Chemical Composition and Biological Activities of Essential Oils from *Alyssoides utriculata* (L.) Medik

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The chemical compositions, antioxidant activities, and antimicrobial activities of the essential oils acquired from the separated parts of air-dried flowers, leaves, and stems of *Alyssoides utriculata* L. plant growing in Turkiye were determined. Three volatile oil components were acquired *via* hydrodistillation using a Clevenger apparatus and analyzed by the Gas Chromatography-Mass spectrometry/Flame Ionization Detection (GC-MS/FID) analysis. A total of 75, 67, and 76 compounds in the volatile oils of flower, leaves, and stem of *A. utriculata* were identified, respectively. The highest percentage of chemical compounds in the essential oils of *A. utriculata* were determined to be monoterpenes in flowers and leaves, (72.4% and 66.5%) and hydrocarbons (29.2%) in stems. While *α*-pinene (62.5% and 46.7%) was defined as the major compound in the flowers and leaves, nonane (21.2%) was determined to be so in the stem essential oil. The antioxidant activity of the obtained essential oils was determined according to free radical scavenging and total phenolic content (TPC), and antimicrobial activity against 12 bacteria and 5 fungi, using the agar dilution method. The amount of TPC and scavenging activity of the flower oil were found to be 440.61 ± 6.26 mg GAE/L and 46.00 ± 1.28%, respectively. Based on the antimicrobial activity results, all the essential oils of *A. utriculata* were determined to have antimicrobial activity against *Escherichia coli* and *Bacillus subtilis.*

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Keywords: Essential oil; Chemical composition; Antioxidant activity; Antimicrobial activity; α-Pinene; Alyssoides utriculata

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INTRODUCTION

Since the beginning of humanity, human beings have been using plants to meet their basic needs such as nutrition, treatment, and warmth. In complementary medicine for treatment purposes, whole plants, including leaves, roots, and flowers, are used, as well as various extracts and essential oils obtained from them (Al Abboud *et al*. 2024; Alghonaim *et al.* 2023). The Brassicaceae (also known as Cruciferae) family has economic, agricultural, nutritional, and medicinal qualities (Preedy 2015). Foods, such as cabbage, broccoli, Bok-choy, and mustard, which have an important place in daily nutrition, are from the Brassicaceae family and contain glucosinolates, minerals, carotenoids, soluble sugars, polyphenols, vitamins, and antioxidant compounds (Preedy 2015; Luo *et al*. 2022). Members of Brassicaceae are widely used in traditional medicine and as veterinary medicines for livestock (Salehi *et al*. 2021).

The popularity and consumption of vegetable Brassicaceae family members are increasing due to their nutritional value and biological effects. Their phytochemical

composition has been studied, as they contain valuable secondary metabolites, such as glucosinolates, phenolic compounds (hydroxycinnamic acids, flavonoids, anthocyanins, tocopherols, and carotenoids), terpenes, and fatty acids, which are directly linked to different biological activities (Favela-González *et al*. 2020). Brassicaceae metabolites are used in the treatment of chronic diseases such as obesity, type-2 diabetes, stroke, hypertension, and cancer (Favela-González *et al*. 2020). Previous studies have reported that the essential oils and extracts of Brassicaceae species are rich in glucosinolates and that they have biological activities such as anticancer, anti-inflammatory, antimicrobial, anti-obesity, cardioprotective, gastroprotective, and antioxidant activities (Favela-González *et al*. 2020; Salehi *et al*. 2021). They are also known to contain high amounts of carotenoids, tocopherol, and ascorbic acid, which have antioxidant effects (Singh *et al*. 2017).

The genus *Alyssoides* Mill., a member of the Brassicaceae family, is represented by two species (The Plant List 2013). *Alyssoides* is morphologically similar to the genus *Physoptychis* Boiss. according to Flora of Turkey (Cullen 1965) and distinguished from *Physoptychis* with less than 10 mm fruit diameter. According to phylogenetic-based studies, the genus *Alyssoides* is not monophyletic, and the members of the genus are grouped with members of *Fibigia* Medik. *Alyssoides utriculata* (L.) Medik is the only species of the genus found in Turkiye, and it is a yellow-flowered ornamental shrubby plant native to the country (Cullen 1965). This species is both an ornamental plant and used in some forms of treatment (rabies and hiccup) (Blazevic *et al*. 2013). *Alyssoides utriculata* var. *utriculata* is the only member of *Alyssoides utriculata* at the variety level in the Flora of Turkey (Cullen 1965; Mutlu 2012).

Essential oils are complex mixtures of low concentrations derived from different parts of plants and evaporate easily at room temperature (Fidan *et al*. 2022). The essential oils exhibit refreshing, pain-relieving, stress-relieving, insecticidal, antimicrobial, antifungal, and antioxidant activities and are used in the food preservation and cosmetic industries (Polatoğlu *et al*. 2013; Yılar *et al*. 2016; Cüce and Basançelebi 2021; Saruhan and Oz 2023). It has been reported that that the essential oils of *Brassicaceae* family members contain interesting natural phytochemicals such as allyl isothiocyanate (*B. juncea, B, nigra*), 1-butene-4-isothiocyanate (*B. juncea*, *B. napus*), benzyl isothiocyanate and 2-phenylethyl-isothiocyanate (*Sinapis alba*) as sulfur-containing compounds, hexahydrofarnesyl acetone (*Arabis alpina*, *Eruca vesicaria*), pulegone, isomenthone (*B. campestris*), phytol (*Capsella bursa-pastoris*), and *β*-elemene as terpene derivatives, and 2,6,10-trimethyldecane, nonacosane (*Arabis alpina*, *Capsella bursa-pastoris*) as hydrocarbons (Singh *et al*. 2015; Hichri *et al*. 2016; Salehi *et al*. 2021; Ucuncu 2021; Gumusok *et al*. 2023).

There is only one study in the literature on the essential oils and biological activities of *A. utriculata*. Blazevic *et al*. (2013) investigated the chemical composition of the essential oil obtained from *A. utriculata* and the acetyl cholinesterase activities of dichloromethane extracts. In the gas chromatography-mass spectrometry (GC/MS) analysis of essential oils of different parts of *A. utriculata*, chemical compounds belonging to the compound classes alcohols, carbonyls, alkanes, sulfur compounds, terpenes, fatty acids and esters, phenols, and phenylpropane derivatives were detected. According to this report, compounds such as but-3-enyl isothiocyanate, erucin, and sulforaphane, which are glucosinolates degradation products, are responsible for the acetylcholinesterase activity exhibited by the essential oil and extracts (Blazevic *et al*. 2013).

