

THE MORPHOLOGICAL, ANATOMICAL AND PALYNOLOGICAL PROPERTIES OF ENDEMIC *HAPLOPHYLLUM MEGALANTHUM* BORNM. (RUTACEAE)

YURDANUR AKYOL, EMİNE ALÇİTEPE, CANAN ÖZDEMİR*

Department of Biology, Faculty of Art and Science, Celal Bayar University, Manisa, Turkey
Corresponding author's e-mail: cozdemir13@gmail.com

Abstract

Haplophyllum megalanthum Bornm. which has been included in the list of EN (Endangered) the IUCN threat category was investigated morphologically, anatomically and palynological. Some different morphological characteristics determined from its description given in the Flora of Turkey for the species. The pollen grains were radial symmetrical, isopolar, tricolporate. Pollen shape differed from subprolate to spheroidal.

Introduction

The *Rutaceae* family has about 150 genus and 900 species distributed throughout temperate and tropical regions, particularly in southern Africa and in Australia (Heywood, 1978). This family is represented by 5 genera (*Ruta* L., *Haplophyllum* A. Juss., *Dictamnus* L., *Citrus* L., *Poncirus* Rafin.) and 30 taxa which have population in Turkey. The *Haplophyllum* genus is represented by 17 taxa in Turkey (Townsend, 1967; Davis, *et al.* 1988; Güner *et al.* 2000).

Haplophyllum megalanthum which is investigated in this study is endemic to Turkey. This species is under the threat due to its limitation to a very small area. So, it has been placed under EN (Endangered) of the IUCN threat category (Ekim *et al.*, 1989; Anon., 1994). It is necessary to be taking various measurements in the protection of endemic plants which are under the threat.

Some researchers have been doing researches on chemical composition some of the *Haplophyllum* species (Patra *et al.*, 1984; Gözler *et al.*, 1994 and 1996; Sağlam *et al.*, 2001; Sağlam, 2002, Sağlam *et al.*, 2003; Ulubelen & Öztürk, 2008; Parhoodh *et al.*, 2012). In addition Navarro *et al.*, (2004) have done morphological, caryological and molecular on a new *Haplophyllum* sp. Salvo *et al.* (2011) have done phylogeny, morphology and biogeography on *Haplophyllum* sp. But, any study on *H. megalanthum* hasn't been found except main knowledge

in Flora of Turkey and essential oil of *H. megalanthum* (Townsend, 1967; Ünver-Somer *et al.*, 2012). So the *H. megalanthum* was investigated from morphological, anatomical and palynological view point in this study.

Materials and Methods

Research materials were collected from natural population the following parts of Turkey during the May-January period both in flowering and fruiting times in the years 2002- 2004 (Fig. 1). Turkey-Manisa: Maldan district, Alitepe environ, 400m (Akyol 923). Description of the plant was done by Prof. Dr. Yasin ALTAN. Specimens were deposited in the herbarium at Celal Bayar University. Anatomic sections of the plants are taken from its root, stem and leaf which were fixed in 70% alcohol for anatomical studies. Sartur reactive were applied to the sections (Çelebioğlu & Baytop, 1949). The Biometric measurements were given in Table 1. Measurements of anatomical and palynological were realized with an ocular- micrometer. For LM study, the pollen slides were prepared according to the technique of Wodehouse (1935) and Erdtman (1960). A Hund microscope was used for examination (ocular ×16, objective ×100). Terminology was used according to Faegri & Iversen (1975). Dimensions and morphological variation in pollen of *H. megalanthum* are given in Table 2.

Table 1. Biometric measurements of *H. megalanthum*

	Broad (µm) min max	Length (µm) min max
Root		
Peridermis cell	30 - 40	15 - 20
Diameter of cortex cell	16.2 - 48.6	
Diameter of trache	20 - 50	
Stem		
Epidermis cell	11 - 27	5.4 -13.5
Diameter of cortex cell	16.2 - 54	
Diameter of trache	16.2- 75.6	
Leaf		
Cuticle	5.4 - 8	
Upper epidermis cell	13.5 - 27	11 - 21.6
Lower epidermis cell	11 - 27	5.4 - 21.6
Palisade parenchyma cell	5.4 - 11	21.6 - 65

