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RESEARCH IN COMPONENTS OF ESSENTIAL OILS FROM FLOWERS AND LEAVES OF THE GENUS ALCHEMILLA L. SPECIES

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The genus Alchemilla L. has about 1000 species in the world flora, of which 35 grow in Ukraine. The most common in Ukraine are A. flabellata Bus., A. phegophila Juz. and A. subrenata Bus. Despite the widespread distribution of species of the genus Alchemilla L. and the publication of a monograph on the herb of the collective species – Herba Alchemillae (Alchemiilla vulgaris L. sensu latiore) in the State Pharmacopoeia of Ukraine, the chemical composition, and pharmacological properties of species of the genus Alchemilla L. are insufficiently studied, therefore, it is advisable to study the chemical composition of the most common species of flora of Ukraine for their introduction into medical and pharmaceutical practice.

The aim *of the work was to study the component composition and quantitative content of essential oils of flowers and leaves of some species of the genus Alchemilla L.*

Materials and methods. *Flowers and leaves of Alchemilla. flabellata Bus., Alchemilla phegophila Juz. and Alchemilla subrenata Bus. harvested in the Ivano-Frankivsk region in 2020–2021.*

The component composition and quantitative content of essential oils were determined by chromato-mass spectrometric method. The compounds were identified by comparing the obtained mass spectra of the chromatographic peak with the mass spectra of the reference compounds and based on comparison with the spectra of the database. Quantitative determination of the content of substances in the raw material was performed in comparison with a standard sample of menthol.

Results. *The essential oil of flowers and leaves of Alchemilla. flabellata Bus., Alchemilla phegophila Juz. and Alchemilla subrenata Bus were obtained. It was found that the highest content of essential oils was characterized by Alchemilla flabellata Bus. flowers (16884.6 mg/kg), and the least essential oil was contained in the leaves of Alchemilla Phegophila Juz. (4895.5 mg/kg). As a result of studying the component composition of essential oils of flowers and leaves of Alchemilla flabellata Bus., Alchemilla Phegophila Juz. and Alchemilla subrenata Bus. 48, 51 and 47 compounds were identified, of which 44, 48 and 43 were identified, respectively. 31 components of essential oil were common in the studied samples of raw materials.*

Conclusions. *Due to the component composition of the essential oil of flowers and leaves of Alchemilla. flabellata Bus., Alchemilla phegophila Juz. and Alchemilla subrenata Bus., as well as considering the known pharmacological activity of its components, it is advisable to conduct further pharmacological studies of raw materials of the genus Alchemilla L. to study their antibacterial, anti-inflammatory and antitumor properties*

Keywords: *Alchemilla, Alchemilla. flabellata Bus., Alchemilla phegophila Juz., Alchemilla subrenata Bus., flowers, leaves, essential oil, chromato-mass spectrometry*

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1. Introduction

Representatives of the genus *Alchemilla L.* of the family *Rosacea* are of great scientific and practical importance for expanding the range of herbal medicines [1, 2]. The genus *Alchemilla L.* has about 1000 species in the world flora [2, 3], of which 35 grow in Ukraine. The genus *Alchemilla L.* is a complex taxonomic complex of plants, that have the ability to polymorphism [2, 4]. Species affiliation of plants is unclear due to the presence of transitional forms [5, 6]. Therefore, botanists use the name *Alchemilla (A.) vulgaris L.* as a collective species consisting of apogamous species [2, 7].

The herb *Alchemilla L.* is included in the State Pharmacopoeia of Ukraine [8]. The raw materials are whole or cut, dried, flowering aboveground parts of *A. vulgaris L. sensu latiare.*

The most common species in Ukraine are *A. flabellata Bus.*, *A. phegophila Juz.* and *A. subrenata Bus.* [9].

Many scientists have identified, isolated, and quantified the biologically active substances of the collective species, grouped under the name *A. vulgaris L.* [9, 10], while other authentic species have not been sufficiently studied. Plants of the genus *Alchemilla L.* contain tannins, flavonoids, hydroxycinnamic acids, lipids, coumarins, bitters, resins, trace elements, vitamins [9].

Despite the widespread distribution of species of the genus *Alchemilla* L. and the publication of a monograph on herb of the collective species – *Herba Alchemi-*

llae (Alchemiilla vulgaris L. sensu latiore) in the State Pharmacopoeia of Ukraine, the chemical composition, and pharmacological properties of species of the genus *Alchemilla L.* are insufficiently studied. At the same time, there is almost no data on the study of the constituent parts of herb species of this genus.

