The Major Components of Essential Oils From *Ocimum* Species of Tanzanian Origin

DEBORAH K.B. RUNYORO

Faculty of Pharmacy, P.O. Box 65013, Dar-es-Salaam, Tanzania

Steam distillation of fresh leaves and flowers of *Ocimum canum* Sims (Syn. *O. americanum* L.) from two localities, Kimara and Muhimbili, gave essential oils in yields of 0.6% and 0.9% respectively. Leaves and flowers of *O. gratissimum* L. gave a 0.26% yield while that of *O. suave* Willd was 0.6%. The oils were analyzed by GC and GC-MS. The oil of *O. gratissimum* and that of *O. suave* had eugenol as their major component. Oils of *O. canum* from the two localities differed considerably in their chemical composition. The oil from plants collected in Kimara gave 1,8-cineole as the major component while ocimenoyl oxide was the major component of the oil from Muhimbili plants. Ocimenoyl oxide which constitutes 60% of the oil has not been reported previously in the genus.

Key Words: *Ocimum canum, O. americanum, O. gratissimum, O. suave,* Essential Oils, Labiatae, ocimenoyl oxide

INTRODUCTION

The genus *Ocimum* consists of about 60 species with a large number of varieties containing both terpene and nonterpene constituents in their essential oils. Members of the genus find a number of uses in African traditional medicine [1-5]. The plants are also reputed for their mosquito repellency and antibacterial activity [1,2,3,6].

Plants containing essential oils as well as the isolated constituents find application as insecticides, insect repellents, antiseptics, disinfectants, carminatives, flavouring agents and for treatment of colds, coughs, sore throat and epilepsy. Although plants containing essential oils have been studied elsewhere, there is only limited information on essential oils containing plants of Tanzania origin. As part of our effort to study Tanzanian odoriferous plants, three *Ocimum* species were investigated. These were *O. canum, O. gratissimum* and *O. suave. Ocimum canum,* known as Hoary or American basil, is indigenous to the tropics and occurs widely in East and West Africa, East India and Sri Lanka [7]. Extracts from the plant have been previously reported to kill *Anopheles gambiae* larvae [8]. It is a general observation that *Ocimum gratissimum* is used in coastal regions of Tanzania as a mosquito repellent. In the Western state of Nigeria the plant is used in treating diarrhoea [9]. The essential oil obtained from the plant was reported to have antifungal activity [10]. *Ocimum suave* is indigenous to Africa and India. Chogo and Crank [1] found that the essential oil had both mosquito repellent and antibacterial activities. Furthermore, the oil from Kenyan plants was reported to repel and kill *Rhipicephalus appendiculatusa* and the oil was effective at growth stages of the tick as well as repelling of maize weevils [11, 12].

EXPERIMENTAL

Plant material and isolation of essential oil

Details on plant, period and place of collection are summarised in table 1. All plant materials were initially identified in the field by a taxonomist. Authentication of the plants was carried out at the University of Dar-es-Salaam and later confirmed at the Royal Botanic Gardens Kew, U.K. where voucher specimens are kept. All plant samples were collected within Dar-es-Salaam area and extracted immediately after collection. The oil content was determined in accordance with the BP (1980) method. Bulk extraction of the essential oil was done by steam distillation using a steam generating kettle. The generated steam was passed through the plant materials contained in a tightly closed round bottomed flask and the steam laden with essential oil was passed through a condenser and the water/oil mixture collected in a separator. The oil layer was separated and dried over anhydrous sodium sulphate.

TABLE 1 : Plant materials under study

Botanical Source	Area of Plant Collection	Month Collected	Plant Part
O. canum	Muhimbili	June	leaves & flowers
O canum	Kimara	July	leaves & flowers
O. gratissimum	Muhimbili	June	leaves & flowers
O. suave	Kimbiji	June	leaves & flowers

GC AND GC-MS ANALYSIS

Terpenes, phenyl propane derivatives and other compounds likely to occur in the essential oils and which are available from commercial sources were used as reference compounds for GC analysis.