The chemical compositions and biological activities of *A. urticulata* with respect to individual parts of the plant, which may be important towards potential use, have not been explored. The goal of the present research is to determine the chemical compositions of essential oils in the air-dried parts (flower, leaf, and stem) of *A. utriculata*, which can be considered as a member of the Brassicaceae family, and has been subject to limited studies, and to investigate their antimicrobial and antioxidant capacities.

EXPERIMENTAL

Plant Materials

Alyssoides utriculata plant was collected from the roadsides between Torul and Kürtün, Gümüşhane: (40° 38' 30" N, 39° 11' 39" E at 800 m above sea level) in Turkiye (A7), a location with dry air and sandy soil, during June 2022. Flowers, leaves, and stems of *A. utriculata* were separated and air-dried at room temperature (20 to 22°C). The botanical identification of the plant was carried out by Prof. Kamil Coşkunçelebi in the Department of Biology, at Karadeniz Technical University (KTU), Trabzon, Turkiye. Voucher specimens were deposited with the number KTUB743 in the Herbarium of KTU.

Separation and Analysis of the Essential Oils

The volatile oils from air-dried plant parts (flower – 85 g, leaves – 54 g, and stems – 124 g) of *A. utriculata* were isolated using a modified Clevenger-type hydrodistillation apparatus (4 h, yields: 0.24%, 0.19%, and 0.08 % (w/w), respectively) (Ucuncu *et al*. 2019). Hydrodistillation for each sample was carried out three times, and the average value of the essential oil percentage (w/w) was used for the final evaluation and was detected on an air-dried weight basis. Essential oil yields were calculated with the following Eq. 1 (Fidan *et al*. 2022):

$$Yield\ (\%) = \frac{(\text{Amount of extracted essential oil (g)})}{(\text{Amount of air-dried plant material (g)})} \times 100 \quad (1)$$

The essential oils obtained from the air-dried plants were taken by dissolving in 1.0 mL high-performance liquid chromatography (HPLC) grade n-hexane, dried over anhydrous sodium sulfate, and filtered (Ucuncu *et al*. 2019; Fidan *et al*. 2022).

A HP-5MS capillary chromatographic apolar column (film thickness 0.2 µm 30 m × 0.25 mm ID) was used for GC-FID (Agilent-7890A) and GC-MS (Agilent 5975C) analyses. These analyses were employed as described previously (Ucuncu *et al*. 2019; Fidan *et al*. 2022; Oz 2022).

The essential oils were analyzed twice. The GC peak areas of essential oil compounds were clarified by comparing the NIST and Willey libraries in the GC-MS device. The retention indices (RI) of components were determined through the retention times (RT) of homolog n-alkanes (C_6-C_{32}) and authentic compounds with linear interpolation. Identification of volatile compounds was determined by matching their RI values with NIST and Willey library data, comparing Kovats indices (KI) and literature value. Quantitative determination of components was performed with regard to peak area integration with GC-FID (Adams 2007; Ucuncu *et al*. 2019; Fidan *et al*. 2022; Oz 2022; Chemdata NIST 2023).

DPPH Assay and the Total Phenolic Content

The antioxidant activities of essential oils were determined by free radical scavenging capacity (DPPH assay), the most frequently used antioxidant/antiradical test, and by total phenolic contents (TPC) analysis, which shows not only total phenolic content but also total reducing capacity of the sample and is widely accepted as an antioxidant test (Sanchez-Moreno *et al*. 1998; Kasangana *et al*. 2015).

The free radical scavenging activities of volatile oils against stable 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH·) were spectrophotometrically determined. For this purpose, a DPPH solution prepared with 4 mL of 0.1 mM methanol was added to the volatile oils of *A. utriculata.* The change in color was measured at 517 nm on a UV-Vis spectrophotometer (Libra S60, Biochrom Ltd, Cambridge UK). The measurements were performed three times, and averaged. Trolox and ascorbic acid were used as standard antioxidants for comparison (Sağdıç *et al*. 2011; Ahmed *et al*. 2015).

The TPC amounts of volatile oils were determined by the Folin-Ciocalteu method. For this purpose, the absorbances of the samples were measured at 765 nm. TPC in essential oils were expressed as gallic acid equivalents (GAE) according to the method described previously (Ucuncu *et al*. 2019). The measurements were performed three times, and averaged (Kasangana *et al*. 2015).

Antimicrobial Activity Assessment

The antimicrobial activities of essential oils were determined using the agar-well diffusion method against 12 bacteria and 5 yeast samples. The antimicrobial tests were made in Gümüşhane University Food Engineering Laboratories. For this purpose, the samples of volatile oils were dissolved in HPLC-grade n-hexane to prepare stock solutions. Measurements were made according to previously described methods (Sağdıç and Özcan 2003; Matuschek *et al*. 2014). The results were expressed as inhibition zones (mm) of test microorganisms. The results of antimicrobial activity are given in Table 3.

Statistical Analysis

Statistical analyses were performed using Microsoft Excel software with XLSTAT (Addinsoft, Version 2024 New York, NY, USA). The consistency of measurements across the analysis was assessed using the relative standard deviation of repeatability (RSDr%) and the predicted relative standard deviation (PRSDr%).

RESULTS AND DISCUSSION

Chemical Composition of Essential Oils

The results of GC-MS and GC-FID analyses performed to determine the chemical compositions of essential oils of *A. utriculata* are given in Table 1, and their chemical class distributions are presented in Fig. 1. About 119 compounds were identified, constituting over 92.77%, 87.66%, and 86.26% total essential oil compositions of flowers, leaves, and stems of *A. utriculata*, respectively. The identified compounds are divided into 10 groups: alcohols, carbonyl compounds, fatty acids, hydrocarbons, monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, diterpene, and ‘other’.