Due to the lack of literature on the presence of essential oils in the herb of species of the genus *Alchemilla L.,* the aim of the work was to study the component composition of essential oils of flowers and leaves of the most common species of the genus *Alchemilla L* of the Ukrainian flora.

2. Research planning (methodology)

Stages of the research of plant raw materials are given in Fig. 1.

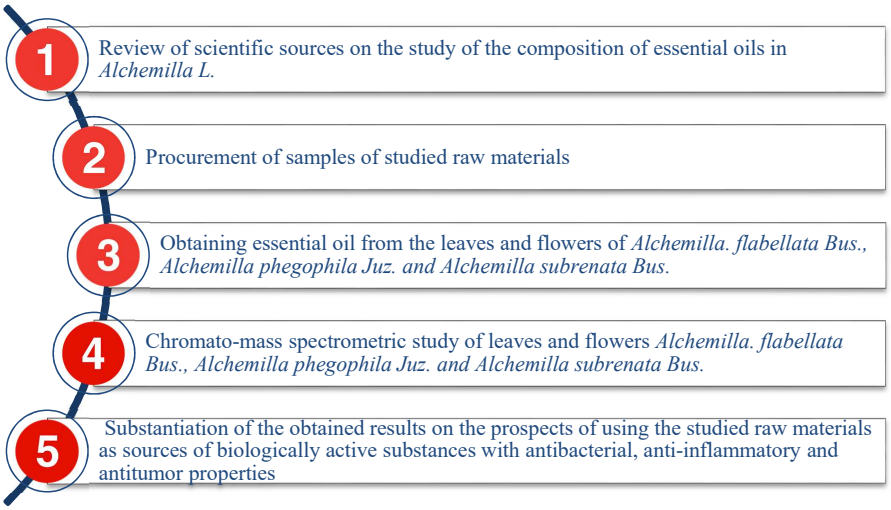

Fig. 1. Planning of the research

3. Materials and methods of the research

Flowers and leaves of *A. flabellata Bus., A. phegophila Juz.* and *A. subrenata Bus.*, harvested during the period of mass flowering in 2020–2021 in the Ivano-Frankivsk region [11].

The component composition and quantitative content of essential oils were determined using the chromato-mass spectrometric method [11, 12]. The retention indices of the components were calculated based on the results of control analyzes of essential oils with the addition of a mixture of normal alkanes (C_{10}–C_{18}) [13, 14].

To study the components of essential oils in medicinal plant raw materials used Vinogradov's method [15, 16], which allows to isolate volatile compounds from a small amount of plant material and fully extract their components for further qualitative and quantitative analysis, which is especially important in the initial assessment of plant projects [17, 18]. For distillation of essential oils from medicinal plant raw materials used 22 ml Agilent vials (part number 5183–4536) with open lids and silicone sealant, through which installed a refrigerator 50 cm long and 5–7 mm in diameter.

The composition of essential oils was investigated on a chromatograph Agilent Technology 6890N (USA) with a mass spectrometric detector 5973N (chromatographic column quartz, capillary HP-5MS; column length – 30 m; inner diameter 0.25 mm; carrier gas – helium; gas velocity carrier – 1 ml/min: sample volume – 0.1–0.5 µl (for solutions of essential oils), the introduction of the sample with a flow separation of 1/50, the temperature of the thermostat 50 °C with programming 4 °C/min to 220 °C, evaporator detector temperature 250 °C) [15, 16].

The obtained spectra were considered both based on general regularities of fragmentation of molecules of organic compounds under the action of electron impact and by comparing the obtained results with the indicators in the mass spectral library of the NIST 02 database (more than 174,000 substances). For each chromatographic peak, the average mass spectrum was calculated from which the background spectrum was subtracted. The compounds were identified by comparing the obtained mass spectra of the chromatographic peak with the mass spectra of the reference compounds and based on comparison with the spectra of the database. Quantitative determination of the content of substances in the raw material was performed in comparison with a standard sample of menthol [10, 11].

4. Research results and their discussion

As a result of the conducted research (Table 1) in flowers and leaves of A. flabellata Bus., *A. phegophila Juz.* and *A. subrenata Bus.* aliphatic and aromatic hydrocarbons, fatty acids, terpenoids and their derivatives were identified.

