

Full Length Research Paper

Analysis of the essential oil from the leaves of *Sesamum radiatum*, a potential medication for male infertility factor, by gas chromatography - mass spectrometry

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Essential oil was extracted from the dried leaves of *Sesamum radiatum* by hydro-distillation and analyzed by combined gas chromatography-mass spectrometry. n-Hexadecanoic acid was found to be the major constituent and with three other fatty acids namely, 9,12,15-octadecanoic acid-(Z,Z,Z), dodecanoic acid and tetradecanoic acid constitute 40.64% of the oil. Other constituents include heptatriacontanol, estra-1,3,5(10)-trien-17 α -ol, 1-(+)-ascorbic acid, 2,6-dihexadecanoate, ethyl iso-allochololate, 18,19-secoyohimban-19-oic acid, 16,17,20,21-tetrahydro-16-(hydroxymethyl)-methyl ester, (15 α , 16E)-trans-(2,3-diphenylcyclopropyl) methylphenyl sulfoxide, 1,1-[2-methyl-2-(phenylthio) cyclopropylidene]bis-benzene and phenolics. The presence of some of these constituents in the essential oil provides the scientific evidences for the observed cardiovascular and estrogenic activity as well as curative properties of the plant for male infertility factor, constipation, fungal and bacterial infections and bruises.

Key words: *Sesamum radiatum*, male infertility factor, essential oil, GC-MS.

INTRODUCTION

Sesamum radiatum is a perennial herb found in the tropical areas of Africa and belongs to the Pedaliaceae family (Purseglove, 1974; Hutchinson and Dalziel, 1954). One of the local names in South-Western Nigeria is "Ewe atura" which means 'leaves that bring relaxation and health to the body' possibly because they relieve constipation and cure other ailments on ingestion (Odugbemi, 2008). The leaves, seeds and oil serve as food especially in farming communities in Nigeria (Akpan-Iwo et al., 2006). The seeds have been reported to have a crude protein content of 22.9% (Oshodi et al., 1999). The leaves are also used for treating various stomach ailments. The decoction of the leaves is used for the treatment of catarrh,

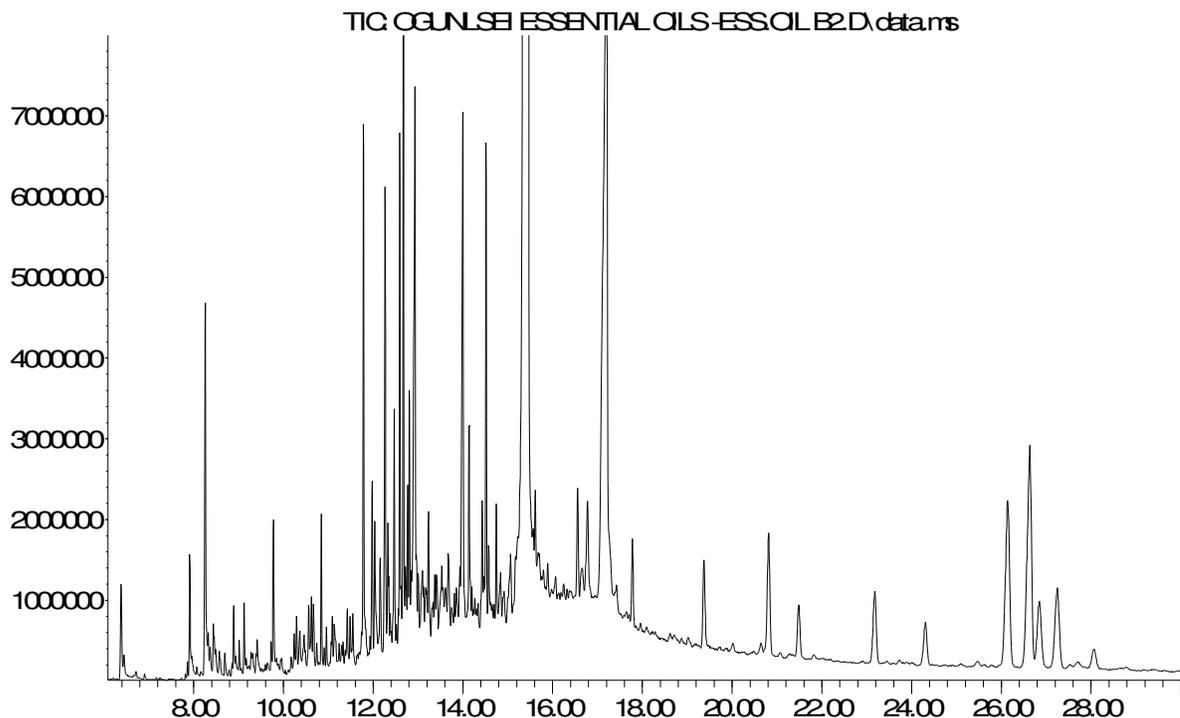
eye pains as well as bruises and erupted skins. The decoction of combined roots and leaves has been reported to have anti-viral and antifungal activity (Gills, 1992).

