Available online at *www.ujpronline.com*
Universal Journal of Pharmaceutical Research
An International Peer Reviewed Journal
ISSN: 2831-5235 (Print); 2456-8058 (Electronic)

RESEARCH ARTICLE

Anatomical investigations on *Haplophyllum cappadocicum* Spach

Hüsniye Kayalar*, Gökay Arar
Ege University, Faculty of Pharmacy, Department of Pharmacognosy, Bornova, İzmir- Türkiye.

Article Info:

Article History:
Received: 10 February 2022
Reviewed: 19 March 2022
Accepted: 28 April 2022
Published: 15 May 2022

Cite this article
Kayalar H, Arar G. Anatomical investigations on *Haplophyllum cappadocicum* Spach. Universal Journal of Pharmaceutical Research 2022; 7(2):16-20.
https://doi.org/10.22270/ujpr.v7i2.753

***Address for Correspondence:**
Dr. Hüsniye Kayalar, Ege University, Faculty of Pharmacy, Department of Pharmacognosy, Bornova, İzmir- Türkiye. Tel- +902323112549; E-mail: *husniye.kayalar@ege.edu.tr*

Abstract

Aim and objective: The aim of this work is to put forward the anatomical features of *Haplophyllum cappadocicum*, an endemic plant for Turkey.
Methods: Plant materials were fixed in 70 % ethyl alcohol. The anatomical studies include transverse sections of roots, stems, leaves, fruits and seeds with illustrations. The investigations were made under microscope and photographs were taken by microphotography apparatus.
Conclusion: *Haplophyllum* genus with potential pharmaceutical interest are worthy of investigation not only for chemical content but also anatomically. This is the first report on anatomical features of *Haplophyllum cappadocicum* collected from Malatya, Türkiye.
Keywords: Anatomy, *Haplophyllum cappadocicum*, Rutaceae.

INTRODUCTION

The Rutaceae family is represented by 153 genera and 1800 species[1]. This family is represented in Turkey by the genera *Ruta* L., *Haplophyllum* A. Juss., *Dictamnus* L., *Citrus* L. and *Poncirus* Rafin. *Haplophyllum*, richest in species, is one of the least known genus of Rutaceae. Turkey is an important gene center for the *Haplophyllum* genus with an endemism rate of 58% (19 taxa, 11 of which are endemic)[2,3]. The genus *Haplophyllum* is represented by about seventy species distributed in an area stretching from the Mediterranean to Eastern Siberia[4]. Members of the genus *Haplophyllum* are known for their use in traditional medicine[5-7], as well as for containing a variety of chemical constituents of diverse structures such as lignans, quinoline alkaloids, coumarins, flavonoids and volatile compounds[8-18]. The leaves of plants belonging to *Haplophyllum* genus are used in traditional medicine for malaria, rheumatoid arthritis and gynecological disorders. *Haplophyllum* species are reported to be used in the treatment of diarrhea, constipation, and skin diseases[19,20,21]. In addition to cardiovascular effects, *Haplophyllum* species are previously shown to exhibit antimalarial, antileishmanial, antioxidant anti-insecticidal, anti-microbial, anti-inflammatory activities whereas *H. sahinii* and *H. vulcanicum* were reported to possess anti-cholinesterase and anti-tyrosinase activities[22,23]. The phytochemical analyses, morphological and palynolo-gical studies have been previously conducted on plants from *Haplophyllum* genus. In respect of anatomical characteristics, *Haplophyllum* species were also inve-stigated but the most important anatomical studies were restricted for species such as *H. megalanthum, H. myrtifolium, H. telephioides* and *H. vulcanicum*[24-36]. In previous studies, phytochemical investigations have been carried out on *H. cappadocicum.* Lignans and quinoline alkaloids isolated from this plant exhibit its potential activity[18,37,41]. Although secondary metabolites of *H. cappadocicum* were extensively examined, comprehensive anatomical features of this species is scarcing in the literature.
The aim of this work was to put forward the compre-hensive anatomical features of *H. cappadocicum* which is endemic to Turkey.