Table 1. Identified Components and Chemical Class Distribution in the Essential Oils of the Aerial Parts (Flower, Leaf, and Stem) of *A. utriculata*

No.	Compounds	Concentration (%)			Exp. RI [b]	N. RI [c]
		Flower [a]	Leaf [a]	Stem [a]		
	Alcohols					
1	1-Octen-3-ol			0.20	977	976
2	(*E*)-2-Decen-1-ol			0.28	1270	1273
3	2-Methyl-Z,Z-3,13-octadecadienol			0.96	2145	MS
	Carbonyl Compounds					
4	Hexanal			0.66	801	800
5	2-(*E*)-Hexenal	0.11	0.42	0.09	854	854
6	2-(*E*)-Heptenal			0.16	959	961
7	Benzaldehyde			0.08	962	966
8	2,3-Octadienone			0.09	982	986
9	6-Methyl-5-hepten-2-one	0.03	0.05	0.15	987	987
10	Octanal			0.11	1002	1001
11	3-Octen-2-one			0.16	1039	1037
12	Benzeneacetaldehyde	0.05			1044	1043
13	Acetophenone[d]	0.38	0.56	0.49	1066	1065
14	2-Nonanone	0.12			1093	1095
15	Nonanal	0.06	0.03	0.42	1105	1102
16	(*E*)-2-Nonenal			0.36	1160	1159
17	2-Methyl-3-phenyl-propanal	0.05	0.06		1241	1244
18	(*E*)-2-Decenal			0.23	1262	1261
19	2-Undecanone	0.06			1294	1294
20	(*E*,*E*)-2,4-Decadienal			0.01	1317	1317
21	(*E*)-2-Undecenal			0.44	1364	1364
22	(*Z*)-Jasmone		0.05		1400	1400
23	*Z*-3-Hexen-1-ol benzoate		0.31		1574	1573
24	Benzyl benzoate	0.03			1768	1770
25	Benzoic acid, octyl ester			0.15	1783	1779
26	Phthalic acid, isobutyl octyl ester			0.72	1871	MS
	Fatty Acids					
27	Nonanoic acid			0.40	1275	1272
28	Dodecanoic acid			0.82	1569	1568
29	Tetradecanoic acid			1.01	1767	1767
30	Pentadecanoic acid			1.04	1867	1867
31	Hexadecanoic acid	0.06		18.37	1965	1968
32	Heptadecanoic acid			0.33	2078	2077
	Hydrocarbons					
33	Nonane[d]	0.36	0.46	21.23	901	900
34	Undecane[d]	0.31	0.25	4.26	1100	1100
35	Tricosane[d]	0.04		0.57	2299	2300
36	Tetracosane[d]			0.22	2400	2400
37	Pentacosane[d]	0.05	0.19	1.11	2500	2500
38	Hexacosane[d]			0.15	2601	2600
39	Heptacosane[d]			0.65	2700	2700
40	Nonacosane[d]			1.02	2900	2900
	Monoterpene Hydrocarbons					
41	*α*-Pinene[d]	62.46	46.69	6.32	939	939
42	Camphene	1.18	3.54	0.26	949	947
43	Verbenene	0.21	0.17		953	951

44	Sabinene	0.78	0.20		974	975
45	*β*-Pinene	1.75	0.72	0.13	978	979
46	*α*-Phellandrene	0.15	0.09		1005	1007
47	*α*-Terpinene	0.46	0.46		1017	1017
48	*o*-Cymene	3.35	6.42	0.89	1023	1021
49	Limonone[d]	2.31	5.46	0.86	1027	1025
50	*β*-Ocimene	0.31	0.11		1038	1037
51	*γ*-Terpinene	0.95	1.47	0.26	1059	1060
52	*α*-Terpinolene	0.51	1.12	0.42	1089	1083
53	Mentha-1,4,8-triene		0.05		1112	1113
	Oxygenated Monoterpenes					
54	(*E*)-Linalool oxide			0.12	1072	1069
55	Fenchol	0.33	0.91	0.23	1114	1115
56	*α*-Thujone		0.05		1117	1114
57	1,3,8-*p*-Menthatriene		0.08		1119	1119
58	*α*-Campholenal	0.65	1.12	0.28	1127	1130
59	(*E*)-Pinocarveol	0.71	0.68	0.34	1140	1140
60	(*Z*)-Verbenol	0.12	0.08		1142	1141
61	Camphore	0.47	0.28		1146	1146
62	(*E*)-Pinocamphone	0.11	0.14		1161	1160
63	Pinocarvone	0.26	0.28	0.13	1164	1164
64	*p*-Mentha-1,5-dien-8-ol	0.25	0.29		1167	1167
65	Borneol	1.24	2.40	0.72	1168	1168
66	4-Terpineol	0.29	0.43	0.44	1179	1178
67	*p*-Cymen-8-ol	0.45	0.39		1187	1188
68	*α*-Terpineol	0.53	1.21	0.60	1193	1190
69	Mrytenol	0.37	0.41	0.33	1198	1198
70	Verbenone	0.76	0.41		1211	1212
71	*trans*-Carveol	0.31	0.42	0.10	1220	1220
72	*α*-Fenchyl acetate	0.03			1230	1228
73	*D*-Carvone	0.08	0.08		1243	1242
74	Carvotanacetone	0.04	0.15		1249	1250
75	3-Carvomenthenone		0.05		1256	1256
76	Thymol	0.03	0.11		1287	1286
77	Carvacrol	0.09	0.21		1321	1317
78	Geranyl acetone			0.18	1451	1452
	Sesquiterpene Hydrocarbons					
79	Bicycloelemene	0.05			1339	1338
80	*α*-Cubebene	0.06			1352	1351
81	Cyclosativene			0.54	1368	1368
82	*α*-Ylangene	0.13	0.03	0.30	1374	1374
83	*α*-Copaene	0.19	0.09	1.05	1378	1378
84	*β*-Bourbonene	0.05		0.10	1386	1386
85	*β*-Cubebene			0.18	1388	1388
86	*β*-Patchoulene	0.06	0.07		1390	1388
87	*α*-Gurjunene			0.12	1403	1408
88	Junipene	0.08			1405	1405
89	*β*-Maaliene		0.21		1413	1414
90	(*E*)-*β*-Caryophyllene	0.47	0.11	0.23	1422	1419
91	γ-Elemene		0.06		1431	1434
92	*α*-Guaiene			0.21	1438	1439
93	Aromadendrene	0.38	0.17	0.88	1442	1443
94	*α*-Humulene	0.07			1457	1456
95	Alloaromadendrene	0.16			1465	1467
96	γ-Gurjunene	0.36	0.51		1478	1479

