcus aureus*, methicilin resistant *Staphyloccocus aureus,* from Bishop Shanahan Hospital, Nsukka, University of Nigeria Teaching Hospital, Ituku/Ozalla Enugu state and Federal Medical Centre Abakaliki, Ebonyi State.

Media : Nutrient Agar (Fluka) Sigma Aldrich UK, Mueller-Hinton Agar (MHA), Oxoid Ltd, England, Mannitol Salt Agar (MSA), Oxoid Ltd, England.

Reagents: PBP2a or PBP2' test kit dr0900a lot. no. 130422. Oxoid Ltd, Japan, staphylase test kit dr0595a., (Oxoid Ltd, Wade Road, Basingstoke, Hants, RG24, UK),Oxoid antimicrobial susceptibility test discs. Hydrogen peroxide (H_2O_2), Dimethylsulfoxide (DMSO),distilled water, silical gel, (Titan Biotech Ltd, India). 0.5 McFarland turbidity standard.

Solvents: Methanol, (Sigma Aldrich, U.K), ethyl acetate, (Sigma Aldrich U.K), dichloromethane (Sigma Aldrich U.K).

Equipment :Test tubes, Petri-dish, pipette, micropipette, micro centrifuge tube, measuring cylinder, flat bottom flask, Soxhlet extractor, glass chromatographic column, autoclave, refrigerator, cotton wool, weighing balance, foil, sterile loops and swabs, incubator, antibiotic disc dispensers, GC-MS

equipment with Agilent technologies 7890B for GC systems and Agilent technologies 5975 series for MS system.[34].

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, and use of documented literature [21]. The plant materials were air-dried in the laboratory for four weeks. The dried samples were grinded to coarse powder with a mechanical grinder; the powdered was stored for future use.

Extraction of the root extract: The pulverized root of *Moringa oleifera* (3 kg) was extracted with 20 litres of methanol for 4 hours using Soxhlet extraction technique to yield methanol crude extract (ME) using established standard procedures by Harborne, Iwu, Trease and Evans [21-23].

The methanol crude extract (ME) was concentrated *in-vacuo* using rotary evaporator and yielded percentage was calculated. The dried extract (ME) (850.60 g) was adsorbed on silica gel (60-200 mesh) in a glass column, was then eluted in succession with dichloromethane and ethyl acetate to yield dichloromethane fraction (DF), ethyl acetate fraction (EAF). [21-23].

Qualitative phytochemical analysis

The ethyl acetate and dichloromethane fractions of the *Moringa oleifera* root bark was subjected to phytochemical tests using established standard procedures by Harborne, Iwu, Trease and Evans [21-23].

Penicilin-binding protein (PBP2$^{1`}$) latex agglutination test for MRSA confirmation

PBP2$^{'}$ extraction procedure as recommended by the Manufacturer (Oxoid,).

Test was performed only on Staphylococcus species (Gram + positive cocci).

Four drops of Extraction Reagent 1 was added to a micro centrifuge tube, an approximately 1.5 x 10^9 (3-5 µl) cells was then suspended into the micro centrifuge tube to obtain a very turbid suspension. The tube was placed into a water bath at temperature over 95^0 C and allowed to heat for three minutes, it was removed and allowed to cool to room temperature before adding a drop of extraction reagent 2 and the mixture was vigorously shook to obtain homogenous mixture. The mixture was centrifuged at 3000 rpm at 15 cm rotation radius for 5 minutes to obtain a supernatant solution containing the extracted PBP2a for MRSA.

Latex agglutination procedure For each supernatant to be tested, one circle of the test card was labeled '**T**' for testing with Test Latex and another with '**C**' for Control Latex. The latex reagent was properly mixed by inversion several times and a drop of test Latex or Control Latex was added to each labeled circle accordingly. 50µl of supernatant was placed on the Test circle and the Control circle and mixed thoroughly with the latex with the aid of the provided sterile plastic mixing stick. The mixing was done for three minutes and observed for agglutination under normal lighting conditions. The results of the Test and Control reactions were recorded before disposing the reaction card safely into disinfectant

