Anatomy, Palynology of Endemic *Cyclamen pseud-ibericum* in Mediterranean Phytogeographic Region of Turkey and Chemical Analysis of Its Tuber Extracts

Türkiye'nin Akdeniz Fitocoğrafik Bölgesi'ne Endemik *Cyclamen pseud-ibericum* Bitkisinin Anatomisi, Palinolojisi ve Tuber Özütünün Kimyasal Analizi

Research Article

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ABSTRACT

By this study anatomical and palynological characters of *Cylamen pseud-ibericum* which is an endemic species for Turkey were researched and anatomical features of this species were described for the first time. Since some species of *Cyclamen* have been used as folk medicines owing to biological activities of their tubers extracts, its tuber extract was analysed by GC-MS (Gas Chromatography-Mass Spectrometry) and qualified according to its chemical composition by this study. The compounds belong to the alcohols, aliphatic acids and their esters, aromatic compounds, carboxylic acids and their esters, hydrocarbons, fatty acids and their ester and sugars groups were determined by chemical analysis of tuber extract. " 2-hydroxy-6-methyl-3-nitro-pyran-4-one" was found in highest ratio (17.31%) in the extract. These results can be a step for new uses of *Cyclamen pseud-ibericum* tubers. Knowingly the chemical content will make easier biological activity tests. The anatomical and chemical results of this study will be guide for pharmacologist and pharmacists to recognise the plant and the chemical composition. So the results will be useful for both taxonomists and pharmacists.

Key Words
Cyclamen pseud-ibericum, endemic, anatomical, palynological, GC-MS

ÖZET

Bu çalışmada Türkiye için endemik bir tür olan *Cyclamen pseud-ibericum*'un anatomik ve palinolojik karakterleri araştırılmış ve türün anatomik özellikleri ilk kez tanımlanmıştır. *Cyclamen*'in bazı türlerinin tuber özütlerinin biyolojik aktivitelerinden dolayı halk arasında kullanılması nedeniyle, türün tuber özütü GC-MS (Gaz Kromatografisi-Kütle Spektrometresi) cihazı ile analiz edilmiş ve kimyasal içeriğine görede değerlendirilmiştir. Tuber özütünün analizi sonucunda alkoller, alifatik asit ve esterleri, aromatik bileşikler, karboksilik asit ve esterleri, hidrokarbonlar, yağ asit ve esterleri ve şekerler gruplarına dahil bileşikler saptanmıştır. "2-hidroksi-6-metil-3-nitro-piran-4-on" bileşiği % 17.31 'lik bir oranla en yüksek miktarda bulunmuştur. Bu sonuçlar *Cyclamen pseud-ibericum* tuberlerinin yeni kullanımı için bir basamak olabilir. Kimyasal içeriğin bilinmesi biyolojik aktivite testlerini de kolaylaştıracaktır. Bu çalışmanın anatomik ve kimyasal sonuçları ise özellikle farmokologlar ve eczacılara hem bitkiyi hem de kimyasal içeriğini tanımlama da rehber niteliğinde olacaktır. Bu yüzden sonuçlar hem taksonomist hem de eczacılar için faydalı olacaktır

Anahtar Kelimeler
Cyclamen pseud-ibericum, endemik, anatomik, palinolojik, GC-MS

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INTRODUCTION

There are more than 500 geophyte species growing naturally in Turkey, and the bulbs of most of these species are exported. In general, they are propagated by vegetative means. However, the horticulturally important genus *Cyclamen* L. is a tuberous perennial geophyte, and seeds are used in its propagation [1]. The species of this genus grow naturally in dry forest or scrub where they are at least partly shaded from intense sunlight [2].

The genus *Cyclamen* (Primulaceae family) includes about 20 species that are perennial and which originated from the Mediterranean coast. It occupies an important position in the world pot plant industry [3].

Cyclamen, with its elegant flowers and attractive leaves, is an ornamental species commercialized as a flowering pot plant [4]. The flowers are white, pink, purple or carmine with scented or unscented flowers and leaves of many *Cylamen* species are beautifully marked [2].

Some species belonging to this genus were used for their biological activities in folk medicine. Antiinflammatory and antinociceptive activities of *Cyclamen repandum* Sm. tubers, antifungal activity of *Cyclamen* mirabile Hildebr and *Cyclamen trochopteranthum* O.Schwarz was also reported [2]. *Cyclamen* tubers have toxic saponins in large amounts. Although the tubers have posinous saponin, wild boars look for them and eat without any post effect [5].

