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Composition of Extract of the *Juniperus oblonga* M. Bieb. Fruits Obtained by Supercritical CO_2 Extraction

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Abstract—Mature *Juniperus oblonga* M. Bieb. fruits growing in Dagestan (Russia) at a height of 1750 m above sea level were extracted using supercritical CO_2 extraction. The extraction was carried out at a constant temperature of 311 K and pressure values of 8, 10, 15, 20, 25, and 30 MPa. The extract, which was obtained at 10 MPa, was analyzed by gas chromatography coupled with mass spectrometry. As a result of the analysis, 44 compounds were revealed, with 43 of these being identified. In the extract, the major components were α-pinene (7.11%), sabinene (19.47%), β-myrcene (11.97%), limonene (2.64%), β-elemene (2.31%), germacrene-D (20.66%), germacrene-D-4-ol (4.90%), bisabolol (2.78%), and linoleic acid (5.28%).

Keywords: *Juniperus oblonga*, supercritical extraction, gas chromatography, mass spectrometry, component analysis

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INTRODUCTION

Juniperus oblonga is an evergreen coniferous shrub-like plant of the Cypress (*Cupressaceae*) family, which is from 1 to 8 m high and grows in temperate climate. At present, there are 68 species and 36 varieties of juniper [1]. In Russia, 14 species of juniper grow in the Ural Mountains; in the mountains of the Siberia and the Far East; and the flat woods of the northern, European, and Asian parts of the country. In the flora of the Caucasus, there are eight species of juniper; six of these occur in the Dagestan, and one of the most widespread is *Juniperus oblonga* [2–4]. Despite the wide circulation of juniper species, only common juniper (*Juniperus communis* L.) is used in medical practice at present. This species of juniper is only used as a diuretic, and it does not meet the requirements of the medical industry for raw materials [5]. In this connection, both the search of additional sources of raw materials and the development of new, hi-tech, harmless, and effective methods able to increase the efficiency of processing of valuable plant raw material have become of current importance. The treatment of medicinal plant raw material with condensed gases and supercritical (SC) fluids is one such new method, an example of which is extraction of raw materials with supercritical carbon dioxide [6, 7].

Supercritical extraction possesses a number of advantages in comparison with other means of extraction: the problem of a residual solvent in an extract is excluded; rapidity and ecological compatibility of the process, a high yield of end product, and low temperature of extraction are provided; and extraction occurs without contact with atmospheric oxygen, which allows one to extract compounds that are unstable in the presence of oxygen.

The purpose of our work is to study the processes of extraction of biologically active substances from *Juniperus oblonga* using supercritical CO_2 and the definition of composition of the received extract. Such data are absent in the literature, but there are results of studies of the essential oil of fruits and needles by chromatography and mass spectrometry and data on the antioxidant activity of fruits and needles [8–15].

In this study, the results of supercritical CO_2 extraction of ripe fruits of *Juniperus oblonga* and the composition of the extract obtained are presented. Analysis of the extract by gas chromatography coupled with mass spectrometry allows us to identify 43 of 44 extracted compounds. The yield of essential oil from *Juniperus oblonga* is determined.

MATERIALS AND METHODS

Ripe fruits of *Juniperus oblonga* were collected in the Republic of Dagestan at a height of 1750 m above sea level in November and dried in a shadowy, ventilated place at a temperature of 308–311 K.

Extraction was performed using an experimental apparatus (Fig. 1) that allowed a complex study to be carried out of extraction processes at values of pressure up to 40 MPa in the range of temperatures of 298–373 K.

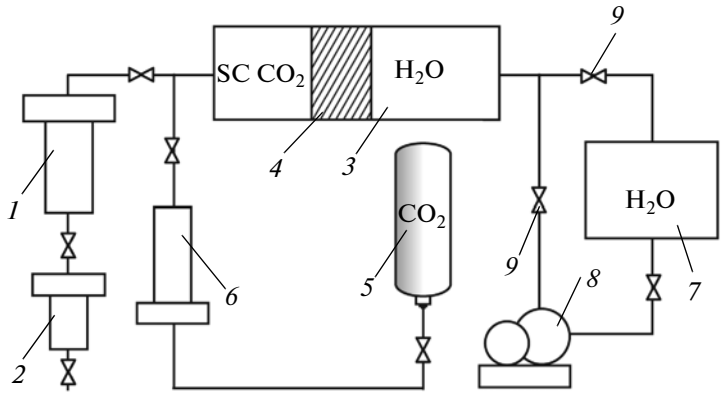

Fig. 1. Circuit diagram of the experimental plant for extraction with supercritical CO_2: (*1*) extractor, (*2*) separator, (*3*) power cylinder, (*4*) separating plunger, (*5*) cylinder with CO_2, (*6*) filter for CO_2 clearing, (*7*) reservoir with distilled water, (*8*) dosing high pressure pump, and (*9*) valves.