The aqueous extract of the leaves has been found to be rich in phenols, lignans and flavonoids. Sterols were also found to be among its constituents. The extract was found to have significant myorelaxant effect in guinea-pig thus supporting the traditional use of the fresh leaves for treating cardiovascular diseases in Cote d'Ivoire (Konan et al., 2008). The leaves are also used in correcting male infertility by enhancing sperm count. This is one of the major uses of the plant in Western Nigeria.

Sesame leaves intake has been found to improve and increase epididymal spermatocytes reserve in adult male Sprague Dawley rat (Shittu et al., 2007). *S. radiatum* seeds have been shown to be estrogenic and/or anti-estrogenic (Collins et al., 1997). The acclaimed success associated with the use of the leaves in treating male infertility factor is the major reason for this study as information about the chemical constituents in the leaves

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Abundance



Time->

Figure 1. Gas chromatogram of the essential oil from the leaves of *S. radiatum*.

will be useful in pharmacological studies. In Africa, high premium is placed on having children and thus several childless marriages end up in separation or divorce. In this report, we present the constituent compounds in the essential oil isolated from the leaves of *S. radiatum* as identified by combined gas chromatography - mass spectrometry.

MATERIALS AND METHODS

Collection of samples

Several batches of the fresh leaves of *S. radiatum* were purchased from Mushin market in Lagos, Nigeria, between October and December, 2005 and were identified by Mr. T. K. Odewo and Mr. Seun Osiyemi of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. A sample with voucher number FHI 107823 was deposited in the Herbarium of the Botany Department at FRIN.

Hydro-distillation of samples

The fresh leaves were air-dried, powdered and the essential oil extracted by hydro-distillation over a period of 4 h using batches of 100 g to which was added 3 L of water for each batch. The essential oil was collected into hexane giving a yellowish colouration. Thereafter, it was separated from water and concentrated by evaporation of the hexane.

GC-MS Analysis of samples

Analysis of the essential oil was carried out on GC-MS model HP 6890 (Agilent Technologies Ltd) fitted with an HP-5 MS (5% phenylmethyl siloxane) capillary column 30 m x 250 μ m x 0.25 μ m. Helium carrier gas was used at a constant flow rate of 1 ml/min. The detector was 5973 inert MSD. 1 μ L of sample was injected. Initial column temperature was maintained at 50°C for 6 min and increased at 25°C/min to 230°C (Xu et al., 2005). Total run time was 30.20 min. Injector and detector temperature was 280°C. Mass spectra were recorded at 70 eV ionization energy. Identification of the ions was by the Chem Office Software and the library of the MS.

RESULTS AND DISCUSSION

The gas chromatogram of the analysis of the essential oil is shown in Figure 1. The structures of some constituents in the essential oil and yohimbine hydrochloride are shown in Figures 2a - d. A total of 63 compounds were identified to be present in the essential oil. The constituents of the essential oil with a minimum percentage of 0.5% of total as well as one compound of interest but with a lower abundance are listed in Table 1. The retention times (R_T) are in minutes. Seven compounds, namely, n-hexadecanoic acid (R_T 15.459 min; 24.7 9%), 9,12,15-octadecatrienoic acid (Z,Z,Z), (R_T 17.209 min; 10.12%)

