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RESEARCH ARTICLE

INVESTIGATIONS ON MORPHO-ANATOMICAL AND VOLATILE COMPOUNDS OF CULTIVATED *Fritillaria persica* L. (LILIACEAE)[#]

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ABSTRACT

In this study, cultivated *Fritillaria persica* L. (Liliaceae) investigated morpho-anatomical and volatile compounds of bulbs. Purple tepals, 12-28 flowers in inflorescence, yellow-green filaments and dark yellow or purple anthers were notable morphological properties. For the anatomical study, sections were prepared from root, stem and leaf. The cortex of the stem was comprised of parenchyma and sclerenchyma. The collateral vascular bundles were scattered. The leaves were unifacial and amphistomatic. The stomata type was anomocytic. The crushed bulb volatiles of *F. persica* were trapped by the headspace-solid phase microextraction technique and determined by analysis by gas chromatography/mass spectrometry. Eight compounds (83.9%) were identified and the major compounds were found as 1,3-dichlorobenzene (60%), limonene (8.1%) and *p*-cymene (5.1%).

Keywords: *Fritillaria persica* L., Morphological, Anatomical, HS-SPME, GC/MS

1. INTRODUCTION

Fritillaria L., belonging to the Liliaceae family, is a genus with about 150 species in the Northern Hemisphere, excluding some parts of North America [1, 2]. In Turkey, the genus *Fritillaria* includes approximately 45 species [2, 3].

"Fritillus" means chessboard and is especially derived from the checkered colors of *Fritillaria meleagris* L. [4]. *Fritillaria* is referred to as the "Ters Lale" in Turkey because its flowers like tulips and look at the ground, some species are grown as ornamental plants [5]. *Fritillaria persica* L. is one of them, it has different names such as "Kırklale, Ağlayan gelin or Acem şahtuğu" (in Turkish) [3, 5, 6].

Fritillaria species have alkaloids, saponins, terpenoids and many different components [7]. In Chinese medicine, Bulbus Fritillaria (Beimu) is used as an expectorant in respiratory tract diseases [8]. Bulbs of *F. pinardii* Boiss. and *F. crassifolia* subsp. *kurdica* (Boiss. & Noë) Rix, are used for external wound healing in Turkey [9]. In Iran, *F. imperialis* L. bulbs are used for digestive diseases and pain treatments [10]. Moreover, it is known that the strong odor emitted by *F. imperialis* has a repulsive effect on some animals such as mice and mole [11]. Among the *Fritillaria* species distributed in North America, *F. agrestis* Greene is another bad odor species, while *F. striata* Eastw. flowers have been reported to be fragrant [12].

F. persica growing naturally in Anatolia and is in the category of Vulnerable (VU) [13]. The aim of this research is to investigate morpho-anatomical features and volatile compounds of the cultivated *F. persica*. As far as we know, this is the first research made on the anatomy and volatiles of the *F. persica* growing in Turkey.

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2. MATERIALS AND METHODS

2.1. Morphological Method

F. persica bulbs were obtained from Şanlıurfa and grown in Anadolu University Faculty of Pharmacy. The samples (ESSE 15403) are kept in the Herbarium of Anadolu University Faculty of Pharmacy. Morphological characters were examined under with the Wild M5A stereomicroscope (with drawing tube) and the results were compared with Flora of Turkey [14], "Resimli Türkiye Florası" [3].

2.2. Anatomical Method

In the anatomical study, superficial (leaf) and cross (root, stem, leaf) sections of the plant parts were made by hand. Methylene blue was used for staining the stem and leaf anatomical sections. Anatomical sections were investigated with the Olympus BX51T microscope and the photographs were taken by the digital camera.

2.3. HS-SPME (Headspace-solid Phase Microextraction) Procedure, GC/MS (Gas Chromatography/Mass Spectrometry) Analysis and Identification of Compounds

SPME fibre precoated with a 65 μm layer of blue fibre (Polydimethylsiloxane-Divinylbenzene, supplied by Supelco Bellefonte, USA) was used. The crushed bulb volatiles of *F. persica* were trapped with HS-SPME technique and analyzed by GC/MS. The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system [15].

3. RESULT

3.1. Morphological Results

Bulb 5 cm diameter, 6 cm height and spindle shaped. Stem 55-82 cm, thick, dull bluish green, smooth, straight. Leaves 50-60, alternate, dull bluish green, lowest leaves 8-9.5 x 1.5-2.5 cm, ovate-lanceolate, acute-acuminate at apex. Bracts 2.1 × 0.4 cm, present or absent. Inflorescence raceme, flowers 12-28. Perianth narrow campanulate, dark purple, tepal segments 14-17 x 7-10 mm, oblanceolate, oblong-oblanceolate, apex and base obtuse. Nectars 3 mm long, 1.5 mm broad, triangular. Filament 8-11 mm, yellow-green; anthers 2 mm, dark yellow or purple. Stylus 7-7.5 mm, smooth; stigma straight (Figure 1).