Raw material is reduced to fragments of 0.3–0.5 mm in size and loaded into extractor *1*, in which carbon dioxide in the SC condition moves from power cylinder *3* and is extracted for 10 min. Thereafter, CO_2 passes with dissolved extract into separator *2*; simultaneously, pure CO_2 moves into the extractor from the power cylinder. In the separator, temperatures not above 243 K and pressure of 0.5 MPa are maintained, at which the extract is separated from gaseous CO_2. The pressure in the system is created by pump *8*, which transports distilled water from tank *7* into the power cylinder, in which the required pressure is reached by CO_2 compression. In the power cylinder, water is separated from CO_2 by plunger *4*.

The extraction was carried out at pressures of 8, 10, 15, 20, 25, and 30 MPa. For each experiment, a fresh portion of raw material from one sample with a humidity of 13.1% was used. The temperature in the extractor was constant and equal to 311 K in all experiments.

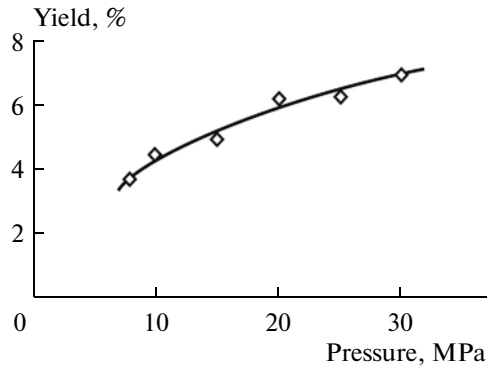

Fig. 2. Dependence of yield of extractive substances from fruits of *Juniperus oblonga* on pressure at the temperature of 311 K.

The composition of the extract was determined by GLC using a Saturn 2000 (Varian, United States) equipped with a mass-spectrometer detector of "ionic trap" type and a Stabilwax column 30 m in length and 0.32 mm in internal. The layer of the immobile phase was 0.5 μm in thickness. The column temperature was raised from 40 (for 2 min) to 190°C at a rate of 5°C/min and then to 280°C at a rate of 3°C/min. The temperature of an injector and heated adapter between the thermostat of the chromatograph and the mass-spectral detector was 250°C. Ionization was performed by an electric strike with an energy of electrons of 70 eV in the regime of automatic setting of ionization time (AGC). The cathode emission current was 10 μA; the range of registered ions was 45–650 m/z. Identification of the components of the fraction was performed using search algorithms in the NIST and WILEY mass-spectra libraries in the standard search regime. The identified compounds had a parameter of coincidence of the analyzed mass spectrum with that of the library of more than 700. The mass spectrometer was calibrated (quantitative data processing) by the method of normalization of the full ionic current of detected compounds. Anethole and cymene were used as standards [16]. Before the analysis, the sample was 1000-fold diluted with methanol. One microliter of the dissolved sample was introduced into the chromatograph with a 1 : 40 stream division.

RESULTS AND DISCUSSION

The results of the determination of the yield of extractive substances from fruits of *Juniperus oblonga* at various values of pressure and constant temperature of 311 K are presented in Fig. 2. The maximum yield of extractive substances from a raw material amounted to 7% at a pressure of 30 MPa. The yield of essential oil was 1.54%.

