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Investigations of Medicinal Aromatic Plants From Cameroon: GC/Fid, GC/MS and Olfactoric Analyses of Essential Oils of *Ocimum suave* Willd. (Lamiaceae)

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Abstract

Ocimum suave Willd. (Lamiaceae) is a plant with insecticidal activities, native in Africa and Asia. The essential oils of flowers, leaves and stems of *O. suave* from Cameroon were analysed by GC, GC/MS and olfactometry. The olfactory characteristics of the oils are: animalic, unpleasant fish notes, fatty train-oil-like (whale), green-fatty sides notes and weak repellent odor. Using GC/FID and GC/MS the oils were characterized by a high percentage of oxygenated aromatic compounds (26.2% - 91.6%), represented especially by elemicine (9.8% - 38.5%), eugenol (1% - 33.1%) and cis-methyl eugenol (6.8% - 19.3%). The oil from dried leaves, dried flowers and dried stems contain a higher percentage of sesquiterpene derivatives (46.9%, 57.3% and 78.2% respectively) with the main compounds β-caryophyllene, β-bisabolene, humulene oxide I and humulene oxide II.

Keywords: *Ocimum suave*, Lamiaceae, essential oil compositions

Introduction

Ocimum suave Willd (Lamiaceae) is a shrub, found in tropical Asia and in West and East Africa (Hutchinson and Dalziel, 1954). The Massai people of East Africa refer to it as Oiamora (Watts and Breyer-Brandwijk, 1962). In Equatorial Africa, *O. suave* is limited to the Congo and Cameroon (Raynald *et al.*, 1979). *O. suave* is a branched, erect, pubescent, perenial, small aromatic shrub which grows at an average of 1 m, with dense spike of small greenish white flowers, leaves pubescent ovate to ovate lanceolate, acutely acuminate (7-8 cm long 2-4 cm broad), petiole 1-3 cm long. The plant is frequently used as mosquitoe repellent (Watts and Breyer-Brandwijk, 1962), branches are burned or placed on the roof and walls of huts. The leaves are also reputed to act as an insecticide towards mosquitoes, flies and other insects. The leaves of *O. suave* in combination with others plants are used by the people of Congo Brazaville to treat fever of children and to counteract menstrual problems (Bouquet, 1969). Insecticidal activities of essential oil of *O. suave* have been tested (Obeng-Ofori and Reichmuth, 1997, Bekele *et al.*, 1996, Hassanali *et al.*, 1990, Mwangi *et al.*, 1995). Antimicrobial activity of plant extracts from Rwanda, have also been tested (Janssen *et al.*, 1989). The anti-ulcer effects of methanol extract of leaves of *O. suave* from Cameroon have recently been investigated (Tan *et al.*, 2002). Some previous investigations has been done on the essential oils of *Ocimum suave*

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plants from various origin, such as West Africa (Pushpangadan *et al.*, 1978), Ethiopia (Rovesti, 1975a, Rovesti, 1975b), Tanzania (Chogo and Crank, 1981), Rwanda (Tetenyi *et al.*, 1986), China (Yu and Cheng, 1986, Wu *et al.*, 1990) and Guinea (Keita *et al.*, 2000).

However to the best of our knowledge, there is no literature on essential oil of *O. suave* from Cameroon. In spite of the great interest in this plant, the aims of this study were to analyse the volatiles of the oils responsable for the significant odor impression, and to give information for a possible use in food preservation, medicinal and cosmetic applications. The investigation was carried out with different parts of the plant: leaves, stems and flowers.

Materials and Methods

The *Ocimum suave* plant parts (flowers, leaves and stems) were collected during the drying season of November to December 1999 as wild plant in the Savannah area of Bini-Ngaoundere (Cameroon) and the plant was identified by Dr. Anacletus Koufani (botanist of the National Herbarium of Yaoundé, Cameroon) and a voucher specimen (No. 24632/SRF/CAM) deposited at the National Herbarium of Yaoundé. A part of the leaves, the flowers and the stems were dried at room temperature of the laboratory for two weeks, before hydrodistillation.

The essential oils were produced by steam-distillation for 4 hours using a Clevenger type apparatus. The yields (v/w) of the oils are as follows: 0.1% of fresh leaves; 0.4% of dried leaves; 0.4% of dried flowers and 0.01% of dried stems.