MATERIALS AND METHODS

Material for *H. cappadocicum* was collected from Boranköy, Malatya. Voucher specimens are deposited in the Herbarium of the Ege University, Faculty of Pharmacy, Department of Pharmacognosy (1178). Plant material was identified by the authors HK and GA. A part of the plant material was fixed in 70%

alcohol for anatomical studies of root, stem, leaf, fruit and seed. The investigations were made under Carl Zeiss Jena microscope and photographs were taken by Carl Zeiss Jena microphotography apparatus with 3.2x, 10x , 40x, 100x magnifications.

***H. cappadocicum* Spach**

Stems moderately to densely crisped-pubescent, 25-50 cm. Leaves usually entire, oblanceolate to linear, rarely lanceolate-obovate, occasionally trisect or incised, long-attenuate and pseudopetiolate below. Infloresence broad and lax, the lower branches much exceeding the terminal cyme, at least the upper branches crisped-pubescent. Sepals broadly to narrowly deltoid, persistent. Petals bright yellow, oblong-elliptic, 5-6 mm. Filaments abruptly expanded and ± parallel-sided in the lower half. Young ovary segments biovulate, each with a pronounced terminal apical corniculus, densely pilose on the upper inner surface, more sparingly so below. Capsule with divergent unbonate appendages on the upper dorsal surface, remaining glands convex, the raised margins not confluent into transverse ridges. Fl. 6-7. Grassy and stony steppe, 700-1500 m[4].

RESULTS AND DISCUSSION

Anatomy of Root

On the outermost side there is a single layer of exodermis made up of suberised cells. The vascular bundles constitute a collateral arrangement. The endodermis is not detected in the transverse sections (Figure 1).

Anatomy of Stem

Stem cortex, with a thin cuticle on the outer side, has xerophytic stomata. On the epidermis, thick cell walled unicellular hairs with dots on cuticle (Figure 2), assume straight or hook-like shapes. In the cortex parenchyma, schizolysigenous oil cavities are just in touch with the epidermal cells (Figure 3). The cortex parenchyma consists of starch sheath cells, schlerenchyma fibers, xylem, phloem and the pith. The pith is composed of parenchymatous cells (Figure 4).

Anatomy of leaf

The leaf is monofacial. As seen in the cross sections of leaf laminae, the outer layers of lower and upper epidermis with thickened walls, have xerophytic stomata (Figure 5). Within the mesophyll tissue, palisade parenchyma is composed of two or three rows whereas the spongy parenchyma takes a little place on the center of the mesophyll tissue. Oil cavities are just in touch with upper epidermis (Figure 6).

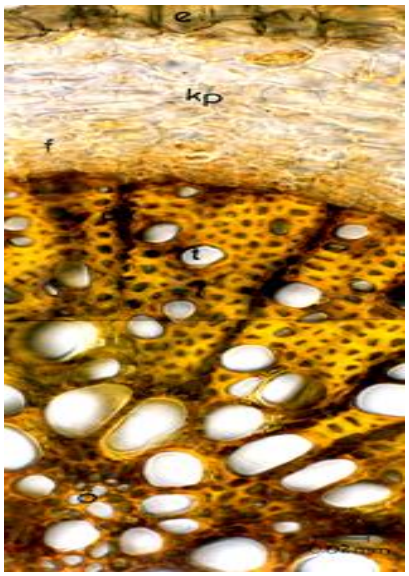

Figure 1: Cross section of the root of *H. cappadocicum.*

e: exodermis, f: phloem, kp: cortex parenchyma, t: trachea, ök: medullary rays, ö: pit

In the cross sections of the mid-rib region, thin-walled parenchymatous cells surround the collateral vascular bundles. Schlerenchyma fibers are detected between starch sheath cells and the phloem (Figure 7). In the surface view of the leaf, on the basis of the arrangement of four to six subsidiary cells relative to stoma, the stomata is found to be as anomocytic type. In the surface view of the epidermis, the epidermal cell walls seem to be thick and unicellular hairs assume straight or hook-like shapes (Figure 8).

