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In vitro evaluation of Anti–MRSA properties and GC-MS bioactive compounds of methanol extract fractions of *Moringa oleifera* Lam. Root Bark

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Abstract
Objectives: Antimicrobial agents are commonly classified into bactericidal and bacteriostatic agents based on their antimicrobial activities. This research is as a result of the emergence of multiple antibiotic resistant *Staphylococcus aureus*, first known as methicillin resistant *Staphylococcus aureus* (MRSA) with potential of cross resistance to other antibiotics of choice like vancomycin and other newly developed antibiotics. The research is aimed to evaluate the anti – MRSA activities and GC-MS bioactive components of the Methanol extract fractions of the root bark of *Moringa Oleifera* on the clinical isolate of methicillin resistant *Staphylococcus aureus* (MRSA).
Methods: *Staphylococcus aureus* isolates from 3 different hospitals in South-east geopolitical region of Nigeria were confirmed by coagulase/staphylase test using Oxoid® reagents kits (DR0595A). The Methicillin resistant *staphylococcus aureus* confirmation was done using Oxoid® DR0900 penicillin binding protein (pbp2') latex agglutination test kits. Pulverised *Moringa oleifera* root bark was defatted with n-hexaneand the dried marc was extracted with methanol using Soxhlet extractor to obtain crude methanol extract (850.60 mg).The marc 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) and ethylacetate fraction (EAF). The Gas Chromatography–Mass Spectrometry (GC-MS) of each fraction was carried out to determine the bioactive compounds. The anti-MRSA activities ofDF and EAF fractions were evaluated with the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) assay. The ratios of antimicrobial substances were determined. Agent is bactericidal when the ratio MBC/MIC ≤ 4 and bacteriostatic when the ratioMBC/MIC is > 4.
Results: Latex agglutination test confirmed 39 strains of the clinical isolates to be MRSA. The MRSAwere sensitive to the extract fractions as EAF MIC (3.5 ± 0.3 to 6.6 ± 0.5 mg/mL) and MBC (4.3 ± 0.5 to 7.3 ± 1.0 mg/mL) and DF MIC (6.3 ± 1.1 to 9.7 ± 1.1 mg/mL) and MBC (7.5 ± 1.1 to 9.6 ± 1.1 mg/mL). The anti-MRSA activities evaluated showed that the bioactive compounds of the fractions were all bactericidal as the ratio MBC/MIC are all less than 4 ranging from DMF (0.12 – 1.27) and EAF (1.00-1.52). GC-MS analysis revealed over 100 distinct compounds, some of which are stigmasterol ($C_{29}H_{48}O$), eugenol ($C_{10}H_{12}O_2$), oxime (C_3H_7NO) and ergosta-4, 22-dien-3-one ($C_{28}H_{44}O$).
Conclusion: The results of this research confirmed the anti-MRSA activities of EAF and DMF fractions of methanol extract of *Moringa oleifera* Lam. root bark against Methicillin Resistant *Staphylococcus aureus* (MRSA) and increased activities in the following order of potency; EAF > DMF considering the values of the MIC and the MBC of the fractions.
Statistical analysis was done with ANOVA followed by Duncan post Hoc test using SPSS v 17 software.

Keywords: GC-MS, bioactive compounds, anti-MRSA, antimicrobial activities, *Moringa oleifera*

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 treating various ailments. 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].

Antibacterial and antifungal activities of Moringa roots bark
Moringa roots have antibacterial activity [1, 3] and are reported to be rich in antimicrobial principles. 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 [2]. The root extract also possesses antimicrobial activity attributed to the presence of 4-α-Lrhamnosyloxybenzyl isothiocyanate [2, 4]. The aglycone of deoxy-niazimicine [N-benzyl, Sethylthioformate] isolated from the chloroform fraction of an ethanol extract of the root barkwas found to be responsible for the antibacterial and antifungal activities [2, 5]. The bark extract has been shown to possess antifungal activity [5], while the juice from

the stem bark showed antibacterial effect against *Staphylococcus aureus* [5].

The discovery of sulfonamides and b-lactam antibiotics in the 1930s had a profound impact on human health by enabling rapid treatment of patients with bacterial infections that previously had often proved fatal [6, 7]. Over the next 40 years, now seen as the ''golden era'' of antibiotic research, the majority of antibiotic drug classes in use today were discovered. Since 1970, most newly approved antibiotics have been based on these known scaffolds, with the exception of linezolid (1), an oxazolidinone; daptomycin (2), a lipopeptide; and the topical antibiotics mupirocin (launched 1985), a pseudomonic acid, and retapamulin (3), a pleuromutilin derivative [8]. The potential for a major antibiotic healthcare crisis is best summarized by the Infectious Diseases Society of America (IDSA) [9] and the European Centre for Disease Prevention and Control [10], both of which report that there are only a few potential drugs in clinical development that (1) offer significant benefits over existing drugs and (2) that target Gram negative, hospital-based infections. Gram-negative bacteria are especially difficult to kill as they have an additional outer membrane permeability barrier that compounds need to surmount to be efficacious, as well as often possessing multiple efflux pumps, and antibiotic and target-modifying enzymes [11, 12, 13].

Methicillin resistant *Staphylococcus aureus* (MRSA)

Staphylococcus aureus is responsible for a broad range of clinical infections, most notable of which are cases of bacteremia and endocarditis [14]. Methicillin-resistant isolates with alterations to existing PBPs have been described. These isolates have been termed 'moderately resistant *S. aureus'* (MODSA). They are not frequently reported, the resistance is low-level and their clinical significance is unclear [15]. Methicillin resistance in *S. aureus* is primarily mediated by the *mec* A gene, which codes for the modified penicillin-binding protein 2a (PBP 2a or PBP 2') [16, 17]. PBP2a is located in the bacterial cell wall and has a low binding affinity for β-lactams. Although all cells in a population of *S.aureus* may carry the *mec*A gene, often only a few of the cells will express the gene [17, 18].

Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography (GC) is an analytical technique for volatile and semi-volatile compounds. Many organic solvents are analysed with GC since impurities in ethanol are basically volatile as well as ethanol itself (Ahmed S. John, Kumar P. (2012) [19]. Gas chromatography-mass spectrometry (GC-MS) is an integrated system of two analytical equipment. Gas chromatography separates analytes and mass spectrometry identifies them [20].

