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Research Article

The essential oil composition of aerial parts of *Artemisia austriaca* Jacq. from three accessions of Northern Kazakhstan

Olessya ISSAYENKO[1], Ashutosh PATHAK[2], Svetlana KABANOVA[3], Yana KREKOVA[3], Andrey KABANOV[3], Vitaliy KIRILLOV[1,3]*

[1]*Shoqan Ualikhanov Kokshetau University, Sadvakasov Agrotechnical Institute, Department of Ecology, Mining and Construction, Kokshetau, Kazakhstan; olesya_5_82@mail.ru*
[2]*University of Rajasthan, Department of Botany, Jaipur 302004, Rajasthan, India; ashutosh.pathak87@gmail.com*
[3]*A.N. Bukeikhan Kazakh Research Institute of Forestry and Agroforestry, Shchuchinsk, Kazakhstan; kabanova.05@mail.ru; yana24.ru@mail.ru; 7058613132@mail.ru; vitaliy.kirillov.82@mail.ru (*corresponding author)*

Abstract

In the flora of Kazakhstan there are many medicinal plants, of which the genus *Artemisia* (Asteraceae) includes 81 species. In the current study, chemical composition of essential oil from aerial parts of *Artemisia austriaca* Jacq. collected from different sites of Northern Kazakhstan was determined using GC-MS analysis. The chemical analysis demonstrated that the oil was dominated by oxygenated monoterpenes amounting to 39.49-59.20% with camphor (7.03-20.52%), 1,8-cineole (8.95-13.55%), α-thujone (3.16-25.78%) and β-thujone (0.87-9.92%) as major constituents. The results also suggested that there was difference in composition as well as amount among different sites depending on pH and organic matter of the soil. Further chemometric analysis using hierarchical cluster analysis (HCA) of *A. austriaca* essential oil compositions from the published literature as well as the composition from present study were used in order to demonstrate geographical variations in the composition of the essential oils. It showed the existence of two main clusters: mixture of α- and β-thujones (32.5±21.6%) / 1,8-cineole (13.9±7.8%) (Cluster I) and camphor (40.5±17.4%) / 1,8-cineole (19.4±9.5%) (Cluster II).

Keywords: 1,8-cineole; *Artemisia austriaca*; essential oil; camphor; thujone

Introduction

The Republic of Kazakhstan has a unique stock of medicinal plants of which genus *Artemisia* (Asteraceae) is the most numerous in the flora of Kazakhstan and has 81 species (Flora of Kazakhstan, 1966). There are currently 23 species of wormwood on the territory of Northern Kazakhstan and the chorological structure is dominated by species of the Western Palearctic and Euro-Siberian areal, the most common group is steppe- 10 species (43%), meadow-steppe- 9 species (39%), synanthropic- 4 species (17%) (Bayanova, 2020). Some representatives of the genus *Artemisia* belong to promising plants that have a sufficient raw material base

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for harvesting in Kazakhstan and are easily introduced into culture. In this aspect, a steppe species of wormwood belonging to the Western Palearctic areal and growing in Kazakhstan is of interest i.e., *Artemisia austriaca* Jacq. (Asteraceae) (Figure 1) which is a subdominant of the steppes of the West Kazakhstan region (Tikhomirova and Kenesarina, 2017). *A. austriaca* is a perennial herbaceous plant with 20-60 cm height. The whole plant is whitish because of full thin, silky hairs and it blooms in July-September. It is found in the steppe zone of Kazakhstan, up to the foothills of the Altai (occasionally in the Almaty region); Western Siberia, the European part of Russia, Crimea, the Caucasus; Western Europe, the Western Mediterranean, the Balkans, Asia Minor, Northern Iran. It grows in steppes, floodplains and river valleys, meadows, forest clearings, edges, sands, chalk outcrops, rocky slopes, rocks, deposits, pastures, garbage places, up to the mid-mountain belt, weed, ruderal (Flora of Kazakhstan, 1966). This plant is used as a folk medicine for decongestant, a remedy for insomnia, bruises and elevated body temperature. It is used in the form of tinctures, infusions, decoctions, as a wound-healing, anticonvulsant, analgesic, appetite-stimulating, anthelmintic, for edema and dysmenorrhea (Konovalov and Konovalov, 2006). The plant contains essential oil, sesquiterpene lactones (matricarin, austricin, hydroxyachillin, anhydroaustricin, arborescin, santonin, artemin), flavonoids (cirsilineol, rutin, quercetin, dihydroquercetin, hesperidin, luteolin-7-glycoside) (Kikhanova *et al*., 2013; Adekenov, 2021).

Figure 1. *Artemisia austriaca* Jacq.

The essential oils from *A. austriaca* are not just used as a perfume (Flora of China, 2011), but they also possess antibacterial, protistocidal and antifungal activities (Sokolov, 1993). In study of Konovalov and Konovalov (2005a, b) they have observed that the essential oil extracted from *A. austriaca* showed antimicrobial activity against various pathogenic bacteria like *Mycobacterium tuberculosis* and fungi such as *Candida*. In another report it has been reported that the oil showed positive effect on treatment of inflammatory gynecological diseases related to anaerobic microorganisms *Peptostreptococcus niger*, *P. anaerobius*, *Bacteroides fragilis* and *Prevotella melaninogenicus* (Levachkova *et al*., 2011). Similarly, many studies also reported antimicrobial (Abdelraheem *et al*., 2012; Badekova and Ahmetova, 2020), antifungal (Badea and Delian, 2014; Razavi *et al*., 2014), hepatoprotective (Mercan *et al*., 2008), antioxidant (Altunkaya *et al*., 2014) and phytotoxic (Razavi *et al*., 2014) activities of essential oil. The major components of essential oil from this plant are 1,8-cineole (eucalyptol), camphor and α- and β-thujones. 1,8-cineole is reported to have pharmacological action such as antiinflammation, anticancer, anesthetic, antioxidant, analgesic etc. (Cai *et al*., 2021). Camphor has antimicrobial, antiviral, antioxidant, analgesic and anticancer activities (Bendi *et al*., 2021). Whereas α- and β-thujones have neurotoxic properties, which makes them limited in practical use. However, some studies supported the use of thujones as the main components of drugs with immunomodulatory, antitumor, antidiabetic and antimicrobial activities (Hold *et al*., 2000; Lachenmeier and Walch, 2011; Zámboriné Németh and Thi Nguyen, 2020; Mohamed Abdoul-Latif *et al*., 2021).

