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Antibacterial Evaluation and GC-MS Analysis of the Essential Oil from Botanically Certified Oleo Gum Resin of *Boswellia Sacra* (Sudanese Luban)

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Abstract

The aim of the present study is to assess the phytochemical screening, investigate the chemical constituents of the Essential Oil from *Boswellia sacra* Seeds and to evaluate its potential antibacterial activity. Using the Soxhlet method to extract the essential oil from *Sudanese Luban* Seeds. The chemical constituents of *Sudanese Luban* Oil were identified and quantified by GC-MS, where the paper disc diffusion assay was employed to evaluate the antibacterial activity. Phytochemical screening showed that Alkaloids, Flavonoids, Triterpens, Streols, Tannins and phenolic compounds are present in Boswellia sacra *(Sudanese Luban)*. Twenty five components have been identified. Nine of them are major namely; Hexadecanoate methyl ester (16.08%); 9,12-Octadecadienoate (Z,Z)-methyl ester (21.03%); 9-Octadecenoate (Z)- methyl ester (12.58%); Methyl stearate (14.40%); E,E-3,13-Octadecadien-1-ol (4.54%); Oxiraneoctanoic acid, Octyl -,methyl ester (3.88%); Eicosanoic methyl ester (7.33%); Docosanoic acid, methyl ester (8.15%); Tetracosanoic acid, methyl ester (3.13%). The antibacterial showed a high inhibitory effect against Escherichia coli (17mm), Pseudomonas aeruginosa (16mm), Bacillus subtilis (15mm), moderate against Bacillus subtilis (14mm) and inactive against Candida albicans. In conclusion, the Essential Oil from *Boswellia sacra* Seeds a good source of natural antibacterial, and justify its uses in folkloric medicines.

Keywords: Phytochemical screening; GC-MS analysis; Antibacterial and antioxidant activities.

1. Introduction

The development of bacterial resistance to treatment, especially the multiple resistance as well as the negative side effects of some treatments and the high costs of their preparation, production and other reasons led to the attention of alternative medicinal to overcome these problems and perhaps the field of medicinal plants are one of the most important alternatives that are studied continuously in the most countries of the world [1]. The frankincense tree spread in Sudan is considered to be an important economic commodity and is considered one of the most famous materials sold in the shops of the tires and medicinal herbs it is used in the treatment of many diseases such as tumors, inflamed sores and cough. It is also has non-medical uses, the most important and the most popular is the manufacture of incense [2]. The resins of *Boswellia serrata* have been used for the treatment of rheumatoid arthritis and their inflammatory diseases [2] such as Crohn's disease [3]. In traditional medicine of many countries. The anti-inflammatory activity has been attributing to the resin's ability in regulating immune cytokines production [4] and leukocyte infiltration [5, 6]. *Boswellia serrata* extract also exhibits anti-bacterial and anti-fungal activities [6] Additionally, extracts from *Boswellia* species gum resins might possess anti-cancer activities, based on their anti-proliferative and pro-apoptotic activities in rat astrocytoma cell lines and Clinically, an extract from the resin reduces the peritumoral edema in glioblastoma patients [7], and in human leukemia cell lines [8] as well as their anti-carcinogenic activity in chemically induced mouse skin cancer models [9]. The pharmacological characteristics and clinical efficacy of *Boswellia serrata* have been studied, with research published and systematically reviewed in the medical literature [10]. These results suggest that frankincense resin contains active ingredients that modulate important biological activities. *B. serrata* flowers and leaves showed significant antibacterial activity [11] In addition *B. serrata* has versatile pharmacological activities [12]. However, there are no enough scientific reports to support these supposed antimicrobial activity. The present investigation was undertaken which deals with the evaluation of antimicrobial activity of frankincense resin extract of *Boswellia serrata*.

2. Materials and Methods

2.1. Extraction of Oil

100 g of the seeds was grounded into a fine powder. Powdered seeds were extracted with n-hexane using Soxhlet extractor for six hours. The volume of hexane was reduced under reduced pressure. The oil of *Boswellia sacra* **(**Sudanese Luban) was obtained by evaporating the reduced hexane by air drying in a steady current. The oil was kept in a refrigerator for further manipulation. The extract was screened for the presence of phenolic compounds, flavonoids, tannins, terpenoids, saponins and alkaloids using standard methods [13].

