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EXTRACTION AND GC/MS ANALYSIS OF THE ESSENTIAL OIL OF *OCIMUM BASILICUM* (COMORO)

M. Riaz, M. Rashid Khalid, M. Hanif and F. M. Chaudhary

PCSIR Laboratories Complex, Lahore-54600, Pakistan

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A combination of GC and GC/MS has been used to characterise chemical composition or essential oil hydrodistilled from comoro variety of *Ocimum basilicum* grown in various parts of Pakistan. Among the forty four compounds identified, methyl chavicol (83.58-87.21%), 1, 8-cineol (2.45-3.37%), linalool (0.04 - 2.69%) and methyl eugenol (1.4 - 2.68%), are predominantly present while other compounds particularly monoterpenes and sesquiterpenes hydrocarbons are found only in traces. Variations in the essential oil composition and yield during hydrodistillation are also reported.

Key words: *Ocimum basilicum,* Essential oil, Sesquiterpenes, GC/MS analysis.

Introduction

Ocimum basilicum (comoro variety) belonging to N.O. Labiateae, commonly known as Niazbo [1, 2] is a scented herb cultivated throughout India and Pakistan. The plant finds numerous uses in local system of medicine and is prescribed as a substitute for *Lallemantia royleana.* The oil is also used in food and perfumery industries.

Though various constituents [3,4] of the basil oil have been identified earlier yet the oils from Pakistan have not been studied earlier. As the variations in the chemical composition of basil oils play an important role in evaluating their utilization and value for various industries, so basil oils from five different places were investigated for their chemical composition using GC and GC/MS techniques. Analyses showed that the various oils were homogenous in composition and methyl chavicol was the most abundant constituent. As 6 harvests/ year of this plant are possible, so this plant can become a viable source of methyl chavicol.

The present studies deal with the physico-chemical characteristics (Table 1) and chemical composition of the basil oils (Table 2).

Materials and Methods

Ocimum basilicum (comoro) plants were grown at five different places namely Lahore, Multan and Muridke in the province of Punjab and Hyderabad and Sajawal in the province of Sindh. Plants at the flowering stage were subjected to steam distillation using Likens Nickerson apparatus [5] for the extraction of basil oil. Oils were dried over anhydrous sodium sulphate, filtered and weighed yielding 0.21–0.23% of basil oils.

Various physico-chemical investigations i.e. acid number, ester number, specific gravity and refractive index (Abbe's) were performed according to the procedures described earlier [6].

Identification by GC and GC/MS. Gas chromatographic analyses were carried out on a Pye-Unicam 104 instrument equipped with a flame ionization detector, using a 25m x 0.22 mm. i.d., SE-30 coated WCOT fused silica column. Hydrogen gas was used as a carrier gas with a flow velocity of 26 cm/sec. and split ratio 1:100. The column temperature programmed at 60° for 0 min with 4°/min. rise to 220°, while detector and injection temperatures of 300°and 250° were used respectively. Various components were identified by their respective retention times and peak enhancement with standards. Percentage composition of individual components was calculated on the basis of peak area using SP-4100 (spectra physics) computing integrator.

Jeol model JMS-AX505H mass spectrometer combined with Hewlett Packard gas chromatograph was used for GC/ MS analysis. Samples were injected on a WCOT fused silica column, coated with SE-30 and helium as carrier gas, split ratio 1:100, EI positive mode, electron energy 70 eV, ionization current 300 μA, ionization source temperature 250°, interface temperature 230°, column temperature programmed

Table 1. Physico-chemical Properties of the Essential Oil Obtained from Different Areas.

	A	B	C	D	E
Yield (%)	0.23	0.22	0.22	0.215	0.21
Wt per ml of oil at 30°	0.9744	0.9616	0.9568	0.9555	0.9675
Acid number	3.41	1.85	0.85	0.63	1.04
Ester number	16.24	6.7	3.69	4.53	4.42
Refractive index at 30°	1.5146	1.5191	1.5124	1.5119	1.5141

TABLE 2. CHEMICAL COMPOSITION (%) OF THE ESSENTIAL OIL OBTAINED FROM FIVE DIFFERENT CULTIVARS OF *OCIMUM BASILICUM* (COMORO).