Cyclamen habitats a wide range of areas of Turkey. Ten *Cyclamen* species grow wild (*C. persicum* Miller, *C. coum* Miller, *C. hederifolium* Aiton, *C.graecum* Link, *C. mirabile* Hildebr., *C. pseud-ibericum* Hildebr., *C. trochopteranthum* O.Schwarz, *C. parvifolium* Pobed, *C. repandum* Sm. in Sibth & Sm and C. *cilicium* Boiss&Heldr), the latter five being endemic to Turkey [6].

The species of the genus share several characteristic features that diagnose them as a monophyletic group, e.g. a well-developed tuberous subterranean bulb formed by swelling of the hypocotyl, conspicuously reflexed corolla lobes and coiled fruiting pedicels [6].

Cyclamen pseud-ibericum is one of the endemic species of the genus from Turkey. We aimed to investigate anatomical and palynological features of this species. Also we determined the chemical composition of tuber extract of this endemic species. Nearly all researches about anatomy and tuber extract of *Cyclamen* belong to the *C. mirabile*. Besides this there is no any research about anatomy or tuber extract of *C. pseud-ibericum*. Also the researches about tuber extract are based on only the biological effects. They don't give any detail information about the chemical composition of tuber extracts. We also determined the chemical composition of tuber extract of *C. pseud-ibericum* by this study.

MATERIALS and METHODS

Plant materials

The specimens of *Cyclamen pseud-ibericum* were collected from their natural habitats, as detailed below:

C6: Osmaniye, Zorkun Plateau, 27.05.2012, 800 m, HA-5924.

Anatomical studies

For anatomical investigations, specimens were kept in 70% ethanol. In anatomical analysis, cross sections of leaves, petiole, root and surface section of leaves were prepared. Slides were observed with a Olympus CX21 microscope light microscope.

Palynological studies

Pollen grains were obtained from mature anthers of fresh flowers. For light microscope (LM) investigation, pollen slides were prepared by glycerine gelatine with basic fuchsin according to the Wodehouse method (1935) [7]. The polar axis (P), equatorial axis (E), colpus length (Clg), colpus width (Clt), exine thickness (Ex), intine thickness (In), apocolpium diameter (t) and AMB diameter (L) were measured from 30 pollen grains of each specimen under Olympus CX21 microscope by using an immersion objective lens (x100).

Table 1. The anatomical characteristics of *Cyclamen pseud-ibericum*.

Species			Length (μm)		Width (μm)	
			Min-max	Mean	Min-max	Mean
Cyclamen pseud-ibericum	Root	Epidermis	11-22	17.6	22-29	25.3
		Cortex	52-74	62.4	55-90	66.4
	Leaf	Upper epidermis	32-50	42.5	35-59	45.8
		Lower epidermis	19-35	24.2	18-32	23.8
		Palisade paranchyma	40-70	58	19-37	30.1
		Spongy paranchyma	20-39	30.2	59-70	64.6

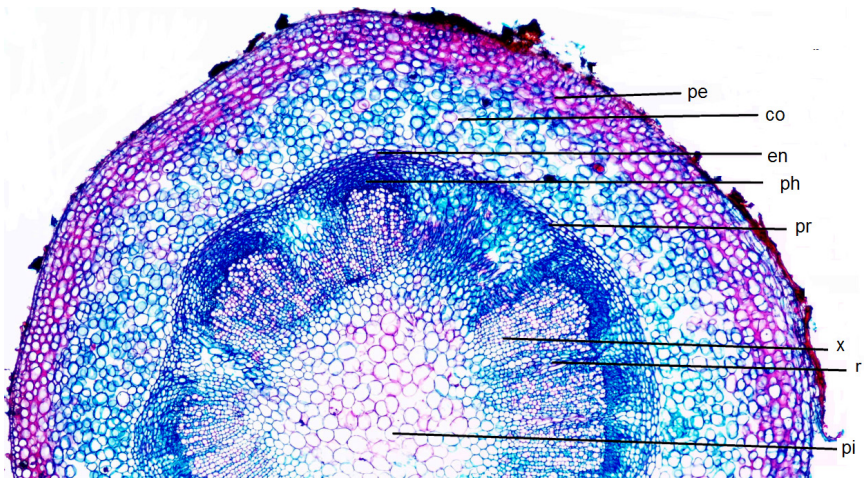

Figure 1. Cross section of root in *Cyclamen pseud-ibericum* (co: cortex, en: endodermis, pe: periderm, ph: phloem, pi: pith, pr: pericycle, r: ray, x: xylem).