It has been noted that in conditions of increased anthropogenic effect, the natural vegetation of the dry steppe is now being displaced by low-eatable and resistant to eating vegetation. A large share in the phytocenosis of agricultural natural lands adjacent to villages located in the natural and climatic zone of the dry steppe of the Akmola region (Northern Kazakhstan) is made up of *A. austriaca* and other species of the family Chenopodiaceae, and due to their bitterness, they are poorly eaten by cattle during the vegetation period (Botabekova *et al*., 2015). It has been noted that the valuable pasture grasses disappear, and their place is immediately taken by *A. austriaca*, being a digression-active species, and under conditions of anthropogenic effects often becomes dominant or subdominant in plant communities, making this species an indicator of the state of pastures and settles where overgrazing has occurred (Kanibolotskaya, 2020). *A. austriaca* plants in disturbed ecosystems can be contaminated with various toxicants, including heavy metals, so this species is an indicator plant species of contamination with chemical elements (Kanibolotskaya, 2020; Chaplygin *et al*., 2020). Despite the widespread distribution of *A. austriaca*, there are not many studies on essential oil. There are various reports which suggested that the chemical composition and content of plant depends on genetic, epigenetic as well as their ecogeographical origin (Srivastava *et al*., 2016; Katakam *et al*., 2019).

Thus, keeping in mind, the aim of this work was- (i) to study the composition of the essential oil of *A. austriaca*, growing wild in Northern Kazakhstan on soils with different pH and organic matter content and (ii) determination the chemical relationship between the various *A. austriaca* essential oil samples by hierarchical cluster analysis (HCA).

Materials and Methods

Plant material

The aerial parts of *Artemisia austriaca* Jacq. were collected from Northern Kazakhstan (Akmola region) in three different places during the flowering time (August-September 2021) (Table 1). The plants were identified by Dr. Anna Ivashchenko at the A.N. Bukeikhan Kazakh Research Institute of Forestry and Agroforestry (Voucher no. 05.09.2021/13).

Table 1. Collection sites for *A. austriaca* in Northern Kazakhstan

Sample No.	Description of collection site	Date of sample collection	GPS coordinates	Altitude (m)	Soil characteristics	
					pH	Organic matter (%)
I	Roadside section of the Kokshetau - Krasny Yar highway, Akmola region. The soil on this place has been transformed by anthropogenic effect, saturated with stony elements. The total projective cover of grass stand - 21-23%, of these *A. austriaca*- 5-6%. Accompanying species: *A. procera* Willd., *A. glauca* Pall. ex Willd., *Stipa* sp. and others. The height of *A. austriaca* - 20-35 cm. The storeyed structure due to sparsity of vegetation cover is practically not expressed.	14.09.2021	53°29'49.6"N 69°32'66.2"E	232	7.80 (Alkaline)	4.78
II	The glade adjacent to the birch forest (*Betula pendula* Roth), Burabay district, Akmola region. Cereal and mixed-grass community with a predominance of *Festuca* sp. and *Artemisia* sp. Edificator- *A. austriaca*, accompanying species- *Festuca sulcata* (Hack.) Beck, *A. absinthium* L. and others. The total projective covers of grass stand- 80-85%, of these *A. austriaca* - 65%. The height of *A. austriaca* - 25-35 cm.	27.08.2021	53°08'96.9"N 70°35'18.1"E	350	6.55 (Neutral)	5.28
III	The glade adjacent to the birch forest (*Betula pendula* Roth), near the settlement of the Zerenda village, Akmola region. Edificator- *A. austriaca*, codominants- *A. dracunculus* L., *A. glauca* Pall. ex Willd., *A. absinthium* L., *F. sulcata* and others. The floristic composition is represented by 12 species. The total projective cover of grass stand- 75-85%, of these *A. austriaca*- 55-65%. The height of *A. austriaca*- 35-45 cm. The storeyed structure is practically not expressed.	27.08.2021	52°89'89.6"N 69°14'17.6"E	396	5.88 (Slightly acidic)	6.04

Soil analysis

Soil analyses were carried out in an accredited laboratory of the Environmental Monitoring Testing Center of EcoLyux-As LLP (Stepnogorsk, Akmola region, certificate of accreditation KZ.T.03.1460 TESTING). The soil solution pH and organic matter content analysed are listed in Table 1. Soil sampling was carried out by the "envelope" method of sampling (Figure S1) (GOST 17.4.3.01-2017, 2018).

At the sample plots, four points were designated at the corners and one in the center, four more small trenchs were made around each point and the surface layer of soil was selected at a depth of up to 10 cm. Prior to the start of soil sampling, the sample plots were cleared of vegetation. The selected point samples were mixed and left in the combined sample. The selected soil samples were air dried, sieved through a sieve (1-2 mm for pH analysis and 0.25 mm for organic matter analysis), and then stored in paper envelopes. The pH of soil solution was measured with a pH meter (pH-150МИ, Russia) (GOST, 26423-85, 2011), whereas the organic matter was determined by oxidation of soil with a solution of potassium bichromate in sulfuric acid and the subsequent determination of trivalent chromium equivalent to the content of organic matter on a photoelectrocolorimeter (KFK-3-01, Russia) (GOST, 26213-91, 1992).

