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VOLATILE COMPONENTS OF *INULA HELENIUM* AND *I. GRANDIS*, GROWING IN UZBEKISTAN*

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The composition of volatile components of essential oils and hexane extracts of the roots and essential oils of leaves of *Inula grandis* and *Inula helenium*, growing in Uzbekistan, was studied using the GC-MS method. In the essential oils of the roots of *Inula grandis* and *Inula helenium*, per 56 compounds were identified, which is 89.75 and 42.72% of the total amount of essential oils of the roots, respectively. In hexane extracts of *Inula grandis* and *Inula helenium* roots, 13 and 33 compounds were identified, accounting for 93.05 and 97.73%, respectively. The major components of the essential oil of *Inula grandis* roots are alantolactone (35.63%), igalan (15.77%), (+)-leden (7.91%), 4,15-dihydroisoalantolactone (7.01%), 6,12-epoxy-11 β -eudesma-4,6-dien-3-one (5.40%), 11 α ,13-dihydroalantolactone (3.33%), а гексанового экстракта alantolactone (54.38%), igalan (17.16%), 6,12-epoxy-11 β -eudesma-4,6-dien-3-one (6.85%), (+)-leden (6.79%), 11 α ,13-dihydroalantolactone (2.93%).

It should be noted that the main components of the essential oil of *Inula helenium* roots with a content of 13.17 and 29.05%, and those of the hexane extract of *Inula helenium* roots were 13.70 and 29.53%, have not been identified.

In the future, these components require isolation in their native form by chromatographic methods. The main components of roots *Inula helenium* essential oil are viridiflorol (3.39%), 1,5-cyclooctadiene, 3,4-dimethyl- (3.41%), 11 α ,13-dihydroele-masteiractinolide (2.85%), 11 α ,13-dihydroalantolactone (2.86%), alantolactone (10.92%), and the main components of roots *Inula helenium* hexane extract are (S*)-1-[(1S*,2S*)-2-ethenyl-1-methyl-2-(phenylthio)cyclopropyl] ethanol (14.77%), alantolactone (14.66%), phosphonic acid, [1-(1,1-dimethylethyl)-4,4-dimethyl-1,2-pentadienyl]- (5.31%), alloalantolactone (3.21%), 1-methyl-3,3-bis(trifluoromethyl) cyclohexene (3.08%).

45 compounds (70.7%) were identified in the essential oil of *Inula grandis* leaves, and 70 compounds (70.81%) were identified in the essential oil of *Inula helenium* leaves. Of the identified components of the essential oil of *Inula grandis* leaves are alantolactone (20.27%), isoalantolactone (7.81%), aromadendrene (3.63%), eudesma-4-en-3-one (3.11%), dihydroactinidi-oide (3.10%), and γ -elemene (2.58%). Of the identified components of the essential oil of *Inula helenium* leaves are δ -3-carene (7.70%), palmitinic acid (7.36%), benzene, 1,3-bis(1,1-dimethylethyl)-2-methoxy-5-methyl- (6.61%), β -ionone (3.90%), dihydroactinidiolide (2.83%), 3,7-benzofurandiol, 2,3-dihydro-2,2-dimethyl- (2.78%), diterpenoid phytene (2.62%).

It has been confirmed that *Inula grandis* and *Inula helenium* are rich sources of sesquiterpenoids, especially sesquiterpene lactones of the germacrane, elemene and eudesmane types.

It was revealed that essential oils of roots and leaves, as well as hexane extracts of *Inula grandis* and *Inula helenium* roots exhibit medium-level antibacterial and antifungal activity.

Keywords: *Inula grandis*, *Inula helenium*, roots, leaves, essential oil, hexane extract, GC-MS analysis, antimicrobial activity, antifungal activity.

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Introduction

The genus *Inula* L. of the *Asteraceae* family in the world flora is composed of approximately 100 species [1]. In Central Asia and Uzbekistan, specifically, there are 9 species [1].

