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Chemical composition of hexane and chloroform extracts from *Artemisia scopaeformis*

Abstract: Research in the field of medicinal products, especially medicinal plants, has been constantly expanding recently. However, to date, health research has not been widely disseminated. This article focus on the analysis of hexane and chloroform extracts of *Artemisia scopaeformis* by Gas Chromatography and Mass Spectroscopy (GC-MS) technique. Detailed chemical constituents of *A. scopaeformis* collected in Kazakhstan were investigated for the first time. The quantitative and qualitative analysis of bioactive constituents of the medicinal plant have been made. *A. scopaeformis* hexane extract contained 4 compounds: methyleugenol (33.87%), hexadecanoic acid, ethyl ester (41.02%), butyl 4,7,10,13,16,19-docosahexaenoate (11.55%), hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl- (13.56%). The chloroform extract of *A. scopaeformis* contained 5 compounds: fluorene, 2,7-bis(1-hydroxyethyl)- (24.28%), p-dimethylaminobenzylidene p-anisidine (14.59%), 3-acetoxy-5-methyl-2-nitro- terephthalic acid, 4-isopropyl ester 1-methyl ester (11.74%), 3,4-diacetyl-2-methyl-4H-thieno [3,2-b] pyrrole-5-carboxylic acid, methyl ester (17.35%), 4H-1,2,4-triazole-3-thiol, 4-(2-fluorophenyl)-5-(1-methylethyl) – (32.04%). The resulting compounds have significant biological properties: antimicrobial, antioxidant, anti-inflammatory.

Key words: *Artemisia scopaeformis*, GC-MS, hexane, chloroform, bioactive constituents, extract.

Introduction

Currently, there is a tendency in the world to increase interest in consuming a wide range of phytochemicals based on environmentally friendly plant materials. In recent decades, there has been an increase in their use not only in the countries of Asia and Africa, where they have been traditionally used for many centuries, but also in Europe, the USA and other countries of the world community. At the same time, not only medicinal plants and fees, but also various galenical and newgalenic preparations, individual biologically active compounds isolated from plants are used as herbal medicines [1]. A report from the World Health Organization disclosed that almost 80% of the world's population relies on nonconventional drug-treatment, particularly of herb, in their primary healthcare [2]. However, despite significant progress is being made in the disclosure of the chemical composition and bioactivities of herbal medicines, their exact biological functions and regulation mechanisms largely remain to be clarified. Medicinal plants with the ancient history of human

use have been considered one of the important and reliable sources to discover promising therapeutic agents in a number of cases, including cancer chemopreventive drugs [3-5].

The *Asteraceae* family includes over 24,000 species, combined in about 1,200 genera. 224 genera and more than 3,500 species grow in the CIS, more than 140 genera and about 790 species of the family are found in the flora of Kazakhstan. The rich chemical composition of the family determines their use as insecticidal and medicinal sources. The genus *Artemisia* unites over 500 species, distributed mainly in the temperate zone of the northern hemisphere. Wormwood species are most often found in the steppes, others grow in semi-deserts and deserts [6-8]. It is known that the pharmacological properties of plants of the genus *Artemisia* are associated with the content of essential oils, terpenoids, flavonoids, coumarins, caffeoylquinic acids and sesquiterpene lactones in them. At the same time, of particular interest are phenolic compounds, in particular flavonoids with a wide spectrum of biological activity [9, 10]. In practical medicine, *Artemisia* species are often used to treat

