

Studies on the essential oil bearing plants of Bangladesh. Part VI. Composition of the oil of *Ocimum gratissimum* L.†

Mohammed Yusuf,¹ Jaripa Begum,¹ Luigi Mondello^{2*} and Ildefonsa Stagno d'Alcontres²

¹BCSIR Laboratories, P.O. Chittagong Cantonment, Chittagong 4220, Bangladesh

²Dipartimento Farmaco-Chimico, Facoltà di Farmacia, Università di Messina, Viale Annunziata, 98168 Messina, Italy

Received 22 March 1997

Accepted 4 July 1997

ABSTRACT: The composition of the essential oil of *Ocimum gratissimum* L. obtained from plants originating from Bangladesh and from plants introduced into Bangladesh from India is reported. The oil obtained from plants native of Bangladesh can be classified as thymol-type according to its thymol content (58.2%), while the oil obtained from plants introduced from India can be classified as eugenol/ocimene type according to its content of eugenol (66.5%) and ocimenes (18.9%). © 1998 John Wiley & Sons, Ltd.

KEY WORDS: *Ocimum gratissimum* L.; essential oil composition; chemotypes; gas chromatography; GC-MS; thymol; eugenol; ocimene

Introduction

Ocimum gratissimum L. (shrubby basil) is a tall, much-branched perennial shrub, 1–2.5 m high, distributed in the tropical areas of Africa, America and Asia. In the Indian subcontinent it is found in the peninsular India (West Bengal and Assam) and in Bangladesh.²

The oils distilled from *O. gratissimum* are characterized, generally, by their high phenol content, consisting mainly of eugenol^{3–23} or thymol.^{24–26} Other chemotypes are also reported: a linalol type;¹⁴ a geraniol type;²⁷ a methyl cinnamate and a eugenol/sesquiterpene type;²⁸ an ocimene type, an ocimene/methyl isoeugenol type and a eugenol/ocimene type;²⁹ an ethyl cinnamate type,³⁰ a citral type;³¹ a sesquiterpene type;³² a eugenol/methyl eugenol type.³³ In the eugenol type, the eugenol content ranges from 18%⁶ to 90%⁸; while in the thymol type the thymol content ranges from 19%²⁵ to 48%²⁴.

In this paper the composition of the essential oil of *O. gratissimum* isolated from the plants originating from Bangladesh and identified by Khan and Khanam² and the oil from the plants of *O. gratissimum* var. *clocicum* evolved in India by Sobti⁵ and introduced into Bangladesh in 1986 is reported.

Experimental

The leaves and the inflorescences were collected from the plants grown in the campus of Bangladesh Council of Scientific and Industrial Research (BCSIR) Laboratories, Chittagong during September 1995 (*O. gratissimum* var. *clocicum*) and May 1996 (Bangladeshi *O. gratissimum*). The oils were isolated from the fresh leaves and inflorescences by hydrodistillation carried out for 2 hours in a modified Clevenger-type apparatus.

Voucher samples of *O. gratissimum* originating from Bangladesh are retained in Bangladesh National Herbarium, Dhaka; while voucher samples of *O. gratissimum* evolved in India are retained in the Herbarium of the Regional Research Laboratories, CSIR, Jammu-Tawi. Both are also retained in the Herbarium of BCSIR Laboratories, Chittagong.

GC Analysis

We used a Fisons chromatograph 5160 Mega Series equipped with a Shimadzu data processor C-R 3A; an SE-52 fused silica column (25 m × 0.32 mm, film thickness 0.40–0.45 µm, Mega, Legnano, Italy); column temperature, 40°C (6 min) to 240°C at 3.0°C/min; injector temperature, 250°C; detector temperature, 280°C; injection mode, split; split ratio, 1:50; volume

* Correspondence to: Luigi Mondello, Dipartimento Farmaco-Chimico, Facoltà di Farmacia, Università di Messina, Viale Annunziata, 98168 Messina, Italy.

† For Part V see Ref. 1.

Contract grant sponsor: Ministero dell'Università e della Ricerca Scientifica e Tecnologica, Italy.