Table 1

Component composition of essential oils of flowers and leaves of *A. flabellata Bus.*, *A. phegophila Bus.* and *A. subrenata Bus.*

No.	Retention index, min	The component of the essential oil	Content, mg/kg					
			A. flabellata		*A. phegophila*		*A. subrenata*	
			Flowers	Leaves	Flowers	Leaves	Flowers	Leaves
1	2	3	4	5	6	7	8	9
1	7.36–7.41	phenylacetaldehyde	14.6	5.7	40.6	10.2	18.2	2.5
2	7.62	unidentified component	8.5	–	–	–	–	–
3	8.84	cis-linalool oxide	–	–	13.8	–	3.3	–
4	9.28	trans-linalool oxide	–	–	16.9	–	5.5	–
5	9.66	nonanal	–	–	10.7	–	12.3	6.7

Continuation of Table 1

1	2	3	4	5	6	7	8	9
6	9.77–9.78	linalool	–	–	15.9	6.1	5.4	–
7	10.30	β-phenethyl alcohol	–	9.8	–	–	–	–
8	11.35–11.45	2-ethylcaproic acid	29.4	20.5	21.0	40.2	–	22.2
9	12.11	terpinene-4-ol	–	–	6.8	–	–	–
10	12.27	para-cymene-8-ol	–	–	2.4	–	–	–
11	12.50–12.53	α-terpineol	7.5	8.2	106.6	18.2	13.8	–
12	12.95–12.96	decanal	4.8	8.7	9.2	30.5	14.1	7.0
13	13.15–13.22	caprylic acid	33.1	26.3	–	–	–	22.3
14	13.21	myrtenol	–	–	30.4	–	–	–
15	15.27	geraniol	–	–	13.3	–	–	–
16	15.53–15.57	indole	–	–	–	–	4.7	6.0
17	16.22	2-methoxy-4-vinylphenol	60.2	37.9	20.7	13.1	8.3	24.4
18	16.38–16.47	nonanoic acid	84.2	75.6	16.9	34.6	81.7	69.3
19	17.27–17.28	γ-nonalactone	5.4	8.2	–	–	–	–
20	17.61–17.62	eugenol	6.6	13.4	9.2	10.8	4.7	–
21	17.97	2-dodecenal	8.1	-	–	9.6		
22	19.27–19.30	capric acid	71.2	85.4	49.4	37.4	74.7	136.6
23	19.27	ethyl caprynate	–	–	–	80.6	–	–
24	19.50	dodecanal	8.9	–	–	–	–	–
25	19.89–19.90	2,3-dehydro-α-ionone	–	–	8.9	12.1	–	–
26	19.99	β-caryophyllene	–	8.9	–	–	–	–
27	20.72	geranylacetone	9.8	11.4	9.0	18.3	5.3	–
28	21.24	β-farnesene	–	14.1	–	–	–	–
29	21.53	β-ionone epoxide	12.8	9.2	–	15.3	–	4.9
30	21.61	β-ionone	17.7	–	7.4	10.5	–	–
31	22.03	undecanoic acid	17.0	21.3	–	–	43.6	14.4
32	22.53	unidentified component	–	–	26.4	–	–	–
33	22.59	tetradecanal	10.1	–	–	18.8	15.3	–
34	24.19–24.20	megastigmatrienon	–	–	–	–	5.3	11.5
35	24.44	spatulenol	–	28.6	–	–	–	–
36	24.47–24.49	caryophyllene oxide	–	–	35.9	20.8	–	–
37	25.10–25.14	lauric acid	153.0	195.0	96.0	87.4	96.1	106.0
38	25.17	benzophenone	47.4	105.0	53.0	162.4	100.1	109.7
39	25.55	unidentified component	–	–	–	–	15.2	–
40	26.32–26.34	β-eudesmol	28.9	45.5	–	–	–	–
41	27.25–27.27	tridecanoic acid	24.3	32.2	18.3	–	37.9	31.9
42	27.86	hexadecanal	13.6	–	–	19.0	51.6	–
43	28.79	11-tetradecenoic acid	–	36.8	–	–	–	–
44	28.8	13-tetradecenoic acid	–	–	29.9	–	–	–
45	29.37–29.44	myristic acid	380.3	507.5	275.1	382.2	498.0	493.1
46	30.25–30.26	hexahydropharnesyl acetone	34.9	44.2	72.4	62.9	274.0	172.7
47	30.43–30.49	14-pentadecenoic acid	37.1	46.5	29.6	–	–	26.3
48	30.86–30.88	pentadecanoic acid	188.9	181.1	149.0	129.3	322.0	163.1
49	31.48	methyl palmitate	–	–	–	–	55.9	–
50	31.88–31.97	palmitoleic acid	305.8	572.5	440.7	350.6	141.7	129.6
51	32.64–32.69	palmitic acid	2705.7	2891.5	2271.3	2948.2	5197.0	2200.8
52	33.32	unidentified component	–	139.9	–	–	–	–
53	33.48–33.50	heptadecanoic acid	60.4	43.7	45.5	51.0	180.0	47.5
54	33.63–33.65	methyl linolenate	69.4	107.4	–	–	–	53.8
55	34.11	phytol	120.9	63.8	–	156.3	228.2	–
56	34.63–34.71	linoleic acid	2801.8	3505.5	1677.9	2241.7	4060.2	2630.5
57	34.80–34.84	stearic acid	148.6	96.9	76.3	98.1	138.2	66.3
58	35.22	unidentified component	–	–	–	–	159.2	–
59	36.21–36.23	tricosan	338.9	114.1	206.8	78.4	1037.3	87.6
60	37.22	tetracosan	–	–	–	54.6	–	–
61	38.13–38.14	unidentified component	95.6	148.8	90.2	73.4	–	143.6
62	38.23–38.24	pentacosane	196.8	58.4	–	–	771.6	115.3
63	40.00–40.02	unidentified component	102.9	153.2	–	–	–	116.3

