Chemical Content of Volatile Oil of *Primula veris* subsp. *columnae,* Obtaining the Methanol Extracts and their Biological Activities

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The proportions and constituents of the essential oils of flowers and leaves of *Primula veris* subsp. *columnae* were determined, and the antioxidant and the antimicrobial (antibacterial and antifungal) properties of their methanol extracts were investigated. Percentage ratios and main components of the *Primula veris* subsp. *columnae* plant, which grows naturally, were detected by extracting flower and leaf volatile oils. The components of the volatile oil were identified with Gas Chromatography Mass Detector-Flame Ionization Detector (GC-MS/FID). A total of 62 compounds were identified in flower volatile oil, and methyl 4-methoxysalicylate (37.1%) was determined as the main compound. While defining the structure of 50 compounds in leaf essential oil, linoleic acid (40.1%) was established as the main compound. As the result of the extraction of flowers and leaves with methanol, the extractive substance was obtained as 34.5% in flowers and as 28.8% in leaves. Methanol extraction and the antioxidant properties of the *Primula veris* subsp. *columnae* plant were quite high. In the antifungal and antibacterial activity test conducted on the volatile oils and methanol extracts of flowers and leaves of the *Primula veris* subsp. *columnae* species, only flower volatile oil showed weak inhibition properties.

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INTRODUCTION

Primula L., consisting of approximately 500 species, is the largest genus of the Primulaceae family. The species spreads commonly in the humid and colder regions of the northern hemisphere (Baasanmunkh *et al.* 2020). A total of 12 taxa of *Primula* genus belonging to 8 species have been identified in Turkey. Aside from *Primula davisii* W.W.Sm, most of the other taxa grow naturally in northeastern Anatolia (Terzioğlu *et al.* 2012). The primrose is a perennial herbaceous plant, growing 10 to 50 cm tall, with clusters of 8 to 12 golden yellow flowers and leaves gathered in a rosette shape at the base. It grows in water meadows. Volatile oil, saponin glycosides, and flavone derivatives are found in its composition. The flowers of the plant have diaphoretic, expectorant, and sedative effects, and it has been used to treat eye diseases. Its fresh leaves have been used to treat furuncles (Baytop 1999). *Primula veris* subsp. *columnae* (Ten.) Maire & Petitm. (Synonym: *Primula veris* subsp. *suaveolens* (Bertol.) Gutermann & Ehrend.) grows

naturally in the provinces of Giresun, Gümüşhane, Rize, and Trabzon (Karapınar 2020).

There are several studies on the *Primula* genus in literature. Borisova and Popov (2012) investigated the qualitative composition and quantitative content of the flavonoids of *Primula officinalis* plant parts in their study. Latypova *et al.* (2015) examined the amount of ursolic acid (they have anti-microbial, anti-tumor, and anti-inflammatory properties) of *Primula veris* and *Primula crocalyx* plants in the research they conducted. Meos *et al.* (2017) investigated the vitamin C (ascorbic acid) amount of *Primula veris* flowers. Bączek *et al.* (2017) examined the phenolic components of *Primula veris* and *Primula elatior* flowers and roots, which they had collected in Poland with the HPLC-DAD method. In the study conducted on *Primula veris* var. *columnae*, Vuko *et al.* (2017) obtained volatile oils from the leaves, roots, and flowers of *Primula veris* var. *columnae* they collected in 2011 in Croatia and determined their components with GC-MS method. Güneş (2016) conducted the chemical composition analysis of volatile oil and chloroform extracts obtained through distillation and extraction from the flower and leaf sections of the *Primula vulgaris* subsp. *sibthorpii* plant collected in Giresun province in a post graduate thesis. Demir *et al.* (2019) investigated the antioxidant properties and cytotoxic effects of the extract prepared with dimethyl sulfoxide from *P. vulgaris* flowers.

Volatile oils, also called essential oils, are oily liquids obtained from trees and plants. Essential oils have pleasant fragrances, are water-insoluble, accumulate on the surface of the water, and can easily evaporate when left uncovered. Volatile oils are found in certain tissues or all organs of the plant, including flower, fruit, petal, leaf, bark, resin, and woody structure (Baltacı *et al.* 2022; Fidan *et al.* 2022). Monoterpene hydrocarbons, sesquiterpene hydrocarbons, and their oxygenated derivatives, aliphatic hydrocarbons, aldehydes, ketones, acids, and esters are the chemical constituents of the volatile essence of aromatic plants (Altaf *et al.* 2020). Gas chromatography coupled with mass spectrometry (GC-MS) leads to a high chromatographic tenacity and a peak intensity and provides compositional information for nearly all volatile and semivolatile materials, including organic acids, amino acids, *etc*. (Qadir *et al.* 2020). The preserving effects of plant spices and herbs are due to the diverse phenolics they contain, which can have antioxidative and antimicrobial properties (Abramovıč *et al.* 2018). Antimicrobial agents obtained from medicinal plants can be used to treat infectious diseases with fewer side effects when compared with synthetic drugs (Liu *et al.* 2021).

In this study, the volatile oil components of the flowers and leaves of the *Primula veris* subsp. *columnae* (*P. v.* subsp. *columnae*) species collected from Gümüşhane, Türkiye were investigated. Chemical characterization was followed by the study of the antioxidant properties of the methanol extracts and the antibacterial and antifungal properties of volatile oils and methanol extracts were determined.

