Asian Journal of Chemistry; Vol. 24, No. 6 (2012), 2799-2801

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Variation in the Composition of the Essential Oils of *Rosmarinus officinalis* L. Grown in Sub-Tropical Region of Jammu and Temperate Region of Kashmir, India

M.K. VERMA*, R. ANAND, A.S. SHAWL, S. KITCHLU, A.K. SHAHI, S. CHANDRA, S. KOUL and R.K. KHAJURIA

Instrumentation Division, Indian Institute of Integrative Medicine (CSIR), Canal Road, Jammu Tawi-180 016, India

*Corresponding author: E-mail: mkvermadr@yahoo.com; mkverma@iiim.res.in

(*Received*: 4 August 2011; *Accepted*: 17 January 2012) AJC-11000

A comparison of constituents of *Rosmarinus officinalis* L. oils, grown in two different climatic regions of Jammu & Kashmir, has been carried out by gas chromatography-mass spectrometry to perform separation and identification of the constituents of the essential oil. The comparative study has resulted in establishing a chromatographic technique for authenticating the variations in chemical composition with change in climatic conditions. Substantial variations in the constituents of essential oil were observed. The technique has also helped in identifying a different Eco-type of rosemary.

Key Words: Rosmarinus officinalis, Essential oil, Chemical composition, GC-MS.

INTRODUCTION

Essential oils and their components are gaining interest due to their relatively safe status as well as their wide acceptance by the consumers and these oils are also being exploited for their potential as medicinal agents. Rosemary (*Rosmarinus officinalis* L.) is a perennial aromatic and medicinal herb of the family Lamiaceae with fragrant evergreen needle like leaves and a native of Mediterranean region. This plant is cultivated for its essential oil in number of countries. The oil of the plant possesses good olfactory properties and is used in perfumes, cosmetic products, food and pharmaceutical industry.

The essential oil of rosemary is used in aromatherapy to enhance both physical and mental health. The plant is also used to maintain beauty and in massage therapy. The plant is getting popular in health spas and resorts in Europe and U.S.A. The plant is used to improve the mood and cognition in healthy adults[1].

The literature survey reveals that rosemary was used in traditional Turkish folk medicine for the treatment of hyperglycemia[2]. The plant also possesses number of medicinal properties such as spasmolytic, antirheumatic, analgesic, carminative, cholagogue, antidepressant, hypertensive, expectorant and diuretic. Rosemary has been recognized and widely accepted as one of the medicinal herb with the highest antioxidant activity[3] these activities are due to the presence of phenolic diterpenes such as carnosol, rosmanol, 7-methyl-epirosmanol, isorosmanol, rosmadial, carnosoic acid, methyl carnoste and phenolic acids such as rosmarinic and caffeic acids.

The essential oil as well as extracts of rosemary has been a subject of various chemical and biological studies[3-11] over the decades from different origins[12-15]. Studies on extraction of essential oil of rosemary have been carried out by different processes[16-19]. This makes it a very significant plant. Keeping in view the importance of the plant the work was undertaken to contribute in a better way about the variations in chemical composition of essential oil of rosemary growing in different climatic regions of Kashmir (temperate region) and Jammu (sub tropical region) of India.

EXPERIMENTAL

Propagation done by cuttings and the saplings were planted at experimental farms at Indian Institute of Integrative Medicine (CSIR), Jammu-Tawi, India under two different agro climatic zones *i.e.* under temperate zone at Srinagar (Latitude 34º 5' N, Longitude 14º 50' E Altitude 1730 m asl) and sub-tropical agro climatic zone at Jammu (Latitude 32º 43' N, Longitude 74º 54' E, Altitude 34º m asl). The fresh aerial parts of *R. officinalis* L. of second year plantation were harvested at flowering stage. Voucher specimen of the plant has been deposited in the herbarium of the institute.

