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STUDY OF THE COMPOSITION OF EXTRACT AND ANTIMYCOTIC PROPERTIES *ANTHEMIS ALTISSIMA* L.

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The qualitative and quantitative composition of chemical components of ethanol extract of the above-ground part of *Anthemis altissima* L. (syn. *Cota altissima* (L.) J.Gay) species (family *Asteraceae*) collected during flowering phase from the flora of Azerbaijan has been studied.

The chemical composition of ethanol extract was analyzed by chromatography-mass-spectrometry methods. 54 components were identified in the extract. As a result of the study, it was found that the *A. altissima* contained benzene aromatic compounds (77.21%). Among them Benzene, (1-butylheptyl) – 7.84%, Benzene, (1-propyloctyl) – 7.71%, Benzene, (1-pentyloctyl) – 7.03%, Benzene, (1-pentylheptyl) – 5.69%, Benzene, (1-butyloctyl) – 5.37%, Benzene, (1-propylnonyl) – 5.02% were the main components.

In addition to the above components in the extract of the aerial part other biological active substances, such as alcohols (6.37% – of them prevail Hop-22(29)-en-3. β.-ol – 1.53%, γ-Sitosterol – 1.32%), esters of acids (5.3% – of them prevail Hexadecanoic acid, ethyl ester – 0.88%; Linoleic acid, ethyl ester – 0.63%) and alkanes (1.64% – of them prevail Eicosane – 0.53%; Pentacosane – 0.40%) were also identified. The share of unidentified components was 8.99%.

The article also presents the results of the study of the antifungal properties of *A. altissima* and its aqueous extract against the culture of pathogenic fungi *Fusarium oxysporium* and *Aspergillus niger*. The aqueous extract of *A. altissima*, in contrast to the plant itself, shows a fungistatic effect on the fungi *Fusarium oxysporum* and *Aspergillus niger*.

Keywords: Anthemis altissima; extract; chromatography-mass-spectrometry; biological active compounds; antifungal; cultures of pathogenic fungi *Fusarium oxysporium* and *Aspergillus niger*.

Introduction

The genus *Anthemis* L. of the tribes *Anthemideae* Cass. of the families *Asteraceae* Bercht. et J. Presl breed is recognized up to 210 species spread throughout Europe, the Mediterranean, West Asia, and Africa. *Anthemis* taxa have been commonly used as folk medicine, insecticides and ornamental plant from ancient times up to now. *Anthemis* is probably, in a phytochemical sense, one of the most repeatedly investigated genera of the *Asteraceae* family. A lot of work has been done on sesquiterpene lactones, polyacetylenes, flavonoids, essential oils [1–15]. Essential oils from flowers *Anthemis* is very useful for pharmaceuticals, and food additives, as well as important sources in food, aromatic and cosmetic industries. The species *Anthemis* has been studied on the chemical composition and diverse biological activities, such as their antioxidant, antifungal, antiplasmodial, antitumor, schistosomicidal, cytotoxic, anthelmintic, phytotoxic, analgesic effects [16–25].

Extracts are used to allay pain and irritation, clean wounds and ulcers and aid prevention, as well as therapy for irradiated skin injuries, treatment of cystitis and dental afflictions. Some species of *Anthemis* are being used as herbal tea to cure anxiety, flatulence stomach disorders and carminative infusions [20].

13 species of *Anthemis* are spread in Azerbaijan [26]. The species are spread mainly in dry clay, stony and torn slopes, in sandy places, clay stones, grasslands, rocks, on stony traps, and sometimes on a

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weed. Some species are known as medicines, decorative, dyes and insecticides plants. Antibacterial, antimicrobial and antifungal properties of these species are also known [6, 21].

As known the plants we use for different purposes (medicines, decorative, food, etc.) are also a good media for many bacteria and fungi. Thus, different mushrooms populate on plants that are favorable to them and cause pathogens to the plant. The intensive development of the disease depends on the interaction of the plant and pathogen with one another (plant resistance), but also on the environment. Diseases caused by fungi weaken the plant by infecting separate plant organs, and ultimately, the plant's total destruction. It should be noted that physiological and structural abnormalities in the herb have a great impact on its productivity. Sometimes the plant has a selective feature, thanks to the biological active compounds contained in the plants, and in particular, prevents any fungus from populating itself, which is antimicrobial.

