ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com
Journal DOI: 10.21474/IJAR01

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

GC-MS Analysis of Fixed Oil from Sudanese *Maerua pseudopetalosa* (Glig and Ben.)De Wolf Roots

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Manuscript Info

Manuscript History:

Received: 19 March 2016
Final Accepted: 26 April 2016
Published Online: May 2016

Key words:
Maerua pseudopetalosa , Fixed oil ,GC-MS analysis.

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Abstract

In view of lack of literature data on the constituents of medicinal plants used in Sudanese ethno medicine , the present study was designed to identify the constituents of the fixed oil from roots of Sudanese *Maerua pseudopetalosa*.This important plant is used traditionally in Sudanese ethno medicine . GC- MS analysis revealed the presence of the following major constituents: oleic acid(24.49%),9,12-octadecadienoic methyl ester((10.64%),9-octadecenoic acid-1,2,3-propanetriyl ester(9.58%), γ-sitosterol(8.60%), cyclododecyne(6.08%). Beside γ-sitosterol, some sterols were also detected, but as minor constituents. These are : β-sitosterol acetate(0.15%), cholest-5-en-3-ol-(3β)-propanoate(0.41%),stigmasta-5,22-dien-3-ol acetate(0.78%) and compesterol(0.40%).

Introduction:-

Different oils and plant extracts have been used throughout history for diverse purposes(Jones,1996) ranging from the use of camphor oil in dental medicine; fennel oil in food industry(Lowless,1995); rosewood in perfumery to the use of lemongrass oil as food crops preservative(Mishra *et.al.*,1994).

Fixed and essential oils are extensively used in ethno medicine. Various phytochemical studies established a rationale for the ethno-medical uses of some plant species via the isolation and characterization of some bioconstituents..Though *in vitro* antimicrobial activity of some oils and plant extracts is well documented now, there are few published data for many medicinal plants(Morris,1979; Ross,1980; Yousef,1980; Deans and Ritchie,1987;Hili,1997).

Few literature data is available on the constituents of medicinal plants employed in Sudanese traditional medicine. Thus, this study was designed to conduct a GC-MS analysis for fixed oil from the medicinally important species *Maerua pseudopetalosa*-known locally as "Kerdala" .

Maerua species comprise 50 species, most of these in the drier areas of tropical Africa, but some extending as well to the middle east and tropical Asia. Several Maerua species in the region are medicinally used(Burkill, 1985).

Maerua bussei(**Glig and Ben.**)**De Wolf** is an evergreen shrub or small tree up to 5m tall occurring in Uganda,Tanzania and Congo. In Tanzania a decoction of the root is rubbed on the chest to cure chest complaints(Burkill,1985; Diallo *et.al*,1999).

Maerua duchesnei (**De Wild**) is a scrambling shrub or small tree up to 8m tall of semi-deciduous forests. It occurs from Sierra- Leone east to Sudan and Uganda. In Congo the sap from the roots is used as ear drops to treat inflammed ear. The wood is fibrous ,hard,white and is used for carving in Nigeria(Burkill, 1985; Diallo *et.al*.,1999).

Maerua oblingifolia (**Forssk.)** is a bushy shrub up to 3m tall. In Ethiopia the leaves are used as wound dressing. In west Africa the plant is used to treat syphilis and in Ghana and Ethiopia the twigs are used as dental sticks. The plant is claimed to possess antibacterial activity .In Sudan the stems are used to treat malaria. The methanolic extract from stems showed antimalarial activity . The alkaloid content is thought to be responsible for the antiplasmodial potential (Burkill, 1985; Diallo *et.al.*,1999).

Maerua crassifolia extends from Mauritania and Senegal east to Somalia. Throughout its area of distribution, this species is used medicinally . Crushed leaves are mixed with butter and given as a cure for diarrhea. Also leaves are applied to the body to alleviate fever. An infusion of the dried leaves is drunk to arrest vomiting and a decoction of leaves is taken as treatment for malaria, jaundice and constipation. The same is used for skin infections (Burkill, 1985).

Maerua angifolia is distrbuted in continental tropical Africa. In Mali , stem bark is applied to wounds, while in Senegal, leaves are used to cure anorexia and asthma. Some African healers treat wounds, abscesses, sores and ulcers with leaves as dressing. In Benin, children suffering from amoebic dysentery are treated with twigs. In Sudan a decoction of bark is used as a remedy for malaria(Nicolas *et.al.*,2009)..

