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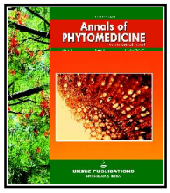

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Anatomical and volatile components investigations on *Primula vulgaris* Huds. subsp. *vulgaris* (Primulaceae)

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Abstract

In this study, the anatomical features and volatile components of *Primula vulgaris* Huds. subsp. *vulgaris* were investigated. Primary growth was observed in sections taken from the root. The leaf and pedicel, only glandular hairs were seen. It was found that the leaf was amphistomatic and the stoma type was anomocytic on both surfaces. The fresh and crushed underground parts of *P. vulgaris* subsp. *vulgaris* were trapped by HS-SPME fiber and analyzed by GC/MS. Fourteen components (97.2%) were characterized. Main components were found as methyl salicylate (51.2%), methyl-4-methoxy salicylate (18.5%) and paeonol (12.4%).

1. Introduction

Primula L., one of the important genera of the Primulaceae family, is distributed in the world with about 426 species (Fico *et al*., 2007). *Primula* genus consists of 9 species, 2 of which are endemic and is known as "Cuhacicegi" in Turkey (Coskuncelebi, 2012). Some species of the family are used for ornamental plants (Simpson, 2012). The HMPC states that *Primula veris* and *P. elatior* (Primulae-radix) are used expectorant (EMA, 2016). *Primula veris* contain saponin glycosides, essential oil, flavone derivatives and especially used as expectorant. Evliya Celebi, in his book of "Seyahatname", refers to the good smell of *Primula* species and the plant's use in the treatment of eye diseases (Baytop, 1999). Many ethnobotanical studies have shown that *P. vulgaris* are used in different form and in the treatment of different target diseases (Jaric *et al*., 2007; Ugulu *et al*., 2009; Koca and Yildirimli, 2010; Saric Kundalic *et al*., 2010; Sarac *et al*., 2013; Akbulut and Ozkan, 2014).

In the literature, anatomical studies (Belaeva and Butenkova, 2019; Ergen-Akcin *et al*., 2019; Ergen-Akcin *et al*., 2021) and volatile components studies (Vitalini *et al*., 2011; Colombo *et al*., 2014) on *Primula* species have been reported. *Primula vulgaris* subsp. *vulgaris* (*Primula acaulis* (L.) Hill subsp. *acaulis*) is one of the 13 taxon of *Primula* genus in Turkey (Coskuncelebi, 2012) and flowers of this species are usually yellow, rarely white (Lamond, 1978). This study investigated the anatomical features and volatile components of *P. vulgaris* subsp. *vulgaris*.

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2. Materials and Methods

2.1 Plant material

P. vulgaris subsp. *vulgaris* was collected during to flowering stage in 2018, Eskisehir. The specimen had been stored in Anadolu University, Faculty of Pharmacy Herbarium (ESSE:15532).

2.2 Morphological and anatomical methods

Plant specimen identification was made by using "Flora of Turkey" (Lamond, 1978). From a part of the material, 70% alcohol sample was prepared for the anatomical study. In anatomical study, cross (root, pedicel, leaf) and superficial (leaf) sections were taken from plant parts. The section photos were taken using a light microscope (Olympus BX51T).

2.3 Headspace-solid phase microextraction (HS-SPME) procedure, gas chromatography/mass spectrometry analysis (GC/MS) and identification of components

SPME fibre precoated with a 75 µm layer of Carboxen ®/ Polydimethylsiloxane (CAR/PDMS) (supplied by Supelco Bellefonte, USA), was used with a sampling time of 30 min (room temperature). The crushed rhizome and root volatile components of *P. vulgaris* subsp. *vulgaris* were captured with HS-SPME fiber and analyzed by GC/MS (Kucuk *et al*., 2018).

3. Results

3.1 Anatomical results

Primary growth is observed in cross sections taken from *P. vulgaris* subsp. *vulgaris* root. Epidermis is made up of a single layer, small and elliptic or round cells. The exodermis layer consists of 1-3 cells. The cortex layer covers a large area and consists of 16-18 rows of thick-walled parenchyma cells. Endodermis layer and

pericycle can be distinguished. The xylem 8-arm (octa arch) or xylem arms can be very close to each other (Figure 1).