Determination of MIC and MBC of the fractions on MRSA clinical isolates

Dilution schemes using formula $C_1V_1 = C_2V_2$ are given as shown in table 1

C_{F1} = Stock concentration of the fractions (EAF & DMF) = 50 mg/ml

V_{F1}= Volume of the fractions (EAF & DMF) in the agar dilution = to be determined

C_{F2}= Concentration of the fractions(EAF & DMF) in agar dilution (1 mg/ml – 10 mg/ml)

V_{F2}= Volume of reaction mixture in MHA plate = 20 ml

Table 1: GC-MS determination of bioactive components the fractions

S/N	C_{F1} (mg/ml)	V_{F1}(ml)	C_{F2}(Conc. of EAF & DMF) (mg/ml)	Volume of MHA (ml)	V_{F2} (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

Gas chromatography-mass spectrometry was performed on the two fractions of the *Moringa oleifera* root bark

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: Percentage yield of the Methanol crude extract and Fractions

Initial weight of ground moringa root bark 3000 g	Final weight of extract (g)	Percentage yield of extract
Methanol crude extract	850.60 ± 20.80	28.35 %
Percentage yield of the fractions from Methanol crude extract		
Ethyl acetate fraction	50.90 ± 8.70	5.98 %
Dichloromethane	150.70 ± 9.50	17.71 %

Qualitative Phytochemical analysis of the fractions of the extract

Table 3: Results of Phytochemical Analysis of the Methanol Extracts and Fractions

Chemical Constituent	Test	Crude methanol extract (ME)	Dichloromethane fraction (DMF)	Ethyl acetate fraction (EF)
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 presently.

Table 4: MIC and MBC of ethyl acetate fraction in mg/ml

S/N	Clinical isolates	MIC	MBC	S/N	Clinical isolates	MIC	MBC
1	SP4	5.6 ± 0.5	6.3 ± 0.5	21	EN390	4.6 ± 0.5	5.3 ± 1.1
2	SS8	4.3 ± 1.1	5.6 ± 1.1	22	SS310	5.3 ± 0.3	6.6 ± 0.5
3	AB20	4.6 ± 1.5	6.6 ± 0.5	23	OW417	4.6 ± 0.3	5.3 ± 0.3
4	SP22	5.3 ± 0.5	6.3 ± 0.5	24	AB570	4.8 ± 0.2	5.6 ± 0.5
5	OW30	5.0 ± 0.5	6.6 ± 0.5	25	OW578	5.3 ± 0.5	6.5 ± 1.0
6	SS33	5.3 ± 0.3	6.3 ± 1.0	26	AB600	5.3 ± 1.1	5.3 ± 1.0
7	EN35	5.6 ± 0.5	7.3 ± 0.5	27	OW620	4.5 ± 0.5	5.0 ± 0.5
8	OW36	5.0 ± 0.5	6.3 ± 0.5	28	SP651	5.5 ± 0.5	6.6 ± 0.5
9	EN38	5.3 ± 0.5	6.6 ± 0.3	29	OW819	3.6 ± 0.3	4.3 ± 0.5
10	SS42	5.6 ± 0.3	6.3 ± 0.5	30	EN831	4.3 ± 0.3	4.6 ± 1.0
11	OW53	6.6 ± 0.5	7.3 ± 0.5	31	AB841	5.6 ± 0.5	6.6 ± 0.5
12	SS57	5.3 ± 0.5	6.6 ± 1.0	32	OW940	4.3 ± 0.5	5.6 ± 0.3
13	AB61	6.6 ± 0.3	7.3 ± 0.5	33	OW947	3.5 ± 0.3	5.3 ± 0.3
14	EN62	5.6 ± 0.5	6.6 ± 0.3	34	AB1009	4.5 ± 0.3	5.6 ± 1.0
15	OW123	5.0 ± 0.5	6.3 ± 1.0	35	OW1104	4.3 ± 0.5	5.6 ± 0.5
16	EN127	6.6 ± 0.3	7.5 ± 0.5	36	SP1172	5.6 ± 0.3	7.3 ± 1.0
17	OW154	5.6 ± 0.5	6.5 ± 0.5	37	OW1420	5.6 ± 0.5	6.3 ± 0.3
18	AB187	6.6 ± 0.3	7.2 ± 1.1	38	OW1827	5.3 ± 0.3	6.0 ± 0.5
19	EN208	5.3 ± 0.5	6.5 ± 0.5	39	AB1956	4.6 ± 0.5	5.6 ± 1.1
20	SS235	5.6 ± 0.3	6.3 ± 0.5				