Anatomy of Fruit and Seed

The exocarp with thick outer cell walls, has thin cuticle. In the tissue of the mesocarp, essential oil cavities are orientated where the neigbouring cells to these cavities are thin-walled. The tissue of the endocarp is entirely sclerenchymatous (Figure 9). Testa, the destroyed cells of nucellus, endosperm and embryo constitutes the seed. In the cross section of the seed, the epidermal cells of the testa, assuming papillae with thicker outer cell walls, have hair-like elongations and crystal-like grains which are destroyed by acidic reagents with gas relase.

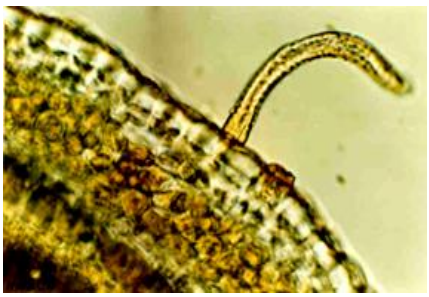

Figure 2: Unicellular hair on the epidermis of the stem.

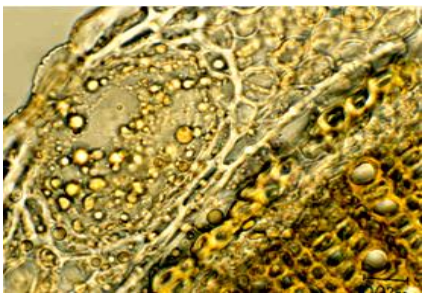

Figure 3: Oil cavity in the cross section of the stem.

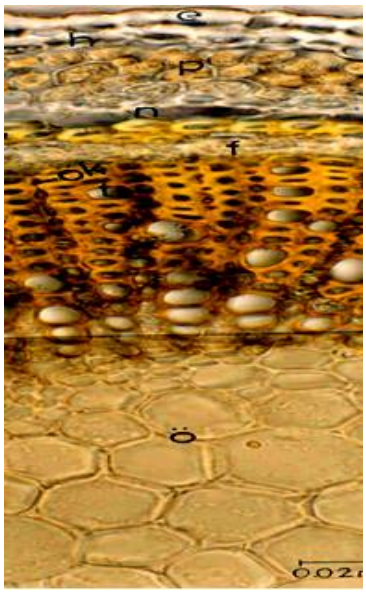

Figure 4: Cross section of the stem of *H. cappadocicum.*

e: epidermis, h: hypodermis, p: parenchymatous cells, n: starch sheath cells, s: schlerenchyma fibers, f: phloem, t: trachea, ök: medullary rays, ö: pith

The lateral and inner cell walls are thinner than the outer walls of the epidermal cells of the testa. The cell walls of the testa parenchyma are thick and seemed to be partially pressed in the mature seeds. The endosperma lying under the destroyed nucellus cells, is composed of thin walled parenchymatous cells and oil droplets as reserve substances are located within the cells (Figure 10 A and Figure 10 B). Rutaceae plants are distinguished from all other families of the order Sapindales by possessing secretory cavities containing aromatic ethereal oils scattered throughout almost all organs, a synapomorphy to the family[42].

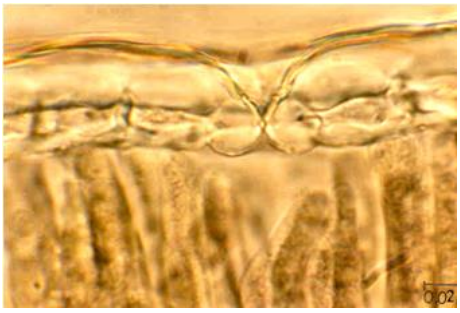

Figure 5: Xerophytic stomata in the cross section of the leaf.