EXPERIMENTAL

Plant Material

Primula veris subsp. *columnae* (Tutya, Primrose) flower and leaf samples were gathered in Zigana Village, Torul District, Gümüşhane Province in the altitude of 1900 m (40°38´16´´N and 39°23´30´´E). The taxonomic identification of plant materials was done by Assoc. Prof. Mutlu Gültepe, in Programme of Forestry, Dereli Vocational School, Giresun University, Giresun, Turkey. The plant was enlisted with the number KTUB Gültepe 661 with a diagnosis from the Herbarium of Karadeniz Technical University,

Faculty of Science, Department of Biology. The location where flower and leaf samples of Primrose (*P. v.* subsp. *columnae*) were gathered is shown in Fig. 1.

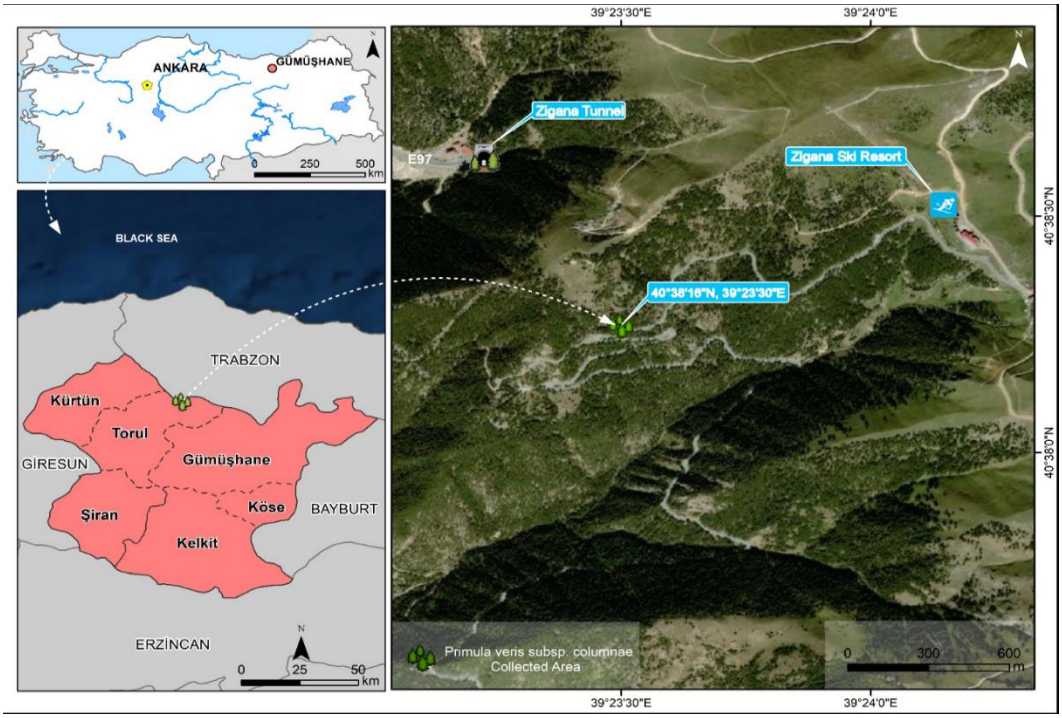

Fig. 1. The location where the plant materials were collected

Extraction of Volatile Oils

Volatile oils were extracted with a modified Clevenger apparatus. Homogenized flower (80 g) and leaf (100 g) samples were placed into a 2 L Clevenger apparatus flask, and 1500 mL of distilled water was added. Then, 2 mL of n-hexane was added to the condenser, and the temperature of the cooler was set to 4.0 °C. Volatile oils were obtained by boiling for 4 hours on the low setting. Percentages of yield for volatile oils by weight were calculated separately for flower and leaf samples according to Eq. 1,

$$Y\,\% = (Q_e / Q_p) \times 100 \quad (1)$$

where Y is the yield (%), Q_e is the quantity of isolated essential oil (g), and Q_p is the quantity of fresh raw plant material (g) (Öz *et al.* 2021).

Analysis of Volatile Oils Components with GC-MS/FID

The volatile oils obtained through water hydrodistillation in the Clevenger apparatus were filtered as dissolved in hexane and were placed in the autosampler by putting them in dark colored bottles. The evaluation of volatile oils was carried out with Gas Chromatography Mass Detector-Flame Ionization Detector (GC-MS/FID). Analyzes were conducted on the GC-FID Agilent-7890A, MS Agilent 5975C model device, and an HP-5MS model nonpolar capillary column was used for analysis (GC-MS conditions: 30. m / 0.32 mm / 0.25 µm, He, 3. K / min; Tstart: 60. °C; Tend: 240. °C). Both detectors are located in the same device. Simultaneous analysis was performed. The mass spectra analysis of the essential oils was carried out in the MS detector, and the quantitative analysis was conducted in the FID detector. The injections were applied in splitless mode

at 240 °C using helium as the carrier gas with a flow rate of 1 mL/min. 1 µL volatile oil solution in hexane (GC class) was injected and stored initially at 60 ° C for 2 min, and then spectra were taken by raising the temperature to 240 °C with an increase of 3 °C/min. The identification of constituents of volatile oils was performed based on a comparison of retention indices (RI) with reference to a homologous series of n-alkanes (C_6 - C_{32}), under the same experimental status. The mass spectrum of each compound was identified through the structure clarification by comparing with the reference constituents of the NIST and Willey libraries, besides that making a comparison of their retention time either with retention times of authentic compounds or with the literature data (Öz *et al.* 2021).