Inula grandis Schrenk ex Fisch. et Mey. (Syn. *Inula macrophylla* Kar. et Kir., *Codonocephalum grande* (Schrenk) B. Fedtsch.). Sariq andis (uzb.), qara-andyz (kaz.), saryndyz, andyz, qaryndyz (kirghiz.), zaboni govak, chuqola (tajik.) – elecampane big or large-leaved elecampane is perennial plant with the height of 35–200 cm, spread

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on the Ugam, Pskem, Chatkal, Kuramin, Fergana, Turkestan, Zarafshan, Hissar and Nurata ranges, and growing on small and small-earthcrushed steppe slopes from foothills to the middle belt of mountains [2].

This species is used in folk medicine for treatment of tuberculosis, diseases of the gastrointestinal tract, brucellosis, and as an anti-helminth agent [3]. According to [4], the decoction of *Inula* roots has been found to cause a decrease in blood sugar levels.

Inula helenium L. Qora andis (uzb.), andoz (tajik.). – elecampane high is a perennial plant 60–250 cm high, distributed on Ugam, Pskem, Chatkal, Kuramin, Fergana, Turkestan, Zarafshan, Hissar ridges, and growing on the banks of rivers in a clump of bushes from the foothills to the middle belt of mountains [2].

The roots and rhizomes of *Inula* have been included in the domestic Pharmacopoeia editions 1–3 of the Commonwealth of Independent States, and they continue to hold official status in Netherlands. The plant has a wide range of pharmacological applications: respiratory diseases, gastrointestinal tract, pancreas, liver, urogenital system, neurosis, diabetes mellitus, epilepsy, atherosclerosis, pneumonia, cardio-respiratory, vascular system diseases, etc. [3]. Based on the research [4], the roots of *Inula* have served as the basis for the development of various preparations with specific therapeutic applications. These include a preparation for treating bronchial asthma complicated by bronchitis and pneumonia, an ointment designed for the treatment of purulent and thrombotic wounds, and an anti-hepatotoxic tea formulated for the management of toxic hepatitis.

The aerial parts of the plants *Inula grandis* and *Inula helenium* are difficult to distinguish, but they differ in the color of the roots and rhizomes. The roots and rhizomes within the *Inula grandis* are yellow, and in the *Inula helenium* the roots and rhizomes are black.

These species have previously been found to contain sesquiterpene lactones related to eudesmanolides, germacranolides, elemanolides, as well as steroids, flavonoids, phenolcarboxylic acids, carbohydrates, etc. [3].

The components of the non-polar fraction of ethanol extract were studied [5]. Otherwise, the volatile components of *Inula grandis* are not sufficiently studied.

In the *Inula helenium* essential oil have been found in the composition such the compounds as alantolactone, isoalantolactone, dihydroisoalantolactone, azulene, camphor, alantonic and acetic acid [3]. However, the composition of the volatile components of *Inula helenium* growing in Uzbekistan has not been investigated.

This material presents the analysis of volatile components by the method of GC-MS extracts and essential oils of roots and leaves of *Inula helenium* and *Inula grandis*, growing in Uzbekistan.

Experimental part

Isolation of essential oils and extracts of roots and leaves of Inula helenium and Inula grandis. The following plant parts were taken for analysis: **1.** *Inula grandis* roots from Tashkent region (04.2020), **2.** *Inula helenium* roots from Tashkent region (11.2020), **3.** *Inula grandis* leaves from Tashkent region (04.2020), **4.** *Inula helenium* leaves from Tashkent region (04.2020).

Plants *Inula helenium* and *Inula grandis* were collected in the Bostanlyk district of the Tashkent region (41.57579°, 70.10106°; 41.617993°, 69.918138°, respectively). The species was identified by doctoral candidate O.M. Nigmatullaev of the Laboratory of Medicinal and Technical Plants, S. Yu. Yunusov Institute of the Chemistry of Plant Substances, AS, RUz.

The samples **1–2** (each per 2 g) were extracted by hexane three times for each extraction time of 8 hours. Each combined extracts were evaporated at room temperature. There were hexane extracts: **1.** 0.0439 g (2.19%), **2.** 0.0994 g (4.97%).

