BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, September 2017. Vol. **14**(3), p. 1025-1031

Chemotyping the Essential Oil in Different Rosemary (*Rosmarinus officinalis* L.) Plants grown in Kashmir Valley

Tawheed Amin, H. R. Naik and Syed Zameer Hussain

Division of Post-Harvest Technology, Sher-e-Kashmir University of Agricultural Sciences & Technology-Kashmir, Shalimar Campus, Srinagar 190 025, Jammu & Kashmir, India.

http://dx.doi.org/10.13005/bbra/2537

(Received: 06 April 2017; accepted: 04 May 2017)

The aim of the present study was to evaluate the yield, chemical constituents and determine the chemotype of the essential oil obtained from different rosemary plants growing in different accessions of rosemary fields. About four plant samples were analyzed for essential oil yield and the essential oil yield varied from 0.88% to 1.2%. The essential oil samples were further analyzed by Gas Chromatography (GC) for the purpose of identification of chemical constituents present in them. It was contended from the results that the selected plants differed from each other in terms of chemical constituents. Camphor content was found in higher amount in all the four samples, thus it could be inferred that the plants are camphor chemotype.

Keywords: Rosemary, Essential oil, Camphor, Gas Chromatography, Terpenoids**.**

Rosmarinus officinalis L. is a perennial herb with an evergreen needle-like leaves that belong to the Lamiaceae (mint) family[1-3]. Rosemary is a widely used aromatic and medicinal plant[4,5]. The leaf of rosemary is an indispensable spice of the French, Italian and Spanish cuisine. Rosemary is cultivated for the valuable oil, which can be extracted from the harvested plants when flowers are in buds[5].

Essential oils are natural, concentrated, hydrophobic liquids containing volatile aroma compounds from plants[1]. They are also known as volatile or ethereal oils. The volatile or essential oils correspond to a mixture of hemiterpenoids, monoterpenoids and some sesquiterpenoids that are in conjunction with oil. These mixtures are highly volatile when exposed to air at room temperature, thus the name ethereal oils. They are almost insoluble in water and soluble in alcohol and usually lighter than water. They have high refractive index and many of them are optically active. Essential oils are generally extracted by distillation. The chemical composition of an essential is quite different from one plant to another and the main chemical constituents present in essential oil determine its aroma, taste and biological activity.

There is a wide range of techniques which have been used for extraction and concentration of essential oils as well as chromatographic separation and identification of chemical constituents present in essential oils. Extraction of volatile terpenoids from plant materials and from a wide variety of other matrices is often carried out using hydrodistillation or steam distillation[6]. Rosemary oil was notified for Generally Recognized as Safe (GRAS) status by the Fragrance and Essence Manufacturers Association of the USA (FEMA) in 1965 and has been listed by the U.S. Food and Drug Administration (FDA) for food use (GRAS)[2]. In 1970, the Council of Europe included rosemary oil in the list of substances, spices and seasonings deemed admissible for use, with a possible limitation of the active principles in the final product[2,7].

* To whom all correspondence should be addressed.
E-mail: tawheed.amin@gmail.com

It is generally known that the components of essential oils from aromatic plants of the same scientific name could be different according to the plant's habitats, or parts and methods for extraction. This variation in composition is called chemotype[8]. Chemotype occurs when aromatic plants grow under different climatic and soil conditions[8,9].

The present study was therefore, carried out to evaluate the yield, chemical composition and determine the chemotype of different rosemary plants grown in Kashmir valley.

MATERIALS AND METHODS

Sample collection

Fresh leaves of *Rosmarinus officinalis* L. were collected 15 minutes prior to distillation of essential oil, from the fields of Indian Institute of Integrative Medicine (IIIM) Sanatnagar, Srinagar, Jammu & Kashmir, India.

Distillation of essential oil

The essential oil was obtained by hydrodistillation in a Clevenger (PERFIT, INDIA) for 3 hours[3,10]. Briefly, 250 g of freshly collected leaves were taken in 500 ml round-bottom flask (PERFIT, INDIA) followed by addition of water in the ratio of 1:6 (w/v) and was distilled for about 3 hours. Essential oil from each of the samples was collected and dried over anhydrous sodium sulphate[3,11]. Oil was then stored at 4°C until analysis with Gas chromatography (GC)[3].