Table 1. Relative percentage as single components and as classes of substances for the oil of *Ocimum gratissimum* L. native to Bangladesh (A) and of *Ocimum gratissimum* L. var. *clocicum* introduced into Bangladesh from India (B)

Compound	Area (%)		Linear retention indices on DB-5		Identification methods			
	A	B			a	b	c	d
(Z)-Hex-3-en-1-ol	<0.1		nd		a		c	d
α -Thujene	2.0		924		a	b	c	d
α -Pinene	0.8		932		a	b	c	
Camphene	0.1		948		a	b	c	d
Sabinene	0.4		971		a	b	c	d
Oct-1-en-3-ol		0.1	977		a			
β -Pinene	0.5		977		a	b	c	d
Octan-3-one		<0.1	985		a			
Myrcene	2.6	0.4	988		a	b	c	d
Octan-3-ol		0.1	996		a	b	c	d
(E)-Hex-3-enol acetate		<0.1	1004		a	b	c	d
α -Phellandrene	0.3		1007		a	b	c	d
δ -3-Carene	0.1		1009		a	b	c	d
α -Terpinene	1.2		1016		a	b		
p-Cymene	7.7		1024		a	b	c	d
Limonene	1.1		1028		a	b	c	d
β -Phellandrene	0.1		1029		a	b	c	d
1,8-Cineole			1031		a	b	c	d
(Z)- β -Ocimene	0.2	18.1	1033		a	b	c	d
(E)- β -Ocimene	0.2	0.8	1043		a	b	c	d
γ -Terpinene	12.3		1058		a	b	c	d
cis-Sabinene hydrate	0.3		1068		a	b		
Terpinolene	1.5		1084		a		c	
p-Cymenene	<0.1		1089		a	b	c	d
Linalol	0.3	0.6	1099		a	b	c	d
1,3,8-p-Menthatriene	<0.1		1111		a			
α -Thujone	0.1		1117		a	b	c	d
allo-Ocimene		0.4	1127		a	b	c	d
cis-p-Menth-2-en-1-ol	<0.1		1127		a	b		
(E)-Myroxide		0.1	1138		a			
Borneol	0.2		1171		a			
p-Menth-1,5-dien-8-ol		0.1	1170		a			
Terpinen-4-ol	1.4		1179		a	b	c	d
p-Cymen-8-ol	0.4		1188		a	b		
trans-p-Mentha-2,8-dien-1-ol		0.7	1201					
Thymol methyl ether	0.2		1284		a	b		
Thymol	58.2		1297		a	b		
Carvacrol	0.9		1302		a	b		
α -Cubebene		<0.1	1344		a	b		
Eugenol	0.1	66.5	1348		a	b		
α -Copaene	<0.1	0.8	1375		a	b	c	d
β -Bourbonene		0.2	1382		a	b	c	d
β -Cubebene		0.4	1387		a	b	c	d
β -Elemene	0.1	0.1	1388		a	b	c	d
Isocaryophyllene		<0.1	1395		a	b	c	d
β -Caryophyllene	0.6	2.1	1416		a	b	c	d
β -Gurjene		0.1	1426		a			
trans- α -Bergamotene	<0.1		1430		a	b	c	
α -Humulene	0.1	0.2	1451		a	b	c	
Germacrene-D	<0.1	2.9	1478		a	b	c	d
β -Selinene	1.1		1485		a	b		
α -Selinene	0.7		1491		a	b	c	d
(E,E)- α -Farnesene		0.1	1503		a	b	c	d
7- <i>epi</i> - α -Selinene	0.2		1514		a			
δ -Cadinene		0.3	1515		a	b	c	d
Spathulenol		0.2	1572		a	b	c	d
Caryophyllene oxide	0.5	0.2	1576		a	b	c	
Humulene epoxide II	0.1		1603		a			
14-Hydroxy-9- <i>epi</i> - β -Caryophyllene		0.2	1663		a			
Isolongifolol	0.1		1716		a			
(E)-Conyopheril alcohol		0.2	1727		a			
Monoterpene hydrocarbons	30.9	19.7						
Sesquiterpene hydrocarbons	2.8	7.2						
Alcohols	2.8	2.0						
Esters		<0.1						
Phenols and derivatives	59.5	66.5						
Eters and oxides	0.6	0.3						
Ketones	0.1	<0.1						

a. Adam's library; b. Perfume library;³⁶ c. FFC bank, co-injection with authentic compounds; d. NIST library; nd, not determined.

injected, 1 µl of a solution 1/20 in pentane of the oil; carrier gas, He, 100 kPa.