There were the essential oils prepared by hydrodistillation method in the amount: **1.** from 101 g of the ground *Inula grandis* roots was obtained 0.6258 g (0.61%); **2.** from 108 g of the ground *Inula helenium* roots was obtained 0.4258 g (0.39%); **3** and **4.** each per 100 g of the ground dry leaves of *Inula grandis* and *Inula helenium* were obtained 0.0052 g (0.005%) and 0.0746 g (0.07%), respectively.

The extracts and essential oils of the roots and leaves of *Inula helenium* and *Inula grandis* were analyzed by GC-MS.

GC-MS analysis. The qualitative and quantitative compositions of the essential oil were determined on an Agilent 5975C inert MSD/7890A GC-MS (Agilent Technologies, USA). Constituents of the mixture were separated on an Agilent HP-INNOWax quartz capillary column (30 m × 250 µm × 0.25 µm). The GC-MS analytical conditions were analogous to the published ones [6]. Constituents were identified based on comparison of mass spectral characteristics with data of electronic libraries and retention indices (RIs) of the compounds determined from the ratio

to retention times of a mixture of *n*-alkanes (C9–C20) and comparisons of their mass spectral fragmentations with those in the literature [7, 8].

In vitro studies of essential oil and hexane extracts of roots and leaves of Inula grandis and I. helenium for antimicrobial and antifungal activity. The examples were screened *in vitro* for antifungal and antibacterial activity using a modified agar-diffusion method [9, 10]. The antimicrobial activity was determined using the following microorganism strains: Gram-positive *Bacillus subtilis* (RKMUz-5), *Staphylococcus aureus* (ATCC 25923) and Gram-negative *Pseudomonas aeruginosa* (ATCC 27879) and *Escherichia coli* (RKMUz-221) and one conditionally pathogenic fungus *Candida albicans* (RKMUz-247).

Results and discussion

Volatile components of Inula grandis. The results of the analysis of the volatile compounds of hexane extract and essential oil of *Inula grandis* roots are presented in table 1 of the electronic supplement.

The main components of the essential oil of *Inula grandis* roots obtained by hydrodistillation were sesquiterpenoids as the alantolactone (35.63%), igalan (15.77%), (+)-leden (7.91%), 4,15-dihydroisoalantolactone (7.01%), 6,12-epoxy-11 β -eudesma-4,6-dien-3-one (5.40%), 11 α ,13-dihydroalantolactone (3.33%).

Major components of hexane extract of *Inula grandis* roots were also sesquiterpenoids as the alantolactone (54.38%), igalan (17.16%), 6,12-epoxy-11 β -eudesma-4,6-dien-3-one (6.85%), (+)-leden (6.79%), 11 α ,13-dihydroalantolactone (2.93%).

In the essential oil, 56 compounds representing 89.75% and belonging to monoterpenoids – 1, to sesquiterpenoids – 43, hydrocarbons – 8, derivatives of benzene – 4 were identified from 69 substances.

In the hexane extract of the roots of *Inula grandis*, 13 compounds (93.05%) of 15 substances have been identified, mainly belonging to sesquiterpenoids.

The results show that essential oil and hexane extract of *Inula grandis* roots are rich in sesquiterpenes and sesquiterpenoids, which are 76.6% for essential oil and 84.55% for hexane extract.

GC-MS analysis of essential oil obtained by hydrodistillation from the leaves of *Inula grandis* was also carried out.

The results of the analysis are presented in table 2 of the electronic supplement.

The results of table 2 of the electronic supplement show that 45 compounds representing 70.7% of 71 compounds have been identified, including 11 monoterpenoids (11.4%), 11 sesquiterpenoids (41.36%), 19 hydrocarbons, 10 benzene derivatives and 1 fatty acid. The major components of the leaves of *Inula grandis* were: alantolactone (20.27%), isoalantolactone (7.81%), aromadendrene (3.63%), eudesma-4-en-3-one (3.11%), dihydroactinidiolide (3.10%), and γ -elemene (2.58%).

An analysis of the literature data shows [11] that the major components of hydrodistilled essential oil for this species growing in Türkiye were borneol (26.4%), β -caryophyllene (15.3%), *p*-cimene (10.2%), bornylacetate (8.9%), α -pinene (4.1%), α -cadinol (3.4%).

