Asian Journal of Chemistry; *Vol. 27, No. 10 (2015), 3895-3899*

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.19062

Composition of Essential Oils of Six Varieties of *Ocimum basilicum* L. Grown in Algeria (Mustaghanem: West of Algeria)

N.B. SLOUGUI[1,*], M. HADJ MAHAMMED[1], Z. RAHMANI[1] and A. BAALIOUAMER[2]

[1]Laboratoire BioGéoChimie des milieu désertiques, université Kasdi Merbah Ouargla, route de Ghardaia, Ouargla 30000, Algeria
[2]Université des Sciences et de la Technologie Houari Boumediene (USTHB), Faculté de Chimie, Laboratoired' Analyse Organique Fonctionnelle, B.P. 32 El Alia, Bab Ezzouar, Algeria

*Corresponding author: Tel: +213 661 712810, E-mail: slougui.nabila@gmail.com

Received: 27 January 2015; *Accepted*: 4 March 2015; *Published online*: 22 June 2015; AJC-17369

Essential oils from six varieties of *Ocimum basilicum* L. grown in the west of Algeria (Mediterranean region) were identified by GC-MS chromatography using two different types of capillary columns: non-polar and polar. Chemotypes identified by the non-polar column are *Ocimum basilicum, Ocimum basilicum* purparescens opal, *Ocimum basilicum* minimum, *Ocimum basilicum* cinnamon, *Ocimum basilicum* (marcellais) and *Ocimum basilicum* citriodora are linalool-eugenol (37.6-33.5 %), linalool-eugenol (40.2-24.4 %), methyl eugenol-farnesene (β, E) (28.4-10.3 %), methyl cinnamate (E)–linalool (37.2-28.2 %), linalool (50.5 %) and geranial-neral (21.5-17.5 %), respectively. The analysis with polar column completes these results and confirms them.

Keywords: *Ocimum basilicum,* Linalool, Methyl cinnamate (E), Methyl eugenol, Eugenol geranial.

INTRODUCTION

Algeria is rich in its natural resources that are not well exploited. The medicinal plants constitute most of these resources. Spontaneous plants and aromatic plants in Algeria present a growing interest for researchers in the world. The traditional uses of plants for medicine were studied in south Algeria. To our best of knowledge, only two varieties of *Ocimum basilicum* are cultivated and marketed on a national scale in Algeria. These are *Ocimum basilicum* and *Ocimum basilicum* minimum.

The genus *Ocimum* belonging to the Lamiaceae comprises annual and perennial herbs and shrubs native to the tropical and subtropical regions of Asia, Africa and South America[1]. Apart from culinary use, basil has been traditionally employed as a medicinal herb in the treatment of headaches, cough, diarhoea and constipation[2-4]. It is also considered a source of aromatic compounds and essential oils containing biologically active constituents that possess insect repellent, nematocidal and antibacterial activity [5-8].

It is also considered to be a source employed as a medicinal herb in the treatment of headaches, cough, diarhoea, and constipation. Pushpangadan and Bradu[9] recognize more than 150 species in this genus. It is important to note that the type of cultivation, the agronomical practices and environmental conditions affect the composition of the sensory important compounds[10,11]. To assist in a classification, a system of standardized descriptors based on volatile oil has been proposed by Lawrence[6] and Grayer *et al.*[12]. They classified the different basil chemo types based on the prevalent aromatic compounds or on components present more than 20 %, respectively.

Many *Ocimum basilicum* varieties contain primarily phenoyl derivatives, such as eugenol, methyl eugenol, chavicol and methyl-cinnamate, which are often combined with various amounts of linalool[13,14]. Several analytical methods have been developed to determine volatile constituents of essential oils present in spices. It is shown that gas chromatography, coupled with mass spectrometry (GC-MS), is the most appropriate for the analysis of these volatile compounds.

In the realm of the natural resources valorization of the Mediterranean regions in Algeria, we have studied the composition of essential oils from several varieties of basil cultivated in the Algerian west (Mustaghanem), using gas chromatography coupled to mass spectrometry (GC-MS). Two types of capillary columns are used *i.e.*, polar and non-polar.