Gas chromatograph: Perkin-Elmer (USA) make gas chromatograph model auto system XL was used to analyse the chemical constituents of essential oil of rosemary. The GC was equipped with FID and Restek (Bellefonte, PA, USA) make fused silica capillary column (30 m × 0.32 mm ID, 0.25 µm film thickness) coated with dimethyl polysiloxane (RTX-1).

Gas chromatograph-mass spectrometer: GC-MS analysis was carried out on a Varian GC-MS 4000 fitted with a Varian Factor Four VF-5 ms fused silica capillary column (30 m × 0.25 mm id, film thickness 0.25 um).

Oil extraction: The aerial parts of the plant were harvested in the year 2008 and were subjected to hydrodistillation in Clevenger's-type apparatus for 3 h. The percentage of the essential oil yielded in different months from April to December was studied and maximum yield was observed in the month of June (1.1 %). Thus, oil collected during this month was subjected to the present studies. The oil was dried on anhydrous Na_2SO_4 and stored in sealed vials.

GC analysis: Column oven temperature was programmed from 50 ºC-240 ºC at 5 ºC per min. Injector and detector temperatures were optimized at 250º and 270 ºC respectively. Nitrogen gas at a flow rate of 1mL/min was used as the mobile phase. Injector split ratio was 1:80.

GC-MS analysis: Temperature programming of oven was from 50 ºC to 240 ºC at 5 ºC/min rising rate. Helium was used as carrier gas at flow rate of 1 mL/min. Mass spectra were recorded over 50-300 amu range at 1 scan per sec with E.I. at 70 eV.

Identification of peaks was carried out by comparing their retention times with authentic samples injected under similar chromatographic conditions. Comparison of kovat retention indices with literature values was carried out. The mass spectra were compared with those reported in the NIST and WILEY computer libraries and those published in literature[20].

RESULTS AND DISCUSSION

The results obtained have been tabulated in Table-1, where area percentage of oil constituents of Srinagar & Jammu plantations has been compared. Out of 35 oil constituents studied, major variations have been observed in at least six compounds.

The most abundant constituent of the essential oil from the plantation of Srinagar, analyzed in the present study was camphor representing 25.38 % of the total oil whereas other constituents were 1,8-cineol (22.68 %), α-pinene (11.98 %) camphene (8.27 %), β- pinene (6.94 %), bornyl acetate (5.56 %), borneol (2.95 %), myrecene (2.59 %), verbenone (1.94 %), linalool (1.82 %), α-terpineol (1.12 %) and (E)-β-caryophylene (1.02 %). The major chemical constituents found in Jammu plantation was α-pinene representing (31.61 %) and other were camphor (13.29 %), β-phellandrene/1,8-cineol (2.28/6.53 %), camphene (8.76 %), β-pinene (3.40 %), bornyl acetate (3.24 %), borneol (7.05 %) myrecene (2.18 %), verbenone (2.1 %), linalool (0.52 %), α-terpineol (1.30 %) and (E)-β-caryophylene (0.33 %).

The reported components were mostly monoterpenes and the major among them were α-pinene, 1,8-cineol and camphor (associated with variable amounts of camphene, limonene, borneol, verbenone and bornyl acetate). Two major types of rosemary oil can be distinguished on the basis of their main constituents: 1,8-cineol rich oil *viz*. oil from Morocco, Tunisia, Turkey and Greece and oils with approximately equal ratios (20-30 %) of 1,8-cineol, camphor and α-pinene *viz*. rosemary oils from Spain, France, Greece and Italy.

