Journal of Medicinal Plants Research Vol. 5(32), pp. 7006-7010, 30 December, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals
DOI: 10.5897/JMPR11.1079

Full Length Research Paper

Analysis of the essential oil of *Vetiveria nigritana* (Benth.) Stapf root growing in Sudan

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Accepted 22 September, 2011

The volatile oil of the roots of *Vetiveria nigritana* (Benth.) Stapf was extracted by hydrodistillation for 36 h to yield 1.35%. The physical and chemical properties were determined according to international standards and were not compatible with published data. Analysis of the oil by gas liquid chromatography/mass spectrometry (GLC-MS) technique revealed that the oil was composed of 50 compounds of which 9 compounds were not identified. The main constituents were longifolene D (25.1%), 2-hydroxycyperol (9.7%) and aromadendrene oxide (1) (8.8%). The different and extremely complex composition of vetiver oil from different geographic regions of the world prompted us to add that the Sudanese vetiver oil is also different and do not comply with the "finger print" of the oil in the literature (α- and β- vetivone plus khusimol).

Key words: *Vetiveria nigritana*, essential oil, composition, gas liquid chromatography (GLC), mass spectrometry (MS).

INTRODUCTION

Vetiver is a common name of the grass of the genus *Vetiveria* (poaceae), which consists of 11 species widely distributed in tropical regions of Asia, Africa, Austrilia and Pacific Islands. *Vetiveria nigritana* is a perennial herb, with an aromatic rhizomes and is considered the main African species found in most sub-saharan areas from Senegal to Mozambique (Dalziel, 1955; Maurice, 1993). The roots have been used for centuries for their fragrance and are woven into fragrant-smelling mats and fans (Evans, 2002). The plant also contains active ingredients used in traditional medicine and as botanical pesticide. The chemical composition of the oil is extremely complex and consists of sesquiterpenes and their derivatives, belonging to 11 structural classes.

The sesquiterpenes α-vetivone, β-vetivone and khusimol are considered as the "finger print" of vetiver oil (Virmani, 1975; Ashour, 1980; Kokwaro, 1991). The different and complex composition of vetiver oil from different geographic locations and the scant data about the chemical composition of Sudanese vetiver oil prompted us to investigate its chemical composition and antimicrobial properties (Jain et al., 1982; Cunnigham et al., 1992; Gurib-Fokim et al., 1993; Chomchalow, 2001). The results of the gas liquid chromatography/mass spectrometry (GLC-MS) analysis of the oil and assessment of its antibacterial activity are reported in the present paper.

MATERIALS AND METHODS

Plant Material

The plant has been collected in the district of southern Niyala, western Sudan and identified as *V. nigritana* (Benth) Stapf at the Medicinal and Aromatic Plants Research Institute (MAPRI), the

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Table 1. Physical and chemical properties of essential oil prepared form *V. nigritana* root.

S/N	Physical and chemical properties of essential oil	
1.	Apparent density	1.001
2.	Relative density	1.002
3.	Refractive index	1.5112
4.	Optical rotation	+40.0
5.	Acid value	46
6.	Saponification value	60
7.	Ester value	14

National Center for Research (NCR), Khartoum, Sudan. The roots were shade dried and pulverized into fine powder.

Extraction of the essential oil

The dried and powdered roots (100 g) were soaked and water distilled in a Clavenger apparatus for 36 h (Wagner and Bladt, 1996). The isolated oil was dried over anhydrous sodium sulphate and the yield was determined in v/w and was kept in the refrigerator.

Determination of physical and chemical properties

Density, refractive index, optical rotation, acid value, ester value and saponification value were determined according to international standards (Ashour, 1980). Results were reported in Table 1.

Assessment of antibacterial activity

The antibacterial activity of the essential oil was assessed by the agar –well diffusion method (Groove and Randall, 1955; Saadabi and Ayoub, 2009). After incubation for 24 h at 37℃, the diameters of the zones of inhibition were measured in mm. Three replicates were performed and results were presented in Table 2.

GLC- MS analysis of Vetiver oil

GLC-MS analysis of the oil sample was performed on Agilent Technology mass spectrometer model 5973 N, combined with Agilent Technologies gas chromatograph model G2577A equipped with mass selective detector using a capillary column 25 m × 0.22 mm internal diameter and film thickness 0.33 mm. The carrier gas was helium with flow rate 1 ml/min. Results were presented in Table 3.

RESULTS AND DISCUSSION

There are distinct geographical differences in the quality and yield of vetiver oil obtained from species growing in different regions of the world. The yield of the oil prepared from the Sudanese *V. nigritana* root was 0.5% at the first five hours of hydrodistillation to reach 1.35% at the end of distillation process lasted for 36 h. The physical and chemical properties of the oil (Table 1) were determined according to international standards and found different from published data in the literature (Ashour, 1980; Chomchalow, 2001).