Schizogenous sac gaps are characteristics for Rutaceae family. In contrast, in this study schizolysigenous secretory cavities were observed in cross sections of *H. cappadocicum.* Taxonomic and systematics studies among *Haplophyllum* A. Juss. and *Ruta* L. Taxa, which are naturally occuring in Turkey, were previously investigated based on morphological, palynological, anatomical and molecular studies. The anatomical characteristics of leaf and stem parts *H. cappadocicum* were also described[43]. Interestingly, thick cell walled unicellular straight or hook like shaped hairs were not mentioned in transverse sections of *H. cappadocicum*. In Rutaceae, in most species stomata are confined to abaxial sides. Conversely the leaves of *H. cappadocicum* are amphistomatic and mesophyll is equifacial as described. Some anatomical features of *Haplophyllum* species were reported before. The most important studies in respect of plant anatomy are restricted for species such as *H. myrtifolium, H. telephioides, H. megalanthum* etc[34-36].

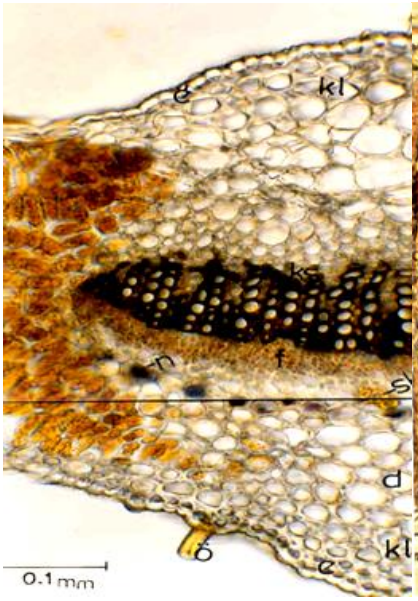

Figure 6: Cross section of the leaf of *H. cappadocicum*.

e: upper epidermis, sc: oil cavity, y: oil droplets, pp: palisade parenchyma, sp: spongy parenchyma, i: vascular bundles, st: stoma, é: lower epidermis

Figure 7: The cross section of the mid-rib region.

e: upper epidermis, é: lower epidermis, kl: collenchyma, ks: xylem, f: phloem, sk: schlerenchyma fibers, n: starch sheath cells, d: parenchyma, ö: unicellular hair

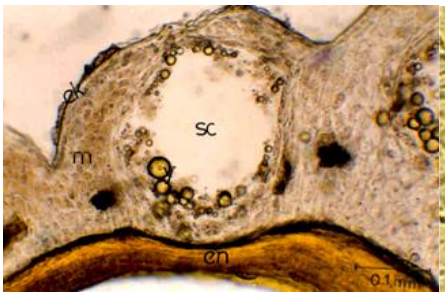

Figure 8: The surface view of the leaf.

Figure 9: Fruit section of *H. cappadocicum*.

In the present work, anatomical characteristics of *H. cappadocicun* did not show significant differences. The anatomy of roots, fruits and seeds of *H. cappadocicum* is reported for the first time whereas these findings might be useful for distinguishing and for comparison with other *Haplophyllu*m species.

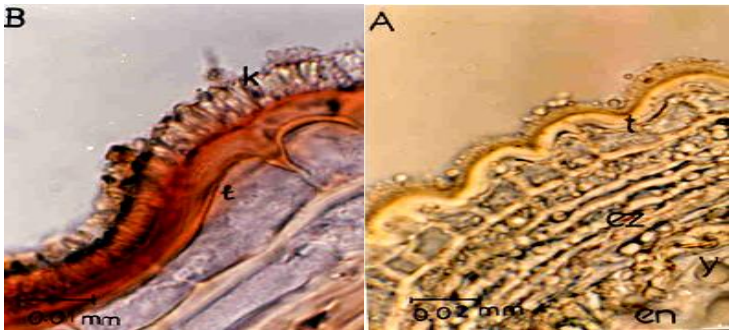

Figure 10: The cross section of the seed of *H. cappadocicum*; A. In Sartur reagent B: In Chloralhydrat reagent.

t: testa, ez: destroyed cells of nucellus, en: endosperma, y: oil droplets, k:crystal-like grains