diseases of the gastrointestinal tract, as a choleric, laxative, diuretic, anti-inflammatory, wound healing [11]. In traditional medicine – as an anti-cancer, anti-inflammatory, wound healing, sedative, choleric, metabolic regulating, diaphoretic, detoxification, bacteriological and fungistatic, antiviral, protistocidal, anthelmintic; with pulmonary tuberculosis and lymph nodes [12]. Currently, sesquiterpene lactones are considered as a promising source of new drugs due to the possibility of easy cultivation of these plants and a high content of lactones in a number of plant species. According to their physicochemical properties, mono – and sesquiterpenes belong to the group of essential oils that have been studied in more detail by the example of *A. princeps*. Unfortunately, to date, drugs based on sesquiterpene lactones produced by pharmaceuticals are insignificant. Some interesting sesquiterpenes lactones including effective antimalarial principle – artemisinin were identified in dichloromethane, chloroform and methanol extracts of perennial herb *A. herba alba*. *Artemisia annua* L. is the main raw material for artemisinin. Medicines based on artemisinin and its derivatives are widely used as new treatments for malaria – a disease that takes at least a million lives annually. It is also important that artemisinin and related compounds showed cytotoxic activity, which allows them to be used in anti-cancer therapy. In addition to artemisinin, *A. annua* is appreciated for the essential oil, which is used in perfumes and cosmetics. The antibacterial effect of essential oils is due to the presence of oxygen-containing compounds (camphor, various acids). Essential oil contains a large number of components of biological value, such as artemisia ketone, 1,8-cineole, borneol, etc. [13]. One of the promising types for use in medical practice is Pontian wormwood (*Artemisia pontica* L.), the essential oil of which has a pronounced anti-inflammatory, wound healing, analgesic effect. Total complexes from *A. absinthium* have an antitoxic effect (in case of mercuric chloride poisoning); exhibit antibacterial, antifungal, antiviral and protistocidal activity. All of the above types of wormwood, being essential oil crops, find their application in various areas of human activity, and most often in cooking, the production of alcoholic and soft drinks, medical supplies and dosage forms. In this regard, wormwood is without exaggeration one of the most studied medicinal plants. [14].

Based on the foregoing, a research of the chemical composition of representatives of the genus *Artemisia*, growing in Kazakhstan, seems to be an urgent task.

Artemisia scopaeformis is an endemic plant that grows in the desert zone on clay and sandy soils, along the margins of meadow. In Kazakhstan, it grows in the Chu-Ili Mountains and Karatau [15]. In our study, constituents in the hexane and chloroform parts of the medicinal plant *Artemisia scopaeformis*, which were grown in the Almaty region of Kazakhstan, were first identified by GC-MS. Over the past 15–20 years in the field of pharmacognosy, qualitative changes have occurred in the technical capabilities of studying the chemical composition of medicinal plants and herbal remedies. This was facilitated by the enrichment of this science with modern spectral and other physicochemical methods. GC-MS method can provide accurate results. It gives a high degree of specificity, good sensitivity and permits the simultaneous determination of a wide range of compounds from phenolic compounds to terpenes in complex matrices. It provides complementary data to LC-MS analysis comprising small polar chemicals such as organic acids, sugars, amino acids, sugar alcohols and many more. GC-MS results can also be recommended for rapid screening of the chemical composition of the main groups of biologically active substances of plant materials used in the development and creation of new herbal medicines [16].

Materials and methods

Plant material. The aerial part of *Artemisia scopaeformis* was collected in Almaty region of Kazakhstan, in 2018. The air dried aerial parts of plant *A. scopaeformis* were cut into small pieces and preserved at room temperature.

Extraction and isolation. The air-dried aerial parts of *A. scopaeformis* (100 g) were pulverised then extracted with 70% ethyl alcohol (1:1) three times (seven days each time) at room temperature. After evaporation of the solvent under reduced pressure, the residues were mixed and suspended in water and then successively partitioned with hexane and chloroform to afford the corresponding extracts. The obtained hexane and chloroform extracts were analyzed by GC-MS method.

Experimental part. The constituents from hexane and chloroform extracts of the medicinal plant were analyzed by using GC-MS method. GC-MS analysis was performed on Agilent 7890A-5975C GC-MS (Gas Chromatograph coupled to Mass Spectrometer) with a HP-5MS fused silica capillary column (30m x 2.5mm; 0.25 µm film thickness). Helium (99.999%) was used as a carrier gas, the column temperature was flashed from 50°C (held for 10min) to 300°C

at 10°C/min. The latter temperature maintained for 40 min. The injector temperature was 310°C. Injection volume was 1µl with the split ratio 5:1. Mass spectra: electron impact (EI+) mode, 70 eV. Mass spectra were recorded over scan range 30-1000 a.m.u.

