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ORIGINAL ARTICLE / ÖZGÜN MAKALE

COMPARATIVE LEAF ANATOMY OF SOME *ASYNEUMA* GRISEB. & SCHENK TAXA

BAZI ASYNEUMA GRISEB. & SCHENK TAKSONLARININ KARŞILAŞTIRMALI YAPRAK ANATOMİSİ

Şeyda YAYLA[1,2]*, Muhammed Mesud HÜRKUL[1]

[1]Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 06560, Ankara, Türkiye

[2]Ankara University, Graduate School of Health Sciences, 06110, Ankara, Türkiye

ABSTRACT

Objective: *The Asyneuma Griseb. & Schenk is represented by 34 accepted species worldwide and its native range covers a wide area from East Central Europe to Japan and Northern Indo-china. The plants of Asyneuma are characterized by herbaceous, simple or branched inflorescences, purple, violet or blue corolla and capsule fruits. Studies show that the Campanulaceae family contains bioactive compounds phenylethanoid (phenylproponoid), alkaloids, cyanogenetic heteroside, flavonoid, triterpene, anthocyanin, phenolic acid, essential oil, coumarin and polysaccharide. In addition, it is known that plants in the family have antioxidant, wound healing, anti-inflammatory, analgesic, antiobesity, expectorant antihepatotoxic, antitumoral, antiatherosclerotic, neuroprotective, antidepressant, tonic and α-glucosidase inhibitory activities. In this study, the anatomical structures of Asyneuma limonifolium subsp. limonifolium, A. limonifolium subsp. pestalozzae (Boiss.) Damboldt, A. linifolium subsp. linifolium and A. linifolium subsp. nallihanicum Kit Tan & Yıldız leaves were examined.*

Material and Method: *Asyneuma limonifolium subsp. limonifolium, A. limonifolium subsp. pestalozzae (Boiss.) Damboldt, A. linifolium subsp. nallihanicum Kit Tan & Yıldız were collected from Ankara. A. linifolium subsp. linifolium was collected from Antalya. Plant parts preserved in alcohol (70%). Microscopic sections were taken using a razor blade. Tissues were stained with Sartur's reagent and examined with a light microscope. Microphotographs were taken with a camera attached to a light microscope.*

Result and Discussion: *The results showed that, the anatomical structures of the basal and cauline leaves of Asyneuma limonifolium subsp. limonifolium and A. limonifolium subsp. pestalozzae were similar. The leaves are bifacial and the palisade parenchyma 1-2 rows. In addition, the unicellular,*

* **Corresponding Author / Sorumlu Yazar:** Şeyda Yayla
e-mail / e-posta: yayla@ankara.edu.tr, **Phone / Tel.:** +903122033113

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non-glandular hairs and anomocytic stomata were observed on both epidermal surfaces. Besides, the leaf of A. linifolium subsp. linifolium is monofacial while that of A. linifolium subsp. nallihanicum is bifacial. Also, unicellular hairs were present on epidermal layer of A. linifolium subsp. linifolium while A. linifolium subsp. nallihanicum were not. The anomocytic stomata were determined on the upper and lower leaf surfaces of both subspecies.
Keywords: *Asyneuma, Campanulaceae, medicinal plants, pharmaceutical botany, plant anatomy*

ÖZ

Amaç: *Asyneuma Griseb. & Schenk, dünya çapında kabul görmüş 34 türle temsil edilmektedir ve doğal yayılışı Doğu Orta Avrupa'dan Japonya ve Kuzey Çin-hindi'ye kadar geniş bir alanı kapsamaktadır. Asyneuma bitkileri, otsu, basit veya dallı çiçekler mor, menekşe veya mavi renkli ve kapsül meyveler ile karakterize edilir. Campanulaceae familyasının feniletanoid (fenilproponoid), alkaloidler, siyanogenetik heterosit, flavonoit, triterpen, antosiyanin, fenolik asitler, uçucu yağ, kumarin ve polisakkarit gibi biyoaktif bileşikler içerdiğini göstermektedir. Ayrıca familya bitkilerinin antioksidan, yara iyileştirici, antienflamatuar, analjezik, antiobezite, balgam söktürücü, antihepatotoksik, antitümöral, antiaterosklerotik, nöroprotektif, antidepresan, tonik ve α-glukosidaz inhibitör aktiviteleri olduğu bilinmektedir. Bu çalışmada Asyneuma limonifolium subsp. limonifolium, A. limonifolium subsp. pestalozzae (Boiss.) Damboldt, A. linifolium subsp. linifolium ve A. linifolium subsp. nallihanicum Kit Tan & Yıldız yapraklarının anatomik yapıları incelenmiştir.*
Gereç ve Yöntem: *Asyneuma limonifolium subsp. limonifolium, A. limonifolium subsp. pestalozzae (Boiss.) Damboldt, A. linifolium subsp. nallihanicum Kit Tan & Yıldız Ankara'dan toplanmıştır. A. linifolium subsp. linifolium Antalya'dan toplanmıştır. Bitki kısımları alkolde (%70) korunmuştur. Jilet kullanılarak mikroskobik kesitler alınmıştır. Dokular Sartur reaktifi ile boyanmıştır ve ışık mikroskobunda incelenmiştir. Işık mikroskobuna bağlı bir kamera ile mikrofotoğrafları çekilmiştir.*
Sonuç ve Tartışma: *Sonuçlar, Asyneuma limonifolium subsp. limonifolium ve A. limonifolium subsp. pestalozzae taban ve gövde yapraklarının benzer olduğunu göstermiştir. Yapraklar bifasiyal ve palizat parenkiması 1-2 sıralıdır. Ayrıca, tek hücreli örtü tüyleri ve anomostik stomalar her iki epidermal yüzeyde de gözlenmiştir. Bunun yanında, A. linifolium subsp. linifolium'un yaprağı monofasiyal, A. linifolium subsp. nallihanicum'un ise bifasiyaldir. Ek olarak, A. linifolium subsp. linifolium'un epidermal tabakasında tek hücreli tüyler bulunurken, A. linifolium subsp. nallihanicum'da ise yoktur. Anomostik stomalar, her iki alt türün yaprak üst ve alt yüzeylerinde tespit edilmiştir.*
Anahtar Kelimeler: *Asyneuma, bitki anatomisi, Campanulaceae, farmasötik botanik, tıbbi bitkiler*