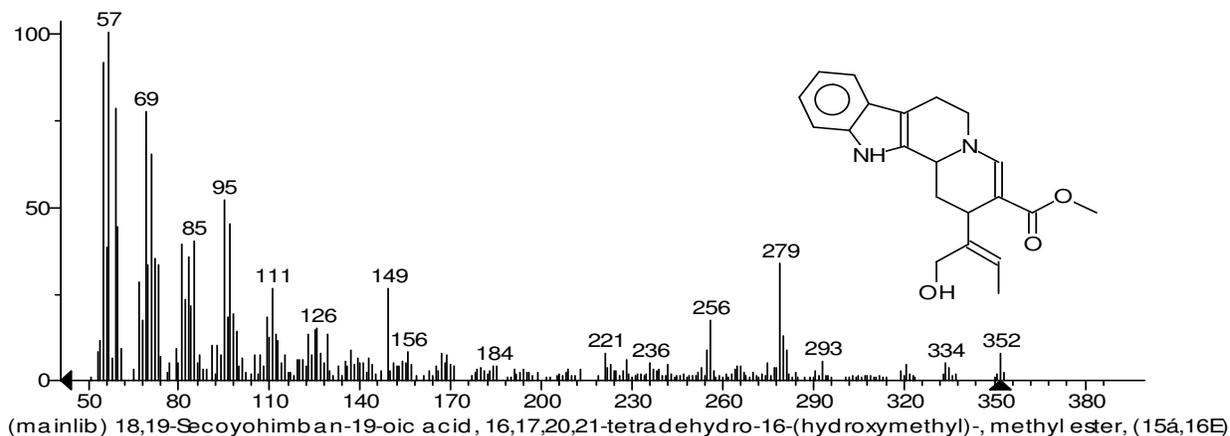


Figure 2a. 18, 19-Secoyohimban-19-oic acid, 16, 17, 20, 21-tetrahydro-16-(hydroxymethyl)-methylester (15á, 16E).

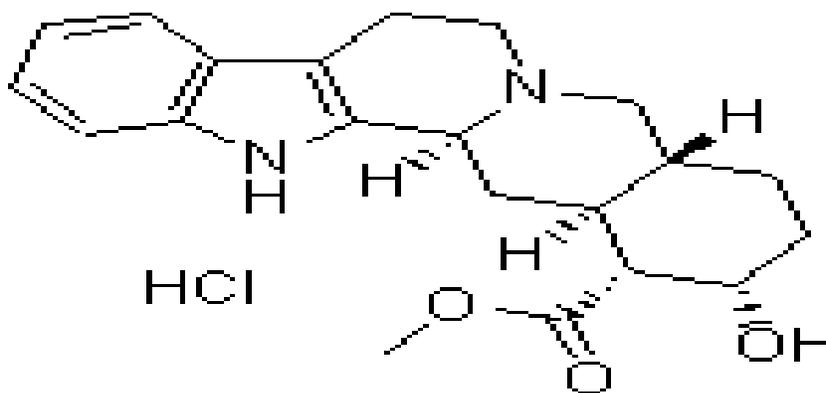


Figure 2b. 17 α -hydroxy-yohimban-16 α -carboxylic acid methyl ester hydrochloride. Yohimbine hydrochloride (CHEMBLINK, 2009).

dodecanoic acid (R_T 12.933 min; 3.05%), tetradecanoic acid (R_T 14.002 min; 2.68%), oleic acid (R_T 16.777 min; 1.76%), (all of which are fatty acids constituting 40.64%), 1,1-[2-methyl-2-(phenylthio) cyclopropylidene] bis benzene eluted at retention times 26.142, 26.632, 26.845 and 27.255 min (with a total of 6.41%) and dasycarpidan-1-methanol, acetate (ester) eluted at retention times 15.679, 15.708, 15.796, 15.979, 16.067, 16.140, 16.250, 16.323, 16.374 and 16.418 min (with a total of 4.34%). All these compounds account for 53.15% of total constituents.

Another set of 11 compounds each of percentage composition of between 1.0 and 2.5% constitute 15.41%. These are 2, 4-bis (1,1-dimethylethyl)-phenol (R_T 12.677 min; 2.04%), eugenol, (R_T 11.791 min; 1.70%), (E-) 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one (R_T 12.596 min; 1.54%), 6, 10, 14-trimethyl-2-pentadecanone (R_T 14.522 min; 1.50%), caryophyllene (R_T 12.274 min; 1.34%), 1-(+)-ascorbic acid, 2, 6-dihexadecanoate (R_T 15.569 and 15.613 min; 1.33%), 2-methyl-1-hexadecanol (R_T 16.557 min; 1.30%), 9-hexadecenoic acid (R_T 15.181

and 15.232 min; 1.26%), 1-octen-3-ol (R_T 8.261 min; 1.24%), heptacosane (R_T 17.780 and 19.376 min; 1.11%), 7-oxo-cyclobuta[a]dibenzo[c,f]cycloheptadiene (R_T 14.141 min; 1.05%).