Identification of the compounds: Interpretation on mass spectrum GC-MS was carried out using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components existed in the NIST library. The Name, Molecular weight and Structure of the components of the test materials were ascertained. Percentage composition was computed from GC peak areas on HP-5MS column without applying correction factors.

Results and discussion

The constituents of hexane and chloroform extracts from the aerial parts of *A. scopaeformis* were

analyzed by GC-MS. GC-MS technology is recognized as the “gold standard” in identifying chemicals in simple and complex mixtures. Besides, the technology is able to recognize substances at a trace level that is unattainable with other technologies. This method allows to selectively and with high sensitivity to determine various types of compounds.

GC-MS chromatogram of the hexane extract from aerial part of *Artemisia scopaeformis* (Fig.1) clearly shows 4 peaks indicating the presence of 4 phytochemical compounds. The identification of the resulting phytochemical compounds was based on the peak area, retention time and molecular formula. The active substances with their retention time, molecular formula, molecular weight and concentration (%) are presented in Table 1 and Fig 1. Analysis of the chloroform extract from *A. scopaeformis* revealed 5 compounds in the studied samples (Fig.2). Detailed data of compounds are shown in Table 2. Their relative contents were determined by area normalization.

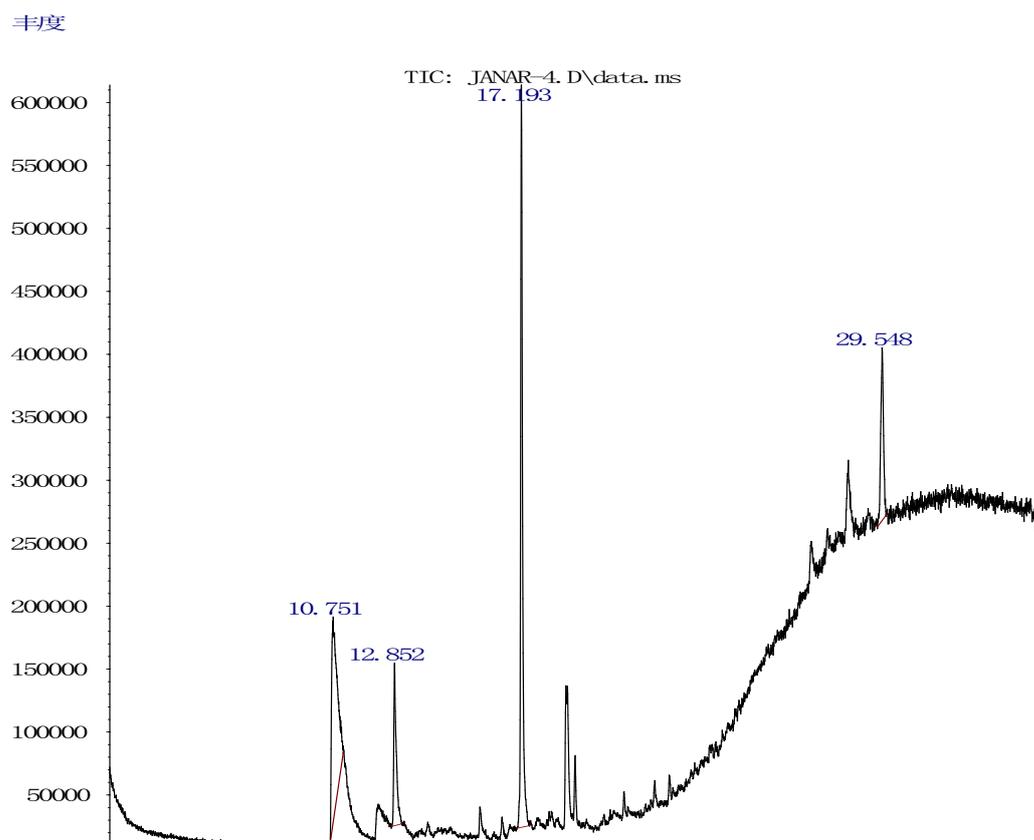


Figure 1 – GC-MS Chromatogram of hexane extract of *Artemisia scopaeformis* plant.