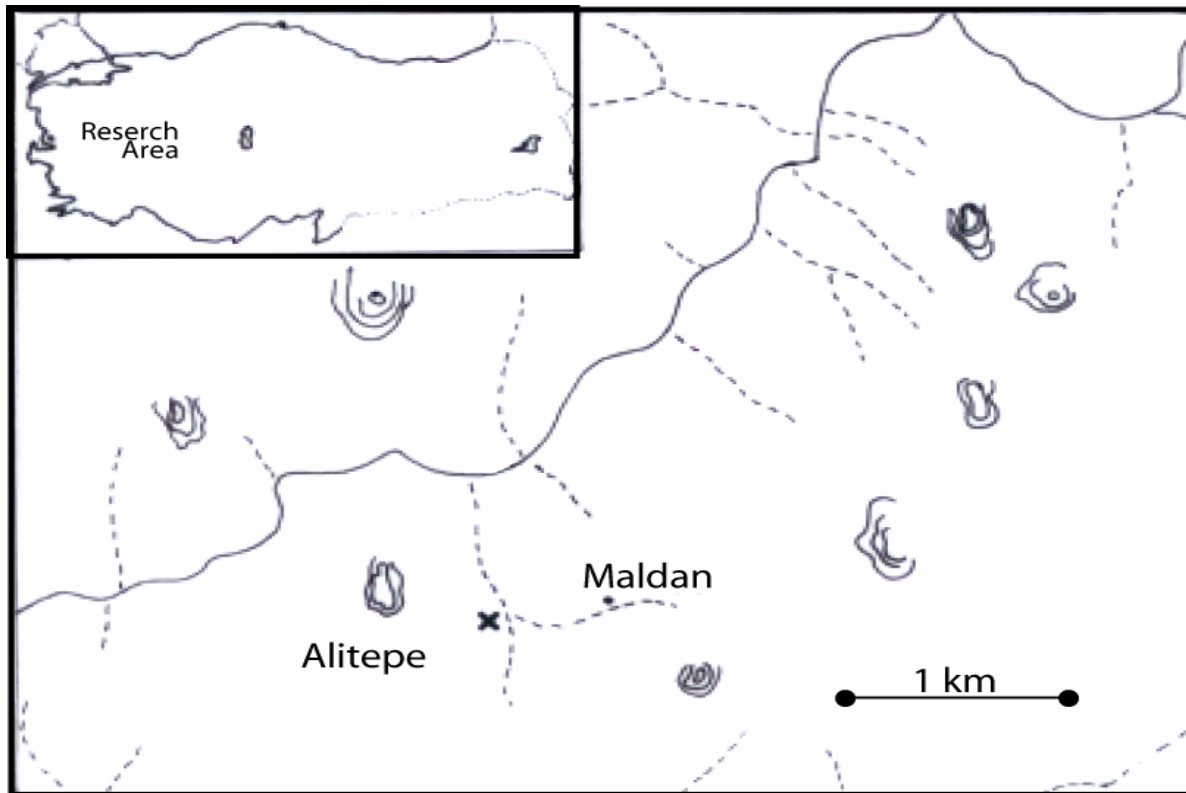


Fig. 1.*Map showing the locality of *H. megalanthum*

Table 2. Pollen morphological data of *H. megalanthum*.

	W		E	
	M (μm)	SD (\pm)	M (μm)	SD (\pm)
Pollen shape	Suboblate-spheroid			
P	32.00	1.88	32.25	1.28
E	32.33	4.43	32.91	3.91
Plg/Plt	8.00	1.77	7.80	1.81
clg	3.09	0.91	3.33	1.00
clt	19.07	5.67	22.93	5.84
Ex	1.19	0.31	1.65	0.51
Int	1.10	0.24	-	-

P, polar axis; E, equatorial axis; Plg/Plt, pore size; clg, colpus width; clt, colpus length; Erdtman, E; Ex, exine; Int, intine; M, mean; SD, standart deviation; Wodehouse, W.

Results

Morphological properties

The plant is 35-45cm length. Its root is taproot in shape. It is covered by pale –brown hard bark. Stem is erect, covered by densely eglandular hairs. This hair gives white color to stem. Leaves are simple, entire and lanceolate in shape. Its upper is rhomboid- lanceolate and narrowed at the apex. The leaves covered by densely eglandular hairs. The hairs of the upper leaves are silky-adpressed. Leaves are 6-27mm in length and 3 -12mm

width. Inflorescence is compact and branches are densely patent- hairy. Sepals are elliptic- lanceolate, green, white – lanate. Its size is 2-3 \times 4-7mm. Petals are elliptic to oblong-ovate in shape, concave, entire and creamy-white. Its size is 4-7 \times 7-16mm. Filaments are narrow gradually attenuate from base to apex and filament is covered by villous in the lower half. They are 4-6mm in length. Capsule lobe is densely hairs. Seed is colored dark brown - black and kidney- shaped with fundamentally longitudinal dorsal ridges. Its size is 1.5 – 2.5 \times 0.25 – 1.5mm. Species is distributed at the 400-2300m height, open limestone hill slopes near pine woods (Figs. 2, 3, 4, 5).