Maerua pseudopetalosa is a perennial woody herb or sub-shrub with ascending branches growing up to 30-60cm tall. In rural area of Sudan, the plant is used to precipitate solids in drinking water. As a traditional medicine, *Maerua pseudopetalosa* root, is used for treatment of pulmonary troubles. Fruits and leaves are commonly used as food (Henry, 1948).Toxic tetramethyl ammonium iodide , known as tetramine, is reported in the tuberous root, shoot and leaves. This substance has proved fatal for humans. The fruits which are rich in iron, potassium and protein are consumed in Senegal in lean times. Fruit are also eaten in Sudan as tonic. Roots as well as fruits are used as tropical application to the chest to treat cough.Masai healers use this species to treat an array of human disorders. The vegatitaive part of the plant contains:14.5%fats(Nicolas *et.al.*,2009).

Materials and Methods:-

Extraction of oil from roots of *Maerua pseudopetalosa*:-

Powdered shade-dried roots of *Maerua pseudopetalosa* (300g) were exhaustively extracted with n-hexane (soxhlet).The solvent was removed under reduced pressure and the oil was kept in the fridge at 4^{o}C till analyzed.

Esterification of oil:-

A stock solution of methanolic sulphuric acid was prepared by mixing (1ml) of concentrated sulphuric acid with (99ml) methanol.A Methanolic solution of sodium hydroxide was prepared by dissolving (2g) of sodium hydroxide in (100ml) methanol. The oil(2ml) was placed in a test tube and (7ml) of alcoholic sodium hydroxide were added followed by (7ml) of alcoholic sulphuric acid. The tube was stoppered and shaked vigorously for five minutes and then left overnight.(2ml) of supersaturated sodium chloride were added, then (2ml) of normal hexane were added and the tube was vigorously shaked for five minutes.The hexane layer was then separated.(5µl) of the hexane extract were mixed with (5ml) diethyl ether . The solution was filtered and the filtrate(1µl) was injected in the GC-MS vial.

GC-MS analysis:-

Oil from *Maerua pseudopetalosa* roots was analyzed by gas chromatography – mass spectrometry.A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 µm, thickness)was used.Helium (99.99 %) was used as carrier gas. Temperature program for oven is depicted in Table 1, while other chromatographic conditions are shown in Table 2.

Table 1: Oven temperature program

Rate	Temperature(^{o}C)	Hold Time (min.$^{-1}$)
-	150.0	1.00
4.00	300.0	0.00

Table2: Chromatographic conditions

Column oven temperature
150.0°C
Injection temperature
300.0°C
Injection mode
Split
Flow control mode
Linear velocity
Pressure
139.3KPa
Total flow
50.0ml/ min
Column flow
1.54ml/sec.
Linear velocity
47.2cm/sec.
Purge flow
3.0ml/min.
Spilt ratio
- 1.0

Results and Discussion:-

Identification of oil constituents:-

The GC-MS spectrum of the studied oil revealed the presence of 45 components (Table 3).The typical total ion chromatogram(TIC) of hexane extract is shown in Fig.1.