Materials and Methods

Materials

Sample collection

A sample size of 2,372 hospitalized patients with skin, throat, open wound, Ear/Nasal infections and Abscess were enrolled for this study. Samples were taken from sputum, open wound, abscess, ear and nasal swab from male and female wards in the orthopedic and intensive care departments of the hospitals. 1,230 samples were taken from University of Nigeria Teaching Hospital, 200 samples from Bishop Shanahan hospital and 942 samples from Federal Medical Centre, Ebonyi. The samples were distributed according to their clinical specimens as sputum (376), skin swab (327), absces swab (466), open wound swab (762) and ear/nasal swab (441) [21].

Reagents

Penicillin binding protein 2 prime (PBP2') test kits lot. no. 130422, Oxoid Ltd, Japan, staphylase test kit (Oxoid Ltd, Wade Road, Basingstoke, Hants, RG24, UK), Oxoid antimicrobial susceptibility test discs. Hydrogen peroxide (H2O2), Dimethylsulfoxide (DMSO), distilled water, silical gel (60-200 mesh), (Titan Biotech Ltd, India). 0.5 McFarland turbidity standard.

Equipment

Soxhlet extractor, glass chromatographic column, autoclave, refrigerator, weighing balance, incubator, antibiotic disc dispensers, GC-MS equipment with Agilent technologies 7890B for GC systems and Agilent technologies 5975 series for MS system.

Methods

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, Nsukka. Taxonomic identity of the plant was authenticated by Mrs. Immanuela Udoma of the Department of Pharmacognosy, Faculty of Pharmacy, University of Uyo, Uyo. The plant materials were air-dried in the laboratory for four weeks. The dried samples were ground to coarse powder with a mechanical grinder; the powder was 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 overnight. The marc was dried and extracted with 20 litres of methanol for 4 hours using Soxhlet extraction technique to yield crude methanol extractusing established standard procedures [22, 23]. The crude methanol extract 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.

Fractionation of crude methanol extract using column chromatography

Crude methanol extract (850.60 g) was adsorbed on silica gel (60-200 mesh) in a glass column, was then eluted in succession with dichloromethane, ethylacetate to yield dichloromethane fraction (DMF), ethyl acetate fraction (EAF). These extract fractions were air dried at room temperature for 24 h [23].

Characterisation of the clinical isolates

The clinical isolates obtained from the clinical samples collected were characterised culturally, based on their shapes, opacity, pigments, surface texture, edge, water emulsification. Microscopically by gram staining reaction and their appearance based on shape, arrangement and spore formation [21].

Biochemical tests
Catalase Test and Mannitol Salt Agar (MSA) Test was also used to characterised the organisms as *Staphylococcus* Spp

Slide coagulase/staphylase test
The test was carried out according to manufacturer's protocol, using the Oxoidstaphylase tests kits [24].

Penicillin-binding protein (PBP2′) latex agglutination test for MRSA confirmation

Preparation of culture
The PBP2' test should be 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. MRSA strain ATCC® 43300 (OxoidCulti – Loops C9022) was used as positive control [24].

Test method
(i) The PBP2′ extraction procedure as recommended by the Manufacturer Oxoid, Four drops of Extraction Reagent 1 was added to a micro centrifuge tube, an approximately 1.5 x 109 (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 ^{o}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 mins to obtain a supernatant solution containing the extracted PBP2′ for MRSA [24].

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. 50 µL 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. If agglutination is seen with test but not with Control Latex within three minutes PBP2′ is positive (MRSA), if no agglutination in either latex test and control test within 3 minutes PBP2′ is negative (MSSA [24].

Determination of MIC and MBC of dichloromethane (DMF) and ethyl acetate (EAF) fractions on the MRSA clinical isolates.
The Minimum Inhibitory Concentration (MIC) of the extract fractions was determined using the agar diffusion method [25].

Table 1: Preparation of extract fractions solution for agar dilution MIC test

S/N	C_1 (mg/ML)	V_1 (mL)	C_2 (gm/mL)	Volume of MHA (mL)	V_2 (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

Twenty ml volume of Muller Hinton Agar (MHA) was used in 9 cm Petri dishes for agar dilution MICs. Dilution schemes using formula $C_1V_1 = C_2V_2$ are given in Tables 2. [352].
C_1 = Stock concentration of the extract and fractions = 50 mg/mL
V_1 = Volume of the extract and fractions in the agar dilution = to be determined
C_2 = Concentration of the extract fraction in agar dilution (1 mg/mL – 10 mg/mL)
V_2 = Volume of reaction mixture in MHA plate = 20 mL.

By means of a sterile calibrated micro pipette, 0.002 mL of the MRSA clinical isolates suspension was streaked with a sterile loop on the surface of the MHA and allowed for 10 minutes for complete absorption of the inoculum by the medium. The plates were incubated in an inverted position at 37 ^{O}C for 24 h before taking the results. The least concentration that inhibits the growth of the organism is taken as the MIC (minimal inhibitory concentration). The control plate without antimicrobial agents was also incubated [25]. 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. 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 ^{O}C for 48h. 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 [16, 24].

Gas Chromatography–Mass Spectrometry (GC-MS) determination of bioactive
Components of methanol extract fractions
Gas chromatography-mass spectrometry was performed on the methanol fractions of ethyl acetate (EAF), and dichloromethane (DMF) fractions of the *Moringa oleifera*

root bark.

This is to identify the constituents of these fractions. The GC-MS analysis of the extract fractions was performed on an Agilent technologies model 7890 Series GC System equipped with an Agilent technologies 5975 MS detector (EI mode, 70 eV).

A column type DB-5 (5 % phenyl methylsiloxane) with a length of 30 m, an inside diameter of 0.25 µm and a film thickness of 320 µm was used. The temperature of the column was programmed to increase after 5 min from 50 ºC to 280 ºC at the rate of 5 ºC/min after which the run was left for 9 min at 280 ºC. Helium was used as a carrier gas at a flow rate of 1.4 ml/min.

The injector and detector temperatures were 300 ºC and 250 ºC, respectively.

The components in the extract samples under investigation were identified by comparing on the basis of gas chromatographic retention indices, mass spectra from National institute of standards and technology (NIST) Standard Reference Database 1A (NIST/EPA/NIH Mass Spectral Database (NIST 11) and NIST Mass Spectral Search Program (Version 2.0 g), Agilent Technologies, Inc. Chem Station Version) [26-30].

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 20 software. Differences were considered significant at $p < 0.05$.

Results

Characterisation of clinical isolates

The 58 clinical isolates confirmed to be *Staphylococcus aureus* from coagulase test were subjected to Penicillin binding protein ($PBP2^{'}$) latex agglutination test.

