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Component composition of supercritical extract of aerial parts of Rosmarinus officinalis from the collection of the Botanical Garden of Pyatigorsk Medico-Pharmaceutical Institute

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Abstract Rosemary (*Rosmarinus officinalis* L.) is a perennial herb from Lamiaceae family, typical of the Mediterranean region. The essential oil of rosemary has been widely used in pharmaceutical and cosmetic industries. Rosemary has important biological properties, such as hepatoprotective, antidiabetic, antioxidant, antiproliferative, antiviral, antimicrobial, antinociceptive. In the literature many studies have reported on the composition of volatile oils isolated from rosemary. The major constituents of essential oil and supercritical (SC) CO_2 extract obtained from this herb were reported to be 1,8-cineole, camphor, borneol, verbenone, bornyl acetate, carnosic acid, oleanolic acid, rosmarinic acid, carnosol. Carnosic acid and carnosol are recognized as the most abundant antioxidants present in rosemary. Numerous studies have shown that the composition of essential oil and SC CO_2 extract of a given herb can vary with genotype, location and climatic conditions. The SC CO_2 extract from the aerial parts of rosemary from the Botanical Garden of Medical and Pharmaceutical Institute was extracted using supercritical fluid extraction (SFE) methods. The yield of the SC CO_2 extract from the aerial parts of rosemary amounted to 4,848%. Fundings of investigations indicated that major compounds identified were 1,8-cineole, camphor, n-triacontane, α-pinene, myrcene, limonene, α-terpineol, caryophyllene, 2,2-bis(4'-methoxyphenyl)-2-ethoxyethane, β-sitosterol. A comparative analysis of the component composition of essential oil and SC CO_2 extract of rosemary carried out. Comparing the extraction levels of these compounds with those of rosemary from other locations suggests that rosemary cultivated in the Botanical Garden of Pyatigorsk Medical and Pharmaceutical Institute could be a special chemotype with a high contents of monoterpene compounds.

Keywords: essential oil; carbon dioxide; *Rosmarinus officinalis* L.; supercritical extraction; supercritical fluid

1. Introduction

Nowadays, the SFE is an environmentally safe and effective technology for dry solid materials extraction. It has been widely studied for the purpose of isolating active compounds from plants. In the last decade the interest in supercritical fluid technology has especially increased because of legal limits regarding solvent residues, mainly in the production of pharmaceutical medicinal products from plant raw materials. Another problem of conventional methods is the high temperatures application, which can cause chemical modifications in the oil components and is often the cause of the loss of the most volatile molecules. Supercritical fluid extraction employing CO_2 is known to establish an innovative industrial process to obtain extracts of high quality with the highest percentage of native extraction of biologically active compounds.

There is an increasing interest in phytocomplexes as multidimensional sources of natural antioxidants and antimicrobials. Rosemary (*Rosmarinus officinalis* L., Lamiaceae) is an aromatic, evergreen, shrub with upright stems, blue flowers and dark green leaves [1]. This spice typical of the Mediterranean region, is widely used in cosmetics, flavoring the food products, in folk medicine and phytopharmacy because of its digestive, diuretic, diaphoretic, hypocholesterolemic, hepatoprotective, antidepressant properties [2]. A great number of compounds, including flavones, diterpenes, steroids and triterpenes, have been isolated from rosemary. The antioxidant activity of rosemary extracts has been primarily related to two phenolic diterpenes: carnosic acid and carnosol. The main compounds responsible for the antimicrobial activity are α-pinene, bornyl acetate, camphor and 1,8-cineole [3, 4]. Supercritical extracts and essential oil of rosemary can become a promising source of metabolites with antibiotic-resistance modifying activity to be used against multiresistant microorganisms [5, 6]. The essential oil of rosemary has a significant rate of antifungal and antibacterial activity against *Escherichia coli*, *Salmonella typhi*, *S. enteritidis*, and *Shigella sonei* [7, 8]. Supercritical rosemary extracts exhibit high anti-inflammatory activity, so the obtained results give grounds for hypothetical increase in the application of supercritical rosemary extracts in dietary supplements for mitigation or prevention of inflammatory diseases [9, 10]. The research conducted by various authors reports that supercritical rosemary extracts and their isolated components show inhibitory effects on the growth of breast, liver, prostate, lung, and leukemia cancer cells [2, 11].

