VOLATILE TERPENOID COMPOSITION OF *ROSMARINUS OFFICINALIS*, “CIM-HARIYALI”: VARIABILITY IN NORTH INDIA DURING ANNUAL GROWTH

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ABSTRACT

To investigate the seasonal influence on essential oil content and composition of rosemary (*Rosmarinus officinalis*) cultivar ‘CIM-Hariyali’ a study was conducted in Kumaon region of western Himalaya. Essential oil content was found to vary from 1.0% to 1.14% during the year. GC and GC-MS analyses enabled to identify thirty components representing 95.33% - 97.03% of the total oil composition. Main components of the oils were 1,8-cineole (22.61% - 23.85%), camphor (24.40% - 25.85%), *α*-pinene (10.74% - 12.59%), verbenone (4.90% - 5.77%), camphene (5.46% - 6.16%), *β*-pinene (3.28% - 4.02%), limonene (2.86% - 3.39%) and *β*-myrcene (1.89% - 1.95%). The study clearly showed that there were no drastic changes in the essential oil content and composition of rosemary due to season. Therefore, the crop may be harvested in any season to get good quality oil in hilly region of north India.

Key words: *Rosmarinus officinalis*, harvesting season, essential oil yield, composition

1. INTRODUCTION

Rosmarinus officinalis L. belonging to family Lamiaceae is commonly known as rosemary. It is found in Mediterranean region and grows wild in Algeria, France, Italy, Portugal, Morocco and Spain and is cultivated in several countries such as USA, Spain, and Yugoslavia for the production of essential oil[1,2]. The plant is reported to possess carminative, stomachic and spasmodic properties and it is also used for the prevention of epilepsy, stimulation of hair growth and dandruff [3,4]. Rosemary is used since ancient times in food, cosmetics, medicinal and pharmaceutical products[5]. In addition to these, the essential oil of this plant possessed antimicrobial properties against yeast[6], mold[4] and gram-positive bacteria[7] and stimulative effect on nervous system[4]. Thus due to immense application for both the herb and its essential oil, rosemary is one of most popular plants in the Europe and North Africa[8]. Consequently, the dried herb, an antioxidative-rich fraction and the essential oil have a real presence in many markets all over the word[9].

The essential oil composition of rosemary has been the subject of considerable study[3, 10-16]. A survey of literature reveals that there are mainly three chemotypes: a 1,8-cineole chemotype from France, Greece, Italy and Tunisia, a camphor-borneol chemotype from Spain and a *α*-pinene and verbenone chemotype from Carsica and Algeria[1,17]. In addition to these chemotypes, myrcene rich rosemary oil is also reported [11,12].

Rosemary was introduced in Uttarakhand hills for commercial cultivation by extensive efforts of CIMAP. Being perennial crop farmers generally harvest it thrice in a year. Essential oil content and composition of aromatic crops are strongly influenced by harvesting season[8,18-21]. Hence, it became imperative to investigate the variability in essential oils of rosemary obtained from different seasons. In present study essential oil of rosemary cultivar ‘CIM-Hariyali’ derived from spring, summer, rain, autumn and winter seasons have been compared for its yield and qualitative composition.

2. EXPERIMENTAL

2.1. Plant material and essential oil isolation procedure

Two month old rooted cuttings of *R. officinalis* cv CIM-Hariyali[22] were transplanted in the experimental field of Central Institute of Medicinal and Aromatic Plants, Research Centre; Purara (Bageshwar) in the month of October. Climatologically, the site falls in temperate zone of western Himalaya, with the monsoon usually breaking in June and continuing up to September. The crop was raised using normal agricultural inputs. The study was started from March and the samples were taken every month from the aerial part (upper 15 cm) of the crop in clear weather conditions. The essential oil was extracted by hydrodistillation for 3 hours, using Clevenger apparatus. The oil content was estimated on the fresh weight basis. The oil samples obtained were dehydrated over anhydrous sodium sulphate and kept in a cool and dark place before analyses.