97	*α*-Amorphene	1.63	0.63	3.25	1480	MS
98	Germacrene D	0.19			1486	1485
99	*β*-Selinene	0.64	0.64	0.80	1490	1489
100	Valencene		0.49	0.33	1496	1496
101	*α*-Selinene	0.73	0.38	0.84	1499	1498
102	*α*-Muurolene	0.15	0.07	0.78	1503	1505
103	Calamenene		0.71		1527	1531
104	*δ*-Cadinene	0.98		2.77	1528	1528
105	*E*-Cadina-1,4-diene	0.06			1536	1537
106	α-Calacorene	0.26	0.28	0.15	1541	1542
107	*α*-Cadinene	0.13		0.27	1544	1544
108	*β*-Calacorene	0.11		0.58	1566	1564
	Oxygenated Sesquiterpenes					
109	Spathulenol	1.10	2.15	0.55	1585	1582
110	Salvial-4(14)-en-1-one	0.18	0.29		1599	1599
111	Viridiflorol	0.19	0.31		1606	1605
112	Isospathulenol		0.15		1640	1640
113	*α*-Cadinol	0.32			1644	1645
114	τ-Muurolol	0.37	0.76	0.30	1646	1648
115	Cadalene	0.25	0.31	0.50	1684	1688
116	Hexahydrofarnesyl acetone	0.17	0.18	1.28	1846	1846
117	(*E,E*)-Farnesyl acetone			0.25	1921	1921
	Diterpene					
118	*ent*-Pimara-8,15-diene			0.13	1941	1942
	Other					
119	2-Pentyl furan			0.32	990	988
	Total (%)	**92.77 (NC: 75)**	**87.66 (NC: 67)**	**86.26 (NC: 76)**		

[a] Percentages obtained by FID peak-area normalization;
[b] Retention index calculated from retention times relative to n-alkanes (C_6-C_{32}) on the non-polar HP-5MS column.
[c] Literature retention indices (RI) on HP-5MS column as seen in NIST, Willey, Kovats Index, and Adams (2007).
[d] Included as authentic compound, NC: Numbers of compounds, and MS: Identification of mass spectrum.

For flower, leaves, and stem essential oils (Table 1), 75, 67, and 76 compounds were determined by GC-MS and GC-FID analyses, respectively Among them, three alcohols, 23 carbonyl compounds, six fatty acids, eight hydrocarbons, 13 monoterpene hydrocarbons, 25 oxygenated monoterpenes, 30 sesquiterpene hydrocarbons, nine oxygenated sesquiterpenes, one diterpene, and one other compound were identified (Fig. 1).

While monoterpene hydrocarbons were the main chemical class of flowers (74.4%), and leaf oils (66.5%), hydrocarbons were the abundant class of stem oils (29.21%). The main compounds were as follows: *α*-pinene (62.5%), *o*-cymene (3.4%), and limonene (2.3%) for flower oils; *α*-pinene (46.5%), *o*-cymene (6.4%), and limonene (5.5%) for leaf oils; nonane (21.2%), hexadecanoic acid (18.4%), and *α*-pinene (6.3%) for stem oils.

Approximately 36 compounds were common to all three essential oils. Nonane, *α*-pinene, *o*-cymene, limonene, borneol, *α*-amorphene, *β*-selinene, *α*-selinene, spathulenol, and hexahydrofarnesyl acetone were common components with relatively high quantities in all parts of *A. utriculata*. A study conducted by Blazevic *et al.* (2013) showed that 31 compounds were detected in the essential oils of the whole plant (*A. utriculata*), of which

hexadecanoic acid (11.5%), nonacosane (10.0%), hexahydrofarnesyl acetone (5.9%), phytol (4.3%), and heptacosane (4.0%) made up the majority (Blazevic *et al.* 2013). When compared to the work of Blazevich e*t al.* (2013) (*E*,*E*)-2,4-decadienal, tricosane, tetracosane, pentacosane, nonacosane, thymol, *β*-caryophyllene, *α*-cadinol, hexahydro-farnesyl acetone, tetradecanoic acid, pentadecanoic acid, and hexadecanoic acid were similarly detected in the current study. In another study, Saka *et al.* (2017) identified 34 and 39 compounds in the essential oils of *Brassica rapa* var. *rapifera* leaves and roots (Saka *et al.* 2017). In this study, methyl-5-hexenenitrile (52.6%), 2-phenylethanol (10.2%), menthol (5.3%), allyl isothiocyanate (4.6%), and hexahydrofarnesyl acetone (3.2%), were abundant compounds in leaf essential oil. When this study is compared with Saka *et al.* (2017), it is seen that terpene derivative compounds, such as geranyl acetone, hexahydrofarnesyl acetone, *α*-pinene, *β*-pinene, camphene, sabinene, *α*-terpinene, limonone, and *α*-terpineol, were similar.

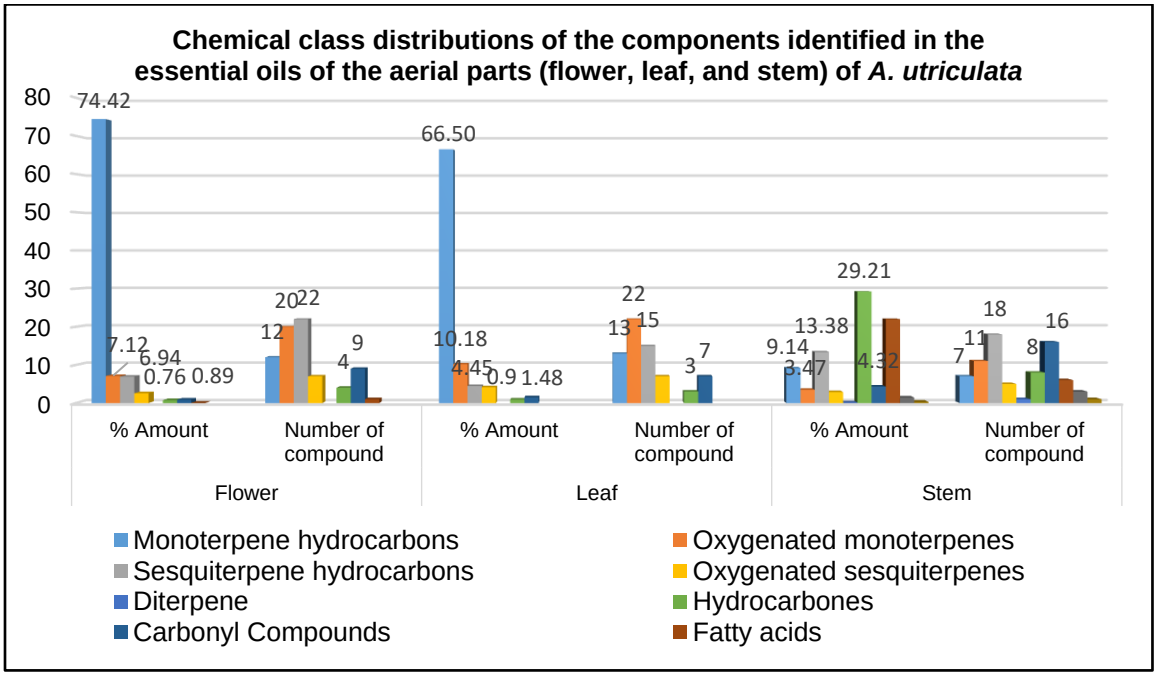

Fig. 1. Chemical class distributions of the components identified in the essential oils of the aerial parts (flower, leaf, and stem) of *A. utriculata*