It is known that vetiver oil has a high specific gravity, negative optical rotation, high vetiverol concentration and higher ester value which make it superior from a perfumery viewpoint (Ashour, 1980; Chomchalow, 2001), but the Sudanese vetiver oil of *V. nigritana* was not compatible with these data.

The prepared oil was screened Primarily by TLC in different solvent systems (Wagner and Bladt, 1996), followed by complete analysis with GLC-MS (Merritt, 1983; Colegate and Molineux, 1993). The apparatus was equipped with an advanced library which enabled a reliable identification of the sample components. The main components of the oil were sequiterpene hydrocarbons and their oxygenated derivatives (Cane, 1999; Dewick, 2001) and from the fifty compounds detected, forty one were identified (Table 2). The main constituent was longifolene- D (25.1%), followed by 2-hydroxycyperol (9.7%) and aromadendrene oxide – (1) (8.8%) (Figure 1).

The antimicrobial activity of the oil was assessed against four pathogenic bacteria. The antibacterial activity was variable, ranging from completely resistant *Escherichia coli* to significant activity of *Pseudomonas aeruginosa* (Goun et al., 2003). Two bacteria, *Bascillus subtitis* and *Staphylococcus aureus*, were less susceptible to the oil. Results were presented in Table 3.

It is known that chemical composition of vetiver oil is extremely complex and some 100 sesquiterpene type of compounds and their derivatives belonging to 11 structural classes were reported in the literature. The results discussed in the present paper are a new addition to the current literature which specifies the different composition of the oil which does not comply with the known "finger print" of vetiver oil.

Table 2. GLC-MS analysis of Vetiver oil prepared from *Vetiveria nigritana* growing in Sudan.

Peak No.	R. T	% of total	Compound
1	2.64	1.270	Methylene chloride (solvent)
2	9.23	0.017	Benzeneacetaldehyde
3	9.46	0.050	3,3,5- Trimethylcyclohexanol
4	10.61	0.010	4- Aminobenzaldehyde oxime
5	11.06	0.011	3.7-Dimethyl-1,6-octadien-3-ol
6	12.59	0.042	(1S) -1,7,7- trimethyl- bicyclo [2.2.1] heptan-2-one
7	13.30	0.024	(1S- endo)-1,7,7-trimethyl –bicyclo [2.2.1] heptan-2-ol
8	13.48	0.021	Unidentified
9	13.70	0.019	4- methyl-1- (1-methylethyl) -3-cyclohexen-1-ol
10	13.94	0.070	1-(4-methylphenyl)-ethanone.
11	14.16	0.037	(S) -α,α, 4- trimethyl-3- cyclohexene-1- methanol
12	14.43	0.095	1- methaxyl-4- (2-propenyl)- benzene.
13	14.65	0.020	6- hydroxymethyl-2,3-dimethylphenyl methanol.
14	15.61	0.006	trans-octahydro-8a-methyl-2(1H)-naphthalenone.
15	15.82	0.009	4-(1-methylethyl)- benzaldehyde.
16	16.30	0.024	3-methyl-6-(1-methylethyl)-2-cyclohexen-1-one.
17	17.00	0.010	trans-Z-α-bisabolene epoxide
18	17.38	0.116	1-methoxy -4- (1-propenyl)-benzene.
19	18.63	0.031	1,2,3b,6,7,8,-hexahydro- 6,6-cyclopenta [1.3] cyclopropa [1,2]cyclohepten-3(3ah)-one
20	18.96	0.041	6-ethenyl-2,4,5,6,7,7a-hexahydro-7-a-hydroxy-3,6-dimethyl-α-methylene -2-oxo-5-benzofuranacetic acid methyl ether.
21	19.21	0.473	5,9,9-trimethyl-spiro[3.6] deca-5,7dien-1- one
22	20.35	4.80	Unidentified
23	20.92	0.533	Unidentified
24	21.72		[3R-(3α,3aβ, 7β, 8aα)] -2,3,4,7,8, 8a-hexa-hydro-3,6,8,8-tetramethyl-1H-3aα,7 methanoazulene
25	22.88	1.325	[1s-(1α,7α,8aα)] -1,2,3,5,6,7,8,8a-Octahydro-1,8a- dimethyl-7-(1-methylethenyl)-naphthalene.
26	23.74	0.75	(1α, 4aβ, 8aα)- 1,2,3,4,4a,5,6-8a-octahydro-7- methyl-4- methylene-1-(1-methylethyl)-Naphthalene.
27	25.12	1.259	(1S-cis)- 1,2,3,5,6,8a- Hexahydro-4,7- dimethyl-1-(1-methylethyl)-naphthalene.
28	25.615	1.994	(1S-cis)- 1,2,3,4,5,6,7,8-Octahydro-1,4-dimethyl-7-(1-methylethylidene)- azulene
29	25.88	2.794	3,9 (1e)-dene-10-peroxy Murolan.
30	26.83	2.232	5,5 – Dimethyl -4- (3-methyl-1,3 -butadienyl)-1- Oxaspiro [2.5]Octane
31	28.64	1.534	2,3,4,4a,5,6,7,8- Octahydro-1,1,4a,7- tetramethyl-1H- benzocyclohepten-7-Ol
32	29.10	3.347	Cedrol
33	29.35	25.147	1S-(1α, 3a β, 4α, 8aβ)]-Decahydro-4,8,8,- trimethyl-9- methylene-1,4- methanozulene (Longifolene- D).
34	31.01	1.675	Aromadendrene oxide-(2)
35	31.64	5.84	[3R-(3α, 3aβ, 7β, 8aα)]- 2,3,4,7,8,8a-hexahydro-3,8,8-trimethyl-1H- 3a,7-methanoazulene-6- methanol.
36	32.39	8.835	Aromadendrene oxide – (1)
37	33.34	6.78	(8S) -1- Methyl-4- isopropyl- spiro-10- (tricyloc[5.50.0(5,9)] decane -7,8 diol) -2- (oxirane).
38	33.44	0.537	3,5,6,7,8,8a-hexahudro-4,8a-dimethyl-6-(1-methylethenyl)-2 (1H) Naphthalene
39	33.7	0.675	2,2,7,7-Tetramethyl-tricyclo[6.2.1.0 (1,6)] undec-4-en-3-one
40	36.26	0.502	Unidentified