Values were expressed as Mean ± SD, N = 3

Key:

SP: Sputum

SS: Skin swab
AB: Abscess
OW: Open wound
EN: Ear/Nasal

Table 5: MIC and MBC of Dichloromethane fraction in mg/ml

S/N	Clinical isolates	MIC	MBC	S/N	Clinical isolates	MIC	MBC
1	SP4	7.5 ± 0.5	8.7 ± 0.5	21	EN390	7.5 ± 0.5	8.6 ± 1.0
2	SS8	6.3 ± 1.1	7.5 ± 1.1	22	SS310	8.7 ± 1.1	9.5 ± 1.3
3	AB20	6.5 ± 1.5	7.8 ± 0.5	23	OW417	8.3 ± 0.5	9.6 ± 1.0
4	SP22	8.2 ± 0.5	8.2 ± 0.5	24	AB570	8.7 ± 1.1	8.8 ± 0.5
5	OW30	7.5 ± 1.5	9.2 ± 0.5	25	OW578	6.7 ± 0.5	8.5 ± 1.1
6	SS33	7.6 ± 0.5	8.6 ± 1.0	26	AB600	7.5 ± 1.1	8.7 ± 1.0
7	EN35	6.6 ± 1.1	7.8 ± 0.5	27	OW620	8.5 ± 1.5	8.8 ± 0.5
8	OW36	8.7 ± 1.1	8.8 ± 0.5	28	SP651	7.6 ± 0.5	8.8 ± 0.5
9	EN38	6.7 ± 0.5	7.8 ± 1.1	29	OW819	6.2 ± 1.1	7.6 ± 0.5
10	SS42	7.5 ± 1.0	8.7 ± 0.5	30	EN831	8.5 ± 0.5	9.5 ± 1.1
11	OW53	8.2 ± 0.5	9.5 ± 0.5	31	AB841	9.3 ± 0.5	9.5 ± 1.1
12	SS57	7.8 ± 0.5	8.8 ± 1.0	32	OW940	8.5 ± 0.5	9.7 ± 1.1
13	AB61	8.8 ± 0.5	9.5 ± 0.5	33	OW947	7.8 ± 1.1	8.5 ± 1.1
14	EN62	8.7 ± 0.5	9.6 ± 1.1	34	AB1009	7.5 ± 1.1	7.6 ± 1.1
15	OW123	7.8 ± 0.5	8.8 ± 1.1	35	OW1104	8.0 ± 0.5	8.5 ± 0.5
16	EN127	8.5 ± 0.5	9.5 ± 0.5	36	SP1172	8.5 ± 1.1	9.6 ± 1.0
17	OW154	7.8 ± 0.5	8.3 ± 0.5	37	OW1420	8.7 ± 0.5	8.8 ± 0.5
18	AB187	8.5 ± 0.5	9.7 ± 1.1	38	OW1827	9.3 ± 1.1	9.5 ± 0.5
19	EN208	7.8 ± 0.5	8.7 ± 0.5	39	AB1956	8.2 ± 0.5	9.6 ± 1.1
20	SS235	8.8 ± 0.5	9.3 ± 0.5				

Values were expressed as Mean ± SD, N = 3
Key:
SP: Sputum
SS: Skin swab
AB: Abscess
OW: Open wound
EN: Ear/Nasal

Table 6: GC-MS report of ethyl acetate fraction

Structural analogue	Rt (min)	Area %	Peak
Stigmast-4-en-3-one , Cholest-4-en-26-oic acid, 3-oxo-Cholest-4-en-3-one	46.49	8.72	1
5-Bromovaleric acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester , Cyclobuta [1,2:3,4]dicyclooctene-1,7(2H,6bH)-dione.	46.58	3.19	2
3-Chloropropionic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester, 2-Amino-4-methyl-3-pyridinol, Pregn-4-en-3-one.	46.62	5.80	3
4,22-Stigmastadiene-3-one, 24S--Ethyl-5.alpha.-cholesta-2-dien-6-one, Spinasterone	48.54	12.29	4
4,22-Stigmastadiene-3-one, 4,22-Cholestadien-3-one , Ergosta-4,22-dien-3-one	48.62	10.73	5