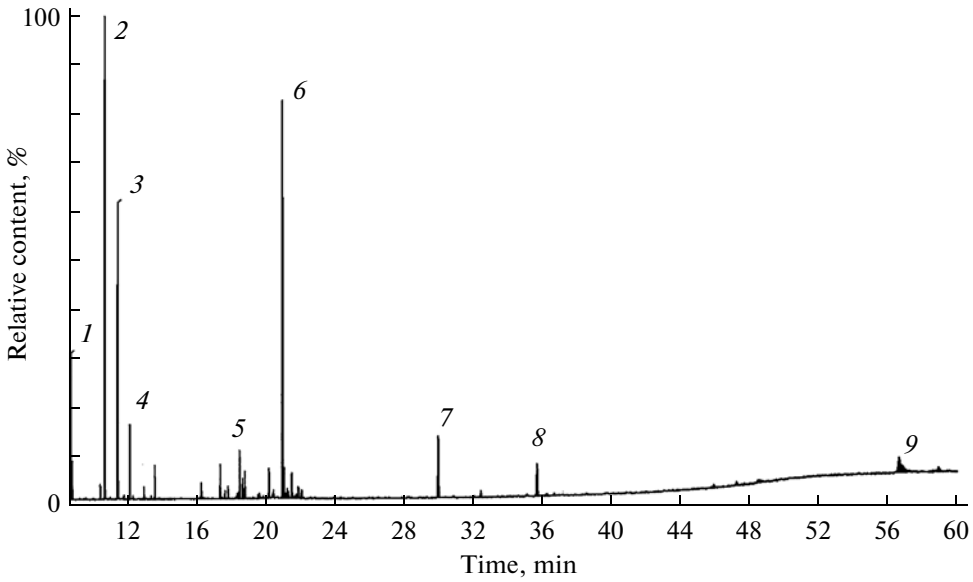

Fig. 3. Chromatogram of the extract of *Juniperus oblonga* fruits: (*1*) α-pinene, (*2*) sabinene, (*3*) β-myrcene, (*4*) limonene, (*5*) β-elemine, (*6*) germacrene-D, (*7*) germacrene-D-4-ol, (*8*) bisabolol, and (*9*) linoleic acid.

Analysis of extract obtained at a temperature of 311 K and pressure of 10 MPa by combined gas chromatography and mass spectrometry allows 44 compounds to be revealed (the chromatogram of investigated extract is presented in Fig. 3); 43 of them have been identified (see table). α-Pinene (7.11%), sabinene (19.47%), β-myrcene (11.97%), limonene (2.64%), β-elimine (2.31%), germacrene-D (20.66%), germacrene-D-4-ol (4.90%), bisabolol (2.78%), and linoleic acid (5.28%) are determined to be the basic components of the extract. The percentages of the compounds, which belong to several basic classes, are shown in Fig. 4.

The structural formulas of the basic constituents of SC CO_2 extract are given in Figs. 5 and 6. A comparison of mass spectra of the α-pinene obtained in (a) this study and (b) the library of standard spectra is presented as an example in Fig. 5.

The compounds revealed in SC CO_2 extract of *Juniperus oblonga* possess a number of unique properties. So, for example, α-pinene has antibacterial and antifungal properties and a dose-dependent antimutagenic effect, that is, prevents mutations caused by UV radiation; this compound is a constituent of preparations for treatment of UV-induced skin cancer, is effective for the treatment of the respiratory tract, and in conjunction with β-pinene is used for the synthesis of pheromones [17–20].

Sabinene is active in the relation of acetylcholine esterase and butyrylcholine esterase [21] and is aeffective for creation of new-generation repellents [22].

β-Myrcene is widely used for production of aromatizers for the cosmetic and food industries, as well as household chemical goods. Pharmacological research on laboratory rodents has shown that β-myrcene can cause nephritic carcinogenesis in quantity [23, 24]. The smell of β-myrcene, α-pinene, and β-phellandrene is rather close to the alarm pheromone of a plant louse, and in this way a plant frightens it off [25].

Limonene is widely used in the perfumery and cosmetic industries and in the manufacture of aromatizers; it is also a good insecticide [26, 27]. β-Elimine is used for treatment of cancer tumors, including brain, liver, and gullet tumors. This substance does not lead to the change of a marrow and does not reduce the

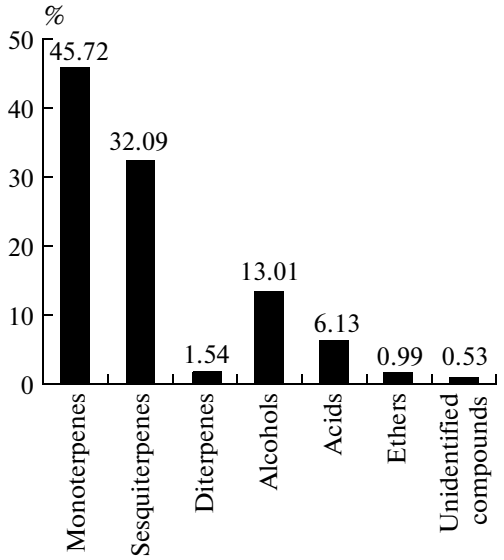

Fig. 4. Content of basic classes of compounds in SC CO_2 extract of *Juniperus oblonga* fruits obtained at 311 K and 10 MPa.

