

Comprehensive study of *Achillea millefolium* L. roots using IR spectroscopy, GC–MS, and biological tests

Aizhan Yelshibayeva¹, Zharkyn Zhumagaliyeva², Aigul Kaldybayeva³, Gulim Mukusheva⁴, Bernadeth Ticar⁵, Roza Jalmakhanbetova^{1*}

¹L.N. Gumilyov Eurasian National University, Astana, Kazakhstan; individ.931@gmail.com, rjalmakhanbetova@gmail.com

²Pedagogical Institute, Astana International University, Astana, Kazakhstan; zharkyn.73@mail.ru

³Asfendiyarov Kazakh National Medical University, Almaty, Kazakhstan; aigul_240873@mail.ru

⁴Karaganda National Research University named after Academician E.A. Buketov, Karaganda, Kazakhstan; mukusheva1977@list.ru

⁵Iloilo Science and Technology University, Iloilo City, Philippines; bernadeth.ticar@isatu.edu.ph

*Correspondence: rjalmakhanbetova@gmail.com

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Abstract: This paper presents a comprehensive study of the underground organs of *Achillea millefolium* L. using gas chromatography–mass spectrometry (GC–MS), infrared (IR) spectroscopy, and biological tests. The GC–MS results show that the chemical composition of the ethanol extract includes aliphatic acids and their esters, phenolic compounds, flavonoids, and carbohydrates. The main components are 1,3-dihydroxyacetone, octanoic acid, ethyl ester of hexadecanoic acid, eupatilin, and jaceosidin. The IR spectra confirm the presence of aliphatic, carbonyl, and phenolic functional groups characteristic of lipophilic and polar metabolites. The extract exhibited moderate antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis*, which is associated with the presence of phenolic and flavonoid compounds, as well as moderate cytotoxicity in the *Artemia salina* test ($LD_{50} \approx 82 \mu\text{g/mL}$). The results indicate that the underground organs of *A. millefolium* accumulate lipophilic and phenolic metabolites that determine the antimicrobial and cytotoxic activities of the plant. These findings expand our understanding of the metabolism of common yarrow and confirm the potential of its roots as a source of natural biologically active substances.

Keywords: *Achillea millefolium*; GC–MS; IR spectroscopy; component composition; antimicrobial activity; cytotoxicity

1. Introduction

The genus *Achillea* L. (yarrow) comprises about 150 species distributed across the Northern Hemisphere, with the greatest diversity found in Europe, Central Asia, and North America (Ehrendorfer & Guo, 2005; Anderberg et al., 2007). Of these, 14 species and one hybrid are found in Kazakhstan (Kulemin, Y., & Kupriyanov, A., 2023). Scientific studies of the *Achillea* genus have shown that the chemical composition

of the plant is rich in sesquiterpene lactones, flavonoids, coumarins, phenolic acids, sterols, and saponins, as well as other components with a lower content (Si et al., 2006).

Among the variety of *Achillea* species, *Achillea millefolium* L occupies a special place. It has been widely used to heal wounds and treat inflammatory diseases (Dorjsembe et al., 2017), as well as an infusion for bleeding, stomach pain, and menstrual cramps (European Medicines Agency [EMA], 2020). It is known that common yarrow is used as a medicinal plant raw material that contains a wide range of biologically active compounds: essential oil, the alkaloid achillein, flavonoids, coumarins, bitter and tannic substances, resins, organic acids, ascorbic acid, phylloquinone, carotene, choline, and mineral components (Komarov, 2018; Shatalina et al., 2002; Gawel-Beben et al., 2020). Among the phenolic acids in common yarrow, 3-, 4-, and 5-caffeoylquinic, chlorogenic, coumaric acids, and cynarin are distinguished, and among flavonoids: quercetin, rutin, luteolin, apigenin (Figure 1), and their glycosides. Their content depends significantly on the plant organ (leaves, flowers, roots) and environmental factors (Gharibi et al., 2015; Benedek & Kopp, 2007). These compounds directly influence the biological activity of the extracts.

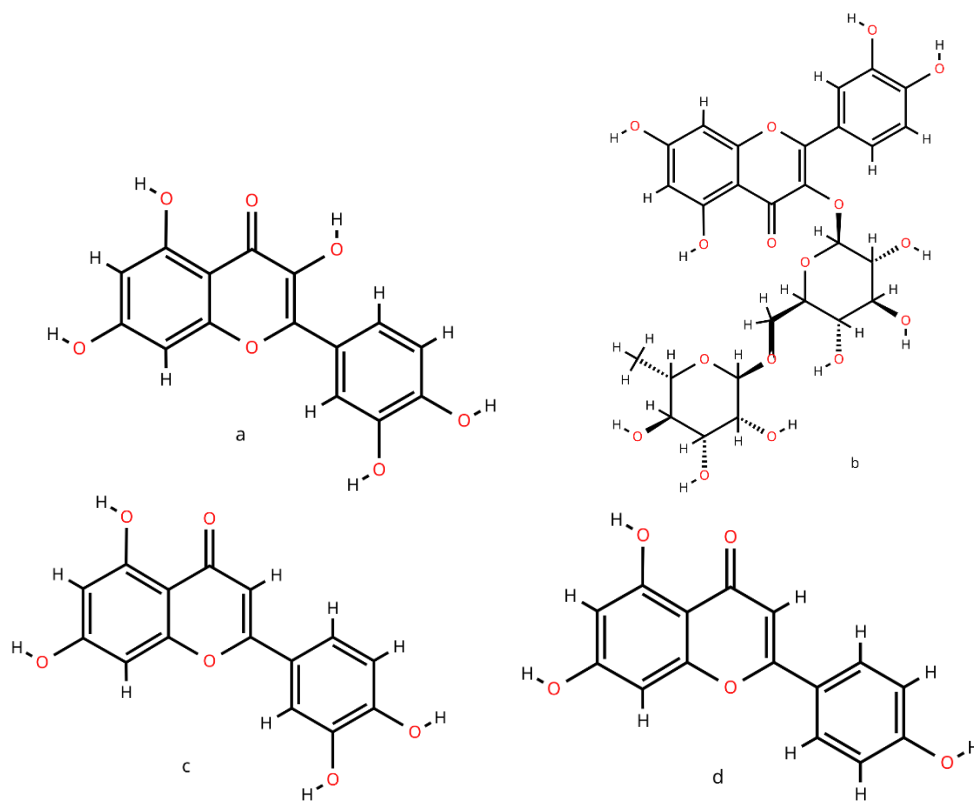


Figure 1. Chemical structures of flavonoids: quercetin (a), rutin (b), luteolin (c), apigenin (d)