2.2. Essential oil analysis

The gas chromatography (GC) analyses of the oil samples was carried out on a Nucon gas chromatograph model 5765 and Perkin-Elmer Auto XL GC equipped with flame ionization detector (FID) and two stationary phases of different polarity viz. BP-20 (coated with a Carbowax 20M, 30 m length × 0.25 mm internal diameter × 0.25 µm film thickness) and PE-5 (coated with 5% phenyl polysiloxane, 60 m length × 0.32 mm internal diameter; 0.25 µm film coating) fused silica columns, respectively. Hydrogen was the carrier gas at 1.0 mL/min. Temperature programming was done from 70°-230°C at 4°C/min with initial and final hold time of 2 min (for BP-20) and from 70°-250°C at 3°C/min (for PE-5). Split ratio was 1: 30. GC-MS recorded on a Perkin Elmer Auto System XL GC and Turbo Mass Spectrometer fitted with fused silica capillary column, PE-5 (50 m × 0.32 mm, film thickness 0.25 µm). The column temperature was programmed 100° - 280°C at 3°C/min, using helium as carrier gas at constant pressure of 10 psi. MS conditions were: EI mode 70 eV, ion source temperature 250°C. Identification of components were done by comparing the retention times, retention indices (determined with reference to homologous series of *n*-alkanes, C_8-C_{24}) and mass fragmentation pattern to literature values[23,24], and by peak enrichment on co-injection with authentic samples wherever possible to corroborate identities. The peak area percentage was computed from the peak areas without applying FID response factor correction.

3. RESULTS AND DISCUSSION

The essential content observed in the fresh herb of rosemary with phenological stage and harvesting season is summarized in Table 1. There was no considerable variation observe in the oil content of rosemary during the year (1.0-1.14%), however, these results are contrary to earlier findings from South India and Spain, where it varied from 0.7% to 1.2% and 1.03% to 1.81%, respectively[8,25]. This might be due to geographical differences of cultivation, harvesting height/method or pedogenetic factors. The essential oils obtained from rosemary herb harvested in different seasons were analyzed by GC and GC-MS and results are presented in Table 2. A typical chromatogram of the essential oil of rosemary cv. CIM-Hariyali is shown in Figure 1. Camphor (24.40% - 25.85%), 1,8-cineole (22.61% - 23.85%), *α*-pinene (10.74% - 12.59%), verbenone (4.90% - 5.77%), *α*-terpineol + borneol (3.73% - 5.32%), camphene (5.46% - 6.16%), *β*-pinene (3.28% - 4.02%) and *β*-myrcene (1.89% - 2.51%) were the major components in all seasons. However, 1,8-cineole and *α*-pinene were slightly higher in the summer season (23.85% and 12.59%, respectively) when compared with other seasons, while camphor and linalool was recorded higher in the rainy season (25.85% and 1.59%). Further,

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verbenone, responsible for herbal note of the oil was 4.90% in autumn and reached its higher value (5.77%) in winter season. *α*-Terpineol + borneol was recorded to be minimum (3.73%) and maximum (5.32%) in summer and spring, respectively. Camphene was observed to be lowest (5.46%) during the rains and thereafter showing gradual increase to the maximum of 6.16% in the winters. The content of *β*-pinene was maximum in spring (4.02%) followed by summer (3.80%) and autumn (3.69%) seasons. It was interesting to note that the concentration of (*Z*)-*β*-ocimene was equal in summer, rainy and autumn seasons (0.82%) while during rest seasons it varied from 0.58% - 0.62%. However, (*E*)-*β*-ocimene was ranged from 0.42% to 0.91% during the year with the highest in the winter season. The other components such as *γ*-terpinene and *p*-cymene varied from 0.14% to 0.20% and 0.66% to 0.84%, respectively with their higher percentages during the rain and lower percentages in winter seasons. Further, 1-octen-3-ol and eugenol were higher in winter (0.64% and 1.0%, respectively) while, (*E*)-sabinene hydrate, chrysanthenone, *β*-caryophyllene and methyl eugenol were higher during springs (0.90%, 0.26%, 0.58% and 0.43%, respectively). *β*-Myrcene (2.51%), terpinen-4-ol (1.11%) and caryophyllene oxide (0.65%) were observed to be highest in summer season. However, *α*-terpinene, limonene, bornyl acetate, *α*-humulene and myrtenol reached their higher value in the autumn season (0.44%, 3.39%, 2.34%, 0.63% and 0.45%, respectively). Thus, the present findings are in good agreement with the earlier results from south India on the essential oil composition of rosemary at different phenological stages with no major changes in the quantitative composition[25]. Since, the result shows no drastic change in the essential content and composition of rosemary throughout the year thus, the crop can be harvested in any season.

Table 1: Essential oil content of *Rosmarinus officinalis* cv. CIM-Hariyali during annual growth.

Season	Phenological stage	Essential oil (%)*
Spring	Flowering	1.00
Summer	Flowering	1.01
Rainy	Fruit ripening	1.09
Autumn	Seed shattering	1.14
Winter	Vegetative	1.12
Average essential oil yield		1.07

* Fresh wt. basis

Table 2: Seasonal influence on essential oil composition of *Rosmarinus officinalis* cv. 'CIM-Hariyali' during annual growth.