The GC analysis was carried out at the University of Bradford (U.K.) using a Perkin Elmer model 88320B GC machine equipped with silica capillary column and a flame ionization detector (FID). One column (non-polar, 50 m x 0.33 mm id) which consisted of BPl phase (SGE Australia) 1 µm coating thickness, was temperature programmed from 70 - 200^{0}C at 2^0/min, 200^{0}C (55 min); carrier gas N_2, 25 ml/sec. The other column (semi-polar, 25 m x 0.33 mm id) which consisted of a BP2O phase (SGE Australia), 0.5 µm coating thickness, was temperature programmed from 60 - 170^{0}C at 20^{0}C/min, the final temperature being maintained for 65 minutes, other conditions were as for the first column.

GC-MS analysis was carried out at the Physico-Chemical Measurement Unit (P.C.M.U.), Hewell (U.K.)

Determination of the percent composition of the oils was based on normalisation of peak areas without application of the response factor. Identification of components was based on comparison of Kovat's Retention Index either with values calculated for authentic reference substances or occasionally from published values on BP 1 [12] and SE30 [13]. The GC-MS results were interpreted by the use of a computer search or visual analysis. The latter method involved comparison of the mass spectra with the published data, either in the Eight Peak Index of Mass [14] or the literature [12, 13, 15-18].

RESULTS AND DISCUSSIONS

Percentage yields of oil from *O. canun* (Muhimbili) *O. canun* (Kiamara), *O. gratissinum* and *O. suave* were 0.6, 0.9, 0.26, 0.6, respectively. Percentage composition of the major chemical compounds in the four oil samples were found to be as shown in Table 2.

TABLE 2 : Percent composition of the major compounds identified in the essential oil from *Ocimum* species

No.	Compound	Percentage Composition			
		1	2	3	4
1.	α-pinene	2.2	0.6	-	-
2.	sabinene	1.2	0.3	-	-
3.	β-pinene	5.8	1.1	-	-
4.	myrcene	2.6	-	0.4	0.3
5.	1,8-cineole	-	14.7		
6.	cis-ocimeme	-	-	12.0	7.9
7.	ocimenoyl oxide	60.0		-	-
8.	trans-ocimene	0.1	0.9	1.0	10.7
9.	linalool	1.0	9.2	2.9	1.8
10.	α-terpineol	3.3	1.3	-	-
11.	nerol	-	3.1	-	-
12.	neral	0.5	3.8	-	-
13.	geraniol	0.2	8.5	-	-
14.	geranial	1.0	6.0	-	
15.	eugenol	0.6	-	46.7	52.4
16.	neryl acetate	-	6.3	-	-
17.	methylcinnamate	-	2.0	0.3	
18.	geranylacetate	0.1	13.2	-	1.6
19.	α-copaene	0.1	-	1.9	-
20.	β-bourbonene	0.6	-	-	1.8
21.	guaiene	-	-	1.6	
22.	b-caryopy1 lene	3.9	3.7	1.3	1.2
23.	unidentified sesquiterpene	-	-	2.6	6.2
24.	β-selinene	2.9	3.1	-	-
25.	α-farnesene	-	-	2.6	-
26.	α-selinene	2.5	2.8	-	-
27.	oxygenated sesquiterpene	-	-	1.3	4.2

1. *O. canum* (Muhimbili)
2. *O. canum* (Kimara)
3. *O. gratissimum*
4. *O. suave*

The two oils from *O. canum* growing in two different localities in Dar-es- Salaam were qualitatively different as shown in Table 2. The plants were collected within fifteen kilometres from each other and had been growing under very similar climatic conditions. According to the Royal Botanical Gardens (Kew, U.K.) there are no recognized varieties within the species which could account for the discrepancy. The most probable explanation for the observed differences is a chemical race phenomenon.

Ocimenoyl oxide which constitutes 60% of the oil from Muhimbili has not been reported in *O. canum* previously. 1 ,8-Cineole, the major constituent of the oil from Kimara, has been previously reported only in trace amount. With the genus, 1 ,8-cineole has been reported as the major constituent of *O kilimandscharicum* oil from Rwanda and *0. keniense* oil from Kenya [19,20]. Linalool, the major constituent of the oil from Rwandese plants [21] was identified in appreciable amounts in the oil from Kimara plants and was only present to the extent of 1% in the oil from Muhimbili plants. Other compounds identified had been reported in oil previously with the exception of *trans* ocimene and guaiene. The oil from Kimara plants had appreciable amounts of citral (ie neral and geranial) (9.8%) which could make it useful in flavour industry.