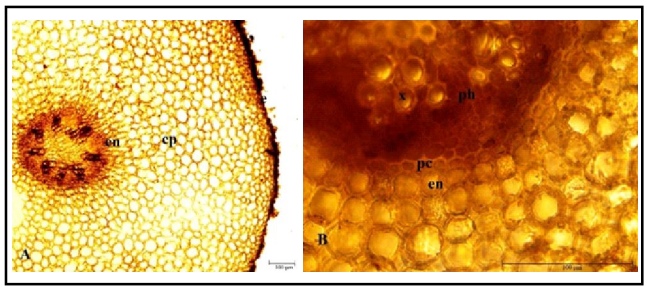

Figure 1: ***P. vulgaris*** **subsp.** ***vulgaris*****; cross-section of root. A: Schematic, B: Anatomic (e: epidermis, cp: cortex parenchyma, en: endodermis, pc: pericycle, x: xylem, ph: phloem).**

The outer part of the pedicel is consists of a corrugation cuticle layer and then a single row of small round or oval epidermis cells. The cortex layer is formed collenchyma and parenchyma cells. Under the epidermis layer, there are 3-4 lines of rarely intercellular spaces with collenchyma cells and then 3-4 rows of generally round parenchyma cells in areas near the central cylinder. There are phloem cells between the xylem arms lined in 5 rows (Figure 2).

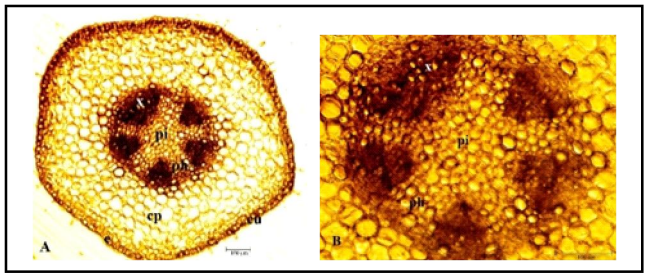

Figure 2: ***P. vulgaris*** **subsp.** ***vulgaris*****; cross-section of pedicel. A: Schematic, B: Anatomic (cu: cuticula, e: epidermis, cp: cortex parenchyma, x: xylem, ph: phloem, pi: pith).**

The following structures are observed in the leaf middle vein, leaf upper and lower superficial sections. The midrib of the leaf is schematically triangular. A row of small epidermis cells is located on the upper and lower surface of the leaf. Mesophyll layer consists of 1-2 rows of palisade parenchyma and 2-3 rows of sponge parenchyma (dorsiventral) (Figure 3).

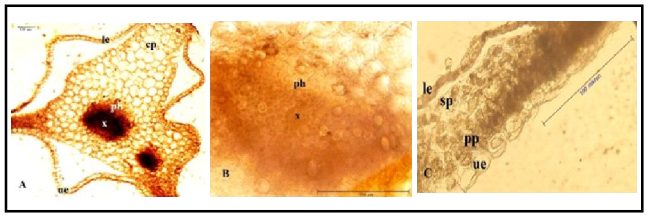

Figure 3: ***P. vulgaris*** **subsp.** ***vulgaris*****; cross-section of leaf. A: Schematic, B-C: Anatomic (ue: upper epidermis, le: lower epidermis, ph: phloem, x: xylem, sp: sponge parenchyma, pp: palisade parenchyma).**

Stoma cells are observed on both surfaces of leaf superficial sections. The stoma on both its upper and lower surfaces is of an anomocytic type and has an oval or round shape. It is seen that lower epidermis cells are more corrugated than upper epidermis cells in superficial sections (Figure 4).

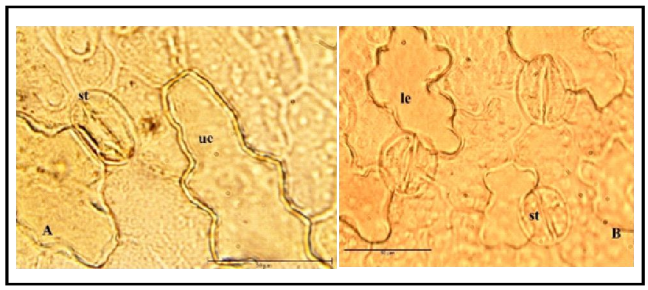

Figure 4: ***P. vulgaris*** **subsp.** ***vulgaris;*** **leaf surface view of upper (A) and lower (B) (ue: upper epidermis, le: lower epidermis, st: stomata).**

Glandular hairs are observed in the cross sections of the leaf and pedicel. Non-glandular hairs were not observed in the sections taken. In addition, glandular hairs have a single head cell and usually a single stalk cell (Figure 5).