The 39 Clinical isolates out of the 58 isolates of *S. aureus* demonstrated resistance as indicated by latex agglutination test for MRSA as shown in table 6,

Table 6: Penicillin binding protein ($PBP2^{'}$) latex agglutination test as attached.

MIC and MBC of the extract fractions on MRSA clinical Isolates and (R) ratio MBC/MIC

Antimicrobial substances are considered as bactericidal agent when the ratio MBC/MIC ≤ 4 and bacteriostatic when the ratio MBC/MIC is > 4 (Joseph *et al*., 2015).

Table 2: MIC, MBC (mg/mL) and MBC/MIC ratio of Ethyl acetate fraction

S/N	Clinical isolates	MIC	MBC	MBC/MIC	S/N	Clinical isolates	MIC	MBC	MBC/MIC
1	SP4	5.6 ± 0.5	6.3 ± 0.5	1.13	21	EN390	4.6 ± 0.5	5.3 ± 1.1	1.15
2	SS8	4.3 ± 1.1	5.6 ± 1.1	1.30	22	SS310	5.3 ± 0.3	6.6 ± 0.5	1.25
3	AB20	4.6 ± 1.5	6.6 ± 0.5	1.43	23	OW417	4.6 ± 0.3	5.3 ± 0.3	1.52
4	SP22	5.3 ± 0.5	6.3 ± 0.5	1.19	24	AB570	4.8 ± 0.2	5.6 ± 0.5	1.17
5	OW30	5.0 ± 0.5	6.6 ± 0.5	1.32	25	OW578	5.3 ± 0.5	6.5 ± 1.0	1.23
6	SS33	5.3 ± 0.3	6.3 ± 1.0	1.19	26	AB600	5.3 ± 1.1	5.3 ± 1.0	1.00
7	EN35	5.6 ± 0.5	7.3 ± 0.5	1.30	27	OW620	4.5 ± 0.5	5.0 ± 0.5	1.11
8	OW36	5.0 ± 0.5	6.3 ± 0.5	1.20	28	SP651	5.5 ± 0.5	6.6 ± 0.5	1.2
9	EN38	5.3 ± 0.5	6.6 ± 0.3	1.20	29	OW819	3.6 ± 0.3	4.3 ± 0.5	1.19
10	SS42	5.6 ± 0.3	6.3 ± 0.5	1.13	30	EN831	4.3 ± 0.3	4.6 ± 1.0	1.07
11	OW53	6.6 ± 0.5	7.3 ± 0.5	1.11	31	AB841	5.6 ± 0.5	6.6 ± 0.5	1.17
12	SS57	5.3 ± 0.5	6.6 ± 1.0	1.25	32	OW940	4.3 ± 0.5	5.6 ± 0.3	1.30
13	AB61	6.6 ± 0.3	7.3 ± 0.5	1.11	33	OW947	3.5 ± 0.3	5.3 ± 0.3	1.51
14	EN62	5.6 ± 0.5	6.6 ± 0.3	1.18	34	AB1009	4.5 ± 0.3	5.6 ± 1.0	1.24
15	OW123	5.0 ± 0.5	6.3 ± 1.0	1.26	35	OW1104	4.3 ± 0.5	5.6 ± 0.5	1.30
16	EN127	6.6 ± 0.3	7.5 ± 0.5	1.14	36	SP1172	5.6 ± 0.3	7.3 ± 1.0	1.30
17	OW154	5.6 ± 0.5	6.5 ± 0.5	1.16	37	OW1420	5.6 ± 0.5	6.3 ± 0.3	1.13
18	AB187	6.6 ± 0.3	7.2 ± 1.1	1.09	38	OW1827	5.3 ± 0.3	6.0 ± 0.5	1.13
19	EN208	5.3 ± 0.5	6.5 ± 0.5	1.23	39	AB1956	4.6 ± 0.5	5.6 ± 1.1	1.22
20	SS235	5.6 ± 0.3	6.3 ± 0.5	1.13					

Values were expressed as Mean ± SD, N = 3

Key: SP: Sputum, SS: Skin swab, AB: Abscess, OW: Open wound, EN: Ear/Nasal

The MBC/MIC ratios of Ethylacetate fraction against all the MRSA tested were less than 4, showing that the activity of the extract faction against the MRSAs was Bactericidal

Table 3: MIC, MBC (mg/mL) and MBC/MIC ratio of Dichloromethane fraction

S/N	Clinical isolates	MIC	MBC	MBC/MIC	S/N	Clinical isolates	MIC	MBC	MBC/MIC
1	SP4	7.5 ± 0.5	8.7 ± 0.5	1.16	21	EN390	7.5 ± 0.5	8.6 ± 1.0	1.15
2	SS8	6.3 ± 1.1	7.5 ± 1.1	1.19	22	SS310	8.7 ± 1.1	9.5 ± 1.3	1.09
3	AB20	6.5 ± 1.5	7.8 ± 0.5	1.20	23	OW417	8.3 ± 0.5	9.6 ± 1.0	1.16
4	SP22	8.2 ± 0.5	8.2 ± 0.5	1.00	24	AB570	8.7 ± 1.1	8.8 ± 0.5	1.01
5	OW30	7.5 ± 1.5	9.2 ± 0.5	1.23	25	OW578	6.7 ± 0.5	8.5 ± 1.1	1.27
6	SS33	7.6 ± 0.5	8.6 ± 1.0	1.13	26	AB600	7.5 ± 1.1	8.7 ± 1.0	1.16
7	EN35	6.6 ± 1.1	7.8 ± 0.5	1.18	27	OW620	8.5 ± 1.5	8.8 ± 0.5	1.04
8	OW36	8.7 ± 1.1	8.8 ± 0.5	1.01	28	SP651	7.6 ± 0.5	8.8 ± 0.5	1.16
9	EN38	6.7 ± 0.5	7.8 ± 1.1	1.16	29	OW819	6.2 ± 1.1	7.6 ± 0.5	1.23
10	SS42	7.5 ± 1.0	8.7 ± 0.5	1.16	30	EN831	8.5 ± 0.5	9.5 ± 1.1	1.12
11	OW53	8.2 ± 0.5	9.5 ± 0.5	1.16	31	AB841	9.3 ± 0.5	9.5 ± 1.1	1.02
12	SS57	7.8 ± 0.5	8.8 ± 1.0	1.03	32	OW940	8.5 ± 0.5	9.7 ± 1.1	1.14