Essential oils from the flowers and leaves of common yarrow exhibit antimicrobial and antioxidant activity. It is noteworthy that essential oil obtained from flowers demonstrated more pronounced antimicrobial and antioxidant properties compared to oil from leaves (Ahmadi-Dastgerdi et al., 2017). The ethanol extract of the aerial parts also showed significant antioxidant (Mohamed et al., 2018) and antifungal activity (Ruiz-Navajas et al., 2013). However, it should be noted that the chemical composition of yarrow essential oils varies greatly depending on geographical and environmental conditions. Studies have revealed the existence of different chemotypes, differing in the ratio of the main terpene components, such as camphor, 1,8-cineole, and borneol (Benedek et al., 2007). This highlights the importance of conducting regional studies on the composition and properties of yarrow, including in Kazakhstan. Such geographically determined variability can be traced not only in the composition of essential oils but also in the phenolic content of *A. millefolium*. Recent studies have shown that plants collected in different climatic regions form significantly

different content of phenolic compounds. The differences affect both the qualitative and quantitative composition of phenolic acids and flavonoids, which directly influence the antioxidant activity of extracts. The authors explain these differences by the adaptation of plants to local environmental factors, such as soil type, light intensity, and temperature regime (Radušienė et al., 2023; Asensio et al., 2020). Taken together, these data highlight the need for comprehensive regional studies aimed at investigating the chemical composition and biological activity of yarrow under different growing conditions.

Scientific studies focused on the roots of common yarrow have been less extensively researched. Among them, it is known that lipophilic amides are present in higher concentrations in the roots than in the above-ground parts (Veryser et al., 2016). An early study (Lourenço et al., 1999) showed that the essential oil studied by GC–MS is rich in sesquiterpenes and, unlike the above-ground parts, azulenes and camphor predominate in the roots.

Despite the existence of publications on the chemical composition and biological activity of *A. millefolium* from Kazakhstan, they mainly cover the above-ground organs during the flowering period. In this regard, this study was the first to investigate the chemical composition using IR spectroscopy and GC–MS methods, as well as the cytotoxic and antimicrobial activities of extracts from the roots (underground parts) of yarrow collected in Kazakhstan.

2. Materials and methods

2.1. Research material

The research material consisted of the roots of *A. millefolium* collected in the outskirts of Astana, Kazakhstan (51.070951° N, 71.334599° E) in June 2023. The sampling site was located in a peripheral green area at a distance from major industrial facilities and high-traffic roads. The underground parts were separated from the above-ground parts and dried in the air at room temperature in the shade until they were air-dry.

2.2. Preparation of the extract

The dried and crushed roots of *A. millefolium* were extracted with ethanol under a reflux condenser. A sample of raw material (20 g) was placed in a 500 ml round-bottom flask, 200 ml of ethanol was added at a ratio of raw material: solvent = 1:10, and heated in a water bath for 2 hours. The extraction was carried out three times. The condensate was returned to the flask through a reflux condenser, ensuring continuous extraction. After cooling, the mixture was filtered, and the filtrate was concentrated on a rotary evaporator at 35–40 °C until a thick extract was obtained. The remaining extract was dried to a constant weight and stored in a sealed container at room temperature until analytical studies were performed.

2.3. GC–MS analysis

Gas chromatography–mass spectrometry (GC–MS) was performed using an Agilent 7890A gas chromatograph with an Agilent 5975C mass-selective detector operating in 70 eV electron impact ionisation mode. The percentage content of the component composition was calculated automatically using the GS-MSDDataAnalysis program, based on the peak areas of the total ion chromatogram. Identification was performed based on mass spectra and retention times using the NIST 08 library.

2.4. IR spectroscopy

The IR spectra of the alcohol extract of common yarrow were recorded on a Bruker Alpha II FTIR spectrometer with an ATR attachment (Platinum Diamond 1 Refl). The measurement range was between 4000 and 400 cm⁻¹.

2.5. Determination of antimicrobial activity

The antimicrobial activity of common yarrow extract was evaluated using standard reference test microorganisms: facultative anaerobic Gram-positive cocci *Staphylococcus aureus* ATCC 6538,

aerobic Gram-positive spore-forming bacilli *Bacillus subtilis* ATCC 6633, Gram-negative facultative anaerobic bacilli *Escherichia coli* ATCC 25922, aerobic Gram-negative rods *Pseudomonas aeruginosa* ATCC 27853, as well as the yeast fungus *Candida albicans* ATCC 10231. The study used the serial dilution method with determination of the minimum inhibitory concentration (MIC) (State Pharmacopoeia of the Republic of Kazakhstan 2015; Mironov A.N. 2012). All test strains were obtained from the American Type Culture Collection. Ceftriaxone (antibacterial control) and nystatin (antimycotic control) were used as reference drugs.

The MIC was determined by serial dilution of ethanol solutions of the test sample in nutrient broth. For the experiment, a suspension of microorganisms with a concentration of 10^6 CFU/ml was prepared from daily cultures grown on skimmed agar at 37 °C (for the yeast fungus *C. albicans*, at 30 °C for 48 hours). The tests were carried out in the concentration range of 1.56–50 µg/ml. 0.1 ml of microbial suspension was added to each test tube with the sample dilution. The control samples contained only microbial suspension and nutrient medium without the addition of the test sample.

