

ANATOMICAL INVESTIGATIONS ON ORIGANUM MINUTIFLORUM*

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S U M M A R Y .

Origanum minutiflorum O. Schwarz & P.H. Davis is an endemic species growing in Isparta, Burdur and Antalya, used as herbal tea and spice by the local people. This species is one of the most important export materials of Turkey, and collecting too much amounts there. In this study the specimens of *O. minutiflorum* collected from Sütçüler (Isparta) are investigated anatomically. In the cross-sections of leaves it is suggested that a few hairs and many glands have occur. Stomata amount is established per mm²: 160 on the lower surface and 80 on the upper one. It is compared by the 2 related, endemic species (*O. bilgeri* P.H. Davis, *O. micranthum* Vogel) from the point view of the investigating characteristics in the Sect. *Chilocalyx* (Briq.) Ietswaart.

Ö Z E T

Origanum minutiflorum O. Schwarz & P.H. Davis, Isparta, Burdur ve Antalya çevresinde yetişen endemik bir türdür, halk arasında çay ve baharat olarak kullanılmaktadır. Önemli ihraç ürünlerimizden olan bu tür, yöreden aşırı miktarda toplanmaktadır. Bu çalışmada Sütçüler (Isparta)'den toplanan *O. minutiflorum* örnekleri anatomik olarak incelenmiştir. Yapraklardan alınan enine kesitlerde az sayıda örtü tüyünün yanında çok sayıda salgı tüyünün varlığı saptanmıştır. Alt yüzde yaklaşık 160, üst yüzde yaklaşık 80 ise mm²'ye düşen stoma sayısıdır. İncelenen özellikleri

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bakımından Sect. *Chilocalyx* (Briq.) Ietswaart'da bulunan diğer 2 yakın, endemik türle (*O.bilgeri* P.H. Davis, *O. micranthum* Vogel) de karşılaştırılmıştır.

Key words: *Origanum minutiflorum*; Labiatae; anatomy; Turkey

I N T R O D U C T I O N

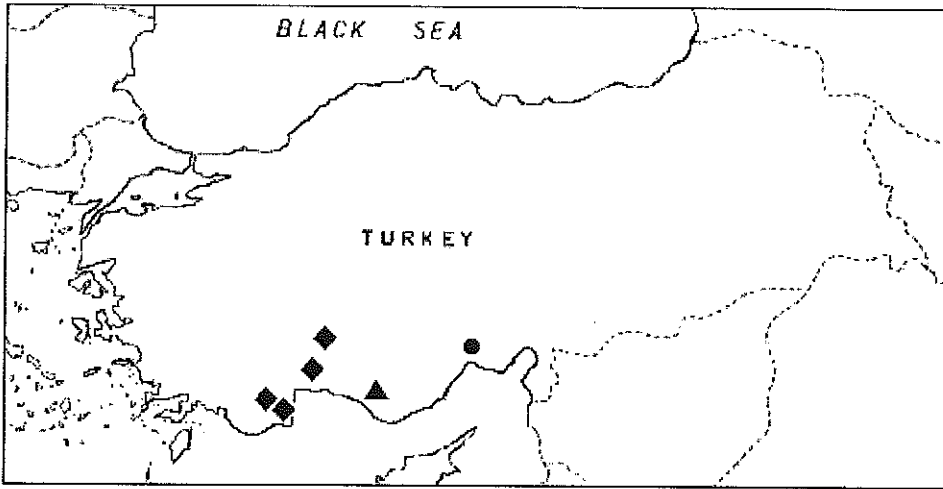
The amount of the natural plants which was exported in the last years shows that the most ones are under the name of "kekik" (1). The plants known as kekik includes generally 5 genera [*Thymus* L., *Thymbra* L., *Coridothymus* Reichb. fil., *Satureja* L. ve *Origanum* L.] in Labiatae family and mostly belong to *Origanum* L. genus. It is recorded that *Origanum* species has been used as kekik in Anatolia since 7th century BC (2). This genus have 45 species in the world and 22 of which occur in Turkey with 13 species being endemic (the endemism rate is 59%) (3, 4, 5, 6).



Figure 1. General view of *O. minutiflorum* (x1) (ISTE 45629).

Origanum minutiflorum O. Schwarz & P.H. Davis (Figure 1) is one of the most using species in commercial (1). Exposing the anatomical characteristics of this endemic species is the aim of this study. And also it is compared with 2 related species by microscopically. These species are very similar, some characteristics not repeated. And only the necessary ones are given.