Eugenol, *cis-* and *trans-* ocimene were the major components of the oil from *O. gratissimum.* Thymol, reported [9] as the major component of the oil from Nigerian species, was not identified in the oil under study. α-farnesene and caryophyllene oxide were identified in the oil for the first time.

Eugenol was found to be the major component of *0. suave* (Table 2). This is in agreement with previous reports [1]. α-Copaene, *trans* ocimene and β-bourbonene had not been identified in the oil before. Several compounds reported to be present in the species were not identified in the oil under study and they include limonene, α- and β-pinene, β-elemene, bisabolene, β-cubebene, α- and β–selinene, carveol, terpinene-4-ol, carvone, linalyl acetate and caryophyllene oxide.

In this study it was found that major components of the species studied were monoterpenoids. Sesquiterpenes were present only in relatively low concentrations. Ocimene, linalool and β–caryopyllene were identified in all samples studied in varying concentrations. Oil from *O. gratissimum* and *O. suave* had some similarities especially in having eugenol as the major component.

REFERENCES

[1] J.B. Chogo and G. Crank. J. Nat. Prod. 44 (1981) 308-11.

[2] C.W. Githinji and J.O. Kokwaro. J. Ethinopharmacol. 39 (1993) 197-203.

[3] A.M. Janssen, J.J. Scheffer, L. Ntezurubanza and A. Baerheim. J. Ethnopharmacol. 26 (1989) 57-63.

[4] G.B. White. East Afr. Med. J. 50 (1973) 249-252.

[5] J.M. Watt, and M.G. Brandwijk, The Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd Edition, E & S Livingstone Ltd, Edinburgh & London (1962).

[6] M.O. Nwosu and J.I. Okafor. Mycoses, 38 (1995)191-5.

[7] E. Guenther, The Essential Oils, D. Van Nostrand, Princeton, New Jersey, Toronto, New York and London. Vol. *5&6.* 1952.

[8] N. Lukwa. Cent. Afr. J. Med. 40 (1994) 306-9.

[9] E.A. Sofowora. Planta. Med. 18 (1970) 73-176.

[10] E.O. Lima, O.F. Gompertz, A.M. Giesbrecht, M.Q. Paulo. Mycoses 36 (1993) 333-6.

[11] E.N. Mwangi, A. Hassanali, S. Essuman, E. Nyandat, L. Moreka and M. Kimondo. Exp. Appl. Acarol. 19 (1995) 11-8.

[12] A.A. Swigar and R.M. Silverstein, Monoterpenes; Infrared, Mass H-NMR and ^{13}C NMR Sepctra and Kovats Indices Adrick Chemical Inc. (1981).

[13] W. Jennings and T. Shibamoto, Qualitative Analysis of Volatiles of Glass Capillary Gas Chromatography. Academic Press, (1980).

[14] Eight Peak Index of Mass Spectra, Mass Spectrometry Data Centre, Aldermaston Reading (U.K.), Vol.1(1970), Vol.2 (1974), Vol.3 (1983).

[15] R. Rhage and E. Von Sydow, Mass Spectrometry of Terpenes I. Monoterpene Hydrocarbons, Acta Chem. Scand. 17 (1963) 2025-2035.

[16] E Von Sydow, Mass Spectrometry of Terpenes II. Monoterpenes Alcohols. Acta Chem. Scand. 17 (1963) 2504-2512.

[17] M.G. Mashonas and E.D. Lund, The Mass Spectrometry of Sesquiterpene Hydrocarbons, Flavour Ind. (1970) 375-378.

[18] S. Dev, A.P.S. Narula and J.S. Yadav, CRC Handbook of Terpenoids 2 CRC Press Inc. (1982).

[19] L. Ntezurubanza, J.J. Scheffer, A. Looman and S.A. Barheim. Planta Med. 50 (1984) 385-388.

[20] J.W. Mwangi. W. Lwande and A. Hassanali. Flavour and Frag. J. 9 (1994) 75-76.

[21] L. Ntezurubanza, J.J. Scheffer and A. Looman. Pharm Week bl. Sci. Ed. 7 (1985) 273-6.