13	AB61	8.8 ± 0.5	9.5 ± 0.5	1.08	33	OW947	7.8 ± 1.1	8.5 ± 1.1	1.09
14	EN62	8.7 ± 0.5	9.6 ± 1.1	1.10	34	AB1009	7.5 ± 1.1	7.6 ± 1.1	1.01
15	OW123	7.8 ± 0.5	8.8 ± 1.1	1.13	35	OW1104	8.0 ± 0.5	8.5 ± 0.5	1.06
16	EN127	8.5 ± 0.5	9.5 ± 0.5	1.12	36	SP1172	8.5 ± 1.1	9.6 ± 1.0	1.13
17	OW154	7.8 ± 0.5	8.3 ± 0.5	1.06	37	OW1420	8.7 ± 0.5	8.8 ± 0.5	1.01
18	AB187	8.5 ± 0.5	9.7 ± 1.1	0.12	38	OW1827	9.3 ± 1.1	9.5 ± 0.5	1.02
19	EN208	7.8 ± 0.5	8.7 ± 0.5	1.12	39	AB1956	8.2 ± 0.5	9.6 ± 1.1	1.17
20	SS235	8.8 ± 0.5	9.3 ± 0.5	1.06					

Values were expressed as Mean ± SD, N = 3

Key: SP: Sputum, SS: Skin swab, AB: Abscess, OW: Open wound, EN: Ear/Nasal

The MBC/MIC ratios of Dichloromethane fraction against all the MRSA tested were less than 4, showing that the activity of the extract faction against the MRSAs was Bactericidal.

Gas Chromatography–Mass Spectrometry (GC-MS) determination of bioactive Compounds of Dichloromethane fraction (DMF)

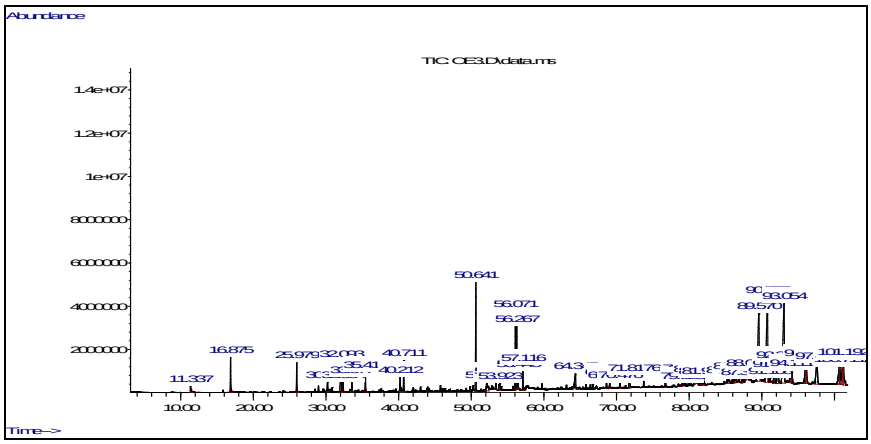

Fig 1: MS fragment of dichloromethane fraction composition Fragmentation pattern for the identified compounds are presented. The abundance of each compound, the peak height, percentage areas and retention time. There is also an overlapping of fragments, showing that some of the functional groups are repeated in another peak as shown in the graph.

Table 4: Bioactive compounds identified in the sample [GC MS study] DCM fraction

S/N	Name of Compounds	Molecular Formula	M.W	Nature	Activity
1	Benzylamine	C_7H_9N Chem Spider ID7223	107	Alkaloids	Antimicrobial
2	Benzyl isocyanate	$C_{10}H_{14}O$ Chem.Bk:2491615	150	Aromatic compound	Antimicrobial, Antitumor, Antiviral
3	Phthalimidin	$C_8H_7NO_2$ Pub. Chem ID171091	149	Organic Compound, phthalic anhydride	Antibacterial, Antifungal
4	3-Methyl-4-isopropylphenol, Thymol, 2-methyl-5-(1-methylethyl) Phenol	$C_{10}H_{14}O$ Lun.Chem:3228-02-2	150	Aromatic Alcohol, Monoterpines, Phenol Derivative	Antiviral, Antibacterial and Antifungal Activities
5	N-Benzylformamide	C_8H_9NO Pub. chem. ID:80654	135	Peptide, Amide Derivatives	Antimicrobial, Antimalaria[
6	Acetamide	C_2H_5NO Pub. Chem: ID 178	59	Peptide, Amide Derivative	Anti-cancer, Anti-inflammatory, Antioxidant
7	N-(phenyl methyl)- 2-Methyl-5-butylpyridine	$C_{12}H_{11}N$,Guide.Chem CAS27012-22-2	169	Esther, Pyridine Derivative	Antimicrobial analogues
8	Dimethyl (1E)-N-hydroxyethanimidoylphosphonate	$C_{10}H_{21}ClN_2O_2$ MFCD08457445	236	Piperidine carboxylic acid, Isoprenoid	Antibacterial and Ant parasitic drug
9	Humulene	$C_{15}H_{24}$ Pub Chem:5281520	204	monocyclic sesquiterpene	Antimicrobial and Antitumor activity
10	1,6-Cyclodecadiene	$C_{10}H_{16}$Chem Spider ID: 4517601	136		Antimicrobial and Antifungal activity
11	1-methyl-5-methylene-8-(1-methylethyl)-1H Cyclopenta[1,3]cyclopropa[1,2]benzene	$C_{15}H_{24}$ Look Chem CAS 13744-15-5	204	Aromatic compound	Antioxidant, Antimicrobial and Insecticidal agent,
12	7 aS*)]-. beta-copaene	$C_{15}H_{24}$ChemSpider ID: 10306774	204	A tricyclic sesquiterpene	Antimicrobial
13	1, 2, 3, 5, 6, 8a-hexahydr-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)-Naphthalene	$C_{15}H_{24}$ChemSpider ID: 389830	204	Aliphatic Alcohol	Antimicrobial and Antifungal Activity
14	alpha-Cadinol	$C_{15}H_{26}O$ ChemSpiderID: 8574094	222	Phenol	Antimicrobial, Antifungal, Anticancer
15	cis-muurola-3,5-diene	C15H24 (CHEBI:61687)	204	carbobicyclic compound, sesquiterpene	Antibacterial, Antioxidants
16	Hexadecanoic acid (Palmitic Acid)	$C_{16}H_{32}O_2$ChemSpider ID:	256	Ester, Fatty Acid	Antibacterial, Anti-inflammatory,