Peak No.	Compound	A	B	C	D	E	m/z
1	α-Pinene	+	+	+	+	+	93, 77,41, 121, 136, 27
1a	Camphene	+	+	+	+	+	93, 121, 107, 79, 43, 68
2	Sabinene	+	+	+	+	+	93, 69, 41, 57, 77, 136
3	β-Pinene	+	+	+	+	+	93, 69, 41, 27, 79, 53
4	48-Cineol	2.86	3.27	2.45	3.24	3.37	93, 81, 68, 55, 41, 111
5	*p*-Menth-1 (7)-en-9-o1	+	+	+	+	+	93, 92, 91, 79, 77, 41
6	Ocimene	1.46	1.75	1.47	+	+	105, 121, 92, 41, 136, 39
6a	$^{3}\Delta$-Carene	+	+	+	+	+	93, 91, 136, 77, 41, 105
6b	Mentha-24(8)-diene	+	+	+	+	+	121, 136, 93, 77, 105, 41
7	Linalool	2.12	2.69	0.04	2.32	1.05	43, 41, 55, 71, 93, 80
8	Fenchol	0.02	0.11	0.26	+	+	81, 80, 69, 41, 55, 111
9	Camphor	0.21	0.28	0.24	+	0.36	81, 95, 108, 41, 152, 69
9a	Borneol	+	+	+	+	+	95, 110, 55, 41, 17, 139
10	4-Terpineol	+	+	+	+	+	71, 111, 93, 86, 154,41
11	Methyl chavicol	85.42	83.58	87.15	87.21	86.31	
12	Octyl acetate	+	+	+	+	+	43, 56, 70, 55, 83, 148
13	Geranyl acetate	0.33	0.31	0.28	+	+	43, 81, 136, 93, 121, 107
14	Propenyl anisole	+	+	+	+	+	148, 147, 117, 133, 105,77
15	α-Bornyl acetate	0.13	0.16	0.19	+	0.28	95, 136, 121, 94, 43, 80
15a	*o*. Isoeugenol	+	+	+	+	+	164, 149, 103, 77, 121, 43
15b	δ-Elemene	+	+	+	+	+	93, 121, 79, 41, 107, 136
15c	α-Ylangene	+	+	+	+	+	105, 161, 119, 91, 32, 41
16	Methyl eugenol	1.65	1.41	2.27	2.41	2.68	51, 65, 91, 115, 151, 178
17	Guaiene	0.17	0.25	0.23	+	+	147, 121, 107, 81, 68, 80
18	Bicyclo-7, 2,o undec-4-ene, 4, 11, 11, trimethyl-8-methylene	0.41	+	0.42	+	0.12	133, 93, 69, 79, 105
19	β-Eudesmene	1.37	0.50	1.15	+	+	120, 107, 69, 55, 41, 91
20	α-Elemene	+	+	+	+	+	161, 105, 119, 204, 81, 41
21	α-Caryophyllene	+	+	+	+	+	80, 93, 121, 147, 41, 107
22	β-Guaiene	+	+	+	+	+	105, 161, 119, 204, 91,81
23	β-Cubebene	+	+	+	+	+	105, 161, 91, 119, 81, 204
24	β-Bisabolene	0.17	+	0.19	+	+	69, 93, 41, 133, 79, 55
25	Eremophilene	0.23	+	0.19	+	+	107, 93, 121, 161, 204, 79
26	α-Bulnesene	0.46	0.18	0.38	+	0.27	107, 93, 189,135, 79, 204
27	γ-Cadinene	+	+	+	+	+	119, 105, 161, 91, 81, 41
28	β-Cadinene	+	+	+	+	+	161, 204, 105, 119, 69, 93
28a	α-Murrolene	+	+	+	+	+	105, 161, 32, 204, 119, 93
28b	Unidentified	+	+	+	+	+	32, 82, 105, 67, 161, 55
28c	α-Himachalene	+	+	+	+	+	93, 69, 43, 55, 107, 121
28d	9-Aristolene-1- o1	+	+	+	+	+	159, 91, 205, 104, 119, 187
28e	Transfarnesol	+	+	+	+	+	91, 41, 105, 55, 121, 79
28f	Unidentified	+	+	+	+	+	161, 91, 107, 69, 133, 77
28g	α-Cadinol	+	+	+	+	+	91, 69, 107, 141, 55, 161
29	δ-Cadinene	0.12	+	0.13	+	+	179, 119, 161, 105, 204, 79
30	Unidentified	+	+	+	+	+	151, 83, 124, 93, 55, 105
31	β-Patchoulene	0.85	+	0.96	0.50	0.96	204, 105, 134, 162, 81, 43
32	Eudesm-7 (11)-en-4-o1	+	+	+	+	+	204, 161, 121, 189, 105, 81
32a	β-Cedrene	+	+	+	+	+	119, 93, 109, 69, 204, 41
	Total	98.16	94.48	98.00	95.68	95.40	