Extraction of the essential oil

The essential oils from dried aerial part of *A. austriaca* were obtained by hydrodistillation using a Clevenger-type apparatus (Clevenger, 1928) for 3 h. The essential oils were dried over anhydrous sodium sulfate and stored in an amber bottle at 4 °C until used. The essential oil content was measured as percentage of the plant dry weight as follows:

$$\text{Essential oil (\%)} = [\text{Essential oil (g)}]/[\text{Biomass (g)}] \times 100 \qquad (1)$$

Qualitative and quantitative analyses

The essential oils were analysed qualitatively and quantitatively by GC-MS on an Agilent Technologies 7890A GC System with an Agilent Technologies 5975C mass selective detector using an HP-5MS column (5% Phenyl Methyl Silox, 30 m × 0.25 mm ID × 0.25 mm film thickness) at carrier gas (He) flow rate 1 mL/min and vaporizer temperature 230 °C. The GC column was held at 40 °C for 10 min with the temperature programmed to 240 °C at 2 °C/min. Then, the temperature was held constant for 20 min and the samples (0.2 μL) were injected with the split ratio 1:100. Mass spectral recording conditions were 70 eV, mass range m/z 10-360. The relative amounts of individual components were calculated based on the GC peak area without correction factors. Identification of the chemical constituents in the analysed essential oils was performed by built-in libraries including software MSD ChemStation, in combination with AMDIS 32 and NIST 2017.

Statistical analysis

A total of 12 *A. austriaca* essential oil compositions from the published literature, as well as the composition from this study were treated as operational taxonomic units (Table S1). Hierarchical cluster analysis (HCA) was done using the TIBCO Statistica, version 14.0.0.15 to evaluate the chemical relationship between the various *A. austriaca* essential oil samples. 1-Pearson r correlation was selected as a measure of similarity, and the Ward's method was used for cluster definition.

Results and Discussion

Yield of the essential oil

In present study, the yield of *A. austriaca* essential oil was found in the range 0.38-0.45% depending on the place of growth in Northern Kazakhstan. The yield of sample I, growing near the road, was 0.40%, whereas in samples II and III, growing at the edges of the forest was 0.38 and 0.45%, respectively. This yield was higher as compared to previous study carried out on *A. austriaca* collected from two different sites of Central Kazakhstan (Auelbekova *et al.*, 2016). They have reported that plants from Ortau mountain system along the road have 0.20% essential oil yield, whereas 0.27% yield was recorded for plants from the mixed forest of the Kesektastinskiy Mountains. The effect of geographical region on essential oil yield has been also documented for plants growing in the conditions of the Northwestern Caspian Sea region (Russian Federation), which averaged 0.30% of the raw mass between year 1991-2000 (Dzhirgalova, 2006a). Similarly, the essential oil yield from *A. austriaca* growing on the lands of the semi-desert Caspian Lowland varied every year: 2001 - 0.20%, 2022 - 0.10%, 2003 - 0.10% and 2004 - 0.20% (Dzhirgalova, 2006b). Further Konovalov (2007) observed variation in essential oil yield and reported that it reached maximum at the end of flower-bud formation (up to 0.46%) and also suggested that the aboveground part of *A. austriaca* with leaves collected during the flower-bud formation is most preferable harvesting material. The highest yield of essential oil (1.10%) was found in *A. austriaca* growing in Iran (Northern Iran) (Delazar *et al.*, 2007; Costa *et al.*, 2009) and the smallest (0.18%) in plants growing in the Russian Federation (Caucasus, Stavropol krai) (Golmov *et al.*, 1948; Konovalov and Konovalov, 2006). Thus, the quantitative content of essential oils in *A. austriaca* significantly fluctuated depending on environmental conditions.

Chemical composition of the essential oil

In present investigation, total 198 compounds were detected of which 101 were identified, which was 59.5-77.83% of the total number of compositions, whereas 97 remained unidentified in three samples of *A. austriaca* collected from different places in Northern Kazakhstan. The chemical compositions are listed in Table 2 according to their retention indices (RI) in the HP-5MS column. Gas chromatograms of the essential oil of the aerial parts from all samples are represented in Figure 2 (A-C). It was observed that the hydrodistillation of the aboveground parts gave blue-green oil which may be due to the presence of the bicyclic sesquiterpenoid chamazulene (0.18-0.59%). It is known that chamazulene is not synthesized by the plant, but is formed during distillation during the thermal decomposition of achillicine and matricine, with the formation of the aromatic azulene system (De Mayo, 1959). Table 3 shows the main components (>1%) present in *A. austriaca* essential oil and it was classified based on their chemical structure.

Table 2. Chemical compositions of essential oil from the aerial parts of *A. austriaca* using GC/MS analysis

No.	Compounds	RI lit	Samples					
			I		II		III	
			RT (min)	%	RT (min)	%	RT (min)	%
1	(E)-1-Butyl-2-methylcyclopropane	790	-	-	5.448	0.01	-	-
2	Mesityl oxide (4-Methyl-3-penten-2-one)	798	-	-	6.054	0.02	-	-
3	5-(1,1-Dimethylethyl)-1,3-cyclopentadiene	833	-	-	6.963	0.07	-	-
4	(E)-2-Hexenal	854	-	-	7.966	0.02	-	-
5	(E)-3-Hexen-1-ol	857	-	-	8.117	tr	-	-
6	Isoamyl acetate	867	-	-	-	-	8.882	0.09
7	2-Methylbutyl acetate	880	8.983	0.01	8.969	0.07	8.969	0.10
8	1-Nonene	889	-	-	9.214	0.02	-	-
9	4-Methylene-5-hexenal	898	-	-	9.452	0.01	-	-
10	Santolina triene	906	9.907	0.04	-	-	-	-
11	Unidentified component	-	10.246	0.06	-	-	-	-
12	Tricyclene	925	-	-	10.267	0.09	10.253	0.08
13	α-Thujene	929	10.534	0.02	-	-	10.549	0.78
14	(+)-α-Pinene	936	10.744	0.93	10.773	5.37	10.758	0.67
15	2,7-Dimethyloxepine	944	-	-	10.902	0.05	-	-
16	1-Methoxy-1,3,5-cycloheptatriene	951	-	-	-	-	10.895	0.01
17	(S)-Camphene	952	11.263	1.55	11.285	2.30	11.278	0.68
18	Dehydrosabinene (2,4(10)-Thujadiene)	956	11.494	0.01	11.516	0.02	11.508	0.02
19	Benzaldehyde	962	11.754	0.02	11.768	0.03	11.761	0.03
20	(+/-)-Sabinene (4(10)-Thujene)	975	12.216	0.10	12.237	0.15	12.223	0.47
21	(-)-β-Pinene	979	12.280	0.26	12.302	0.69	12.295	0.16
22	Unidentified component	-	12.533	0.12	-	-	-	-
23	β-Myrcene	991	12.901	1.29	12.923	1.33	12.908	2.05
24	Unidentified component	-	13.110	0.01	-	-	13.139	0.01
25	Decane	1000	13.182	0.01	13.204	0.01	-	-
26	α-Phellandrene	1005	13.291	0.04	13.312	0.14	13.305	0.22
27	Isobutyl isovalerate	1007	-	-	13.507	0.01	13.495	0.03
28	(E,E)-Hepta-2,4-dienal	1012	-	-	-	-	13.608	0.02