		960			Antitumor
17	Pentadecanoic acid	$C_{15}H_{30}O_2$ChemSpider ID: 13249	242	Saturated Fatty Acid	Antibacterial, Antifungal activity
18	Cyclohexane, 1-ethenyl-1-methyl-2- (1-methylethenyl)-4-(1-methylethylidene)-Epiglobulol	C_6H_{12} ChemSpider ID: 7787	84	Aromatic Phenols	Cytotoxic, Antimicrobial, Antifungal, Antioxidant
19	Cyclopropaneoctanoic acid	$C_{22}H_{38}O_2$ NIST CAS10152-71-3	334	Natural alicyclic fattyacids	Antimicrobial [370]
20	2-methyl ester 9-Octadecenoic acid, Methyl stearate	$C_{19}H_{36}O_2$, NIST CAS 1937-62-8	296	Ester	Antioxidant activity, Ant carcinogenic, -exist in human blood and urine and serve as endogenous peroxisome proliferatoractivated receptor ligand, dermatitigenic flavor
21	Heptadecenoic acid	$C_{17}H_{32}O_2$. Pub. Chem CID 5312435	268	Fatty Acid	Antifungal
22	Urea	CH_4N_2O Chem Spider ID: 1143	60	Organic Compound	Antimicrobial
23	N,N'-bis(phenyl methyl)-Benzeneacetamide	C14H21 N O, Guide Chem CAS. 34251-46-2	219	Endogenous Peptide	Antimicrobials, Herbicidal
24	alpha.-amino-Benzene acetic acid	C8H9NO2. Chemical Book CAS 2835-06-5	151	Carboxylic Acid, Amino Acid, Ester	Antibacterial and Antifungal
25	Docosanoic acid (Behenic Acid)	$C_{22}H_{44}O_2$ Pub. Chem ID 8215	340	Saturated Fatty Acid	Antibacterial
26	Tricosanoic acid	$C_{23}H_{46}O_2$. Pub. Chem CID 17085	354	Long Chain Fatty Acid	Antimicrobial and Insecticidal activity
27	5-Fluoro-1,3-bis[phenylmethyl]-2,4 (1H,3H)-pyrimidinedione,	$C_{18}H_{15}FN_2O_2$ ChemSpider ID: 309662	310	Peptides	Antiglioma, Antimicrobial, Cytotoxic
28	5-Benzyloxy-6-methoxy-8-nitroquinoline	$C_{17}H_{14}N_2O_4$. Pub. Chem 563944	310	Quinolines, Ethyl Esther	Antitumor Antibiotic, Antimalarial, Antileishmanial, Antimicrobial
29	Ethisterone	$C_{21}H_{28}O_2$ Pub Chem 5284557	312	Steroid Hormone	Antibacterial and Antitumor
30	2-Thiazolamine	$C_3H_4N_2S$ CAS 96-50-4	100	Hydrazine derivatives, Heterocyclic Amine	Antimicrobial, Anti-infective and Antioxidant
31	]-(1,2,6)-phosphadiazine	$C_{16}H_{13}N_2PS$ Chem Spider 544268	296	Hydrazine derivatives	Antimicrobial(Topical) Agents
32	Dimethyl(ethenyl)silyloxy-3-phenylpropane	$C_{13}H_{20}OS$ Pub. Chem 00576332	220	Pyridine Derivatives	Antimicrobial
33	Didodecylphthalate	$C_{24}H_{38}O_4$ChemSpider ID: 8043	390	Carboxylic Acid	Antimicrobial, Food Preservative
34	Cedran-diol	$C_{15}H_{26}O_2$ Pub Chem 536384	238	Flavonoids	Antimicrobial anti-inflammatory, anticancer
35	Inner salt	$C_{11}H_{23}COHNSO_3$ EC N0 226-003-9	292	sulfonium salts	Antimicrobial and Antifungal
36	2-fluoro-4-(4'-propyl l[1,1'-bicyclohexyl]-4-yl)-Oxazole	$C_{26}H_{40}O_5$, CAS 913258-34-1	432	Carboxylic Acids, Diterpines	Antimicrobial
37	Benzonitrile	C_7H_5N Chem Spider ID: 7224	103	Benzo and naphthonitrile derivatives.	Antibacterial and Antifungal
38	Benzene	C6H6	78	Aromatic Compound	Antibacterial and Antifungal
39	2-(3-methoxyphenyl)-5-phenyl-Cyclopropan-1- carboxamide	$C_{24}H_{20}FNO_4$ Pub. ChemID 59206456	405	Carboxylic Acid	Anticonvulsant, Antituberculosis
40	N-(phenylmethyl)-Acetamide,	$C_9H_{11}NO$ ChemSpider ID: 11016	149	Peptide Amide Derivative	Trypanosidal, Antibacterial Antifungal
41	(5-chloro-2-oxo-1,2-dihydro-indo l-3-ylidene)-hydrazide			Isatin, Imidazoline	Antibacterial, antifungal, Anticancer, Antihelmintic
42	Stigmastan-3,5-diene	$C_{29}H_{48}$ Pub Chem ID 00525918	396	Steroid compound	Antifungal, Antibacterial Antioxidant
43	beta.-Sitosterol acetate	$C_{29}H_{50}O$ CAS 83-46-5	414	Plant Sterols	Benign prostatic hyperplasia, Antimicrobial
44	Campesterol	$C_{28}H_{48}O$	400	Steroidal compound	Antimicrobial, Antileishmanial
45	(3.beta.)-Ergost-7-en-3-ol,	$C_{28}H_{48}O$ Pub. Chem ID 86509	400	Fatty Alcohols, Steroid compound	Antibacterial, Anticandidasis, Antioxidant.
46	Stigmasterol	$C_{29}H_{48}O$ Chem Spider ID: 504066	412	Fatty Alcohols, Steroid compound	Antimicrobial Antioxidant Anti-inflammatory, Antiarthritic Antiasthma Diuretic
47	Pyridine-3-carboxamide	$C_8H_7N_3O$ Pub.Chem CID 80322	161	Nicotinamide, Nicotine Amines.	Antiviral, Anticancer, antiprotozoa
48	Oxime	C_3H_7NO ChemSpider ID: 60524	73	Natural Phenolic flavonoid	Antioxidant, Antibacteria and anticancer agent
49	N-(2-trifluoromethylphenyl)-3-n-Heptyl-7-			Alkyl Esthers	Useful for inhibiting the delta isoform