Sect. *Chilocalyx* (Briquet) Ietswaart have 4 species in the world, 3 of them occur in Turkey (Map 1). *O. minutiflorum* grows in Antalya, Burdur and Isparta, it is collected too much amounts (600-800 tonnes per year) there, for export and to use as spice and herbal tea by the local people (1). The other species *O. bilgeri* Davis grows in Antalya, it is collected and used by the local people and is sold in the local spice shops. *O. micranthum* Vogel grows in Adana and İçel and is used by the local people.



Map 1. Distribution of ◆ *Origanum minutiflorum*, ▲ *O. bilgeri*, and ● *O. micranthum*.

The local names of *O. minutiflorum* are yayla kekiği, eşek kekiği, beyaz kekik, boz kekik, cıngıllı kekik, toka kekiği and Sütçüler kekiği.

There are a few studies about the *Origanum* species investigated by morphological and anatomical characteristics, known as kekik and used as remedies and herbal tea in Turkey, except unpublished thesis and proceeding summaries (7, 8, 9, 10). There are some morphological and anatomical studies about the other species used as kekik which belong to Labiatae family (11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21).

M A T E R I A L A N D M E T H O D

Examined specimens:

Origanum minutiflorum C3 Isparta: Sütçüler, Beydili-Çimenova, 11.08.2001, 1390 m, N. Sadıkoğlu, ISTE 80558!; Antalya: Çalbalı Dağı, Fesleğen Yaylası yakını, 04.08.1980, 1830 m, N. Özhatay, E. Tuzlacı, B. Çubukçu, A. Meriçli, ISTE 45629!

Origanum bilgeri C3 Antalya: Akseki, Murtiçi, Kocaoluk Yaylası, 06.07.2002, 1600 m, E. Akalın, ISTE 80579!; C4 Antalya: Alanya, Yerköprü-Turbalınaz Yaylası, kaya üzeri, 10.08.1994, 1200-1300 m, H. Duman 5623 GAZI! (ESSE 10696!, ISTE 80576!)

Origanum micranthum C5 İçel: Tarsus, Gülek Boğazı, harabeler yanı, 17.07.1994, G. Tümen *s.n.*GAZI! (ISTE 80575!); Muğla: Datça, Cumalı (Çeşme) köyü - kültür- 14.05.1984, 400 m, E. Tuzlacı, ISTE 53707!

The specimens are kept in ISTE.

Anatomical sections were taken manually from the stems and the leaves of the plants both fresh and fixed in 70% ethanol, were stained by Sartur and chloralhydrate (for removing chloroplasts) reactive. Stem photograph was taken by Olympus trinocular BH-2 photomicroscope and leaf illustrations were prepared with Leitz SM-LUX binocular microscope with Leitz Weitzler drawing tube. The less hairy specimens are chosen for a simple view of the cross-sections.

R E S U L T S

STEM

Origanum minutiflorum (Figure 2)

The stem is hairy, reddish brown and quadrangular.

Epidermis: Single layer of cells are elongated, oval or isodiametric. The outer walls of the epidermal cells are thicker than the anticlinal walls. An undulate cuticular layer occurs on it. Various type of glandular and non-glandular hairs are observed. Glands have one celled stalk and head, and are in Labiatae type which is sessile and has 8 celled head, sunken non-glandular hairs are simple, 1-6 celled, 1 celled ones are prickly hair.

Cortex: Below the epidermis, collenchyma tissue occurs, which was 4-6 layered on the corners, and 2-3 layered between the corners. There is parenchymatous tissue which has crashed and damaged somewhere with a few layered cells below the

collenchyma. Endodermis is the layer of the cortex and usually has single layered cells, in a continuous ring. The cells are big, regular walled, and elongated. Cambium is indistinct.

Vascular region: There are 4 big vascular bundles on the corners with sclerenchymatous pith rays. Phloem 4-5 layered, usually has crashed cells in a discontinuous ring. Sclerenchymatous tissue which has 5 layered, flat or round cells, surrounds vascular cylinder in a continuous ring at the phloem pole. The xylem tracheas are oval or roundish, tracheids are irregular. Single or rarely 2 layered pith rays are occur in the xylem tissue. Pith is made of big and roundish parenchymatous cells and contains starch.