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2.2. GC -MS Method

The qualitative and quantitative analysis of the sample was carried out by using GC MS technique model (GC /MS-QP2010-Ultra) from japan "Simadzu Company ,with capillary column (Rtx-5ms -30 m × 0.25 mm ×0.25 µm).The sample was injected by using split mode ,helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60 C with rate 10 C /min to 300 c as final temperature degree , the injection port temperature was 300c, the ion source temperature was 200 c and the interface temperature was 250 c .The sample was analyzed by using scan mode in the range of m/z 40 – 550 charge to ratio. Identification of component for the sample was achieved by comparing their retention times and mass fragmentation patent with those available in the library, the National Institute of Standards and Technology (NIST).,results were recorded.

2.3. Testing of Antibacterial Susceptibility

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines [14]. Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on the surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

3. Results and Discussion

The preliminary phytochemical screening of *Boswellia sacra* ethanolic extracts showed the presence of various secondary metabolites such as, Alkaloid, flavonoid, triterpenes, sterol, and tannins which are the bioactive principles responsible for medicinal values of the respective plants were all present. These phytoconstituents were detected in varied concentrations in the methanol extracts were presented in Table1.

Table-1. Phytochemical screening of *Boswellia sacra* extract

No	Constituents	Test	Results
1	Alkaloids	Mayer's,Wanger's reagent	+
2	Flavonoid	Alkaline reagent	+
3	Saponins	Forth	-
4	Triterpen,Streol	Liberman	+
5	Tannins,phenolic	Ferric hloride,/Alminum chloride	+

(+) present ; (-) -indicates absent

The oil extracted from *Boswellia sacra* seeds was investigated by GC-MS analysis. Identification of the oil constituents was based on retention times and the observed fragmentation pattern and twenty five components were detected. The typical total ion chromatogram (TIC) is presented in Figure 1**.** The chemical constituents of the oil are outlined in Table 2**.** The spectra of the compounds are matched with Wiley 9.0 and NIST libraries.