EXPERIMENTAL

Our study was conducted in the west of Algeria under Mediterranean conditions. The seeds of six varieties of *Ocimum basilicum* L.: *O. basilicum* (V_1), *O. basilicum* purpurascens (V_2), *O. basilicum* minimum (V_3), *O. basilicum* cinnamon (V_4), *O. basilicum* (marceillais) (V_5) and *O. basilicum* citriodora

(V_6), obtained from European markets (Belgium) were sown and grown for 3 months at 20 to 35 °C. Cultivars used for this study represent most of the commercially available basil plants in Europe.

To examine essential oils composition of the basil varieties without environmental influences, the plants were grown under identical conditions (environmental and soil conditions). Plants at the flowering stage were collected and the aromatic compounds from fresh material were extracted by steam distillation after they were analyzed by GC-MS.

General procedure

Essential oils extraction: Samples (1 kg) of fresh material were extracted by steam distillation for 2 h. The distillate was then extracted with dichloromethane. The solvent was removed at room temperature. The essential oils were stored in dark glass bottles at 4 °C until the beginning of the analyses[15].

Physical indices: The physical indices of the oils were determined following ISO Regulations: ISO 279: 1981 for specific gravity, ISO280: 1976 for refractive index, ISO 592: 1981 for optical rotation, ISO875: 1999 for miscibility in ethanol.

GC-FID and GC-MS analysis: Essential oil components were analyzed using a 6890 Agilent gas chromatograph equipped with HP-1 capillary column (50 m × 0.2 mm, film thickness 0.33 µm). Helium was the carrier gas, at a flow rate of 1.3 mL/min. The oven temperature was held at 60 °C for 2 min and then increased from 60 to 300 °C at a rate of 4 °C/min and maintained at 300 °C for 10 min. Injector and detector (FID) temperatures were 250 °C and 250 °C, respectively. Diluted samples (in dichloromethane) of 1 µL were injected in the split/splitless (100:1 split) mode.

GC-MS analysis was performed using an Agilent System comprising a model 6890 with 5975 (C series) mass selective detector which was equipped, for the first analysis, with a polar capillary column HP-Innowax (50 m × 0.2 mm, film thickness 0.5 µm). Helium was the carrier gas at a flow rate of 1.4 mL/min. The oven temperature was held at 60 °C for 2 min and then increased from 60 to 245 °C at a rate of 4 °C/min and maintained at 245 °C for 25 min. In the second analysis, the Mass selective detector was equipped with a non-polar capillary column HP-1 (50 m × 0.2 mm, film thickness 0.33 µm). Helium was the carrier gas at a flow rate of 1.3 mL/min. The oven temperature was held at 60 °C for 2 min and then increased from 60 to 300 °C at a rate of 4 °C/min and maintained at 300 °C for 10 min. For GC-MS detection, an electron ionization system with ionization energy of 70 eV, was used. Injector temperature was: 250 °C. Diluted samples (in dichloromethane) of 1 µL were injected in the split/splitless (100:1 split) mode.

Identification of oil components analyzed by the two capillary columns was accomplished based on comparison of their retention index (Ir), calculated from GC-FID analysis, with those of literature[16] and by a comparison of their mass spectral fragmentation patterns with those of WILEY and NIST02 databases.

RESULTS AND DISCUSSION

Algerian mediterranean regions contain various medicinal plants. Therefore, we studied the volatile composition of six varieties of *Ocimum basilicum,* cultivated in the west of Algeria under mediterranean conditions. V_1 and V_3 are the most commonly utilized species in Algeria while V_2, V_4, V_5 and V_6 have never been cultivated or studied in Algeria before.

Plants have been grown under the same conditions and the leaves used for GC and GC-MS analysis were of the same age and at a comparable developmental stage.

Table-1 shows geographical conditions of Mustaghanem varieties, their scientific and common names, the total yields of essentials oils and their physical properties.

Table-2 shows the different compounds identified in the extracts obtained from varieties used in our study and analyzed by two capillary columns: a non-polar and a polar one.

In the first analysis (non-polar column), we identified 31 compounds in V_1, 51 compounds in V_2, 44 compounds in V_3, 33 compounds in V_4, 24 compounds in V_5 and 37 compounds in V_6. Oxygenated monoterpenes were the most important ones followed by the aromatic compounds. In V_3, V_5 and V_6 sesquiterpenes hydrocarbons were present with a great percentage.