Chromatography

The analysis of the oil was carried out by following the method of Amin et al. (2013) on a gas chromatograph Perkin Elmer – Auto XL equipped with head space analyzer and FID, using a fused-silica capillary column (30 m x 0.32 mm; 0.25 ¼m film thickness). The oven temperature was programmed from 60°C to 250°C at 5°C per minute. The injector and detector temperatures were set at 250°C and 270°C, respectively. Nitrogen at a pressure of 8 psi was used as the carrier gas. The identification was done on the basis of retention time, Kovats index, MS Library search (NIST & WILEY). Retention indices (RI) of the chemical components of samples and authentic compounds were determined. The relative amounts of the identified compounds were calculated based on GC peak areas without using correction factors.

RESULTS AND DISCUSSION

Yield of essential oil

The yield of essential oil from each of the four samples ranged from 0.88% to 1.2% (Figure 1). It is clear from Figure 1 that the highest essential

Table 1. Composition of essential oil of Rosemary (*Rosmarinus officinalis* L.) (P_{55}/B_2)

Compound	RT (min)	RI or KI	Peak Area (%)
Alpha-pinene	6.546	937.3.0	7.2730
Sabinene	7.284	961.8	5.3293
Beta pinene	7.643	972.6	6.9296
Mycrene	8.083	988.0	1.9276
Alpha phellandrene	8.530	1001.0	0.2754
Alpha terpinene	8.844	1012.2	7.6594
1,8-cineole	9.473	1032.0	13.6826
(E)- beta ocimene	9.870	1042.8	1.5131
(Z) – sabinene hydrate	0.925	1069.7	0.5493
Camphor	13.946	1146.0	38.6226
Borneol	14.693	1165.1	5.0088
Terpinen-4-ol	15.075	1173.7	0.6782
Alpha terpineol	15.870	1190.5	1.6530
Verbenone	16.601	1204.0	0.4285
Bornyl acetate	19.323	1287.0	4.7110
Beta caryophyllene	23.830	1417.0	0.2279
Alpha humulene	25.217	1453.2	0.1997
Caryophyllene oxide	30.832	1582.5	0.5527
Total identified			97.2217

oil content was found from plant – P_{55}/B_2 (1.2%) and lowest yield from plant P_{57}/B_2 (0.88%).

Identification of compounds

Gas chromatographic analysis of the essential oil resulted in the identification of 18 different components representing about 97.2217% of the essential oil of P_{55}/B2; 19 components representing 97.6266% of the essential oil of P_{57}/B_2; 17 components representing about 76.5109% of the essential oil of P_{67}/B_2 and 22 components representing about 86.0686% of the essential oil of P_{178}/B_4. All of the identified compounds and their percentage present in each of the plant are summarized in Tables 1, 2, 3 & 4. Camphor was found to be present in the highest concentration (53.3871%) which is much higher than that reported by Verma *et al.*[10] followed by 1, 8- cineole (11.6516%), alpha-pinene (5.6862%) and camphene (5.4258%). It is thus clear from the results that all the plants are camphor chemotype.

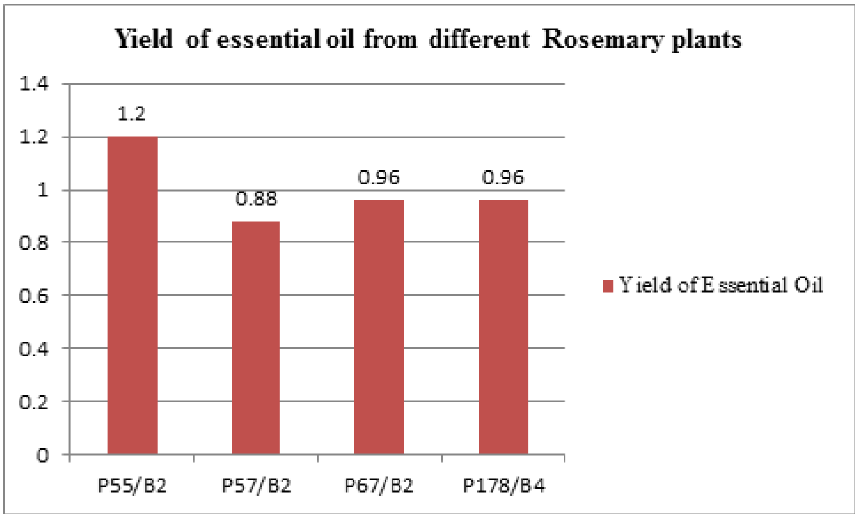

Fig. 1. Percentage yield of essential oil from different Rosemary (*Rosmarinus officinalis* L.) plants