Figure-1. The typical GC chromatogram of Boswellia sacra seeds oil

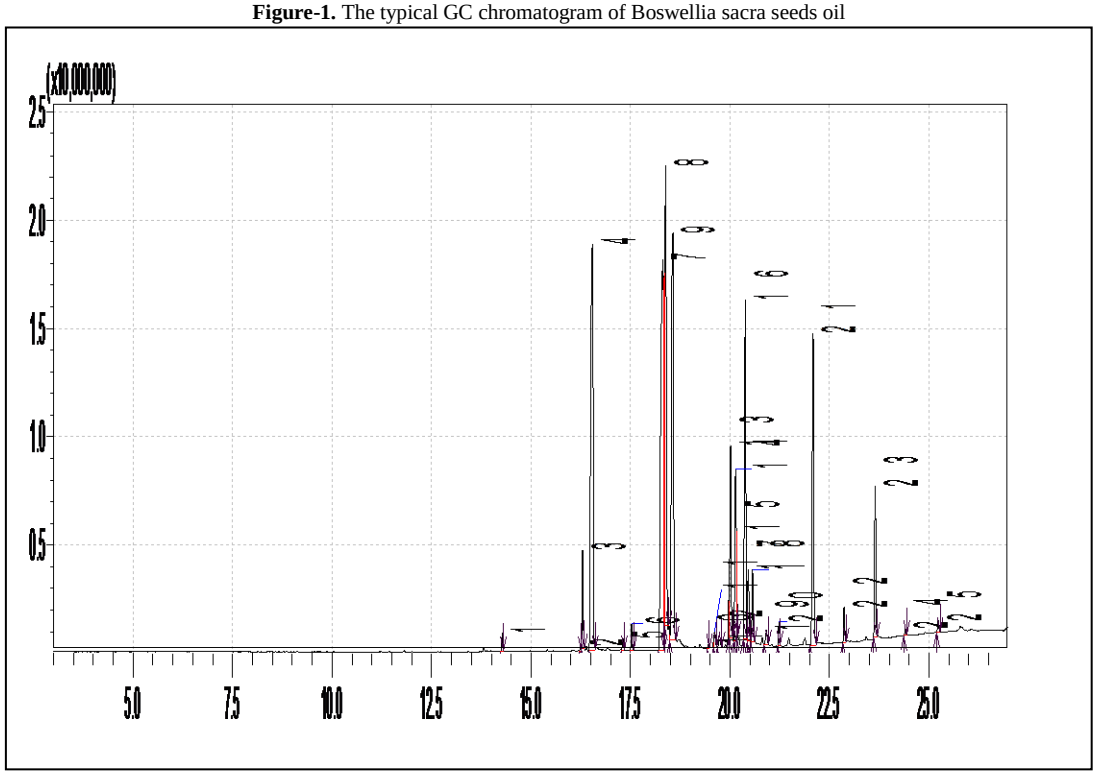


Table-2. Chemical constituents of *Boswellia sacra* Oil

Peak report TIC					
Compound	**R.T**	**M+**	**BP**	**Key fragment ions**	**Name of compound**
1	14.270	284	74	199 185 143 87 41	Methyl tetradecanoate
2	16.248	158	55	264 222 123 98 84 74 7 41	6-Octadecenoic acid, methyl ester
3	16.296	172	55	236 194 152 98 87 69 41	9-Hexadecenoic acid, methyl ester, (Z)-
4	16.540	168	74	227 199 171 143 87 57 41	Hexadecanoic acid, methyl ester
5	17.311	196	55	250 208 166 98 69 41	cis-10-Heptadecenoic acid, methyl ester
6	17.527	198	74	241 199 185 143 129 87 57 41	Heptadecanoic acid, methyl ester
7	18.303	214	67	263 164 150 109 95 81 55 41	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
8	18.384	192	55	222 180 123 98 87 69 41	9-Octadecenoic acid (Z)-, methyl ester
9	18.563	298	74	267 255 199 143 87 57 41	Methyl stearate
10	19.458	242	74	281 269 143 129 87 57 43	Nonadecanoic acid, methyl ester
11	19.614	256	79	261 175 150 121 107 93 67	Methyl9.cis.,11.trans.t,13.trans. octadecatrienoate
12	19.740	268	79	194 150 135 107 93 67 55 41	.gamma.-Linolenic acid, methyl ester
13	20.014	268	55	248 222 194 149 135 109 81	E,E-3,13-Octadecadien-1-ol
14	20.138	270	55	199 171 155 109 87 74 69 41	Oxiraneoctanoic acid, Octyl -,methyl ester
15	20.165	280	67	248 150 136 109 95 81 55	cis-11-Eicosenoic acid, methyl ester
16	20.384	282	74	295 283 227 199 143 87 57	Eicosanoic acid, methyl ester
17	20.458	144	99	208 177 164 93 80 67 55 41	7,10,13-Eicosatrienoic acid, methyl ester
18	20.564	294	67	262 109 95 81 55 41	6,9-Octadecadienoic acid, methyl ester
19	20.918	296	79	205 180 161 106 93 67 55	cis-5,8,11-Eicosatrienoic acid, methyl ester
20	21.230	298	74	297 241 143 87 57 43	Heneicosanoic acid, methyl ester
21	22.088	282	74	311 143 87 57 43 41	Docosanoic acid, methyl ester
22	22.868	292	74	325 269 143 87 57 43 41	Tricosanoic acid, methyl ester
23	23.650	294	74	339 283 143 87 55 41	Tetracosanoic acid, methyl ester
24	24.404	310	74	353 297 199 185 143 87 43	Pentacosanoic acid, methyl ester
25	25.230	392	74	367 311 199 185 143 87 57	Hexacosanoic acid, methyl ester

The Oil of *Boswellia sacra* showed high inhibitory effect against Escherichia coli (17mm), Pseudomonas aeruginosa (16mm), Bacillus subtilis (15mm), moderate against Bacillus subtilis (14mm) and inactive against Candida albicans (-). The observations results were recorded in Table (3).