Sesquiterpene lactones igalan, alantolactone and isoalantolactone, isolated from the roots of *Inula grandis* by chromatographic methods in native form, have been analyzed by chromatomass spectral method as standard samples.

Thus, 43 compounds were first identified in *Inula grandis* root essential oil, 7 compounds in hexane extract and 39 compounds in essential oil of the leaves. It should be noted that the essential oils of the roots and leaves of *Inula grandis* are more likely to contain sesquiterpenes and sesquiterpenoids.

Volatile components of Inula helenium. The results of the analysis of the extract and essential oils of the roots and leaves of *Inula helenium* were presented in tables 3 and 4 of the electronic supplement.

In the essential oil of the roots *Inula helenium* of 74 compounds identified 56, constituting 42.72%. Hexane extract of 42 compounds identified 33 (97.73%). However, major compounds of essential oil and hexane extract of roots have not been identified (numbers 64 (13.17 and 13.70%, respectively) and 65 (29.05 and 29.53%, respectively) in table 3 of the electronic supplement), and their prospective research will be isolating them in native form by chromatographic methods.

Among the identified compounds in relatively larger quantities in the essential oil of the roots of *Inula helenium*, it should be pointed out viridiflorol (3.39%), 1,5-cyclooctadiene, 3,4-dimethyl- (3.41%), 11 α ,13-dihydroelemestearactinolide (2.85%), 11 α ,13-dihydroalantolactone (2.86%), alantolactone (10.92%), and in hexane extract of

the roots have (*S*^{*})-1-[(1*S*^{*},2*S*^{*})-2-ethenyl-1-methyl-2-(phenylthio)cyclopropyl]ethanol (14.77%), alantolactone (14.66%), phosphonic acid, [1-(1,1-dimethylethyl)-4,4-dimethyl-1,2-pentadienyl]- (5.31%), alloalantolactone (3.21%), 1-methyl-3,3-bis(trifluoromethyl)cyclohexene (3.08%).

The content of monoterpenoids in essential oil is 4 compounds and 0.79%, sesquiterpenoids (41 compounds) – 34.82%, and hexane extract of roots have monoterpenoids (1 compound) contains 0.09%, and sesquiterpenoids (20 compounds) – 27.43%.

According to [12], the chloroform fraction of the methanolic extract of the roots of *Inula helenium* (Romania) contains 2-methoxyethanol, 2-ethyl-1-hexanol, 2-methyl-cyclohexyl ester of 2-propenoic acid, benzaldehyde, β -elemene, 3-methyl-5-propylnonane, methyl benzoate, acetophenone, α -muurolene, 2-phenyl-2-propanol, β -selinene, α -selinene, azulene, 2,2-dimethyl-1,3-propandiol, benzyl alcohol, 2-ethyl- hexanoic acid, 1-dodecanol, phenol, 1-tetradecanol, palmitic acid methyl ester, 2,4-di-tert-butylphenol, 1,2-benzodicarboxylic acid mono(2-ethylhexyl) ester.

The authors [13] examined the essential oil of the roots of *Inula helenium* (Serbia), obtained by hydrodistillation, and found a large amount of alantolactone (55.8%), isoalantolactone (26.3%) and diplophyllin (5.1%).

In the roots of *Inula helenium* subsp. *turcorasemosa* (Turkiye) (hydrodistillation) as major components were found alantolactone (9.16%), 1-deoxyivangustin (8.42%), isoalantolactone (7.03%), β -elemene (1.96%) [14].

In the French population of *Inula helenium*, the authors [15] found alantolactone (56.64%), isoalantolactone (37.31%), α -amorphene (1.88%), β -elemene (1.10%), junipene (0.40%), α -selinene (0.33%), 1,4-pentadiene (0.27%).

Essential oil from the roots of *Inula helenium*, native to Italy, contains mainly β -elemene, valencene, alantolactone and isoalantolactone [16].

Comparative analysis showed that populations from Turkiye, Serbia and France were closer in composition of compounds of the Uzbek population of *Inula helenium* in terms of major components.