Gas chromatography with Flame Ionization Detection (GC/FID)

GC/FID analyses were carried out using a GC-14A with FID and C-R6A-Chromatopac integrator (Shimadzu, Japan), and a GC-3700 with FID (Varian, Germany) and C-R1B-Chromatopac integrator (Shimadzu, Japan). The carrier gas was hydrogen; injector temperature, 250°C; detector temperature, 320°C. The temperature programme was: 40°C/5 min to 280°C/5 min, with a heating rate of 6°C/min. The columns were 30 m x 0.32 mm bonded FSOT-RSL-200 fused silica, with a film thickness of 0.25 µm (Biorad, Germany) and 30 m x 0.32 mm bonded Stabilwax, with a film thickness of 0.50 µm (Restek, USA). Quantification was achieved using peak area calculations (GC/FID). The identification of single compounds was performed by comparison of retention-indices with reference data (Adams, 2001, Davies, 1990, Jennings and Shibamoto, 1980, Kondjoyan and Berdaque, 1996, Tudor, 1996).

Gas chromatography with Mass Spectrometry (GC/MS)

For GC/MS measurements a GC-17A with QP5050 (Shimadzu, Japan), and data system ProLinea (Compaq, software class5k), a GC-17A with QP5000 (Shimadzu, Japan), data system ProLinea (Compaq, software class5k), a GC-HP5890 with HP5970-MSD (Hewlett-Packard, USA) and the data system on a Pentium-PC (Böhm, Austria; MSD-ChemStation software), a GCQ (Finnigan, USA) with data system Gateway-2000-PS75-PC (Siemens-Nixdorf, Germany, GCQ-software) were used. The carrier gas was helium; injector temperature, 250°C; interface-heating at 300°C, ion-source-heating at 200°C, EI-mode was 70 eV, and the scan-range was 41-450 amu. Temperature programm and column see GC/FID part. Mass spectra correlations were done using Wiley, NBS, NIST and private library spectra, as well as published data (Adams, 2001, Joulain and König, 1998).

Results and Discussion

The olfactory evaluation of the investigated essential oils was given by professional perfumers as follows: animalic, unpleasant fish notes, fatty train (wheal) oil like, smokey, green-fatty sides notes with weak repellent odor.

Using gas chromatographic-spectroscopic methods, more than 60 compounds were identified in the five *Ocimum suave* samples, which represent about 98% of the total peaks area (see Table

1). The GC and GC/MS analysis showed that the oils from fresh leaves of this *Ocimum* species contain a high percentage of oxygenated aromatic compounds (91.6%), while the monoterpene derivatives concentrations were lower (1.8%). The distribution of the components of the essential oil of dried leaves of *O. suave* is drastically different from that one of the oil of fresh leaves. The oxygenated aromatic compounds in the oils of fresh leaves are represented mainly by elemicine (93.5%), eugenol (33.1%) and cis-methyl eugenol (19.7%). Further monoterpenes and sesquiterpenes were identified as α-farnesene (3.7%) and limonene (1.2%).

The essential oil of dried leaves contains a higher concentration of sesquiterpene derivatives (43.9%) and aromatic compounds (35.9%). Cis-methyl eugenol was found to be the main compound (19.3%), followed by β-bisabolene (12.2%), humulene oxide (13.1%) and elimicine (9.8%). Eugenol was observed as component of the essential oil of fresh leaves only in a lower percentage (4.3%), in correlation to the dried leaf oil.

The essential oil of dried flowers of *O. suave* contains also a higher percentage of sesquiterpene derivatives (57.3%), represented by β-bisabolene (19.4%), humulene oxide I (12.5%) and humulene oxide II (10.2%). Additionally, in this oil the highest percentage of elemicine (19.1%) was found. Other sesquiterpene hydrocarbons with a concentration higher than 1.0% are as follows: α-copaene (2.2%), germacrene D (1.6%) and β-caryophyllene (1.3%).

The yield of the essential *O. suave* oil from stems is very low (<0.01%), this oil contains a high percentage of sesquiterpene hydrocarbons (56.0%) and lowest percentage of monoterpene derivatives (2.9%). The main components were found to be β-bisabolene (25.1%), elemicine (18.9%), β-caryophyllene (18.1%), humulene oxide I (6.8%) and humulene oxide II (6.8%) as well as cis-methyl eugenol (6.8%).