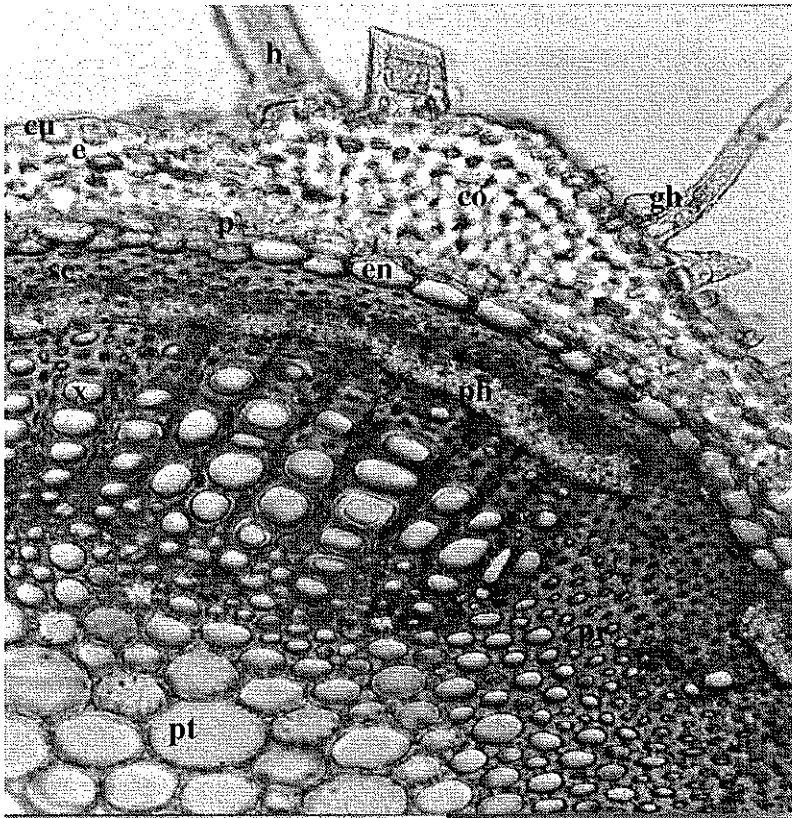


Figure 2. Cross-section of the *O. minutiflorum* stem (10x40) cu, cuticle; e, epidermis; h, hair; gh, glandular hair; co, collenchyma; p, parenchyma; sc, sclerenchyma; en, endodermis; ph, phloem; x, xylem; pr, pith rays; pt, pith

Origanum bilgeri

The stem is hairy, brown and quadrangular. There is an undulate cuticular layer followed by a single layer of epidermis of cubic cells with many hairs. Collenchyma tissue is 5-8 layered at the four edges of the stem, but reduced to 2-3 layers in the parts between the corners. A single layer of endodermis has cubic cells are found below it. Vascular bundles are on the corners with sclerenchymatous tissue.

Origanum micranthum

The stem is rather hairy, purplish brown and quadrangular but has irregular margins. There is a cuticular layer followed by a single layer of epidermis cells are elongated. Collenchyma tissue is multilayered. 1-2 layer of endodermis has big and elongated cells. 3 layered sclerenchymatous tissue is surrounded vascular cylinder.

LEAVES

Origanum minutiflorum (Figure 3)

Leaves are green, pilosellous, ovate and isobilateral. In *O. minutiflorum* like the other kekik species (7, 8, 9, 10, 11, 13, 14, 16, 17, 18, 19, 20) thick lateral veins are occur along the blade, both side of the midrib. The midrib and the lateral veins are made a deep promontory to the outside. Anatomically, the lateral veins have the same structure with the midrib but the vascular bundles are reduced.

Epidermis: Single layer of cells are elongated or isodiametric. There is a thick cuticular layer on it and the cells of the upper epidermis are bigger than the lower ones. On the superficial section, walls of the epidermis cells are undulate, that upper ones are slightly, lowers are prominent. Glandular and non-glandular hairs are similar to these of the stem and observed on both epidermises. Non-glandular hairs are 1-6 celled. Glandular hairs are sunken, occur in deep hollows both on the upper and the lower epidermis and observed less to very dense on the specimens. The parenchymatous cells on which the glandular hairs entered to the blade are lost or changed morphologically. Stomata are present on both upper and lower epidermis (amphistomatic), and denser on the lower surface. On the superficial section, stomata amount is established per mm²: 160 on the lower surface, 80 on the upper one. On the cross-section, guard cells are upper than the epidermis cells (higromorf stomata). They are oval and the subsidiary cells -one of them is smaller then the other- are 2 (diacytic), rarely 3 (anisocytic).