The dominant constituent in V_1, V_2, V_4 and V_5 was linalool ranging between 28.2 and 50.5 % of total oils. An excellent agreement has been found between our results and those obtained by other authors[15]. Eugenol was also prevalent in these

TABLE-1
PHYSICAL PROPERTIES OF ESSENTIAL OILS *Ocimum basilicum* VARIETIES GROWN IN WEST OF ALGERIA

	MUSTAGHANEM					
Climate	Mediterranean					
Latitude	35° 94'					
Longitude	0° 09'					
Altitude (m)	55					
Varieties	V_1	V_2	V_3	V_4	V_5	V_6
Scientific name	O. *basilicum*	*O. basilicum* purparescens	*O. basilicum* minimum	*O. basilicum* cinnamon	*O. basilicum*	*O. basilicum* citriodora
Common name	Basilic grand vert	Basilic purple	Basilicnain compact	Basiliccannelle	Basilicmarcellais	Basilic citron
Percentage of essential oil	0.34	0.31	0.28	0.18	0.26	0.23
Moisture content (%)	83.13	84.86	86.86	78.56	77.87	81.33
Specificgravity (20 °C)	0.942	0.962	0.915	0.924	0.928	0.933
Refractive index	1.5010	1.4971	1.4805	1.5113	1.5045	1.4578
Optical rotation (°)	-2.25	-9.87	-7.814	-6.45	-8.85	-9.25
Miscibility in ethanol 90° (v/v)	1.7/1	1.5/1	2.7/1	2.1/1	2/1	2.5/1

TABLE-2
COMPOSITION OF SIX CULTIVARS OF *Ocimum basilicum* GROWN IN MUSTAGHANEM (WEST OF ALGERIA)