Significant is also the good agreement of gas chromatographic-spectroscopic results with the olfactory evaluation of single compounds (Arctander, 1969, BACIS, 1999, Bauer *et al.*, 1997, Sigma-Aldrich, 2001) as follows: the animalic unpleasant note could be attributed to elimicine derivatives and some sesquiterpene oxides. The green side note to sesquiterpene hydrocarbones and the fatty side note to the higher percentage of fatty acids. In correlation to investigations of volatile analyses of other *Ocimum suave* samples, it is remarkable, that in an essential oil from leaves of *O. suave* from Guinea a higher percentage of p-cymene (56.8%) was found (Keita *et al.*, 2000), whereas our results show a majority of oxygenated aromatic compounds and sesquiterpene derivatives, such as elemicine, eugenol, β-bisabolene and β-caryophyllene (main components of the *O. suave* essential oils from Cameroon). The aromatic compounds eugenol (71.5-84.5%) and methyl eugenol have also been identified in a higher percentage in essential oils of *O. suave* from Ethiopia-Eritrea (Rovesti, 1975a, Rovesti, 1975b), from Tanzania (Chogo and Crank, 1981), from Rwanda (Tetenyi *et al.*, 1986) and from China (Yu and Cheng, 1986, Wu *et al.*, 1990). In contrast to our investigations, an essential oil of *O. suave* from West Africa with a significantly higher percentage of sesquiterpenes and with a lower amount of aromatic derivatives has been described (Pushpangadan *et al.*, 1978).

In conclusion, we can report that essential oils of different plant parts of *Ocimum suave* from Cameroon seem to be a new chemotype, beside the known eugenol-type (Rovesti, 1975a, Rovesti, 1975b, Chogo and Crank, 1981, Tetenyi *et al.*, 1986, Yu and Cheng, 1986, Wu *et al.*, 1990) and sesquiterpene type (Keita *et al.*, 2000), and may be a potential source for the identified main compounds of these oils, like elemicine and methyl isoeugenol.

The composition of the essential oils are changing with the used part plant and when the plant is dried. Therefore, a possible alternative to known fragrances with repellent odor impressions and of interest for the food industry, was found. In the future these essential oils of *O. suave* from Cameroon may be valuable for the food preservation and medicinal applications, after developing an effective production in an industrial scale.

Table 1. Compounds of the essential oils of *Ocimum suave* from Cameroon in order of their retention indices (RI, using a non-polar column)

	RI	Compounds	Fresh Leaves	Dried Leaves	Stem	Dried Flowers
		Hydrocarbons	-	**0.45**	tr	**0.00**
1	951	Oct-4-ene-3-one	-	tr	-	-
2	977	6-Methyl-5heptene-2-one	-	0.39	tr	-
3	1052	(Z)-3-Hexene-1-ol	-	0.06	-	-
		Monoterpene hydrocarbons	**1.38**	**7.80**	**2.91**	**1.36**
4		α-Thujene	-	0.07		
5	936	α-Pinene	tr	1.48	0.23	0.26
6	946	Camphene	-	0.04	-	-
7	973	β-Pinene	0.15	5.90	1.53	1.03
8	1023	α-Terpinene		tr	-	-
9	1035	Limonene	1.23	0.28	1.14	0.07
10	1092	Terpinolene	-	0.03	0.01	-
		Oxygenated monoterpenes	**0.42**	**4.81**	**tr**	**2.16**
11	1032	1,8-Cineol	tr	0.21	tr	tr
12	1064	(E)-ß-Ocimene	0.12	0.04	tr	-
13	1074	(Z)-Linalool oxide, furanoid		0.06	-	tr
14	1080	Fenchone	tr	0.11	-	tr
15	1087	(E)-Linalool oxide, furanoid	tr	0.06	-	-
16	1098	Piperitone		0.08	-	-
17	1103	Linalool	tr	0.18	tr	
18	1112	Fenchol	-	tr	-	tr
19	1118	α-Pinene oxide	-	0.24	-	-
20	1127	Neral	-	-	tr	-
21	1140	Pinocarveol	tr	0.77	-	0.51
22	1146	(Z)-Limonene oxide		tr	-	-
23	1150	Verbenol	-	0.18	-	-
24	1157	Pinocarvone	-	0.34	-	0.16
25	1173	(E)-Limonene oxide	tr	0.17	-	tr
26	1188	Terpinen-4-ol	tr	0.18	tr	tr
27	1195	Myrtenal	-	0.57	tr	0.37
28	1203	α-Terpineol	0.30	0.51	tr	0.54
29	1209	Myrtenol	-	0.51	tr	0.37
30	1231	Carveol, cis	-	tr	-	-
31	1254	Piperitone oxide	-	0.20	-	tr
32	1302	2-Hydroxipiperitone	-	0.14	-	tr
33	1381	Damascenone	tr	0.26	-	0.21
		Aromatic compounds	**91.59**	**35.88**	**27.03**	**26.16**
34	1026	p-Cymene	tr	0.05	tr	-
35	1307	Carvacrol	-	0.23	-	-
36	***1357***	***Eugenol***	***33.09***	***4.34***	***tr***	***0.96***