2 Materials and methods

2.1 *Raw material characterization and preparation*

The aerial parts (leaves and stems) of cultivated rosemary were collected in May of 2017 in the Botanical Garden of Pyatigorsk Medical and Pharmaceutical Institute, (Pyatigorsk, Russia). The harvested plant parts were weighed and then dried up to the constant weight in air. The drying period at atmospheric pressure and temperature 25□C was 8 days. The humidity was determined from the loss of water and volatile substances in the drying oven at 105 °C. The initial humidity of the aerial sample parts was 71.43 % and the constant humidity at the end of drying was 7.56%. Before the extraction, the dried material sample were crushed to powder with particle sizes of 0.5 to 1.0 mm in the electric coffee-grinder. Hereby liquid carbon dioxide (99.5 wt %) was used. In contrast to organic solvents extraction, the technology of the supercritical extraction has a number of advantages: high process speed, low-temperature extraction, an oxygen-contact-free extracting which allows to obtain compounds that break down during heating, and environmental friendliness [12].

2.2 *Supercritical fluid extraction*

The measurements were made through the use of an experimental CO_2 apparatus. The apparatus provided an opportunity to make complex researches of extracting processes at pressures up to 40 MPa and temperature intervals from 25 to 100°C with the maximum flow of supercritical fluid of 1.7(±0.05) kg/h. Raw material grinded was loaded into a cylindrical extractor 1 (70 mm in inside diameter and 0.993 l of internal volume), to where SC CO_2 was fed through a power cylinder 3, and infused for 10 min. After that, CO_2 with dissolved extract was delivered into a separator 2 simultaneously feeding pure CO_2 into the extractor from the power cylinder. In the separator, a temperature and a pressure were supported within -20°C and 1 MPa being optimum for separation of an extract from gaseous CO_2. A pressure in the system was provided by a pump 8 which feed distilled water from a reservoir 7 into the power cylinder, where CO_2 was compressed to achieve a needed pressure. In the power cylinder, a plunger 4 divided water from CO_2 [12,13].

To determine a quantitative yield (wt%) of BAS from grinded aerial parts of rosemary were exposed to the SC extraction by CO_2 at pressure 30 MPa. The extraction time (2 h), a rate of SC CO_2 flow (1.5 ± 0.05 kg/h), and temperature (50°C) were constant for all processes.

2.3 *Compositional analysis of extracts*

The compositional analysis of extracts obtained was carried out using Shimadzu GCMS-QP2010plus chromatograph-mass spectrometer in Supelco SLB TM-5 ms column (30 m × 0.25 mm × 0.25 µm) in "split" mode. High-purity helium (99.9999%) with a flow rate of 1 mL/min was used as a carrier gas. The column temperature was raised from 60ºC (the hold time was 4 min) to 150°C at a rate of 10ºC/min after that up to 280ºC at a rate of 5ºC/min. The temperature of an injector was 280ºC, an interface, and a detector was 250ºC. The ionization was performed by an electron impact with electron energy of 70 eV. The cathode emission current was 150 µA, a range of recorded ions was 45–500 m/z. The identification of components was performed by means of NIST08 and FFNSC mass-spectra libraries. Before analysis, a test portion was diluted in n-hexane by a factor of 500. 1 mL of diluted test portion was injected with split of 1:40 [13].