Methanol Extraction

For analysis, 80 and 100 grams were taken from the grounded flower and leaf parts, respectively. Then, 750 mL of methanol was added to the samples and were stirred at room temperature at 200 rpm for 24 h. At the end of 24 h, they were centrifuged at 4000 rpm for 10 min by filtering through Whatman 1 filter twice, and plant extracts were acquired. The section, which stayed on the top at the end of centrifugation, was placed in a beaker and the extracts were acquired by completely evaporating methanol at 40 °C. Percentages of yield for methanol extract with respect to weight were calculated separately for flower and leaf samples by the following Eq. 2,

$$Y\,\% = (A_e / A_p) \times 100 \quad (2)$$

where Y is the yield (%), A_e is the amount of extracted substance (g), and A_p is the amount of fresh raw plant material (g) (Karapınar 2020).

Determination of Antioxidant Activity

DPPH Free radical scavenging activity

Samples were prepared from 0.20% methanol extracts obtained from flower and leaf sections. Methanol was HPLC purity grade, and DPPH, Trolox, and L-Ascorbic acid were analytical grade. Trolox and ascorbic acid working solutions in methanol were prepared at 25, 50, 100, 200, and 400 µg/mL. The mixture was vortexed and stored in the dark for 30 min. The absorbance of the obtained solution was read in a UV-Vis spectrophotometer (Optizen MECASYS) at 517 nm. Triplicate analyses were performed for each specimen. Methanol was utilized as the blank solution. The same procedures were conducted by taking from the standards (Ascorbic acid and Trolox). Results were provided as mg AA eq./g, mg Trolox eq./g and % inhibition (Ahmed *et al.* 2015).

Ferric (III) ion reducing antioxidant power (FRAP)

Samples were prepared from 0.04% methanol extracts obtained from flower and leaf sections. Research standards were as follows: solutions were prepared at concentrations of 5, 10, 25, 50, 100, and 200 µL/mL. from the main stock ferric (II) sulphate heptahydrate ($FeSO_4$. $7H_2O$) solution. The mixture was vortexed and kept in the dark for 30 min. The absorbance of the obtained solutions was read in a spectrophotometer at 593 nm. Triplicate analyses were performed for each specimen. Pure water was used as the blank. Total ferric reducing antioxidant capacity was determined as mg $FeSO_4$ eq./g (Ahmed *et al.* 2015).

Total phenolic substance

Samples were prepared from 0.20% methanol extract obtained from the flower and leaf sections. First, 0.50 mL methanol and 200 µL Folin-Ciocalteu reagent were added to the mixture. The mixture was vortexed and incubated under room conditions for 10 min, and 600 µL 10% (m/ v) Na_2CO_3 solution was added. After the final mixture was vortexed again, it was incubated in the dark at room conditions for 120 min and the absorbance of the mixture at 760 nm was read at the end of the incubation period. Triplicate analyses were performed for each specimen. A mixture of 3.7 mL water, 500 µL methanol, 100 µL Folin-Ciocalteu reagent, 600 µL Na_2CO_3 was used as a blank. The phenolic substance amounts in the samples were declared as the total phenolic mg GA eq./g by using the correct equation of the calibration graph obtained with the solution of gallic acid (10, 20, 30, 40, 60, and 80 µg/mL) (Kasangana *et al.* 2015).

Total antioxidant substance

Samples were prepared from 0.20% methanol extracts obtained from flower and leaf sections. Ascorbic acid was utilized for the calibration curve. The mixture was vortexed and incubated in a 95 °C water bath with caps closed for 90 min. It was recovered from the water bath and was kept for 20 to 30 min until it reached room temperature. Distilled water was used as the blank sample. The absorbance of the obtained reaction mixtures was read as 695 nm in the spectrophotometer. Triplicate analyses were performed for each specimen. The same procedures were conducted by taking 500 µL from the standards. Total antioxidant amount from flower and leaf samples was determined as mg AA eq./g by using the correct equation of the calibration graph obtained with the solution of ascorbic acid (25, 50, 100, 150, 250, and 500 µg/mL) (Kasangana *et al.* 2015).

Total flavonoid substance

Samples were prepared from 0.20% methanol extracts obtained from flower and leaf sections. The mixture was vortexed and 150 µL of 0.5 M sodium nitrite solution was added and then 150 µL 0.3 M aluminum chloride was added. It was kept for 5 min. 1 mL of 1 M NaOH solution was added. The mixture was vortexed again and after storing for 10 min, and then its absorbance was read at 506 nm in the spectrophotometer. Triplicate analyses were performed for each specimen. 500 µL distilled water was used as the blank. The same procedures were carried out by taking 500 µL from the standards. The total flavonoid was determined as mg catechin eq./g by using the correct equation of the calibration graph obtained with standard solutions at 5, 10, 25, 50, and 100 mg/L (Kasangana *et al.* 2015).

Determination of Antimicrobial Activity

The antimicrobial (antibacterial and antifungal) activities of the methanol extracts and volatile oils were determined with two different methods in accordance with agar diffusion and disk diffusion methods (Sağdıç *et al.* 2006; Matuschek *et al.* 2014). Four different concentrations of samples (100%, 50%, 25%, and 10%) were prepared for both methods. Volatile oil samples were prepared by dissolving in hexane, methanol extracts were dissolved in methanol.