The chemical differences of the essential oils obtained in the present study can be used as alternative additives in foods, medicines, and cosmetic preparations. Terpene and terpene-related compounds in the essential oils in the current study are known for their important biological activities in humans (Saka *et al.* 2017). For example, among the identified compounds, *α*-pinene was an abundant compound of flower, leaf, and stem essential oils in ratios 62.46%, 46.49%, and 6.32%, respectively. There are studies in the literature showing that *α*-pinene is used for its anti-inflammatory, antimicrobial, anticancer, antiulcerogenic, and gastroprotective properties, and its ability to aid memory retention (Salehi *et al.* 2021). Possessing high amounts of *α*-pinene in its volatile oils, *A. utriculata* appears to be a potentially good source of biological effects.

According to a literature survey, attractive natural phytochemicals, such as isothiocyanates, thymol, limonene, 1,5-heptadiene, 3-methyl-3-butenenitrile, *α*-farnesene, and linalool, have been reported from essential oils of Brassicaceae with wide bioactivities

(Salehi *et al.* 2021). The essential oils in this family generally contain characteristic sulfur and nitrogen compounds (Salehi *et al.* 2021; Ucuncu 2021). However, sulfur and nitrogen compounds were not found in any of the essential oils in the current study. Some differences within the chemical composition of volatile oils from Brassicaceae and in the present study were observed, and it is probably related to different species, agronomical factors, extraction techniques, and several physical and chemical environmental factors (Salehi *et al.* 2021).

Biological Activities

The antioxidant capacities of essential oils (flowers, leaves, and stems) were investigated using TPC and DPPH. The antioxidant analysis results of the essential oils of *A. utriculata* are given in Table 2. The TPC and DPPH analyses of essential oils demonstrated very good repeatability. The indices showed excellent consistency (RSDr% < PRSDr%) across the values obtained from three separate analytical trials. The repeatability values (RSDr% < RSDr%) of the flower, leaf, and stem EO samples for TPC and DPPH tests were determined to be 1.42 < 4.23, 1.99 < 4.59, 2.29 < 4.72, and 1.11 < 4.28, 2.86 < 4.08, 2.25 < 4.20, respectively. The values of TPC and DPPH scavenging of flower oil were higher than those of other oils. *Brassicaceae* family members are known to have antioxidant properties (Golkar and Moattar 2019).

Table 2. Total Phenolic Content and Reducing Activity of the Essential Oils from Aerial Parts of *A. utriculata*

Sample	Total Phenolic Content (mg GAE/L)	DPPH Scavenging Activity (%)	DPPH IC_{50} (µg/mL)
Flower EO	440.61 ± 6.24	% 46.00 ± 1.28	478.92 ± 5.82
Leaf EO	255.04 ± 5.11	% 24.10 ± 0.98	529.13 ± 15.34
Stem EO	210.20 ± 4.83	% 21.65 ± 0.82	485.45 ± 12.43
Ascorbic Acid	-	%98.66 ± 1.39	119.46 ± 11.93
Trolox	-	%98.88 ± 1.47	148.45 ± 15.44

EO: Essential oil, and GAE/L: Gallic acid equivalent per L, ±: Standart deviation

In this study, the TPC of the flower, leaf, and stem essential oil samples were found to be 440.61 ± 6.24, 255.04 ± 5.11, and 210.20 ± 4.83 mg GAE/L, respectively. According to the results, the TPC of the sample oils were similar to those reported by Ucuncu (2021) in the essential oils of the flower (485.60 ± 7.28 mg GAE/L) and aerial parts (140.00 ± 3.24 mg GAE/L) of *Arabis alpina*. In another study, the TPC of *Iberis amara* essential oils were 32.9 ± 0.7 (mg/g GAE/g DW) and 28.3 ± 1.7 (mg/g GAE/g DW) in leaf and bud explants, respectively (Golkar and Moattar 2019).

In the present study, DPPH values of the samples were defined as 46.00 ± 1.28, 24.10 ± 0.98, and 21.65 ± 0.82%, respectively. The percentage DPPH scavenging values of trolox and ascorbic acid were found to be 98.66 ± 1.39% and 98.88 ± 1.47%, respectively, at a 200 µg/mL concentration. Ucuncu (2021) also determined the DPPH scavenging activity in flower (as 49.85 ± 1.22) and in aerial parts (as 23.20 ± 0.76%) in the essential oils of *A. alpina*. In another study, Balpinar (2018) detected DPPH scavenging activity (as 76.3%) in the flower-fruit-seed ethanol extract of *Arabis alpina* L. subsp. *brevifolia*. In a study by Xiao *et al.* (2019), the DPPH radical scavenging capacity of different varieties of the *Brassicaceae* ranged from 157.3 to 806.3 µmol of Trolox equivalents (TE)/100 g of fresh vegetable. These values correspond to TPC ranging from 88.6 to 811.2 mg of gallic acid equivalents (GAE)/100 g of fresh vegetable (Xiao *et al.*

2018). The DPPH and TPC values obtained in the present study are consistent with the literature.

According to the results of antioxidant analysis, essential oils showed reduction in the stable violet DPPH radical to the yellow-colored diphenylpicryl hydrazine, reaching 50% of reduction with IC_{50} values ranging 478.92 ± 5.82, 529.13 ± 15.34, and 485.45 ± 12.43 µg/mL for flower, leaves and stem, respectively (Table 2). The DPPH-radical scavenging activities of essential oils were lower compared to standard antioxidants ascorbic acid and trolox (IC_{50} 119.46 ± 11.93 and 148.45 ± 15.44, respectively). According to the IC_{50} values it can be suggested that components present in the studied essential oils that are capable to scavenge DPPH radicals.