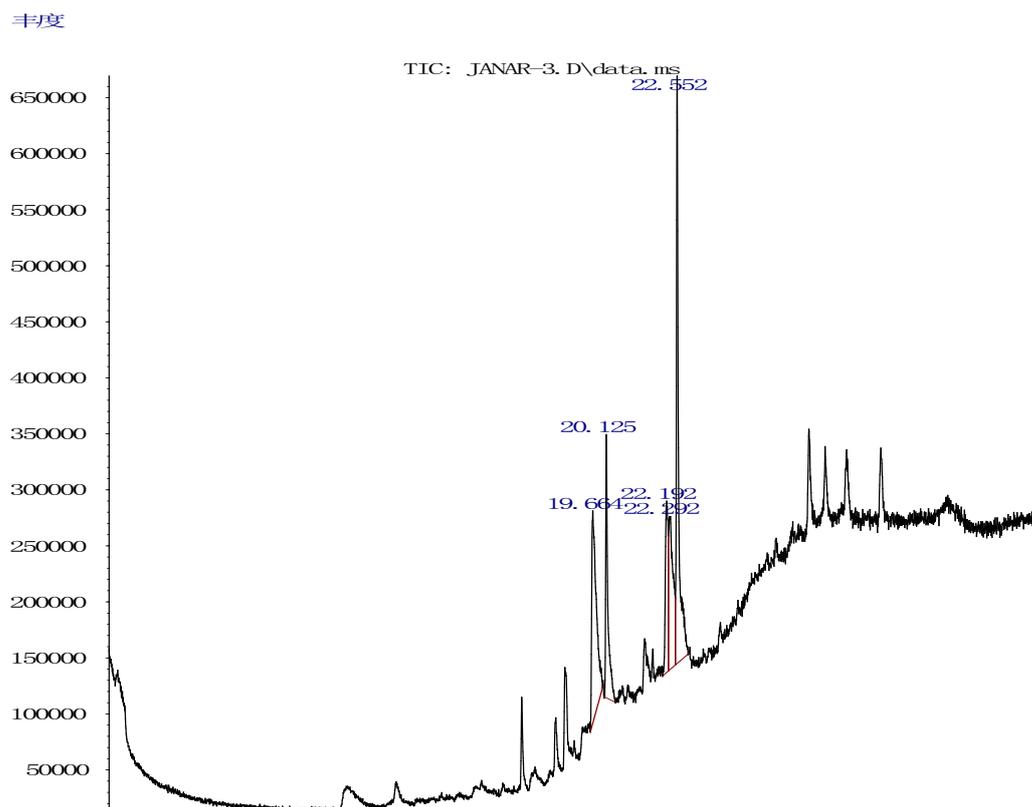
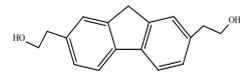
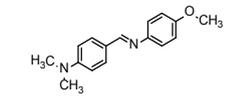
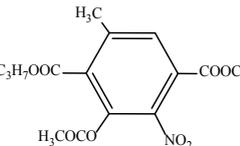
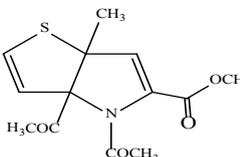
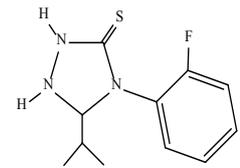


Figure 2 – GC-MS Chromatogram of chloroform extract of *Artemisia scopaeformis* plant

Table 1 – Constituents identified in hexane extract of *Artemisia scopaeformis*.