Table 2. Composition of essential oil of Rosemary (*Rosmarinus officinalis* L.) (P_{57}/B_2)

Compound (%)	RT (min)	RI or KI	Peak Area
Alpha-pinene	6.556	936.0	5.6862
Camphene	7.294	954	5.4258
Beta pinene	7.713	976	0.7221
Mycrene	8.094	983.9	2.7746
Delta-3-carene	8.867	1007.7	5.3275
1,8-cineole	9.566	1030.2	11.6516
(E)- beta ocimene	9.904	1040	0.4748
(Z) – sabinene hydrate	10.927	1065.8	0.7116
Linalool	12.108	1092.8	0.9980
Camphor	14.064	1141	53.3871
Borneol	14.731	1165	1.6864
Terpinen-4-ol	15.13	1174	0.6502
Alpha terpineol	15.88	1186	1.33
Verbenone	16.343	1202	0.0366
Nerol	17.529	1233.5	1.6855
Bornyl acetate	19.365	1287	4.2267
Beta caryophyllene	23.823	1417	0.6217
Alpha humulene	25.248	1453.5	0.0484
Caryophyllene oxide	30.923	1580.7	0.1818
Total identified			97. 6266

RT: Retention time; KI: Kovat index (relative to *n*-alkane)

It is clear from the results that the yield of essential oil from samples of different plants varied considerably. The composition of the essential oil varies from plant to plant as can be seen in the tables 2, 3, 4 and 5. The camphor content of the essential oil for all the 4 samples is higher than the value of 15.64% and 22.01% reported by Verma *et al.*[10] and Shawl[12], respectively. The camphor content also varies within the samples. Since the camphor content of essential oil is higher, therefore, all the samples are camphor chemotype. The chemical composition of oil depends on how and where the plant was grown, harvested and distilled. When the conditions cause permanent variation in the chemical composition of essential oil of rosemary plants, such plants are called chemotypes. Three principal chemotypes of *R. officinalis* L. have been reported which include camphor/borneol, cineole and verbenon[13]. Typical components of rosemary are 1,8-cineole, ±- pinene and camphor[14] and the relatively stable ratio of these components defines each chemotype. It is known that the rosemary oils are widely divided into two chemotypes by the ratio of major components; one with more than 40% of 1,8-cineole and the other with almost the same percentage of 1,8-cineole, ±-pinene and camphor[15]. These findings are in concomitance with our present results.

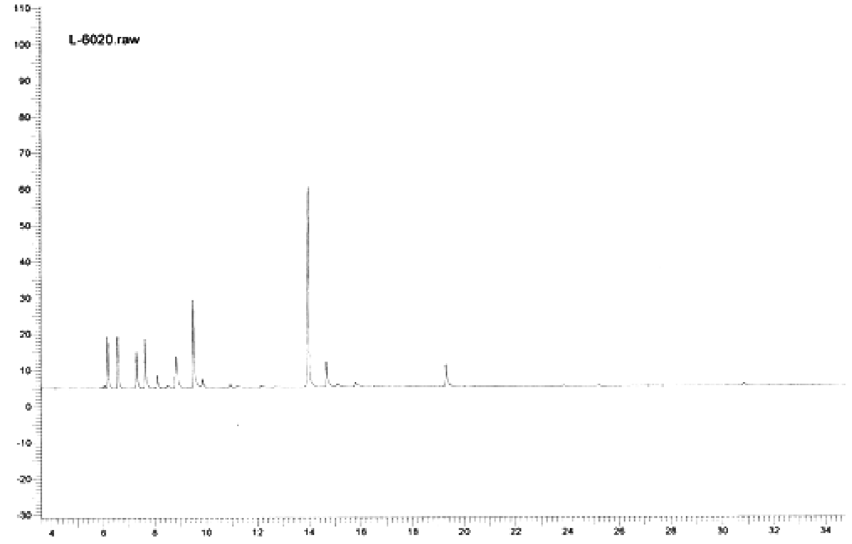

Fig. 2. Gas chromatogram of essential oil *Rosmarinus officinalis* L. (P_{55}/B_2)