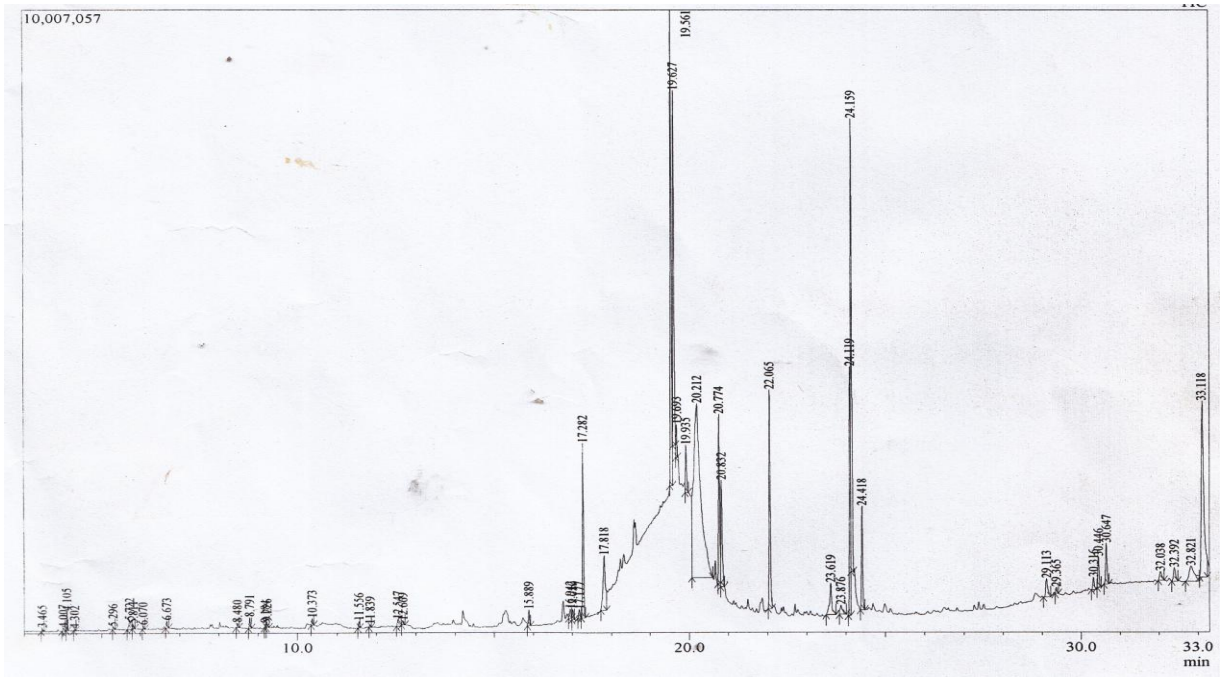


Fig.1: Total ion chromatogram of hexane extract

Table 3: Peak Report- TIC

Peak#	R. Time	Area	Area %	Name
1	3.465	64368	0.04	Hexanoic acid, methyl ester
1.	4.007	116955	0.08	Hexanoic acid
2.	4.105	381153	0.26	Hexanal dimethyl acetal
3.	4.302	47379	0.03	Furan, 2-pentyl-
4.	5.296	76291	0.05	Heptanoic acid
5.	5.722	589431	0.40	5-Undecene, 4-methyl-
6.	5.811	131147	0.09	Nonanal
7.	6.070	83770	0.06	Octanoic acid, methyl ester
8.	6.673	211873	0.14	Nonanoic acid
9.	8.480	124964	0.09	2,4-Decadienal, (E,E)-
10.	8.791	297002	0.20	2,4-Decadienal
11.	9.184	104781	0.07	4,4,6-Trimethyl-cyclohex-2-en-1-ol
12.	9.226	177117	0.12	2-Hexen -1-ol,2-ethyl-
13.	10.373	186284	0.13	Nonanoic acid, 9-oxo-,methyl ester
14.	11.556	191608	0.13	Butylated Hydroxytoluene
15.	11.839	81113	0.06	Oxiraneoctanoic acid, 3-octyl-,methyl ester
16.	12.547	459388	0.31	Tetradecanal
17.	12.669	167643	0.11	Octanoid acid, 6,6-dimethoxy-, methyl ester
18.	15.889	467167	0.32	Pentadecanoic acid , methyl ester
19.	16.942	383267	0.26	2-Nonadecanone
20.	17.010	312452	0.21	9-Hexadecanoic acid, methyl ester (Z)-
21.	17.177	303802	0.21	Hexadecanal
22.	17.282	5596909	3.81	Hexadecanoic acid, methyl ester
23.	17.818	3681884	2.51	n-Hexadecanoic acid
24.	19.561	15618117	10.64	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
25.	19.627	11302449	7.70	9-Octadecenoic acid, methyl ester, (E)-
26.	19.693	831502	0.57	11-Octadecenoic acid, methyl ester
27.	19.935	1585276	1.08	Methyl stearate
28.	20.212	35960142	24.49	Oleic acid
29.	20.774	5506320	3.75	Iso-Propyl 9-.cis.,11-.trans.-octadecadienoate
30.	20.832	3928108	2.68	Elaidic acid, isopropyl ester
31.	22.065	7437053	5.07	1-(+)-Ascorbic acid 2,6-dihexadecanoate
32.	23.619	2013474	1.37	Phenol,2,2'-methylenebis[6-(1,1-dimethyl
33.	23.876	600972	0.41	E,E,Z-1,3,12-Nonadecatriene-5,14-diol
34.	24.119	8922185	6.08	Cyclododecyne
35.	24.159	14063640	9.58	9-Octadecenoic acid,1,2,3-propanetriyl ester
36.	24.418	3510723	2.39	Tristearin
37.	29.113	1135399	0.77	Cis-9-Hexadecenal
38.	29.365	216224	0.15	.beta,-Sitosterol acetate
39.	30.316	604409	0.41	Cholest-5-en-3-ol(3.beta)-,propanoate
40.	30.446	1140539	0.78	Stigmasta-5,22-dien-3-ol,acetate, (3.beta
41.	30.647	1700176	1.16	Stigmast-5-en-3-ol, oleate
42.	32.038	584066	0.40	Campesterol
43.	32.392	834438	0.57	Stigmasterol
44.	32.821	2459598	1.68	E,E.Z-1,3,12-Nonadecatriene-5,14-diol
45.	33.118	12632841	8.60	.gamma.-Sitosterol
		146825399	100.00	