Composition of extract of *Juniperus oblonga* M. Bieb. fruits obtained by supercritical CO_2 extraction

Compound	Time of retention	Percentage
α-Pinene	8.74	7.11
α-Thujene	8.83	1.67
(+)-β-Pinene	10.45	0.63
(+)-Sabinene	10.71	19.47
β-Myrcene	11.46	11.97
α-Terpinolene	11.82	0.16
Limonene	12.16	2.64
β-Phellandrene	12.34	0.11
γ-Terpinene	12.97	0.55
p-Cymene	13.40	0.12
Terpinolene	13.60	1.29
cis-β-Terpineol	16.29	0.73
cis-Sabinene hydrate acetate	17.39	1.45
4-Tuyanol stereoisomer	17.65	0.39
Methyl citronellate	17.83	0.50
Bornyl acetate	18.37	0.30
β-Elemine	18.50	2.31
4-Terpineol	18.68	1.01
α-Caryophyllen	18.80	1.30
Citronellol acetate	19.57	0.19
β-Fernesene	19.64	0.23
α-Caryophyllen	20.20	1.57
α-Terpineol	20.42	0.25
Copaene	20.46	0.40
Germacrene-D	20.98	20.66
β-Bisabolene	21.08	1.56
α-Muurolene	21.18	0.39
8-Isopropilene-1,5-dimethil-cyclodeca-1,5-diene	21.24	0.60
α-Selinen	21.33	0.44
Bicyclogermacrene	21.52	1.46
α-Farnesine	21.78	0.28
β-Cadinene	21.90	0.66
γ-Cadinene	22.04	0.23
Unidentified	22.09	0.53
Germacrene-D-4-ol	29.98	4.90
Spathulenol	32.45	0.68
Bisabolol	35.69	2.78
T-muurolol	36.27	0.47
19-D-torulosol	36.68	0.26
Abietatriene	45.91	0.39
1-(4-hydroxi-7-isopropil-4-methil-octhydro-1h-indene-1-il)ethanol	47.21	0.35
Oleic aid	48.61	0.85
Linoleic acid	56.61	5.28
Cryptopinone	58.90	0.89

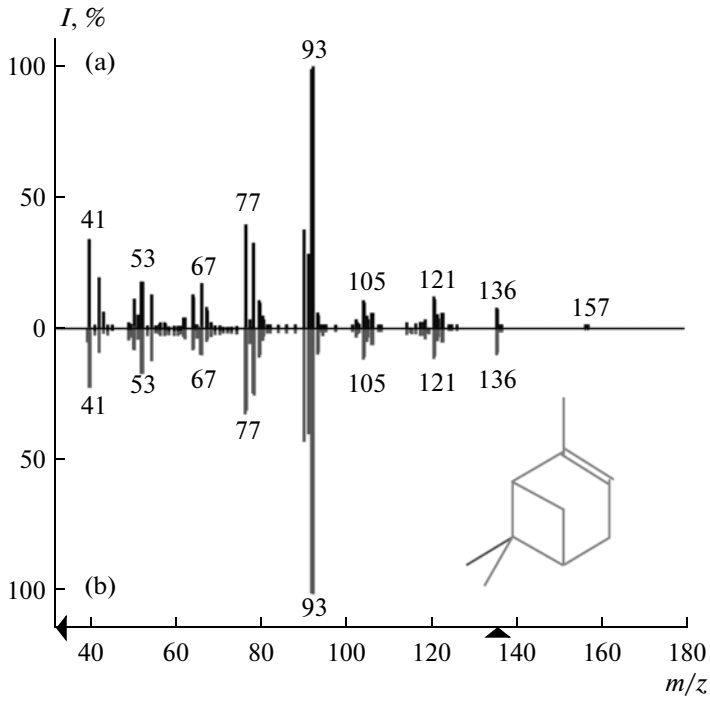

Fig. 5. Structural formula and mass spectra of α-pinene: (a) obtained in this work; (b) standard (obtained from databases).