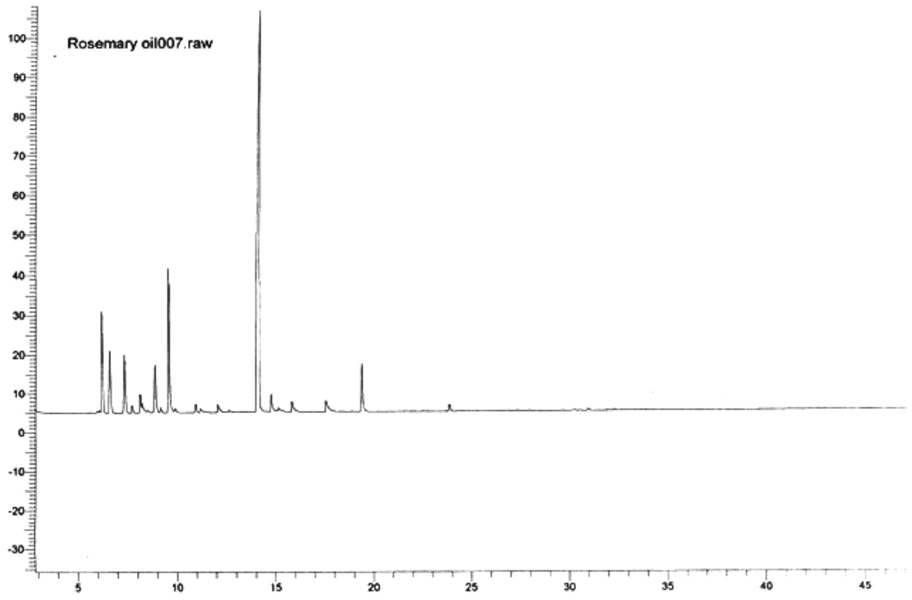

Fig. 3. Gas chromatogram of essential oil *Rosmarinus officinalis* L. (P_{57}/B_2)

Table 3. Composition of essential oil of Rosemary (*Rosmarinus officinalis* L.) (P_{67}/B_2)

Compound	RT (min)	RI or KI	Peak Area (%)
Alpha-pinene	6.598	936.0	3.2764
Sabinene	7.309	967.4	3.2513
Beta pinene	7.663	973.6	2.4618
Alpha phellandrene	8.135	1001.0	1.7835
Alpha terpinene	8.556	1012.7	0.3062
1,8-cineole	8.874	1032.2	6.4457
(E)- beta ocimene	9.482	1043.3	4.2923
(Z) – sabinene hydrate	10.971	1069.8	1.2216
Linalool	12.102	1095.0	0.3874
Camphor	13.959	1146.0	34.3253
Borneol	14.662	1164.3	5.0162
Terpinen-4-ol	15.066	1173.5	0.7620
Alpha terpineol	15.737	1188.0	3.2681
Nerol	17.555	1277.0	1.5419
Bornyl acetate	19.285	1287.0	6.7744
Beta caryophyllene	23.854	1417.6	0.4346
Caryophyllene oxide	30.856	1582.5	0.9622
Total identified			76.5109

RT: Retention time; KI: Kovat index (relative to n-alkane)