Another set of 7 compounds having a range of 0.70 to 0.92% of total are 1-heptatriacontanol, a long chain unsaturated fatty acid alcohol (R_T 15.063 min, 0.89%), ethyl iso-allocholate (R_T 17.421 min; 0.92%), 3-(1,5-dimethyl-hexyl)-3a,10,10,12b-tetramethyl-1,2,3,3a,4,6,8,9,10,10a,11,12,12a,12b-tetradecahydrobenzo[4] (R_T 20.819 min; 0.88%), α caryophyllene (R_T 12.472 min; 0.79%), cis-hexahydro-8a-methyl-1,8(2H,5H)-naphthalenedione (R_T 12.808 min; 0.78%), geranyl isovalerate (R_T 13.101 min; 0.78%) and 9,10-dihydro-11,12-diacetyl-9,10-ethanoanthracene (R_T 14.522 min; 0.70%). These constitute 5.74% of total.

The following set of 11 compounds each has an abundance of between 0.50 and 0.67%. They are trans-(2,3-diphenylcyclopropyl) methylphenyl sulfoxide (R_T 23.184 min; 0.64%), estra-1,3,5 (10)-trien-17a-ol (R_T 15.891 min; 0.62%), phthalic acid, butyl tetradecyl ester (R_T 14.749 min;

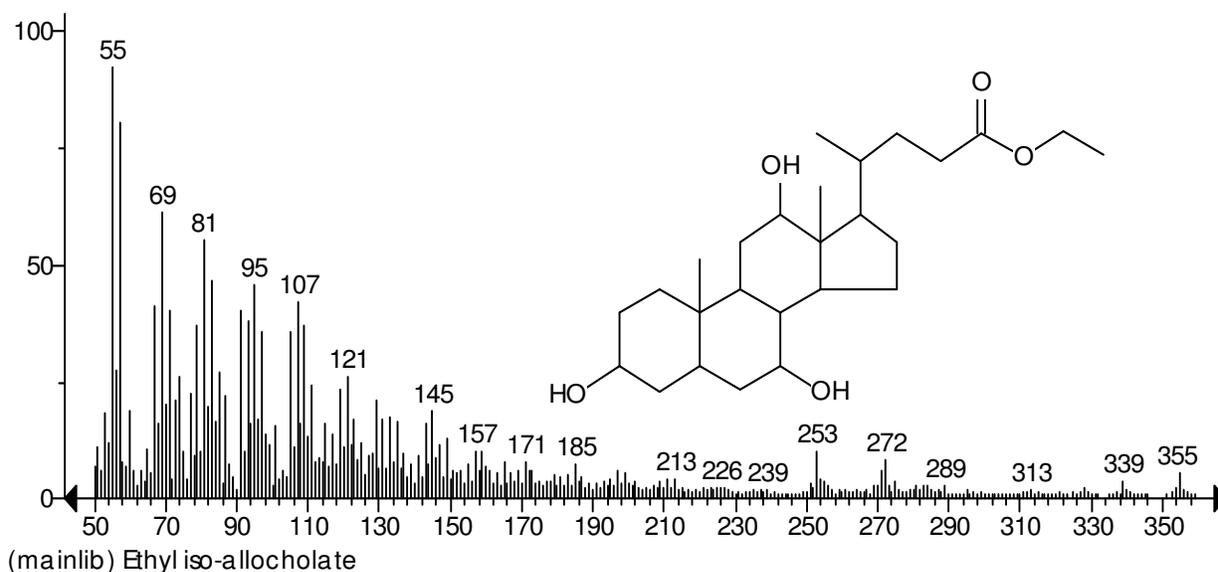


Figure 2c. Ethyl iso-allocholate.

0.60%), 2-[4-methyl-6-(2,6,6-trimethyl cyclohex-1-enyl) hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde (R_T 13.233 min; 0.60%), (E)-1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one (R_T 11.981 min; 0.59%), [a R-(1aá, 4aá, 7aá, 7bá)] decahydro-1,1,7-trimethyl-4-methylene-1H-cycloprop[e]azulene (R_T 12.040 min; 0.59%), 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)-hexa-1,3,5-trienyl]-cyclohex-1-en-1 (R_T 13.680 min; 0.59%), tert-hexadecanethiol (R_T 14.844 min; 0.54%), 7-methyl-Z-tetradecen-1-ol acetate (R_T 14.917 min; 0.54%), (E)-6,10-dimethyl-5,9-undecadien-2-one (R_T 12.332 min; 0.52%) and 1,7-dimethyl-naphthalene (R_T 12.157 min; 0.51%). These add up to 6.34%.