Parenchyma tissue: Under the upper and lower epidermis, single layered palisade parenchyma including many chloroplast and 3-5 layered spongy parenchyma between them (isobilateral leaf) are occur. On the superficial section, the cells of

palisade parenchyma are elongated, uppers are closer than the lowers, but spongy parenchyma cells are in disorder and lax. There is no broad intercellular space in the mesophyll tissue.

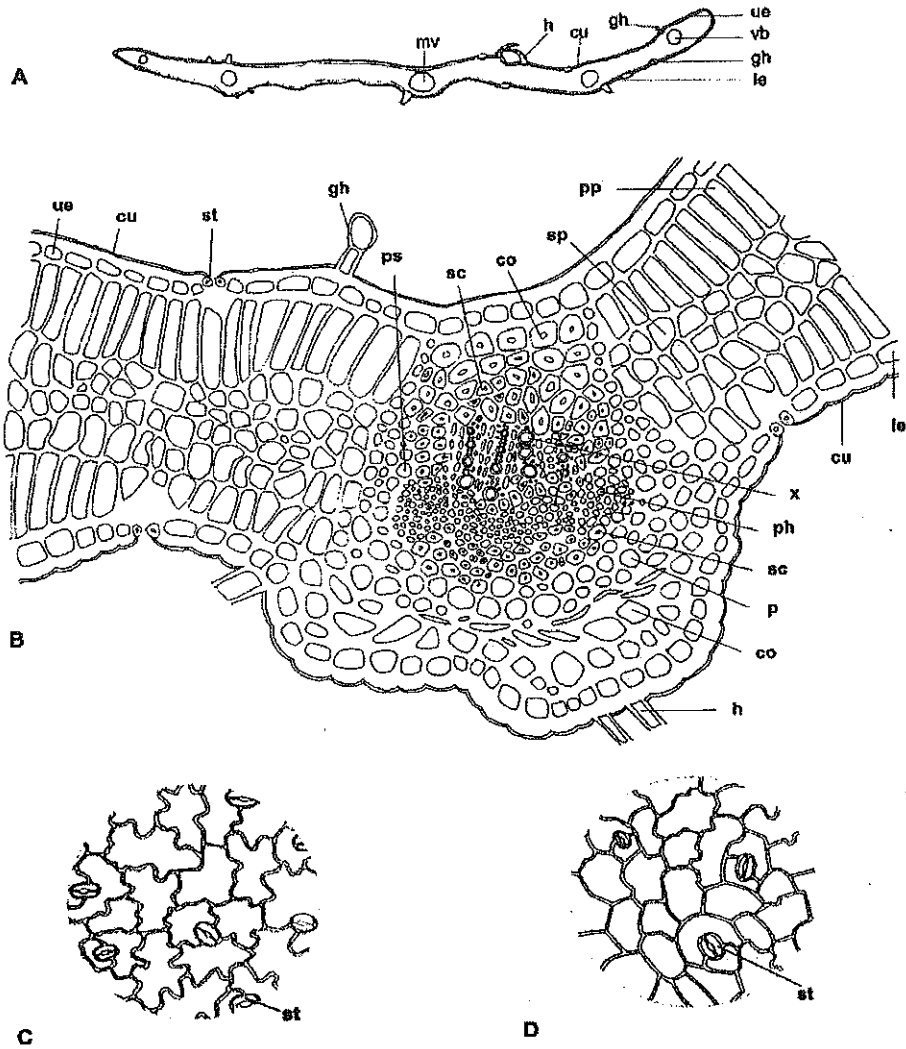


Figure 3. *O. minutiflorum*. A, cross-section of the leaf (x22,5); B, midrib (x450); C, surface view of the lower epidermis with stomata (x225); D, surface view of the upper epidermis with stomata (x225). cu, cuticle; ue, upper epidermis; le, lower epidermis; h, hair; gh, glandular hair; vb, vascular bundle; mv, middle vessel; pp, palisade parenchyma; sp, spongy parenchyma; sc, sclerenchyma; co, collenchyma; ps, parenchymatous sheath; p, parenchyma; x, xylem; ph, phloem; st, stomata.

Vascular cylinder: Upper and lower parts of the vascular bundle of the old leaves and most of the young ones have been surrounded by sclerenchymatous tissue. After the lower epidermis, 4-5 layered collenchyma occurs. Xylem is found towards the upper side and, phloem is towards the lower. Thin walled parenchymatic cells are found between tracheary elements in the xylem. Under the upper epidermis, 2-3 layered collenchyma occurs. A parenchymatous sheath covers the bundle.