Agar diffusion method

The agar diffusion method was executed in 3 stages: preparation of microorganisms, preparation of agar medium, and incubation. The samples were prepared as 108 CFU/mL at the end of the second activation for 18 h after the first activation of the bacteria in nutrient broth (Merck) medium at 36 °C for 24 h. Yeast and molds were prepared as 108 CFU/mL at the end of the second activation for 24 h after the first activation at 25 °C for 48 h. Nutrient Agar (Merck) medium was prepared for bacteria, and Malt Extract Agar (Merck) medium was prepared for yeast-mold. Afterwards they were sterilized in the autoclave. Then, 1% of the activated microorganisms were added to the medium which was brought to casting temperature, and they were put in petri dishes to enable solidification. Wells with a diameter of 4 mm were drilled on the agar medium. A total of 20 µL from sample extracts were added to these drilled wells. Bacteria were incubated at 36 °C for 24 h, and yeast-molds were left at 25 °C for 48 h. After the incubation, the diameter of the transparent zone formed around the wells was measured (Sağdıç *et al.* 2006).

Disc diffusion method

Microorganisms were prepared at the density of 108 CFU/mL in disc diffusion method. Bacteria whose densities were arranged by activating on the sterile Nutrient Agar and Malt Extract Agar media were spread as 1 mL. Sterile discs were soaked with 20 µL sample extracts and placed on the media. Bacteria were incubated at 36 °C for 24 h, and yeast-molds were incubated at 25 °C for 48 h. After the incubation, the diameter of the transparent zone formed around the discs was measured (Matuschek *et al.* 2014).

RESULTS AND DISCUSSION

Yield of Obtained Volatile Oil

The yields of the volatile oil obtained from the flowers and leaves of *P. v.* subsp. *columnae* species were determined as 0.33% and 0.12% respectively. The volatile oil amount and ratio in flowers was detected to be higher. In a study carried out on leaves, roots, and flowers of *Primula veris* var. *columnae* in Croatia in 2011, Vuko *et al.* (2017) noted that the total volatile oil yield was found to be 0.03% with respect to the dry weight of the samples.

GC-MS/FID Analysis Results of Volatile Oil Components Obtained from Flower and Leaf

The GC-MS/FID analysis identified 62 compounds belonging to the flowers of the *P. veris* subsp. *columnae*, but 3 compounds could not be identified. While the structure of a total of 50 compounds belonging to the leaves of the *P. veris* subsp. *columnae* has been defined, 4 compounds could not be identified. The GC-MS/FID analyses results of the volatile oil obtained from the flowers and leaves of the *P. veris* subsp. *columnae* (Tutya, Primrose) plant are given in Table 1.

Table 1. GC-MS/FID Analysis Results of Volatile Oil Obtained from the Flowers and Leaves of *Primula veris* subsp. *columnae*

No	Compounds	Percentage Composition		RI	LRI /MS
		PvcF	PvcL		
1	2,5-Dimethyltetrahydrofuran		0.05	713	727
2	Tropilidene		0.09	765	765
3	Toluene	0.05		765	765
4	2-Hexanone	0.15	0.21	789	789
5	Hexanal	0.26	0.15	801	801
6	Heptanal	0.05		902	902
7	*α*-Pinene	0.09	0.38	933	933
8	Benzaldehyde	0.47	0.24	960	960
9	*β*-Pinene	0.11	0.37	976	976
10	1-Octen-3-ol	0.09	0.07	980	980
11	4-Carene	0.04		1011	1011
12	*p*-Cymene	0.12		1025	1025
13	*o*-Cymene		0.33	1025	1025
14	Limonene	2.23	5.76	1029	1029
15	Benzeneacetaldehyde		0.11	1044	1044
16	*γ*-Terpinene	0.41	1.09	1059	1059
17	1-Octanol	0.05	0.04	1067	1067
18	*α*-Terpinolen	0.03	0.09	1089	1089
19	Linalool	1.10	1.04	1101	1101
20	Nonanal	0.29	0.21	1105	1105
21	(*E,E*)-2,6-Nonadienal	0.03		1154	1153
22	*o*-Acetylphenol	0.04	0.15	1164	1167
23	1-Nonanol	0.06	0.26	1172	1172
24	Terpinen-4-ol	0.04	0.28	1179	1179
25	Naphthalene	0.10	0.26	1184	1184
26	Methyl salicylate	0.58	0.14	1196	1196
27	Decanal	0.06		1206	1206
28	*cis*-Geraniol	0.08	0.20	1230	1230
29	Carvone		0.39	1246	1246
30	D-Carvone	0.16		1247	1246
31	Thymol	0.10	0.20	1294	1294
32	Carvacrol	0.04	0.10	1304	1304
33	(*E,E*)-2,4-Decadienal	0.15		1318	1318
34	Eugenol	0.23	0.38	1360	1360
35	Diphenyl ether		0.05	1404	1396
36	Methyleugenol		0.08	1406	1406
37	*α*-Copaene	0.05		1413	1416
38	Aromadendrene	0.11		1436	1436
39	Methyl 2,6-dihydroxy-4-methylbenzoate	12.2	3.41	1447	1474
40	*cis*-Geranylacetone		0.07	1455	1455
41	Humulene	0.20	0.06	1460	1460