The antioxidant capacity values of the essential oils found in this study are moderate. Monoterpene hydrocarbons, which are the main group components of the flower and leaf essential oils of *A. utriculata*, are known to act as radical scavenging agents (Golkar and Moattar 2019). The terpenic compounds, such as *α*-pinene, limonene, and *o*-cymene, play a significant role in electron transfer/hydrogen donating ability, and these compounds were found in the essential oils of *A. utriculata* (Pandey and Rizvi 2009). The antioxidant capacity of *α*-pinene (62.46% and 46.69%), which is abundant in flower and leaf essential oils, is well known (Bouzenna *et al.* 2017; Wang *et al.* 2019).

The volatile oils of *A. utriculata* exhibited different inhibition levels against selected four gram (+), eight gram (-) bacteria as well as five fungi, as shown in Table 3.

Five different concentrations (50, 100, 200, 500, and 1000 ppm) of essential oils were tested in this study. No antimicrobial activity of essential oils was observed at 50, 100, and 200 ppm concentrations. The inhibition zone increased with an increased concentration of *A. utriculata* volatile oils. A 10-ppm streptomycin sulfate was used as a standard antimicrobial. A 30-ppm nistasine was used as a standard antifungal. All the essential oils showed antibacterial activity against gram (-) *ESC* and gram (+) *BS* at 1000 ppm. The flower, leaves, and stem essential oil showed good antimicrobial activity against *ESC* (inhibition zone (mm) > 5.20 mm, 5.39 mm, and 5.30 mm, respectively). In contrast, flower oil exhibited moderate inhibition activity against *KP* and *LM*. The leaf and stem essential oils were effective fungi SC, whereas they showed no antifungal activity for the fungi studied in the present work. The antimicrobial activities of the samples were lower than the standards. For the antimicrobial activity analysis of the essential oils (RSDr% < RSDr%), repeatability was very good.

According to the literature, Brassicaceae plants have shown good antimicrobial activity against bacteria and fungi (Balpinar 2018; Favela-González *et al.* 2020; Salehi *et al.* 2021; Ucuncu 2021). In one of the studies, the ethanol extracts of *Brassica oleracea* showed the maximum zone of inhibition for *Aspergillus fumigatus*, *Citrobacter divergens* and *Klebsiella pneumonia* at a concentration of 200 µg/200 µL (Paul *et al.* 2012). In another study, the essential oils of black mustard (*Brassica nigra*) exhibited antifungal activity against *Botrytis cinera, Aspergillus niger*, *Aspergillus ochraceus,* and *Penicillium citrinum* (Salehi *et al.* 2021). In a different study, *B. rapa* var. *rapifera* root and leaves essential oils showed great antimicrobial activity against *Listeria monocytogenes and Candida albicans*, moderate great activity against *Staphylococcus aureus*, *Escherichia coli*, and *Aspergillus flavus*, and moderate activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Saka *et al.* 2017). Especially, the results obtained for *Klebsiella pneumoniae*, *Aspergillus niger*, and *Penicillium* in the current study agree with the literature.

Table 3. Screening Results for Antimicrobial Activity of the Essential Oil from the Aerial Parts (Flower, Leaf, and Stem) of *A. utriculata* (expressed as inhibition zone diameter in mm)

Gram (-) Bacteria	Flower EO		Leaf EO		Stem EO			
	1000 ppm	500 ppm	1000 ppm	500 ppm	1000 ppm	500 ppm	Streptomsin Sulfate 10 ppm	Nistasine 30 ppm
AH	-	-	-	-	-	-	17.15 ± 0.05	NT
EC	-	-	-	-	-	-	-	NT
ESC	5.20± 0.05	-	5.39 ± 0.05	-	5.30 ± 0.05	-	7.10 ± 0.05	NT
ECO	-	-	-	-	-	-	15,10 ± 0,05	NT
KP	6.39 ± 0.05	4.00 ± 0.05	-	-	-	-	16.18 ± 0.05	NT
PV	-	-	-	-	-	-	14.02 ± 0.05	NT
PA	-	-	-	-	-	-	17.00 ± 0.05	NT
ST	-	-	-	-	-	-	17.88 ± 0.05	NT
Gram (+) Bacteria								
BC	-	-	-	-	-	-	16.00 ± 0.05	NT
BS	6.29 ± 0.05	-	6.45 ± 0.05	4.30 ± 0.05	4.19 ± 0.05	-	19.20 ± 0.05	NT
LM	4.14 ± 0.05	-	-	-	-	-	19.25 ± 0.05	NT
SA	-	-	-	-	-	-	11.95 ± 0.05	NT
Fungus								
SC	-	-	7.24 ± 0.05	5.27 ± 0.05	4.10 ± 0.05	-	NT	18.10 ± 0.05
CA	-	-	-	-	-	-	NT	12.20 ± 0.05
AN	-	-	4.00 ± 0.05	-	-	-	NT	14.28 ± 0.05
AP	-	-	-	-	-	-	NT	11.10 ± 0.05
P	-	-	-	-	-	-	NT	12.08 ± 0.05

EO: Essential oil, —: No activity observed, NT: Not tested, AH: *Aeromonas hydrophila* ATCC 35654, EC: *Enterobacter cloacae* ATCC 13047, ESC: *Escherichia coli* ATCC 25922, ECO: *Escherichia coli* O157: H7 ATCC 35150, KP: *Klebsiella pneumoniae* ATCC 13883, PV: *Proteus vulgaris* FMC, PA: *Pseudomonas aeruginosa* ATCC 27853, ST: *Salmonella typhimurium* ATCC 23566, BC: *Bacillus cereus* ATCC 9634, BS: *Bacillus subtilis* ATCC 6633, LM: *Listeria monocytogenes* ATCC 7644, SA: *Staphylococcus aureus* ATCC 25923, SC: *Saccharomyces cerevisiae* S288C, CA: *Candida albicans* ATCC 10231, AN: *Aspergillus niger*, AP: *Aspergillus flavus* ATCC 46283, P: *Penicillium*

The main component in the essential oils obtained in the current investigation is α-pinene. In one study, this compound has broad potential in antimicrobial therapy to inhibit the growth of bacteria as a synergist of antibiotics (Borges *et al.* 2022).