3. Results and discussion

The data on the compositional analysis for SC extract are presented in Table 1. As it can be seen from Table 1, the SC extract of the aerial parts of rosemary contains the following basic components: 1,8-cineole, camphor, n-triacontane, α-pinene, myrcene, limonene, α-terpineol, caryophyllene, 2,2-bis(4'-methoxyphenyl)-2-ethoxyethane, β-sitosterol. 85 compounds were found, 73 of which were identified. Analyzing the results, it can be said that the SC extract is rich in bioactive compounds. It is characterized by a high percentage up to 53.85% monoterpenes and the major constituents are oxygenated monoterpenes: 1,8-cineole (12.23%), camphor (8.60%), α-terpineol (2.45%), monoterpene hydrocarbons α-pinene (6.31%), myrcene (3.49%), limonene (3.38%). The percentage of diterpenes in the extract is 1.16%. The share of sesquiterpenes reaches 7.25%. The major oxygenated sesquiterpene is caryophyllene (2.18%). Other important constituents - n-alkane (16.24%), fatty acids (2.76%), triterpenes 2.45% are represented in considerable amounts. The fatty acids were methyl-linolenate (1.59%), palmitic acid (0.93%), cis,cis-linoleic acid (0.24%). The extract also includes a high percentage of β-sitosterol (3.45%) However, 9.97% of substances have been not identified.

Table 1 Compositional analysis of SC CO_2 extract derived from aerial parts of *Rosmarinus officinalis* L.

Name	Ret. Index	Area %	Percentage composition according to the literature
Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl	729	0.09	
α-Thujene	927	0.09	
α-Pinene	933	6.31	0.37-1.68
Camphene	953	2.15	
Benzaldehyde	960	0.08	
Sabinene	972	0.06	
β-Pinene	978	1.76	
Myrcene	991	3.49	
n-Caproic acid ethyl ester	1003	0.10	
α-Phellandrene	1007	0.64	0.43-0.58
α-Terpinene	1018	0.66	
para-Cymene	1025	1.81	

Limonene	1030	3.38	0.55-2.70
1,8-Cineole	1032	12.23	61.00-67.00
γ-Terpinene	1058	1.41	
Name	Ret. Index	Area %	Percentage composition according to the literature
cis-Sabinene hydrate	1069	0.05	
Terpinolene	1086	0.50	0.32-0.54
para-Cymenene	1092	0.04	
Linalool	1101	0.68	
trans-Sabinene hydrate	1099	0.09	
Fenchyl alcohol	1123	0.04	
cis-, para-Menth-2-en-1-ol	1124	0.05	
Camphor	1149	8.60	1.82-7.72
Camphene hydrate	1156	0.08	
trans-Pinocamphone	1160	0.09	
Δ-Terpineol	1170	0.45	
Borneol	1173	1.84	0.54-2.97
cis-Pinocamphone	1176	0.08	
Terpinen-4-ol	1180	0.88	0.60-1.13
trans-Verbenol	1185	0.27	
α-Terpineol	1192	2.45	0.67-2.70
Verbenone	1208	1.00	2.42-13.02
2-Hexanoylfuran	1275	0.12	
Thymol	1293	0.07	
Bornyl acetate	1295	1.15	0.70-3.53
Carvacrol	1300	0.05	0.22-0.30
Phenol, 2-ethyl-4,5-dimethyl	1340	0.09	
Eugenol	1357	0.06	
Ylangene	1371	0.05	
α-Copaene	1375	0.26	
methyl-Eugenol	1403	0.15	
Caryophyllene (E)	1424	0.54	
Caryophyllene	1494	2.18	2.11-8.95

