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Some morphological and phenological characteristics and volatile components of *Campanula rapunculus* L. var. *rapunculus*

Abstract. *Campanula* is one of the largest genus of the *Campanulaceae* family, represented by approximately 115 species in Turkey and is spread through Iran-Turan, Eastern Mediterranean and Mediterranean phytogeographical regions. The aim of the current study was to determine some morphological features, phenological characteristics and leaf-flower volatile components of *Campanula rapunculus* var. *rapunculus* collected in different sites of Uludag Mountain (Bursa). The average plant height was calculated as 37.21 cm, number of corolla was 2.4, the length of the corolla was 2.0 cm, calyx lobe length was 1.36 cm, leaf width was 1.7 cm and leaf length was 4.31 cm. Considering phenological features, it was measured that the first flowering ends in May, full flowering ends in June and late flowering ends in late July. A total of 32 different volatile components were detected in *Campanula rapunculus*. With the aid of gas chromatography-mass spectroscopy (GC-MS) of volatile components by solid phase microextraction technique (SPME), 3-Methylbutanal (10.87%), cis-3-Hexene-1-ol (9.85%) and 3-Octanol (9.62%) were identified as major components. In addition to its medicinal benefits, studies on *Campanula* genus, considered as food and ornamental plants, should be increased and its value should be revealed.

Key words: *Campanula*, solid phase microextraction technique, volatile components, morphological and phenological features.

Introduction

Plants demonstrate an extensive range of positive features, including antibacterial, anthelmintic, antihypertensive, antimutagenic, carminative, choleretic, sedative, antispasmodic, insecticide [1-8]. Medicinal and aromatic plants, which have never lost their importance throughout the history of mankind, are preferred extensively today with the rise of living standards [9,10].

Rich in medicinal and aromatic plants *Campanulaceae* is mostly presented by herbaceous or woody climber plants, rarely consisting of thick-bodied trees and bushes. *Campanula*, one of the largest genera of the *Campanulaceae* family, comprises around 300 species across the globe, especially in the northern hemisphere and Mediterranean region [11]. However, according to some records, the number of species of this genus is reported to be approximately 500 [12].

Leaves of *Campanula* are altered but can sometimes vary. Flowers are violet or blue, big and showy. Seeds are endospermic. Fruit is a capsule, it can rarely be a capsule with caps in some breeds, meaty grapes or dry and unopened [13-16].

The genus *Campanula* is represented by about 115 species in our country and has 43 taxa endemic at the species level [17]. *Campanula rapunculus* is a 2-year plant with a length of 50-100 cm. The lower stem and leaves are obovate, while the top ones are reduced. Flowers are unique but rarely, can be similar to 2-3 paniculae and long spica. Corolla is funnel-shaped, pale blue or whitish, slightly longer than calyx lobes. It has 3 stigmas. The taxon that blooms in May-July is 1500-2200 m in Uludag. It spreads between stunted junipers, shrubs and under the *Pinus-Fagus-Abies* forest at altitudes [18].

Bursa city and Uludag are one of the most important centers of plant diversity in Turkey. Different vegetation zones starting from the skirts of Uludag mountain to the summit and are located at different altitudes are very rare in the world and are of particular importance [18]. Under the focus of the current study were morphological characteristics, phenological features and leaf-flower volatile components of *Campanula rapunculus* var. *rapunculus* collected from different zones of Uludag.

Materials and methods

Object. Campanula rapunculus var. *rapunculus* collected from different points of Uludag mountain served the object of the study. During the field stud-

ies within 2018-2019 vegetation period, coordinates of distribution points of *Campanula rapunculus* var. *rapunculus* were taken with Garmin GPS Map 64 S. 20 different points, 20 x 20 m in size identified as sample areas representing different growing conditions (Figure 1; Table 1).

