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QUANTITATIVE AND QUALITATIVE ANALYSIS OF THE PHYTOCHEMICAL COMPOSITION OF *ARTEMISIA RUTIFOLIA*

Artemisia rutifolia Steph. ex Spreng. has a long history of folk medicinal usage in Kazakhstan, although rigorous study into its chemical makeup is still restricted. This study used the aerial portions of Artemisia rutifolia in Almaty Region, Kazakhstan, to determine the amounts of basic physicochemical indicators, mineral elements, and important secondary metabolites using the Kazakh National Pharmacopoeia's procedures. Chemical components in the petroleum ether and dichloromethane phases were separated and identified using gas chromatography-mass spectrometry (GC-MS), followed by compound comparison and qualitative identification using the NIST14 and Wiley standard mass spectrometry databases. The analysis showed that the plant sample extracted with 50% ethanol contained 7.42% moisture, 4.58% total ash, and 35.76%. Coumarin showed the highest concentration (2.53%), followed by polysaccharides (1.30%), and saponins (1.16%). The mineral elements were primarily K (65.41 mg/100 g) and Ca (39.41 mg/100 g), with heavy metals Pb, Cd, and Ni being below WHO limits. The petroleum ether fraction contained twenty-eight compounds, the majority of which were methoxybenzene derivatives (43.80%), while the dichloromethane fraction contained 63 compounds, including methoxybenzene derivatives (25.82%), scopoletin (3.00%), sesquiterpene lactones such as ambrosin (3.36%), and various phenolic acids. In summary, A. rutifolia from this location has a variety of bioactive components having therapeutic properties. The petroleum ether fraction consists primarily of aromatic and steroidal chemicals, whereas the dichloromethane fraction is rich in sesquiterpene lactones and phenolic acids. More research into its chemical composition and active ingredients will follow.

Keywords: Artemisia rutifolia, chemical components, GC-MS, macro and micro elements, sesquiterpene lactones.

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Artemisia rutifolia фитохимиялық құрамын сандық және сапалық талдау

Артемисия рутифолия, Стеф. бұрынғы Спренг. Қазақстанда халықтық дәрілік заттарды қолданудың ұзақ тарихы бар, дегенмен оның химиялық құрамын мұқият зерттеу әлі де шектеулі. Бұл зерттеуде қазақстанның Алматы Облысындағы Artemisia rutifolia әуе бөліктері қазақ Ұлттық Фармакопеясының процедураларын пайдалана отырып, негізгі физика-химиялық көрсеткіштердің, минералды элементтердің және маңызды қайталама метаболиттердің мөлшерін анықтау үшін

үшін пайдаланылды. Мұнай эфирі мен дихлорметан фазаларындағы химиялық компоненттер газ хроматографиясы-масс-спектрометрия (GC-MS) көмегімен бөлініп, анықталды, содан кейін nist14 және Wiley стандартты масс-спектрометрия дерекқорлары арқылы қосылыстарды салыстыру және сапалы сәйкестендіру жүргізілді. Талдау көрсеткендей, 50% этанолмен алынған өсімдік үлгісінде 7,42% ылғал, 4,58% күл және 35,76% күл бар. Кумариннің ең жоғары концентрациясы (2,53%), одан кейін полисахаридтер (1,30%) және сапониндер (1,16%) байқалды. Минералды элементтер негізінен K (65,41 мг/100 г) және Ca (39,41 мг/100 г) болды, ауыр металдар PB, Cd және Ni ДДҰ шегінен төмен болды. Мұнай эфирінің фракциясында жиырма сегіз қосылыс болды, олардың көпшілігі метоксибензол туындылары (43,80%), ал дихлорметан фракциясында 63 қосылыс, соның ішінде метоксибензол туындылары (25,82%), скополетин (3,00%), амброзин (3,36%) сияқты сесквитерпен лактондары және әртүрлі фенол қышқылдары. Қорытындылай келе, A. rutifolia осы жерден емдік қасиеттері бар әртүрлі биоактивті компоненттерге ие. Мұнай эфирінің фракциясы негізінен хош иісті және стероидты химиялық заттардан тұрады, ал дихлорметан фракциясы сесквитерпен лактондары мен фенол қышқылдарына бай. Оның химиялық құрамы мен белсенді ингредиенттері туралы қосымша зерттеулер жүргізілетін болады.

Түйін сөздер: Artemisia rutifolia, химиялық компоненттер, GC-MS, макро және микроэлементтер, сесквитерпен лактондары.

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Количественный и качественный анализ фитохимического состава Artemisia rutifolia

Artemisia rutifolia Steph. ex Spreng. имеет долгую историю народного лекарственного применения в Казахстане, хотя тщательное изучение ее химического состава все еще ограничено. В этом исследовании использовались надземные части Artemisia rutifolia в Алматинской области, Казахстан, для определения количеств основных физико-химических показателей, минеральных элементов и важных вторичных метаболитов с использованием процедур Национальной фармакопеи Казахстана. Химические компоненты в фазах петролейного эфира и дихлорметана были разделены и идентифицированы с помощью газовой хроматографии-масс-спектрометрии (GC-MS) с последующим сравнением соединений и качественной идентификацией с использованием баз данных масс-спектрометрии NIST14 и Wiley standard. Анализ показал, что растительный образец, экстрагированный 50%-ным этанолом, содержал 7,42% влаги, 4,58% общей золы и 35,76% спирта. Наибольшая концентрация была у кумарина (2,53%), за ним следовали полисахариды (1,30%) и сапонины (1,16%). Содержание минеральных элементов в основном составляло K (65,41 мг/100 г) и Ca (39,41 мг/100 г), а содержание тяжелых металлов Pb, Cd и Ni было ниже допустимых значений ВОЗ. Петролейная эфирная фракция содержала двадцать восемь соединений, большинство из которых были производными метоксибензола (43,80%), в то время как дихлорметановая фракция содержала 63 соединения, включая производные метоксибензола (25,82%), скополетин (3,00%), сесквитерпеновые лактоны, такие как амброзин (3,36%), и различные фенольные кислоты. Таким образом, A. rutifolia, произрастающий в этих местах, содержит множество биологически активных компонентов, обладающих терапевтическими свойствами. Петролейная эфирная фракция состоит в основном из ароматических и стероидных соединений, в то время как дихлорметановая фракция богата сесквитерпеновыми лактонами и фенольными кислотами. Более подробно о его химическом составе и активных ингредиентах будет рассказано ниже.