Continuation of Table 1

1	2	3	4	5	6	7	8	9
64	40.08–40.09	heptacosan	91.2	63.6	108.0	116.7	559.5	117.6
65	41.10–41.13	squalene	759.6	950.5	813.9	599.3	900.4	689.2
66.	41.81–41.83	nonacosane	–	–	49.2	45.6	204.6	34.3
67.	42.83–42.84	1,27-octacosadiene	–	–	92.8	135.7	221.4	–
68.	43.44	triacontanol	100.5	53.1	192.2	288.3	511.7	86.5
69.	44.98	tritriacontanol	189.5	109.0	396.8	799.7	806.6	141.9
Total:			9405,9	10658,9	7877,4	4895,5	16884,6	8091,4

Note: "–" – the substance is not detected.

5. Discussion of research results

For the first time the essential oil of flowers and leaves of *A. flabellata Bus., A. phegophila Juz.* and *A. subrenata Bus.* It was found that the highest content of essential oils was characterized by flowers of *A. subrenata Bus.* (16884.6 mg/kg), somewhat inferior to the leaves of *A. flabellata Bus.* (10658.9 mg/kg). The least essential oil was contained in the leaves of *A. phegophila Juz.* (4895.5 mg/kg). The obtained results indicate that the content of volatile compounds in the leaf of *A. flabellata Bus.* is slightly higher than in flowers – 10658.9 mg/kg and 9405.9 mg/kg, respectively; essential oil content in the flowers of *A. phegophila Juz.* prevails over the content in the leaves 1.6 times – 7877.4 mg/kg and 4895.5 mg/kg; the content of essential oil of *A. subrenata Bus.* in flowers prevailed over the content in the leaves in 2 times – 16884.6 mg/kg and 8091.4 mg/kg.

As a result of studying the component composition of essential oils of flowers and leaves of *A. flabellata Bus., A. phegophila Juz.* and *A. subrenata Bus.* 48, 51 and 47 compounds were identified, of which 44, 48 and 43 were identified, respectively.

31 components of essential oil were common in the studied samples of raw materials. In particular, these are substances of terpene nature: monocyclic monoterpenoid – α-terpineol, β-ionone epoxide; diterpenoids – geranyl acetone, phytol; sesquiterpene – hexahydropharnesylacetone; acyclic triterpenoid – squalene; aromatic compounds: alcohols – eugenol, 2-methoxy-4-vinylphenol; aldehyde – enyl acetaldehyde; ketone - benzophenone; aliphatic compounds: saturated hydrocarbons – tricosan, pentacosan, heptacosan, triacontane, tritriacontane; aldehydes – decanal, tetradecanal, hexadecanal; fatty acids: lauric, myristic, palmitoleic, palmitic, linoleic, stearic, pelargonium, 2-ethylcaproic, capric, tridecanoic, 14-pentadecenoic, pentadecanoic, margarine.