Incubation was carried out at 37 °C for 24–48 hours, depending on the type of microorganism. The presence or absence of growth was determined visually by the turbidity of the medium. The minimum concentration of the sample at which no growth was observed (the medium remained clear) was taken as the MIC. To increase the reliability of the results, the values were averaged based on the results of three independent experiments

2.6. Determination of cytotoxicity

The cytotoxic activity of the sample was assessed in a survival test using *Artemia salina* larvae (Sarah et al., 2017). Two-day-old larvae obtained by culturing eggs in artificial seawater for 48 hours at 37 °C were used for the experiments.

The initial sample weighing 2 mg was dissolved in 2 mL of ethanol. Aliquots of 500 µl, 50 µl, and 5 µl (three parallels) were taken from this solution, and after evaporation of the solvent, 5 ml of artificial seawater was added to each. Thus, the final concentrations were 100, 10, and 1 µg/ml, respectively (each in triplicate).

Ten two-day-old *A. salina* larvae were placed in each test tube. Incubation was carried out at room temperature in the light for 24 hours. At the end of the experiment, the number of live and dead larvae was counted. Based on the data on the upper and lower toxic limits, the median toxic dose (LD₅₀) was calculated.

Equivalent volumes of DMSO were used as a negative control, and dactinomycin (actinomycin D), which has pronounced cytotoxic activity, served as a positive control. Statistical processing of the data was performed using the FIN programme. All experiments were carried out in triplicate (n = 3). The results are presented as mean ± standard deviation (SD).

3. Results

Gas chromatographic analysis of the ethanol extract of *A. millefolium* roots revealed 25 volatile and semi-volatile compounds of various chemical natures. Table 1 shows the main components with retention times and relative peak areas.

Table 1. GC–MS results for ethanol extract of *A. millefolium* roots

Peak No	Retention time (RT, min)	Area (%)	Name of compound
1	4.803	5.20	carbonyl sulfide
2	5.177	3.71	dimethyl sulfoxide
3	5.705	22.74	1,3-dihydroxypropan-2-one

4	7.081	4.97	2-fluoro-2-methylpropane
5	9.314	1.02	2-hydroxy-3,4-dimethylcyclopent-2-en-1-one
6	10.051	3.77	chlorocyanide
7	10.733	2.32	2,4-difluorobenzenecarbohydrazide
8	11.811	0.61	benzene-1,3,5-triol (phloroglucinol)
9	12.075	2.14	3-methyl-1,3-thiazolidine
10	12.824	1.04	1-methoxy-2,4-difluorobenzene
11	13.869	3.22	n'-(2-ethoxybenzylidene)-4-methylbenzenesulfonohydrazide
12	15.233	1.15	1-chloro-2-methoxybenzene
13	15.299	2.64	β -d-fructofuranosyl α -d-glucopyranoside (sucrose)
14	15.772	2.95	4-(furan-2-yl) but-3-en-2-one
15	17.411	5.13	3-methyltetrahydrothiophene
16	17.543	3.81	1-o-dodecyl- α -d-ribofuranoside
17	17.609	4.73	octanoic acid
18	17.851	4.03	formohydrazide
19	20.228	0.53	1,1-diphenylethene
20	23.859	1.09	ethyl hexadecanoate
21	24.607	6.04	(e)-2,4-dimethoxy-5-ethoxy-1-ethenyl-3-methylbenzene
22	26.917	4.20	4',5,7-trihydroxy-3',6-dimethoxyflavone (jaceosidin)
23	28.017	7.03	5,7-dihydroxy-3',4',6-trimethoxyflavone (eupatilin)
24	30.856	1.11	1',4'-dimethoxy-[2,2']-binaphthalene-1,4-dione
25	31.318	4.80	5,5'-dihydroxy-3,3'-dimethyl-[2,2']-binaphthalene-1,1',4,4'-tetrone

According to GC–MS analysis, the chemical profile of *A. millefolium* extract is characterized by the predominance of primary metabolites, including carbohydrates, low-molecular oxygen-containing compounds, fatty acids, and sulfur-containing metabolites. The major primary metabolite was 1,3-dihydroxypropan-2-one (22.74 %), while octanoic acid (4.73 %) and 3-methyltetrahydrothiophene (5.13 %) were also detected in significant amounts.

At the same time, several secondary metabolites were identified, mainly represented by methoxylated flavonoids. Among them, jaceosidin (4.2 %) and eupatilin (7.03 %) were the dominant secondary metabolites (Figure 2). The presence of these flavonoids confirms the contribution of secondary phenolic metabolism to the biological activity of the extract.

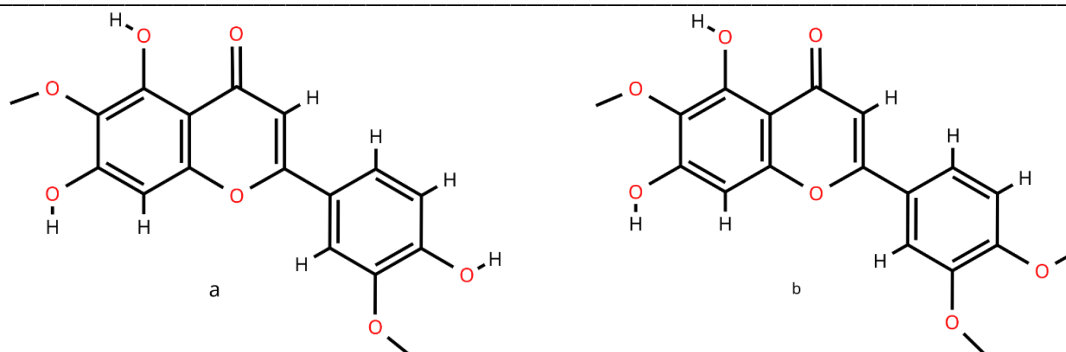


Figure 2. Chemical structures of the secondary metabolites jaceosidin (a) and eupatilin (b)