Fig. 2. General appearance of *H. megalanthum* (Akyol 923).



Fig. 3. General appearance of *H. megalanthum* in its natural habitat (Akyol 923).



Fig. 4. General appearance of *H. megalanthum* in its natural habitat (Akyol 923).

Anatomical properties

Root: Lignified periderm layer is 5 -8 layered on the outer surface on of root. Its cells are dark colored, crushed and break up. Cortex is multilayered and parenchymatic. Cortex cells are bigger on outer of cross- section of root than on inner of cross-section of root. There are glad sacs between

cortex cells. Cambium cells are distinguishable and 2-4 layered. Under the cortex layer phloem forms a thin layer followed by xylem which covers a large area. There isn't pith because of xylem occupies region of pith (Fig. 6).

Stem: In the upper part of cross-section of stem there is a thick cuticular layer followed a single layer of epidermis

below the epidermis layer cortex tissue which is 8-12 layered were observed. Its cells are small at the outer parts of the stem but are big at the inner parts of stem. And this cells have intercellular space. There are sclerenchymatous ring consists of sclerenchyma cells at the upper part of the phloem region. Phloem region is large. Cambium is 3-5 layered and distinguishable. The pith region is very small. Its cells are ovoidal and they have intercellular space (Fig. 7).

Leaf: There are single layered epidermises on upper and lower surface of leaf. Its cells are rectangular in shape. Epidermis is covered by a thick cuticle. Palisade parenchyma 2-3 layered and very compact. Spongy parenchymatic region is very narrow. Stoma cells are present on both upper and lower epidermis. There is sclerenchyma sheath on the median vein. Further more in cross-section of leaf we observed small transparent points along the median axis of the leaf (Fig. 8).