No.	Compound	Retention index		Percentage					
		HP1	HP INNOVAX	V_1	V_2	V_3	V_4	V_5	V_6
01	(*Z*) 3-Hexene-1-ol	837	-	0.4	0.9	-	-	-	-
02	α-Pinene	931	-	-	-	0.1	-	-	-
03	Comphene	944	-	-	-	t	-	-	-
04	Sabinene	969	1132	-	-	0.1	-	-	-
05	β-Pinene	973	-	0.1	t	0.2	-	-	-
06	6-Methyl 5-hepten-2-one	974	-	-	-	-	-	-	0.5
07	1-Octen-3-ol	975	1121	-	0.6	-	-	-	-
08	3-Careen	1010	-	-	-	0.7	-	-	-
09	Eucalyptol	1030	1224	9.1	9.6	7.0	2.2	0.5	0.1
10	γ-Terpinene	1054	-	-	t	-	-	-	-
11	(*Z*) Sabinene hydrate	1066	1558	0.1	0.1	0.1	0.1	-	-
12	Fenchone	1084	1417	-	1.6	-	0.1	-	0.3
13	α-Terpinolene	1086	1286	-	-	0.4	-	-	-
14	Linalool	1103	1550	37.6	40.2	5.5	28.2	50.5	5.9
15	Fencholendo	1120	-	-	1.9	-	-	-	-
16	(Z/Z) Photocitral	1127	-	-	-	-	-	-	0.1
17	(Z) Epoxy ocimène	1130	1493	-	-	-	-	-	0.4
18	Camphor	1141	1538	0.4	2.0	1.1	0.5	-	-
19	(E) Chrysanthemal	1146	-	-	-	-	-	-	0.3
20	Isoborneol	1155	-	-	-	-	0.1	-	
21	Phellandral	1161	-	-	-	-	-	-	0.7
22	δ-Terpineol	1162	1682	-	0.5	0.1	-	-	-
23	Borneol	1164	1713	0.6	t	0.1	0.2	-	-
24	Terpinene-4-ol	1175	1615	0.2	0.5	0.1	1.1	0.7	-
25	(S,Z) Verbanol	1179	-	-	-	-	-	-	1.0
26	α-Terpineol	1188	1707	0.9	2.0	0.6	0.4	0.6	0.7
27	Methyl chavicol	1195	-	-	-	-	t	-	-
28	γ-Terpinene	1197	-	-	-	-	0.1	-	-
29	Exo-2-hydroxycineole	1207	1869	-	0.1	-	-	-	-
30	5-Nonen-2-one	1211	-	-	0.1	-	-	-	-
31	Fenchyl acetate	1214	-	-	0.2	-	0.1	-	-
32	1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-6-one	1220	-	-	0.1	-	-	-	-
33	Nerol	1229	1804	-	0.2	0.1	-	-	9.7
34	Neral	1241	-	-	0.2	0.1	-	-	17.5
35	(Z) Anethol	1247	-	-	-	-	t	-	-
36	Geraniol	1253	1850	-	1.6	-	-	4.6	6.3
37	Geranial	1270	-	-	0.3	0.2	-	-	21.5
38	Endo-bornyl acetate	1278	1595	0.5	0.4	1.9	0.4	0.3	-
39	(Z) Methyl cinnamate	1301	1973	-	-	-	3.5	-	-
40	δ-Elemene	1329	-	-	t	0.1	-	-	-
41	Exo-2-hydroxycineol acetate	1334	1742	0.1	0.2	0.1	-	-	-
42	α-Cubebene	1342	1468	-	t	0.1	0.1	-	0.1
43	Eugenol	1357	2183	33.5	24.4	9.7	4.6	2.2	-
44	Neryl acetate	1359	-	-	-	-	-	-	0.6
45	Eugenol (dihydro)	1367	-	-	-	-	-	0.6	
46	α-Copaene	1374	1504	-	-	0.7	0.5	-	0.7
47	2-Methoxy-3-(2-propenyl)phenol	1375	-	0.4	0.4	-	-	-	-
48	(E) Methyl cinnamate	1376	2101	-	-	-	37.2	3.0	-
49	Geranyl acetate	1377	1763	-	-	-	-	-	t
50	2-Ethenyl-6-methyl-5-hepten-1-ol	1379	-	-	-	-	-	-	2.0
51	β-Cubebene	1381	-	0.3	-	t	0.6	0.8	t
52	β-Elemene	1383	1603	1.4	1.3	2.5	-	8.1	t
53	Methyl eugenol	1399	2024	1.3	1.3	28.4	-	-	0.2
54	(E) Caryophyllene	1415	-	0.8	0.6	4.8	1.0	-	8.1
55	Calarene	1429	1611	0.1	t	1.0	-	-	-
56	(α,E) Bergamotene	1431	1595	3.2	1.9	7.2	1.1	1.3	2.2
57	α-Guaiene	1437	-	-	-	-	-	0.9	-
58	Aromadendrene	1438	1736	-	-	-	-	1.1	-
59	Guaia-1(5),11-diene	1439	-	-	t	-	1.1	-	-
60	α-Humulene	1450	1688	0.3	t	t	0.4	0.6	1.3

61	(β,E) Farnesene	1452	1670	1.2	0.9	10.3	1.1	1.0	-
62	epi-Bicyclosesquiphellandrene	1454	-	-	0.2	-	-	-	-
63	Alloaomadendrene	1456	1686	-	t	-	-	-	-
64	γ-Muurolene	1478	-	-	0.2	-	-	-	-
65	D-Germacrene	1481	1728	1.6	1.5	6.3	4.3	4.5	7.0
66	Valencene	1494	-	-	t	-	-	-	-
67	Bicyclogermacrene	1499	1652	0.5	0.7	1.7	-	1.8	-
68	(α,Z) Bisabolene	1505	-	0.3	0.2	3.0	t	0.3	7.5
69	Guaia-1(10),11-diene	1506	1731	0.4	0.6	1.3	1.8	1.7	0.1
70	(γ,Z) Bisabolene	1512	1697	-	-	t	-	-	0.4
71	γ-Cadinene	1515	1776	0.7	0.7	0.9	1.8	2.8	-
72	β-Sesquiphellandrene	1519	1781	0.2	-	-	-	-	-
73	δ-Cadinene	1520	-	-	0.3	-	0.3	0.4	0.6
74	Ledene	1521	-	-	-	0.2	-	-	-
75	Germacrene B	1558	1752	-	-	-	1.4	-	0.3
76	(E) Nerolidol	1559	2044	-	0.1	0.2	-	-	-
77	Caryophyllene oxide	1580	-	-	-	0.2	-	-	0.5
78	Humulenepeoxide II	1606	-	-	-	-	-	-	0.1
79	Cubenol (1-epi)	1625	-	0.3	0.2	0.1	0.4	0.6	-
80	τ-Muurolol	1635	-	0.2	0.2	0.1	-	-	-
81	Agarospirol	1643	2196	0.1	0.1	-	-	-	-
82	β-Eudesmol	1647	-	-	-	0.3	-	-	0.3
83	τ-Cadinol	1649	-	2.2	1.5	0.9	3.1	5.3	0.1
84	α-Bisabolol	1680	-	-	-	-	-	-	0.3
85	(β,Z) Santalol	1711	-	-	-	-	-	-	1.0
	Total identified (%)			98.6	99.5	97.5	96.0	97.2	97.3
	Monoterpene hydrocarbons (%)			0.1	-	0.1	-	-	-
	Oxygenated monoterpenes (%)			49.1	61.8	17.4	33.5	57.2	65.1
	Sesquiterpenes hydrocarbons (%)			11.4	9.5	40.1	13.7	28.3	29.7
	Oxygenated sesquiterpenes (%)			2.8	2.1	1.8	3.5	5.9	2.3
	Aromatic compounds (%)			35.2	26.1	38.1	45.3	5.8	0.2