CONCLUSION

Haplophyllum genus possessing biologically active natural substances of potential pharmaceutical interest, are worthy of investigation not only for chemical content but also anatomically. To the best of our knowledge, comprehensive anatomical studies have been carried out on *H. cappadocicum*, an endemic plant to Iran-Turan phytogeographical region, from Boranköy-Malatya, Turkey, for the first time in this report. Unfortunately there is a lack of academic education in taxonomy and properly trained pharmaceutical botanists. Endemic medicinal plants that might be of potential economic and pharmacological importance should also be investigated not only for secondary metabolites but also for their morphological and anatomical properties.

ACKNOWLEDGEMENTS

We thank lecturer Proffessor M. Ali Önür (Ege University Faculty of Pharmacy Department of Pharmacognosy) for his guidance in preparing cross-sections and for taking photos.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

AUTHOR'S CONTRIBUTION

Kayalar H: plant material collection, anatomical studies, drafted the manuscript. **Arar G: critical review, supervision.** The final manuscript was read and approved by all authors.

CONFLICT OF INTEREST

None to declare.

REFERENCES

1. Simpson MG., Plant Systematics, 2006, 1st ed., Elsevier, Academic Press, Amsterdam.
2. Ulukuş D, Tugay O, Celep F. Morphology, anatomy and palynology of Turkish endemic species *Haplophyllum myrtifolium*, *H. vulcanicum*, *H. megalanthum* (Rutaceae) and their systematics implications. Phytotaxa 2016; 247: 197-209. *https://doi.org/10.11646/phytotaxa.247.3.3*
3. Ulukuş D, Tugay O. *Haplophyllum ermenekense* (Rutaceae), a new species from Turkey. Phytokeys 2018; 8(111): 119–131. *https://doi.org/10.21597/jist.596057*
4. Willis, JC. A Dictioanary of Plants and Ferns, 8th ed., revised by Airy H. K. Shaw, Cambridge University Press, Cambridge, 1980:532.
5. Bessonova IA, Batsuren D, Yunusov S. Yu. Alkaloids of *Haplophyllum dauricum*. Chem Nat Comp 1984; 20: 68-70.
6. Bessonova IA, Kurbanov D, Yunusov S.Yu. Components of *Haplophyllum ramosissimum*. Chem Nat Comp 1989; 25 (1): 39-40.
7. Ali MB, Mohamed AH, Bashir AK, Salih AM. Pharmacological investigation of *Haplophyllum tuberculatum*. Int J Pharmacog 1992; 30(1): 39-45.
8. Kostova I, Ivanova A, Bozhanka M, Vitkova A. Lignans and alkaloids from *Haplophyllum suaveolens*. Monatsh Chem 2000; 131:191-194.