9,19-Cycloergost-24(28)-en-3-ol, 4 ,14-dimethyl-,(3.beta.,4.alpha.,5 .alpha.)-7-(1,3-Dimethylbuta-1,3-dienyl)-1,6,6-trimethyl-3,8-dioxatricyclo[5.(2,4)]Octane 2(1H)-Naphthalenone.	49.97	2.89	6
11,13-Dimethyl-12-tetradecen-1-ol, Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl(E,E,E)-3,7,11,15-Tetramethylhexadeca1,3,6,10,14-pentaene.	50.49	2.28	7
9,19-Cycloergost-24(28)-en-3-ol ,14-dimethyl-(3.beta.,4 .alpha.,5.alpha.)-9,19-Cyclolanostan-3-ol,	50.52	2.57	8
Stigmast-4-en-3-one, Cholest-4-en-26-oic acid, 3-oxo- 1,4-Benzenediol,	51.34	28.23	9
Supraene, Octasiloxane, 1,1,3,3,5,5,7,7,9,92511,11,13,13,15,15-hexadecamethyl-17-(1,5-Dimethylhexyl)-10,13 dimethyl4vinylhexadecahydrocyclopenta [a]phenanthren-3-ol	52.51	1.39	10
17-(1,5-Dimethylhexyl)-10,13-dimethyl4vinylhexadecahydrocyclopenta [a]phenanthren-3-ol, Stigma sterol , 2,2-Dimethylpropanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester	52.52	1.18	11
Cholestan-3-one, 4,4-dimethyl-, (5.alpha.)-beta.-Alanine, N-(2-furoyl)-, hep tyl ester, 5.beta.,6.beta.-Epoxy-7.alpha.-bromocholestan-3.beta.-ol	54.48	3.57	12
4-Oxatricyclo[20.(7,16)]triac onta-1(20)7(16)-diene , 17-(1,5-Dimethylhexyl)-10, 13-dimethyl-4-vinylhexade cahydrocyclopenta [a]phenanthren-3-ol, Cholestan-3-one,	54.51	3.39	13
Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl) Cyclopropane carboxamide, 2-cyclopropyl-2-methyl-N-(1-cyclopropylethyl)-Benz[e]azulene-3,8-dione.	55.43	1.49	14
1,19-Eicosadiene, 9-Methyl-Z-10-tetradecen-1-ol, 1-Naphthalenepropanol.	56.02	3.51	15
2(1H)-Naphthalenone, octahydro-4a- methyl-7-(1-methylethyl)-, (4a.alpha. 7. beta.,8a.beta.)- 9-Methyl-Z-10-tetradecen-1-ol , 1-Naphthalenepropanol,	56.05	2.03	16
3a-Cholesterol acetate, Cholest-7-en-3-ol, Citrost-7-en-3-ol	59.18	3.96	17
Citrost-7-en-3-ol, Stigmastane-3,6-dione, Cholest-8-en-3.beta.-ol,	59.21	2.78	18