IR spectroscopic analysis of the *A. millefolium* roots extract revealed a number of characteristic absorption bands reflecting the presence of major classes of organic compounds. An intense broad band at 3286 cm^{-1} corresponds to the stretching vibrations of hydroxyl groups ($-\text{OH}$), indicating a high content of phenolic compounds and alcohols. Peaks in the range $2918\text{--}2850\text{ cm}^{-1}$ are attributed to C–H stretching vibrations of methyl and methylene groups characteristic of aliphatic structures, including fatty acids and their esters. A pronounced absorption band at 1712 cm^{-1} corresponds to C=O stretching vibrations, indicating the presence of esters, aldehydes, ketones, and phenolic acids. Bands at 1600 cm^{-1} and in the $1516\text{--}1378\text{ cm}^{-1}$ region are associated with aromatic ring vibrations, confirming the presence of flavonoids and other aromatic phenolic compounds. Intense bands at 1251 and 1041 cm^{-1} correspond to C–O stretching vibrations characteristic of alcohols, phenols, and simple esters. Absorptions in the low-frequency region ($877\text{--}816$ and 419 cm^{-1}) are attributed to deformation vibrations of aromatic and aliphatic fragments, indicating structural diversity of the detected metabolites (Figure 3).

Consequently, spectral analysis of the underground part of *A. millefolium* indicates a high content of phenolic compounds, fatty acids and their esters, as well as flavonoid derivatives. These results are consistent with the GC–MS data, which show the predominance of aliphatic acids and flavonoid compounds in the root extract.

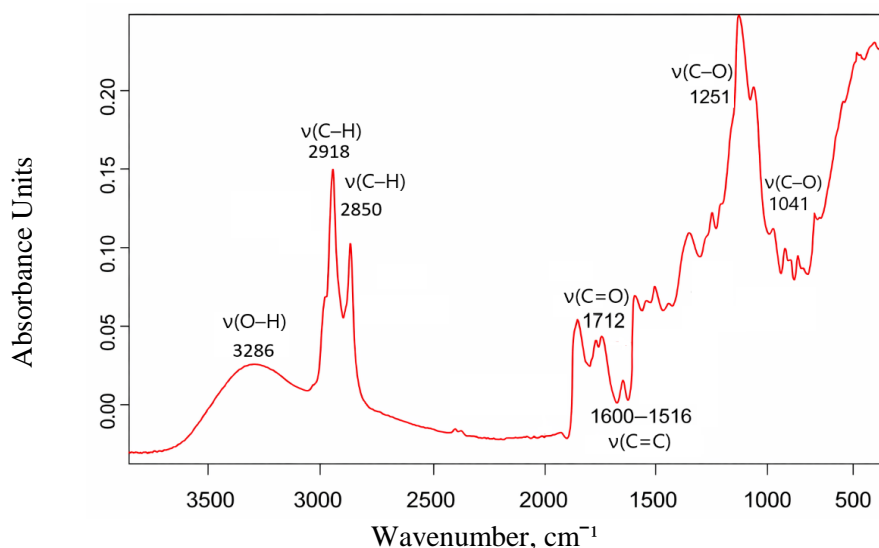


Figure 3. IR spectrum of the extract from the underground part of *A. millefolium*

The antimicrobial activity of the *A. millefolium* plant sample demonstrates selective activity mainly against Gram-positive microorganisms, especially *B. subtilis* ($\text{MIC} = 12.5 \pm 0.1\ \mu\text{g/ml}$), as well as against *S. aureus* ($\text{MIC} = 25 \pm 0.02\ \mu\text{g/ml}$). The activity against *E. coli* is weaker ($\text{MIC} = 50$

$\pm 0.25 \mu\text{g/ml}$), while there is no activity against *P. aeruginosa* and *C. albicans* (Table 2). In this analysis, the antibacterial drug ceftriaxone and the antifungal drug nystatin were used as comparators.

Table 2. Antimicrobial activity of *A. millefolium* extract expressed as minimum inhibitory concentration (MIC, mg/mL) against reference bacterial and fungal strains

Sample	<i>Staphylococcus aureus</i> ATCC 6538	<i>Bacillus subtilis</i> ATCC 6633	<i>Escherichia coli</i> ATCC 25922	<i>Pseudomonas aeruginosa</i> ATCC 27853	<i>Candida albicans</i> ATCC 10231
extract	25 \pm 0.02	12.5 \pm 0.1	50 \pm 0.25	-	-
ceftriaxone	6.3 \pm 0.05	12.5 \pm 0.2	6.3 \pm 0.04	12.5 \pm 0.16	-
nystatin	-	-	-	-	12.5 \pm 0.02

Results of testing the cytotoxic activity of the sample against *A. salina* larvae under *in vitro* cultivation conditions. *A. millefolium* extract demonstrated moderate cytotoxic activity in the *A. salina* larvae test. The LD₅₀ value was 82.4 \pm 3.3 $\mu\text{g/ml}$, indicating a pronounced but less potent effect compared to the reference drug dactinomycin (LD₅₀ = 45.8 \pm 1.2 $\mu\text{g/ml}$).

4. Discussion

The obtained GC–MS data are consistent with literature reports indicating that the phytochemical composition of *A. millefolium* is strongly organ-dependent, with different morphological parts accumulating distinct classes of secondary metabolites (Ali et al., 2017; Radušienė et al., 2023). According to Platonov et al. (2020), GC–MS analysis of the n-hexane extract of the aerial part allowed the identification of 110 compounds, with a predominance of hydrocarbons, terpenoids, sterols and other lipophilic constituents, whereas phenolic compounds were present only in trace amounts. In contrast, the present study demonstrates that the root extract is characterized by a higher contribution of fatty acids and their esters, together with phenolic compounds and methoxylated flavonoids. The detection of eupatilin and jaceosidin in underground organs is particularly significant, as these flavonoids exhibit pronounced anticancer and anti-inflammatory activities (Nageen et al., 2021; Mohamed et al., 2018; Lee et al., 2024), and their presence suggests common biosynthetic pathways of phenolic metabolites throughout the plant, confirming the involvement of roots in antioxidant protection. In addition, fatty acids and their esters, including hexadecanoic acid ethyl ester, contribute to membrane stabilization and energy storage, which is typical for underground organs.