29	α-Terpinene	1015	13.724	0.18	13.745	0.35	13.738	0.44
30	2-Methylbutyl isobutyrate	1016	13.817	0.02	-	-	13.846	0.11
31	1,2,3-Trimethylbenzene	1020	13.889	0.01	13.911	0.09	-	-
32	*p*-Cymene	1025	13.998	0.78	14.027	1.26	14.027	2.46
33	D-(+)-Limonene	1030	14.135	0.10	14.171	0.32	14.149	0.12
34	1,8-cineol (Eucalyptol)	1032	14.250	10.01	14.301	8.95	14.265	13.55
35	Unidentified component	-	14.560	0.03	-	-	14.597	0.06
36	Phenylacetaldehyde	1045	14.683	0.01	14.708	0.01	14.708	0.01
37	(Z)-β-ocimene	1048	14.849	0.01	14.885	0.05	-	-
38	Unidentified component	-	15.015	0.01	-	-	-	-
39	γ-Terpinene	1060	15.167	0.43	15.203	1.71	15.195	0.84
40	Unidentified component	-	15.325	0.01	-	-	-	-
41	4-Terpinenyl acetate	1064	15.477	0.99	-	-	15.499	0.23
42	Unidentified component	-	15.614	tr	-	-	-	-
43	Unidentified component	-	15.967	0.02	-	-	-	-
44	Unidentified component	-	-	-	16.025	0.06	-	-
45	α-Terpinolene	1089	16.141	0.10	16.177	0.23	16.170	0.22
46	Unidentified component	-	-	-	16.271	0.02	-	-
47	Unidentified component	-	16.350	0.01	-	-	-	-
48	Unidentified component	-	16.501	0.89	-	-	-	-
49	Unidentified component	-	16.588	0.08	-	-	16.548	0.26
50	α-Thujone	1103	16.732	3.16	16.704	4.47	16.689	25.78
51	2-Methylbutyl isovalerate	1107	-	-	16.891	0.12	16.877	0.09
52	β-Thujone	1121	17.079	1.05	17.108	0.87	17.115	9.92
53	Unidentified component	-	17.194	0.28	-	-	-	-
54	Unidentified component	-	17.252	0.31	-	-	17.245	0.27
55	Unidentified component	-	17.353	0.18	-	-	17.367	0.18
56	4-Acetyl-1-methylcyclohexene	1137	17.555	0.06	-	-	17.555	0.06
57	Unidentified component	-	-	-	-	-	17.678	0.10
58	Unidentified component	-	-	-	-	-	17.844	1.74
59	(+)-Camphor ((+)-2-Bornanone)	1145	17.988	17.72	17.966	20.52	17.880	7.03
60	Unidentified component	-	-	-	-	-	18.089	0.09
61	Unidentified component	-	-	-	-	-	18.132	0.08
62	Unidentified component	-	18.341	0.10	18.334	0.10	18.298	0.10
63	Sabine ketone	1156	-	-	-	-	18.385	0.02
64	Unidentified component	-	-	-	-	-	18.456	0.10
65	Unidentified component	-	18.507	2.35	-	-	-	-
66	Unidentified component	-	18.565	4.30	-	-	-	-
67	endo-Borneol	1167	18.652	4.84	-	-	18.616	0.49
68	Unidentified component	-	-	-	-	-	18.659	0.38
69	Unidentified component	-	-	-	-	-	18.897	0.35
70	Terpinen-4-ol	1182	18.976	2.21	18.947	4.52	18.976	2.29
71	α-Thujenal	1190	19.214	0.16	19.258	0.22	19.207	0.25
72	α-Terpineol	1198	-	-	-	-	19.294	0.14
73	Unidentified component	-	19.380	1.11	19.431	1.24	19.373	0.80
74	Unidentified component	-	-	-	-	-	19.517	0.24
75	Unidentified component	-	19.553	1.19	-	-	19.705	0.05
76	γ-Terpineol	1203	19.878	0.50	19.835	1.41	19.877	0.14
77	Unidentified component	-	19.958	0.16	-	-	-	-
78	(-)-Carveol	1225	20.203	0.35	20.160	0.33	20.203	0.15