Specific compounds for the essential oil of *A. flabellata Bus.* leaves were β-phenylethyl alcohol and 11-tetradecenoic acid; for the essential oil of *A. phegophila Juz.* flowers – substances of terpene nature: terpinene-4-ol, para-cimen-8-ol, myrtenol, geraniol; 13-tetradecenoic acid; for the essential oil of the leaves of *A. phegophila Juz.* – tetracosane hydrocarbon; for essential oils of flowers and leaves of *A. phegophila Juz.* – substances of terpene nature: 2,3-dehydro-α-ionone, caryophyllene oxide; for essential oils of flowers and leaves of *A. subrenata Bus.* – base indole and triterpene megastigmatrienone.

Analyzing the data obtained we could conclude that for raw materials *A. flabellata Bus.* specific markers are β-phenylethyl alcohol, γ-nonalactone, β-caryophyllene, β-farnesene, spatulenol, β-eudesmol and 11-tetradecenoic acid; for *A. phegophila Bus.* – terpinene-4-ol, para-cimen-8-ol, myrtenol, geraniol, 2,3-dehydro-α-ionone, caryophyllene oxide, 13-tetradecenoic acid and tetracosane; for *A. subrenata Bus.* – megastigmatrienone and methyl palmitate.

The dominant compounds of essential oils of flowers and leaves of all studied samples were palmitic and linoleic fatty acids, as well as triterpene squalene. Of particular scientific interest are biologically active compounds: α-terpineol, geranyl acetone, phytol, hexahydropharnesyl acetone, eugenol, spatulenol, β-ionone, linalool, geraniol and squalene.

The biological activity of essential oils is not the sum of the activities of its constituent components but is manifested in the combined effect of one component on another. In particular, BAS *squalene* exhibits hypolipidemic, hypocholesterolemic, immunomodulatory, antioxidant, anti-inflammatory and antitumor activity [19, 20]. *Linalool* has antibacterial, wound healing, anti-inflammatory, sedative and diuretic activities [20, 21]. The aromatic compound *eugenol* has bactericidal, antiseptic, anti-inflammatory, expectorant, antifungal, analgesic, antispasmodic, antioxidant and sedative effects [20, 22]. The diterpenoid *geranylacetone* has a cytoprotective effect. Cyclic terpenoid *β-ionone* reduces the growth of tumors and metastases [23]. Terpene *α-terpineol* in the experiment shows a pronounced antifungal activity against mycelial and yeast-like fungi, as well as antioxidant properties in the model oxidation system of methyl linoleate at the level of 80–95 % [24, 25]. Monounsaturated diterpene alcohol *phytol* has anti-inflammatory and antioxidant effects, is part of chlorophyll, vitamin E and K [15, 26]. Sesquiterpene *hexahydropharnesylacetone* exhibits anti-inflammatory activity [27, 28].

In connection with the establishment of the presence of such biologically active substances as squalene, phytol, linalool, α-terpineol, geranyl acetone, hexahydropharnesyl acetone, eugenol, β-ionon and geraniol, it is advisable to conduct further pharmacological studies of herbs of the genus *Alchemilla L.* antibacterial, anti-inflammatory and antitumor properties.

Study limitations. As chromato-mass spectrometric determination of the component composition of essential oils was performed by comparing the obtained mass spectra of the chromatographic peak with the mass spectra of reference compounds and based on comparison with the spectra of the database, identified compounds whose spectra were in the register. Therefore, not

all compounds for which quantitative content was determined by this method could be identified.

Prospects for further research. Detection of such biologically active substances as squalene, phytol, linalool, α-terpineol, geranyl acetone, hexahydropharnesyl acetone, eugenol, β-ionon and geraniol indicates the prospects of research into antibacterial, anti-inflammatory and antitumor properties of the study.

6. Conclusions

For the first time the component composition of essential oils of flowers and leaves of the most common species of the genus *Alchemilla L.* flora of Ukraine was studied by chromato-mass spectrometric method. Among the components of essential oils, aliphatic, aromatic hydrocarbons, fatty acids, terpenoids and their derivatives have been identified. In the essential oils of flowers and leaves of *A. flabellata Bus., A. phegophila Juz.* and *A. subrenata Bus*. 48, 51 and 47 compounds were identified, of which 44, 48 and 43 were identified, respectively.

Comparative analysis of the component composition of biologically active substances in the essential oil of flowers and leaves *A. flabellata Bus., A. phegophila Juz.* and *A. subrenata Bus.* showed the prospects for further pharmacognostic and pharmacological research of these plants as sources of medicinal raw materials.

Conflict of interests

The authors declare that they have no conflicts of interest.

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