42	Tetradecane, 4,11-dimethyl	2.07	0.73	1465	1464
43	Methyl 4-methoxysalicylate	37.1	7.49	1488	1490
44	2-Tridecanone	0.09		1497	1497
45	Valencene	0.20	0.55	1498	1499
46	*β*-Bisabolene	0.06		1512	1512
47	Dodecanoic acid, methyl ester	0.54	0.24	1525	1525
48	*cis*-Calamenene	0.07		1529	1529
49	*α*-Selinene	0.13		1555	1534
50	2-Tetradecanone	0.27	0.28	1562	1569
51	Dodecanoic acid	0.70	0.70	1570	1570
52	Benzophenone	0.08	0.17	1634	1635
53	Tetradecanoic acid, methyl ester	0.08		1725	1725
54	Tetradecanoic acid	0.26	0.65	1766	1766
55	Hexahydrofarnesyl acetone	2.30	0.79	1846	1846
56	Benzoic acid, 2-hydroxy-, phenylmethyl ester	0.09		1875	1876
57	Nonadecane	0.16		1899	1900
58	Hexadecanoic acid, methyl ester	0.31	0.29	1927	1927
59	2-Methylnonadecane	0.27		1963	1966
60	Hexadecanoic (Palmitic) acid	0.24	9.78	1976	1976
61	Eicosane		0.16	1998	2000
62	Eicosane, 2-methyl-	0.23		2063	2063
63	9,12,15-Octadecatrienoic acid, methyl ester		0.79	2086	2086
64	Linolenic acid, methyl ester	0.13	0.28	2096	2096
65	Heneicosane	2.07	0.34	2100	2100
66	Phytol	0.26		2114	2114
67	Methyl stearate	0.43	0.10	2128	2128
68	Linoleic acid ethyl ester	0.08		2141	2144
69	Linoleic acid		40.1	2161	2159
70	Heneicosane, 3-methyl-	0.32		2172	2172
71	Docosane	3.10	0.43	2200	2200
72	Docosane, 4-methyl	0.22		2261	2261
73	Tricosane	23.5	14.1	2302	2300
Esters (No. 26,39,43,47,53,56,58,63,64,67,68)		51.6	12.6		
Oil acids (No. 51,54,60,69)		1.20	51.2		
Hydrocarbons (No. 2,3,25,42,57,59,61,62,65,70-73)		32.1	16.1		
Monoterpenes (No. 7,9,11-14,16,18)		3.03	8.02		
Sesquiterpenoids (No. 55)		2.30	0.79		
Monoterpenoids (No. 35,38,40,43,44,48,51,52,59)		1.52	2.28		
Aldehydes (No. 5,6,8,15,20,21,27,33)		1.31	0.71		
Sesquiterpenes (No. 37,38,41,45,46,48,49)		0.82	0.61		
Ketones (No. 4,44,50,52)		0.59	0.66		
Others (No. 1,22,34,36)		0.27	0.66		
Diterpenoids (No. 66)		0.26	-		

Alcohols (No. 10,17,23)	0.20	0.37		
Ethers (No. 35)	-	0.05		
Total percentages (%)	95.2	94.1		
Unidentified constituents	4.80	5.93		

PvcF: *Primula veris* subsp. *columnae* flower; PvcL: *Primula veris* subsp. *columnae* leaf; RT: Retention time; RI: Retention index, hydrocarbons with (C_6-C_{32}) carbon numbers were taken as standard, LRI: Literature retention indices based on Adams (2007), NIST and WILLEY.

A total of 62 of the compounds with the defined structures were found to belong to *P. veris* subsp. *columnae* flowers; 50 of them belonged to *P. veris* subsp. *columnae* leaves and constitute 95.2% and 94.1% of the total sample, respectively. While the number of compounds with the unidentified structures was 3 in flower volatile oil, it was 4 in leaf volatile oil; their ratios were 3.52% and 3.66%, respectively. Results of the analyses on the volatile oil of flowers show that the number of identified compounds and the percentage of these compounds in the total sample were found to be higher than the leaf volatile oil samples. The main compounds in volatile oils, which were isolated from flowers, were determined as methyl 4-methoxysalicylate (37.1%), tricosane (23.5%), methyl 2,6-dihydroxy-4-methyl benzoate (12.2%), and docosane (3.10%). The main compounds in volatile oils, which were isolated from the leaves, were determined as linoleic acid (40.1%), tricosane (14.1%), methyl 4-methoxysalicylate (7.49%), hexadecenoic acid (9.87%), and limonene (5.76%) (Table 1).

Vuko *et al.* (2017) reported that the main components were methyl 4-methoxysalicylate, pentacosane, and benzoic acid, 2-hydroxy-methyl ester (methyl salicylate) in their research, which they conducted on leaves, roots, and flowers volatile oil of *Primula veris* var. *columnae*. Güneş (2016) specified that while the main components of *Primula vulgaris* Huds. subsp. *sibthorpii* flower volatile oil were tricosane and hexadecenoic acid, the main components of leaf volatile oil were tricosane and hexadecenoic acid. Yaylı *et al.* (2016) specified that the main components of *Primula vulgaris* Huds. subsp. *vulgaris* volatile oil are methyl-4-methoxy salicylate, (*Z,Z,Z*)-7,10,13-hexadecatrienal, flavone, docosane, tricosane, and tetracosane, depending on the altitude (300 to 2100 m) in their study on *Primula vulgaris* Huds. subsp. *vulgaris* (Pvv) and *P. vulgaris* Huds. subsp. *sibthorpii* (Pvs). In the same study, the main compounds of *P. vulgaris* Huds. subsp. *sibthorpii* volatile oil were stated as methyl-4-methoxy salicylate, flavone, docosane, tricosane, tetracosane, and pentacosane depending on the altitude (100-1300 m). In another study, Nan *et al.* (2002) stated that the main components in the *Primula obconica* leaves, stems and flowers volatile oils were found as methyl 2,4-dihydroxy-5-methyl benzoate, methyl 2,6-dihydroxy-4-methyl benzoate, hypnone, and methyl salicylate.