Extractions of Tubers

Tubers are extracted by ethanol and analysed by GC-MS.

RESULTS AND DISCUSSION

Morphological properties

According to Davis (1978); *Cyclamen pseud-ibericum*'s tuber is 2-3 cm diameter, depressed globose, rather fissured and corky, rooting from base. Leaves appearing before flowers in early spring, broadly cordate, 2.5-5 (-8) cm long and about as wide, usually marbled and often rather glossy above, margins frequently with well marked teeth. Corolla vivid magenta with a very conspicuous dark basal blotch surrounding a large, wild or pallid, basal eye, exauriculate, lobes 15-25 mm, broadly ovate, acute or obtuse. Style distinctly exserted. Fruiting pedicel coiling from apex [8].

Anatomical characteristics

Root anatomy

The root was found a secondary structure; the primary structure was found to be diarch. The secondary xylems occupy the central part of the root while primary xylems are still visible. Cortex is 11-16 layered and consists of scarce fibres and numerous parenchyma cells. Vascular cylindir is poliarch. Pericycle and endodermis are single layered. At the root periphery, a well developed periderm was observed (Figure 1). It is 6-7 layered.

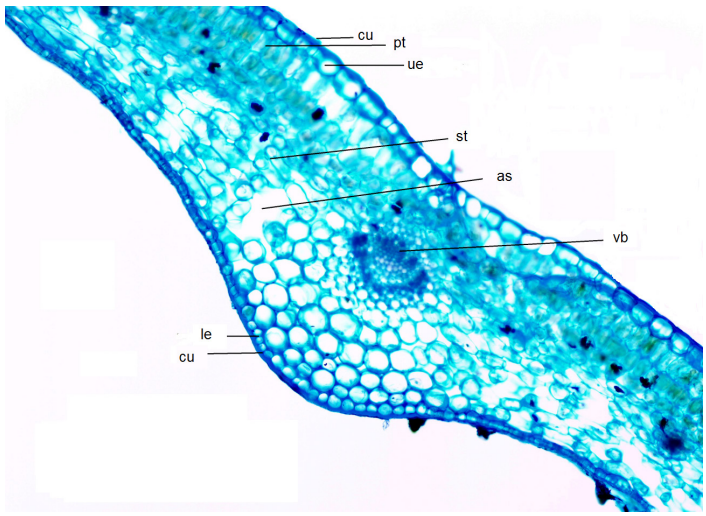

Figure 2. Cross-section of leaf in *Cylamen pseud-ibericum* (as: air space, cu: cuticula, le: lower epidermis, pt:palisade tissue, st:spongy tissue, ue: upper epidermis, vb: vascular bundle.