Figure 1 – Sampling areas within the study area – Uludag mountain, Bursa (Source: Google Earth®)

Table 1 – Information on the determined sample areas in the study area

Sample No.	Sampling Locations	Altitude	Aspect
	40°06,712' N-29°06,162' E	1707 m	NW
	40°06,751' N-29°06,243' E	1674 m	NE
	40°06,674' N-29°06,277' E	1649 m	NE
	40°06,821' N-29°06,805' E	1793 m	NW
	40°06,883' N-29°06,501' E	1677 m	SW
	40°07,009' N-29°06,454' E	1646 m	SW
	40°07,137' N-29°06,525' E	1685 m	W
	40°07,108' N-29°06,817' E	1744 m	NW
	40°08,006' N-29°06,971' E	1608 m	NW
	40°06,970' N-29°07,029' E	1701 m	N
	40°06,830' N-29°06,985' E	1830 m	NE
	40°06,675' N-29°07,008' E	1871 m	N
	40°06,174' N-29°07,541' E	1961 m	E

Table 1 continued

Sample No.	Sampling Locations	Altitude	Aspect
	40°06,035' N-29°07,854' E	1949 m	N
	40°06,389' N-29°07,656' E	1901 m	NE
	40°08,055' N-29°08,707' E	1671 m	W
	40°07,386' N-29°09,284' E	1741 m	NW
	40°06,889' N-29°09,475' E	1852 m	W
	40°06,764' N-29°04,957' E	1440 m	NW
	40°07,303' N-29°05,534' E	1521 m	W

Samples were taken from 20 areas with altitudes varying from 1440 to 1961 m above the sea level.

Analysis of morphological properties. Samples of *Campanula rapunculus* var. *rapunculus* were collected from sampling areas for determining of morphological features and storage in herbarium. In order to calculate the morphological measurements, digital diameter meter and meter, 30 plant length, number of corolla, length of corolla, calyx lobe length, leaf width and height were measured (Figure 2).

Figure 2 – Images of some morphological measurements on *Campanula rapunculus* var. *rapunculus*.
Note: a – plant height, b – corolla height, c – leaf width, d – leaf length. Photo by A.G. Sarıkaya

Estimation of phenological time. Field studies started on March, 2018. Along with some features of *Campanula rapunculus* characteristics of the spreading area were considered, such as topographic, soil, climate, other vegetation and altitude.

Analysis of volatile components. Leaves and flowers of *Campanula rapunculus* collected from the testing site, were dried at room temperature (25°C), leaves and flowers components were determined by the Peak Cavity-Solid Phase Micro Extraction (HS-SPME) technique combined with gas chromatography/mass spectrometry (GC/MS).

Samples of leaves and flowers taken as 2.5 g of each sample were placed in a 10 mL glass vial closed with a silicone cap and kept at 60°C for 30 minutes. The SPME apparatus was passed through 75 µm thin Carboxen/Polydimethylsiloxane (CAR/PDMS) coated fused silica fiber (Merck, Germany) to adsorb volatile compounds and then was directly injected into the capillary column (Rtx®-5MS 30 m x 0.25 mm I.D. df=0.25 µm, Restek, USA).

Device was connected to the same brand mass selector detector operated in hand mode (70 eV).

This process was repeated three times and the accuracy of the results was compared and the results were given as average. Helium with a flow rate of 1.61 mL per minute was used as the carrier gas. Injection and detection temperatures were set at 250°C. Retention Indices (RI) of the volatile components were calculated according to the C7-C30 alkane mixtures standard under the above-mentioned chromatographic conditions. The identification of the compounds was determined by comparison of mass spectra and compounds found in the spectral library (Wiley 7th edition, NIST 20, Tutor, FFNSC 3).

Results and discussion

Analysis of morphological properties and phenological time. Mean plant height of *Campanula rapunculus* collected from different points of Uludag was determined as 37.21 cm, number of corolla – 2.4, corolla length – 2.0 cm, calyx lop length – 1.36 cm, leaf width – 1.7 cm, leaf length – 4.31 cm (Table 2).

It was noted that first flowering started in early May, full flowering was in June and late flowering ended in late July. The color of the flowers varied from whitish and pale blue to bluish-purple and lilac-blue (Figure 3).