Ключевые слова: Artemisia rutifolia, химические компоненты, GC-MS, макро- и микроэлементы, сесквитерпеновые лактоны.

Introduction

Artemisia L. belongs to the Asteraceae family and is one of its largest genera. According to the literature, about 500 species of *Artemisia* plants have been recorded globally, with the majority of them found in temperate regions of Asia, Africa, Europe, and North America (Nurlybekova et al., 2022). Central Asia has historically been an important source of variation for *Artemisia* plants. For example, Kazakhstan possesses 81 *Artemisia* plant species, 19 of which are indigenous to the region (Nurlybekova et al., 2022). *Artemisia* plants contain many bioactive components, including sesquiterpene lactones, flavonoids, coumarins, and volatile oils. Related pharmacological research have also revealed that these plants contain a variety of therapeutic properties, including antimalarial, anticancer, anti-inflammatory, and antibacterial actions(Adekenov, 2016; Nurlybekova et al., 2022).

Artemisia rutifolia Steph. ex Spreng. is a perennial subshrub from the *Artemisia* genus in the Asteraceae family. It is commonly found in dry and semi-arid regions such as Central Asia, Siberia, Mongolia, and Northwest China (Dylenova et al., 2023). Razzakova (2024) also identified A. rutifolia as a common essential oil plant on rocky slopes in the Kyrtashtau region, emphasizing its adaptability to arid environments. The plant is typically 20 to 80 cm tall. The stems can grow either erect or slant upward. The leaves are bipinnately compounded. The capitula are spherical or hemispherical, grouped in racemes or compound racemes at the branch tips (World Flora Online, 2024). Razzakova (2024) describes A. rutifolia as a shrub with silver-gray leaf and aromatic perfume on rocky slopes in the Kyrtashtau region. *Artemisia rutifolia* is a traditional medicinal plant with a long history in Mongolia, Kazakhstan, Kyrgyzstan, and Pakistan. It is widely used in folk medicine for pain relief, antibacterial and anti-inflammatory properties, and to treat respiratory and digestive system diseases (Dylenova et al., 2023; Hussain et al., 2019; Sharopov et al., 2011, 2015). Modern study has discovered that *A. rutifolia* contains numerous volatile oils, sesquiterpene lactones, flavonoids, and phenolic acids, as well as antibacterial, antioxidant, anti-inflammatory, and cytotoxic properties (Nurlybekova et al., 2022). This suggests it has a lot of potential for development and study, and it deserves more in-depth examination.

Flavonoids, one of the several active components of *Artemisia*, have been shown to help maintain nitric oxide levels in the body, improve vascular endothelial function, and regulate blood pressure, as well as limit oxidative damage and inflammatory reactions (Nurlybekova et al., 2022). Alkaloids have pharmacological effects, including anesthetic, cardioprotective, and anti-inflammatory properties (Yu et al., 2025). Coumarins exhibit a variety of actions, including anti-inflammatory, anti-ulcer, anticancer, antibacterial, and anticoagulant properties. Sesquiterpene lactones (particularly guaiacolane, eucalyptane, and gimaran types) are identified as key *Artemisia* components, with strong anti-inflammatory and cytotoxic properties (Nurlybekova et al., 2022).

However, the majority of current study on *A. rutifolia* focuses on single or a few components in specialized production areas. Because the chemical makeup of this type of plant changes greatly with geographical location, and there is a major chemotype differentiation phenomenon (Rustaiyan & Masoudi, 2011), quality control and standardized development and exploitation of *A. rutifolia* are hampered. Kazakhstan is located in the heart of *A. rutifolia*'s natural distribution area and offers a wealth of wild resources, however systematic chemical composition study on *A. rutifolia* in Kazakhstan is still limited.

In order to provide a reference basis for the resource evaluation, quality standard formulation, and related pharmacodynamic studies of *A. rutifolia*, this study chose *A. rutifolia* from Almaty Region, Kazakhstan as the research object, systematically determined its physicochemical indicators and mineral element content, and analyzed various secondary metabolites. At the same time, the chemical components in the petroleum ether and dichloromethane extracts were identified in conjunction with GC-MS technology.

Methodology

The qualitative and quantitative analysis of bioactive components of *A. rutifolia* in this study was mostly conducted in line with the applicable general principles and monographs of the 2015 edition of the State Pharmacopoeia of the Republic of Kazakhstan (Gosudarstvennaya Farmakopeya Respubliki Kazakhstan, 2015).

Plant Material

The aerial parts of *A. rutifolia* were obtained from the Almaty Region in Kazakhstan. Fresh samples were naturally dried in a cold environment, then cut into little pieces and stored at room temperature. The samples were crushed before being passed through a 40-mesh sieve.