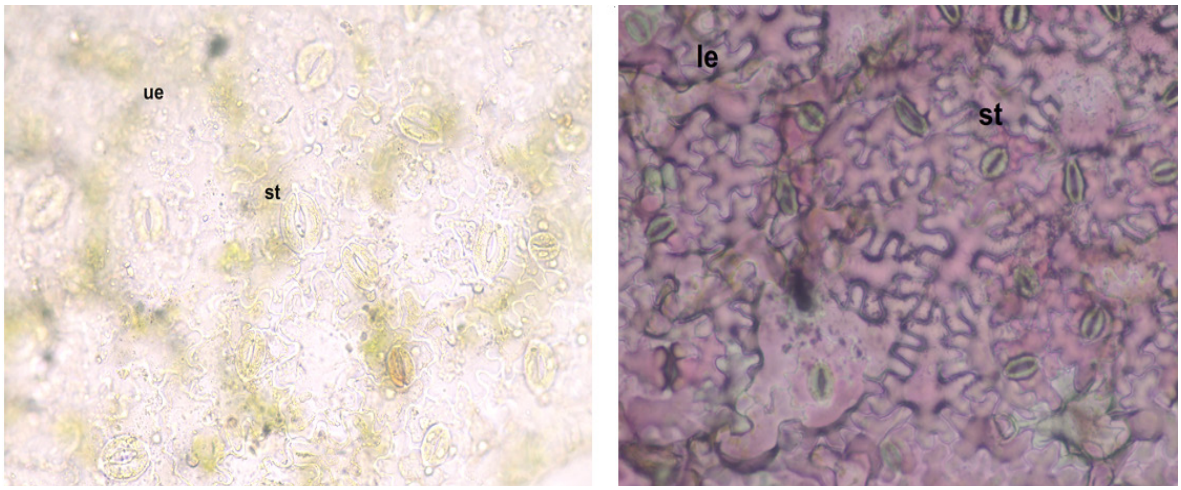

Figure 3. Surface sections of leaf (le: lower epidermis, up:upper epidermis, st: stomata).

Anatomical measurements related to root are presented in Table 1.

Leaf anatomy

The leaf's lamina has bifacial structure. The stomata cells are present on the surfaces of both epidermis and are often located at the same level as the epidermal cells. The transverse section of the leaf shows a thin cuticle on the upper and lower epidermis. Anisositic stoma is placed on both sides of the leaves (amphistomatic). Number of stoma in 1 mm^2 is 120-150 (mean: 130). There are glandular hairs on both epidermal surfaces (Figures 2 and 3). Mesophyll cells are more or less uniform in shape. Vascular bundles lie between the palisade and the spongy tissue. Anatomical measurements are given in Table 1.

Petiole anatomy

Cross sections of the petiole has a circular shape. The epidermis is unilayered, the cortex is thick (4-5 cells layers). Vascular bundle is only one

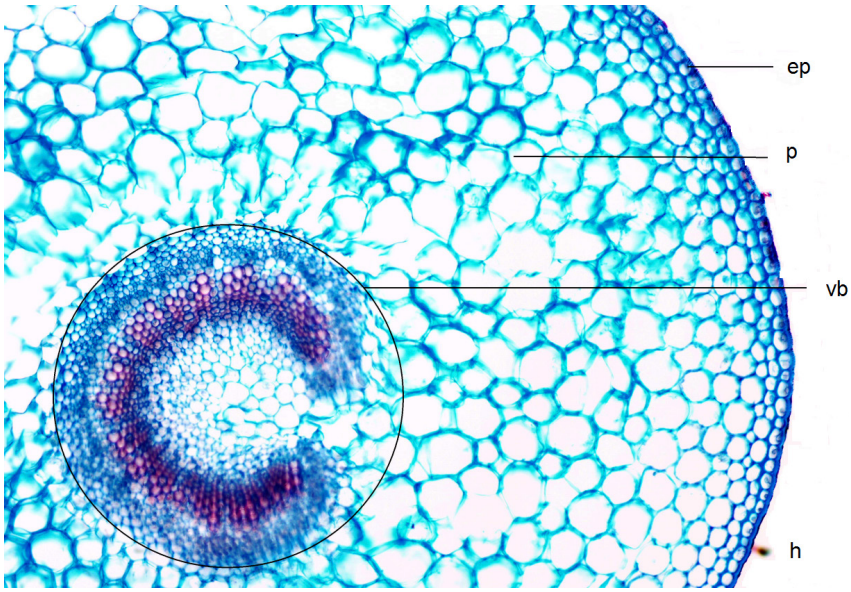

Figure 4. Cross section of petiole in C.*pseud-ibericum* (ep: epidermis, h: hair, p: parenchyma tissue, vb: vascular bundle).

and located in the centre of petiole. The vascular tissues have secondary structure. In the center it has paranchymatic pith (Figure 4).

Pollen characteristics

Pollen grains of *Cyclamen pseud-ibericum* are radially symmetric, isopolar, tricolporate, spheroidal and circular in polar view. Colpi are long and with distinct margin. Pori are suboblate and also with distinct margin. Colpus edges are acute. Porus latitude is wider than the colpus latitude. Exine is getting thicker from colpus margin to mesocolpium. Sexine is thinner than nexine. In light microscope exine ornamentation is microreticulate (Figure 5). The palynological features of the *Cyclamen pseud-ibericum* are summarized in Table 2.

GC-MS analysis of tuber extracts

The chemical analysis results are given in Table 3. According to the Table 3 the compounds belong to the alcohols (1.88%), aliphatic acids and their esters (27.28%), aromatic compounds (2.62%), carboxylic acids and their esters (8.36%), hydrocarbons (4.93%), fatty acids and their ester (1.34%) and sugars (2.79%) groups were determined by chemical analysis of tuber extract. "2-hydroxy-6-methyl-3-nitro-pyran-4-one" was found quite high with a ratio of 17.31%.