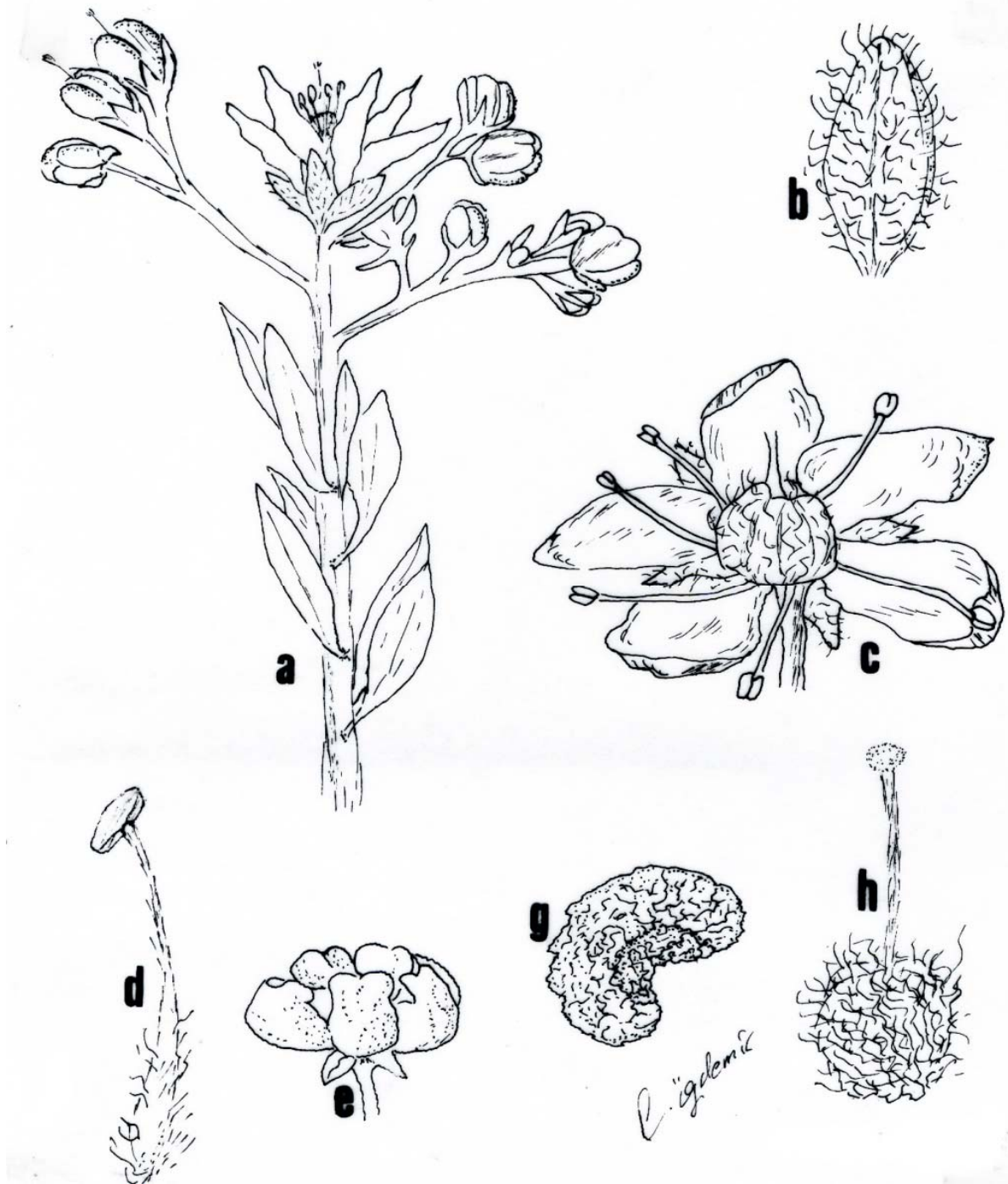


Fig. 5. *H. megalanthum* a. general appearance x 2; b. sepal x 8; c. flower x 4; d. stamen x 15; e. fruit x 7; g. seed x 8; h. ovarium x 10.

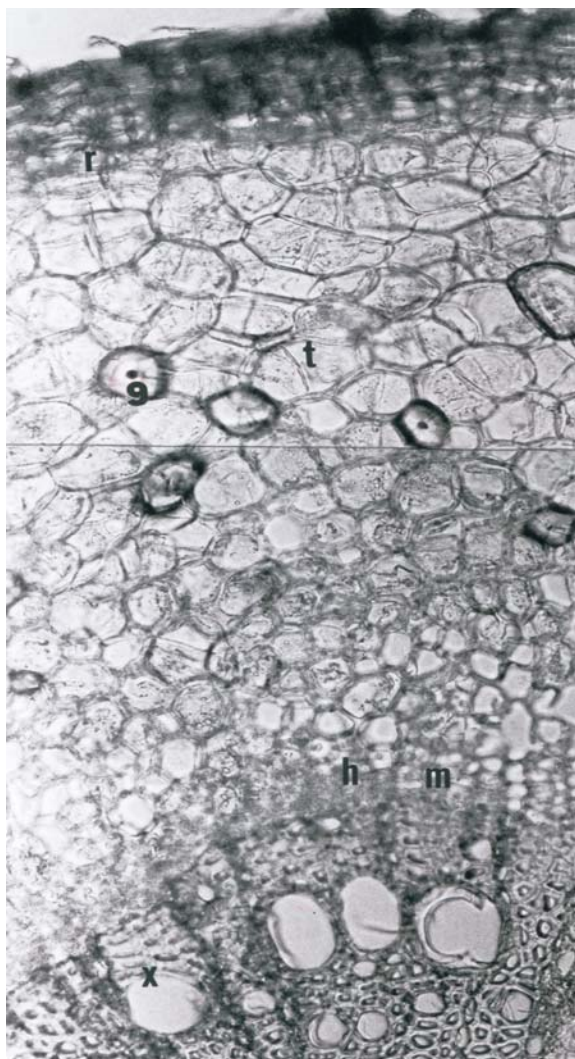


Fig. 6. Cross-section of root of *H. megalanthum* (LM x40), r. peridermis; t. cortex; m. cambium; g. gland sac; h. phloem; x. xylem

Palynological properties

The pollen grains are radial symmetrical, isopolar, tricolporate. Pollen shape differs from subprolate to spheroidal (Fig. 9). Pollen diameter 32 μ m (W), 32.25 μ m