Moisture content

The moisture content was estimated using the General Chapter "Determination of Moisture Content in Plant Materials" of the Kazakh National Pharmacopoeia (SP RK, 2015). One gram of

crushed sample was precisely weighed and placed in a pre-weighed flat weighing bottle. The material was dried at 100-105 ℃ to a constant weight and moisture content was measured.

Determining the Total Ash Content

The total ash content was estimated using the "Determination of Total Ash Content in Plant Materials" section from the General Chapter of the Kazakh National Pharmacopoeia (SP RK, 2015). Weigh 3 g of sample, place it in a pre-weighed crucible, slowly heat until totally carbonized, and ash in a muffle furnace at 550 ℃ to achieve a constant weight.

Determining the Extractive Content

The extractive content was evaluated using the hot extraction method from the "Determination of Extractive Content in Plant Materials" part of the General Chapter of the Kazakh National Pharmacopeia (SP RK, 2015). Weigh 1 g of sample, then extract with 50%, 70%, and 96% ethanol for 2 hours. Filter, evaporate 25 mL of the filtrate to dryness, and dry at 100-105 ℃ for a consistent weight.

Analysis of macro- and micro-elements

3.0 g of dry powder was correctly weighed and placed in a precisely weighed crucible. It was fully ashed at 550 °C with a steady mass. After calcination, the crucible was cooled in a desiccator, and the resulting ash was re-burned at 600 °C until it was uniformly gray. The plant ash was heated and dissolved in 10.0 mL of 40% nitric acid. The resultant solution was then heated, yielding wet salt. It was then dissolved in 15.0 mL of 1N nitric acid and poured into a 25.0 mL volumetric flask. K, Ca, Mg, Na, Fe, Zn, Cu, Mn, Pb, Cd, Ni, and other elements were detected using a Shimadzu 6200 series spectrometer (Amarjargal et al., 2017).

Determining the Secondary Metabolite Content

The material was calculated based on applicable monographs from the Kazakh National Pharmacopoeia (SP RK, 2015). The procedures for determining different components are as follows:

Analysis of Flavonoid Content

The aluminum chloride colorimetric technique was utilized. Weigh 1 g of material accurately, then extract three times with 1% HCl and 90% ethanol under reflux, combining the filtrates to make a final volume of 100 ml. Take 2 mL of the extract and add 1 mL of 1% $AlCl_3$ ethanol solution to make a total volume of 25 mL. After 20 minutes, measure absorbance at 430 nm. Calculate the flavonoid content with quercetin as the reference standard.

Determining Tannin Content

The potassium permanganate titration method was utilized. Weigh 1 g of sample precisely, extract twice with 50 mL of hot water, then combine the extracts to get a total volume of 100 mL. Take 10 mL of the extract, 100 mL of water, and 10 mL of sodium indigosulfonate solution, then titrate with 0.02 M $KMnO_4$ until golden yellow. At the same time, create a blank control and calculate the tannin content.

Analysis of Alkaloid Content

The acid-base titration method was applied. Weigh 10 g of material accurately, then add 100 mL of chloroform and 5 mL of pure ammonia. Shake and extract for 2 hours before filtering. Take 50 mL of the filtrate, evaporate until dry, dissolve the residue in 10 mL of 0.1 M HCl, filter, take 10 mL of the following filtrate, add methyl red indicator, and back-titrate with 0.1 M NaOH to compute the alkaloid content (calculated as aconitine).

Measurement of Polysaccharide Content

A gravimetric approach was used. Weigh 5 g of sample accurately, then add water and reflux three times to extract. Combine the extracts and dilute to 250 mL. Take 75 mL of the extract, add 95% ethanol to a concentration of 75%, heat at 60 ℃ for 5 minutes, centrifuge, wash the precipitate with 96% ethanol, and dry at 100-105 ℃ to constant weight, then calculate the polysaccharide content.

Measurement of Coumarin Content

The UV spectrophotometric technique was applied. Weigh 2 g of material accurately, then extract by reflux with chloroform for 2 hours and filter. Evaporate 20 mL of the filtrate until dry, then dissolve the residue in 10 mL of 96% ethanol to a volume of 25 mL. Dilute 1 mL of the solution to 50 mL, measure the absorbance at 272 nm, and calculate the concentration with the coumarin reference standard.

Determining the Organic Acid Content

The acid-base titration method was applied. Weigh 10 g of sample accurately, add 80 mL of water, then extract in a boiling water bath for 2 hours before filtering and diluting to 100 mL. To quantify the organic acid content, take 10 mL of the filtrate and add phenolphthalein indicator, then titrate with 0.1 M NaOH (calculated as malic acid).

Measurement of Saponin Content

The UV spectrophotometric technique was applied. Weigh 2 g of sample precisely, extract with 3% acetone-acetic acid solution, adjust the pH to 8.3-8.6 with ammonia water, filter the precipitate, wash with 30 mL of acetone, dissolve the precipitate in 50 mL of water, and dilute to 100 mL. Take 30 mL of precipitate, dilute it to 100 mL, and measure the absorbance at 258 nm. Calculate the saponin content with glycyrrhizic acid as a reference standard.

Extraction and Separation

Due to the significant amount of plant material, the maceration process was used for extraction.