cultivars. Methyl eugenol and geranial are predominant constituents of essential oil of V_3 and V_6, respectively.

Five different chemotypes were identified which are: (1) linalool-eugenol, (2) methyl eugenol-farnesene (β, E), (3) methyl cinnamate- linalool, (4) linalool and (5) geranial-neral.

Chemotype I: The major compounds in V_1 and V_3 are linalool (37.6 and 40.2 %, respectively) and eugenol (33.5 and 24.4 %, respectively). Labra *et al.*[17] reported that eight cultivars of *Ocimum basilicum* from Italy contained linalool and eugenol as major components.

Chemotype II: The major compounds in V_3 are: methyl eugenol (28.4 %) and farnesene (β, E) (10.3 %). Methyl eugenol has been detected as the main component in several *O. basilicum* varieties[12] and in some *Ocimum* species, such as *O. tenuiflorum* (syn: *Ocimum sanctum)*[18].

Chemotype III: The major compounds in V_4 are methyl cinnamate (E) (37.2 %) and linalool (28.2 %). It was found that methyl cinnamate was the main component in the tropical chemotype[19] and in some *Ocimum basilicum* cultivars, such as cinnamon basil in Australia[20] and minimum basil in Brazil[21] and in some *Ocimum* species, such as *O. canum* from S. Tome and Principe islands[22]. Simon and Vieira[23] and Ozcan and Chalchat[24] reported *Ocimum basilicum* chemotypes with methyl cinnamate and linalool contents from USA, Brazil and Turkey, respectively[23-25].

Chemotype IV: The major compounds in V_5 are linalool with 50.5 % and elemene β with 8.1 %. HadjKhlifa *et al.*[26] reported that the major compound in *Ocimum basilicum* leaves grown in northen region of Algeria is linalool with 32.83 %.

Chemotype V: the major compounds in V_6 are geranial with 21.5 % and neral with 17.5 %.

We have observed that the second analysis using polar capillary column confirms the results obtained with the non-polar column and completes them.

Conclusions

The culture of six varieties of basil *i.e.*, *O. basilicum, O. basilicum* purpurascens, *O. basilicum* minimum, *O. basilicum* cinnamon, *O. basilicum* (marcellais) and *O. basilicum* citriodora in the west of Algeria has been successfully accomplished. Essential oils of the six samples are rich with sesquiterpenes hydrocarbons, aromatic compounds and oxygenated sesquiterpenes. Five chemotypes were identified: linalool-eugenol, methyl eugenol- farnesene (β, E), methyl cinnamate-linalool, linalool and geranial-neral. High contents of methyl cinnamate (E) in *Ocimum basilicum* cinnamon could be considered as tropical countries originated chemotypes with some influence of the North African (the Egyptian) chemotypes.

Because methyl eugenol have structural resemblance to carcinogenic phenyl propanoids such as safrole, chemotypes with linalool, methyl cinnamate, eugenol and related mixture are preferable for cultivation when they are used in food and perfume industries.

Analysis with two capillary columns with different polarity has enabled us to have a clear and complete idea on the composition of the essential oils from different varieties of basil grown under mediterranean conditions.