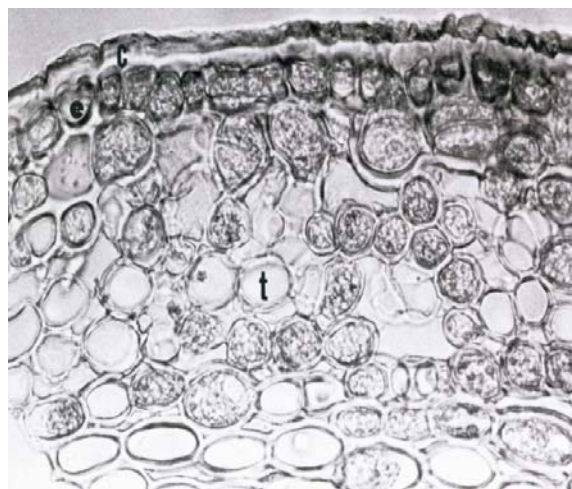


Fig. 7. Cross-section of stem of *H. megalanthum* (LM x40), c. cuticle; e. epidermis; t. cortex.



Fig. 8. Cross-section of leaf of *H. megalanthum* (LM x40), e. epidermis; p. palisade; v. vascular bundle; g. schyzogenic gland sac.

(E); equatorial diameter 32.33 μ m (W), 32.91 μ m (E), Plg/Plt : 8.00 μ m (W), 7.80 μ m (E); colpus length 19.07 μ m (W), 22.93 μ m (E); exine 1.19 μ m (W), 1.65 μ m (E), intine 1.10 μ m (W) thick. The *H. megalanthum* pollen morphological observations have given in Table 2.



Fig. 9. Pollen grains of *H. megalanthum* (LM)A. Polar view (LMx100); B. Equatorial view (LMx100).

Discussion

This is the first study on *H. megalanthum* except main knowledge in "Flora of Turkey". Some different morphological characteristics have been determined from its description given in Flora of Turkey for the species. In this study, long of plant and petal was determined respectively as 35-45 cm and 7-16 mm. While this measures were given as 20-30 cm and 10-14 mm in Flora of Turkey (Townsend, 1967). The size of filament, sepal and leaf of *H. megalanthum* are new observations which have been determined in this study. Another new findings are the morphological characteristics of seed concerning their shapes, sizes and colour of testa. *H. megalanthum* is endemic and taxon has been placed under EN of the IUCN threat category. This species extends to a very small area where is rested environment of stream. So it is under the threat of erosion (Fig. 3, 4). After having taken into consideration above mentioned threatening factors it is necessary to be taking various measurements in the protection.

Schyzogenic gland sac which present on leaves as are characteristics for *Rutaceae* family (Zeybek and Zeybek, 1994). The same feature was observed on leaves of *H. megalanthum*. Few chemical studies have been done on the *Haplophyllum* genus (Gözler *et al.*, 1994 and 1996; Sağlam *et al.*, 2001 and 2003). But, any anatomical, palynological study on *Haplophyllum megalanthum* has not been found. In the view of the pollen grain morphology; the types of the pollens the measurements in polar have been carried out using the Wodehouse and Asetolysed methods for the first time. The pollen grains were radial symmetrical, isopolar, tricolporate. Pollen shape differed from subprolate to spheroidal. The same findings were mentioned by Navarro *et al.* (2004) on a new *Haplophyllum* sp. and Perveen & Qaiser (2005) on pollen of Pakistan. El Nagger & Abdel Hafez (2003) and Perveen & Qaiser (2005) investigated *Haplophyllum* pollens with Scanning Electron Microscope. We aimed to give detailed knowledge about the *Haplophyllum megalanthum* that belong to *Rutaceae* which has economical value.