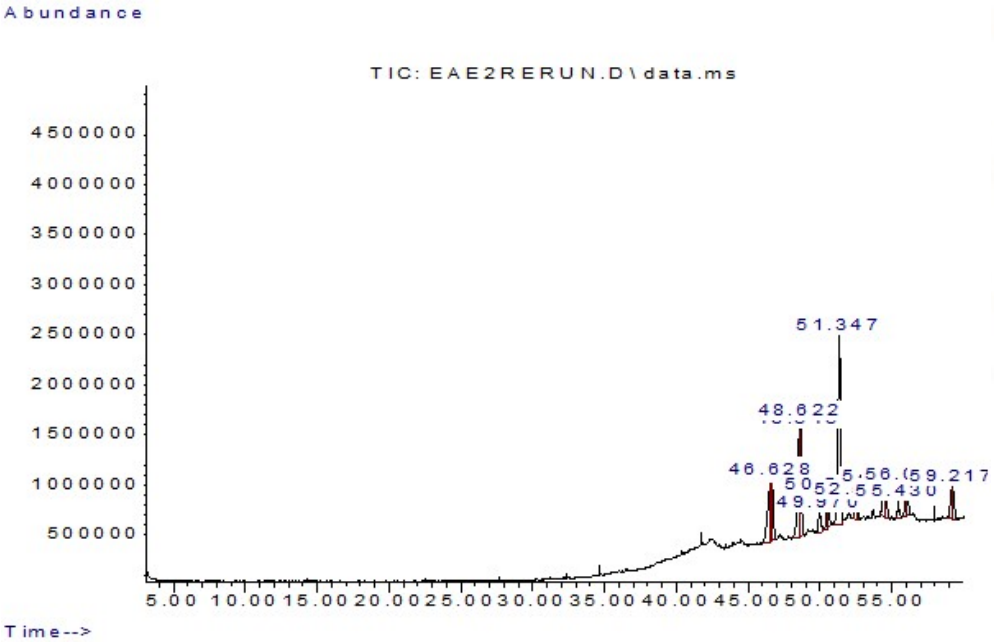

Fig. 1: MS fragment of ethyl acetate fraction composition

Fragmentation pattern for the identified compounds are presented in Fig. 1. The abundance of each compound, the peak height, percentage area and retention time are all shown in the graph. There is overlapping of fragments, showing that some of the functional groups can be repeated in another peak.

Table 7: GC-MS report of dichloromethane fraction

Structural analogue	Rt(min)	Area %	Peak
Benzylamine	11.33	0.46	1
Benzyl isocyanate, Phthalimidin	16.87	1.96	2
3-Methyl-4-isopropylphenol , Thymol , 2-methyl-5-(1-methylethyl) Phenol	25.97	1.57	3
N-Benzylformamide	30.21	0.68	4
Acetamide, N-(phenyl methyl)- 2-Methyl-5-butylpyridine , Dimethyl (1E)-N-hydroxyethanimidoy lphosphonate	32.09	2.78	5
Humulene	32.35	0.56	6
1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-1H Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-[3aS-(3a alpha.,3b.beta.,4.beta.,7.alpha.,7 aS*)]-.beta.-copaene	33.57	0.77	7
1, 2, 3, 5, 6, 8a-hexahydr-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)-Naphthalene.	35.41	1.06	8
tau.-Muurolol, alpha-Cadinol , cis-muurola-3,5-diene	40.21	0.99	9
.alpha-Cadinol, Cyclohexane, 1-ethenyl-1-methyl-2- (1-methylethenyl)-4-(1-methylethylidene)-Epiglobulol	40.71	1.70	10
Hexadecanoic acid, Pentadecanoic acid, 14-methyl-,methyl ester	50.64	5.89	11
n-Hexadecanoic acid	52.16	1.42	12
Cyclopropaneoctanoic acid, 2- methyl ester 9-Octadecenoic acid, (Z)-methyl Ster,cis-10-Heptadecenoic acid.	53.39	0.67	13
Heptadecanoic acid, Hexadecanoic acid.	53.92	0.39	14
9,12-Octadecadienoic acid, 10,13-Octadecadienoic acid,	56.07	4.29	15
9-Octadecenoic acid , 8-Octadecenoic acid, methyl ester	56.26	3.32	16
9-Octadecenoic acid, 11-Octadecenoic acid,	56.44	0.96	17
Methyl stearate	57.11	1.28	18
Urea, N,N'-bis(phenyl methyl)-Benzeneacetamide, alpha.-amino-Benzene acetic acid,	64.34	1.87	19
Docosanoic acid, methyl ester, Tricosanoic acid, methyl ester	68.62	0.53	20
Bis(2-ethylhexyl) phthalate, Di-n-octyl phthalate	21.69	0.37	21
5-Fluoro-1,3-bis[phenylmethyl]-2,4 (1H,3H)-pyrimidinedione, 5-Benzyloxy-6-methoxy-8-nitroquinoline, Ethisterone	70.47	0.39	22
2-Thiazolamine, 4-(4-methoxyphenyl)-N-(4-methylphenyl)-Naphtho[1,8-cd]-(1,2,6)-phosphadiazine, 2-phenyl-2-thioxo-1,3(2H)-di hydro-1-Dimethyl(ethenyl)silyloxy-3-phenylpropane	23.71	1.20	23
Benzene, 1-isocyanato-3-methoxy- 1H-S-Triazolo[1,5-a]pyridin-4-ium, 2-hydroxy-1-methyl-, hydroxide, inner salt, Didodecylphthalate,	76.72	0.73	24
Benzonitrile, 2-fluoro-4-(4'-propyl l[1,1'-bicyclohexyl]-4-yl)-Oxazole, 2-(3-methoxyphenyl)-5-phe nyl-Cyclopropane , carboxamide,	79.02	0.39	25
N-(phenylmethyl)- Acetamide,	80.83	0.69	26
Cedran-diol, (8S,14)- Phthalic acid, hexyl 1-phenylpropy l este, Benzo[1,3]dioxole-5-carboxylic aci d (5-chloro-2-oxo-1,2-dihydro-indo l-3-ylidene)-hydrazide	81.51	0.51	27
Stigmastan-3,5-diene; .beta.-Sitosterol acetate	81.97	0.57	28
Campesterol, (3.beta.)-Ergost-7-en-3-ol,	85.32	0.92	29
Stigmasterol	86.28	1.13	30