ACKNOWLEDGEMENTS

The authors thank Prof. Istvan E. Marko, Director of CHOM Unity, UCL, Louvain la neuve, Belgium, for his assistance and fruitful discussion.

REFERENCES

1. H.H. Darrah, The Cultivated Basil, Missouri Buckeye Printing Company, MO, Independence (1988).
2. R.J. Grayer, R.F. Vieira, A.M. Price, G.C. Kite, J.E. Simon and A.J. Paton, *Biochem. Syst. Ecol.*, **32**, 901 (2004).
3. M. Ozcan, D. Arslan and A. Unver, *J. Food Eng.*, **69**, 375 (2005).
4. O. Politeo, M. Jukic and M. Milos, *Food Chem.*, **101**, 379 (2007).
5. S.R. Chavan and S.T. Nikam, *Indian J. Med. Res.*, **75**, 220 (1982).
6. B.M. Lawrence, Mother Nature's Chemical Factory in Essential Oils: Labiatae Oils, Allured Publishing, Carol Stream, IL (1993).
7. R.S. Deshpande and H.P. Tipnis, *Pesticides*, **11**, 11 (1977).
8. Current Awareness in Flavour and Fragrance, *Flavour Fragr. J.*, **23**, 360 (2008).
9. P. Pushpangadan and B.L. Bradu, in eds.: K.L. Chandha and R. Gupta, Advances in Horticulture, Medicinal and Aromatic Plants, New Delhi (1995).
10. L. Jirovetz, G. Buchbauer, M.P. Shafi and M.M. Kaniampady, *Eur. Food Res. Technol.*, **217**, 120 (2003).
11. A. Vina and E. Murillo, *J. Braz. Chem. Soc.*, **14**, 744 (2003).
12. R.J. Grayer, G.C. Kite, F.J. Goldstone, S.E. Bryan, A. Paton and E. Putievsky, *Phytochemistry*, **43**, 1033 (1996).
13. E. Weker, E. Putievsky and U. Ravid, *Ann. Bot. (Lond.)*, **71**, 43 (1993).
14. M. Miele, R. Dondero, G. Ciarallo and M. Mazzei, *J. Agric. Food Chem.*, **49**, 517 (2001).
15. M. Marotti, R. Piccaglia and E. Giovanelli, *J. Agric. Food Chem.*, **44**, 3926 (1996).
16. R.P. Adams, Identification of Essential Oils Components by Gas Chromatography/Quadrupole Mass Spectrometry, Allured Publishing Corporation, Carol Stream, IL, USA, edn 4 (2007).
17. M. Labra, M. Miele, B. Ledda, F. Grassi, M. Mazzei and F. Sala, *Plant Sci.*, **167**, 725 (2004).
18. S.K. Kothari, A.K. Bhattacharya and S. Ramesh, *J. Chromatogr. A*, **1054**, 67 (2004).
19. G. Vernin and J. Metzger, *Perfum. Flavor.*, **9**, 71 (1984).
20. K.J. Lachowicz, G.P. Jones, D.R. Briggs, F.E. Bienvenu, M.V. Palmer, V. Mishra and M.M. Hunter, *J. Agric. Food Chem.*, **45**, 2660 (1997).
21. M.G. de Vasconcelos Silva, F.J. de Abreu Matos, M.I. Lacerda Machado and A.A. Craveiro, *Flavour Fragrance J.*, **18**, 13 (2003).
22. A. Martins, L. Salgueiro, R. Vila, F. Tomi, S. Canigueral, J. Casanova, A. da Cunha and T. Adzet, *Planta Med.*, **65**, 187 (1999).
23. R.F. Vieira and J.E. Simon, *Econ. Bot.*, **54**, 207 (2000).
24. M. Ozcan and J.C. Chalchat, *Czech. J. Food Sci.*, **20**, 223 (2002).
25. J. Janick, J.E. Simon, J. Quinn and R.G. Murray Simon, Basil: A Source of Essential Oils. In: Advanced in new Crops, Timber Press, Portland, OR, pp. 484-489 (1999).
26. L. HadjKhelifa, M. Brada, F. Brahmi, D. Achour, M.L. Fauconnier and G. Lognay, *J. Herbal Med.*, **1**, 25 (2012).