Table-3. Antibacterial activity of the *Boswellia sacra* oil

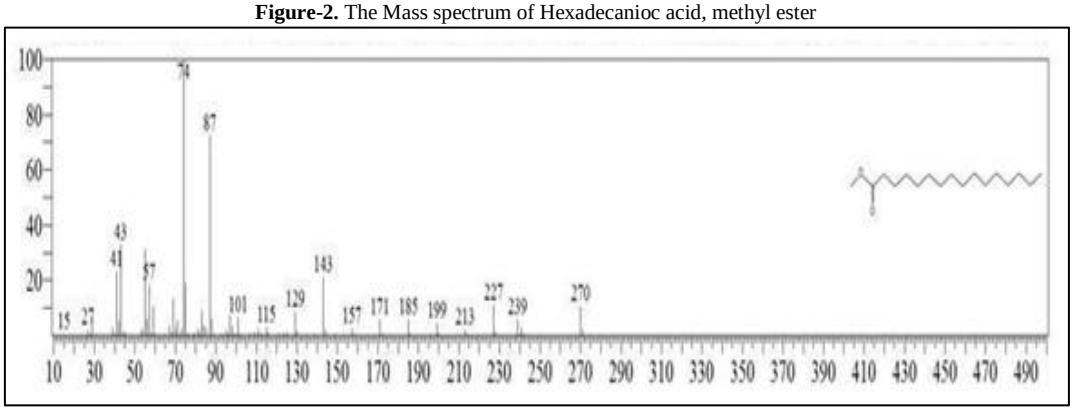
Sample conc. 100 µl/ml	**Zone of inhibition (mm)**				
	Ec	Ps	Sa	Bs	Ca
Boswellia sacra seeds oil	17	16	15	14	-

Ec=Escherichia coli, ps = Pseudomonus aureus; Sa=Staphylococcus Bs =Bacillus subitus, aeregnosia ; Ca=Candida albicans

Twenty five components have been identified *Boswellia sacra* seed oil. Nine of them are major namely Hexadecanoic acid, methyl ester (16.08%), 9,12-Octadecadienoic acid (Z, Z)-, methyl ester (21.03%), 9-Octadecenoic acid (Z)-, methyl ester (12.58%), Methyl stearate (14.40%) , E, E-3,13-Octadecadien-1-ol (4.54%) , Oxiraneoctanoic acid, Octyl -methyl ester (3.88%), Eicosanoic acid, methyl ester (7.33%), Docosanoic acid, methyl ester (8.15%), Tetracosanoic acid, methyl ester (3.13%). Main constituents are: Hexadecanoic acid, methyl ester, appeared at 14.270 min in the GC chromatogram with peak aria 16.08 %, it was produced molecular ion m/z at 270 $[M]^+$ corresponded to an elemental composition $C_{17}H_{34}O_2$ in it mass spectra Figure 2 as well as the following fragment ions: 227, 143, 87 and 74 (base peak) which are in agreement with literature [15]. This compound had been

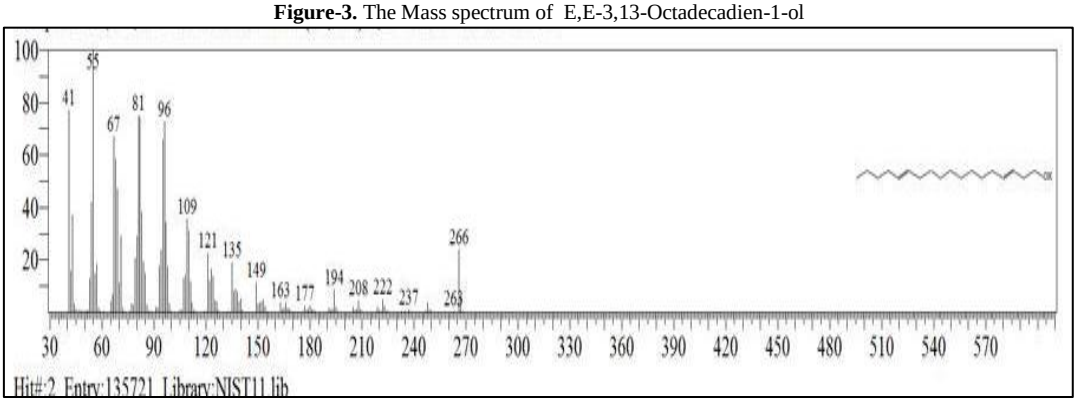
reported to cause autolysis of membranous structures, induce significant aortic dilation, and inhibit phagocytic activity and nitric oxide production of certain cells [16].