Table 2. Pollen characteristics of *Cyclamen* pseud-ibericum.

Pollen characteristics	***Cyclamen pseud-ibericum***
Pollen shape	Spheroidal
P (μm)	16-18 (mean: 16.70±0.65)
E (μm)	16-20 μm (mean: 17.66±1.06)
P/E	0.94
AMB (μm)	14-16 (mean: 5.93±1.04)
Exine (μm)	1.5-2 (mean: 1.6±0.2)
Clg (μm)	13-16 (mean: 14.50±0.97)
Clt (μm)	3-5 (mean: 3.6±0.56)
T (μm)	5.93±1.04
Plg (μm)	5-6 (mean: 5.33±0.47)
Plt (μm)	5-8 (mean: 6.36±0.71)
Plg/Plt	0.83 suboblate
Pollen type	3-colporate

Table 3. GC-MS analysis results of *Cylamen pseud-ibericum* tuber.

Compounds	**Ratio%**
Alcohols	
2-[2-(2-butoxyethoxy)ethoxy]ethanol	1.88
Total	1.88
Aliphatic acids and their esters	
2-hydroxy-6-methyl-3-nitro-pyran-4-one	17.31
Methylene butanedioic acid	3.20
2,3-Dihydroxybutanoic acid	0.31
n-propyl pentanoate	4.18
Nonadecanoic acid	2.28
Total	27.28
Aromatic compounds	
Quinoline	2.62
Total	2.62
Carboxylic acids and their esters	
(5-acetoxy-2,3-dimethoxy-6-methyl-tetrahydropyran-4-yl) acetate	4.09
2(1H)-Oxoquinoline-4-carboxylic acid	0.96
Diethyl pimelate	3.31
Total	8.36
Fatty acids and their esters	
2,3-bis(trimethylsilyloxy)propyl dodecanoate	1.34
Total	1.34
Hydrocarbons	
1,3,7,7-Tetramethyl-9-oxo-2-oxabicyclo[4.4.0]dec-5-ene	0.56
Nonane	4.37
Total	4.93
Sugars	
2-O-Mesyl arabinose	2.79
Total	2.79
Others	
2-hexa-2,5-diynoxyoxane	0.62
5-Butyl-5-ethyl-4,6-dihydroxy-2(5H)-pyrimidinone	0.64
3,4-bis(diethylamino)-2-phenyl-1H-pyrido[2,1-b]benzothiazol-1-on	1.12
4,5-Dihydro-2-methylthiazole	1.84
cis- 4-ethyl-2,5-dimethyl-isoxazolidine	0.78
Cis-2,3-diisopropyl-triazirdin-carbonsaure-ethylester	7.24

Table 3. GC-MS analysis results of *Cylamen pseud-ibericum* tuber. (continue)

2-O-[2,4-Di-O-methyl-3-O-(2,3,4-tri-O-methylpentopyranosyl) pentopyranosyl]-1,3,4,5-tetra-O-methylpentitol	6.33
1,2,3,4-tetramethoxy butane	2.98
4,5-Bis (4-methoxyphenyl)isoxazole	0.53
2,5-Dibromo-1,4-benzoquinone	2.38