When the main components of essential oil obtained in the present study were compared with the studies in the literature, it was seen that compounds such as methyl 4-methoxysalicylate, tricosane, methyl 2,6-dihydroxy-4-methyl benzoate, docosane, methyl 4-methoxysalicylate were similar. In addition, it was understood that differences occurred in compounds such as linoleic acid, limonene, tetracosane, pentacosane, methyl salicylate, flavone, (*Z,Z,Z*)-7,10,13-hexadecatrienal, and hypnone. The chemical composition and the number of essential oils vary according to their type, species, growing environment, climatic characteristics, genetic differences, collection time, storage method, and analysis parameters of the plants (Öz *et al.* 2021; Karataş *et al.* 2022).

The 62 compounds with identified structures in flower samples were classified into

13 groups. The identified structures from these classes and the number of compounds they contained were determined, respectively, as 4 ketones, 7 aldehydes, 3 alcohols, 10 esters, 3 oil acids, 11 hydrocarbons, 7 monoterpenes, 6 monoterpenoids, 7 sesquiterpenes, 1 sesquiterpenoid, 1 diterpenoid, and 2 others. Fifty compounds with the identified structures in leaf samples were classified into 13 groups. The identified classes in leaf samples were, respectively, 3 ketones, 4 aldehydes, 3 alcohols, 8 esters, 1 ether, 4 oil acids, 7 hydrocarbons, 6 monoterpenes, 7 monoterpenoids, 2 sesquiterpenes, 1 sesquiterpenoid, and 4 others. In flower volatile oil samples, hydrocarbons have the highest number of compounds with 11 compounds, while in the leaf volatile oil samples, esters were the most frequent, with 8 compounds.

As the result of flower essential oil analysis, the chemical class with the highest % amount was esters with 51.6% and it was oil acids with 51.2% in leaf volatile oil analysis. While 22 compounds and 7.93% terpene class compounds were identified in flower volatile oil samples, monoterpenes (3.03%) were determined as the highest terpene class. While 16 compounds and 11.7% terpene class compounds were identified in the leaf volatile oil, the highest terpene class was found as monoterpenes (8.02%) in flowers. The chemical classification of compounds found in flowers and leaves of *P. v.* subsp. *columnae* are given in Fig. 2.

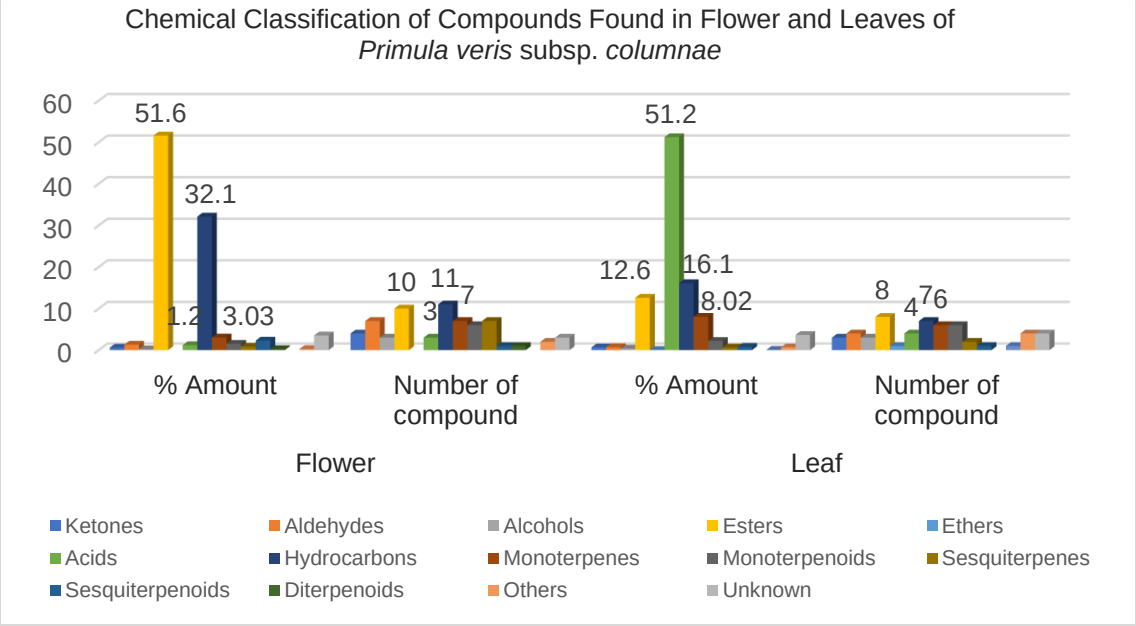

Fig. 2. Chemical classification of compounds found in flowers and leaves of *Primula veris* subsp. *columnae*

The Extractive Substance Amount Obtained as the Result of Methanol Extraction

As a result of the methanol extraction analysis, the percentage of extractive substance amount in the flower was determined as 34.5%. The extractive substance amount in the leaf was detected as 28.8%.

Antioxidant Activity Results

Antioxidant activity results of methanol extracts obtained from flowers and leaves of the *P. v.* subsp. *columnae* plant are given in Table 2.