Figure-2. The Mass spectrum of Hexadecanioc acid, methyl ester



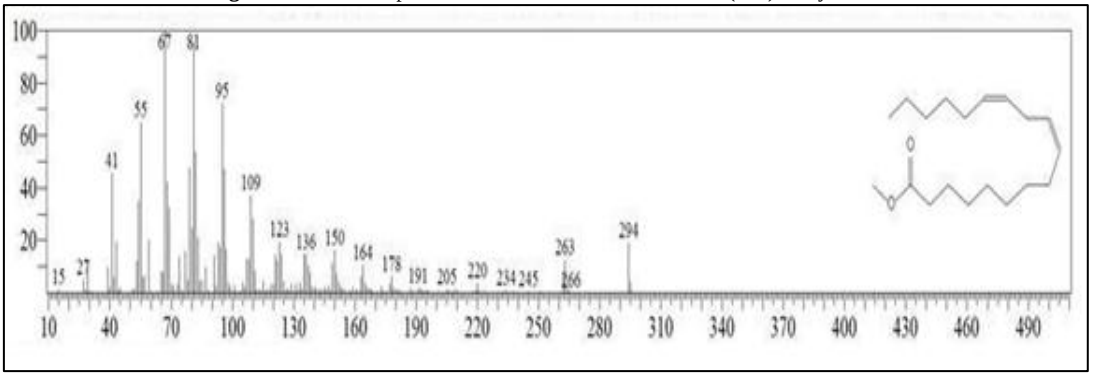
The peak at 20.014 min with area 4.54%, and has Ms ions *m/z* 266 [M] + correspond to formula C18H34O2 Figure 3 as well as the following fragment ions: 248 , 222 , 194 ,149 , 135, 109, 81 and 55 (base peak) which are in agreement with E, E-3, 13-Octadecadien-1-ol.

Figure-3. The Mass spectrum of E,E-3,13-Octadecadien-1-ol



The peak at 18.303 min with Area (21.03%) on GC chromatogram, produced molecular ion peaks *m/z* at 294 $[M]^+$ corresponded to formula $C_{19}H_{34}O_2$ in it MS spectra Figure 4, in addition of fragment ions: 263 , 164, 150, 109, 95 ,81, 55, 41 and 67 (base peak) by direct comparison with the Standard Mass Library spectral data and those reported in Sledzinski, *et al.* [16], this compound identified to be 9, 12 octadecadienoic acid (Z,Z), methyl ester which is known to has antifungal potential [17, 18].

Figure-4. The Mass spectrum of 9,12- octadecadienioc acid (Z-Z)methyl ester



There is another peak appeared at 16.296 min with area (12.58%) it mass spectra produced molecular ion m/z at 296 $[M]^+$ corresponded to molecular formula $C_{19}H_{36}O_2$ Figure 5 as well as the following fragment ions: 236 , 194, 152, 98, 87, 69, 41 and 55 (base peak) which are in agreement with 9- octadecenioc acid, methyl ester [19], which possess Antioxidant, anti-cancer. The GC chromatogram showed peak at 18.563with area (14.40%), it Mass spectrum showed ions *m/z* 298 $[M]^+$ correspond to formula $C_{19}H_{38}O_2$ Figure 6 as well as the following fragment ions: 267 , 255 , 199 , 143, 87, 57 , 41 and 74 (base peak) which are good agreement with methyl stearate. The chromatogram also showed four peaks at 20.138 , 20.384 , 22.088 and 23.650 with area 3.88% , 7.33 , 8.15 and 3.13%) % respectively, their masses spectrum Figures 7, Figure 8, Figure 9 and Figure 10 produced molecular ion peaks *m/z* at 312 $[M]^+$, 326 , 354 and *m/z* at 382 $[M]^+$, these compounds identified to be Oxiraneoctanoic acid,

Octyl -methyl ester $C_{19}H_{36}O_2$; Eicosanoate methyl ester $C_{21}H_{42}O_2$; Docosanoate methyl ester $C_{23}H_{46}O_2$; and Tetracosanoate methyl ester $C_{25}H_{50}O_2$ respectively.