	methyl-9-(2,6, thylcyclohex-1-enyl)nona-2,4,6,8- etraenal				of PI3K, and for treating disorders mediated by lipid kinases such as inflammation, immunological, and cancer.
50	1,3-Dioxolane	C3H6O2 CB 5712494	74	Aprotic solvent, Ether	Intermediate for the preparation of Acyclovir-d4, Antibacterial
51	14b-Octamethyl-octadecahydro-2H-picen-3-one	$C_{30}H_{48}O$ Pub.Chem ID 612782	424	Triterpine	Antibiotic Prototype, Antioxidant
52	14b-Octamethyl-octadecahydro-2H-picen-3-one	$C_{30}H_{48}O$ Pub.Chem ID 612782	424	Triterpine	Antibiotic Prototype, Antioxidant
53	2(1H)Naphthalenone	$C_{10}H_{10}O$ Chem Spider ID: 21106584	146	Aliphatic Alcohol, ally amines	Antibacterial and antifungal.
54	8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-.gamma.-Sitosterol			Steroid compound	Frangrance Ingredient
55	5-Bromovaleric acid	$C_5H_9BrO_2$, CAS No.: 2067-33-6	181	B-Aroylacrylic acids, Polyether.	Antimicrobial
56	2,6-dimethylnon-1-en-3-yn-5-yl ester	$C_{16}H_{24}O_2$ Pub. Chem ID 00530940	248	Ester	Moderate Antibacterial and Antifungal, Vector control
57	6-Bromohexanoic acid	$BrCH_2(CH_2)_4COOH$	195	Carboxylic Acid	As a conjugate in Antitumor drug
58	Cyclohexanecarboxylic acid	$C_6H_{11}CO_2H$ CAS Num 98-89-5	128	Carboxylic acid, Ester	Food and Flavor Ingredient, Antifungal
59	4,22-Stigmastadiene-3-one	$C_{29}H_{46}O$ Pub.Chem: ID5364563	410	Steroid Compound	Antimicrobial Antioxidant Anti-inflammatory, Antiarthritic Antiasthma Diuretic
60	Ethyl-5.alpha.-cholesta -dien-6-one	$C_{29}H_{48}O$ Chem Spider ID: 504066	412	Steroid compound	Antimicrobial Antioxidant Anti-inflammatory, Antiarthritic Antiasthma Diuretic
61	Ergosta-4,22-dien-3-one	$C_{28}H_{44}O$ Chem Spider ID:4941415	396	Triterpenoids and Steroids, Pyridine	Antimicrobial and Antitumor, Anti-inflammatory, Anti-viral.
62	Cholest-7-en-3-one	$C_{27}H_{44}O$ Pub. Chem.ID 27296	384	Steroid Compound, Peptide	antibacterial, antifungal, antiviral, and antiprotozoal
63	Ergost-25-ene-3,5,6,12-tetrol	$C_{28}H_{48}O_4$ ChemSpider ID: 473378	448	Steroid Compound, Peptide	Cytotoxic effect
64	Benzofran-3-one	$C_{15}H_{10}O_5$ ChemSpider ID: 4444681	270	Triterpenoids and Steroids, Pyridine	Antileishmanial, Antimicrobial
65	2-[3,4-dihydroxybenzylidene]-6-hydroxy-Stigmasterol	$C_{29}H_{48}O$ Chem Spider ID: 504066	412	Fatty Alcohols, Steroid compound	Antimicrobial Antioxidant Anti-inflammatory, Antiarthritic Antiasthma Diuretic
66	Ergosta-4,6,22-trien-3-one	$C_{28}H_{42}O$ ChemSpider ID: 4526809	394	Aromatic Steroids	Antimicrobial
67	C(14a)-Homo-27-nor-14.beta.-gammaceran-3.alpha.-ol	$C_{30}H_{52}O$ Pub. ChemID 550124	428	Fatty Alcohols, Steroid compound	Peptide Receptor Targeting in Cancer
68	Dimethylhexyl)-10,13-dimet hyl-4 vinylhexadecahydrocyclopenta[a]phenanthren-3-ol	$C_{29}H_{50}O$ ChemSpider ID: 467796	414	Steroid compound	Antibiofilm, Insecticidal activity
69	9,19-Cyclo-25,26-epoxyergostan-3-ol4,4,14-trimethyl-, acetate	$C_{33}H_{54}O_3$ Pub Chem 565753	498	Steroid compound	Anti inflammatory
70	Stigmast-4-en-3-one	$C_{29}H_{48}O$ Chem Spider ID: 504066	412	Steroid compound	Antimicrobial Antioxidant Anti-inflammatory, Ant arthritic Antiasthma Diuretic
71	Testosterone, Androst-4-en-3-one	$C_{19}H_{28}O_2$ Pub. Chem:ID 6013	288	Androgenic Steroid	Anti-inflammatory
72	Nickel, cyclopentadienyl-(dicyclohexylphosphino)benzyl-o-yl-	$C_{24}H_{34}NiP^-$ Pub. Chem ID 11987286	412	Isoflavones, Flavonoids	Chiral Langand in synthesis of drugs
73	Stigmasta-4, 6, 22-trien-3.alpha.-ol, Stigmasta-4, 6, 22-trien-3.beta.-ol, Stigmasta-3, 5-dien-7-one, 4, 4-dimethyl-, (alpha.)-Stigmastan-7-one	$C_{29}H_{46}O$ Pub. Chem ID 5379793	410	Fatty Alcohols, Steroid compound	Antimicrobial Antioxidant Anti-inflammatory
74	Cholestan-3-one	$C_{27}H_{46}O$ ChemSpider ID: 77468	386	Fatty Alcohols, Steroid compound	Antimicrobial Antioxidant Anti-inflammatory
75	C(14a)-Homo-27-nor-14.beta.-gammaceran-3.alpha.-ol	$C_{30}H_{52}O$ Pub. Chem ID 550124	428	Chenodeoxycholic acid, steroid acid	cholagogue, a choleretic laxative
76	(5.alpha.)- Lanostane	$C_{30}H_{54}$ Chem Spider ID:7827588	414	Triterpines compound	Antibacterial, Antiinflammatory
77	10,13-dimethylhexadec ahydrocyclopenta[a]phenanthren-3-one	$C_{23}H_{35}NO_3$ Chem. Book	373	Phenyl Ester	Antibiofilm Agent
78	5. Alpha.)- anost-7-en-3-one	$C_{19}H_{28}O$ CB1139907	272	Androgenic Steroid	Antimicrobial, Anti-inflammatory
79	Benzenepropanoic acid-dimethylethyl)-4-hydroxy-,octadecyl ester	$C_{37}H_{66}O_3$ Chem. Net CAS56823-64-4	558	Acid Ester	Ester Prodrugs and Antiviral Activity
80	beta.-Tocopherol	$C_{28}H_{48}O_2$. Pub.hem86052	416	Beta Carotene	Cancer Prevention, Antioxidants