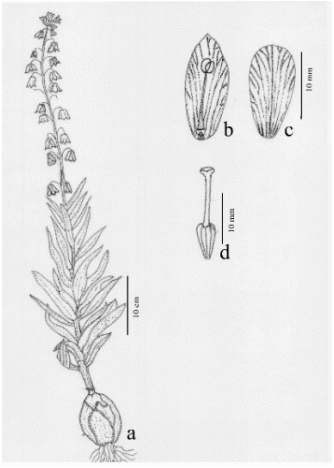

Figure 1. *F. persica*, a: general view, b: inner segment and stamen, c: outer segment, d: pistil.

3.2. Anatomical Results

3.2.1. Root

In the outer region there are small and one row of epidermis cells. Exodermis cells just below the epidermis. The cortex layer usually consists of irregularly shaped parenchyma cells. The endodermis and pericycle cells are evident. In the vascular bundles the metaxylem consists of 6-arms and there are phloem cells between them (Figure 2).

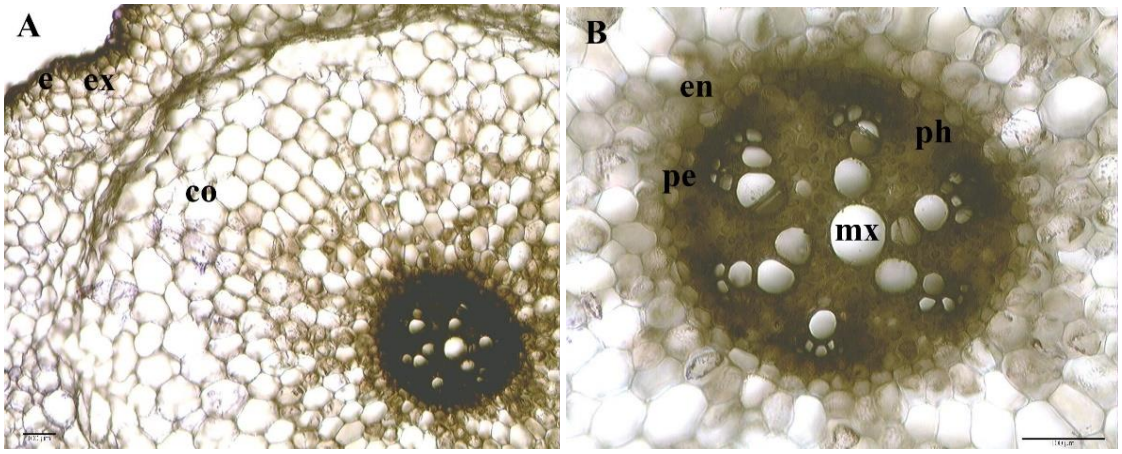

Figure 2. Cross section of *F. persica*; A-B: Root (e: epidermis, ex: exodermis, co: cortex, en: endodermis, pe: pericycle, mx: metaxylem, ph: phloem).

3.2.2. Stem

In the stem section, there is a cuticle followed by a single layer of small, rectangular epidermis cells. Between epidermis and sclerenchyma layers, there are 5-6 rows of round or oval shaped parenchyma cells. They are more oval shape in 1-3 raw under the epidermis. The sclerenchyma layer consists of 5-6 rows of cells. The vascular bundles are numerous and collateral. There are round or oval shaped parenchyma cells in the pith region, but the parenchyma cells in this part are more rounded (Figure 3).