160 g dried powder was mixed with 1.5 L of 96% ethanol and extracted five times at room temperature for 24 hours each. The extracts were mixed and concentrated under vacuum with a rotary evaporator at 45-50 °C until there was no alcohol odor left, providing an ethanol extract (8.0056 g). The extract was dispersed in 200 mL of distilled water and extracted three times in succession with equal amounts of petroleum ether (60-90 °C), dichloromethane, ethyl acetate, and n-butanol (water-saturated). The extracts were mixed, and the solvent was recovered at decreased pressure, providing the following fractions: petroleum ether (1.4971 g), dichloromethane (3.1087 g), ethyl acetate (0.3903 g), n-butanol (2.001 g), and water (0.8787 g). The yields of each fraction were determined as a percentage of the crude drug mass.

GC-MS Analysis

The petroleum ether and dichloromethane fractions were dried with anhydrous sodium sulfate, diluted in acetone at a concentration of 1 mg/mL, and filtered through a 0.22 μm microporous membrane before GC-MS analysis.Agilent 7890A/5975C gas chromatograph-mass spectrometer, DB-17ms capillary column (30 m × 0.25 mm × 0.25 μm); carrier gas was high-purity helium (99.999%), flow rate 1.0 mL/min; injection port temperature 280 ℃; petroleum ether fraction was split (1:5), dichloromethane fraction was injected splitlessly, injection volume 2 μL; temperature program: initial temperature 40 ℃, held for 2 min, increased to 300 ℃ at 5 ℃.

Mass spectrometry conditions include an EI ionization source with electron energy of 70 eV, an ion source temperature of 230 ℃, a quadrupole temperature of 150 ℃, and a mass scan range of 34–750 m/z. Compounds were discovered by searching the NIST 11 and Wiley 11 libraries, and their relative % composition was determined using peak area normalization.

Results and discussion

The moisture percentage of the aerial sections of *A. rutifolia* was 7.42%, whereas the total ash content was 4.58%. The concentrations of 50%, 70%, and 96% ethanol extracts were 35.76%, 10.95%, and 7.32%, showing that more polar components were present (Table 1). Table 2 presents the results of content of Secondary Metabolites. The *A. rutifolia* samples utilized in this study had a moisture content of 7.42%. This number is consistent with the general moisture control requirement for medicinal plants. The total ash percentage was 4.58%; its value can be influenced by a variety of factors, including plant material properties, soil conditions, harvesting season, and drying procedure. When extracted with 50% ethanol, the extract yield reached 35.76%, which was significantly greater than the extraction results obtained with 70% and 96% ethanol, respectively. This pattern shows that highly polar chemical components (such as polysaccharides, saponins, and phenolic glycosides) predominate in *A. rutifolia*, as smaller concentrations of ethanol aqueous solution facilitate the dissolution of highly polar macromolecules. This finding is consistent with Nurlybekova et al. (2022) analysis of *Artemisia* species in Central Asia, which concluded that *Artemisia* species in this region are generally rich in medium to polar components.

Table 1
Authenticity Parameters of A. rutifolia

Parameter	Content (%)
Moisture	7.42
Total ash	4.58
50% ethanol extract	35.76
70% ethanol extract	10.95
96% ethanol extract	7.32

Coumarin was the most abundant compound in *A. rutifolia*, accounting for 2.53%, followed by polysaccharides (1.30%), saponins (1.16%), tannins (0.57%), alkaloids (0.16%), flavonoids (0.03%), and organic acids. Detailed results are shown in Table 2. A quantitative examination of secondary metabolites revealed that the overall coumarin concentration was 2.53%, much greater than that of flavonoids (0.03%) and tannins (0.57%).

Table 2
Secondary Metabolites in A. rutifolia

Component type	Content (%)
Flavonoids (as quercetin)	0.03
Tannins (as tannic acid)	0.57
Alkaloids (as aconitine)	0.16
Polysaccharides	1.30
Coumarins (as coumarin)	2.53
Free organic acids (as malic acid)	0.05
Saponins (as glycyrrhizic acid)	1.16

Macro- and Micro-element Composition

A. rutifolia had the highest macro-element K content (65.41 mg/100 g), followed by calcium (39.41 mg/100 g), sodium (16.38 mg/100 g), and magnesium (8.88 mg/100 g). Among the microelements, Fe (2.34 mg/100 g), Zn (0.075 mg/100 g), and Mn (0.307 mg/100 g) were prevalent. Heavy metals Pb, Cd, and Ni levels were 0.026, 0.0032, and 0.0011 mg/100 g, respectively, falling below WHO criteria for medicinal plants (Pb < 10 mg/kg, Cd < 0.3 mg/kg) (World Health Organization, 2007). Table 3 shows the detailed composition.

Table 3
Macro- and Microelement Content in A. rutifolia

Element	Concentration in ash (mg/g)	Content in plant (mg/100 g)
Cu	0.0082	0.0347
Mn	0.0723	0.3068
Zn	0.0176	0.0748
Fe	0.5519	2.3408
Ca	9.2914	39.4070
Mg	2.0932	8.8777
Na	3.8618	16.3788
K	15.4228	65.4113
Pb	0.0061	0.0261
Cd	0.0008	0.0032
Ni	0.0003	0.0011

A quantitative examination of mineral components in *A. rutifolia* revealed that potassium (K), calcium (Ca), sodium (Na), and magnesium (Mg) were abundant. Amarjargal et al. (2017) determined *A. rutifolia* from Mongolia using X-ray fluorescence (XRF), and our finding is broadly consistent with that trend. The K content in the sample examined in this study was 65.41 mg/100g, and the Ca content was 39.41 mg/100g; the former is involved in fluid balance and nerve signal transmission (Weaver, 2013), whilst the latter is important for bone health (Cormick & Belizán, 2019). In addition, trace metals like iron (Fe), zinc (Zn), and manganese (Mn) were relatively abundant. These components serve important roles in the human hematopoietic, immunological, and antioxidant systems (Dong et al., 2024).