The literature contains a fairly large number of research papers devoted to the study of the antifungal activity of various plant species (*Funtumia elastica*, *Mallotus oppositifolius*, *Trachystemon orientalis*, *Smilax excelsa*, *Rhododendron ponticum*, *Phytolacca Americana*, *Prunus laurocerasus*, *Artemisia herba alba*, *Cotula cinerea*, *Asphodelus tenuifolius*, *Euphorbia guyoniana* and etc.) and extracts, essential oils, saponins, flavonoids, and other biologically active substances obtained from them [27–30].

Considering the beneficial properties of species of the genus *Anthemis*, we decided to investigate the chemical composition and antifungal properties of *Anthemis altissima* L. (syn. *Cota altissima* (L.) J.Gay), common in the territory of Azerbaijan.

In composition *A. altissima* identified of essential oils, sesquiterpenoids, steroids, aliphatic carbohydrates, aromatic compounds, high fatty acids, aldehydes, ketones, phenolcarbonic acids and others chemical components [6].

Materials and methods

The investigated materials were the above-ground part of species *A. altissima*. They were gathered in flowering phase in the village Avaxil of Shamakhi region, Pirqulu settlement, Pirsaat river, yard areas and grassland, as well as Shamakhi city. Here *A. altissma* forms large spots with an abundance mark of 3 among the forbs.

A. altissima is annual plant with a height of 20–80 sm. The leaves are oblong egg-shaped. The basket are one by one. The flowers are white. Seeds are light-brown and compressed. Flowering time of plant from May to July, seeds – June to August.

A. altissima is found everywhere in Azerbaijan except Nakhchivan. From lowland to middle mountain range, it spreads along the road between fields, plantings, gardens, bushes and grass stones. Weed, forest-meadow is a representative of plant species. It is xeromezophyte plant. Geographical type – xerophytes, class – intermediate sea [31].

The harvested plant material of the above-ground part of *A.altissima* was dried and crushed. The 300 g of plant raw material *A. altissima* was extracted in 3 days 3 times with 95% ethanol (alcohol) 3 times a day.

The chemical composition of ethanol extract of *A. altissima* was analyzed by using chromatography-mass-spectrometry methods.

The component composition of the obtained plant extract *A. altissima* was investigated by gas chromatography-mass spectrometry (GC/MS) on an Agilent Technologies gas chromatograph 6890N Network CG System, 5975 inert Mass Selective Detector mass spectrometer as a Split/Splitless detector, Injection – Split, Inlet pressure 60.608 kPa, Split/ Splitless detector 100 Low Mass – 40, High Mass – 400, Thresold 150. A 30-meter capillary quartz column "HP-5MS 5% Metil Siloxane" with an internal diameter of 0.25 mm and a slick of a stationary phase of 0.25 μ was used. Analyzes were carried out in temperature programming mode from 50 °C to 280 °C at a speed of 15 °C/min. Column temperature: initial temperature 50 °C – 2 minutes is stable; raising the temperature 15 °C/min. up to 200 °C – 6 minutes stable; raising the temperature 15 °C/min. up to 280 °C – 10 minutes stable. Vacuum – HiVac – 3.38×10^{-5}. Extract diluted with methanol : chloroform (1 : 2).Velocity of carrier gas (He) 1 ml/min. Input samples with current division (1 : 50). The analysis time is 45 minutes. To identify the connection using the standard mass spectrometric library NIST (the NIST Mass Spectral Seach Proqramfo the NIST/EPA/NIH/Mass Spectral Library Version 2.0.g.buld May 19 2011) [32].

The antifungal activity of the *A. altissima* and aqueous extracts obtained from them was studied by conventional methods against the culture of microscopic pathogenic fungi *Fusarium oxysporum* and *Aspergillus niger,* stored in the museum of the Institute of Microbiology ANAS [33].

Antifungal activity was measured in two steps as follows: development of fungus in solid media and development of fungus in aqueous extract of different concentrations from *A. altissima*.