Table 2. Antioxidant Activity of Methanol Extracts from *P. veris* subsp. *columnae*

	Flower Avg.* ± SD.**	Leaf Avg.* ± SD.**
DPPH (Inhibition %)	81.97 ± 0.73	61.03 ± 2.64
DPPH (mg AA eq./g) (mg Trolox eq./g)	24.75 ± 0.10 25.74 ± 0.09	6.42 ± 0.07 7.28 ± 0.34
FRAP (mg $FeSO_4$ eq. /g)	667.59±41.30	165.83±2.62
TAC (Total Antioxidant Content) (mg AA eq./g)	127.05±9.55	74.63±5.80
TPC (Total Phenolic Content) (mg GA eq./g)	78.62±2.25	38.43±0.68
TFC (Total Flavanoid Content) (mg Catechin eq./g)	1.96±0.06	1.00±0.01

*: Results are given as average, **±: Standard deviation, AA: Ascorbic acid, GA: Gallic acid

DPPH Free radical scavenging activity

As shown in Table 2, the radical scavenging amount (DPPH) of the samples were defined as 24.75 ± 0.10 mg AA eq./g and 25.74 ± 0.09 mg Trolox eq./g in methanol extract of flowers. The amount of DPPH was found to be 6.42 ± 0.07 mg AA eq./g and 7.28 ± 0.34 mg Trolox eq./g in leaves. DPPH % inhibition rates of the same samples were determined as between 81.97 ± 0.73 and 61.03 ± 2.64%, respectively. When the radical scavenging amount and % inhibition ratios of flower and leaf extracts were examined, the flower methanol extract demonstrated more inhibition properties when compared with the leaf. It is considered to originate from the existence of more organic compounds in the flower sections when compared with the leaf sections. In the literature, DPPH inhibition ratios were found out as 86.65% ± 1.11 and 88.46% ± 0.11 in the study on 10 and 20 *Primula veris* L. flower samples with 70% ethanol extract. It has been acknowledged that the inhibition ratio increased depending on the concentration (Tünde *et al.* 2015). In the literature, it has been stated that the main active compounds of primula flowers and roots are phenolic compounds, flavonoids (about 3% in flowers), phenolic acids, and phenolic glycosides, as well as triterpene saponins. Saponins are responsible for secretolytic and expectorant activity; phenolic compounds in *Primula* flowers are responsible for antioxidant, antimicrobial and cytostatic properties (Tokalov *et al.* 2004; Demir *et al.* 2014). When compared in terms of DPPH inhibition rates, it was understood that *P. veris* subsp. *columnae* species has similar values with other *Primula* species.

Ferric (III) ion reducing antioxidant power (FRAP)

Total ferric reduction antioxidant capacity (FRAP) amounts of the *P. v.* subsp. *columnae* samples were found out as 677.59 ± 41.30 mg $FeSO_4$ eq./g in the flower section and 165.83 ± 2.62 mg $FeSO_4$ eq./g in the leaf section (Table 2). In the study conducted with the fresh above ground sections of *Primula veris* L., the total ascorbic acid content was determined as 118 mg/100g. In the same study, the FRAP value of *Primula veris* L. plant was determined in terms of 757 µmol Fe II/g (Murathan 2018). When compared with *Primula veris* L. values in the literature, the FRAP values of *P. v.* subsp. *columnae* methanol extract were determined as significantly different, it was identified that especially flower sections have high ferric reduction properties.

Total phenolic substance

Total phenolic substance amounts prepared from 0.20% methanol extracts obtained from flower and leaf sections of *P. v.* subsp. *columnae* species have been found as 78.62 ± 2.25 mg GA eq./g in the flower section in terms of gallic acid and as 38.43 ± 0.68 mg GA eq./g in the leaf section (Table 2). In the literature, phenolic compounds, especially hyperoxide, primeverin, and primulaverin, have been specified as chemical markers for the determination of *Primula* species in a study on *Primula veris*. Bączek *et al.* (2017) found it as 1.73 ± 0.16 mg/g in their total phenolic substance study conducted on *Primula veris* L. in 2017. In a study, the total phenolic substance contents of different *Primula veris* L. extracts were determined as between 5.10 and 17.3 mg/g, respectively (Rudhani *et al.* 2017). It can be said that *P. v.* subsp. *columnae* methanol extract is significantly different in terms of phenolic substance when looking at the values of *Primula veris* L. plant in the literature. On this basis, it can be declared as quite rich in terms of phenolic content.

Total antioxidant substance

According to ascorbic acid standards, the total antioxidant substance was found as 127.05 ± 9.55 mg AA eq./g in the flower section and 74.63 ± 5.80 mg AA eq./g in the leaf section in samples with 0.20% methanol extracts obtained from flower and leaf sections of *P. v.* subsp. *columnae* species (Table 2). *Primula veris* L. is a plant species of the Primulaceae family. According to the literature data, it has been stated as a plant rich in saponins (about 60%) including primulic acid, as well as many flavonoid compounds and flavanols, which means rutin, catechin, kaempferol, and luteolin (Okrslar *et al.* 2007; Colombo *et al.* 2017). *Primula veris* L. is a source of phenolic and flavonoid compounds. Since they are rich in rutocide, they express effects in terms of many pharmacological hyperoxide activities, which means anti-inflammatory, antioxidant, and antimicrobial activities (Başbülbül *et al.* 2008).