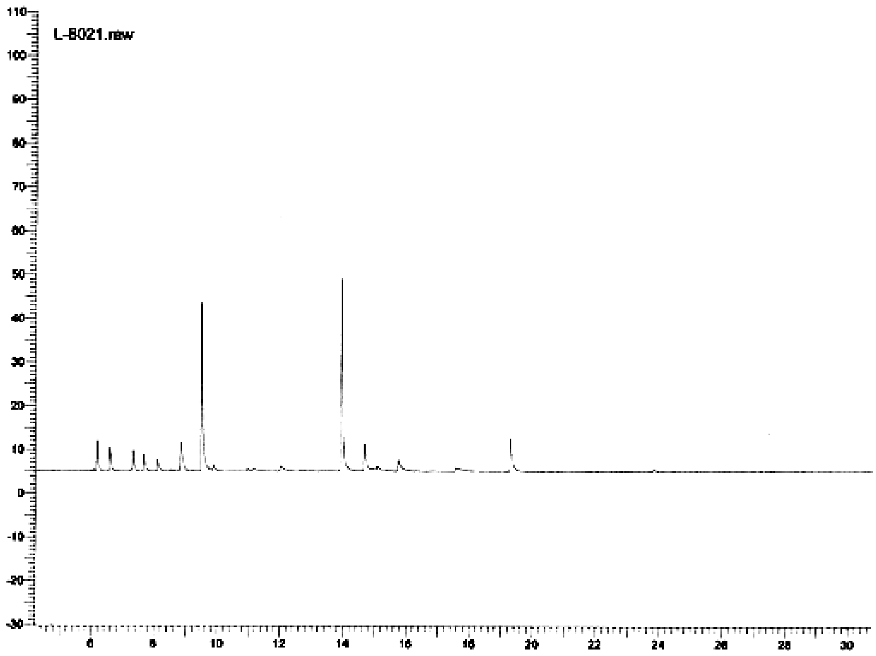

Fig. 4. Gas chromatogram of essential oil *Rosmarinus officinalis* L. (P_{67}/B_2)

Table 4. Composition of essential oil of Rosemary (*Rosmarinus officinalis* L.) (P_{17}/B_4)

Compound	RT (min)	RI or KI	Peak Area (%)
Alpha-pinene	6.574	936.	6.0510
Camphene	6.822	947.0	0.1545
Sabinene	7.299	967.0	4.7442
Beta pinene	7.658	973.4	4.2248
Mycrene	8.078	987.8	2.1758
Alpha phellandrene	8.480	1001.0	0.5840
Alpha terpinene	8.857	1013.6	0.6961
1,8-cineole	9.505	1032.9	11.5797
(E)- beta ocimene	9.872	1042.9	1.3948
(Z) – Sabinene hydrate	10.933	1069.9	0.8736
Linalool	12.025	1095.0	1.5214
Chrysanthenone	13.314	1129.1	0.1002
Camphor	14.004	1146.0	28.2261
Borneol	14.719	1165.8	3.8690
Terpinen-4-ol	15.012	1174.0	1.4618
Alpha terpineol	15.797	1188.9	2.3279
Verbenone	16.663	1204.0	0.1357
Nerol	17.432	1227.6	7.4616
Bornyl acetate	19.323	1287.0	7.2866
Beta caryophyllene	23.829	1417.0	1.0323
Alpha humulene	25.231	1453.6	0.1675
Caryophyllene oxide	30.827	1581.9	2.9385
Total identified			86.0686

RT: Retention time; KI: Kovat index (relative to *n*-alkane)

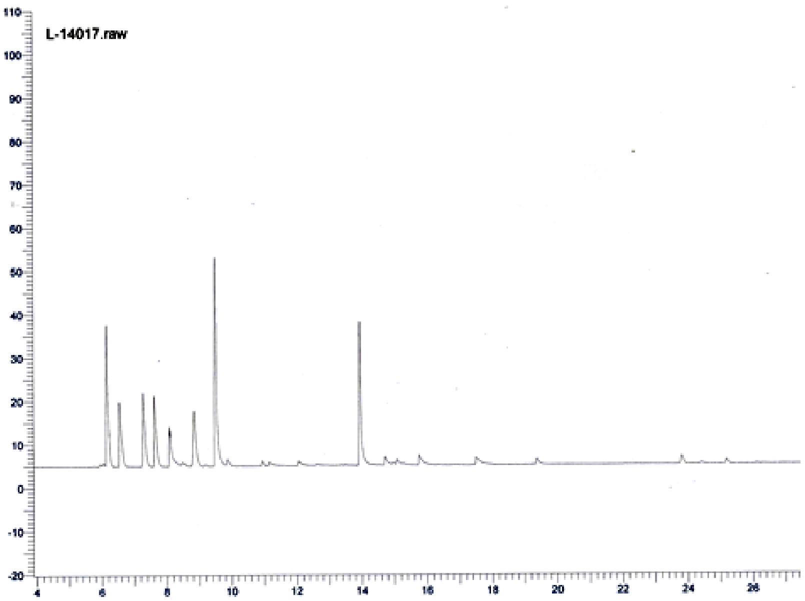

Fig. 5. Gas chromatogram of essential oil *Rosmarinus officinalis* L. (P17/B4)