Development of fungi in solid media. The material obtained from dried *A. altissima* plants was ground into small pieces of 0.5 to 1 cm in size and wetted with 55–60% water. Therefore, fungi can develop in a solid medium. The pH level was relatively constant in the range of 6.5 to 7. The substrate, widely washed with culture, was placed in Petri dishes and sterilized at 1 atm pressure for 45 minutes. After sterilization, the fungal biomass was also inoculated into Petri dishes and incubated at 25–27 °C. The number of colonies was performed on each of the dishes 3, 5 and 7 days after the inoculation.

The development of fungi in different extracts. For study antifungal effects of extracts from aboveground parts species *A. altissima* was taken various coagulation extracts (5, 10, 15 q) and it has been heated. The extraction pour into glass bottles. Then, extraction was cooled, filtered and poured into glass bottles. It was sterilized at 0.5 atm pressure for 30 minutes. The test was conducted at pH levels of 6, 5 and 7. Fungi strains were inoculated into extract inside a test tube. Then they were incubated at 25–27 °C for seven days. Czapek's media was used for comparing results.

Experimental part

As mentioned above, 300 grams of plant raw material *A. altissima* was extracted with ethanol and total of 26 grams of extractable ingredients were obtained, which means 8.7% of the production. It was identified 54 components from the ethanol extract of the *A. altissima* by using chromato-mass-spectroscopy method. The results are given in the table.

As can be seen from the table, the extract contains aromatic compounds, alcohols, acids, alkanes, lactone, coumarine, triterpenoids and terpenes. Preference is given to benzene-cyclic aromatic compounds – 77.21%. Among them are Benzene, (1-butylheptyl) – 7.84%, Benzene, (1-propyloctyl) – 7.71%, Benzene, (1-pentyloctyl) – 7.03%, Benzene, (1-pentylheptyl) – 5.69%, Benzene, (1-butyloctyl) – 5.37%, Benzene, (1-propylnonyl) – 5.02% the main components.

In addition to the above components in the extract of the aerial part other biological active substances, such as alcohols (6.37% – of them prevail Hop-22(29)-en-3 β-ol – 1.53%, γ-Sitosterol – 1.32%), esters of acids (5.3% – of them prevail Hexadecanoic acid, ethyl ester 0.88%; Linoleic acid, ethyl ester 0.63%) and alkanes (1.64% – of them prevail Eicosane – 0.53%; Pentacosane – 0.40%) have been identified. The share of unidentified components was 8.99%.

As we noted *A. altissima* species antifungal activity was measured in two steps as follows: development of fungus in solid media and development of fungus in aqueous extract of different concentrations from *A. altissima*.

Figure 1 shows the results of the growth of phytopathogen fungi *Fusarium oxysporum* and *Aspergillus niger* on *A. altissima*.

As shown in figure 1 in solid food environment consisting of *A. altissima*, the size of fungal colonies of *Fusarium oxysporium* on 3-rd day were 1.0 sm, 5-th day 2.6, and 7-th day 5.6 sm, the colonies of *Aspergillus niger* fungi, respectively, were 4.2; 6.9; 8.0 sm.

The control options size of fungi colonies was 8.0 sm.

Thus, the results show that aboveground parts *A. altissima* can not be used as an antifungal vehicle.

When analyzing literature sources, we encountered similar studies on the antifungal activity of water extract of *A. altissima*. Aqueous extract *A. altissima* growing in the Kermanshah acts antifungal against *Phytophthora drechsleri* and prevented the growth [28]. Out of 54 identified components of the extract, 20 components make up the aromatic compounds. besides, among the components found in the extract there are no compounds that are contained in large quantities, and therefore it is not possible to connect the antifungal properties with any predominant components. According to our assumptions, the antifungal activity of the extract is probably due to the synergistic active components of the aromatic compounds.

Figure 2 presents the results of the effect of different concentrations of water extract of *A. altissima* on the development of the fungi *Fusarium oxysporum* and *Aspergillus niger*.