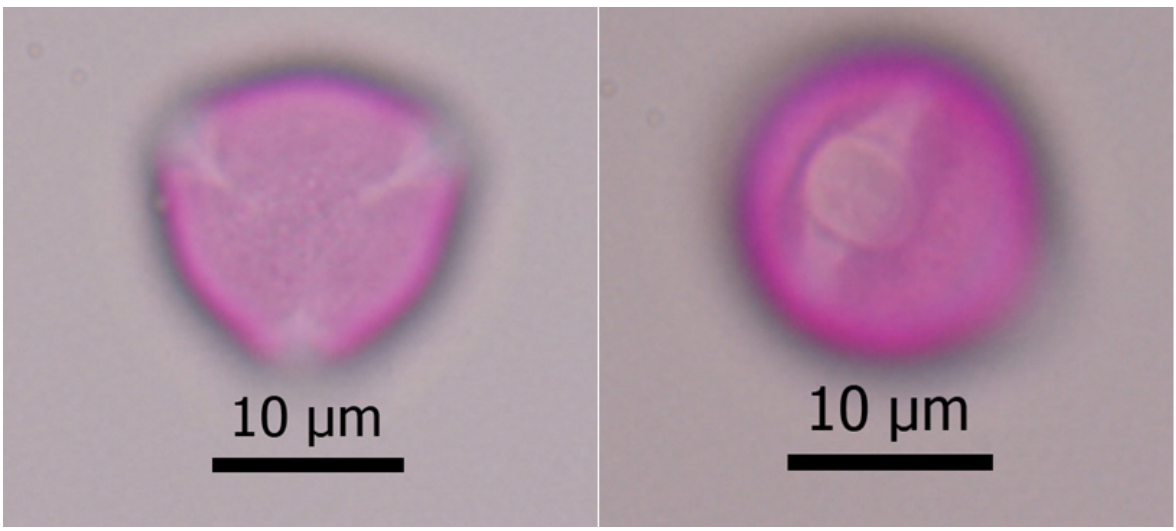

Figure 5. Light microscope micrographs of pollen of C.*pseud-ibericum* (a.polar view of pollen, b.equitorial view of pollen).

DISCUSSION and CONCLUSION

In this study, morphological, anatomical and palynological properties of *Cyclamen pseud-ibericum* have been investigated. The anatomical investigations of *Cyclamen pseud-ibericum* are reported for the first time by this study. The cross sections of root, leaf and petiole were studied. Besides these palynological properties and chemical composition of *Cyclamen pseud-ibericum* tuber were determined by this study.

If we compare to the Dusen et al. (2014) results about *C. mirabile* Hildebr. anatomy; epidermal cells of *C. pseud-ibericum* root are smaller than *C. mirabile*'s. Besides this, *Cyclamen pseud-ibericum* has larger paranchyma cells in cortex compare to *C. mirabile*. Leaf anatomy of two species is similiar.

But while *C. mirabile*'s leaves are covered by a thick cuticular layer on both upper and lower surfaces, *Cyclamen pseud-ibericum*'s leaves are covered by a thin cuticular layer on both surfaces. This can be a distictive anatomical feature for *C. pseud-ibericum*. At this point more species must be investigated.

If we compare the pollen shape of *Cyclamen pseud-ibericum*, we can say that it is similiar with the shape of *C. africanum*, *C.neapolitanum* and *C. purpurascensand C. mirabile* pollen. Also aperture type of *C. pseud-ibericum* pollen is similar to *C. africanum*, *C. neapolitanum* and C. purpurascens (9), but different from *C. mirabile*. As *C. mirabile* pollen is tetracolporate [5], *Cyclamen pseud-ibericum* pollen is tricolporate. Polar axis (P) is 13.86±1.30 µm (12.82-16.66 µm), equatorial axis (E) 13.16±0.78 µm (12.82-15.38 µm) in *C. mirabile*. Polar axis (P) of *C. pseud-ibericum* is 16-18 (mean: 16.70±0.65), equatorial axis (E) is 16-20 µm (mean: 17.66±1.06). But the ornemantation of two species are the same. While comparing this two species number of apertures and pollen size has an importace in taxonomy and phylogenetic classification.

Some researchers had investigated the biological effects of *Cyclamen* tubers. Oz et al., (2013) evaluated the larvicidal activity of the tuber extracts isolated from two species of *Cyclamen* (*Cyclamen mirabile* Hildebr. and *Cyclamen alpinum* Dammann ex. Sprenger) against Culex pipiens L. (Diptera: *Culicidae*). They found that *C. mirabile* extract was more toxic than *C. alpinum* extract on young and older larval instars [2].

In another study *C. mirabile* tuber extracts tested to determine potential antibacterial activities against *S. aureus* and CNS pathogens isolated from subclinical cow mastitis. They suggest that *C. mirabile* has significant antibacterial activity and it could be very useful in the discovery of novel antibacterial agents of plant origin [10].

Çalış et al. (1997) isolated six saponins, cyclaminorin, deglucocyclamin, cyclacoumin, cyclamin, isocyclamin, and mirabilin from the tubers of *C. mirabile* [11].