A noteworthy feature of the chemical profile is the predominance of 1,3-dihydroxypropan-2-one (22.74 %), together with readily soluble carbohydrates (sucrose and monosaccharides), indicating adaptive metabolic strategies related to energy supply and osmotic regulation under soil-related stress conditions (Ciriminna et al., 2018). Dihydroxyacetone is industrially produced mainly via glycerol fermentation (Zhou et al., 2016); therefore, the relatively high proportion detected in the root extract suggests that *A. millefolium* underground organs may represent a potential alternative natural source of this compound. However, further quantitative validation and isolation studies are required to confirm its actual content and evaluate its practical feasibility.

To verify the GC–MS identification of the major classes of compounds, the IR spectral data were correlated with the detected metabolites in Table 3.

Table 3. Correlation of IR spectral features with GC–MS-identified compounds in the root extract of *A. millefolium*

FT-IR absorption band (cm ⁻¹)	Vibrational assignment	Functional groups	Compound classes identified by GC–MS	Representative compounds (GC–MS)	Interpretative relevance
3286	v(O–H) stretching	Phenolic and alcoholic hydroxyl groups	Phenolic compounds, flavonoids, polyphenols	Phloroglucinol; jaceosidin; eupatilin	Confirms the presence of phenolic and flavonoid structures
2918, 2850	v(C–H) stretching	Aliphatic – CH ₂ and – CH ₃ groups	Fatty acids, esters, lipophilic glycosides	Octanoic acid; ethyl hexadecanoate; ribofuranoside derivatives	Indicates dominance of lipophilic metabolites
1712	v(C=O) stretching	Carbonyl groups of acids and esters	Fatty acids, esters, ketones, quinones	Octanoic acid; ethyl hexadecanoate; binaphthalene-diones	Supports GC–MS identification of carbonyl-containing compounds
1600–1516	v(C=C) stretching	Aromatic ring vibrations	Flavonoids and aromatic phenolic compounds	Jaceosidin; eupatilin	Confirms aromatic and polyphenolic structures
1251	v(C–O) stretching	Phenolic and ether C–O bonds	Methoxylated flavones, phenols, esters	Eupatilin; jaceosidin; dimethoxy aromatic derivatives	Indicates methoxy-substituted phenolic compounds
1041	v(C–O) stretching	Alcohols and glycosidic bonds	Carbohydrates and glycoside derivatives	Sucrose; ribofuranoside derivatives	Confirms presence of carbohydrate-related components

The consistency between IR absorption bands and GC–MS-identified compounds confirms the predominance of lipophilic and phenolic metabolites in the roots of *A. millefolium*.

The ethanol extract of *A. millefolium* roots showed moderate antimicrobial activity against the Gram-positive bacteria *S. aureus* and *B. subtilis*, while its effect on *E. coli*, *P. aeruginosa*, and *C. albicans* was insignificant. A similar activity ratio was previously observed by Candan et al. (2003) for methanol and ethanol extracts of the aerial parts of *A. millefolium*. The predominant sensitivity of Gram-positive strains is explained by the structure of their cell wall and greater accessibility of targets for phenolic and flavonoid compounds. According to the literature (Lee et al., 2023), the flavones eupalatin and jaceozidin, as well as phenolic acids characteristic of the genus *Achillea*, have a membrane-tropic effect, causing destabilisation of the lipid layer of *S. aureus* cell membranes. This is consistent with the results of GC–MS analysis of the roots, where these compounds were identified.

The absence of antimicrobial activity against *E. coli* and *P. aeruginosa* correlates with the high resistance of Gram-negative bacteria to lipophilic metabolites (Özkan et al., 2016). The results

obtained confirm that the biological activity of the root extract of *A. millefolium* is mainly due to the presence of flavonoids and phenolic compounds that act on Gram-positive microorganisms.

In a toxicity test with *A. salina* crustaceans, *A. millefolium* root extract showed moderate toxicity: the LD₅₀ value was approximately 82 µg/ml. This indicates significant biological activity, but not pronounced toxicity. Similar results have been reported by other authors: methanol and ethanol extracts of *A. millefolium* showed toxicity in the range of 50–100 µg/ml, which is associated with the presence of phenolic and flavonoid compounds (García-Risco et al., 2017).

Phenolic compounds and flavones can disrupt cell membranes, cause oxidative stress, and thus exhibit moderate cytotoxicity. According to GC–MS data, eupalatin and jaceosidin, flavones known as active components of the *Artemisia* and *Achillea* genera, were found in the composition of common yarrow extract (Lee et al., 2023). These compounds have been shown in cell models to induce apoptosis and inhibit tumour cell growth (Nageen et al., 2021). Therefore, it can be assumed that they contribute to the observed toxicity in the *A. salina* test.

5. Conclusion

This study is the first to examine the underground parts of *A. millefolium* collected in Astana (Kazakhstan). Gas chromatography and IR spectroscopy analysis of the ethanol extract of the roots showed that its composition consists of aliphatic acids, their esters, phenolic compounds, and flavonoids. The main components are 1,3-dihydroxyacetone, octanoic acid, ethyl ester of hexadecanoic acid, eupatilin, and jaceosidin. The identified phenolic and flavonoid metabolites provide antimicrobial activity, mainly against Gram-positive bacteria. The extract showed moderate cytotoxicity (LD₅₀ ≈ 82 µg/mL), indicating the presence of biologically active but non-toxic compounds. The obtained data indicate that the underground organs of *A. millefolium* are a source of lipophilic and phenolic metabolites that determine the antimicrobial and cytotoxic activities of the extract and are of interest for further pharmacological studies.

6. Supplementary Materials: No supplementary material.

7. Author Contributions

Conceptualization – Y.A., R.J.; methodology – Y.A., R.J.; software – Y.A.; validation – R.J., G.M.; formal analysis – Y.A.; investigation – Y.A., R.J., G.M., Zh.Zh., A.K.; resources – Y.A., R.J.; data curation – R.J., G.M., B.T; writing - original draft preparation – Y.A.; writing - review and editing – R.J., G.M., B.T., Zh.Zh., A.K.; visualisation – Y.A. R.J.; supervision – R.J. G.M., B.T; project administration – R.J., G.M. All authors have read and agreed to the published version of the manuscript.