A list of ten compounds with abundances in the range of 0.30 to 0.48% is as follows: nonanal (R_T 9.777 min; 0.48%), 3,7,11-trimethyl-1-dodecanol (R_T 12.772 min; 0.47%), hexadecanoic acid, ethyl ester (R_T 14.580 min; 0.46%), 4-(1,5-dihydroxy-2,6,6-trimethylcyclohex-2-enyl)-but-3-en-2-one, (R_T 13.174 min; 0.43%), 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde (R_T 10.846 min; 0.39%), 1-oxaspiro[2,5] octane,5,5-dimethyl-4-(3-methyl-1,3-butadienyl) (R_T 12.728 min; 0.38%), hexanoic acid, 2-ethyl-, oxybis (2,1-ethanedioxy-2,1-ethanedioyl) ester (R_T 13.006 min; 0.36%), benzaldehyde (R_T 7.917 min; 0.35%), 2-methylene-(3á,5á)-cholestan-3-ol (R_T 13.753 min; 0.32%) and 6,9-octadecadiynoic acid, methyl ester (R_T 13.379 min; 0.30%). These add up to 3.99%.

One compound with rather low abundance, but of interest, was identified. This is 18,19-secoyohimban-19-oic acid, 16,17,20,21-tetrahydro-16-(hydroxymethyl) methyl ester (15á,16E) (R_T 27.701 min; 0.097%).

The compounds discussed add up to 83.84% of all the compounds recorded in the integration.

The major constituents in the essential oil are fatty acids, namely n-hexadecanoic acid, 9,12,15-octadecatrienoic

acid-(Z,Z,Z) and dodecanoic acid. Tetradecanoic acid and oleic acid are also present as minor constituents. Many fatty acids such as hexadecanoic acid and oleic acid have been reported to exhibit antibacterial and antifungal activity (Russel, 1991; McGraw et al., 2002; Seldel and Taylor, 2004; Dilika et al., 2000). Concentrations as low as 0.7% of oleic acid have been found to be fungistatic against a wide spectrum of moulds and yeasts (Davidson et al., 1999). Free fatty acids including long chain unsaturated fatty acids present in the extract from *Tinospora smilacina* Benth were suggested to be responsible for its anti-inflammatory activity (Li et al., 2004). Thus the combination of all the saturated and unsaturated fatty acids such as hexadecanoic acid, dodecanoic acid, tetradecanoic acid, 9-hexadecenoic acid, oleic acid and 9,12,15-octadecanoic acid-(Z,Z,Z), present in the essential oil may be useful for the management of eye pains and inflammatory conditions arising from bacterial and fungal infections as well as bruises and erupted skins.

A long chain fatty acid alcohol, heptatriacontanol, is present in the oil. Octacosanol, a long chain fatty alcohol, was reported to suppress lipid accumulation in rats fed on a high-fat diet (Kato et al., 1995) and to inhibit platelet aggregation (Arruzazabala et al., 1994). Heptatriacontanol may exhibit such activities.

The use of policosanol has been demonstrated to show modest advantages over pravastatin in the treatment of patients with type II hypercholesterolemia (Bentez et al., 1997). Policosanol, a natural mixture of higher primary unsaturated aliphatic alcohols isolated and purified from sugar cane wax contains C28, C26 and/or C30 alcohols, has been proven to exhibit cholesterol-lowering effects in patients with type II hypercholesterolemia and dyslipidemia due to type 2 diabetes mellitus and was demonstrated