GC-MS Analysis

We used a CE Instruments MD 800 equipped with Adams' library,³⁴ FFC bank,³⁵ Parfume library (CE Instruments)³⁶ and NIST library; DB-5 fused silica column (30 m × 0.25 mm, film thickness 0.25 µm, J & W, Folsom, California, USA); column temperature, 60–240°C at 3°C/min, to 300°C at 30.0°C/min; injector temperature, 250°C; injection mode, split; split ratio, 1:20; volume injected, 1 µl of a solution 1/20 in pentane of the oil; carrier gas, He, 83 kPa; linear velocity, 40 cm/sec at 60°C; interface temperature, 250°C; source temperature, 200°C, EI⁺ acquisition with mass range of 41–300 amu.

Results and Discussion

O. gratissimum native to Bangladesh yielded 0.75% (volume/fresh wt.) of essential oil. Table 1 shows the relative percentage as single components and as classes of substances for this oil. Forty-two components were identified that represent about 97% of the whole oil. The main component was thymol (58.2%), followed by γ-terpinene (12.3%) and *p*-cymene (7.7%). The total content of phenol compounds was 59.5%. Monoterpene hydrocarbons represents 30.9%, sesquiterpene hydrocarbons 2.8% and alcohols 2.8%. The oil also contained three oxides: 1,8-cineole, caryophyllene oxide and humulene oxide II, and small quantities of a ketone, α-thujone.

According to the thymol content, this oil could be classified as thymol-type oil, but it shows a thymol content higher than those reported in literature for *O. gratissimum* thymol-type oils.

Table 1 also shows the relative percentage as single components and as classes of substances for the oil of *O. gratissimum* var. *clocimum*, an Indian native introduced into Bangladesh. This yielded 0.72% of oil. Here 29 components were identified that represent about 96% of the whole oil. The main component was eugenol (66.5%), followed by (Z)-β-ocimene (18.1%). Among the other components only two sesquiterpene hydrocarbons, germacrene-D (2.9%) and β-caryophyllene (2.1%) occur at a percentage higher than 1%. According to the content of the main components, this oil could be classified as eugenol/ocimene type. Moreover, the sesquiterpene hydrocarbon fraction of the oil is qualitatively and quantitatively rich.

Acknowledgements — The analytical part of this research was supported by Ministero dell'Università e della Ricerca Scientifica e Tecnologica of Italy (60% and 40% Research funds). Coordinator of the research group: Prof. Giovanni Dugo.