Continuation ofGC-MS report of dichloromethane fraction

Structural analogue	Rt(min)	Area %	Peak
Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)-3-n-Heptyl-7-methyl-9-(2,6, thylcyclohex-1-enyl)nona-2,4,6,8- etraenal ,1,3-Dioxolane.	87.33	0.38	31
4,4,6a,6b,8a,11,12,14b-Octamethyl-octadecahydro-2H-picen-3-one, 2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-.gamma.-Sitosterol.	88.06	1.63	32
5-Bromovaleric acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester, 6-Bromohexanoic acid,4-methoxyphenyl ester Cyclohexanecarboxylic acid, 4-methoxyphenyl ester	10.14	89.57	33
4,22-Stigmastadiene-3-one, Ethyl-5.alpha.-cholesta -dien-6-one, Ergosta-4,22-dien-3-one	90.75	11.91	34
Cholest-7-en-3-one, 4, 4-dimethyl-(5.alpha.)- Stigmasterol, Ergost-25-ene-3,5,6,12-tetrol	91.10	0.44	35
Benzofran-3-one, 2-[3,4-dihydroxybenzylidene]-6-hydroxy- Stigmasterol, Ergosta-4,6,22-trien-3-one	91.70	1.60	36
C(14a)-Homo-27-nor-14.beta.-gammaceran-3.alpha.-ol, 17-(1,5-Dimethylhexyl)-10,13-dimet hyl-4 vinylhexadecahydrocyclopenta[a]phenanthren-3-ol, 9,19-Cyclo-25,26-epoxyergostan-3-o l, 4,4,14-trimethyl-, acetate	92.21	2.77	37
Stigmast-4-en-3-one, Testosterone , Androst-4-en-3-one,	93.05	12.33	38
Nickel, cyclopentadienyl-(dicycloh exylphosphino)benzyl-o-yl- Stigmasta-4,6,22-trien-3.alpha.-ol, Stigmasta-4,6,22-trien-3.beta.-ol	94.09	1.53	39
-4,6,22-trien-3.beta.-ol , Stigmasta-4,6,22-trien-3.alpha.-ol, Stigmasta-3,5-dien-7-one	94.11	1.02	40
Cholestan-3-one, 4,4-dimethyl-, (alpha.)-Stigmastan-7-one, 17 (1,5Dimethylhexyl)-10,13-dimethyl-4vinylhexadecahydrocyclopenta[a]phenanthren-3-ol	96.05	2.17	41
Cholestan-3-one, 4,4-dimethyl-Dihydrosarsasapogenin-5,17(20)-die Phenol	96.08	1.98	42
C(14a)-Homo-27-nor-14.beta.-gammaceran-3.alpha.-ol 22-Stigmasten-3-one ,4-Diethyl thiophosphoryl-3-thiometh 1 yl allophanate	97.57	3.62	43
Stigmastane-3,6-dione, (5.alpha.)- Lanostane, 11,18-epoxy-, (11.beta. 17-(1,5-Dimethylhexyl)-2-(1-hydrox yethylidene)-10,13-dimethylhexadec ahydrocyclopenta[a]phenanthren-3-one	100.66	1.80	44
Stigmastane-3,6-dione , (5.alpha.)- anost-7-en-3-one, (9.beta.,,17.alpha.)- 3,12 Diazatetrabenzo[a,cd,j,lm] per ylene	100.71	1.62	45
Benzenepropanoic acid, 3,5-bis(1,1 -dimethylethyl)-4-hydroxy-, octade Benzenepropanoic acid, -dimethylethyl)-4-hydroxy-,octade cyl ester, beta.-Tocopherol,	101.15	2.59	46
Benzenepropanoic acid, 3,5-bis(1,1 -dimethylethyl)-4-hydroxy-, octade cyl ester	101.19	2.43	47