GC-MS analysis of oil revealed the following major constituents:

Oleic acid(24.49%)

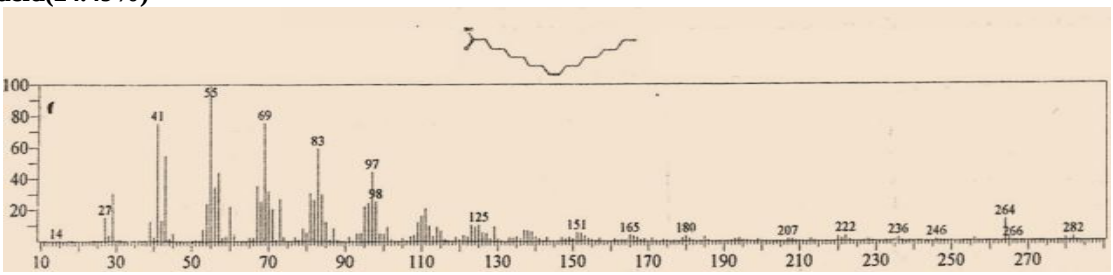


Fig. 2: Mass spectrum of oleic acid

The EI mass spectrum of oleic acid is shown in Fig.2.The peak at m/z 282, which appeared at R.T. 20.212 in total ion chromatogram, corresponds to $M^{+}[C_{18}H_{34}O_2]^{+}$.

9,12-Octadecadienoic acid methyl ester((10.64%)

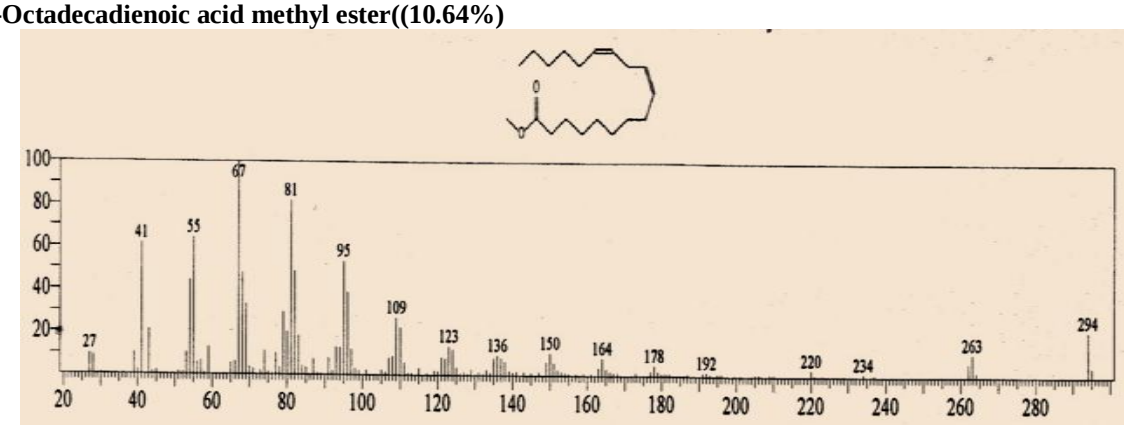


Fig.3: Mass spectrum of 9,12-octadecadienoic acid

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig. 3.The peak at m/z 294 , which appeared at R.T. 19.561 in total ion chromatogram, corresponds $M^{+}[C_{19}H_{34}O_2]^{+}$.The peak at m/z 263 correspond to loss of a methoxyl function.

9-Octadecenoic acid,1,2,3-propanetriyl ester (9.58%)