Compound	BP	RI[a]	RI[b]	Content (%)/Season				
				Spring	Summer	Rainy	Autumn	Winter
α-Pinene	93	1026	935	10.74	12.47	11.66	12.59	11.85
Camphene	93	1065	951	5.83	5.48	5.46	6.07	6.16
β-Pinene	93	1105	980	4.02	3.80	3.28	3.69	3.57
Sabinene	93	1116	974	t	-	-	t	-
Myrcene	93	1163	998	1.89	2.51	2.31	2.45	1.95
α-Terpinene	93	1181	1021	0.23	0.31	0.33	0.44	0.30
Limonene	68	1206	1030	2.86	3.38	3.27	3.39	3.20
1,8-Cineole	43	1210	1035	23.47	23.85	23.45	22.61	23.32
β-Phellandrene	93	1212	1037	t	t	t	t	t
(*Z*)-*β*-Ocimene	93	1216	1038	0.58	0.82	0.82	0.82	0.62
γ-Terpinene	93	1240	1062	0.17	0.14	0.20	0.17	0.19
(*E*)-*β*-Ocimene	93	1247	1054	0.76	0.42	0.44	0.59	0.91
p-Cymene	119	1271	1025	0.82	0.75	0.84	0.66	0.83
α-Terpinolene	93	1279	1090	-	-	t	-	-
(*Z*)-3-Hexenol	67	1391	841	t	t	-	t	-
1-Octen-3-ol	57	1411	995	0.22	0.30	0.49	0.44	0.64
(*E*)-Sabinene hydrate	43	1463	1095	0.90	0.54	0.62	0.58	0.53
Camphor	95	1506	1146	25.24	24.40	25.85	24.59	25.76
Chrysanthenone	107	1510	1029	0.26	0.22	0.21	0.19	0.24
Linalool	71	1554	1098	1.03	1.12	1.59	1.22	0.96
Bornyl acetate	95	1588	1285	2.22	2.23	1.90	2.34	1.19
β-Caryophyllene	41	1598	1419	0.58	0.44	0.28	0.32	0.29
Terpinen-4-ol	71	1601	1177	0.91	1.11	0.99	1.08	1.06
α-Humulene	93	1670	1462	0.40	0.39	0.46	0.63	0.61
α-Terpineol+borneol†	59	1704	1189	5.32	3.73	4.33	4.90	5.09
Verbenone	107	1723	1205	5.75	5.65	5.44	4.90	5.77
Myrtenol	79	1787	1195	0.24	0.13	0.18	0.45	0.31
Caryophyllene oxide	41	1995	1584	0.45	0.65	t	0.18	0.49
Methyl eugenol	178	2020	1405	0.43	0.32	0.33	0.36	0.19
Eugenol	164	2178	1354	0.78	0.17	0.53	0.58	1.00
Total identified (%)				96.10	95.33	95.34	96.33	97.03

BP: m/e of base peak in mass spectrum; [a]Retention Indices on polar column (BP-20); [b]Retention Indices on non-polar (PE-5 column); t: trace (<0.10%); †base peak of borneol: 95

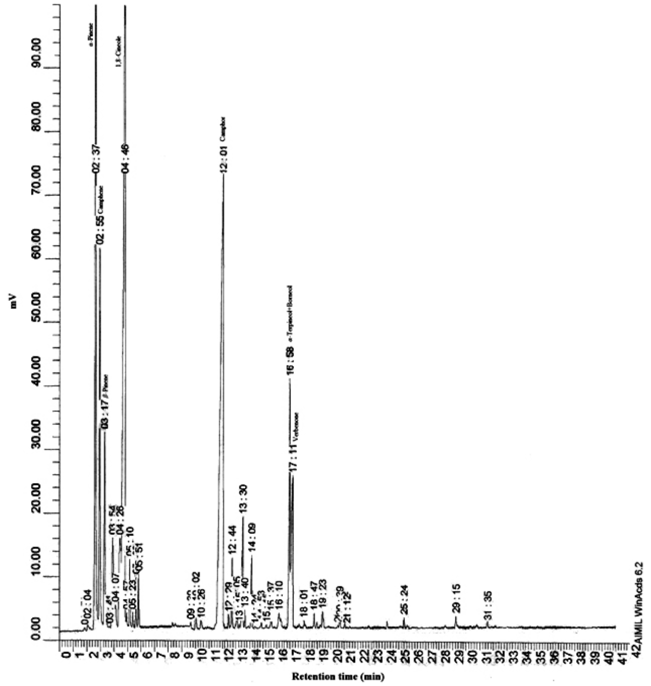

Figure 1: Gas chromatogram of *Rosmarinus officinalis* cv. 'CIM-Hariyali' essential oil.

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