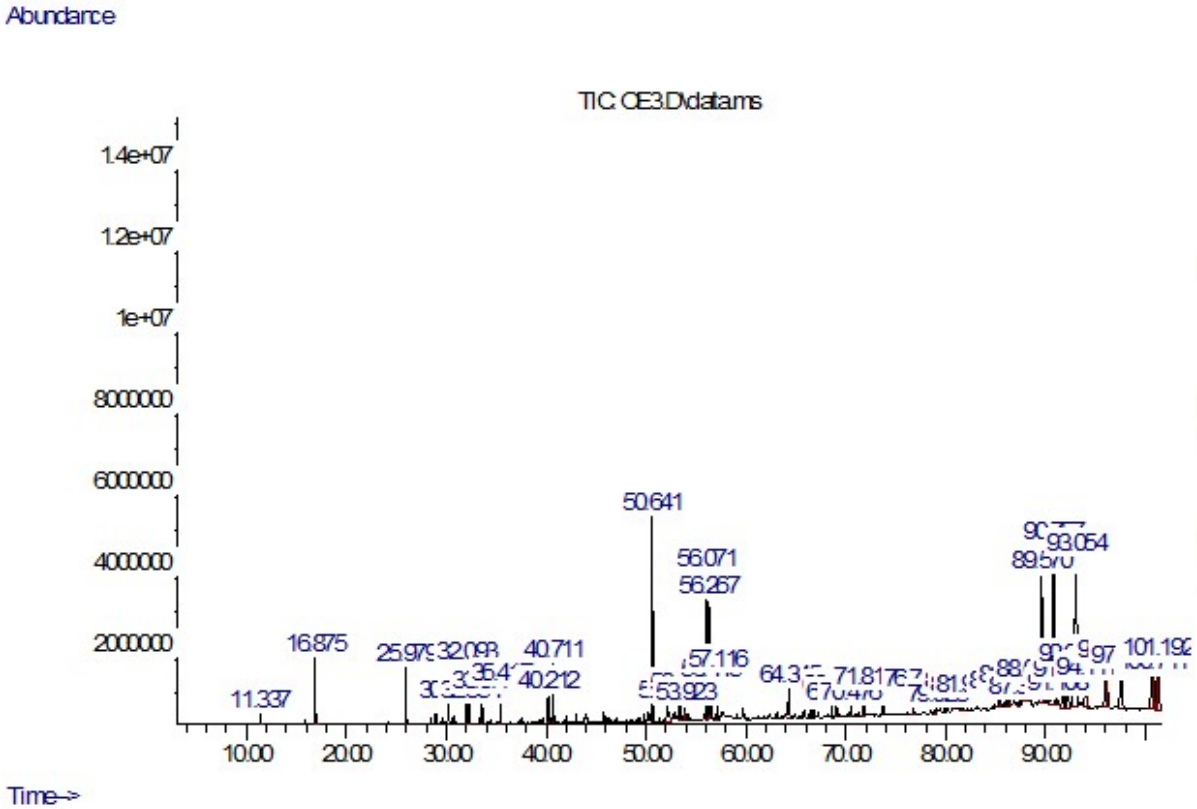

Fig. 2: MS fragment of dichloromethane fraction composition

Fragmentation pattern for the identified compounds are presented in Fig. 2.

