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CHEMICAL COMPONENTS OF PEROVSKIA KUDRJASCHEVII

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Abstract: The composition of the essential oil and hexane extract of air-dried roots of Perovskia kudrjaschevii was studied using the GC-MS method. It was found that the main component of the essential oil is 1,8-cineole. Five natural biologically active compounds were isolated from the methanol extracts of the roots and above-ground part. Based on the study of UV, 1H, 13C NMR, HSQC and HMBC spectra, the isolated compounds were identified with coffee, rosemary and oleanolic acids, β-sitosterol and stigmasterol.

Keywords: *Perovskia kudrjaschevii,* Lamiacaeae, essential oil, 1,8-cineole, coffee, rosmarinic and oleanolic acids, β-sitosterol, stigmasterol.

Introduction. The genus *Perovskia* Kar. belongs to the family Lamiacae and has only 9 species of subshrubs, most of which grow wild in the mountainous regions of Southwest and Central Asia[1-3]. Four species of this plant grow in Uzbekistan, some of which are used by locals to treat scabies, sunburn, skin diseases, and to remove intestinal parasites from the body. Flavonoids, catechols, phenylpropanoids, lignans, sesqui- tenopenoids, diterpenoids, dinorsesterpenoids, sterols, triterpenoids, and their glycosides have been isolated from plants of this genus[2-8].

Perovskia kudrjaschevii Gorschk. & Pjataeva – a subshrub growing on gravel along river beds, on rocky slopes of gorges from the foothills to the middle mountain belt. Medicinal, essential oil, dye and honey plant. It is found in the Pskem, Chatkal, Fergana ranges of the western Tien Shan, the Nurata, Turkestan, Gissar ranges of the Pamir-Alay in Uzbekistan, Tajikistan and

Kyrgyzstan[1]. The chemical composition of the components of *P. kudrjaschevii* has not been studied.

In order to search for biologically active compounds and rational use of local plant materials, we studied the chemical composition of the roots of P. kudrjaschevii.

Methodology & empirical analysis. The roots used in this work were collected during the period of death of the aboveground part (November, 2024) in the Bostandyk district of the Tashkent region. The species was identified by PhD in Biology O.M. Nigmatullaev in the laboratory of medicinal and technical plants of the Institute of Chemistry of Plant Substances named after academician S.Yu. Yunusov of the Academy of Sciences of the Republic of Uzbekistan.

Silica gel of the KSK brand (100/200 μm, Tianjin Sinomed Pharmaceutical, China) was used for column chromatography (CC). Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden) was used to separate individual eluates. Fluka plates (Sigma-Aldrich, Germany) were used for thin-layer chromatography. The plates were viewed in ultraviolet light in a UFS-254/365 chromatographic irradiator at 254 and 365 nm. UV spectra were recorded on EPS-3T "Hitachi", Specord UV-Vis and SF-26 spectrophotometers in ethanol. NMR spectra ^{1}H and ^{13}C were recorded on a JNM-ECZ600R spectrometer (Jeol, Japan) at an operating frequency of 600 MHz and 150 MHz.

GC-MS analysis. The qualitative and quantitative composition of the essential oil was determined on an Agilent 5975C Inert MSD/7890A GC chromatograph-mass spectrometer. The components of the mixture were separated on an Agilent HP-INNOWax quartz capillary column (30 m × 250 μm × 0.25 μm).

Isolation of essential oil and hexane extract. The essential oil was isolated from the crushed air-dried roots (350 g) by hydro distillation at atmospheric pressure for 3 hours. The resulting distillate was extracted with dichloromethane, the extract of the essential oil was dried with anhydrous sodium sulfate. The crushed roots (5.0 g) were extracted at room temperature with hexane for 24 hours, the extract was filtered, evaporated at room temperature and transferred for study by GC-MS.

Isolation of root components. Ground air-dried roots (4.83 kg) were extracted six times with methanol at room temperature. The combined extract was evaporated in vacuum to obtain 498 g of extract, which was mixed with silica gel (500 g), dried at room temperature, then for two hours in a drying cabinet at a temperature of 50-60 0C, placed in a column and successively washed with gasoline, chloroform, ethyl acetate and n-butanol. After distilling off the solvents, 47.8 g of gasoline, 53.4 g of chloroform, 39.9 g of ethyl acetate and 75.0 g of n-butanol fraction were obtained. The gasoline fraction (47.8 g) was chromatographed on a column with silica gel (600 g), washing successively with gasoline and a gradient mixture of gasoline-ethyl acetate solvents. Washing with a mixture of gasoline and ethyl acetate solvents (19:1) yielded 127 mg of β-sitosterol (4) and 94 mg of stigmasterol (5).