Origanum bilgeri

Leaves are roundish, greyish and bifacial, covered by a cuticular layer on both surfaces and pubescent or tomentose hairs, followed by a single layered epidermis. Stomata are amphistomatic type, 90/110 per mm² on upper/lower surfaces and diacytic. Upper and lower parts of vascular bundle surrounded by collenchymatous cells, and multilayered sclerenchymatous tissue below them.

Origanum micranthum

Leaves are whitish and isobilateral, covered by a cuticular layer on both upper and lower surfaces and dense tomentose hairs, followed by a single layered epidermis and palisade cells. Spongy parenchyma cells are between them. Stomata are amphistomatic type, 200/210 per mm² on upper/lower surfaces and diacytic. Below them, multilayered collenchyma tissue occurs. Vascular bundles are surrounded by sclerenchymatous tissue.

D I S C U S S I O N

O. minutiflorum is an endemic species that grows in Isparta, Burdur and Antalya. This plant is an important economical material for the local people. But early harvesting material has no enough rate of volatile oil and could not find any customer. People also couldn't sell non-dried material. Kekik harvesting is increasing rapidly. For this reason the local people built a cooperative system. Both Local Forestry Administration and Cooperatives choose the best harvesting time. Kekik must be collect when the seeds are thrown away by a special cutting method without damaging the roots. This application would be prevented this endemic plant's dying out and also damaging the nature.

O. minutiflorum is investigated anatomically and compared with the endemic species belong to Sect. *Chilocalyx*. It is never seen any publication about them before and this is a part of a continuing study.

O. minutiflorum, has a quadrangular stem, as the both species. There is a cuticular layer followed by a single layer of epidermis contains various type of

glandular and non-glandular hairs. Collenchyma tissue is multilayered at the four edges of the stem, but reduced to 2-3 layers in the parts between the corners. Endodermis has 1-2 layered cells in a continuous ring. There are 4 big vascular bundles on the corners with sclerenchymatous pith rays. Phloem usually has crashed cells, pith rays occur in the xylem tissue. Pith is made of big parenchymatous cells and contains starch. Leaves are hairy, usually ovate, covered by a cuticular layer on both surfaces, followed by a single layered epidermis. Stomata are higromorf and amphistomatic type. The cells of palisade parenchyma are closer, but spongy parenchyma cells are lax. There is no broad intercellular space in the mesophyll tissue. Upper and lower parts of the vascular bundle surrounded by collenchymatous cells and multilayered sclerenchymatous tissue below them. A parenchymatous sheath covers the bundle.

Anatomical characteristics of the 3 species occur in the same section are summarised as seen in the Table 1.

Table 1: Differences of anatomical characteristics

Part of plant	<i>O. minutiflorum</i>	<i>O. bilgeri</i>	<i>O. micranthum</i>
Stem	isodiametric epidermis cells, oval or elongated, collenchyma 2-3 layered, 4-6 layered on the corners, big, elongated endodermis cells	cubic epidermis cells and undulate margins, collenchyma 2-3 layered, 5-8 layered on the corners, cubic endodermis cells	isodiametric epidermis cells oval or elongated, collenchyma 2-3 layered, 4-5 layered on the corners, big, elongated endodermis cells
Leaf	isobilateral, stomata amount per mm ² lower/upper surfaces: 160/80	bifacial, stomata amount per mm ² lower/upper surfaces: 110/90	isobilateral, stomata amount per mm ² lower/upper surfaces: 210/200