The synergistic action of macro and micro elements may promote the normal function of the circulatory, immunological, and metabolic systems, establishing a solid foundation for the use of *A. rutifolia* as a traditional medicine and dietary supplement. In terms of safety, this investigation found 0.026 mg/100 g of lead (Pb) and 0.0032 mg/100 g of cadmium. Both readings fall well below WHO standards for medicinal plants (Pb < 10 mg/kg, Cd < 0.3 mg/kg)(World Health Organization, 2007). As a result, the *A. rutifolia* sample is believed to be safe based on the heavy metal markers listed above.

Extraction Yields

Each polar fraction yielded (as a percentage of crude drug mass) 0.94% petroleum ether, 1.94% dichloromethane, 0.24% ethyl acetate, 1.25% n-butanol, and 0.55% water. The overall yield of the ethanol extract was 5.00%. The dichloromethane fraction had the highest yield, followed by n-butanol and petroleum ether, and the ethyl acetate fraction had the lowest yield.

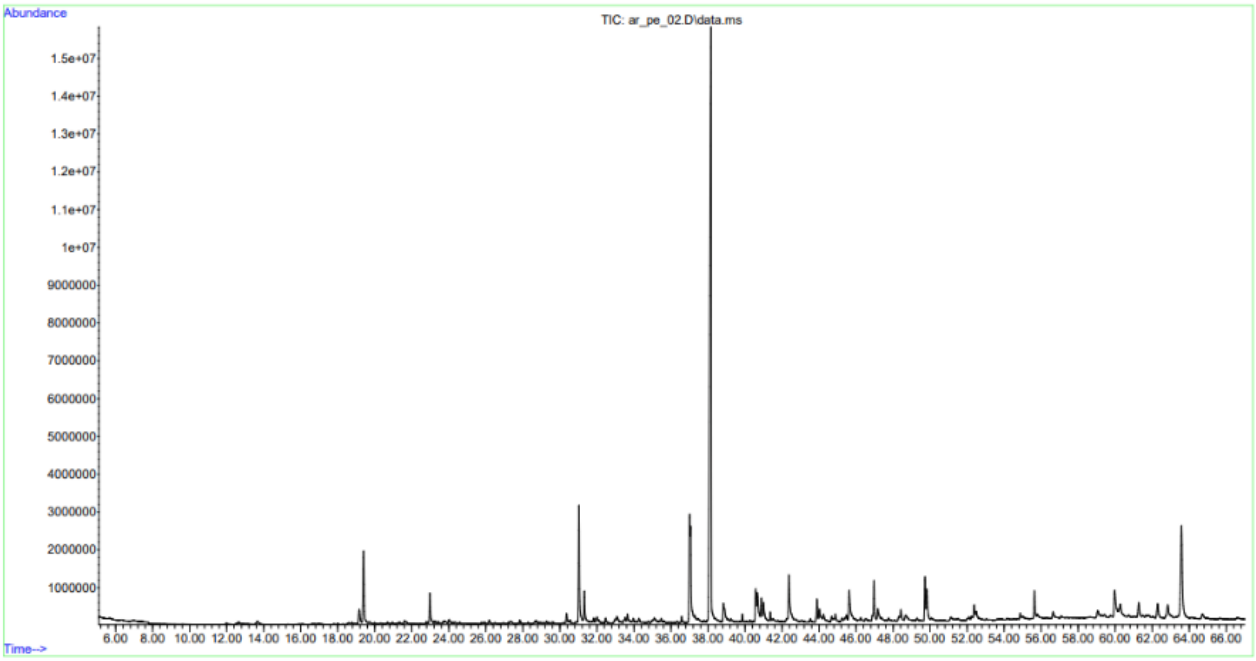
GC-MS Analysis Results

Petroleum Ether Fraction

The total ion chromatogram of the petroleum ether fraction (Fig. 1) revealed 28 peaks, which identified 28 compounds and accounted for more than 99% of the total peak area. The primary constituents were aromatic chemicals and fatty acid esters. The highest content (43.80%) was found in a methoxybenzene derivative (1,4-dimethoxy-2-methyl-5-isopropylbenzene), followed by γ-sitosterone (8.57%), 3',4'-(methylenedioxy)acetophenone (5.41%), ethyl palmitate (4.91%), palmitic acid (3.85%), behenol (3.70%), ethyl tetracosanoate (1.77%), γ-sitosterol (2.57%), and Stigmasta-3,5-dien-7-one (1.49%). Table 4 shows a list of chemicals.

Furthermore, GC-MS analysis of the dichloromethane extract identified and quantified the major coumarin monomer, scopoletin, at a concentration of 3.00%. The following results confirm that coumarin components are one of *A. rutifolia* distinctive metabolites. scopoletin has a variety of pharmacological activities, including anti-inflammatory, antioxidant, and antibacterial properties (Sharopov et al., 2015). The anti-inflammatory, antibacterial, and neuroprotective properties of scopoletin were further described in a thorough review by Gao et al. (2024), supporting its medicinal potential. Based on this, it is speculated that this compound may be one of the material basis for *A. rutifolia* traditional anti-inflammatory use. This sample contained 1.30% polysaccharides and 1.16% saponin, both of which achieved a specified amount. Dong et al. (2024) reported that polysaccharides found in medicinal plants in Kazakhstan have immunomodulatory properties, implying that the polysaccharides found in *A. rutifolia* may also have similar effects, but more activity investigations are needed to confirm this.