8. Author Information

Yelshibayeva, Aizhan – PhD student of the educational program: "D089-Chemistry", L.N. Gumilyov Eurasian National University, Kazhymukan st., 13, Astana, Kazakhstan, 010000; individ.931@gmail.com, <https://orcid.org/0000-0003-0830-2946>

Zhumagaliyeva, Zharkyn – Candidate of Chemical Sciences, Associate Professor, Astana International University, Kabanbay Batyr Avenue, 8, Astana, Kazakhstan, 010000; zharkyn.73@mail.ru, <https://orcid.org/0009-0002-0993-008X>

Kaldybayeva, Aigul - Candidate of Chemical Sciences, Professor, Asfendiyarov Kazakh National Medical University, Tole bi st., 94, Almaty, Kazakhstan, 050000; aigul_240873@mail.ru, <https://orcid.org/0000-0002-9328-457X>

Mukusheva, Gulim – Candidate of Chemical Sciences, Associate Professor, E.A. Buketov Karaganda National Research University, Mukanov st., 41, Karaganda, Kazakhstan, 100024; mukusheva1977@list.ru, <https://orcid.org/0000-0001-6706-4816>

Ticar, Bernadeth – PhD, Professor, Iloilo Science and Technology University, Burgos St, La Paz, Iloilo City, Philippines, 5000; bernadeth.ticar@isatu.edu.ph, <https://orcid.org/0000-0001-7151-9107>

Jalmakhanbetova, Roza – Doctor of Chemical Sciences, Associate Professor, L.N. Gumilyov Eurasian National University, Kazhymukan st., 13, Astana, Kazakhstan, 010000; rjalmakhanbetova@gmail.com, <https://orcid.org/0000-0001-9937-275X>

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12. References

1. Ahmadi-Dastgerdi, A., Ezzatpanah, H., Asgari, S., Dohani, S., & Rahimi, E. (2017). Phytochemical, antioxidant and antimicrobial activity of essential oil from flowers and leaves of common yarrow (*Achillea millefolium* subsp. *millefolium*). *Journal of Essential Oil Bearing Plants*, 20(2), 395–409. <https://doi.org/10.1080/0972060X.2017.1280419>
2. Ali, S. I., Gopalakrishnan, B., & Venkatesalu, V. (2017). Pharmacognosy, phytochemistry and pharmacological properties of *Achillea millefolium* L.: A review. *Phytotherapy Research*, 31(8), 1140–1161. <https://doi.org/10.1002/ptr.5840>
3. Anderberg, A. A., Baldwi, B. G., Bayer, R. G., Breitwieser, J., Jeffrey, C., Dillon, M. O., Eldenäs, P., Funk V., Gar-cia-Jacas, N., Hind, D. J. N., Karis, P. O., Lack, H. W., Nesom, G., Nordenstam, B., Oberprieler, Ch., Panero, J. L., Puttock, C., Robinson, H., Stuessy, T. F., Susanna, A., Urtubey, E., Vogt, R., Ward, J., & Watson, L. E. (2007). Flowering Plants: Eudicots - Asterales. In *Compositae. The families and genera of vascular plants. Vol. VIII. Flowering plants. Eudicots. Asterales* (Vol. 8, pp. 61–558). Springer. https://doi.org/10.1007/978-3-540-31051-8_7
4. Asensio, E., Vitales, D., Pérez, I., Peralba, L., Viruel, J., Montaner, C., Vallès, J., Garnatje, T., & Sales, E. (2020). Phenolic compounds content and genetic diversity at population level across the natural distribution range of bearberry (*Arctostaphylos uva-ursi*, Ericaceae) in the Iberian Peninsula. *Plants*, 9(9), 1250. <https://doi.org/10.3390/plants9091250>
5. Benedek, B., & Kopp, B. (2007). *Achillea millefolium* L. s.l. revisited: Recent findings confirm the traditional use. *Wiener Medizinische Wochenschrift*, 157(13–14), 312–314. <https://doi.org/10.1007/s10354-007-0431-9>
6. Candan, F., Unlu, M., Tepe, B., Daferera, D., Polissiou, M., Sökmen, A., & Akpulat, H. (2003). Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. *millefolium* Afan. (Asteraceae). *Journal of Ethnopharmacology*, 87(2–3), 215–220. [https://doi.org/10.1016/s0378-8741\(03\)00149-1](https://doi.org/10.1016/s0378-8741(03)00149-1)
7. Ciriminna, R., Fidalgo, A., Ilharco, L. M., & Pagliaro, M. (2018). Dihydroxyacetone: an updated insight into an important bioproduct. *ChemistryOpen*, 7(3), 233–236. <https://doi.org/10.1002/open.201700201>
8. Dorjsembe, B., Lee, H. J., Kim, M., Dulamjav, B., Jigjid, T., & Nho, C. W. (2017). *Achillea asiatica* extract and its active compounds induce cutaneous wound healing. *Journal of Ethnopharmacology*, 206, 306–314. <https://doi.org/10.1016/j.jep.2017.06.006>
9. Ehrendorfer, F., & Guo, Y. P. (2005). Changes in the circumscription of the genus *Achillea* (Compositae-Anthemideae) and its subdivision. *Willdenowia*, 35(1), 49–54. <https://doi.org/10.3372/wi.35.35102>
10. European Medicines Agency. (2020). *European Union herbal monograph on *Achillea millefolium* L., herba (Revision 1) (EMA/HMPC/376415/2019)*. <https://www.ema.europa.eu/en/medicines/herbal/millefolii-herba#documents>