Metin et al. (2013) determined the antioxidant activities of various solvent extracts (methanol, ethanol, acetone and petroleum benzine) obtained from tubers of *Cyclamen graecum* Link. All extracts of *C. graecum* tubers had effective free radical scavenging activity. A positive correlation was observed between antioxidant activity and amount of phenolic contents of the extracts. In this study, all types of extracts obtained from *C. graecum* have showed strong antioxidant activity. The researchers mentioned that, this species can be used as a natural antioxidant in food processing and pharmaceutical industries. [12].

Sarıkurkcu (2011) indicate that, the extracts of *C. mirabile* tubers and leaves possessed high antioxidant activity in vitro and can be easy accessible source of natural antioxidant. The researcher dealed that the components responsible for antioxidant activity of the extracts of *C. mirabile* are unclear. Future studies will be aimed at investigating the effects of different parts of *C. mirabile* upon isolating and identifying the substances responsible for the antioxidant effects of the solvent extracts [13].

Cyclamen pseud-ibericum is an andemic species for Turkey. It is also "endangered (EN)" according to Red Data Book of Turkish Plants [14]. Therefore it is important to recognize this species. As seen from the literature there is no any anatomical research for *Cyclamen pseud-ibericum*. Despite there are so many research about tuber extract of *C.mirabile*, there is no any research about *Cyclamen pseud-ibericum* tuber. The investigations about tuber extracts are based on biological activities, not about detailed chemical composition. By this study the chemical composition of *Cyclamen pseud-ibericum* tuber is investigated by the first time. This will be a step for further researches.

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References

1. B.G. Taşkın, N. Vardareli, E. Doğacı, R. Mammadov, V.Taşkın, Genetic diversity of natural *Cyclamen alpinum* populations, Turk J Biol, 36 (2012) 413-422.
2. E. Oz, S. Koc, O.D. Dusen, R. Mammadov, H.Cetin, Larvicidal activity of *Cyclamen* (Myrsinaceae) extracts against the larvae of West Nile virus vector Culex pipiens L. (Diptera: Culicidae), Asian Pacific of Tropical Medicine, (2013) 449-452.
3. M. Kocak, T. Izgu, B. Sevindik, M. Tutuncu, P. Curuk, O. Simsek, Y.A. Kacar, J.A.T. Silva, Y.Y. Mendi, Somatic embryogenesis of Turkish *Cyclamen persicum* Mill., Scientia Horticulturae, 172 (2014) 26-33.
4. N. Jalali, R. Naderi, A. S. Gharahlar, J.A.T. Silva, Tissue culture of *Cyclamen* spp. Scientia Horticulturae, 137 (2012) 11-18.
5. O.D. Dusen, B. Gurcan, R. Mammadov, Morphology, Anatomy and Palynology of Endemic *Cyclamen* Mirabile Hıldebr. (*Primulaceae*) In South-west Turkey, Bangladesh J. Bot, 43 (2014) 243-247.
6. T. Ekim, M. Koyuncu, A. Güner, S. Erik, B. Yıldız, M. Vural, Taxonomic and Ecological Research on Economically Important Geofits in Turkey, Publication and Publicity 669,65. The Ministry of Agriculture and Forestry (1991). Ankara, pp.111.
7. R.P Wodehouse, Pollen Grains, (1935). New York: McGraw-Hill.
8. Flora of Turkey And the East Aegean Islands, Volume 6:132 (1978) Edinburgh at the University Press. Ed.Davis PH.
9. www.paldat.org/pub/Cyclamen. (2015)

10. G. Okmen, P. Erdal, D. Isık, D.Bayrak, The antibacterial activities against mastitis pathogens of *Cyclamen mirabile* Hildebr. tubers and its non-enzymatic antioxidant activities, European Journal of Experimental Biology, 4 (2014) 370-374.
11. T. Çalıs, M.E. Satana, A. Yürüker, P. Kelican, R. Demirdamar, R. Alaçam, N. Tanker, H. Rüegger, O.Sticher, Triterpene saponins from Cyclamen mirabile and their biological activities, J Nat Prod., 60 (1997) 315-8.
12. H. Metin, Ç. Aydın, C. Ozay, R. Mammadov, Antioxidant Activity of the Various Extracts of *Cyclamen graecum* Link Tubers and Leaves from Turkey, J.Chem.Soc. Pak., 35 (2013) 1332-1336.
13. C.Sarıkurkcu, Antioxidant activities of solvent extracts from endemic *Cyclamen mirabile* Hildebtubers and leaves, African Journal of Biotechnology, 10 (2011) 831-839.