α-Humulene	1454	0.34	0.79-1.77
γ-Muurolene	1478	0.23	
α-Muurolene	1497	0.07	
Name	Ret. Index	Area %	Percentage composition according to the literature
γ-Cadinene	1512	0.24	
Δ-Cadinene	1518	0.58	
Cubenol (1,10-di-epi-)	1554	0.11	
Caryophyllene oxide	1587	0.14	
Myristaldehyde	1614	0.24	
Methyl jasmonate	1647	0.67	0.52-2.86
Stearaldehyde	1701	0.25	
Palmitic acid	1977	0.93	
Phytol	2106	0.38	0.69-3.65
cis,cis-Linoleic acid	2183	0.24	
methyl-Linolenate	2098	1.59	
Humulane-1,6-dien-3-ol	2157	0.30	
Ferruginol	2225	0.76	0.83-10.22
2,2-Bis(4'-methoxyphenyl)-2-ethoxyethane	2134	3.50	
Isocarnosol	2225	0.78	19.77-69.39
2-Phenanthrenol, 4b,5,6,7,8,8a,9,10-octahydro-4b,8,8-trimethyl-1-(1-methylethyl)-, (4bS-trans) (trans-Totarol)	2227	0.37	
1-Bromotriacontane	2249	1.18	
n-Triacontane	2300	1.16	
n-Nonacosane	2904	0.61	
Heptacosyl acetate	2910	0.67	
Squalen	2914	0.70	
β-Sitosterol	2931	3.45	
Dotriacontane	3202	6.87	
n-Tritriacontane	3301	5.81	
n-Tetratriacontane	3401	0.28	
n-Octacosyl acetate	3471	0.47	
Hexatriacontane	3600	0.94	

Table 1 shows the percentage of some components of rosemary SC extract described in the literature on essential oil and SC extract, in which terpenes are represented as the prevalent compounds of the volatile profile [14]. The rosemary SC extracts and essential oil with a similar component composition showed antibacterial activity against Gram-positive bacteria (*S. aureus* and *B. cereus*) as well as the effect against Gram-negative bacteria (*E. coli* and *P. aeruginosa*) [3].

Previously, we (Nikitina et al) published the data on the composition of components and antimicrobial activity of rosemary essential oil [15]. Our results are consistent with the data obtained in respect of rosemary essential oil, in which the compounds of volatile profile of the terpenes 1,8-cineole (19.63%), α-pinene (7.99%), caryophyllene (3.35%) dominate. The SC extract contains large quantities of such compounds as 1,8-cineole, camphor, α-pinene, myrcene, limonene, α-terpineol, caryophyllene, which have a pronounced antimicrobial activity against *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Candida albicans и Streptococcus mutans* [16]. Oxygenated monoterpenes such as camphor, 1,8-cineole, terpinen-4-ol and borneol, which were detected in rosemary SC extract as major components, have antibacterial activity against *B. cereus, B. subtilis* and *S. epidermidis.* Such components as terpinen-4-ol and linalool show antibacterial activities against a penicillin-resistant bacterium *E. coli.* Linalool, terpinen-4-ol, *α*-terpineol showed a broad spectrum of antibacterial activity against causal bacterial agents of plant diseases *Agrobacterium tumefaciens*, *Clavibacter michiganense*, *Erwiniaamylovora*, *Erwinia carotovora*, *Pseudomonas* sp. and *Xanthomonas* sp. [17, 18].

4. Conclusion

The compounds found in rosemary SC extract possess a variety of unique features. It is known that, α-pinene has antibacterial and antifungal properties and exerts a dose-dependent antimutagenic effect, i.e. prevents UV-radiation induced mutations. Myrcene is widely used in the production of flavors for cosmetic and food industries as well as household chemicals. Limonene is also widely used in perfumery, cosmetics and fragrance additives production; it is a good insecticide. α-Humulene and its derivatives are used in brewing as a corrigent of beer taste. Alpha-humulene and (-)-trans-caryophyllene are effective in reducing the edema formation caused by the histamine, that is important for treatment of inflammatory diseases [19, 20]. Ferruginol has also been found to have antibacterial activity and also gastroprotective effects [21].

Our results shows that rosemary cultivated in the Botanical Garden of Pyatigorsk Medical and Pharmaceutical Institute is a special chemotype with a high contents of monoterpenes. Finally, the development of natural medicines will help to decrease the negative effects of synthetic medicines, as natural medicines, for instance antimicrobials, can also be effective, selective, biodegradable and less toxic to the environment.

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