11. García-Risco, M. R., Mouhid, L., Salas-Pérez, L., López-Padilla, A., Santoyo, S., Jaime, L., Ramírez de Molina, A., Reglero, G., & Fornari, T. (2017). Biological activities of Asteraceae (*Achillea millefolium* and *Calendula officinalis*) and Lamiaceae (*Melissa officinalis* and *Origanum majorana*) Plant Extracts. *Plant foods for human nutrition (Dordrecht, Netherlands)*, 72(1), 96–102. <https://doi.org/10.1007/s11130-016-0596-8>
12. Gawel-Beben, K., Strzemska-Gomulka, M., Chop, M., Sakipova, Z., Glowniak, K., & Kukula-Koch, W. (2020). *Achillea millefolium* L. and *Achillea biebersteinii* Afan. hydro-glycolic extracts - bioactive ingredients for cosmetic applications. *Molecules*, 25(15), 3368. <https://doi.org/10.3390/molecules25153368>
13. Gharibi, S., Tabatabaei, B. E. S., Saeidi, G., Goli, S. A. H., & Talebi, M. (2013). Total phenolic content and antioxidant activity of three Iranian endemic *Achillea* species. *Industrial Crops and Products*, 50, 154–158. <https://doi.org/10.1016/j.indcrop.2013.07.038>
14. Komarov, B. A. (2018). The elemental composition of yarrow (Elementnyi sostav tsysyachelistnika in Russian). *Drug development & registration*, 3, 158–161.
15. Konarska, A., Weryszko-Chmielewska, E., Materska, M., Sulborska-Różycka, A., Dmitruk, M., & Chilczuk, B. (2025). Phenolic compounds in flowers and herb of *Achillea millefolium* L.: histochemical and phytochemical studies. *Molecules*, 30(9), 2084. <https://doi.org/10.3390/molecules30092084>
16. Kulemin, Y., & Kupriyanov, A. (2023). Botanical-geographical analysis of yarrows (*Achillea* L. - *Asteraceae*) of Siberia and Kazakhstan (Botaniko-geograficheskiy analiz tsysyachelistnika (*Achillea* L. - *Asteraceae*) Sibiri i Kazakhstana in Russian). *Natural and Technical Sciences*, 12(187). <https://doi.org/10.25633/etn.2023.12.09>
17. Lee, B. E., Park, S. J., Kim, G. H., Joo, D. C., & Lee, M. W. (2024). Anti-inflammatory effects of eupatilin on *Helicobacter pylori* CagA-induced gastric inflammation. *PLoS ONE*, 19(11). <https://doi.org/10.1371/journal.pone.0313251>
18. Lee, S., Won, H. J., Ban, S., Park, Y. J., Kim, S. M., Kim, H. S., Choi, J., Kim, H., Lee, J. H., & Jung, J. H. (2023). Integrative analysis of metabolite and transcriptome reveals biosynthetic pathway and candidate genes for eupatilin and jaceosidin biosynthesis in *Artemisia argyi*. *Frontiers in Plant Science*, 14. <https://doi.org/10.3389/fpls.2023.1186023>
19. Lourenço, P., Figueiredo, A., Barroso, J., Pedro, L., Oliveira, M., Deans, S., & Scheffer, J. (1999). Essential oils from hairy root cultures and from plant roots of *Achillea millefolium*. *Phytochemistry*, 51(5), 637–642. [https://doi.org/10.1016/s0031-9422\(99\)00083-7](https://doi.org/10.1016/s0031-9422(99)00083-7)
20. Mironov, A. N., & Bunatyan, N. D. (2012). Guidelines for conducting preclinical studies of drugs - Part 1 (Rukovodstvo po provedeniyu doklinicheskikh issledovaniy lekarstvennykh sredstv. Chast' pervaya). *GRIF-K: Moscow, Russia*, 510.
21. Mohamed, D. A., Hanfy, E. A., & Fouda, K. (2018). Evaluation of antioxidant, anti-inflammatory and anti-arthritic activities of yarrow (*Achillea millefolium*). *Journal of Biological Sciences*, 18(7), 317–328. <https://doi.org/10.3923/jbs.2018.317.328>
22. Mostafa, S., Wang, Y., Zeng, W., & Jin, B. (2022). Floral scents and fruit aromas: functions, compositions, biosynthesis, and regulation. *Frontiers in Plant Science*, 13. <https://doi.org/10.3389/fpls.2022.860157>
23. Nageen, B., Rasul, A., Hussain, G., Shah, M. A., Anwar, H., Hussain, S. M., Uddin, M. S., Sarfraz, I., Riaz, A., & Selamoglu, Z. (2021). Jaceosidin: a natural flavone with versatile pharmacological and biological activities. *Current pharmaceutical design*, 27(4), 456–466. <https://doi.org/10.2174/1381612826666200429095101>
24. Platonov V.V., Dunaev V.A., Sukhikh G.T., Volochaeva M.V., Frankevich V.E., Datieva F.S. (2020). Chromato-Mass-Spectrometry of hexane extract of common yarrow (*Achillea millefolium* L., Asteraceae family - Asterace) (*Khromato-mass-spektrometriya geksanovogo ekstrakta tsysyachelistnika obyknovennogo (Achillea millefolium L., semeystvo astro-vye - asterace) (soobshchenie I)*). *Journal of New Medical Technologies*, 27(4), 82-86.