Figure-5. The Mass spectrum of 9- octadecenioc acid, methyl ester

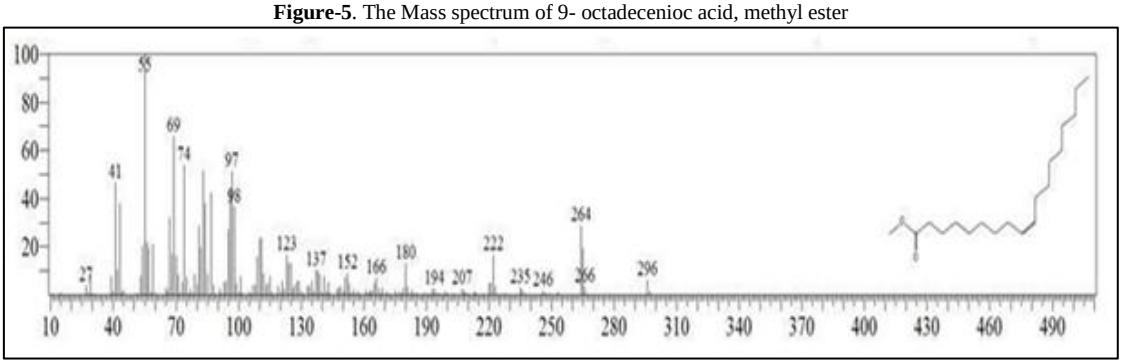


Figure-6. The Mass spectrum of Methyl stearate

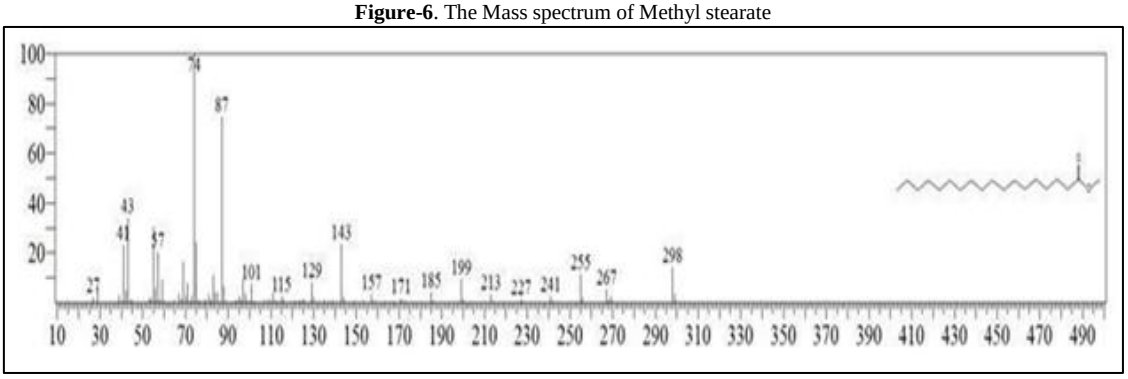


Figure-7. The Mass spectrum of Oxiraneoctanoic acid, Octyl -,methyl ester

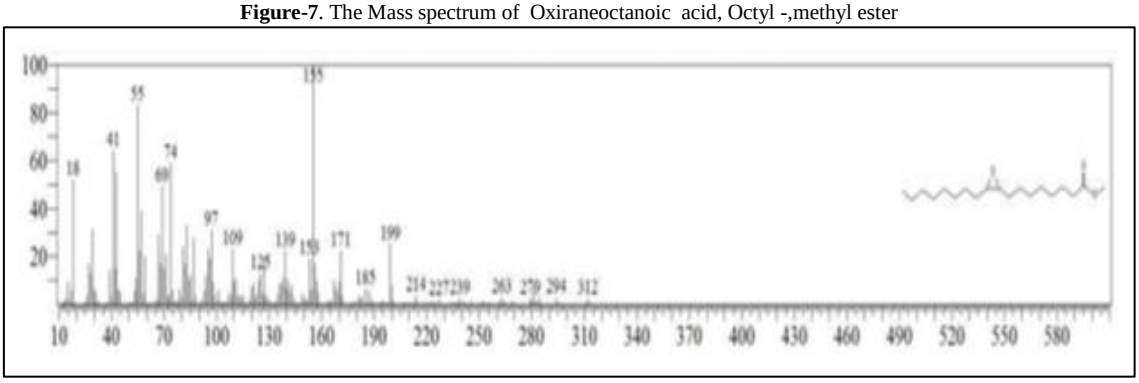


Figure-8. The Mass spectrum of Eicosenoic acid,methyl ester

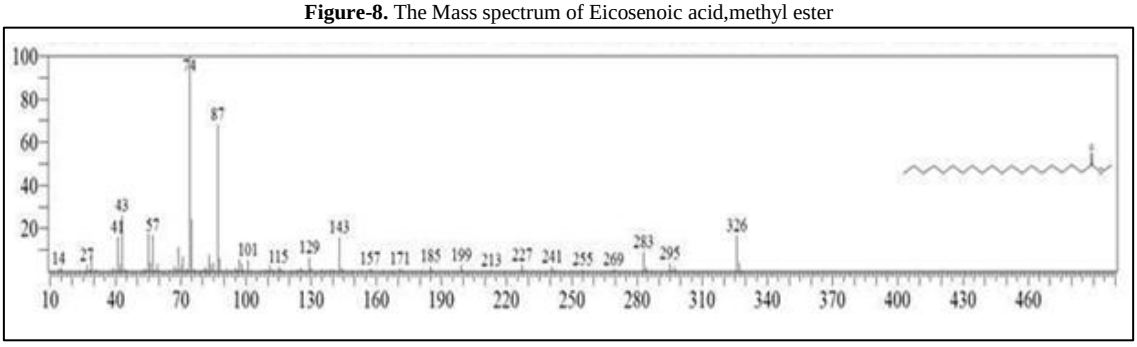


Figure-9. The Mass spectrum of Docosanoic acid, methyl ester

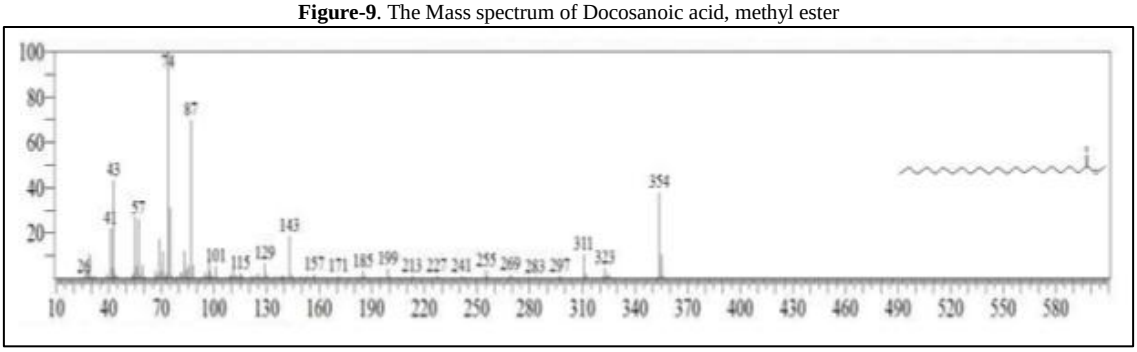