79	*m*-Cumenol	1227	-	-	20.340	0.02	-	-
80	*p*-Cumenol	1227	20.463	0.13	-	-	20.463	0.09
81	Unidentified component	-	-	-	20.482	0.12	-	-
82	Unidentified component	-	-	-	20.571	0.14	20.549	0.07
83	Unidentified component	-	20.607	0.10	20.636	0.04	-	-
84	Unidentified component	-	-	-	-	-	20.730	0.03
85	Cuminaldehyde	1239	20.802	0.13	-	-	20.809	0.15
86	Unidentified component	-	20.888	0.03	-	-	-	-
87	Carvone	1244	20.924	0.04	20.953	0.12	20.939	0.06
88	(+)-Carvotanacetone	1246	-	-	-	-	21.047	0.02
89	Piperitone (3-Carvomenthenone, p-Menth-1-en-3-one)	1253	21.227	0.11	21.256	0.33	21.235	0.21
90	Unidentified component	-	-	-	-	-	21.336	0.06
91	Chrysanthenyl acetate	1256	-	-	21.379	2.40	-	-
92	Unidentified component	-	21.422	0.42	-	-	21.429	0.13
93	Unidentified component	-	21.696	0.05	-	-	-	-
94	Unidentified component	-	21.797	0.11	-	-	-	-
95	Phellandral	1276	-	-	-	-	21.826	0.04
96	Unidentified component	-	21.971	0.02	21.992	0.03	21.978	0.03
97	Bornyl acetate	1281	22.122	0.46	22.144	0.55	22.129	0.10
98	Unidentified component	-	22.281	0.81	22.288	0.52	22.310	0.83
99	3,4-Diethylphenol	1284	-	-	22.389	0.04	-	-
100	Cumic alcohol (*p*-Cymen-7-ol)	1295	-	-	-	-	22.461	0.04
101	Carvacrol	1299	22.533	0.19	22.555	0.24	22.548	0.34
102	Undecanal	1310	22.678	0.10	-	-	22.685	0.07
103	2,4,6-trimethylbenzaldehyde (Mesitaldehyde)	1323	-	-	22.966	0.05	-	-
104	Unidentified component	-	23.204	0.07	23.219	0.04	-	-
105	Unidentified component	-	23.284	0.01	-	-	-	-
106	(E,E)-2,4-Decadienal	1327	-	-	-	-	23.937	0.06
107	Unidentified component	-	-	-	-	-	23.981	0.08
108	Unidentified component	-	-	-	-	-	24.111	0.03
109	Unidentified component	-	-	-	24.298	0.02	24.291	0.07
110	Unidentified component	-	24.435	0.02	-	-	-	-
111	Unidentified component	-	24.529	0.01	-	-	-	-
112	Unidentified component	-	-	-	24.609	0.02	-	-
113	Unidentified component	-	24.652	0.06	-	-	-	-
114	Unidentified component	-	-	-	-	-	24.767	0.03
115	Unidentified component	-	24.839	0.03	24.861	0.06	24.847	0.43
116	Eugenol	1357	25.049	0.08	25.078	0.61	25.056	0.20
117	(E,Z)-Nepetalactone	1365	25.178	0.04	-	-	25.180	0.08
118	(+)-Cyclosativene	1370	25.323	0.03	-	-	-	-
119	(-)-α-Copaene	1376	25.568	0.29	25.583	0.14	25.575	0.07
120	Unidentified component	-	25.676	0.01	-	-	-	-
121	Unidentified component	-	-	-	25.727	0.03	-	-
122	(-)-β-Bourbonene	1384	25.813	0.11	25.828	0.11	25.821	0.08
123	Unidentified component	-	-	-	-	-	25.936	0.15
124	β-Elemene	1391	25.987	0.43	-	-	26.001	0.60
125	(Z)-Jasmone	1394	-	-	26.181	0.08	26.174	0.14
126	Methyleugenol	1402	26.282	0.44	26.290	0.51	26.282	1.02

127	Unidentified component	-	-	-	26.586	0.04	26.578	0.03
128	β-Caryophyllene	1419	26.715	0.32	26.737	0.65	26.730	0.33
129	β-Copaene	1432	26.961	0.04	26.968	0.05	26.971	0.03
130	Aromandendrene	1440	27.343	0.16	27.220	0.02	-	-
131	Unidentified component	-	-	-	27.350	0.03	-	-
132	Unidentified component	-	27.502	0.03	-	-	-	-
133	Humulene	1454	27.588	0.10	27.596	0.10	27.596	0.04
134	Alloaromadendrene	1461	27.769	0.14	27.716	0.03	27.747	0.17
135	Unidentified component	-	27.928	0.55	27.942	0.30	27.993	0.06
136	Unidentified component	-	28.007	0.06	28.029	0.09	-	-
137	Unidentified component	-	28.122	0.21	28.144	0.15	28.122	0.33
138	Unidentified component	-	-	-	-	-	28.180	0.11
139	Germacrene D	1481	28.281	2.63	28.303	2.43	28.281	0.73
140	(+)-β-Selinene (β-Eudesmene)	1486	28.411	0.91	28.425	0.57	28.411	1.08
141	(E)-α-Bergamotene	1491	-	-	28.541	0.07	-	-
142	Capillene	1501	28.627	1.73	28.671	0.78	28.656	0.38
143	(-)-γ-Himachalene	1505	28.728	0.07	28.736	0.06	28.728	0.04
144	β-Bisabolene	1509	28.894	0.32	28.902	0.18	28.894	0.22
145	(+)-γ-Cadinene	1513	29.075	0.05	29.082	0.06	29.075	0.05
146	γ-Muurolene	1515	29.111	0.03	-	-	-	-
147	Unidentified component	-	-	-	29.169	0.02	-	-
148	Cadina-1(10),4-diene ((+)-δ-Amorphene)	1524	29.277	0.29	29.284	0.15	29.284	0.07
149	Unidentified component	-	29.529	0.06	-	-	-	-
150	α-Muurolene	1538	-	-	29.630	0.01	-	-
151	Unidentified component	-	-	-	29.703	0.03	-	-
152	Unidentified component	-	29.897	0.13	-	-	-	-
153	Unidentified component	-	-	-	29.977	0.03	-	-
154	Unidentified component	-	-	-	-	-	30.056	0.30
155	Unidentified component	-	30.193	0.54	30.200	0.29	30.193	0.25
156	Unidentified component	-	30.460	0.06	30.467	0.11	-	-
157	Unidentified component	-	30.612	1.03	30.619	0.58	30.604	0.49
158	Unidentified component	-	30.749	0.87	30.736	0.58	-	-
159	9,10-Dehydro-isolongifolene	1558	30.842	0.31	30.843	0.10	-	-
160	Unidentified component	-	-	-	-	-	30.929	0.15
161	Unidentified component	-	-	-	-	-	30.980	0.12
161	Unidentified component	-	31.153	0.10	-	-	-	-
162	Unidentified component	-	31.232	0.46	31.239	0.16	-	-
163	Unidentified component	-	-	-	31.390	0.24	31.384	0.15
164	Unidentified component	-	31.766	0.56	-	-	31.752	0.06
165	Unidentified component	-	31.874	0.22	31.860	0.28	31.845	0.37
166	Unidentified component	-	31.968	0.52	-	-	31.961	0.26
167	Unidentified component	-	-	-	-	-	32.040	0.18
168	Unidentified component	-	32.170	0.34	32.177	0.16	32.163	0.08
169	Unidentified component	-	-	-	32.293	0.38	32.278	0.67
170	Unidentified component	-	32.372	1.20	32.372	0.28	32.365	0.60
171	Unidentified component	-	-	-	-	-	32.473	0.44
172	Unidentified component	-	32.574	0.14	-	-	-	-
173	Unidentified component	-	32.726	0.91	32.726	0.41	32.726	0.30
174	Unidentified component	-	32.978	2.54	32.978	0.56	32.964	0.23
175	Unidentified component	-	-	-	-	-	33.072	0.14