Thus, 43 compounds in essential oil and 26 compounds in the roots of hexane extract were first identified in the roots of *Inula helenium*.

GC-MS analysis of essential oil obtained by hydrodistillation from *Inula helenium* leaves collected in the Tashkent region was also carried out. The results are presented in table 4 of the electronic supplement.

The results of table 4 of the electronic supplement show that out of 99 compounds, 70 compounds representing 70.81% were identified. The essential oil of *Inula helenium* leaves includes 3 hemiterpenoids (0.63%), 28 monoterpenoids (33.74%), 8 sesquiterpenoids (4.47%), 5 fatty acids and their derivatives (10.86%), 25 hydrocarbons (18.52%) and 1 dieterpenoid (2.62%).

The major components of the leaves of *Inula helenium* growing in Uzbekistan were large, among the identified compounds were δ -3-carene (7.70%), palmitinic acid (7.36%), benzene, 1,3-bis(1,1-dimethylethyl)-2-methoxy-5-methyl- (6.61%), β -ionone (3.90%), dihydroactinidiolide (2.83%), 3,7-benzofurandiol, 2,3-dihydro-2,2-dimethyl- (2.78%), diterpenoid phytene (2.62%).

Thus, in the leaves of *Inula helenium*, that growing in Uzbekistan, were determined 65 compounds for the first time, mostly monoterpenoids.

Antimicrobial and antifungal activity of *Inula grandis* and *Inula helenium* essential oils and extracts

Previously, work was carried out on the analysis of the antimicrobial and antifungal activity of the essential oil of the roots of *Inula helenium* [13, 16–20], however, tests have not been carried out for non-polar fractions.

In vitro antimicrobial and antifungal activity of the obtained essential oils and extracts at a dose of 2 mg per disc were conducted at the department of Molecular genetics. The results are presented in Table 5 of the electronic supplement.

The essential oil of the roots of *Inula grandis* showed significant activity against gram-positive bacteria *Bacillus subtilis* (11.08±0.12) and *Staphylococcus aureus* (11.04±0.10), as well as weak antifungal activity against *Candida albicans* (6.04±0.10). Hexane extract of the roots of *Inula grandis* also showed significant activity against gram-positive bacteria *Bacillus subtilis* (10.08±0.12).

As can be seen from table 5 of the electronic supplement, the essential oil of the leaves of the *Inula helenium* exhibits noticeable activity against bacteria *Bacillus subtilis* (10.08±0.12) and fungi *Candida albicans* (10.04±0.10). Hexane extract of the roots has shown significant activity against gram negative bacteria *Escherichia coli* (10.12±0.13).

Conclusion

The component composition of essential oils and hexane roots and essential oils of leaves of *Inula grandis* and *Inula helenium* growing in Uzbekistan was studied using the GC-MS method. *Inula grandis* and *Inula helenium* have been confirmed to be sources of biologically active sesquiterpene lactones.

The antibacterial and antifungal activities *in vitro* of essential oils and hexane extracts of roots and essential oils of the leaves of *Inula grandis* and *Inula helenium* were studied. The essential oil of *Inula grandis* roots showed a noticeable effect against the gram-positive bacteria *Bacillus subtilis* (11.08 ± 0.12) and *Staphylococcus aureus* (11.04 ± 0.10), as well as an antifungal effect against *Candida albicans* (6.04 ± 0.10). Hexane extract of *Inula grandis* roots also showed significant activity against the gram-positive bacteria *Bacillus subtilis* (10.08 ± 0.12).

The essential oil of *Inula helenium* leaves shows a visible effect against the bacteria *Bacillus subtilis* (10.08 ± 0.12) and fungi *Candida albicans* (10.04 ± 0.10). Hexane root extract showed significant activity against gram-negative bacteria *Escherichia coli* (10.12 ± 0.13).

Supplementary Information

The electronic supplement to the article (DOI: <http://www.doi.org/10.14258/jcprm.20240313541s>) provides additional experimental material that reveals the main points set out in the article

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Conflict of Interest

The authors of this work declare that they have no conflicts of interest.

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