Thus, as shown in Figure 2, the aqueous extract of *A. altissima* in different concentrations (5, 10, 15 g) had a fungistatic effect on both fungi (*Fusarium oxysporium* and *Aspergillus niger*), inhibition was observed to one degree or another. In contrast to the control (5.12–5.52 g/l), biomass ranged from 0.09 to 0.58 g/l.

The results obtained from the aqueous extracts of *A. altissima* can be used in the preparation of new antifungal drugs.

The content of etanol extract of the above-ground part of *Anthemis altissima*

No.	Retention time	Name of the compound	Molecular formula	Peak (%)
1	7.169	2-Hydroxy-gamma-butyrolactone	$C_4H_6O_3$	1.44
2	8.216	Thymine	$C_5H_6N_2O_2$	0.49
3	8.492	Butanoic acid, 3-amino-	$C_4H_9NO_2$	0.60
4	9.039	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	0.67
5	9.516	Catechol	$C_6H_6O_2$	0.24
6	9.775	Benzofuran, 2,3-dihydro-	C_8H_8O	0.17
7	11.557	Naphthalene, 2-methyl-	$C_{11}H_{10}$	0.45
8	11.851	2,1,3-Benzothiadiazole	$C_6H_4N_2S$	1.16
9	12.098	Silane, dimethyl(4-acetylphenoxy)isobutoxy-	$C_{14}H_{22}O_3Si$	1.42
10	12.692	Benzene, (1-butylhexyl)-	$C_{16}H_{26}$	1.29
11	12.780	Benzene, (1-propylheptyl)-	$C_{16}H_{26}$	0.97
12	12.951	Benzene, (1-ethyloctyl)-	$C_{16}H_{26}$	1.24
13	13.133	Sorbitol	$C_6H_{14}O_6$	0.82
14	13.263	Benzene, (1-methylnonyl)-	$C_{16}H_{26}$	2.00
15	13.374	Benzene, (1-pentylheptyl)-	$C_{18}H_{30}$	0.25
16	13.521	Benzene, (1-butylheptyl)-	$C_{17}H_{28}$	7.84
17	13.621	Benzene, (1-propyloctyl)-	$C_{17}H_{28}$	7.71
18	13.839	Benzene, (1-ethylnonyl)-	$C_{17}H_{28}$	3.60
19	14.221	Benzene, (1-methyldecyl)-	$C_{17}H_{28}$	3.87
20	14.304	Bicyclo[6.3.0]undeca-1(8),9-diene, 11,11-dimethyl-	$C_{13}H_{20}$	0.24
21	14.439	Benzene, (1-pentylheptyl)-	$C_{18}H_{30}$	5.69
22	14.492	Benzene, (1-butyloctyl)-	$C_{18}H_{30}$	5.37
23	14.639	Benzene, (1-propylnonyl)-	$C_{18}H_{30}$	5.02
24	14.915	Benzene, (1-ethyldecyl)-	$C_{18}H_{30}$	4.23
25	15.421	Benzene, (1-methylundecyl)-	$C_{18}H_{30}$	4.74
26	15.645	Benzene, (1-pentyloctyl)-	$C_{19}H_{32}$	7.03
27	15.739	Benzene, (1-butylnonyl)-	$C_{19}H_{32}$	4.79
28	15.939	Benzene, (1-propyldecyl)-	$C_{19}H_{32}$	3.85
29	16.315	Benzene, (1-ethylundecyl)-	$C_{19}H_{32}$	3.55
30	16.939	Benzene, (1-methyldodecyl)-	$C_{19}H_{32}$	3.45
31	17.692	Scopoletin	$C_{10}H_8O_4$	0.38
32	18.151	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	0.88
33	19.992	Phytol	$C_{20}H_{40}O$	0.72
34	20.786	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	0.63
35	20.874	Ethyl 9,12,15-octadecatrienoate	$C_{20}H_{34}O_2$	0.62
36	22.827	3-Oxatricyclo [4.1.1.0 (2,4)] octane, 2,7,7-trimethyl-	$C_{10}H_{16}O$	0.25
37	22.903	Heptadecane	$C_{17}H_{36}$	0.39
38	23.550	3-Cyclohexene-1-methanol, 6-methyl	$C_8H_{14}O$	1.08
39	24.162	Benzylcarbamate	$C_8H_9NO_2$	0.84
40	24.421	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	$C_{23}H_{32}O_2$	0.35
41	24.556	2,3,4-Trimethylpyrrole	$C_7H_{11}N$	0.85
42	25.709	Pentacosane	$C_{25}H_{52}$	0.40
43	25.815	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	0.59
44	26.079	Phthalic acid, di(2-propylpentyl) ester	$C_{24}H_{38}O_4$	0.51
45	28.103	9,12-Octadecadienoic acid (Z,Z)-2,3-dihydroxypropyl ester	$C_{21}H_{38}O_4$	0.36
46	28.209	Linolenic acid, 2-hydroxy1- (hydroxymethyl) ethyl ester	$C_{21}H_{36}O_4$	0.46
47	28.285	Heneicosane	$C_{21}H_{44}$	0.32
48	29.432	Decanedioic acid, bis(2-ethylhexyl) ester	$C_{26}H_{50}O_4$	0.30
49	30.661	Eicosane	$C_{20}H_{42}$	0.53
50	35.544	Stigmasterol	$C_{29}H_{48}O$	0.57
51	36.726	γ-Sitosterol	$C_{29}H_{50}O$	1.32
52	37.561	β-Amyrin	$C_{30}H_{50}O$	0.62
53	40.743	Taraxasterol	$C_{30}H_{50}O$	0.82
54	41.049	Hop-22(29)-en-3 β-ol	$C_{30}H_{50}O$	1.53