176	Unidentified component	-	-	-	-	-	33.137	0.57
177	Unidentified component	-	-	-	33.231	0.08	-	-
178	Unidentified component	-	33.332	0.08	33.325	0.06	-	-
179	6-Methyl-2-(4-methylcyclohex-3-en-1-yl)hepta-1,5-dien-4-ol	1614	33.418	0.16	33.426	0.06	-	-
180	Tetradecanal	1617	-	-	-	-	33.563	0.18
181	Unidentified component	-	33.772	0.70	33.772	0.10	33.772	0.06
182	Unidentified component	-	-	-	33.851	0.05	33.851	0.10
183	Chamazulene	1725	34.024	0.33	34.025	0.18	34.025	0.59
184	Unidentified component	-	-	-	34.118	0.09	-	-
185	Unidentified component	-	34.356	0.23	34.356	0.34	34.465	0.12
186	Unidentified component	-	-	-	-	-	34.825	0.22
187	Methyl isocostate	1792	35.157	0.12	35.164	0.10	-	-
188	Unidentified component	-	-	-	-	-	35.359	0.02
189	Hexadecanal	1817	35.720	0.18	35.727	0.05	-	-
190	Gerany-*p*-cymene	1951	38.652	0.37	37.548	0.10	37.505	0.22
191	Eicosane	2000	39.684	0.16	-	-	-	-
192	Heneicosane	2100	40.182	0.15	-	-	40.182	0.09
193	Phytol	2116	-	-	-	-	40.449	0.09
194	Docosane	2200	41.899	0.12	-	-	-	-
195	8-n-Hexylpentadecane	2245	43.595	0.32	-	-	-	-
196	Tricosane	2300	43.660	0.19	-	-	43.667	-
197	Tetracosane	2400	45.276	0.40	-	-	-	-
198	Pentacosane	2500	-	-	-	-	47.174	0.40
Total				83.66		74.67		90.99

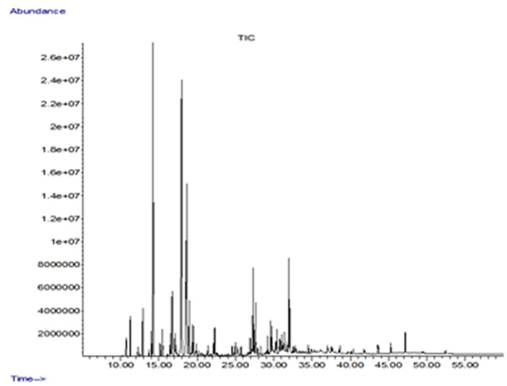
RI lit: Retention index from literature data

RT: Retention time

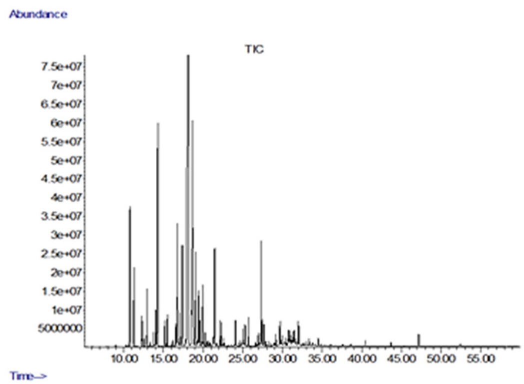
%: Calculated from TIC data

Table 3. Main compounds (>1%) present in the essential oils of *A. austriaca*

No.	Compound	Composition (%)						Groups of components
		I		II		III		
1	α-Pinene	0.93	4.98	5.37	11.97	0.67	6.70	Monoterpenes
2	Camphene	1.55		2.30		0.68		
3	β-Myrcene	1.29		1.33		2.05		
4	*p*-Cymene	0.78		1.26		2.46		
5	γ-Terpinene	0.43		1.71		0.84		
6	1,8-cineole (Eucalyptol)	10.01	39.49	8.95	40.74	13.55	59.20	Oxygenated monoterpenes
7	α-Thujone	3.16		4.47		25.78		
8	β-Thujone	1.05		0.87		9.92		
9	(+)-Camphor	17.72		20.52		7.03		
10	endo-Borneol	4.84		-		0.49		
11	Terpinen-4-ol	2.21		4.52		2.29		
12	γ-Terpineol	0.50		1.41		0.14		
13	Germacrene D	2.63	3.54	2.43	3.00	0.73	1.81	Sesquiterpenes
14	(+)-β-Selinene	0.91		0.57		1.08		
15	Capillene	1.73	2.17	0.78	3.69	0.38	1.40	Others
16	Methyleugenol	0.44		0.51		1.02		
17	Chrysanthenyl acetate	-		2.40		-		
Total		50.18		59.40		69.11		



(A)



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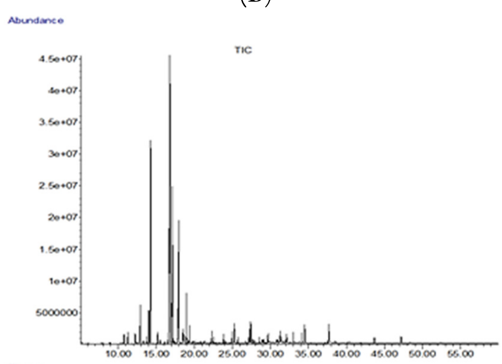

(C)

Figure 2. Gas chromatograms of the essential oil of the aerial parts of *A. austriaca*: (A) sample I; (B) sample II; (C) sample III

The essential oil of all three samples was rich in oxygenated monoterpenes (39.49-59.20%). In samples I-III, the predominant components were camphor (7.03-20.52%) and 1,8-cineole (Eucalyptol, 8.95-13.55%), whereas α-thujone (25.78%) and β-thujone (9.92%) were majorly present in sample III. Monoterpenes were present in smaller amounts (4.98-11.97%) with predominance of α-pinene (5.37%) in sample II. Germacrene D prevailed among sesquiterpenes (0.73-2.63%) whereas terpene ester chrysanthenyl acetate (2.40%) was only recorded in sample II. Similarly, oxygenated monoterpenes were the major constituents of the essential oils in *Rosa damascena* (Yassa *et al*., 2009; Ghavam, 2021).