References

- Anonymous. 1994. Species Survival Commission IUCN Red List Categories. Gland, Switzerland.
- Çelebioğlu, S. and T. Baytop. 1949. *A new reagent for microscopical investigation of plant*. Publication of the Institute of Pharmacognosy, 10(19): 301.
- Davis, P.H., R.R. Mill and T. Kit. 1988. *Flora of Turkey and the East Aegean Islands*. (Suppl 1) Edinburgh Univ Press Vol. 10. Edinburgh.
- Ekim, T., M. Koyuncu, S. Erik and R. İlarıslan. 1989. Tehlike Altındaki Nadir ve Endemik Bitki Türleri, Türkiye Tabiatı Koruma Derneği, *IUCN Red Data Book*. 18: 1-214.
- El Nagger, S.M. and S.I.I. Abdel Hafez. 2003. Leaf surface, pollen morphoogy, fungal biodiversity and leaf spots of some wild plants in Sinai, Egypt. *Feddes Repertorium*, 114(1-2): 74-90.
- Erdtman, G. 1960. The asetolysis method. A revised description. *Svensk. Bot. Tidskr.*, 51: 561-564.
- Faegri, K. and J. Iversen. 1975. *Textbook of Pollen Analysis* (3rd ed.), Hafner Press. New York.
- Gözler, B., M.A. Önür, T. Gözler, G. Kadan and M. Hesse. 1994. Lignans and Lignan Gyyosides from *Haplophyllum cappadocium*. *Phytochemistry*, 37: 1693-1698.
- Gözler, B., T. Gözler, N. Ünver, D. Rentsch and M. Hesse. 1996. Lignans, Alkoloids and coumarins from *Haplophyllum vulcanicum*. *Phytochemistry*, 42: 695-699.
- Güner, A., N. Özhatay, T. Ekim and K.H.C. Başer. 2000. *Flora of Turkey and the East Aegean Islands*. (Suppl 2) Edinburgh Univ Press Vol. 11. Edinburgh.
- Heywood, V. H. 1978. *Flowering Plants of The World*. London: Oxford University Press.
- Navarro, F.B., V.N. Suarez-Santiago and G. Blanca. 2004. A new species of *Haplophyllum* A. Juss. (Rutaceae) From The Iberian Peninsula: Evidence from morphological, caryological and molecular analyses. *Annals of Botany*, 94(4): 571-582.
- Parhoodeh, P., M. Rahmani, N. Mohd, H. Mohd, A. Sukari and G. Cheng Lian Ee. 2012. Alkoloid Constituents Of *Haplophyllum laeviusculum* (Rutaceae). *Sains Malaysiana* 41(1): 47-52.
- Patra, A., E. Valencia, R.D. Minard, M. Shamma, N. Tanker, B. Gözler, M.A. Önür and T. Gözler. 1984. Furoquinoline alkaloids from *Haplophyllum vulcanicum*". *Heterocycles*, 22(12): 2821-2825.
- Perveen, A. and M. Qaiser. 2005. Pollen Flora of Pakistan-XLV. Rutaceae. *Pak. J. Bot.*, 37(3): 495-501.
- Sağlam, H. 2002. *Haplophyllum myrtifolium* Boiss. Türü Üzerinde İzolasyon Ve Yapı Aydınlatma Çalışmaları. 14. *Bitkisel İlaç Hammaddeleri Toplantısı Bildiriler* 29-31 Mayıs, Eskişehir, 203-205.
- Sağlam, H., T. Gözler and B. Gözler. 2003. A new prenylated aryl-naphthalene lignan from *Haplophyllum myrtifolium* Boiss. *Fitoterapia*, 74(6): 564-569.
- Sağlam, H., T. Gözler, B. Kıvrak, B. Demirci and K.H.C. Başer. 2001. Volatile compounds from *Haplophyllum myrtifolium* Boiss. *Chemistry of Natural Compounds*, 37(5): 442-444.
- Salvo, G., M. Sara, G. Farrokh, K. Tojibaev, L. Zeltner and E. Conti. 2011. Phylogeny, morphology and biogeography of *Haplophyllum* (Rutaceae) A species-rich genus of the Irano-Turanion Floristic Region. *Taxon*. Vol. 60: 15 pp. Electronic Supplement To www.systbot.uzh.ch/.../Salvo_Conti_2011...
- Townsend, C.C. 1967. *Haplophyllum* A. Juss. In: (Ed.): P.H. Davis. *Flora of Turkey and The Aegean Islands*. Vol: 2 496-506. Edinburgh Univ. Press. Edinburgh.
- Ulubelen, A. and M. Öztürk. 2008. Alkoloids, Coumarins And Lignans From *Haplophyllum* Species. *Rec. Nat. Prod.*, 2(3): 54-69.
- Ünver Somer, N., G.I. Kaya, B. Sarıkaya, M.A. Ömür, C. Özdemir, B. Demirci and K.H.C. Başer. 2012. Composition on the essential oil endemic *Haplophyllum megalanthum* Bornm. From Turkey. *Rec. Nat. Prod.*, 6(1): 80-83.
- Wodehouse, R.R. 1935. *Pollen Grains*, Mc-Graw Hill Press. New York.
- Zeybek, N. and U. Zeybek. 1994. *Farmasötik Botanik*, Ege Üniversitesi Basımevi, İzmir.