Isolation of components of the above-ground part. The dried and crushed aerial parts of *P. kudrjaschevii* (6.9 kg) were extracted eight times with methanol at room temperature. The extract concentrated in a vacuum was diluted with water in a 1:1 ratio and subjected to successive liquid-liquid extraction with gasoline (6 times 1 l), chloroform (6x2 l), ethyl acetate (6x2 l) and n-butanol

(5x1 l). Washing with chloroform in the interphase medium resulted in the formation of a precipitate, which was filtered, dried and treated three times with 400 ml of methanol. Evaporation of the methanol solution yielded a precipitate of 24.0 g of rosmarinic acid (2). Evaporation of the mother liquor yielded 4.7 g of residue, which was chromatographed on a Sephadex LH-20 column in methanol. From the individual eluates, 0.57 g of caffeic acid (1) and 0.24 mg of oleanolic acid (3) were isolated.

Results. Using the GC-MS method, 63 substances were identified in the composition of the essential oil of the roots, which amounted to 95.2% of the total amount of essential oil [9,10]. Sesquiterpenes (46.9%) and oxidized monoterpenes (30.7%) predominate in the composition of the essential oil. The main components of the essential oil are 1,8-cineole (16.0%), camphor (7.4%), β-caryophyllene (8.3%), alloaromadendrene (8.0%), β-bisabolene (7.0%), endo-borneol (6.5%) α-caryophyllene (5.9%). 1,8-Cineole (11.7%) and cyclopentadecane (19.6%) were found in the composition of the hexane extract. 1,8-Cineol (eucalyptol) is a bicyclic monoterpene and has moderate antiexudative and cytotoxic activity, as well as significant analgesic and antitumor properties[9].

The isolated compounds were identified by studying UV, ^{1}H and ^{13}C NMR spectral data, as well as HSQC and HMBC experiments, followed by comparison with literature data for these compounds, as well as direct comparison with authentic samples of the substances.

Compound 1. UV λ_{max} (MeOH) 325, 299, 235 nm; ^{1}H NMR (DMSO-d_6+CCl_4, δ, ppm, 600 MHz): 6.09 (d, 16.2 Hz, H-8), 6.72 (d, 8.2 Hz, H-5), 6.85 (dd, 8.2 and 2.1 Hz, H-6), 6.97 (d, 2.1 Hz, H-2), 7.38 (d, 16.2 Hz, H-7). ^{13}C NMR spectrum (DMSO-d6+CCl4, δ, ppm, 150 MHz): 114.21 (C-8), 115.03 (C-2), 115.51 (C-5), 120.75 (C-6), 125.74 (C-1), 144.35 (C-3), 145.39 (C-7), 147.88 (C-4), 167.76 (C-9). Based on the study of ^{1}H, ^{13}C NMR, HSQC, and HMBC spectra, compound 1 was identified with caffeic acid[11].

Compound 2. The UV spectrum exhibited two absorption maxima (λ_{max} MeOH) at 290 and 330 nm. ^{1}H NMR (DMSO-d_6+CCl_4, δ, ppm, 600 MHz): 2.92 (dd, 14.4 and 8.3 Hz, H-7'), 2.99 (dd, 14.4 and 4.2 Hz, H-7'), 5.01 (dd, 8.3 and 4.2 Hz, H-8′), 6.18 (d, 15.8 Hz, H-8), 6.50 (dd, 8.2 and 2.1 Hz, H-6′), 6.62 (d, 8.0 Hz, H -5′), 6.67 (d, 2.1 Hz, H-2′), 6.73 (d, 8.2 Hz, H-5), 6.90 (dd, 8.2 and 2.1 Hz, H-6), 7.01 (d, 2.1 Hz , H-2), 7.45 (d, 15.8 Hz, H-7). ^{13}C NMR spectrum (DMSO-d_6+CCl_4, δ, ppm, 150 MHz): 36.22 (C-7′), 72.53 (C-8′), 113.16 (C-8), 114.38 (C-2), 115.19 (C-5′), 2), 115.54 (C-5), 116.53 (C-2′), 119.82 (C-6′), 121.25 (C-6), 125.30 (C-1), 127.08 (C-1′), 143.85 (C-4′), 144.74 (C-3′), 145.43 (C-3), 145.63 (C-7), 148.39 (C-4), 165.57 (C-9), 170.59 (C-9′). Based on the study of ^{1}H, ^{13}C NMR, HSQC, and HMBC spectra, compound 2 was identified with rosmarinic acid[11,12].