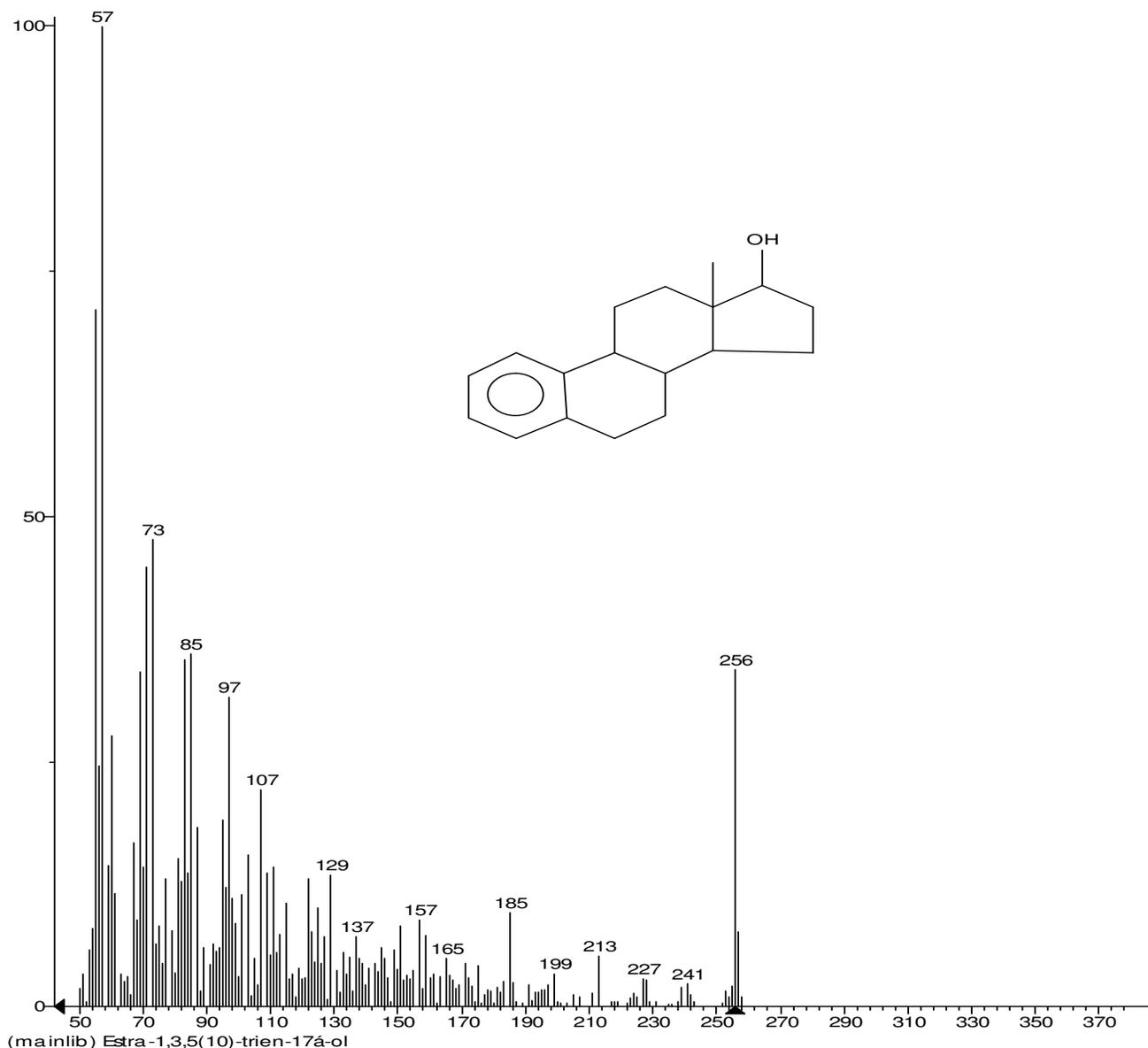


Figure 2d. Estra-1, 3, 5(10)-trien-17-ol.

to have good safety and tolerability profiles with no evidence of drug-related adverse events (Castano et al., 2002). Heptatriacontanol, a C37 aliphatic alcohol may exhibit such activity.

Carboxylic acids having a thioether, a sulphoxide or sulphone function in dermatological or cosmetic compositions have been found to promote skin exfoliation and/or stimulating epidermal regeneration. They are also useful for controlling intrinsic and extrinsic skin ageing (Maignan, 1998). The constituent eluted at R_T 23.184 min, trans-(2,3-diphenylcyclopropyl) methylphenyl-sulfoxide and the carboxylic acids present may therefore contribute to the healing of bruises and erupted skins. It is also pertinent to note that other sulphur-containing constituents, such as tert-hexadecanethiol as well as 1,1-[2-

methyl-2-(phenylthio)cyclopropylidene]bis-benzene with possible isomeric forms eluted at R_T 26.142, 26.632, 26.845 and 27.255 min, constituting 6.41% of total may also be useful for the management of bruises and erupted skins. The multiple and distinct peaks for this latter constituent may be due to impairment of column efficiency associated with high retention time.

