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DETERMINATION OF ESSENTIAL OIL COMPOSITION OF *Rosmarinus officinalis* GROWING AS EXOTIC SPECIES IN KASHMIR VALLEY

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The Lamiaceae is a large family, rich in aromatic species and used as culinary herbs, folk medicines, fragrances, etc. Many species of this family possess essential oils secreted by glandular trichomes. In the family Lamiaceae, there are two main types of trichomes: peltate and capitates. Studies of these glandular trichomes comprise morphological, structural, and histochemical analysis. *Rosmarinus officinalis* L. is an evergreen, perennial shrub native to the Mediterranean Sea and areas [1]. *R. officinalis* L., is a diploid (2n = 2x = 24) and allogamous species. It is the most exploited species for its essential oil and phenolic biological properties [2]. These variations are mostly correlated to differences in the chemical composition of the oils according to regions [3–8]. Dried rosemary leaves are used as seasoning for soups, meat, fish, and poultry. The oil extracted from this plant is used in food products, perfumes, and cosmetics such as soaps, creams, deodorants, hair tonics, and shampoos. The plant and extracts possess antibacterial and antioxidant activity and are considered as a good source of nectar for insects. As a medicinal plant, rosemary has been used as an external stimulant and as a relaxant of nervousness, muscle spasms, and headaches. It has also been used in the treatment of cancer and is categorized today as a therapeutic emmenagogue [9]. It has been used as an analgesic, antirheumatic, carminative, cholagogue, diuretic, expectorant, and antiepileptic. It has effects on human fertility and is hepatoprotective and antimutagenic [10–12]. The antioxidant activity of rosemary extracts has been associated with the presence of several phenolic diterpenes such as carnosic acid, carnosol, rosemariquinone, and rosemaridiphenol, which break free radical chain reactions by hydrogen donation [13, 14]. Various extracts of rosemary are used for retarding lipid oxidation in various foods [15].

The essential oil of *Rosmarinus officinalis* L. contains 1,8-cineole, camphor, α-pinene, and borneol as major compounds [16]. Our objectives in the present work were, first, to further determine the chemical composition of the essential oils of *Rosmarinus officinalis* L. growing in this temperate Western Himalyan range. After analyzing the oil, the major compounds were indentified and characterized as camphor (22.00 %), α-pinene (16.33%), 1,8-cineole (14.32%), camphene (9.28%), β-pinene (5.97%), β-phellandrene (5.9%), bornyl acetate (4.59%), myrecene (4.31%), and borneol (3.35%).

Rosmarinus officinalis L. is found in the Mediterranean Sea and as an exotic species in the Himalayas. The chemical composition of a plant essential oil depends upon a number of parameters, such as environmental conditions, seasonal collection of plant material, the dehydration procedure, the storage material under which the collected plants were kept until their essential oil extraction, and the applied method of essential oil and essential oil analysis (column, programmed conditions) used for the identification of the compounds. The plant material collected from F/S, Bonera, Pulwama (Kashmir) on hydrodistillation yielded about 0.12% white viscous oil on a moisture-free basis (MFB) with a characteristic woody smell. Upon analysis, the oil was found to be a complex mixture of mono- and sesquiterpenes, and 38 compounds were identified by RI and GC/MS, which includes 98% of the oil. Identified chemical constituents are shown in Table 1.

The major constituents of *Rosmarinus officinalis* L. were mono- and sesquiterpenes such as camphor (22.00%), α-pinene (16.33%), 1,8-cineole (14.32%), camphene (9.28%), β-pinene (5.97%), β-phellandrene (5.9%), bornyl acetate (4.59%), myrecene (4.31%), borneol (3.35%), and (*E*)-β-caryophyllene oxide (2.88%). Qualitatively and quantitatively, the GC pattern of the oil is 80% similar to reported earlier, but this report shows some new constituents [16].

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TABLE 1. Chemical Composition of Essential Oil of *Rosmarinus officinalis*

Compound	RI (BP-5)	%	Compound	RI (BP-5)	%
Tricycline	920	0.046	1-Octanol	1064	0.146
α-Thujene	924	0.281	(*Z*)-Sabinene hydrate	1076	0.648
α-Pinene	932	16.331	Linalool	1087	1.163
Camphene	948	9.284	1-Oct-3-en-yn acetate	1091	0.027
1-Octen-3-ol	954	0.210	α-Campholenal	1107	0.005
Sabinene	973	0.108	Chrysanthenone	1109	0.175
β-Pinene	976	5.971	Camphor	1122	22.006
Verbenene	981	0.067	Borneol	1150	3.353
Myrcene	986	4.317	Terpinen-4-ol	1162	1.112
α-Phellandrene	1001	0.985	α-Terpineol	1177	1.029
Hexyl acetate	1005	0.006	Myrtenol	1186	0.027
Δ^3-Carene	1006	0.017	α-Campholenol	1191	0.008
α-Terpinene	1012	0.511	Verbenone	1205	1.389
p-Cymene	1015	0.005	Bornyl acetate	1270	4.598
Limonene	1019	0.951	Methyl eugenol	1368	0.105
β-Phellandrene	1023	5.192	(*E*)-β-Caryophylene	1420	2.888
1,8-Cineole	1028	14.329	α-Humelene I	1453	0.759
(*E*)-β-Ocimene	1039	0.005	Germacrene D	1471	0.012
γ-Terpene	1050	1.048	Caryophyllene oxide	1566	0.124

Method of identification – MS, RI.

Plant Material. The fresh aerial parts of *Rosmarinus officinalis* L. were collected at the flowering stage in July 2008 from IIIM, field station Bonera, Pulwama Kashmir (India). The plant was identified and authenticated by the taxonomist at the Center of Plant Taxonomy (COPT), University of Kashmir, and a voucher specimen (2008/RM-L-02) was deposited at the Indian Institute of Integrative Medicine (IIIM), Srinagar, India.

The essential oil was obtained by the hydro-distillation of fresh plant material in a Clevenger type apparatus for four hours. The sample afforded a white viscous oil with characteristic floral woody flavor (yield 0.12%). The oil was dried over anhydrous Na_2SO_4 and placed at a low temperature in the refrigerator until analysis.

GC Analysis. The composition of the oil was studied by GC on a Perkin–Elmer 8500 gas chromatograph with flame ionization detector (FID), using a fused-silica column (30 m × 0.32 mm i.d.; 0.25 μm film thickness) coated with 5% diphenyl and 95% polysiloxane (BP-5). Oven temperature was programmed from 60–220°C (injector temperature 240°C; detector temperature 270°C; carrier gas nitrogen at 8 psi, split ratio 1:80). Retention indices (RI) of the sample components and authentic compounds were determined on the basis of homologous *n*-alkane hydrocarbons under the same conditions.

GC/MS. GC/MS data were obtained on a Varian Mass spectrometer using a VF-5 column (60 m × 0.32 mm i.d.; 0.25 μm film thickness). Column temperature was programmed at 5°C/min at 60°C, rising 2 and 3°C up to 240°C (injector temperature 240°C; ion source temperature 250°C; interface temperature 270°C; acquisition mass range 700–40 amu; ionization energy 70 eV). Helium was used as carrier gas, with flow rate 1 mL/min.

The peaks were identified by comparison of the mass spectra with those reported in the NIST library [17]. The oil components was also identified by comparison of their linear RI with those from the Mass Finder library.

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