Figure 1
Chromatogram of the Petroleum Ether Fraction



Gas chromatography-mass spectrometry (GC-MS) is a widely used technology for qualitative and quantitative detection of active components in medication analysis, quality control, and phytochemical research. GC-MS analysis indicated considerable chemical variations between the petroleum ether and dichloromethane fractions. The petroleum ether fraction was primarily composed of aromatic chemicals and steroidal molecules. Monomethoxybenzene derivatives were the most abundant aromatic chemical, accounting for 43.80% of the total content; steroidal substances comprised γ-sitosterone (8.57%) and γ-sitosterol (2.57%). Additionally, camphor (4.37%), palmitic acid and its esters (8.76%), and behenol (3.70%) were discovered in this fraction. Mukhamatkhanova et al. (2024) confirmed the presence of these bioactive compounds in A. rutifolia by isolating camphor, casticin, and daucosterin from the same species. Monomethoxybenzene compounds are relatively infrequent among *Artemisia* species and may be unique to this origin; nevertheless, this conclusion requires additional confirmation by comparison investigations of samples from other origins. Steroidal compounds (such as sitosterol and stigmasterol derivatives) have been shown in the literature to have anti-inflammatory and cholesterol-lowering properties (El Omari et al., 2024), therefore they can be considered as potential active components in the petroleum ether fraction.

Table 4
GC-MS identification of chemicals in the petroleum ether fraction (relative content > 1%)

Ppeak No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)	Match factor
2	19.40	(+)-2-Bornanone	$C_{10}H_{16}O$	4.37	96
3	22.98	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-	$C_{10}H_{16}O$	1.63	94
5	31.04	3’,4’-(Methylenedioxy)acetophenone	$C_9H_8O_3$	5.41	77
6	31.33	4’,6’-Dihydroxy-2’,3’-dimethylacetophenone	$C_{10}H_{12}O_3$	1.69	78
8	37.01	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	4.91	83

Continuation of the table

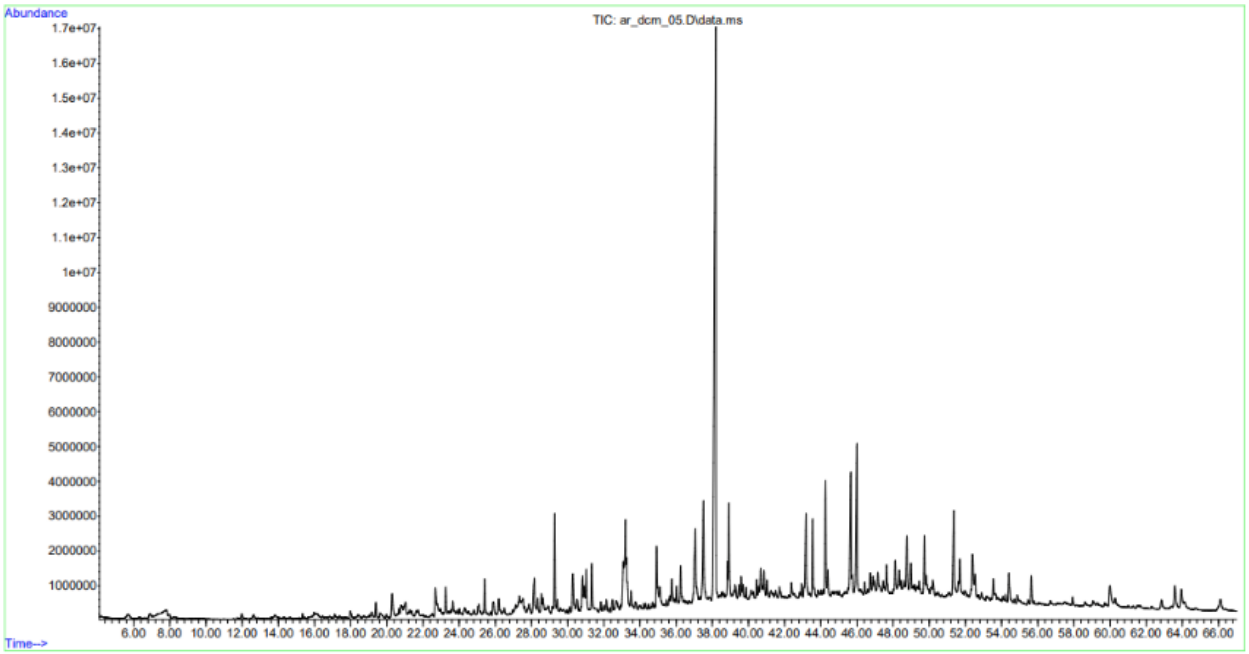
Ppeak No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)	Match factor
9	37.07	Hexadecanoic acid	$C_{16}H_{32}O_2$	3.85	93
10	38.17	Benzene, 1,4-dimethoxy-2-methyl-5-isopropyl-	$C_{12}H_{18}O_2$	43.80	76
12	40.58	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	1.49	76
13	40.67	Octadecanoic acid	$C_{18}H_{36}O_2$	2.48	79
14	40.89	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	1.40	86
15	41.01	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	1.22	82
16	42.37	Behenic alcohol	$C_{22}H_{46}O$	3.70	90
17	43.89	Eicosanoic acid, ethyl ester	$C_{22}H_{44}O_2$	1.06	88
19	46.97	Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	1.83	89
21	49.83	Ethyl tetracosanoate	$C_{26}H_{52}O_2$	1.77	85
24	59.98	γ-Sitosterol	$C_{29}H_{50}O$	2.57	86
25	61.27	Stigmastan-7-one	$C_{29}H_{50}O$	1.04	71
26	62.29	Spinasterone	$C_{29}H_{46}O$	1.16	80
27	62.84	Stigmasta-3,5-dien-7-one	$C_{29}H_{46}O$	1.49	78
28	63.59	γ-Sitostenone	$C_{29}H_{48}O$	8.57	91