+ = in traces, A = Lahore. B =Multan, C= Muridke. D= Hyderabad. E= Sajawal. F = The six most intense peaks are represented. G= Percentages calculated from the peak areas. H = Peak numbers are given in the order of appearance.

at 60° for 4 min with a 6°/min rise to 230°, data acquisition and reprocessing were performed by Jeol JMA-DA 5500 system with MS-4BTK library search system.

Results and Discussion

The plant was grown at five different places to study the effect of soil and climate in chemical composition and yield of essential oils. Volatile substances of the five cultivars identified by GC and GC/MS are presented in Table 2. A review of Table 1 and 2 indicated that practically there was no appreciable change in the composition and yield of basil oils. Out of the forty seven compounds detected in the oil, forty four have been identified. Among the 44 compounds identified the quantitative distribution indicated that methyl chavicol was the most abundant (83-87%) in the oils. These Pakistani *Ocimum basilicum* (comoro) essential oils resemble those from Malagasy (India) and Ife (Nigeria) having 74-87% and 84% methyl chavicol, respectively. The chemical constituents identified by the above mentioned techniques consist of 7 monoterpene hydrocarbons, 8 alcohols, 4 esters, 1 carbonyl, 5 ethers, 18 sesquiterpenes hydrocarbons and 3 unidentified sesquiterpenoids. Monoterpenes hydrocarbons constitute (traces to 1.46%), alcohols (1.05% to 2.8%), ester (traces to 0.47%) carbonyls (traces to 0.36%), ethers (88.26 to 92.86%) and sesquiterpene hydrocarbons (0.5 to 3.66%) of the essential oil. Among the 18 sesquiterpenoids identified, α-ylangene, β-cubebene, α-bulnesene, eremophilene, α-himachalene, β-cedrene and β-patchoulene have been identified for the first time. Among the other compounds that had not previously been reported are *p*-menth-1 (7)-en-9-ol, transfarnesol, octyl acetate, *p*-propenyl anisol, 9-aristolene 1-α-ol and Eudesm-7 (11) en-4-o1.

The compound producing peak 5 was tentatively identified as *p*-menth-1 (7)-en-9-ol. Its MS showed characteristics fragments at m/z (rel. int.):154 $[M]^+$ (10), 93 (100), 92 (36), 79 (32), 121 (12),77 (26), 105 (16), 41 (25) in accordance with the expected fragments of this structure. Peak 6b was due to a compound tentatively identified as *p*-mentha-2,4 (8)-diene. Its MS showed important peaks at m/z (rel. int): 136 $[M]^+$ (89), 121 (100), 93 (83), 77 (23), 107 (20) and 41 (10). The compound producing peak 18 was tentatively identified as bicyclo-7-2-o-undec-4-ene, 4,11,11 trimethyl-8-methylene. Its MS showed characteristic peaks at m/z (rel.int): 204 $[M]^+$ (26), 133 (100), 93 (99), 69 (89), 41 (78), 105 (61), 120 (54), 79 (62). The compound in peak 24 was tentatively identified as β-bisabolene. Its MS showed important peaks at m/z (rel.int): 204 $[M]^+$ (15), 69 (100), 93 (71), 41 (61), 133 (43), 79 (39), 55 (22), 107 (22). The peak at 28 d was tentatively identified as 9-aristolene-1-α- ol. Its MS showed characteristic fragments at m/z (rel.int.): 220 $[M]^+$ (20), 159 (100), 91 (94), 205 (83), 105 (80), 119 (70), 187 (67), 131 (61).

Studies on the other varieties of *Ocimum basilicum* are in hand and will be reported elsewhere.

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