Table 2 – Morphological measurements of *Campanula rapunculus* var. *rapunculus*

Features	Smallest	Biggest	Mean
Plant height	25 cm	55.5 cm	37.21 cm
Number of corolla	2	3	2.4
Corolla length	1.4 cm	3 cm	2.0 cm
Calyx lop length	0.5 cm	2.4 cm	1.36 cm
Leaf width	0.5 cm	2.7 cm	1.37 cm
Leaf length	2.3 cm	7 cm	4.31 cm

Figure 3 – *Campanula rapunculus* var. *Rapunculus* at Uludag, Bursa
Note: a – general view, b – flower. Photo by S.A. Kavaklı

In the study by Davis [17], plant height of *Campanula rapunculus* was determined as 50-100 cm, number of corolla as 2-3, the length of the corolla as 1.2-2 cm, the calyx lobe length 0.9-1.6 cm, leaf width as 2 cm and the length as 4 cm. It was determined that morphological measurements other than the number of corolla differed from our study. Kaynak et al. [18] assessed the plant height of *Campanula rapunculus* as 50-100 cm, and the length of the corolla as slightly longer than the calyx lobes. The height of the plant differed from our study, though the fact that the length of the corolla and slightly longer than the calyx lobes supports our study.

In our study it was observed that first flowering began in early May, full flowering was observed in June, and flowering ended in late July. The color of the flower was determined to be whitish, pale blue, bluish-purple and lilac-blue. Kaynak et al. [18] determined the flowering time as May-July and color of the flower as whitish to pale blue. It was determined that the phenological time and flower color were the same in both studies, and in our study, the color of the flower was bluish-purple and lilac-blue.

Analysis of volatile components. Volatile components of *Campanula rapunculus* were determined by GC-MS using solid phase microextraction technique (SPME). A total of 32 different components were detected in *Campanula rapunculus*. 3-Methylbutanal (10.87%), cis-3-Hexene-1-ol (9.85%) and 3-Octanol (9.62%) were identified as the main components. Among volatile components, aromatic aldehydes were found to be high (Figure 4; Table 3).

Peak values seen on Figure 4 revealed the presence of 32 components.

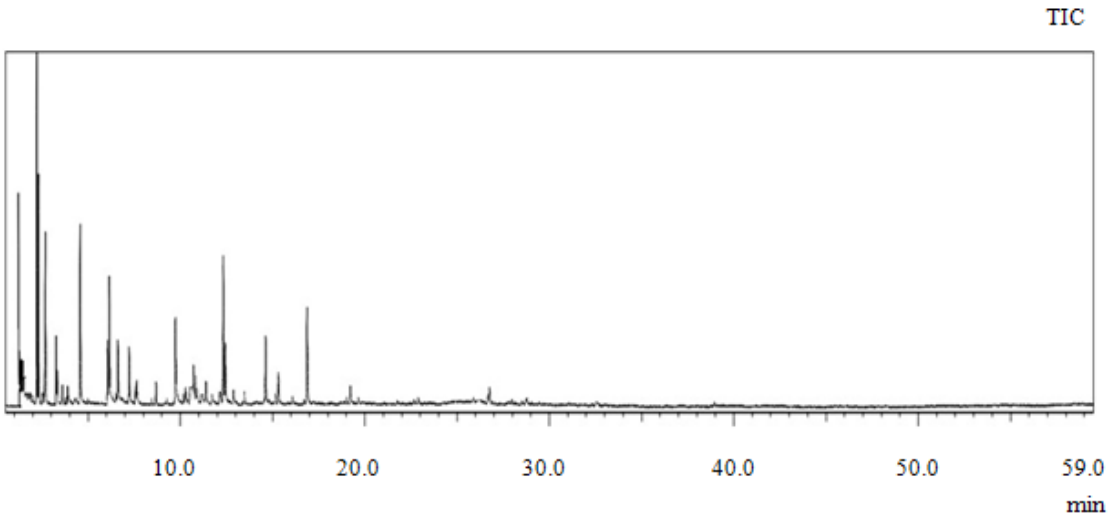

Figure 4 – Gas chromatogram of volatile components for *Campanula rapunculus* var. *rapunculus*

Table 3 – Leaf-flower volatile components of *Campanula rapunculus* var. *rapunculus*

	Retention time	Components	%	Formula	Class
	1.434	2-methyl-Pentanal	1.66	$C_6H_{12}O$	AA
	1.660	2-Methylpropanal	0.68	C_4H_8O	AA
	2.215	3-Methylbutanal	10.87	$C_5H_{10}O$	AAI
	2.304	2-Methylbutanal	7.36	$C_5H_{10}O$	AAI
	2.684	Sorbaldehyde	6.14	C_6H_8O	AA
	3.264	3-Methyl-1-butanol	1.82	$C_5H_{12}O$	AA
	3.331	2-methyl-1-Butanol	1.09	$C_5H_{12}O$	AA
	3.615	(E)-2-Pentenal	0.71	C_5H_8O	AAI
	3.888	1-Pentanol	0.42	$C_5H_{12}O$	AA