The abundance of each compound, the peak height, percentage areas and retention time are all shown in the graph. There is also an overlapping of fragments, showing that some of the functional groups are repeated in another peak.

Discussion

Percentage yield of extractand fractions

The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent [24] (Ozarkar) [25]. The percentage yield of the extract and fractions indicates a good yield. The methanol crude extract 850.60 ± 20.8 g (28.35 %). The fact that dichloromethane fraction yield is more thanethyl acetate fraction means there are more polar phytochemical constituents in the roots of *Moringa oleifera* than non-polar [25, 26]

Qualitative phytochemical analysis of methanol extract and fractions

Phytochemical compounds may inhibit bacterial growth by mechanisms different from presently used treatment regimens, and could therefore be of clinical value in the treatment of resistant bacteria, including MRSA. Tannins, flavonoids, alkaloids, essential oils and many phenolic compounds serve as plant defense mechanisms against predation by insects, herbivores and infection by microorganisms (Cowan) [120].

MIC and MBC of the fractions

The results of the antibacterial testing of the fractions against all the MRSA clinical isolate as display in table 12 and 13, revealed that ethyl acetate fraction (EF) showed better activity than dichloromethane (DMF) fraction, possibly because of the presence of high concentration of flavonoids and other secondary metabolites eluted by the solvent, which has been shown to enhance antimicrobial property of a plant [13-15]. The MRSA isolates for this study restricted all the popular antibiotics used in the hospitals in varying degrees, but was inhibited by ethyl acetate fraction at concentrations of 3.5 mg/ml as the least MIC, 4.3 mg/ml as the least MBC and 6.6 mg/ml and 7.5 mg/ml as the highest MIC and MBC, while dichloromethane fraction (DMF) . This confirms the presence of bioactive principles in both fractions but the most potent component against the strain of MRSA is found in ethyl acetate fraction which can be formulated for the treatment of infections by the MRSA isolates.

Gas chromatography-mass spectrometry (GC-MS) of the fractions

A large numbers of compounds were identified in the fractions with these three compounds having the highest percentages, Stigmast-4-en-3-one (36.95%), molecular formula $C_{29}H_{48}O$, M.W 412, a ketone steroid with antimicrobial activities, antioxidant, anti-inflammatory, antiarthritic, antiasthma and diuretic activities (S. John and P. Kumar) [355], (Jennings and shibamoto)[357], Cholest-4-en-26-oic acid (36.95%) Molecular formula $C_{27}H_{42}O_4$, M.W 430, Aliphatic acid sesterterpenes that regulates the metabolism of cholesterol and homeostasis [356] , 3-oxo- 1,4-Benzenediol (28.23%) , $C_{18}H_{18}N_2O_5$, M.W 342, Oxygenated aldehyde with antimicrobial and antioxidant properties, antimycobacterial activity [353]. Other significant constituents with bioactivities are Spinasterone 12.29%, a seroid compound with antibacterial and antifungal activities [354], 5-Bromovaleric acid 3.19%, 2-Amino-4-methyl-3-pyridinol 5.8%, Ergosta-4,22-dien-3-one 10.73%, Pregn-4-en-3-one 5.8%, 2(1H)-Naphthalenone 2.89%, Pentaene 2.28%, (5.alpha.)-beta.-Alanine 3.57%, Supraene 1.39%, Oxime 1.49% and Citrost-7-en-3-ol 6.7% [354,355, 356, 357, 358].

CONCLUSION

Moringa oleifera is already highly esteemed by people in the tropics and sub-tropics for the many ways it is used nutritionally by people and medicinally by local herbalist. In recent years, laboratory investigations have confirmed the efficacy of some of these applications as found in leaves, flowers, pods, roots, root bark and stem bark, gum, seeds and seed oil [116-120].Ethyl acetate (EAF) fraction is more potent than dichloromethane fraction; this indicates that the most active compound against the MRSA can be isolated from the EAF for further analysis.

Conflict of Interest

No conflict of interest

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