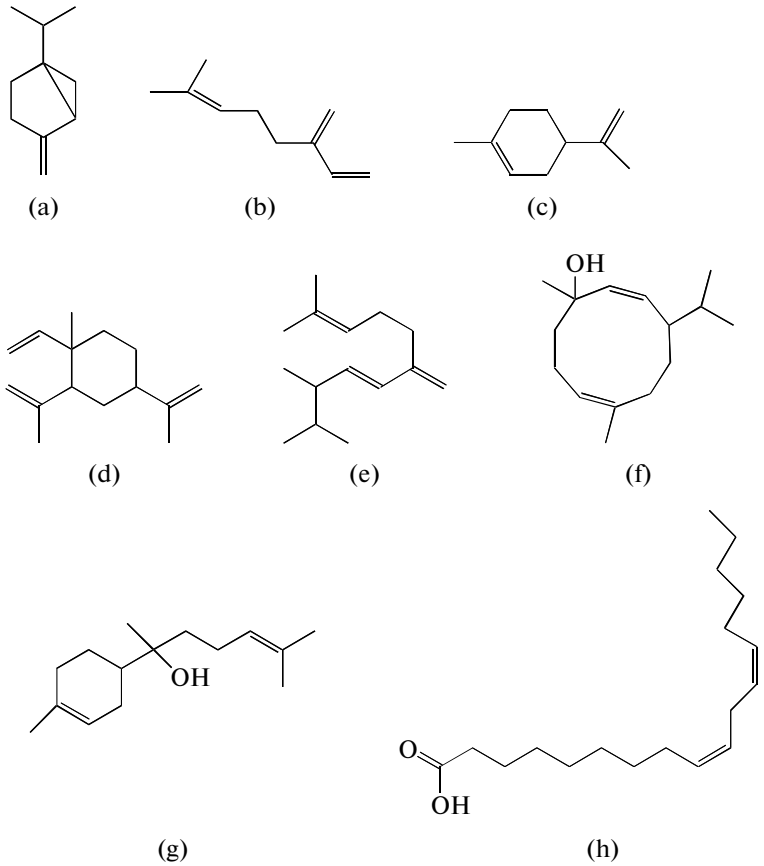

Fig. 6. Structural formulas of components of extract of *Juniperus oblonga* fruits: (a) sabinene, (b) myrcene, (c) imonene, (d) β-elemine, (e) germacrene-D, (f) germacrene-D-4-ol, (g) bisabolol, and (h) linoleic acid.

quantity of leukocytes, which are the general collateral actions of usual anticancer preparations. β-elimine inhibits apoptosis (spontaneous death) of cells [28].

According to the literature data, germacrene-D is the precursor of sesquiterpenes such as cadinene and selinen [29, 30]. The smell of germacrene-D attracts one species of insects and scares others away, providing chemical regulation of interaction with insects [31, 32]. This compound also possesses antibacterial property and is used as an aromatizer [33].

Bisabolol is used as an anti-inflammatory and antibacterial substance that calms irritated skin, as well as in perfumery [34, 35]. In recent years, bisabolol has been used for medical treatment of malignant tumors and leucosis [36, 37].

Linoleic and oleic acids possess a smell that frightens insects away [38, 39]. These substances are also widely used in the cosmetics industry. Conjugated linoleic acid prevents and hampers the development of breast cancer [40].

CONCLUSIONS

Extract of the *Juniperus oblonga* fruits obtained by extraction with supercritical CO_2 contains monoterpenes, sesquiterpenes, diterpenes, alcohols, acids, and ethers. In the extract, the following compounds are found to be basic components: α-pinene, sabinene, β-myrcene, limonene, β-elimine, germacrene-D, germacrene-D-4-ol, bisabolol, and linoleic acid. Due to its rich chemistry, SC CO_2 extract of the *Juniperus oblonga* fruits may be used in medicine (including treatment and prophylaxis of oncological diseases) and in the food and cosmetics industries, as well as for creation of the effective, nonpolluting new-generation repellents.

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