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REFERENCES

1. Özhatay, N., Koyuncu, M., Atay, S., Byfield, A., Türkiye'nin Doğal Tıbbi Bitkilerinin Ticareti Hakkında Bir Çalışma. Doğal Hayatı Koruma Derneği, İstanbul (1997).
2. Kıtıkı, A., Status of cultivation and use of oregano in Turkey. In Padulosi, S. (ed.) Oregano, Promoting the conservation and use of underutilized neglected crops. 14. Proceedings of the IPGRI International Workshop on Oregano, (Valenzano (Bari), 8-12 May 1996), CIHEAM, p.121-131, International Plant Genetic Resources Institute, Rome, Italy (1997).
3. Davis, P.H., Flora of Turkey and the East Aegean Islands, vol. 7. University Press, Edinburgh (1982).
4. Davis, P.H., Mill, R.R.& Tan, K., Flora of Turkey and the East Aegean Islands, vol.10 (supplement I). University Press, Edinburgh (1988).
5. Güner A., Özhatay, N., Ekim, T. & Başer, K.H.C., Flora of Turkey and the East Aegean Islands, vol.11 (supplement II). University Press, Edinburgh (2000).
6. Ietswaart, J.H., A Taxonomic Revision of The Genus *Origanum* (Labiatac). Leiden University Press, Netherlands (1980).
7. Baytop, A., Melikoğlu, G., Une drogue Anatolienne: Les inflorescences de l'*Origanum acutidens*. *İstanbul Ecz. Fak. Mec.*, **21**:128-136 (1985).
8. Gönüz, A., Özörgücü, B., An investigation on the morphology, anatomy and ecology of *Origanum onites* L. *Tr. J. of Botany*, **23**(1):19-32 (1999).
9. Sezik, E., Demirezer, Ö., Türkiye'de halk ilacı ve çay olarak kullanılan bitkiler üzerinde morfolojik ve anatomik araştırmalar IV. *Origanum saccatum* P.H. Davis. *DOĞA TU Tıp ve Ecz. D.*, **11**(2):304-309 (1987).
10. Tanker, M., Deux succédanés du thym: *L'Origanum heracleoticum* L. et la *Majorana onites* (L.) Benth. *İstanbul Ecz. Fak. Mec.*, **1**:32-48 (1965).
11. Alan, S., Eskişehir Çevresinin *Thymus* L. Türleri Üzerinde Morfolojik ve Anatomik Araştırmalar. Yüksek Lisans Tezi. AÜ Sağlık Bilimleri Enstitüsü, Eskişehir (1997).
12. Cutler, D.F., Applied Plant Anatomy. Longman Group Limited, London (1978).

13. Kaya, A., Bařer, K.K.C., Koca, F., Özdemir, A., Eskiřehir çevresi *Nepeta* türleri üzerinde morfolojik ve anatomik arařtırmalar. IX. Bitkisel İlaç Hammaddeleri Toplantısı (Eskiřehir, 16-19 Mayıs 1991) Bildirileri, p. 311-317, Anadolu Üniversitesi Basımevi, Eskiřehir (1992).
14. Kaya, A., Koca, F., Bařer, K.H.C., Tümen, G., *Satureja cuneifolia* türü üzerinde morfolojik, anatomik, palinolojik çalıřmalar. XII. Ulusal Biyoloji Kongresi (Edirne, 6-8 Temmuz 1994) Bildirileri, p.208-216, Edirne (1994).
15. Rudall, P., Anatomy of Flowering Plants. University Press, Cambridge (1994).
16. Sezik, E., Saracođlu, İ., Türkiye’de halk ilacı ve çay olarak kullanılan bitkiler üzerinde morfolojik ve anatomik arařtırmalar V. *Thymus eigii* (M. Zohary et P.H. Davis) Jals. *DOĐA TU Tıp ve Ecz. D.*, **12(1)**:32-37 (1988).
17. Tanker, M., Sever, B., Çitođlu, G., Tanker, N., Englert, J., Anton, R., *Ballota saxatilis* subsp. *saxatilis* üzerinde farmakognozik arařtırmalar. XI. Bitkisel İlaç Hammaddeleri Toplantısı (Ankara, 22-24 Mayıs 1996) Bildirileri, p.167-177, Ankara Üniversitesi Basımevi, Ankara (1997).
18. Tanker, N., İlisulu, F., Türkiye’de kekik olarak kullanılan bitkilerden *Thymus capitatus* (L.) Hoffm. et Link. *Ankara Ecz. Fak. Mec.*, **11**:127-135 (1981).
19. Tavukçuođlu, S., Kaynak, G., Tuyji, O., Uludađ’da yayılıřı olan *Thymus* L. türleri üzerinde morfolojik ve anatomik arařtırmalar. *Tr. J. of Botany*, **20(ek)**:50-71 (1996).
20. Tümen, G., Satıl, F., Duman, H., Bařer, K.H.C., Two new records for Turkey: *Satureja icarica* P.H. Davis, *Satureja pilosa* Velen. *Tr. J. of Botany*, **24(3)**:211-214 (2000).
21. Yentür, S., Bitki Anatomisi, İstanbul Üniversitesi Fen Fakültesi Yayınları, No: 227, İstanbul (1995).