The result of present study was compared with previous reports on extraction of essential oils by hydrodistillation from aboveground parts (Table S1). It was observed that except samples from Zhytomyr (Ukraine) (Ivashchenko *et al*., 2014), Stavropol (Russia) (Golmov *et al*., 1948) and Miyaneh (Iran) (Jafari *et al*., 2018), all other samples indicated presence of camphor as the main component of the oils. The main components of essential oil detected in present investigation were camphor (15.09%), α-thujone (11.14%), 1,8-cineole (10.84%) and β-thujone (3.95%). Previous report on same plant from the Almaty region (South-East of Kazakhstan) documented similar compounds only the amount varied i.e., thujone (31.50%), camphor (20.50%) and 1,8-cineole (16.20%) as the main components, (Goryaev *et al*., 1967). In earlier reports from samples collected from Russia (Golmov *et al*., 1948; Konovalov and Konovalov, 2006; Velikorodov *et al*., 2011) and Iran (Miyaneh) (Jafari *et al*., 2018) also reported presence of thujones (α-thujone and β-thujone). Whereas essential oil extracted from aerial parts during the flowering phase (August-September) from *A. austriaca* growing in Tabriz (Iran) showed the presence of camphor, 1,8-cineole, camphene and others, but thujones were completely absent. However, α-thujone was detected in volatile organic compounds released from the flowers only and not in the leaves, and the content of β-thujone was 20 times less in the leaves than flowers. Thus, it can be concluded that raw materials without the presence of thujones or their minimum content in the essential oil of *A. austriaca* is during the vegetation period (June-July) (Delazar *et al*., 2007). Later on, in another report from same place on volatile organic compounds released from leaves and flowers of *A. austriaca*, using the modified pencil lead as a fibre of solid phase microextraction showed same main components. The flowers (during flowering- September) (in descending order) contained β-thujone, camphor, 1,8-cineole, carvacrol, α-thujone, α-terpinolene, camphene, γ-terpinene, pinocarvone, carvenone and myrtenol; whereas leaves (during vegetation period- early June) showed presence of camphor, 1,8-cineole, camphene, β-thujone, α-terpinolene, borneol and carvacrol (Torbati *et al*., 2016).

Chemometric analysis using hierarchical cluster analysis (HCA) is a technique that measures either the difference or the similarity among the samples to be clustered. According to literature there are only 13 essential oil compositions of *A. austriaca* growing in 6 countries. The percentage composition of 67 essential oil components (2-methylbutan-1-ol, α-pinene, camphene, α-thujene, β-myrcene, *o*-cymene, *p*-cymene, 1,8-cineole (eucalyptol), γ-terpinene, α-thujone, β-thujone, (+)-camphor [(+)-2-bornanone], borneol, terpinen-4-ol, γ-terpineol, germacrene D, chrysanthenyl acetate, chamazulene, sabinene, β-pinene, α-terpinene, trans-sabinene hydrate, α-terpinolene, amyl-isovalerate, isoamyl isovalerate, β-fenchyl alcohol, α-terpinyl acetate, copaene, β-eudesmol, 1,6-octadecadiene oxide, β-caryophyllene, α-humulene, carvone, carveol, caryophyllene oxide, humulene epoxide, spathulenol, patchoulane, pinocarvone, myrtenol, *trans*-pinocarveol, *cis*-sabinene hydrate, α-terpineol, *cis*-β-terpineol, isoborneol, bornyl acetate, r-elemene, *trans*-pinocarvyl acetate, thujyl alcohol, thujyl acetate, phellandrene, *cis*-sabinol, menthone, bornane, aromadendrene, β-selinenol, *trans*-ocimene, verbenole, *p*-mentha-1,5-dien-8-ol, sabinil acetate, β-selinene, capillene, methyleugenol, *p*-cymol, limonene, 1-octen-3-ol and eugenol) was used to determine the chemical relationship between the various *A. austriaca* essential oil samples. The dendrogram was formed based on relationship between the samples (Figure 3). They were grouped according to the similarity of the predominant chemical components (cluster analysis) into two clusters: cluster I formed by samples Ua-1, Kz-1, Kz-2, Ru-1, Ru-2 and Ir-4 and cluster II consisting of samples Ro-1, Ru-3, Tr-2, Tr-1, Ir-2, Ir-1 and Ir-3. HCA allowed classifying samples into two main clusters: mixture of α- and β-thujones (32.5±21.6%) / 1,8-cineole (13.9±7.8%) (cluster I) and camphor (40.5±17.4%) / 1,8-cineole (19.4±9.5%) (cluster II). Oxygenated monoterpenes like camphor, α- and β-thujones and 1,8-cineole were responsible for the characterization of the two clusters due to their high percentage values, which suggested that they were main components. In cluster I, the Ua-1 sample is allocated to a separate group, since the predominant components in this sample are *trans*-verbenole (30.77%), sabinil acetate (18.16%), *trans*-ocimene (12.10%) and pinocarvone (10.77%), which are not found in other samples. On the contrary, camphor is not detected in this sample and a mixture of α- and β-thujones and 1,8-cineole were detected at concentrations of 0.72 and 7.31%, respectively. Albeit the samples were collected from different places of Kazakhstan (South-East and North) with over a period of 50 years, they showed great similarity in the chemical composition of essential oils and are in the same group of clusters I (Figure 3).