A steroid, estra-1,3,5(10)-trien-17-ol (Figure 2d), present in the essential oil differs from estradiol, a sex hormone, in the absence of the C3-OH. Steroids though similar in basic structure, have extreme specificity hence this steroid cannot be said to function like estradiol (Bruce, 1998). However, it may be responsible for the observed estrogenic and/or anti-estrogenic activity (Collins et al., 1997). A derivative of ascorbic acid,

Table 1. Composition of the constituents of the essential oil from the dried leaves of *S. radiatum*.

S/N	Time (min)	Compound	(%)
1	7.917	Benzaldehyde	0.35
2	8.261	1-Octen-3-ol	1.24
3	9.777	Nonanal	0.48
4	10.846	2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde	0.39
5	11.791	Eugenol	1.70
6	11.981	(E)-1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-Buten-1-one	0.59
7	12.040	[aR-(1aá, 4 aá, 7áá, 7bá] decahydro-1,1,7-trimethyl-4-methylene-1H-cycloprop[e]azulene	0.59
8	12.157	1,7-dimethyl-naphthalene	0.51
9	12.274	Caryophyllene	1.34
10	12.332	(E)-6,10-dimethyl-5,9, undecadien-2-one	0.52
11	12.472	á Caryophyllene	0.79
12	12.596	(E)-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	1.54
13	12.677	2,4-bis (1,1-dimethyl ethyl)-phenol	2.04
14	12.728	1-Oxaspiro [2,5] octane, 5,5-dimethyl-4-(3-methyl-1,3-butadienyl)	0.38
15	12.772	3,7,11-trimethyl-1-dodecanol	0.47
16	12.808	cis-hexahydro-8a-methyl-1,8(2H,5H)-naphthalenedione	0.78
17	12.933	Dodecanoic acid	3.05
18	13.006	Hexanoic acid, 2-ethyl-, oxybis (2,1-ethanedioxy-2,1-ethanedioyl) ester	0.36
19	13.101	Geranyl isovalerate	0.78
20	13.174	4-(1,5-dihydroxy-2,6,6-trimethylcyclohex-2-enyl) but-3-en-2-one	0.43
21	13.233	2-[4-methyl-6-(2,6,6-trimethyl cyclohex-1-enyl) hexa-1,3,5-trienyl] cyclohex-1-en-1-carboxaldehyde	0.60
22	13.379	6,9-Octadecadiynoic acid, methyl ester	0.30
23	13.533	2-methyl-1-hexadecanol	0.65
24	13.680	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl) hexa-1,3,5-trienyl] cyclohex-1-en-1	0.59
25.	13.753	2-methylene-(3á, 5á)-cholestan-3-ol	0.32
26	14.002	Tetradecanoic acid	2.68
27	14.141	7-Oxo-cyclobuta [a] dibenzo [c,f] cycloheptadiene	1.05
28	14.434	9,10-dihydro-11,12-diacetyl-9, 10-ethanoanthracene	0.70
29	14.522	6,10,14-trimethyl-2-pentadecanone	1.50
30	14.580	Hexadecanoic acid, ethyl ester	0.46
31	14.749	Phthalic acid, butyl tetradecyl ester	0.60
32	14.844	tert-hexadecanethiol	0.54
33	14.917	7-methyl-Z-tetradecen-1-ol acetate	0.54
34	15.063	1-Heptatriacontanol	0.89
35	15.181	9-Hexadecenoic acid	0.66
36	15.232	9-Hexadecenoic acid	0.60
37	15.459	n-Hexadecanoic acid	24.79
38	15.569	1-(+)-Ascorbic acid, 2,6-dihexadecanoate	0.54
39	15.613	1-(+)-Ascorbic acid, 2,6-dihexadecanoate	0.79
40	15.679	Dasycarpirdan-1-methanol, acetate (ester)	0.49
41	15.708	Dasycarpirdan-1-methanol, acetate (ester)	0.45
42	15.796	Dasycarpirdan-1-methanol, acetate (ester)	0.47
43	15.891	Estra-1,3,5(10)-trien-17a-ol	0.62
44	15.979	Dasycarpirdan-1-methanol, acetate (ester)	0.49
45	16.067	Dasycarpirdan-1-methanol, acetate (ester)	0.60
46	16.140	Dasycarpirdan-1-methanol, acetate (ester)	0.42
47	16.250	Dasycarpirdan-1-methanol, acetate (ester)	0.53
48	16.323	Dasycarpirdan-1-methanol, acetate (ester)	0.35

Table 1. continues...