ACKNOWLEDGMENT

Author is highly grateful to Indian Institute of Integrative Medicine, Sanatnagar, Srinagar, Jammu & Kashmir for providing an opportunity to work in their lab.

REFERENCES

1. Amin, T., Sharma. N., Bhat, S.V. A general overview on *Rosmarinus officinalis* L. (Rosemary) as a medicinal plant. *Med. Plant: Int. J. Phytomed. Related Ind.*, 2012a; **4**(3): 177-81.
2. Amin, T., Gulleria, S.P., Bhat, S.V. Potential applications of Rosemary (*Rosmarinus officinalis* L.) as a natural food additive. *Res. J. Agric. Sciences (An Int. J.)*, 2012b; **3**(6): 1165-9.
3. Amin, T., Bhat, S.V., Shrma, N. Antimicrobial properties of Rosemary (*Rosmarinus officinalis* L.) essential oil against *Sacharomyces cereviseae*. *Asian J. Microbiol., Biotech. Environ. Sci.*, 2013; **15**(4): 107-10.
4. Joseph, S. Advances in essential oils analysis using comprehensive two-dimensional GC and time-of-flight mass spectrometer (GCxGC-TOFMS) Detection. *Life Sci. Chem. Anal. Sol.*, 2008.
5. Éva Stefanovits-Bányai, M. H. Antioxidant effect of various rosemary (*Rosmarinus officinalis* L.) clones. *Acta Biologica Szegediensis*, 2003; 111-13.
6. Guenther, E.: Oil of Bay. In *The Essential Oils*. Van Nostrand: New York, 1966; pp 378-96.
7. Opdyke, D.L.J. Monographs on fragrance raw materials. *Food Cosmet. Toxicol.*, 12 supplement, 807.
8. Stewart, E. The chemistry of essential oils made simple: God's love manifest in molecules. Care Publications, Marble Hill. 2005.
9. Mills, S.Y. The scientific foundation for herbal medicinal products. 2nd Edition, European Scientific Cooperative on Phytotherapy, Exeter. 2003.
10. Verma, R.S., Sashidhara, K.V., Yadav, A. Essential oil composition of 'blue flower rosemary' (*Rosmarinus officinalis* L.) from subtropical India. *Acta Pharmaceutica Sciencia*, 2010; **52**: 427-30.
11. Salehi, P., Fakhari, A.R., Ebrahimi, S.N., Heydari, R. Rapid essential oil screening of *Rosmarinus officinalis* L. by hydrodistillation–headspace solvent microextraction. *Flavour Fragrance J.*, 2007; **22**(4): 80-285.
12. Shawl, A., Nisar, S., Kumar, T., Nawchoo, I.A. Essential oil composition of *Rosmarinus officinalis* L. cultivated in Kashmir valley - India. *Indian Pertumer.*, 2008; 47-9.
13. Guetat, A., Al-Ghamdi, F.A., Osman, A.K. 1, 8-Cineole, ±-Pinene and Verbenone chemotype of essential oil of species *Rosmarinus officinalis* L. from Saudi Arabia. *Int. J. Herbal Med*. 2014; **2**(2): 137-41.
14. Flamini, G., Cioni, P.L., Morelli, I., Macchia, M. and Ceccarini, L. Main agronomic-productive characteristics of two ecotypes of *Rosmarinus officinalis* L. and chemical composition of their essential oils. *J. Agric. Food Chem.*, 2002; **50**: 3512-17.
15. Özcan, M.M., Chalchat, J.C. Chemical composition and antifungal activity of rosemary (*Rosmarinus officinalis* L.) oil from Turkey. *Int. J. Food Sci. Nutr.,* 2008; **59**: 691-98.