Compound 3. White amorphous powder, mp 308-310°C. ^{1}H NMR (600 MHz, CD_3OD+CCl_4, δ, ppm, J/Hz): 0.71 (1H, dd, J=11.4, 1.6, H-5), 0.74 (3H, s, H-24), 0.78 (3H, s, H-26), 0.91 (3H, s, H-29), 0.92 (3H, s, H-25), 0.94 (3H, s, H-30), 0.95 (3H, s, H-23), 0.96 (1H, m, H-1a), 1.04 (1H, ddd, J=13.7, 3.4, 3.3, H-15a), 1.13 (3H, s, H-27), 1.14 (1H, m, H-19a), 1.19 (1H, m, H-21a), 1.31 (1H, m, H-7a), 1.34 (1H, m, H-21b), 1.38 (1H, m, H-6a), 1.44 (1H, m, H-7b), 1.53 (1H, m, H-22a), 1.53 (1H, m, H-9), 1.54 (1H, m, H-6b), 1.56 (1H, m, H-2), 1.58 (1H, m, H-16a), 1.62 (1H, m, H-19b), 1.62 (1H, m, H-1b), 1.72 (1H, m, H-15b), 1.72 (1H, m, H-22b), 1.87 (1H, m, H-11), 1.93 (1H, m, H-16b), 2.80 (1H, dd, J=13.8, 4.5, H-18), 3.13 (1H, dd, J=11.2, 5.0, H-3), 5.23 (1H, dd, J=3.6, 3.6, H-12). ^{13}C NMR spectrum (150 MHz, CD3OD+CCl4, δ, ppm): 39.57 (C-1), 27.45 (C-2), 79.16 (C-3), 39.57 (C-4), 56.34 (C-5), 19.21 (C-6), 33.72 (C-7), 40.17 (C-8), 48.62 (C-9), 37.86 (C-10), 24.23 (C-11), 123.20 (C-12), 144.58 (C-13), 42.53 (C-14), 28.52 (C-15), 23.77 (C-16), 47.08 (C-17), 42.13 (C-18), 46.87 (C-19), 31.53

(C-20), 34.81 (C-21), 33.34 (C-22), 28.79 (C-23), 16.36 (C-24), 16.02 (C-25), 17.54 (C-26), 26.63 (C-27), 181.10 (C-28), 33.90 (C-29), 24.32 (C-30). Compound 3 was identified with oleanolic acid[13].

Compounds 4 and **5** were identified as β-sitosterol and stigmasterol, respectively, based on spectral data and direct comparison with authentic samples[14,15].

Caffeic acid has antioxidant properties and is used to prevent inflammation, cancer, neurodegenerative diseases and diabetes [16]. Rosmarinic acid has immunomodulatory, anti-inflammatory, antimicrobial, antioxidant, neuroprotective, and antidiabetic properties[17].

All the above compounds from *P. kudrjaschevii* were isolated for the first time. To study the antibacterial and antifungal properties of the hexane extract and essential oil from the roots of *P. kudrjaschevii,* a modified agar diffusion method was used [18], and the following strains of microorganisms were used as test cultures: gram-positive bacteria *Bacillus subtilis* (RKMUz - 5), *Staphylococcus aureus* (ATCC 25923); gram-negative bacteria - *Pseudomonas aeruginosa* (ATCC 27879), *Escherichia coli* (RKMUz - 221) and one fungal strain *Candida albicans* (RKMUz - 247), as a positive control - disks with ampicillin, ceftriaxone and fluconazole (Himedia Laboratories). The results of in vitro antimicrobial tests showed that the hexane extract exhibited a significant antibacterial effect observed against gram-positive strains of bacteria *S. aureus* and *P. aeruginosa* with inhibition zone diameters of 15.04±0.10 and 17.04±0.10, respectively.

The antibacterial and antifungal properties of essential oil and hexane extract were studied by Doctor of Biological Sciences S.A. Sasmakov in the laboratory of molecular genetics.

Conclusions. In order to search for biologically active compounds and rational use of local plant materials, the composition of the essential oil, hexane extract and components of the methanol extract of the roots and above-ground part of *P. kudrjaschevii* were studied for the first time. The qualitative and quantitative compositions of the essential oil and hexane extract, as well as their dominant components, were determined using GC-MS. The roots of *P. kudrjaschevii* can serve as a rich source of 1,8-cineole, which has immunomo-dulatory, anti-inflammatory, antimicrobial, antioxidant, neuroprotective and antidiabetic properties. Coffee, rosemary and oleanolic acids, β-sitosterol and stigmasterol were isolated and identified for the first time from methanol extracts of the roots and above-ground parts of the plant.

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