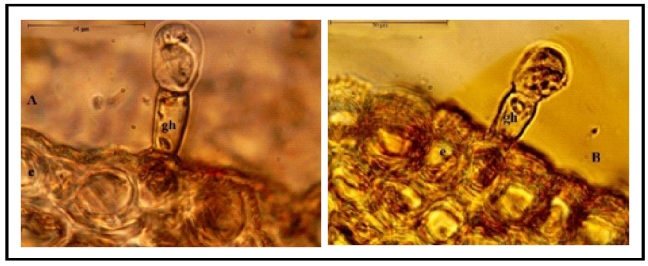

Figure 5: ***P. vulgaris*** **subsp.** ***vulgaris;*** **hairs of leaf (A) and pedicel (B).(e: epidermis, gh: glandular hair).**

3.2 SPME results

Volatile components of *P. vulgaris* subsp. *vulgaris* fresh and crushed underground parts were captured by HS-SPME technique and analyzed by GC/MS. The results are given in Table 1.

Table 1: Volatile components of ***P. vulgaris*** **subsp.** ***vulgaris***

RRI	Compounds	%	ID
1203	Limonene	1.9	t_R, MS
1255	γ-Terpinene	0.2	t_R, MS
1265	3-Octanone	2.3	t_R, MS
1280	*p*-Cymene	5.5	t_R, MS
1412	1,2-Dichloro benzene	1.7	MS
1495	2-Ethyl hexanol	0.2	MS
1532	Camphor	0.2	t_R, MS
1541	Benzaldehyde	0.2	MS
1611	Terpinen-4-ol	1.9	t_R, MS
1637	1,2-Dichloro octane	0.1	MS
1798	Methyl salicylate	51.2	MS
1878	Guaiacol	0.9	t_R, MS
2219	Methyl-4-methoxy salicylate	18.5	MS
2255	Paeonol	12.4	MS

RRI: Relative retention in dices calculated against n-alkanes. Identification method (ID): MS, identified on the basis of computer matching of the mass spectra with those of the Wiley, Adams and Mass Finder libraries and comparison with literature data. t_R: identification based on the retention times of genuine components on the HP Innowax column.

4. Discussion

In this study, the anatomical features of the *P. vulgaris* subsp. *vulgaris* collected from Eskisehir have been investigated with the sections taken from the root, pedicel and leaf parts.

Luna *et al.* (2017), in the leaf anatomical study of 5 genera and 33 taxa belonging to the Primulaceae family, were stated that a row epidermis layer and dorsiventral mesophyll are common features. Belaeva and Butenkova (2019) reported that *Primula denticulata* Sm., *P. macrocalyx* Bunge and *P. pallasii* Lehm. have anomocytic stoma type.

Ergen-Akcin *et al.* (2021) reported that in *P. acaulis* subsp. *rubra*, the root is xylem penta arch, the mesophyll layer in the leaf consists of 1-2 layers of palisade parenchyma, 2-3 layers of sponge parenchyma and stoma type is anomocytic stomata on both surfaces.

The anatomy of the *P. acaulis* subsp. *acaulis* was previously examined using the samples collected from Ordu province. In this study, it was reported that the pericycle layer in the root was evident, xylem tetra arch and the stomata in the leaf surface sections were of an anomocytic type (Ergen-Akcin *et al*., 2019). Unlike this study, root xylem was different and non-glandular hairs were not found in the leaf and pedicel sections in our study.

Fourteen volatile components were identified of the crushed rhizome and root representing 97.2% of *P. vulgaris* subsp. *vulgaris*. Main components were found as methylsalicylate (51.2%), methyl-4-methoxy salicylate (18.5%) and paeonol (12.4%).

Colombo *et al*. (2014) reported that a high amount of paeonal (98.0%) was found in *Primula albenensis* Banfi and Ferl leaves. Involatile components or essential oil studies on *Dionysia diapensifolia* Boiss. (Primulaceae) and some *Primula* species, methylacetate or methyl-4-methoxy salicylate components were found (Javidnia *et al*., 2010; Vitalini *et al*. 2011; Yayliet al., 2016).

According to Yayli *et al*. (2016) reported that the ratios of methyl-4-methoxy salicylate and (Z, Z, Z)-7,10,13-hexadecatrienal components in essential oils of *P. vulgaris* subsp. *vulgaris* and *P. vulgaris* subsp. *sibthorpii* collected from different altitudes varied significantly. Methyl-4-methoxy salicylate was one of main components in our study.

5. Conclusion

In this study, the anatomical features of the *P. vulgaris* subsp. *vulgaris* collected from Eskisehir have been investigated with the sections taken from the root, pedicel and leaf parts. Only glandular hairs were seen in the cross sections taken from the leaf and pedicel parts. It was observed that the leaf was amphistomatic and the stoma type was anomocytic on both surfaces. As a result, anatomical properties of *P. vulgaris* subsp. *vulgaris* are parallel within the literature studies. Regarding *P. vulgaris* subsp. *vulgaris*, 14 compounds have been identified in the rhizome and root. These are monoterpene (7.6%), oxygenated monoterpene (2.1%) and others (87.5%).

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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