Total flavonoid substance

The total amount of flavonoid substance was determined as 1.96 ± 0.06 mg catechin eq./g in the flower and as 1.00 ± 0.01 mg catechin eq./g in the leaf section of 0.20 % methanol extract samples obtained from the flower and leaf sections of the *P. v.* subsp. *columnae* species, regarding to the catechin standards (Table 2). In a study, Rudhani *et al* figured out that the total flavonoid substance contents of different *Primula veris* L. extracts were between 12.2 and 31.4 mg/g, respectively (Rudhani *et al.* 2017). In another study, the total flavonoid substance contents of above ground sections of *Primula veris* L. were determined as 22.88±2.7 mg/g (Murathan 2018). In the literature, it has been acknowledged by the public that the leaves, roots, and flowers of *Primula veris* L. have various health properties lasting for a long time. Therefore, it is used as a diuretic, antimicrobial, antifungal, sedative, anti-inflammatory and expectorant remedy among the public. Since the plant is also rich in phenolic acids and flavonoids in its flowers and roots, it is used against bronchitis, cough, and flu (Başbülbül *et al.* 2008). When compared with the literature data, it can be said that the antioxidant properties of the *P. veris* subsp. *columnae* plant are quite high.

Antimicrobial Test Results of Volatile Oil and Methanol Extracts

In the antimicrobial activity test conducted on flower and leaf volatile oils and methanol extracts, it was determined that among them, only flower essential oil showed weak activity against *Escherichia coli* bacteria, and it did not show any antifungal and

antibacterial activity apart from that. The antimicrobial activity test conducted on flower and leaf volatile oils and methanol extracts are shown in Table 3.

Başbülbül *et al.* (2008) found with the well diffusion method that among the tested microorganisms: *Enterococcus faecalis*, *Bacillus cereus*, and *Pseudomonas fluorescens* are inhibited by all extracts in their study on water, ether, and ethanol extracts of *Primula veris* L. flowers. Besides, ether and water extracts had higher inhibitory activity than ethanol extract and it has been stated that none of the tested extracts showed a activity against *Staphylococcus aureus*, *Proteus* sp. and *Listeria* sp. Najmus-Saqib *et al.* (2009) stated in their study that antibacterial activity was carried out on *Escherichia coli*, *Bacillus subtilis*, *Shigella flexneri*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi* by using the raw extract of *Primula macrophylla* and agar well diffusion method, and the samples did not show any activity at the end of the study.

Yaylı *et al.* (2016) stated that *Primula vulgaris* Huds. subsp. *vulgaris* (Pvv) and *P. vulgaris* Huds. subsp. *sibthorpii* (Pvs) volatile oils showed weak activity against *Bacillus cereus* and strong activity against *Mycobacterium smegmatis* in their antimicrobial study with agar diffusion method. The resulting activity can be explained by the high concentration of hydrocarbons and aromatic esters found in essential oils (Yaylı *et al.* 2016).

In the literature, it is emphasized that carboxylic acids, aldehydes, and terpenes (from the main chemical classes of essential oils) have antimicrobial properties (Swamy *et al.* 2016; Baltacı *et al.* 2022).

Table 3. Antimicrobial Activity Test Conducted on Flower and Leaf Volatile Oils and Methanol Extracts

	Volatile Oils				Methanol Extracts			
	Flower		Leaf		Flower		Leaf	
	100 %	50 %	100 %	50 %	100 %	50 %	100 %	50 %
Bacteria sp.								
Aeromonas hydrophila ATCC 7965	-	-	-	-	-	-	-	-
Bacillus cereus ATCC 33019	-	-	-	-	-	-	-	-
Bacillus subtilis ATCC 6633	-	-	-	-	-	-	-	-
Enterobacter cloacae ATCC 13047	-	-	-	-	-	-	-	-
Escherichia coli ATCC 11230	4.50± 0.10	-	-	-	-	-	-	-
Escherichia coli O157: H7 ATCC 33150	-	-	-	-	-	-	-	-
Klebsiella pneumoniae ATCC 13883	-	-	-	-	-	-	-	-
Listeria monocytogenes ATCC 7644	-	-	-	-	-	-	-	-
Proteus vulgaris ATCC 13319	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa ATCC 17853	-	-	-	-	-	-	-	-
Salmonella typhimurium ATCC 14028	-	-	-	-	-	-	-	-
Staphylococcus aureus ATCC 25923	-	-	-	-	-	-	-	-
Yeasts and Mold								
Aspergillus niger ATCC 20611	-	-	-	-	-	-	-	-
Candida albicans ATCC 1223	-	-	-	-	-	-	-	-
Aspergillus flavus ATCC 9807	-	-	-	-	-	-	-	-
Penicillium expansum ATCC 74414	-	-	-	-	-	-	-	-
Saccharomyces cerevisiae BC 5461	-	-	-	-	-	-	-	-

CONCLUSIONS

1. In the conducted study, 62 volatile components were found in the flower section of the plant and 50 volatile components were found in the leaf section, which belong to acid, hydrocarbon, alcohol, aldehyde, ketone, terpenoids, and ester groups. As the result of the analyses on the volatile oil of the flowers, the number of identified compounds and the percentage of these compounds with respect to the total sample were found to be higher than the leaf volatile oil samples.
2. As the result of the extraction of the flowers and leaves of the plant with methanol, it was identified that the ratio of extractive substance amount in the flower was higher than extractive substance amount in the leaf.
3. When the *P. veris* subsp. *columnae* plant was examined generally, its antioxidant properties were determined as quite high. It has been revealed that the plant is rich in terms of phenolic substances in samples prepared from methanol extracts, which were obtained from the flower and leaf sections of the plant. It can be stated that its phenolic components can be investigated in future studies.
4. In the antimicrobial activity test, which was carried out on flowers and leaves of the *P. veris* subsp. *columnae* species, only flower volatile oil showed weak activity against *Escherichia coli* bacteria. Apart from that, no activity against test microorganisms was evident in any one of the used concentrations of flower and leaf volatile oils and methanol extracts.

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