Dichloromethane Fraction

The dichloromethane segment's total ion chromatogram revealed more than 60 peaks (Fig. 2), which identified 63 chemicals and accounted for 89.2% of the peak area. The primary constituents were aromatic chemicals, phenolic acids, coumarins, and sesquiterpene lactones. The highest content (25.82%) was found in a methoxybenzene derivative (1,4-dimethoxy-2-methyl-5-isopropylbenzene), followed by 6,7-dimethoxy-1,4-dihydro-2,3-quinoxalindione (9.56%), (E)-4-(3-hydroxypropyl-1-en-1-yl)-2-methoxyphenol (4.06%), palmitic acid (3.88%), ambrosin (3.36%), scopoletin (3.00%), monoethyl azelaic acid (2.85%), vanillin (2.47%), vanillic acid (2.18%), and 4-hydroxy-3-methoxyphenylpropanol (2.05%). Table 5 shows a list of chemicals.

Compared to the previously stated petroleum ether fraction, the dichloromethane extract of *A. rutifolia* exhibits a more complex and varied chemical makeup. Methoxybenzene derivatives were also detected in this fraction (relative content 25.82%), and it was also rich in three main classes of compounds: phenolic acids (including vanillin 2.47%, vanillic acid 2.18%, 4-hydroxy-3-methoxyphenylpropanol 2.05%, ethyl ferulic acid 0.51%, etc.), coumarins (mainly scopoletin, content 3.00%), and sesquiterpene lactones (ambrosin 3.36%, austricin 1.72%, santamarine 0.97%, reynosin 1.23%). Previous research has shown that sesquiterpene lactones are key active components of *Artemisia* species, with guaiacol-type lactones having substantial anticancer activity (Tang et al., 2025). The ambrosin found in this study is a guaiacolane lactone with anti-inflammatory (Tang et al., 2025), cytotoxic (El-Sawy et al., 2025), and antiparasitic properties (Adekenov, 1995); austricin, another guaiacolane lactone, has anti-tumor properties. After screening 114 Mongolian plant extracts, Oidovsambuu et al. (2024) consistently discovered that an ethanol extract of A. rutifolia showed strong cytotoxicity against HepG2 (liver) and HCT116 (colon) cancer cells with IC_{50} values below 1.5 μg/mL, directly supporting the sesquiterpene lactones' potential anticancer role. Given the existence of the aforementioned lactone components and their recognized pharmacological effects, it is fair to infer that these chemicals may collectively serve as one of the material underpinnings for *A. rutifolia* anticancer potential. It should be highlighted that this speculation requires further verification by in vitro or in vivo activity tests.

Figure 2
Chromatogram of the dichloromethane fraction



Notably, the dichloromethane fraction had a high concentration of 6,7-dimethoxy-1,4-dihydro-2,3-quinoxaline dione (9.56%). This chemical is a quinoxaline alkaloid. It could be a derivatization product from sample preparation or something that occurs naturally, and it needs to be validated using NMR. In addition, phenolic components such as monoethyl azelaic acid (2.85%) and (E)-4-(3-hydroxypropyl-1-en-1-yl)-2-methoxyphenol (4.06%) show antioxidant and anti-inflammatory properties (Petrovici et al., 2025). Ayurzana et al. (2022) verified the antioxidant potential of *A. rutifolia* by reporting a DPPH IC_{50} of 39 µg/mL for the ethanol extract, which is similar to the activity of our dichloromethane fraction.

Compared to Dylenova et al. (2023) and Trendafilova et al. (2010), the camphor level in the petroleum ether fraction examined in this investigation was only 4.37%, with no detection of α/β-thujone or 1,8-cineole. This compositional feature differs from the normal "thujone type" or "cineole type" essential oils mentioned in the preceding research. This could be due to the extraction method: this study used 96% ethanol extraction followed by petroleum ether extraction, which differs from the essential oil composition obtained through steam distillation; it could also be due to the fact that *A. rutifolia* in Almaty, Kazakhstan, has a distinct chemotype, primarily composed of aromatic compounds and steroids. The occurrence of geographic chemotype diversity in this species is further supported by a recent study by Dylenova et al. (2023) that found a "Buryat-Mongol" chemotype of A. rutifolia from Russia, which is characterized by 4-phenyl-2-butanone and camphor without thujone. Furthermore, our analysis discovered a range of sesquiterpene lactones in the dichloromethane fraction, which is consistent with observations in the literature that "*Artemisia* species are generally rich in sesquiterpene lactones" (Nurlybekova et al., 2022; Rustaiyan & Masoudi, 2011).