The use of plant-derived antimicrobials and antioxidants is increasing continually, and plants are a great source of bioactive metabolites. The natural antimicrobials and antioxidants are a good option for the development of new alternative antimicrobials and antioxidants against resistance caused by the abuse of conventional synthetic drugs (Favela-González *et al*. 2020). The different antimicrobial and antioxidant effects of the

essential oils obtained in this study may be due to the different phytochemicals they contain. The reason for these differences can be attributed to agronomical, physical, chemical and environmental factors.

Minor (*α*-pinene, *o*-cymene, limonene, hexadecanoic acid, *etc*.) or trace compounds in the present essential oils might also give rise to the exhibited biological activities. Possible synergistic effects of compounds in essential oils should also be considered.

CONCLUSIONS

This study characterized the identities, phytochemical content, antimicrobial and activities, and antioxidant activities of essential oils of *Alyssoides utriculata* L. plant.

1. The results indicated that essential oils had moderate antimicrobial and antioxidant activities. The essential oils obtained in this study were effective against 4 microorganisms and 2 fungi at different doses. The findings suggest that the essential oils of *A. utriculata* contain a valuable source of bioactive compounds such as *α*-pinene, *o*-cymene, and limonene. These essential oils can be used as effective tools to control foodborne pathogenic microorganisms.
2. The structures of a total of 119 components in essential oils obtained from three different parts of *A. utriculata* were elucidated. Sulfur-containing compounds, such as glucosinolate, thiocyanate and isothiocyanate, which are characteristic compounds of *Brassicaceae* family members, were not found in the essential oils. In further studies, various extracts of *A. utriculata* can be obtained and their different biological activities and secondary metabolite contents can be investigated. The chemical differences of the essential oils obtained in the current study represent an alternative set of additives for foods, medicines, and cosmetics.
3. In future studies, the use of the essential oil obtained in present study as a preservative additive in foods can be investigated.

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REFERENCES CITED

Adams, R. P. (2007). *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, 4th Ed., Allured Publishing, Carol Stream, IL, USA.

Ahmed, D., Khan, M. M., and Saeed, R. (2015). "Comparative analysis of phenolics, flavonoids, antiooxidant and antibacterial potential of methanolic, hexanic, and aqueous extracts from *Adiantum caudatum* leaves," *Antioxidants* 4(2), 394-409. DOI: 10.3390/antiox4020394

Al Abboud, M. A., Shater, A. R. M., Moawad, H., Mashraqi, A., Yahya, A., and Ghany, T. M. A. (2024). "Assessment of sesame and sweet almond oils efficacy against food-

borne and human illness microorganisms with molecular docking study," *International Journal of Pharmacology* 20(3), 403-425. DOI: 10.3923/ijp.2024.403.425

Alghonaim, M. I., Alsalamah, S. A., Alsolami, A., and Ghany, T. M. A. (2023). "Characterization and efficiency of ganoderma lucidum biomass as an antimicrobial and anticancer agent," *BioResources* 18(4), 8037-8061. DOI: 10.15376/biores.18.4.8037-8061

Balpinar, N. (2018). "The biological activities of *Arabis alpina* L. subsp. *brevifolia* (DC.) Cullen against food pathogens," *Open Chem.* 16(1), 930-936. DOI: 10.1515/chem-2018-0104

Blazevic, I., Burcul, F., Ruscic, M., and Mastelic, J. (2013). "Glucosinolates, volatile constituents, and acetylcholinesterase inhibitory activity of *Alyssoides utriculata*," *Chem. Nat. Comp.* 49(2), 374-378.

Borges, M. F. A., Lacerda, R. S., Correia, J. P. A., Melo, T. R., and Ferreira, S. B. (2022). "Potential antibacterial action of alpha-pinene," *Med. Sci. Forum*. 12(11), 1-5. DOI: 10.3390/eca2022-12709

Bouzenna, H., Hfaiedh, N., Giroux-Metges, M. A., Elfeki, A., and Talarmin, H. (2017). "Potential protective effects of alpha-pinene against cytotoxicity caused by aspirin in the IEC-6 cells," *Biomed. Pharmacother.* 93, 961-968. DOI: 10.1016/j.biopha.2017.06.031

Chemdata NIST (2023). "NIT 23 GC method / retention index library," Chemdata NIST, (https://chemdata.nist.gov/dokuwiki/doku.php?id=chemdata:ridatabase), Accessed 24 March 2024.

Cüce, M., and Basançelebi, O. (2021). "Comparison of volatile constituents, antioxidant and antimicrobial activities of *Thymus leucotrichus* (Lamiaceae) stem and leaves essential oils from both natural resources and in vitro derived shoots," *J. Essent. Oil Bear. Pl.* 24(5), 1097-1112. DOI: 10.1080/0972060X.2021.2003256

Cullen, J. (1965). "Alyssoides (L.) Medik.," in: *Flora of Turkey and East Aegean Islands*, Vol. 1, P. H. Davis (Ed.), Edinburgh University Press, Edinburgh, Scotland, pp. 355-356.

Favela-González, K. M., Hernández-Almanza, A. Y., and Fuente-Salcido, N. M. D. (2020). "The value of bioactive compounds of cruciferous vegetables (Brassica) as antimicrobials and antioxidants: A review," *J. Food Biochem.* 44(10), 1-21. DOI: 10.1111/jfbc.134114

Fidan, M. S., Öz, M., Üçüncü, O., Baltacı, C., and Karataş, S. M. (2022). "Composition of antimicrobial and antioxidant activities and chemical components of essential oil from flowers and leaves of *Pyrus elaeagrifolia* Pallas in Turkey," *Fresenius Environ. Bull*. 31(4), 4106-4117.

Golkar, P., and Moattar, F. (2019). "Essential oil composition, bioactive compounds, and antioxidant activities in *Iberis amara* L.," *Nat. Prod. Commun.* 14(5), 1-8. DOI: 10.1177/1934578X1984635

Gümüşok, S., Kırcı, D., Demirci, B., and Kılıç, C. S. (2023). "Essential oil composition of *Capsella bursa-pastoris* (L.) Medik. aerial parts," *Turk. J. Pharm. Sci.* 20(5), 341-344. DOI: 10.4274/tjps.galenos.2022.15098

Hichri, A. O, Mosbah, H., Majouli, K., Hlila, B. M., Jannet, H. B., Flamini, G., Aouni, M., and Selmi, B. (2016). "Chemical composition and biological activities of *Eruca vesicaria* subsp. *longirostris* essential oils," *Pharm Biol.* 54(10), 2236-2243. DOI: 10.3109/13880209.2016.1151445