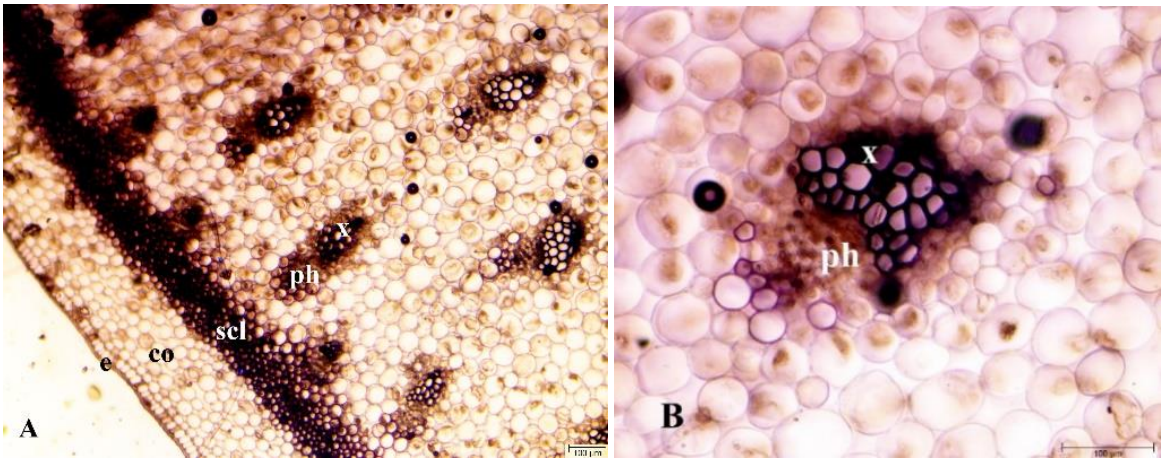

Figure 3. Cross section of *F. persica*; A-B: Stem (e: epidermis, co: cortex, scl: sclerenchyma; ph: phloem, x: xylem).

3.2.3. Leaves

In the leaf section, there is a thick cuticle layer on both surfaces. Epidermis cells are long quadrilateral or asymmetrical and cell walls are undulate. There is not any differentiation as palisade parenchyma and spongy parenchyma (unifacial leaf) and there are large spaces between the parenchyma cells.

In surface sections made from the leaf, there are anomocytic type stomata on both abaxial and adaxial surfaces (amphistomatic). The stomata cells are oval-rounded (Figure 4).

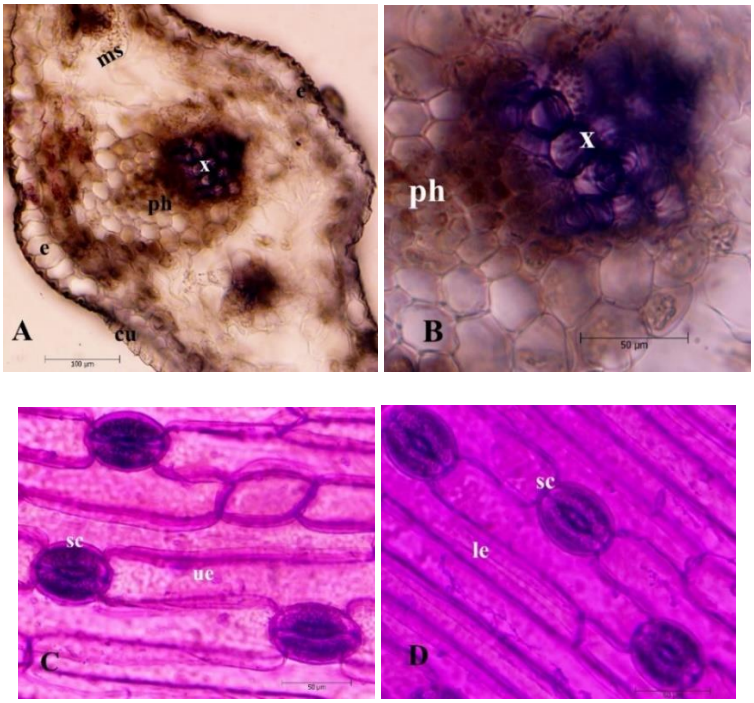

Figure 4. Cross (A-B) and superficial (C-D) section of *F. persica* leaves (cu: cuticula, e: epidermis, ms: mesophyll, x: xylem, ph: phloem, ue: upper epidermis, le: lower epidermis, sc: stomata cell).

3.3. HS-SPME Results

Volatile compounds of *F. persica* were determined by trapping with HS-SPME technique and analyzing by GC/MS. In this study, eight compounds were identified (Table 1).

Table 1. Chemical composition of crushed *F. persica* bulbs.

RRI	Compounds	%	IM
1203	Limonene	8.1	t_R, MS
1272	Styrene	1.9	MS
1280	*p*-Cymene	5.1	t_R, MS
1409	Rosefuran	2.9	t_R, MS
1429	1,3-Dichloro benzene	60.0	MS
1495	2-Ethyl hexanol	3.8	MS
1532	Camphor	1.2	t_R, MS
1896	Benzyl alcohol	0.9	MS

%; calculated from FID data. IM; Identification method. RRI; Relative retention indices calculated against n-alkanes. t_R; identification based on the retention times (t_R) of genuine compounds on the HP Innowax column. 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.