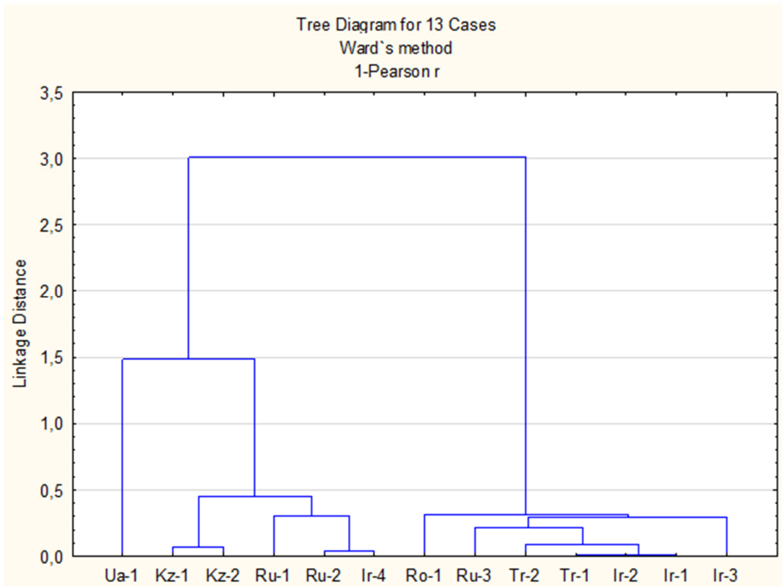

Figure 3. Dendrogram obtained by hierarchical cluster analysis (HCA) for essential oils extracted from *A. austriaca* collected from different regions, obtained by the Ward's method and 1-Pearson r measure

Another factor which is known to affect the essential oils biosynthesis in plant is the soil characteristics which take part in the biochemical reactions in plants and ultimately affects the essential oil composition (Habibi *et al*., 2007; Vaičiulytė *et al*., 2017; Feng *et al*., 2021). The observation of present study also revealed that there was significant difference in the quality as well as in the quantitative ratio of components of the essential oil within the same region. Figure 4 shows that with a decrease in the pH of the soil solution and an increase in the content of soil organic matter, the percentage of the main components of essential oils particularly oxygenated monoterpenes was increased.

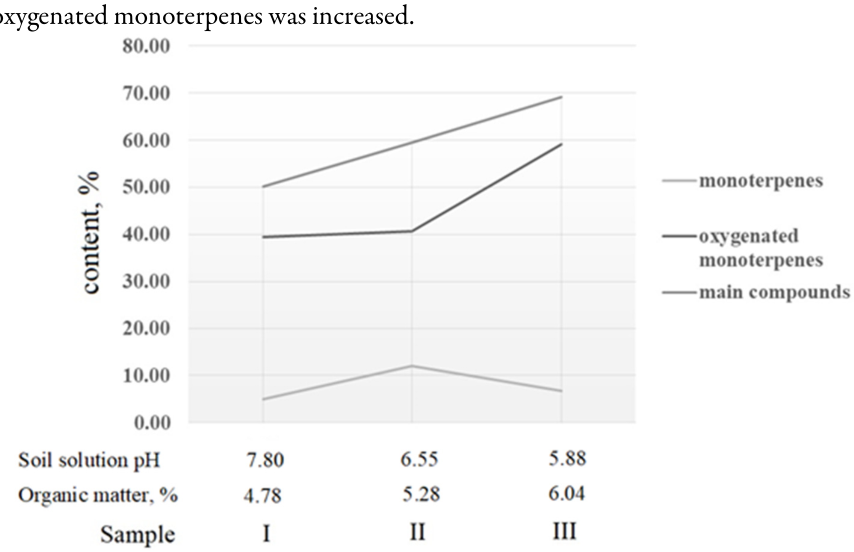

Figure 4. Dependence of the content of the main components of *A. austriaca* essential oil samples, including monoterpenes and oxygenated monoterpenes, on pH and organic matter of soil

Within the same region and same climatic conditions, but with different pH values and organic matter content, samples I and II by the composition of the predominant components of essential oil can be attributed to cluster II, and sample III to cluster I. In present study, the highest yield of essential oil (0.45%) was observed on soil richer in organic matter and lower pH for sample III and the qualitative composition of essential oil differed due to an increase in the content of α- and β-thujones by 20 and 9%, respectively, and a decrease in the camphor by 10-13% compared with samples I and II. In corroboration with present study, the soils richer in organic matter and minerals produced higher essential oil but with less quality in *Lavandula latifolia* (Fernández-Sestelo and Carrillo, 2020), whereas soil texture and K_2O content are correlated with some components of *Salvia desoleana* essential oil (Rapposelli *et al*., 2015). It has been reported that the high organic content of the soil leads to desirable water retention which enhances root growth and the gradual release of nitrogen increases its uptake and it ultimately increase the essential oil synthesis (El-Gohary *et al*., 2015).

Conclusions

In this work, GS-MS analysis and chemometric methods was carried out to compare the essential oils of *A. austriaca* from three different sites of Northern Kazakhstan. It was found that majority of volatiles belonged to the oxygenated monoterpenes classes. The oils of the plant are dominated by camphor (17.72%) and 1,8-cineole (10.01%) in sample I, camphor (20.52%) and 1,8-cineole (8.95%) in a sample II, and α-thujone (25.78%) and 1,8-cineole (13.55%) in a sample III. The results suggested that the difference in quantity of essential oil will help in identification of elite plant material for harvesting as well as on breeding programmes. The selection of camphor, 1,8-cineole and α, β-thujone as chemical marker for quality control and standardization will be useful as multimarker approach will give more reliability and robustness for quality assurance.

Authors' Contributions

O.S.: collection of plant material samples, description of collection sites, methodology, data analysis; A.P. and V.K.: methodology, literature review, writing and editing the manuscript; S.K., Y.K. and A.K.: planning of the research, literature review, participation in the experiment of this study, data analysis. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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