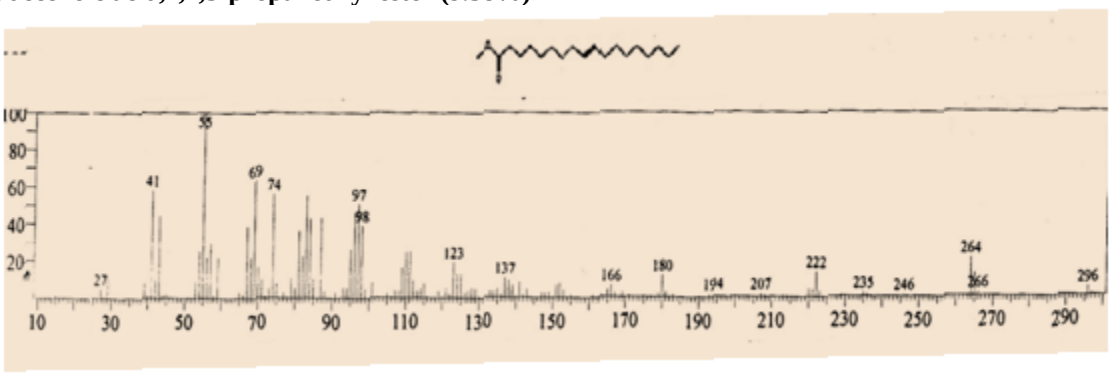


Fig.4: Mass spectrum of 9-octadecanoic acid methyl ester

The EI mass spectrum of 9-octadecenoic acid,1,2,3-propanetriyl ester is shown in Fig. 4.The peak at m/z 296 , which appeared at R.T. 24.159 in total ion chromatogram, corresponds M^{+} $[C_{19}H_{36}O_2]^{+}$.The peak at m/z 265 correspond to loss of a methoxyl function.

γ-sitosterol(8.60%)

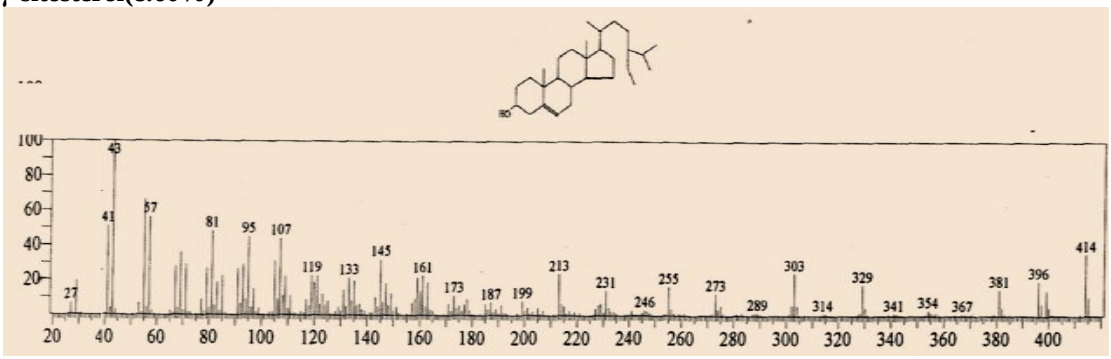
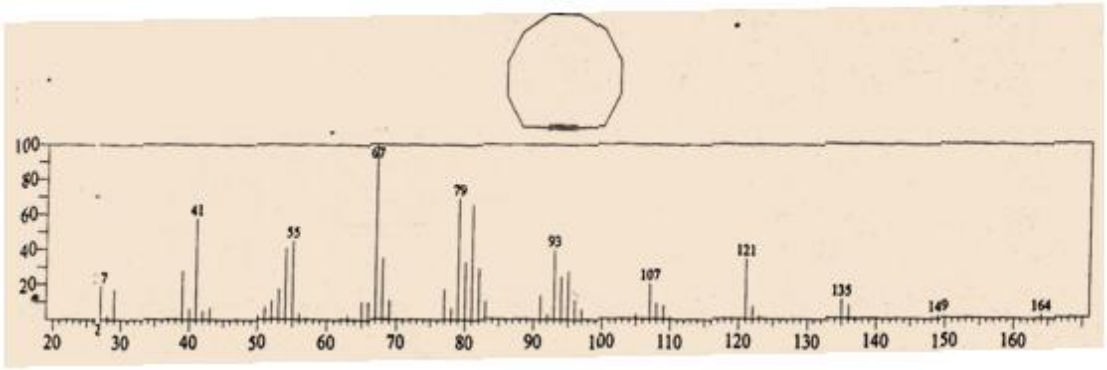


Fig.5:GC-MS spectrum of γ-sitosterol

The EI mass spectrum of γ-sitosterol is shown in Fig. 5.The peak at m/z 414, which appeared at R.T.33.118 in total ion chromatogram, corresponds M^+ $[C_{29}H_{50}O]^+$ .The peak a m/z 396 correspond to loss of a hydroxyl function**.**

Cyclododecyne(6.08%)



. Fig.6:GC-MS spectrum of cyclododecyne

The EI mass spectrum of cyclododecyne is shown in Fig.6.The peak at m/z164, which appeared at R.T. 24.119 in total ion chromatogram ,corresponds M^+ $[C_{12}H_{20}]^+$ .The peak a m/z 149 correspond to loss of a methyl function**.**

Beside **γ**-sitosterol(8.60%) some sterols were also detected,but as minor constituents. These are : β-sitosterol acetate(0.15%), cholest-5-en-3-ol-(3β)-propanoate(0.41%),stigmasta-5,22-dien-3-ol acetate(0.78%),compesterol(0.40%).

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