Gas Chromatography–Mass Spectrometry (GC-MS) determination of bioactive Components of Ethylacetate fraction (EAF)

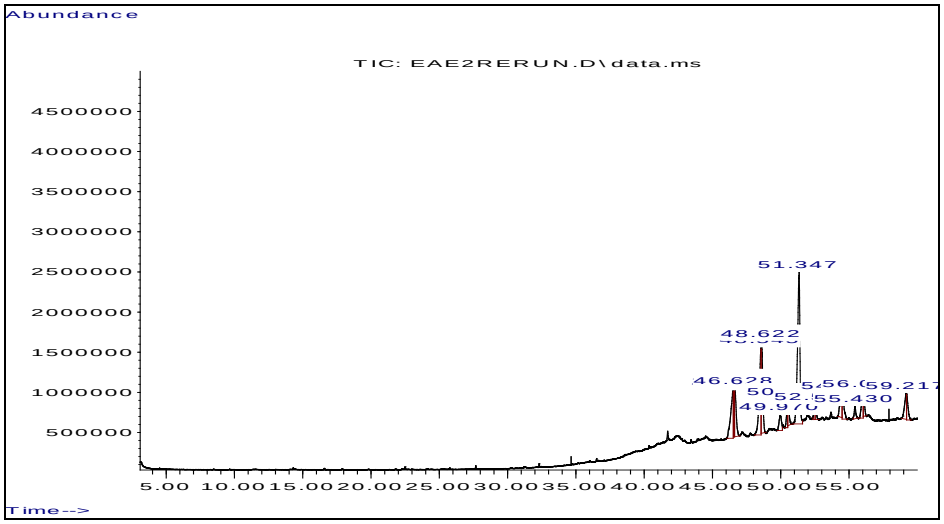

Fig 2: MS fragment of ethylacetate fraction composition Fragmentation pattern for the identified compounds are presented. The abundance of each compound, the peak height, percentage areas and retention time. There is also an overlapping of fragments, showing that some of the functional groups are repeated in another peak as shown in the graph.

Table 5: Active compounds identified in the sample [GC MS study] of ethyl acetate fraction

S/N	Name of compounds	Molecular.formula	M.W	Nature	Activity
1	Stigmast-4-en-3-one,	$C_{29}H_{48}O$ Chem Spider ID: 504066	412	Ketone, Steroid compound	Antimicrobial, Antioxidant Anti-inflammatory, Antiarthritic Antiasthma Diuretic
2	4,22-Stigmastadiene-3-one	$C_{29}H_{46}O$ Pub.Chem: ID5364563	410	Ketone, Steroid Compund	Antimicrobial Antioxidant Anti-inflammatory, Antiarthritic Antiasthma Diuretic
3	Cholest-4-en-26-oic acid	$C_{27}H_{42}O_4$ Chem Spider ID:20566404	430	Aliphatic Acid, Sesterterpenes	Metabolism and regulation of cholesterol homeostasis
4	5-Bromovaleric acid	$C_5H_9BrO_2$, CAS No.: 2067-33-6	181	B-Aroylacrylic acids, Polyether.	Antimicrobial
5	2,6-dimethylnon-1-en-3-yn-5-yl ester,	$C_{16}H_{26}O_2$, NIST MS No 299336	250	Oxime esther derivatives	Antimicrobial, Virucidal activity
6	Cyclobuta [1, 2:3, 4] dicyclooctene-1, 7(2H, 6bH)-dione.	C16H24O2 Pub. Chem. ID: 579875	248	Hydrocarbons	No Activity reported
7	3-Chloropropionic acid	$C_3H_5ClO_2$ Chem. Spider ID: 7611	108	chlorinated aliphatic acids	protocols in organic synthesis including Antibacterial agents
8	2-Amino-4-methyl-3-pyridinol	$C_5H_6N_2O$ ChemSpider ID: 26152	110	Pyridine derivatives, Alcohol	Antimicrobial, antitumor agents and herbicide
9	-bis (Methylene-dioxin)-Pregn-4-en-3-one	C25H34O6 ChemSpider ID: 4444479	486	Endogenous steroid hormone	Metabolic Intermediate in Production of other endogenous steroid, Treatment of Brain Injury
10	24S--Ethyl-5.alpha.-cholesta-2-dien-6-one, Spinasterone	$C_{29}H_{46}O$ PubChemID: 5364566	410	Steroid compound	Antibacterial and antifungal activities
11	2,2-Dimethylpropanoic acid (Pivalic acid)	$C_5H_{10}O_2$ ChemSpider ID: 7611		Pivalic (Carboxylic) acid	Antimicrobial
12	4vinylhexadecahydrocyclopenta[a] phenanthren-3-ol	$C_{29}H_{50}O$ ChemSpider ID: 467796	414	Steroidal compound	Antibiofilm, Insecticidal activity
13	Ergosta-4,22-dien-3-one	$C_{28}H_{44}O$ ChemSpiderID:4941415	396	Triterpenoids and Steroids, Pyridine	Antimicrobial and Antitumor, Anti inflammatory, Anti viral.
14	2(1H)-Naphthalenone.	$C_{10}H_{10}O$ Chem Spider ID: 21106584	146	Aliphatic Alcohol, ally amines	Antibacterial and antifungal.
15	11,13-Dimethyl-12-tetradecen-1-ol	$C_{18}H_{34}O_2$ NIST: MS NO: 130810	282	Fatty Alcohols, Phenolic	Antioxidant
16	Bicyclo[5.2.0] nonane	C_9H_{16}, Pub. ChemID:524792	124	Sesquiterpene compound	Antimicrobial,Anti-inflammatory, Anti Hyperlipidemic
17	Pentaene	$C_{20}H_{32}$ Chem Spider ID:391697	272	Polyene Lipids	Antimicrobial
18	3. beta., 4. Alpha. 5. alpha.)-9,19-Cyclolanostan-3-ol,	$C_{32}H_{54}O_2$ PubChemID 00537304	470	Triterpenes	Antimicrobial, Antioxidant
19	3-oxo- 1, 4-Benzenediol	$C_{18}H_{18}N_2O_5$ Chem Spider 677762	342	Oxygenated Aldehyde Derivatives, Indole analogues	Antimicrobial, Antioxidant
20	Supraene	$C_{30}H_{50}$ Pub. Chem.ID: 638072	410	Sesquiterpenoid and triterpenoid	Anticancer, antimicrobial, antioxidant, chemo preventive pesticide, anti- tumor sunscreen
21	Octasiloxane	$C_{40}H_{74}O_{13}$ Sigma Alreich	987	Sesquiterpenoid,	Antibacterial, Antifungal