Table 1. (continued)

	RI	Compounds	Fresh Leaves	Dried Leaves	Stem	Dried Flowers
37	1392	Methyl eugenol	tr	-	tr	-
38	1465	(E)-Methyl isoeugenol	0.31	-	0.27	2.32
39	1484	Methyl vanillin	-	0.13	0.43	-
40	*1523*	*(Z)-Methyl isoeugenol*	*19.70*	*19.26*	*6.78*	*8.06*
41	*1600*	*Elemicine*	*38.49*	*9.76*	*18.88*	*13.19*
42	1709	(E)-Isoelemicine	tr	2.11	0.67	1.63
		Sesquiterpene hydrocarbons	**4.66**	**23.10**	**51.97**	**28.50**
43	1367	α-Cubebene	-	0.24	0.10	0.22
44	1390	α-Copaene	tr	1.62	1.04	2.18
45	1398	β-Bourbonene	tr	1.01	0.38	1.34
46	1408	β-Elemene	tr	0.19	0.90	0.43
47	1434	α-Gurjunene	-	0.15	0.23	-
48	*1447*	*β-Caryophyllene*	*0.67*	*1.59*	*18.08*	*1.33*
49	1460	(E)-β-Bergamotene	-	-	-	0.13
50	1473	(E)-β-Farnesene	-	4.37	0.09	0.37
51	1479	β-Cubebene	-	0.18	0.43	0.21
52	1491	α-Humulene	0.07	0.25	1.51	0.21
53	1495	γ-Muurolene	0.14	0.16	0.88	-
54	1502	Germacrene D	-	0.43	0.65	1.65
55	1528	Bicyclogermacrene	tr	0.43	1.93	-
56	1546	Zingiberene	tr	0.09	0.07	0.43
57	*1569*	*β-Bisabolene*	*3.70*	*12.22*	*25.20*	*19.46*
58	1581	α-Muurolene	0.08	0.17	0.48	0.54
		Oxygenated sesquiterpenes	**1.08**	**23.83**	**16.26**	**28.85**
59	1608	Caryophyllene oxide I	-	0.46	0.25	0.78
60	1615	Caryophyllene oxide II	-	0.21	-	0.31
61	1620	Spathulenol	tr	0.23	-	-
62	1629	Nerolidol	tr	tr	0,35	0,24
63	*1648*	*Humulene oxide I*	*0.48*	*13.10*	*6.83*	*12.47*
64	*1651*	*Humulene oxide II*	*0.41*	*6.70*	*6.80*	*10.18*
65	1656	γ-Eudesmol	tr	0.29	0.16	0.56
66	1663	t-Muurolol	tr	0.44	0.29	0.38
67	1677	t-Cadinol	0.06	2.11	1.24	3.36
68	1716	α-Cadinol	0.13	0.29	0.34	0.57
		Diterpenes	**0.00**	**0.08**	**0.00**	**0.21**
69	2225	Phytol	-	0.08	-	0.21
		Total Identified	**99.13**	**95.95**	**98.17**	**87.24**
		Unknown oxygenated sesquiterpenes	**0.35**	**2.94**	**1.11**	**6.95**
		Fatty acids	**0.24**	**0.00**	**0.00**	**4.06**

tr trace compound
bold/italic identified main compounds

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