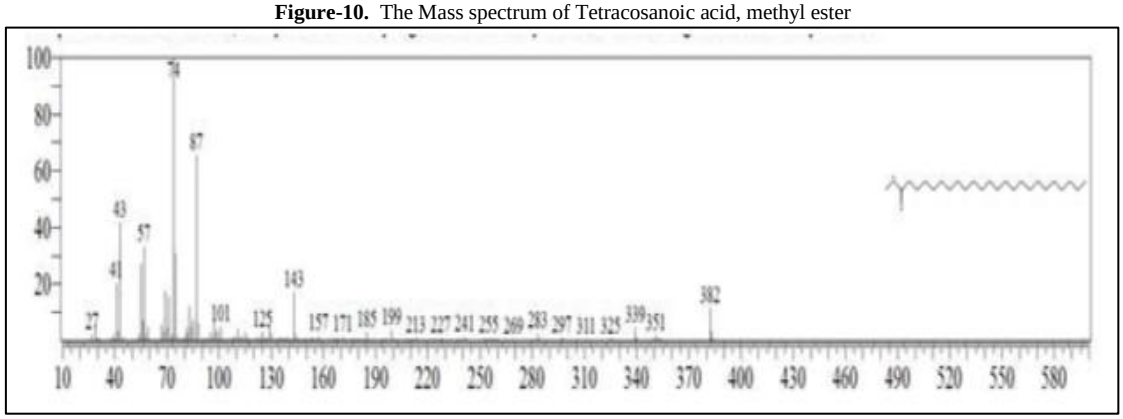


Figure-10. The Mass spectrum of Tetracosanoic acid, methyl ester



4. Conclusion

Phytochemical screening of ethanolic extract of the *Boswellia sacra* showed to contain alkaloids, flavonoids, tannins and triterpenes , antibacterial activity of the *Boswellia sacra* oil was given moderately activities against all bacterial organisms, GC-Ms analysis of the *Boswellia sacra* oil reveals twenty five chemical constituents have been identified.

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