		CAS Number 352538-83-1		organosilicon compound	
23	Pyridine-3-carboxamide	$C_6H_6N2_{18}O$ ChemSpider ID: 9334423	124	Nicotinamide, Nicotine Amines.	Antiviral, Anticancer, antiprotozoa
24	Oxime	C_3H_7NO ChemSpider ID: 60524	73	Natural Phenolic compound	Antioxidant, Antibacteria and anticancer agent
25	N-(2-trifluoromethylphenyl) Cyclopropane carboxamide,	$C_{15}H_{18}F3NO$ Pub. Chem ID 00722477	285	Aliphatic Hydrocarbon	Pesticides in domestic animals
26	Citrost-7-en-3-ol	$C_{30}H_{52}O$ Pub. Chem.ID: 541368	428	Aliphatic Alcohol Compound	Antimicrobial, Antioxidants
27	1-Naphthalenepropanol	$C_{13}H_{15}NO$ CAS No.: 19352-04-6	201	Phenolic compound	Antimicrobial and Antifungal

Table 6: Latex agglutination test for MRSA

S/N	Ear/Nasal	LT	Inf.	Abscess	LT	Inf.	Sputum	LT	Inf.	Skin swab	LT	Inf.	Open wound	LT	Inf.
1	EN35	PBP2'+ve	MRSA	AB20	PBP2'+ve	MRSA	SP4	PBP2'+ve	MRSA	SS8	PBP2'+ve	MRSA	OW30	PBP2'+ve	MRSA
2	EN38	PBP2'+ve	MRSA	AB61	PBP2'+ve	MRSA	SP22	PBP2'+ve	MRSA	SS33	PBP2'+ve	MRSA	OW36	PBP2'+ve	MRSA
3	EN62	PBP2'+ve	MRSA	AB187	PBP2'+ve	MRSA	SP651	PBP2'+ve	MRSA	SS42	PBP2'+ve	MRSA	OW53	PBP2'+ve	MRSA
4	EN127	PBP2+ve	MRSA	AB570	PBP2'+ve	MRSA	SP1172	PBP2'+ve	MRSA	SS57	PBP2'+ve	MRSA	OW123	PBP2'+ve	MRSA
5	EN208	PBP2'+ve	MRSA	AB600	PBP2'+ve	MRSA				SS235		MRSA	OW154	PBP2'+ve	MRSA
6	EN390	PBP2'+ve	MRSA	AB841	PBP2'+ve	MRSA				SS310		MRSA	OW417	PBP2'+ve	MRSA
7	EN831	PBP2'+ve	MRSA	AB1009	PBP2'+ve	MRSA							OW578	PBP2'+ve	MRSA
8				AB1956	PBP2'+ve	MRSA							OW620	PBP2'+ve	MRSA
9													OW819	PBP2'+ve	MRSA
10													OW940	PBP2'+ve	MRSA
11													OW947	PBP2'+ve	MRSA
12													OW1104	PBP2'+ve	MRSA
13													OW1420	PBP2'+ve	MRSA
14													OW1827	PBP2'+ve	MRSA
15															
16															
17															
18															
19															
T.T	7			8			4			6			14		39 MRSA

Discussion

MIC and MBC of ethyl acetate fraction

The results of the antibacterial testing of the ethyl acetate fractionagainst all the MRSA clinical isolate as shownin table 2, ethyl acetate fraction (EAF) showed better activity than DMF, Inhibited MRSA at concentrations of 3.5 mg/ml as the MIC and 4.3 mg/ml as the MBC. This confirms the presence of bioactive principles that can be formulated for the treatment of infections by the MRSA acquired in the hospitals. The results also confirm that the plantcontains more bioactive compounds inthe polar solvent with higher potency than the compounds in DMF which is less polar [23, 27].

MIC and MBC of dichloromethane fraction (DMF)

The results of the antibacterial testing of the dichloromethane fraction (DMF) against all the MRSA isolates seems to show low activity compared to theethyl acetate fractions, this can be due to reduction in the concentrations of active principles [23] in the less polar fraction. The antibacterial results in table 3 indicates that the (DMF) still maintain some level of activities against the MRSA clinical isolates because of the presence of antibacterial compounds from the GC-MS report known as benzyl isocyanate [27,28] in agreement with previous study of (Shafiqur Rahman and Laila Zerin (2008) [4] that the compound has potent antibacterial property. The MIC and MBC of the fraction ranges from6.3 ± 1.1 - 9.3 ± 0.5 and 7.5 ± 1.0 - 9.7 ± 1.1, respectively.

Gas chromatography-mass spectrometry (GC-MS) of ethyl acetate fraction

In this study a total of twenty seven (27) compounds were identified in the fraction 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) [29], (Jennings and shibamoto) [20, 30], 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 [29], 3-oxo- 1,4-Benzenediol (28.23%), $C_{18}H_{18}N_2O_5$, M.W 342, Oxygenated aldehyde with antimicrobial and antioxidant properties [20, 29, 30]. Other significant constituents with bioactivities are Spinasterone 12.29%, a seroid compound with antibacterial and antifungal activities [29], 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% [20, 27, 29, 30].

Gas chromatography-mass spectrometry (GC-MS) of dichloromethane fraction

In this study DMF contains the highest number of bioactive principles 80, but not as active as ethyl acetate fraction in the antimicrobial susceptibility test, showing that there is a potent compound in EAF that is probably not in DMF or a synergistic effect of the compounds in EAF but not exhibited in DF, it may be overlapping of derivatives of same compounds.

The compounds with high percentages in this fraction are 5-Bromovaleric acid 89.57%, 2, 6-dimethylnon-1-en-3-yn-5-yl ester 89.57%, Stigmast-4-en-3-one 12.33%, Testosterone 12.33%, Ergosta-4, 22-dien-3-one 11.91%, Hexadecenoic acid 7.31% nature and activities of the compound are in agreement with references [27, 28, 29, 30].

Conclusion

The rapid identification of these bio-active compounds, however, is critical if this tool of drug discovery is to compete with developments in technology. Plant preparations are distinguished from chemical drugs due to their complexity-mixtures containing large numbers of bio-active compounds. This brings about the challenge of drug discovery from natural sources. Approximately 9000 different flavonoids have been reported from plant sources, and with almost certainty many more are still to be discovered, as they continue to capture the interests of scientists from numerous disciplines. Based on the 10-carbon skeleton o flavonoids, they can be substituted by a range of different groups, *viz*. hydroxyl, methoxyl, methyl, isoprenyl and bezyl substituents.

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