The high α-pinene content oil of rosemary form Jammu plantation is similar to the previously investigated essential oils of rosemary from Morocco[21], Lebanon[22], Sardinia[14,23] and Corsica[14]. Rosemary oil from Lebanon[22] was characterized by α-pinene (18.8-39.0 %) and 1,8-cineole (19.1-23.4 %), the Moroccan essential oil by α-pinene (37.0-40.0 %), the Sardinian oils[14,23] by α-pinene (14.7-20.2 %) and (19.09-25.82 %) respectively and the Corsican essential oil[23] by α-pinene (13.7-24.6 %). Chemical composition of essential oil from Attiki and Zakynthos Island in Greece were studied by Daferera *et al.*[24] and Pitarokili *et al.*[25] respectively and were characterized by 1,8-cineole (31.5 %), borneol (14.2 %) and α-pinene (12.7 %) and α-pinene (24.1 %), 1,8-cineole (9.3 %), camphor (14.9 %) and borneol (8.0 %) respectively.

TABLE-1
COMPARISON OF AREA PERCENTAGE OF CONSTITUENTS OF ROSEMARY OIL OBSERVED IN SRINAGAR & JAMMU PLANTATION

S. No.	Name of component	Area percentage	
		Srinagar	Jammu
1.	Tricyclene	0.26	0.37
2.	α-Thujene	0.40	0.27
3.	α-Pinene	11.98	31.61
4.	Camphene	8.27	8.76
5.	Verbenene	-	0.44
6.	Sabinene	0.11	-
7.	1-Octen-3-ol	0.19	0.27
8.	β-Pinene	6.94	3.40
9.	Myrecene	2.59	2.18
10.	α-Phellandrene	1.39	2.44
11.	Δ^3-Carene	0.04	0.06
12.	α-Terpinene	-	2.44
13.	Limonene	0.69	3.80
14.	β-Phellandrene/1,8-cineole	22.68	2.28
15.	(*E*)-β-ocimene	0.05	-
16.	γ-Terpinene	0.95	1.18
17.	1-Octanol	0.25	-
18.	(*Z*)-Sabinene-hydrate	0.82	-
19.	Linalool	1.82	0.52
20.	1-Oct-3-en-yn-acetate	-	-
21.	α-Campholenal	-	-
22.	Chrysnthenone	0.03	0.36
23.	Camphor	25.38	13.29
24.	Borneol	2.95	7.05
25.	Terpinen-4-ol	-	0.76
26.	α-Terpineol	1.12	1.3
27.	Myrtenol	-	-
28.	α-Campholenol	-	-
29.	Verbenone	1.94	2.10
30.	Bornyl acetate	5.56	3.24
31.	Methyl eugenol	-	-
32.	(E)-β-Caryophyllene	1.02	0.33
33.	α-Humelene	0.27	0.37
34.	Germacrene-D	-	0.05
35.	Caryophyllene oxide	0.11	-

The chemical constituents of essential oil rosemary have been the subject of considerable study -reviewed by Lawrence[26] and Bolens[27].

The essential oil of rosemary from various geographic origins have been extensively studied for their chemical composition and antimicrobial and antioxidant activity[28-38].

Most significant variation was observed in β-phellandrene/1,8-cineole. The plantation of Rosemary cultivated in sub-tropical region (Jammu) had about ten times less amount of β-phellandrene/1,8-cineole. On the other hand, α- pinene was three times more in the Jammu plantation. The sub-tropical plantation has less amount of camphor as compared to temperate plantation. The Jammu plantation is rich in limonene and borneol. All these variations point to the fact that the climatic conditions and altitude of the place of cultivation has influenced the constituents of the Rosemary essential oil, to the extent that totally new ecotype has been observed. It can be safely concluded that quantitative variation in chemical composition of essential oil of rosemary depends upon number of factors such as environmental conditions, location, elevation, harvesting period and storage conditions.

The chemical composition of rosemary oil described in European Pharmacopoeia[39] from Spain, Morocco and Tunisia reveal that Spanish oil consists of α-pinene (18-26 %), 1,8-cineole (16.0-25.0 %), camphor (13.0-21.0 %) and borneol (2.0- 4.50 %) where as Moroccan and Tunisian rosemary oil contain α-pinene (9.0-14.0 %), 1,8-cineole (38.0-55.0 %), camphor (5.0-15.0 %) and borneol (1.5-5.0 %).