25. Radušienė, J., Karpavičienė, B., Raudone, L., Vilkickyte, G., Çırak, C., Seyis, F., Yayla, F., Marksa, M., Rimkienė, L., & Ivanauskas, L. (2023). Trends in phenolic profiles of *Achillea millefolium* from different geographical gradients. *Plants*, 12(4), 746. <https://doi.org/10.3390/plants12040746>
26. Ruiz-Navajas, Y., Viuda-Martos, M., Sendra, E., Perez-Alvarez, J. A., & Fernández-López, J. (2013). In vitro antioxidant and antifungal properties of essential oils obtained from aromatic herbs endemic to the Southeast of Spain. *Journal of Food Protection*, 76(7), 1218–1225. <https://doi.org/10.4315/0362-028x.jfp-12-554>
27. Sarah, Q. S., Anny, F. C., & Misbahuddin, M. (2017). Brine shrimp lethality assay. *Bangladesh Journal of Pharmacology*, 12(2). <https://doi.org/10.3329/bjp.v12i2.32796>
28. Shatalina, N. V., Pervishina, G. G., Efremov, A. A., Gordienko G.P., Agafonova E.A., & Goncharov D.V. (2002). Content of some biologically active substances in common yarrow herb (*Achillea millefolium*) growing in Krasnoyarsk region (Soderzhanie nekotorykh biologicheskii aktivnykh veshchestv v trave tysyachelistnika obyknovennogo (*Achillea millefolium*), proizvodstvennykh v Krasnoyarskom krae in Russian). *Khimiya Rastitel'nogo Syr'ya*, 3, 13–16.
29. Si, X., Zhang, M., Shi, Q., & Kiyota, H. (2006). Chemical constituents of the plants in the genus *Achillea*. *Chemistry & Biodiversity*, 3(11), 1163–1180. <https://doi.org/10.1002/cbdv.200690119>
30. *State Pharmacopoeia of the Republic of Kazakhstan* (Gosudarstvennaya farmakopeya Respubliki Kazakhstan in Russian). (2015). In publishing House “Zhibek Zholy” (2nd ed., Vol. 1, p. 720).
31. Veryser, L., Taevernier, L., Wynendaele, E., Verheust, Y., Dumoulin, A., & De Spiegeleer, B. (2016). N-alkylamide profiling of *Achillea ptarmica* and *Achillea millefolium* extracts by liquid and gas chromatography–mass spectrometry. *Journal of Pharmaceutical Analysis*, 7(1), 34–47. <https://doi.org/10.1016/j.jpha.2016.09.005>
32. Zhou, X., Zhou, X., Xu, Y., & Yu, S. (2016). Improving the production yield and productivity of 1,3-dihydroxyacetone from glycerol fermentation using *Gluconobacter oxydans* NL71 in a compressed oxygen supply-sealed and stirred tank reactor (COS-SSTR). *Bioprocess and biosystems engineering*, 39(8), 1315–1318. <https://doi.org/10.1007/s00449-016-1595-8>

***Achillea millefolium* L. тамырларын ИҚ–спектроскопиясы, ГХ–МС және биологиялық сынақтар арқылы кешенді зерттеу**

Айжан Елшібаева, Жарқын Жұмағалиева, Айгуль Калдыбаева, Гулим Мукушева, Бернадет Тикар, Роза Джалмаханбетова

Аңдатпа: Берілген жұмыста *Achillea millefolium* L. өсімдігінің жер асты бөліктері газды хромато-масс-спектрометриясы (ГХ–МС), инфрақызыл (ИҚ) спектроскопия және биологиялық сынақтар әдістерімен кешенді түрде зерттелді. ГХ–МС нәтижелері бойынша этанолдық экстракттың химиялық құрамы алифаттық қышқылдар мен олардың эфирлерін, фенолды қосылыстарды, флавоноидтарды және көмірсуларды қамтитыны анықталды. Негізгі компоненттер ретінде 1,3-дигидроксиацетон, октан қышқылы, гексадекан қышқылының этил эфиірі, эупатилин және яцеозидин табылды. ИҚ–спектрлер липофильді және полярлы метаболиттерге тән алифаттық, карбонилді және фенолдық функционалды топтардың бар екенін растады. Экстракт құрамында фенолдық және флавоноидтық қосылыстардың бар болуына байланысты *Staphylococcus aureus* және *Bacillus subtilis* бактерияларына қарсы орташа антимикробтық белсенділік көрсетті. Сонымен қатар, *Artemia salina* асшаяндарымен жүргізілген тест нәтижесі бойынша орташа цитоуыттылық ($LD_{50} \approx 82$ мкг/мл) байқалды. Алынған деректер *A. millefolium* өсімдігінің жер асты бөліктері антимикробтық және

цитоуыттылық қасиеттерін айқындайтын липофильді және фенолдық метаболиттерді жинақтайтынын дәлелдейді. Нәтижелер мыңжапырақтың метаболизмін тереңірек түсінуге мүмкіндік беріп, оның тамырларын табиғи биологиялық белсенді заттардың көзі ретінде қолданудың болашағын көрсетеді.

Түйін сөздер: *Achillea millefolium*; ГХ–МС; ИҚ–спектроскопиясы; компоненттік құрамы; микробқа қарсы белсенділік; цитоуыттылық.

Комплексное исследование корней *Achillea millefolium* L. методами ИК–спектроскопии, ГХ–МС и биологических тестов

Айжан Елшібаева, Жаркын Жумагалиева, Айгуль Калдыбаева, Гулим Мукушева, Бернадет Тикар, Роза Джалмаханбетова

Аннотация: В данной работе представлено комплексное исследование подземных органов *Achillea millefolium* L. с использованием газовой хромато-масс-спектрометрии (ГХ–МС), инфракрасной (ИК) спектроскопии и биологических тестов. Результаты ГХ–МС показывают, что химический состав этанольного экстракта включает алифатические кислоты и их эфиры, фенольные соединения, флавоноиды и углеводы. Основными компонентами являются 1,3-дигидроксиацетон, октановая кислота, этиловый эфир гексадекановой кислоты, эупатилин и джацеозидин. ИК-спектры подтверждают наличие алифатических, карбонильных и фенольных функциональных групп, характерных для липофильных и полярных метаболитов. Экстракт проявил умеренную антимикробную активность в отношении золотистого стафилококка (*Staphylococcus aureus*) и сенной палочки (*Bacillus subtilis*), что связано с наличием фенольных и флавоноидных соединений, а также умеренную цитотоксичность в тесте с *Artemia salina* ($LD_{50} \approx 82$ мкг/мл). Результаты показывают, что подземные органы *A. millefolium* накапливают липофильные и фенольные метаболиты, определяющие антимикробную и цитотоксическую активность растения. Полученные данные расширяют наши представления о метаболизме тысячелистника обыкновенного и подтверждают потенциал его корней как источника природных биологически активных веществ.

Ключевые слова: *Achillea millefolium*; ГХ–МС; ИК–спектроскопия; компонентный состав; антимикробная активность; цитотоксичность.