9. Gözler B, Rentsch D, Gözler T, Ünver N, Hesse M. Lignans, alkaloids and coumarins from *Haplophyllum vulcanicum*. Phytochemistry 1996;42(3): 695-699.
10. Gözler T, Gözler, B, Linden A, Hesse M. Vulcanine, a beta-carboline alkaloid from *Haplophyllum vulcanicum*. Phytochem 1996; 43(6):1425-1426. *https://doi.org/10.1016/S0031-9422(96)00444-X*
11. Prieto JM, Recio MC, Giner RM, *et al*. Topical Anti-Inflammatory lignans from *Haplophyllum hispanicum*. Z. Naturforschung [C] 1996; 51(9-10):618-622. *https://doi.org/10.1515/znc-1996-9-1002*
12. Bessonova IA. Componenets of *Haplophyllum bucharicum*. Chem Nat Comp 2000; 36(3): 323-324.
13. Al-Rehaily AJ, Al-Howiriny TA, Ahmad MS, *et al*. Alkaloids from *Haplophyllum tuberculatum*. Phytochem 2001; 57(9):597-602. *https://doi.org/10.1016/s0031-9422(01)00041-3*
14. Evcim Ü, Gözler B, Freyer AJ, Shamma M. Haplomyrtin and (-)-haplomyrfolin: two lignans from *Haplophyllum myrtifolium*. Phytochem 1986; 25(8): 1949-1951. *https://doi.org/10.1016/S0031-9422(00)81181-4*
15. Ulubelen A, Gil, RR, Cordell GA, Meriçli AH, Meriçli F. Prenylated lignans from *Haplophyllum ptiliostlum*. Phytochem 1995; 39(2): 417-422.
16. Saglam H, Gozler T, Gozler B, Kıvçak B. A new prenylatedarylnaphthalene lignan from *Haplophyllum myrtifolium*. Fitoterapia 2003; 74(6): 564-569. *https://doi.org/10.1016/s0367-326x(03)00147-3*
17. Saglam H, Gozler T, Kıvçak B, Demirci B, Başer KHC. Volatile compounds from *Haplophyllum myrtifolium*. Chem Nat Comp 2001; 37(5): 442-444. *https://doi.org/10.1023/A:1014467225815*
18. Arar G, Gözler, T. Malatyamine, a 4-quinolone alkaloid from *Haplophyllum cappadocicum*. J Nat Prod 1985; 48(4): 642-643.
19. Mossa JS, Al-Yahya MA, Al-Meshal IA: Medical plants of Saudi Arabia. 1st ed. Riyadh: King Saud University Libraries; 1987.
20. Al-Yahya MA, Al-Rehaily AJ, Mohammed SA, Mansour S, Farouk S. New alkaloid from *Haplophyllum tuberculatum*. J Nat Prod 1992, 55:899–903. *https://doi.org/10.1021/np50085a008*
21. Mohamed AH, Ali MB, Bashir AK, Salih AM. Influence of *Haplophyllum tuberculatum* on the cardiovascular system. Pharm Biol 2008; 34(3):213-217. *https://doi.org/10.1076/phbi.34.3.213.13208*
22. Ulubelen A, Öztürk M. Alkaloids, coumarins and lignans from *Haplophyllum* Species. Rec Nat Prod 08; 202(3):54-69.
23. Mohsen ZH, Jaffer HI, Alsaadi M, Ali ZS. Insecticidal Effects of *Haplophyllum tuberculatum* against *Culex quinquefasciatus*. Int J Crude Drug Res 1989; 1: 17-21. *https://doi.org/10.3109/13880208909053931*
24. Östan I, Sağlam H, Limoncu ME, *et al*. *In vitro* and *in vivo* activities of *Haplophyllum myrtifolium* against *Leishmania tropica* L. New Microbiol 2007; 30:439-445. PMID: 18080680
25. Mahmoud AB, Danton O, Kaiser M, *et al*. Lignans, amides, and saponins from *Haplophyllum tuberculatum* and their antiprotozoal activity. Molecules 2020; 25:2825. *https://doi.org/10.3390/molecules25122825*
26. Ashfaq S, Hussain M, Bibi N, Alam J, Junaid M, Rehman SU. Antimicrobial and cytotoxic potential of *Haplophyllum gilesii* (Hemsl.) C.C. from Northern Pakistan. Pak J Agric Res 2019; 33(1):146-153. *https://doi.org/10.17582/journal.pjar/2020/33.1.146.153*
27. Al-Burtamani SK, Fatope MO, Marwah RG, Onifade AK, Al-Saidi SH. Chemical composition, antibacterial and antifungal activities of the essential oil of *Haplophyllum tuberculatum* from Oman. J Ethnopharmacol 2005; 96(1-2): 107-112. *https://doi.org/ 10.1016/j.jep.2004.08.039*