The quality rosemary oil from Spain usually consists of 50 % of Camphoraceous constituents like Camphor + 1,8-cineole, borneol/isoborneol and bornyl acetate along with 17-25 % of α-pinene. In the present study, Rosemary oil obtained from temperate region of Kashmir, India was found to contain about 56.0 % of camphoraceous constituents (Camphor + 1,8-cineole, borneol/isoborneol and bornyl acetate) *i.e.* nearly 6 % more than Spanish Rosemary oil along with 11.9 % of α-pinene. On the other hand oil obtained from Rosemary plantation of sub-tropical region of Jammu contained only 26.0 % of camphoraceous compounds with much higher content of α-pinene (31.6 %), making it very inferior essential oil. The agro-climatic conditions of both places are tabulated in Table-2, which shows a lot of variation.

TABLE-2
AGRO-CLIMATIC CONDITION DURING 2008

S. No.	Name of agro–climatic condition	Name of the place	
		Jammu	Srinagar
1.	Soil pH	7.80	6.95
2.	Average rain fall	1106.7 mm	649.7 mm
3.	Temperature: Summer	< 40 ºC	29.9 ºC
	Winter	> 4.9 ºC	-2.5 ºC

ACKNOWLEDGEMENTS

The authors are grateful to Director, IIIM (CSIR), Jammu, India, for providing necessary facilities and encouragement during the course of the study.