4. DISCUSSION

In this study, the morphological, anatomical, and volatile compounds of *F. persica* was investigated. The bulbs produced in Şanlıurfa were grown in Eskişehir and studies were carried out with these samples. Tekşen and Aytaç [16] reconsidered the *Fritillaria* of the Mediterranean region in Turkey. According to the work "Resimli Türkiye Florası", *F. persica* and *F. imperialis* the bulbs of are at least 4 cm in diameter, unlike other *Fritillaria* species in Turkey. The differences between these two species are that the flowers of *F. persica* are purple, greenish-purple and the inflorescence is raceme, the flowers of *F. imperialis* are orange to red and the inflorescence is umbella [3]. Morphological properties in this study are similar to comparative studies (Table 2) [3, 14]. But our samples did not contain capsules.

Table 2. Cultivated *F. persica* comparison with the morphological characteristics of the Flora of Turkey [14] and "Resimli Türkiye Florası" [3].

Characteristics	Flora of Turkey (1984)	Resimli Türkiye Florası (2018)	This study
Bulb	3-5 cm diam, 6 cm height	2.5-6 × 2.5-5 cm	5 cm diam, 6 cm height
Stem	20-100 cm	20-100 cm	55-82 cm
Number of leaves	10-25	10-70	50-60
Leaf	15 × 3 cm	4.4-15 × 1.0-3.0 cm (lower) 5.1-10 × 0.5-1.1 cm (middle)	8-9.5 × 1.5-2.5 cm (lowest)
Bracte	-	1.6-6.4 × 0.4-0.5 cm	2.1 × 0.4 cm
Tepal	15-20 × 6-7 mm Purplish, greenish-grey	1-2 × 0.5-0.8 cm (outer) 1-2 × 0.5-0.9 cm (inner) Purple, greenish-purple	14-17 × 7-10 mm Purple
Number of flowers	7-20	3-28	12-28
Nectar	1.5 mm broad	1.5-3 × 1.5-3.5 mm	3 × 1.5 mm
Filament	5-6 mm	5-10 mm, yellow	8-11 mm, yellow-green
Anther	4 mm	1.5-4 mm, brownish or purple	2 mm, dark yellow or purple
Style	6-8 mm	5.5-10 mm	7-7.5 mm

Namazi et al. [17] reported that they had defined a dichotomous key based on anatomical features for *Fritillaria* species in Iran and according to this key, phloem fiber is absent in the anatomy of the stem of *F. persica*. Corneanu and Popescu [18] and Akyol et al. [19], reported that there was no distinction between parenchyma cells in the leaf section of the anatomy of *Fritillaria* species. In some anatomical studies related to the *Fritillaria* species, the stomata type was determined as anomocytic [20, 21] and other studies stated stomata surrounded by four neighboring cells [22, 23] or do not have by-cells [18]. Many of the anatomical features in our study were like other species of *Fritillaria*.

In the *F. persica* volatile components study with the HS-SPME technique, eight compounds corresponding to 83.9% were identified and a high rate of 1,3-dichlorobenzene (60%) was found and other major compounds are limonene (8.1%) and *p*-cymene (5.1%). Compounds consist of monoterpene hydrocarbons (13.2%), oxygenated monoterpenes (4.1%) and others (66.6%). Seyed Saleh et al. (2018), α-bisabolol oxide A, camphor, chamazulene, trans-thujone, α-bisabolone oxide A, δ-3-carene and α-pinene by hydrodistillation, and α-bisabolol oxide A, chamazulene, α-bisabolone oxide A, camphor, trans-thujone, δ-3-carene, α-pinene were reported as major constituents of *F. imperialis* oil obtained by HS-SPME from Iran [24]. Has (2019), volatile compounds of *F. imperialis* bulbs were captured with HS-SPME fiber, analyzed by gas chromatography/mass spectrometry from Turkey. Thirteen compounds represented by 93.6% were identified in the 30 minutes study with the blue fiber, and the main compounds were *p*-cymene (23.6%), 1,8 cineol (22.4%) and limonene (15.2%). Ten compounds represented by 98.2% were identified the at 50°C and 15 minutes study with black fiber, and the main compounds were hexanal (29.8%), styrene (22.5%) and 1-hexanol (21.8%) [21, 25].

As a result, morphological and anatomical properties of cultivated *Fritillaria persica* are parallel within the literature within some size or number variations. Volatile compounds of crushed bulbs of *Fritillaria persica* by HS-SPME technique were reported for the first time in this study. Eight compounds (83.9%) were identified and the main components were found as 1,3-dichlorobenzene (60%), limonene (8.1%) and *p*-cymene (5.1%). Compounds consist of monoterpene hydrocarbons (13.2%), oxygenated monoterpenes (4.1%) and others (66.6%).

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CONFLICT OF INTEREST

The authors stated that there is no conflicts of interest regarding the publication of this article.

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