Kasangana, P. B., Haddad, P. S., and Stevanovic, T. (2015). "Study of polyphenol content and antioxidant capacity of *Myrianthus arboreus* (Cecropiaceae) root bark extracts," *Antioxidants (Basel)* 4(2), 410-426. DOI: 10.3390/antiox4020410

Luo, S., An, R., Zhou, H., Zhang, Y., Ling, J., Hu, H., and Li, P. (2022). "The glucosinolate profiles of *Brassicaceae* vegetables responded differently to quick-freezing and drying methods," *Food Chem.* 383, article ID 132624. DOI: 10.1016/j.foodchem.2022.132624

Matuschek, E., Brown, D. F. J., and Kahlmeter, G. (2014). "Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories," *Clin. Microbiol. Infect.* 20(4), 255-266. DOI: 10.1111/1469-0691.12373

Mutlu, B. (2012). *Türkiye Bitkileri Listesi (Damarlı Bitkiler) [List of Plants of Turkey (Vascular Plants)]*, NGBB Press, İstanbul, Turkiye.

Öz, M. (2022). "Chemical composition and antimicrobial properties of essential oils obtained from the resin of *Picea orientalis* L.," *J. Essent. Oil Bear. Pl.* 25(2), 326-337. DOI: 10.1080/0972060X.2022.2077140

Pandey, K. B., and Rizvi, S. I. (2009). "Plant polyphenols as dietary antioxidants in human health and disease," *Oxid. Med. Cell. Longev*. 2(5), 270-278. DOI: 10.4161/oxim.2.5.9498

Paul, S., Bin Emran, T., Saha, D., and Zahid, S. H. (2012). "Phytochemical and antimicrobial activity of the plant extracts of *Brassica oleracea* against selected microbes," *Asian J. Pharm. Med. Sci.* 2(2), 30-34.

Polatoğlu, K., Karakoç, Ö. C., and Gören, N. (2013). "Phytotoxic, DPPH scavenging, insecticidal activities and essential oil composition of *Achillea vermicularis*, *A. teretifolia* and proposed chemotypes of *A. biebersteinii* (Asteraceae)," *Ind. Crops Prod.* 51, 35-45. DOI: 10.1016/j.indcrop.2013.08.052

Preedy, V. (2015). "Brassica composition and food processing," In: *Processing and Impact on Active Components in Food*, Academic Press, Cambridge, MA, USA, pp. 17-25.

Sağdıç, O., and Özcan, M. (2003). "Antibacterial activity of Turkish spice hydrosols," *Food Cont.* 14(3), 141-143. DOI: 10.1016/S0956-7135(02)00057-9

Sağdıç, O., Özturk, I., Özkan, G., Yetim, H., Ekici, L., and Yılmaz, M. (2011). "RP-HPLC-DAD analysis of phenolic compounds in pomace extracts from five grape cultivars: Evaluation of their antioxidant, antiradical and antifungal activities in orange and apple juices," *Food Chem.* 126(4), 1749-1758. DOI: 10.1016/j.foodchem.2010.12.075

Saka, B., Djouahri, A., Djerrad, Z., Terfi, S., Aberrane, S., Sabaou, N., Baaliouamer, A., and Boudarene, L. (2017). "Chemical variability and biological activities of *Brassica rapa* var. *rapifera* parts essential oils depending on geographic variation and extraction technique," *Chem. Biodiversity* 14(6), article e1600452. DOI: 10.1002/cbdv.201600452

Salehi, B., Quispe, C., Butnariu, M., Sarac, I., Marmouzi, I., Kamle, M., Tripathi, V., Kumar, P., Bouyahya, A., Capanoglu, E., *et al*. (2021). "Phytotherapy and food applications from *Brassica* genus," *Phytotherapy Research* 35(7), 3590-3609. DOI: 10.1002/ptr.7048

Sanchez-Moreno, C., Larrauri, J. A., and Saura-Calixto, F. A. (1998). "A procedure to measure the antiradical efficiency of polyphenols," *J. Sci. Food Agr.* 76(2), 270-276. DOI: 10.1002/(SICI)1097-0010(199802)76:2<270::AID-JSFA945>3.0.CO;2-9

Saruhan, E., and Öz, M. (2023). "Chemical content and antimicrobial activities of essential oils obtained from plant parts of *Juniperus excelsa* M. Bieb.," *Drvna Industrija* 74(3), 347-357. DOI: 10.5552/drvind.2023.0082

Singh, S., Das, S. S., Singh, G., Perroti, M., Schuff, C., and Catalán, C. A. N. (2017). "Comparison of chemical composition, antioxidant and antimicrobial potentials of essential oils and oleoresins obtained from seeds of *Brassica juncea* and *Sinapis alba*," *MOJ Food Process Technol.* 4(4), 113-120. DOI: 10.15406/mojfpt.2017.04.00100

The Plant List (2013). "The plant list 2018, Version 1.1.," The Plant List, (http://www.theplantlist.org/), Accessed 20 March 2024.

Üçüncü, O., Karataş, Ş. M., Baltacı, C., Karaköse, M., and Türkuçar, S. A. (2019). "Volatile constituents and biological properties of essential oils from aerial parts of *Gentiana gelida* BIEB.," *J. Oleo Sci.* 68(10), 1011-1017. DOI: 10.5650/jos.ess18113

Üçüncü, O. (2021). "Chemical composition and biological activities of volatile oils of *Arabis alpina* L. ssp. *alpina*," *J. Anim. Plant Sci.* 31(5), 1501-1510. DOI: 10.36899/JAPS.2021.5.0352

Xiao, Z., Rausch, S. R., Luo, Y., Sun, J., Yu, L., Wang, Q., Chen, P., Yu, L., and Stommel, J. R. (2019). "Microgreens of Brassicaceae: Genetic diversity of phytochemical concentrations and antioxidant capacity," *LWT-Food Sci. Technol.* 101, 731-737. DOI: 10.1016/j.lwt.2018.10.076

Wang, C. Y., Chen, Y. W., and Hou, C. Y. (2019). "Antioxidant and antibacterial activity of seven predominant terpenoids," *Int. J. Food Prop.* 22(1), 230-238. DOI: 10.1080/10942912.2019.1582541

Yılar, M., Bayan, Y., and Onaran, A. (2016). "Chemical composition and antifungal effects of *Vitex agnus-castus* L. and *Myrtus communis* L. plants," *Nat. Bot. Horti. Agrobo.* 44(2), 466-471. DOI: 10.15835/nbha44210399

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