REFERENCES

1. M. Moss, J. Cook, K. Wesnesand and P. Duckett, *Int. J. Neurosci.*, **113**, 15 (2003).
2. T. Bakirel, U. Bakirel, O.U. Keles, S.G. Ulgen and H. Yardibi, *J. Ethnopharmacol.*, **116**, 64 (2008).
3. T. Atsumi and K. Tonosaki, *Psychiatry Res.*, **150**, 89 (2007).
4. T. Bosnic, D. Softic and V.J. Grujic, *Acta Medica. Academica.*, **35**, 19 (2006).
5. M. Estevez and R. Cava, *Meat Sci.*, **72**, 348 (2006).
6. Y. Fu, Y.G. Zu, L.Y. Chen, X.G. Shi, Z. Wang, S. Sun and T. Efferth, *Phytotherapy Res.*, **21**, 989 (2007).
7. A.R. Hras, M. Hadolin, Z. Knez and D. Bauman, *Food Chem.*, **11**, 220 (2000).
8. P. Ramirez, F.J. Senorans, E. Ibanez and G. Reglero, *J. Chromatogr. A*, **1057**, 241 (2004).
9. I. Rasooli, M.H. Fakoor, D. Yadegarinia, L. Gachkar, A. Allameh and M.B. Rezaei, *Int. J. Food Microbiol.*, **122**,135 (2008).
10. D. Soyal, A. Jindal, I. Singh and P.K. Goyal, *Phytomedicine,* **14**, 701 (2007).
11. W. Wang, N. Wu, Y.G. Zu and Y.J. Fu, *Food Chem.*, **108**, 1019 (2008).
12. O.Y. Celiktas, E.E.H. Kocabas, E. Bedir, V. Sukan, T. Ozek and K.H.C. Baser, *Food Chem.*, **100**, 553 (2007).
13. G.R. Mallavarapu, M. Singh, R.S. Chandra Shekhar, S. Ramesh and S. Kumar, *J. Med. Arom. Plant Sci.*, **22**, 298 (2000).
14. G. Pintore, M. Usai, P. Bradesi, C. Juliano, G. Boatoo, F. Tomi, M. Chessa, R. Cerri and J. Casanova, *Flav. Fragr. J.*, **17**, 15 (2002).
15. G. Sacchetti, S. Maietti, M. Muzzoli, M. Scaglianti, S. Manfredini, M. Radice and R. Bruni, *Food Chem.*, **91**, 621 (2005).
16. C. Boutekedjiret, F. Bentahar, R. Belabbes and J.M. Bessiere, *Flav. Fragr. J.*, **18**, 481 (2003).
17. C. Boutekedjiret, R. Belabbes, F. Bentahar, J.M. Bessia-re and S.A. Rezzoug, *J. Essent. Oil Res.*, **163**, 195 (2004).
18. R.N. Carvalho, L.S. Moura, P.T.V. Rosa and M.A.A. Meireles, *J. Supercrit. Fluids*, **35**, 197 (2005).
19. S.A. Rezzoug, C. Boutekedjiret and K. Allaf, *J. Food Eng.*, **71**, 9 (2005).
20. R.P. Adams, Identification of Essential Oil Composition by Gas Chromatography and Mass Spectrometry. 4th Edition-Allured Pub. Corp. Carol. Stream, Illinois, USA (2006).
21. A. Elamrani, S. Zrira and B. Benjilali, *J. Essent. Oil Res.*, **12**, 487 (2000).
22. Y. Diab, L. Auzovz, H. Chebib, J.-C. Chalcat and G. Figueredo, *J. Essent. Oil Res.*, **17**, 449 (2002).
23. A. Angioni, A. Barra, E. Cereti, D. Barile, J.D. Coisson, M. Arlorio, S. Dessi, V. Coroneo and P. Cabras, *J. Agric. Food Chem.*, **52**, 3530 (2004).
24. D.J. Daferera, B.N. Ziogas and M.G. Polissiou, *Crop. Protec.*, **22**, 39 (2003).
25. D. Pitarokili, O. Tzakou and A. Loukis, *J. Essent. Oil Res.*, **20**, 457 (2008).
26. B.M. Lawrence, *Perfumer & Flavourist*, **20**, 47 (1995); **22**, 71 (1997); **26**, 44 (2001).
27. M.H. Bolens, *Perfumer & Flavourist*, **10**, 21 (1985).
28. N. Arnold, G. Valentini, B. Bellomaria and L. Hocine, *J. Essent. Oil Res.*, **9**, 167 (1997).
29. C. Boutekedjiret, R. Bellales, F. Bentahar and J.M. Bessiere, *J. Essent. Oil Res.*, **10**, 680 (1998).
30. J.C. Chalchat, R. Ph. Garry, A. Michet, B. Bemjilali and J.L. Chabart, *J. Essent. Oil Res.*, **5**, 613 (1993).
31. J. Domokos, E. Hethelyi, J. Palinkas, S. Szirmai and M.H. Tulok, *J. Essent. Oil Res.*, **9**, 41 (1997).
32. J.A. Garbarino, N. Troncoso, P. Delpiano, L. Carvajal and A. Russo, *Nat. Prod. Commun.*, **1**, 1123 (2006).
33. K.H. Kubeczka and V. Formacek, Essential oil Analysis by Capillary Gas Chromatography and Carbon-13 NMR Spectroscopy. 2nd Edition - John Wiley & Sons, New York, USA (2002).
34. E. Guazzi, S. Maccioni, G. Monti, G. Flamini, P. L. Cioni and I. Morelli, *J. Essent. Oil Res.*, **13**, 231 (2001).
35. Z. Marzouk, A. Neffati, B. Marzouk, I. Chraief, K. Fathia, L.C. Gredira and K. Boukef, *J. Food Agric. Environ.*, **4**, 61 (2006).
36. G. Pintore, M. Marchetti, M. Chessa, B. Sechi, N. Scanu, G. Mangano and B. Tirillini, *Nat. Prod. Commun.*, **4**, 1685 (2009).
37. E. Serrano, J. Palma, T. Tinoco, F. Venancio and A. Martins, *J. Essent. Oil Res.*, **14**, 87 (2002).
38. C.I.G. Tuberoso, M. Satta, P. Cabras and V.L. Garau, *J. Essent. Oil Res.*, **10**, 660 (1998).
39. European Pharmacopoeia, **5.0**, 2378.