Table 5
GC-MS identification of chemicals in the dichloromethane fraction (relative content > 1%)

Peak No.	Retention time (min)	Compound name	Molecular formula	Relative content (%)	Match factor
5	20.30	Dihydro-3-methylene-5-methyl-2-furanone	$C_6H_8O_2$	1.00	84
11	25.41	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	1.01	89
19	29.29	Vanillin	$C_8H_8O_3$	2.47	95
22	30.29	Ethanone, 1-(2-hydroxyphenyl)-	$C_8H_8O_2$	1.56	72
24	30.84	L-Pyroglutamic acid	$C_5H_7NO_3$	1.26	86
26	31.03	4-Acetylbenzoic acid	$C_9H_8O_3$	1.09	77
27	31.33	4’,6’-Dihydroxy-2’,3’-dimethylacetophenone	$C_{10}H_{12}O_3$	1.40	74
28	33.08	Diethyl azelate	$C_{13}H_{24}O_4$	1.61	70
29	33.19	Azelaic acid, monoethyl ester	$C_{11}H_{20}O_4$	2.85	90
30	33.25	Vanillic acid	$C_8H_8O_4$	2.18	83
33	34.93	Benzenepropanol, 4-hydroxy-3-methoxy-	$C_{10}H_{14}O_3$	2.05	87
36	36.25	Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	$C_9H_{10}O_4$	1.39	79
37	37.07	Hexadecanoic acid	$C_{16}H_{32}O_2$	3.88	90
38	37.51	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	$C_{10}H_{12}O_3$	4.06	89
39	38.19	Benzene, 1,4-dimethoxy-2-methyl-5-isopropyl-	$C_{12}H_{18}O_2$	25.82	75
40	38.85	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	1.14	80
46	40.68	Octadecanoic acid	$C_{18}H_{36}O_2$	1.12	81
48	43.18	Scopoletin	$C_{10}H_8O_4$	3.00	93
50	45.66	6,7-Dimethoxy-1,4-dihydro-2,3-quinoxalinedione	$C_{10}H_{10}N_2O_4$	9.56	80
53	47.64	Reynosin	$C_{15}H_{20}O_3$	1.23	90
54	48.35	Phenol, 2,2’-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	$C_{27}H_{40}O_2$	1.13	77
56	51.36	Ambrosin	$C_{15}H_{18}O_3$	3.36	69
57	51.69	Austricin	$C_{15}H_{20}O_3$	1.72	80
58	54.40	10,11-Dihydro-10-hydroxy-2,3-dimethoxydibenz(b,f) oxepin	$C_{18}H_{18}O_4$	1.15	74
60	59.99	γ-Sitosterol	$C_{29}H_{50}O$	1.23	85
62	63.59	γ-Sitostenone	$C_{29}H_{48}O$	1.12	75

This study comprehensively examined the chemical contents of *A. rutifolia* from Kazakhstan utilizing phytochemical analysis and GC-MS, preliminarily verifying its therapeutic potential. The detected active compounds partially support the plant’s traditional medicinal uses (such as anti-inflammatory and antibacterial capabilities) and establish the groundwork for future pharmacological and chemical research. This study includes the following significant limitations: GC-MS identification was primarily based on library searching; the structures of some compounds (particularly sesquiterpene lactones) require additional NMR confirmation; and only samples from one origin were analyzed, so the results do not reflect the intraspecific chemical diversity of this species across different geographical or ecological environments. To address these limitations, future research should broaden the sample reach (to include numerous sources and growth seasons) and combine in vitro and in vivo activity screening with the requisite NMR structural analysis to more thoroughly investigate the therapeutic usefulness of this plant. Furthermore, combining traditional medicinal wisdom with current scientific

analytical methods will aid in determining the scientific worth of medicinal plants.

Conclusion

This is the first study to comprehensively examine the physicochemical parameters, mineral elements, secondary metabolites, and GC-MS analysis of *A. rutifolia* in Almaty, Kazakhstan. The results showed that the plant is rich in coumarin (2.53%), polysaccharides (1.30%), saponins (1.16%), and phenolic components; the mineral element composition is reasonable; and the heavy metal lead and cadmium content is lower than the WHO limit standard (World Health Organization, 2007), indicating its safety. Further analysis revealed that the petroleum ether fraction was primarily made up of aromatic compounds (43.80%) and steroids (15%), while the dichloromethane fraction was high in phenolic acids (vanillin, vanillic acid, etc.), coumarins (scopoletin 3.00%), and sesquiterpene lactones (ambrosin 3.36%, austricin 1.72%, etc.). The statistics presented above give fundamental information for the development, exploitation, and quality control of *A. rutifolia*, as well as a preliminary foundation for the screening of high-quality medicinal resources and the scientific transmission of traditional medicinal knowledge. It should be noted that, while many of the components discovered in this study have known biological activities (such as anti-inflammatory, antibacterial, and anticancer effects), their precise mechanisms of action in *A. rutifolia* still need to be clarified through future experiments. As medicinal plants gain popularity due to their excellent safety profile, broad therapeutic spectrum, and suitability for long-term use, incorporating these basic research discoveries into modern medical practice offers enormous promise. As a result, a thorough investigation of the clinical application value of *A. rutifolia* is expected to enhance the organic integration of traditional knowledge and modern science in the field of herbal medicine.

Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization, J.J. and U.J.; Methodology, Ye.S., C.Z. and S.D.; Software, C.Z., U.J. and Ye.S.; Validation, Ye.S., H.Y. and U.J.; Formal Analysis, U.J., C.Z. and J.J.; Investigation, U.J., Ye.S. and N.M.; Resources, J.J.; Data Curation, J.J., H.Y. and S.D; Writing – Original Draft Preparation, U.J., H.Y., J.J.; Writing – Review & Editing, N.M. and Ye.S.; Visualization, Ye.S., U.J. and J.J.; Supervision, J.J.; Project Administration, J.J.; Funding Acquisition, J.J.

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