Peak No	Constituents	t_R (min)	Molecular Formula	Structure	MW	Content (%)
1	Methyleugenol	10.75	$C_{11}H_{14}O_2$		178	33.87
2	Butyl 4,7,10,13,16,19-docosahexanoate	12.857	$C_{26}H_{40}O_2$		384	11.55
3	Hexadecanoic acid, ethyl ester	17.198	$C_{18}H_{36}O_2$		284	41.02
4	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecylmethyl-	29.55	$C_{12}H_{38}O_5Si_6$		430	13.56

Table 2 – Constituents identified in chloroform extract of *Artemisia scopaeformis*.

Peak No	Constituents	t _R (min)	Molecular Formula	Structure	MW	Content (%)
1	Fluorene, 2,7-bis(1-hydroxyethyl)-	19.661	C ₁₇ H ₂₀ O ₂		254	24.28
2	p-Dimethylaminobenzylidene p-anisidine	20.128	C ₁₆ H ₁₈ N ₂ O		254	14.59
3	3-Acetoxy-5-methyl-2-nitro-terephthalic acid, 4-isopropyl ester 1-methyl ester	22.193	C ₁₅ H ₁₇ NO ₈		339	11.74
4	3,4-Diacetyl-2-methyl-4H-thieno[3,2-b]pyrrole-5-carboxylic acid, methyl ester	22.295	C ₁₃ H ₁₃ NSO ₄		279	17.35
5	4H-1,2,4-Triazole-3-thiol, 4-(2-fluorophenyl)-5-(1-methylethyl)-	22.55	C ₁₀ H ₁₄ N ₃ SF		237	32.04

The GC-MS analysis of hexane extract revealed the presence of 4 compounds: methyleugenol (33.87%), hexadecanoic acid, ethyl ester (41.02%), butyl 4,7,10,13,16,19-docosahexaenoate (11.55%), hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl- (13.56%), main contents of them were methyleugenol and hexadecanoic acid, ethyl ester. The principal chemical constituents of chloroform extract were found to be fluorene, 2,7-bis(1-hydroxyethyl)- (24.28%), p-dimethylaminobenzylidene p-anisidine (14.59%), 3-acetoxy-5-methyl-2-nitroterephthalic acid, 4-isopropyl ester 1-methyl ester (11.74%), 3,4-diacetyl-2-methyl-4H-thieno[3,2-b]pyrrole-5-carboxylic acid, methyl ester (17.35%), 4H-1,2,4-triazole-3-thiol, 4-(2-fluorophenyl)-5-(1-methylethyl)- (32.04%). Almost one third (34.13%) of the extract consists of the substance 4H-1,2,4-triazole-3-thiol, 4-(2-fluorophenyl)-5-(1-methylethyl)-.

Identified compounds have been found to possess a wide range of biological activities. Their various activities are also mentioned in Table 3. These properties determine the use of the plant for antimicrobial, antioxidant, anti-inflammatory and pesticidal and

other activities. The major component of hexane extract from *A. scopaeformis*, namely the hexadecanoic acid, ethyl ester (41.02%), have been reported to have antioxidant, hypocholesterolemic, nematocidal, antifungal, hemolytic, flavor, pesticide, lubricant activities [17]. Hexadecanoic acid, ethyl ester is also found in plant extracts from *Artemisia austro-yunnanensis*, *Artemisia frigida* Willd *Carbonisatus* [18]. The other compound, methyleugenol (33.87%) is an aromatic terpene, which is a colorless oily liquid with a faint pleasant odor. This substance is an integral part of many essential oils, such as: citronella, laurel, pink, pine and fennel oils. Citronella oil, unlike most other essential oils, has a rather limited list of beneficial properties. Moreover, its main advantage is the ability to scare away all kinds of insects. Many studies show that the oil has quite good antifungal properties. Methyleugenol refers to insect-attracting substances. A mixture of methyleugenol with parathion and pyrolane are effective in controlling insects. It is also used as a flavouring agent in jellies, baked goods, soft drinks, chewing gum, sweets, puddings, condiments and ice cream. In addition methyleugenol is also being applied as a fragrance ingredient in per-