Table 3 continued

	Retention time	Components	%	Formula	Class
	4.573	Hexanal	5.74	$C_6H_{12}O$	AA
	6.057	(E)-2-Hexenal	3.13	$C_6H_{10}O$	AA
	6.149	cis-3-Hexene-1-ol	9.85	$C_6H_{12}O$	AA
	6.505	(Z)-2-Hexen-1-ol	0.84	C_6H_{12}	AA
	6.610	n-Hexanol	3.36	$C_6H_{14}O$	AA
	7.220	2-Heptanone	2.06	$C_7H_{14}O$	AA
	7.635	Heptanal	1.08	$C_7H_{14}O$	AA
	8.691	α-pinene	0.76	$C_{10}H_{16}$	MH
	9.740	Benzaldehyde	4.69	C_7H_6O	AAI
	9.840	3-Octanol	9.62	$C_8H_{16}O$	AAI
	10.397	3-Octanone	3.62	$C_8H_{14}O$	AAI
	10.650	(Z)-6-Octen-2-one	0.75	$C_8H_{14}O$	AAI
	11.145	2.4-Heptadienal	0.97	$C_7H_{10}O$	AA
	11.387	Octanal	0.81	$C_8H_{16}O$	AAI
	12.327	Limonene	5.67	$C_{10}H_{16}$	MH
	12.436	1.8-Cineole	2.49	$C_{10}H_{18}O$	OM
	12.885	Benzeneacetaldehyde	0.53	C_8H_8O	OC
	13.469	γ.-Terpinene	0.56	$C_{10}H_{16}$	MH
	14.622	Fenchone	2.66	$C_{10}H_{16}O$	OM
	15.320	Nonanal	1.87	$C_9H_{18}O$	AAI
	16.872	Camphor	6.96	$C_{10}H_{16}O$	OM
	19.212	Decanal	0.65	$C_{10}H_{20}O$	OM
	26.764	Caryophyllene	0.58	$C_{15}H_{24}$	SH
Total			100		
Component number			32		
AA: Aromatic alcohol			38.84		
AAI: Aromatic aldehyde			40.30		
MH: Monoterpene hydrocarbon			6.99		
OC: Other components			0.53		
OM: Oxygen monoterpene			12.76		
SH: Sesquiterpene hydrocarbon			0.58		

3-Methylbutanal (10.87%), cis-3-Hexene-1-ol (9.85%) and 3-Octanol (9.62%) were identified as the main components. 3-Methylbutanal component is used as a reagent for the production of pharmaceuticals and pesticides.

No study was found on the volatile components of *Campanula rapunculus* species. However, there are some researches on other *Campanula* species. In previous studies on volatile components of *Campanula* genus, Politeo et al. [19] identified the presence of 53 different components in *Campanula portenschlagiana*, and identified labda-13 (16), 14-dien-8-o as the main components. Chenxing et al. [20] identified 57 different components in *Campanula colorata* Wall. with 1,2-benzenedicarboxylic acid, butyloctyl ester, cedar camphor, hexadecanoic acid, dibutyl phthalate, tetradecanoic acid, caryophyllene oxide,

6,10,14-trimethyl-2-pentadecanone as main components. These results differ from the results presented in the current study.

Conclusion

Fresh leaves and roots of *C. rapunculus* are used raw (salad, etc.) or cooked (soup, tea, etc.) [21-24]. *Campanula rapunculus* is begining to appear more frequently in local street markets in the Umbria Region, Central Italy and used to treat inflammation of the oral cavity, also leaves used to treat warts, infusion of flowers used as a gargle [25]. It is known that leaves and roots were used as milk enhancer and stone reducer in Turkey, and today it is used in wound healing and constipation disorders [26-28]. *Campanula* species are especially rich in flavone and flavone glycosides. These species, which have decorative flowers, also find use as ornamental plants [29]. In addition to its medical benefits, it is recommended to increase the anatomical, physiological, molecular and biochemical studies of *Campanula* taxa, which are considered as food and ornamental plants. Thus, the value and importance of *Campanula* genus, which spread naturally in our country, can be revealed.

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