References

- Part V: L. Mondello, A. Verzera, I. Bonaccorsi, J. U. Chowdhury, M. Yusuf and J. Begum, accepted for publication by *J. Essent. Oil Res.*
- M. S. Khan and M. M. Khanam, *Bangladesh J. Bot.*, **4**, 37 (1975).
- N. A. Kekelidze and L. V. Baradze, *Subtrop. Kul't.*, 103 (1975); in *C.A.*, **84**, 1405984 (1976).
- S. N. Sobti, P. Pushpangadan and C. K. Atal, *Indian Perf.*, **20**(1A), 59 (1976); in B. M. Lawrence, *Perf. Flav.*, **12**(4), 80 (1987).
- S. N. Sobti, P. Pushpangadan, B. L. Bradu and B. B. Jain, *Indian Perf.*, **23**(1), 16 (1979); in B. M. Lawrence, *Perf. Flav.*, **12**(4), 80 (1987).
- Y. Cheng and M. Liu, 9th International Congress of Essential oils, Fragrances and Flavors, Singapore (1983). In B. M. Lawrence, *Perf. Flav.*, **12**(4), 80 (1987).
- R. D. Tripathi, R. Banerji, M. L. Sharma, V. R. Balasubrahmanyam and S. K. Nigam, *Agric. Biol. Chem.*, **49**, 2277 (1985); in *C.A.*, **103**, 137003t (1985).
- V. A. Zamurenko, N. A. Klyuev, L. B. Dimitriev, S. G. Polyakova and I. I. Grandberg, *Izv. Timiryazevsk S-Kh Akad.*, **2**, 172 (1986); in B. M. Lawrence, *Perf. Flav.*, **12**(4), 80 (1987).
- P. T. Son, N. Van Dau, P. L. Chau and N. Q. Chien, *Tap Chi Hoa Hoc*, **25**(2), 23 (1987); in *C.A.*, **108**, 137683t (1988).
- B. M. Lawrence, *Perf. Flav.*, **12**(4), 80 (1987).
- M. K. Khosla and S. N. Sobti, *Indian Perf.*, **81**(2), 54 (1987).
- N. Maheshwari, H. G. Krishnamurti, M. Chien and M. L. Maheshwari, *Indian Perf.*, **32**(1), 7 (1988).
- R. K. Khanna, O. S. Sharma, M. L. Sharma, P. N. Misra and A. Singh, *Parfuem Kosmet.*, **69**, 564–568 (1988); in *C.A.*, **110**, 28901 (1989).
- B. M. Lawrence, 11th International Congress of Essential Oils, Fragrances and Flavors, New Delhi, India, November 11–16 (1989).
- B. L. Bradu, S. N. Sobti, P. Pushpangadan, M. K. Khosla, B. L. Rao and S. G. Gupta, 11th International Congress of Essential Oils, Fragrances and Flavors, New Delhi, India, November 11–16 (1989).
- M. K. Khosla, B. L. Bradu and R. K. Thapa, *Herba Hung.*, **28**(1,2), 13 (1988).
- B. Gulati and G. K. Sinha, 11th International Congress of Essential Oils, Fragrances and Flavors, New Delhi, India, November 11–16 (1989).
- M. Colson, P. Tetenyi and A. Perrin, *Herba Hung.*, **30**(3), 5 (1991).
- K. Umeha, K. M. Bojappa, A. A. Farooqi and T. Subhaiah, *Indian Perf.*, **35**(1), 53 (1991).
- D. De Medici, S. Pieretti, G. Salvatore, M. Nicoletti and P. Rasoanaino, *Flavour Fragr. J.*, **7**, 275 (1992).
- S. N. Choudhury and D. N. Bordoloi, *Indian Perf.*, **37**(1), 51 (1993).
- K. Umeha, K. M. Bojappa, N. S. Suresh, A. A. Farooqi and S. Jagannath, *Indian Perf.*, **37**(4), 311 (1993).
- S. C. Gupta, *J. Essent. Oil Res.*, **8**, 275 (1996).
- M. Sainsbury and E. A. Sofowora, *Phytochemistry*, **10**, 3309 (1971).
- A. S. Dro and F. W. Hefendehl, *Planta Med.*, **24**, 353 (1973).
- L. Ntezurubanza, J. J. C. Scheffer and A. B. Svendsen, *Planta Med.*, **53**, 421 (1987).
- D. J. Charles and J. E. Simon, *J. Essent. Oil Res.*, **4**, 231 (1992).
- C. E. Fun and A. B. Svendsen, *Flavour Fragr. J.*, **5**, 173 (1990).
- A. K. Vimalan, K. Sadanandan, M. P. Philip, N. Natrangan, M. Murugesan and N. P. Damodaran, 11th International Congress of Essential Oils, Fragrances and Flavors, New Delhi, India, November 11–16 (1989).
- M. E. Ali and L. A. M. Shamsuzzaman, *Sci. Res. (Dacca)*, **5**, 91–4 (1968); in *C.A.*, **70**, 60746c (1969).
- R. Hegnauer, *Chemotaxonomic der Pflanzen*, **4**, p. 314, Birkhäuser Verlag Basel (1966); in *C.A.*, **66**, 102603d (1967).
- V. A. Zamurenko, V. Ya Tokareva, N. A. Klyuev, T. I. Karpova and I. I. Grandberg, *Izv. Timiryazevsk S-Kh Akad.*, **4**, 153 (1981); in *C.A.*, **95**, 103133m (1981).

33. O. Vostrowsky, W. Garbe, H.-J. Bestmann and J. G. S. Maia, *Z. Naturforsch.*, **45**(c), 1073 (1990); in *C.A.*, **113**, 229893t (1990).
34. R. P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*. Allured Publ. Corp., Carol Stream, IL, USA 1995.
35. L. Mondello, P. Dugo, A. Basile, G. Dugo and K. D. Bartle, *J. Microcol. Sep.*, **7**, 581 (1995).
36. *Flavor and Fragrance Library*, CE Instruments, Rodano, Milan, Italy.