49	16.374	Dasycarpirdan-1-methanol, acetate (ester)	0.30
50	16.418	Dasycarpirdan-1-methanol, acetate (ester)	0.34
51	16.557	2-methyl-1-hexadecanol	1.30
52	16.777	Oleic acid	1.76
53	17.209	9,12,15-Octadecatrienoic acid (Z,Z,Z)	10.12
54	17.421	Ethyl iso-allocholate	0.92
55	17.780	Heptacosane	0.61
56	19.376	Heptacosane	0.50
57	20.819	3-(1,5-dimethyl-hexyl)-3a, 10,10,12b-tetramethyl-1,2,3,3a,4,6,8,9,10,10a,11,12,12a,12b tetradecahydro-benzo[4]	0.88
58	23.184	trans-(2,3-diphenylcyclopropyl) methyl phenylsulfoxide	0.64
59	26.142	1,1-[2-methyl-2-(phenylthio) cyclopropylidene] bis-benzene	1.97
60	26.632	1,1-[2-methyl-2-(phenylthio) cyclopropylidene] bis-benzene	2.68
61	26.845	1,1-[2-methyl-2-(phenylthio) cyclopropylidene] bis-benzene	0.80
62	27.255	1,1-[2-methyl-2-(phenylthio) cyclopropylidene] bis-benzene	0.96
63	27.701	18,19-Secoyohimban-19-oic acid, 16, 17, 20, 21-tetrahydro-16-(hydroxymethyl)-methylester (15a, 16E)	0.097

vitamin C, namely 1-(+)-ascorbic acid, 2-6-dihexadecanoate eluted at R_T 15.569 and 15.613 (1.33%) min is present in the essential oil. Vitamin C is an antioxidant and belongs to the class of compounds identified to enhance sperm quality and prevent sperm agglutination, thus making them more motile with forward progression and thus would promote male fertility (Glennville, 2008; Dawson et al., 1992). This constituent may be responsible for the enhancement of sperm production in males claimed by herbal medical practitioners and also explain the reported improvement and increase in epididymal spermatocytes reserve in adult male Sprague Dawley rat (Shittu et al., 2007). An aqueous extract of the leaves is mucilaginous and pro-motes bowel action and relieves constipation. This is a general observation in some items of the Nigerian diet that are mucilaginous vegetables and fruits such as *Coc-corhus olitorius*, okro and the stipules of the umbrella tree *Musanga cercropiodies* which have been found to promote bowel action and thereby give relief in constipation. Ethyl iso-allocholate (Figure 2c) present in the essential oil is the ester of a bile acid and can act as an emulsifying agent so that fats and oils can be digested by water-soluble digestive enzymes in the small intestines (Bruce, 1998). Thus this constituent may be responsible for the relief of constipation or indigestion experienced on ingesting the stewed leaves.

Phenolic compounds present in the essential oil such as eugenol and 2,4-bis(1,1-dimethylethyl)-phenol, are antibacterial agents and may cure eye infections.

18,19-Secoyohimban-19-oic acid, 16,17,20,21-tetrahydro-16-(hydroxymethyl)-methylester; (15á, 16E), whose structure is shown in Figure 2a is present in the essential oil and belongs to the same family as yohimbine which is 17 α -hydroxy-yohimban-16 α -carboxylic acid methyl ester having the structure shown in Figure 2b (Chemblink, 2009). Yohimbine has been found to be active on

endocrine and reproductive systems; for example, it was reported to be effective in relieving male impotency (Temple and Smith, 1992; Sobotka, 1969). Yohimbine has also been found to exhibit cardiovascular activity (Henauer et al., 1984). Thus, 18,19-seco-yohimban-19-oic acid, 16,17,20,21-tetrahydro-16-hydro-xymethyl)-methyl ester-(15á,16E) may be responsible for the reported cardiovascular activity (Konan et al., 2008) and spermatogenic effect of the leaf extract (Shittu et al., 2007).

Conclusion

The study has been found useful in the identification of several constituents present in the essential oil from the leaves of *S. radiatum*. Some of these have been found to be useful in explaining the scientific basis of the role of the plant in treating male infertility factor, constipation, inflammations, eye pains and skin infections and explaining its cardiovascular bioactivity. The results of this study would lead to pharmacological testing of the plant extract on animal models.

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