28. Hamdi A, Majouli K, Flamini G, Marzouk B, Marzouk Z, Heyden YV. Antioxidant and anticandidal activities of the Tunisian *Haplophyllum tuberculatum* (Forssk.) A. Juss. essential oils, S Afr J Bot 2017; 112: 210-214. *https://doi.org/10.1016/j.sajb.2017.05.026*
29. Awaad A, Alothman E. Antiulcer and anti-ulcerative colitis activities of *Haplophyllum tuberculatum* (Forsskal). Int J Pharmacol 2017; 14:31-38. *https://doi.org/10.3923/ijp.2018.31.38*
30. Zengin G, Aktumsek A, Ramazan C, Olcay C. A comprehensive study on phytochemical characterization of *Haplophyllum myrtifolium* Boiss endemic to Turkey and its inhibitory potential against key enzymes involved in Alzheimer, Skin diseases and type II diabetes. Ind Crops Prod 2014; 53:244-251. *https://doi.org/10.1016/j.indcrop.2013.12.043*
31. Yaman C, Tugay O, Ulukuş D. The effect of location and species differences on antioxidant activities of endemic *Haplophyllum* A. Juss. JSIT 2020; 10(1): 648-657. *https://doi.org/10.21597/jist.596057*
32. Muddathir AM, Yamauchi K, Batubara I, Mohieldin EAM, Mitsunaga T. Anti-tyrosinase, total phenolic content and antioxidant activity of selected Sudanese medicinal plants. S Afr J Bot 2017, 109: 9-15. *https://doi.org/10.1016/j.sajb.2016.12.013*
33. Karahisar E, Tugay O, Erdoğan Orhan İ, *et al*. Metabolite Profiling by Hyphenated Liquid Chromatographic Mass Spectrometric Technique (HPLC-DAD-ESI-Q-TOF-MS/ MS) and Neurobiological Potential of *Haplophyllum sahinii* and *H. vulcanicum* extracts. Chem Biodivers 2019; 16(9): 1-14. *https://doi.org/10.1002/cbdv.201900333*
34. Tekin M, Eruygun N. The structural studies on the medicinal plant *Haplophyllum telephioides*. Rev Bras Farmacogn 2016; 26(5):544-552. *https://doi.org/10.1016/j.bjp.2016.05.007*
35. Akyol Y, Alçıtepe E, Özdemir C. The morphological, anatomical and palynological properties of endemic *Haplophyllum megalanthum* Bornm (Rutaceae). Pak J Bot 2012; 44(3): 1121-1126.
36. Ulukuş D, Tugay O, Celep F. Morphology, anatomy and palynology of Turkish endemic species *Haplophyllum myrtifolium, H. vulcanicum, H. megalanthum* (Rutaceae) and their systematics implications. Phytotaxa 2016; 247 (3): 197-209. *https://doi.org/10.11646/phytotaxa.247.3.3*
37. Gözler B, Arar G, Gözler T, Hesse M. Isodaurinol, an arylnaphtalene lignan from *Haplophyllum capadocium*. Phytochem 1992; 31(7) 2473-2475.
38. Gözler B, Gözler T, Sağlam H, Hesse M. Minor lignans from *Haplophyllum cappadocicum*. Phytochem 1996; 42(3): 689-693.
39. Gözler B, Güneş S, Hesse M. Further lignans from *Haplophyllum cappadocium*. J Fac Pharm Gazi 1995; 12(1): 9-15.
40. Gözler B, Kıvçak B, Arar G, Gözler T, Hesse M. (-)-Padocin: A novel epoxylignan from *Haplophyllum cappadocicum*. Heterocycles 1994; 39(1): 243-249. *https://doi.org/10.1002/chin.199526256*
41. Gözler B, Önür MA, Gözler T, Kadan G, Hesse M. Lignans and lignan glycosides from *Haplophyllum capadocium*. Phytochem 1994; 37(6):1693-1698.
42. Groppo M, José RP, Maria LF, Salatino SRB, Jacquelyn AK. Phylogeny of rutaceae based on two noncoding regions from CpDNA. Am J Bot 2008; 95 (8): 985–1005.
43. Ulukuş D, Tugay O. Taxonomic and systematics studies among *Haplophyllum A.* Juss. and *Ruta L.* taxa which are naturally occuring in Turkey, PhD thesis, Selcuk University, Institute of Sciences 2015, Konya, Turkey.