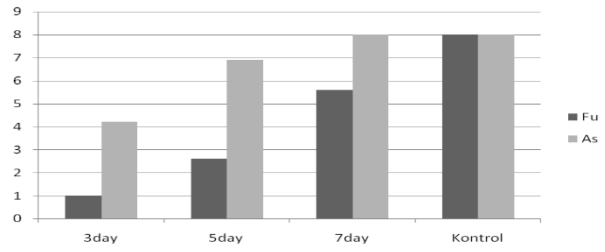

Fig. 1. Development processes for *Fusarium oxysporum* and *Aspergillus niger* colonies in solid media prepared with *A. Altissima* by abscissa – developments of colonies fungus, sm; by horizontals – days

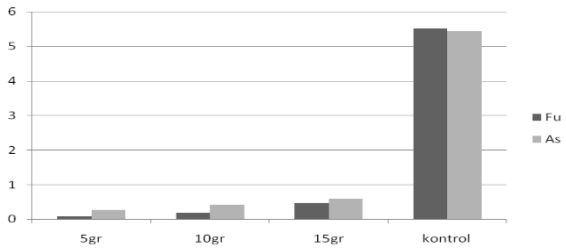

Fig. 2. Effects of extracts from *A. altissima* on *Fusarium oxysporum* and *Aspergillus niger* fungal colonies by abscissa – dry biomass, q/l; by horizontals – aqueous extact

Conclusion

1. The above-ground part of species *A. altissima* were gathered in flowering phase from the village Avaxil of Shamakhi region, Pirqulu settlement, Pirsaat river, yard areas and grassland, as well as Shamakhi city.

2. After ethanol extracting of 300 g of *A. altissima* plant, was obtained 26 g of extractable ingredients, which means 8.7% of output.

3. 54 components from the ethanol extract of the *A. altissima* were identified by the chromato-mass-spectroscopy method.

4. The extract of *A. altissima* contains aromatic compounds, alcohols, esters of acids, alkanes, lactone, coumarine and terpenes. Preference is given to benzene-cyclic aromatic compounds – 77.21%. Among them Benzene, (1-butylheptyl) – 7.84%, Benzene, (1-propyloctyl) – 7.71%, Benzene, (1-pentyloctyl) – 7.03% are the main components. Of the identified components can be also noted alcohols (6.37%), esters of acids (5.3%), and alkanes (1.64%). The share of unidentified components was 8.99%.

5. Unlike the control variant, water extracts of *A. altissima* have a rather high fungistatic effect on the pathogenic fungi *Fusarium oxysporium* and *Aspergillus niger*, which allows them to be used for the preparation of new antifungal drugs.

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