Table 2. Contd.

41	36.29	9.757	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-2,3-diol (2-hydroxycyperol)
42	36.63	7.318	4-(3,3- Dimethyl-but -1-ynyl) -4-hydroxy-2,6,6-trimethylcyclohex-2- enone
43	40.14	0.007	[3a5-(3a α, 6β, 6aα, 9aβ, 9bα)] -2- metyl-(decahydro-6a-hydroxy- 9a- methyl-3- methylene-2,9- dioxoazuleno [4,5-b] furan-6yl) propanoic acid methyl ester.
44	39.97	0.033	Dotriacontane
45	49.12	0.007	Unidentified
46	50.01	0.006	3-Ethyl-5-(2-ethylbutyl)- octadecane
47	51.53	0.008	Unidentified
48	56.61	0.364	Unidentified
50	56.93	0.082	Unidentified

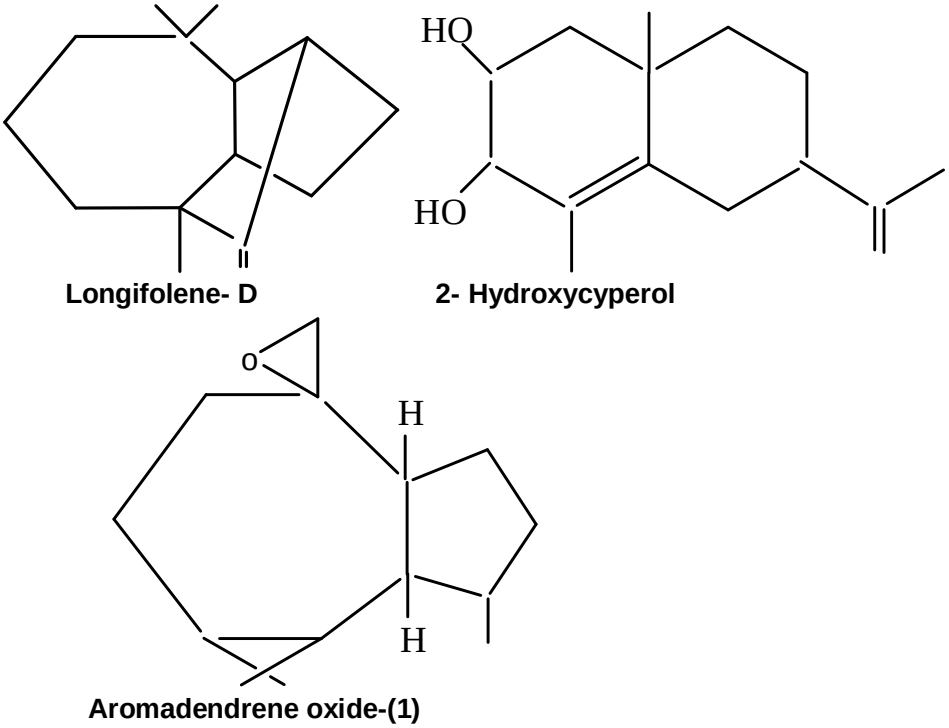

Figure 1. The main constituent of oil were, longifolene- D (25.1%), 2-hydroxycyperol (9.7%) and aromadendrene oxide –(1) (8.8%).

Table 3. Assessment of antibacterial activity of vetiver oil.

Microoorgnism	Concentration (mg/ml)	Inhibition zone (mm)	
		Range	Mean
Bacillus subtilis	10	15-16	15.5
Escherichia coli	10	0	0
Pseudomonas aeruginosa	10	22-24	23
Staphylococcus aureus	10	11-12	11.5
Inhibition zone ≥ 15: sensitive, <15: resistant			

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