fumes (0.3–0.8%), creams and lotions (0.01–0.05%), toiletries and detergents (0.02–0.2%). The presence of methyleugenol in the plants of *Artemisia dracunculoides* L., *A. scoparia*, *A. capillaris* was also detected. [19]. Methyleugenol is reported as relaxant, antispasmodic, anesthetic, antinociceptive active principal and can be used as insecticide [20]. Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl- (13.56%) is a plasticizer compound, it may be act as an antimicrobial, antiseptic, antifouling drug, hair conditioning agent, skin-conditioning agent-emollient and solvent, whereas p-dimethylaminobenzylidene p-anisidine (14.59%) can be represented as an antihistaminic, analgesic drug [21]. Several studies have attributed the antidiabetic, anti asthma and anticancer activities to butyl 4,7,10,13,16,19-docosahexaenoate (11.55%) shows [22]. It should be noted that 4H-1,2,4-triazole-3-thiol, 4-(2-fluorophenyl)-5-(1-methylethyl)- (32.04%) has antitubercular properties. Its antimicrobial and antioxidant activities have also

been demonstrated. Antioxidants have the ability to stabilize free radicals, which leads to cytoprotection from the harmful effects of free radicals. Antioxidants have the ability to stabilize free radicals, which leads to cytoprotection from the harmful effects of free radicals [23].

Thus, as a result of a qualitative analysis of the aerial parts of the plant *A. scopaeformis*, the presence of the main biologically active substances, which determine the pharmacological effect and nutritional value of the studied plant, was established. It is obvious that the plant contains complexes of antioxidant, antifungal, antimicrobial, and also anti-inflammatory effects. Nowadays, the anti-inflammatory properties of medicinal plants and preparations from them are not widely used, differing, perhaps, with a slightly less pronounced effect, but with better tolerance and less toxicity. In this regard, the search for new effective anti-inflammatory herbal preparations is relevant.

Table 3 – Reported activities of the identified bioactive compounds from *A. scopaeformis*

№	Compound	Activity	References
1	Butyl 4,7,10,13,16,19-docosahexaenoate	Antidiabetic, anti asthma, anticancer, anti heart disease	[22]
2	4H-1,2,4-Triazole-3-thiol, 4-(2-fluorophenyl)-5-(1-methylethyl)-	Antioxidant, antitubercular and antimicrobial	[23]
3	Methyleugenol	Relaxant, antispasmodic, anesthetic, flavouring agent, insecticide, shows antinociceptive effect	[24, 25]
4	Hexadecanoic acid, ethyl ester	Antifungal, antioxidant, aypocholesterolemic nematocide, aesticide, antiandrogenic flavour, hemolytic, 5-Alpha reductase inhibitor, potent antimicrobial activity Antimicrobial, antiseptic, hair conditioning agent, skin-conditioning agent-emollient, solvent	[26]
5	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	Antimicrobial, antiseptic, hair conditioning agent, skin-conditioning agent-emollient, solvent	[27]
6	p-Dimethylaminobenzylidene p-anisidine	Analgesic, anti-inflammatory activity	[28]

Conclusion

Thus, using GC-MS, the chemical composition of the plant *Artemisia Scopaeformis*, growing in the Almaty region of Kazakhstan, was first studied. Among this study, there are 4 compounds were identified from hexane extract of *A. scopaeformis*. From chloroform extract of *A. scopaeformis*, 5 compounds were separated. GC-MS analysis is the first step in identifying and understanding the nature of medicinal plants. Isolation of a individual phytochemical constituent and further study of its biological activity will definitely yield fruitful results. It could be con-

cluded that *A. scopaeformis* contains various bioactive compounds. Though, further studies are needed to determine its bioactivity and toxicity profile.

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