INTRODUCTION

Folk medicine and medicinal plants have a long history in the Anatolia and many traditional practices are still used today. Studies have documented the diversity of plant species used in folk medicine and their traditional uses in the treatment of many diseases in Türkiye [1-7]. Overall, these studies and many others highlight the richness and traditional uses of medicinal plants in Türkiye. More research is needed to better understand the therapeutic potential of these herbs and ensure their sustainable use.

Campanulaceae Juss. family includes 94 accepted genera worldwide [8]. The family has a native range from the tropics to the subtropics and includes herbs, trees and shrubs [9]. Leaves are alternate and exstipulate; the flowers are hermaphrodite, epigynous, actinomorphic; inflorescence 1-many flowered spicate, racemiform or paniculate; the fruit is an erect or nodding capsule; seeds are numerous, small and usually shiny [10].

Studies show that the Campanulaceae family contains bioactive compounds phenylethanoid (phenylproponoid), alkaloids, cyanogenetic heteroside, flavonoid, triterpene, anthocyanin, phenolic acid, essential oil, coumarin and polysaccharide [11-22]. In addition, it is known that family plants have antioxidant, wound healing, anti-inflammatory, analgesic, antiobesity, expectorant, antihepatotoxic, antitumoral, antiatherosclerotic, neuroprotective, antidepressant, tonic and α-glucosidase inhibitory activities [23-35].

The genus *Asyneuma* Griseb. & Schenk is represented by 34 accepted species worldwide and its native range covers a wide area from East Central Europe to Japan and Northern Indo-china [8]. The

plants of *Asyneuma* are characterized by herbaceous, simple or branched inflorescences, purple, violet or blue corolla and capsule fruits [36].

Light microscopy is an effective and practical method that is frequently used in the analysis and diagnosis of herbal drugs. Elucidation of plant tissues is very important to correctly describe and determine of medicinal plants [37-39]. In this study, the anatomical structures of *Asyneuma limonifolium* subsp. *limonifolium*, *A. limonifolium* subsp. *pestalozzae* (Boiss.) Damboldt, *A. linifolium* subsp. *linifolium* and *A. linifolium* subsp. *nallihanicum* Kit Tan & Yıldız leaves were examined.

MATERIAL AND METHOD

Asyneuma limonifolium subsp. *limonifolium* (*mh23012*) (Figure 1A), *A. limonifolium* subsp. *pestalozzae* (Boiss.) Damboldt (*mh23013*) (Figure 1B), *A. linifolium* subsp. *nallihanicum* Kit Tan & Yıldız (*mh23019*) (Figure 1D) were collected from Ankara. *A. linifolium* subsp. *linifolium* (*mh23023*) (Figure 1C) was collected from Antalya. Plant parts were preserved in alcohol (70%). Microscopic sections were taken using a razor blade. Tissues were stained with Sartur reagent and examined with a light microscope. Microphotographs were taken with a camera attached to a light microscope.

Figure 1. A: *Asyneuma limonifolium* subsp. *limonifolium*, B: *A. limonifolium* subsp. *pestalozzae*, C: *A. linifolium* subsp. *linifolium*, D: *A. linifolium* subsp. *nallihanicum* (photo: Şeyda Yayla)

RESULT AND DISCUSSION

Asyneuma limonifolium subsp. *limonifolium*

The basal leaf is bifacial (Figure 2). The midrib is domed outward in both sides. It is more protruding in the abaxial side. The upper and lower epidermis layers of the midrib are composed of square-rectangular cells. The cuticle covering the epidermis layer is thicker at the bottom. Both epidermal layers of the midrib are covered with unicellular non-glandular hairs, it is observed more intensely especially in the lower epidermis of the midrib. In the midrib, the vascular bundle is embedded in thin-walled, round-shaped parenchymatous cells. The xylem is surrounded by the phloem on the

abaxial side. The upper and lower epidermal cells of the leaf lamina are observed as square-rectangular. The palisade parenchyma is in 1-2 rows and located below the upper epidermis. The spongy parenchyma is observed below the palisade parenchyma. The unicellular non-glandular hairs and anomocytic stomata with 3-5 subsidiary cells were seen in the both surface sections of the leaf.

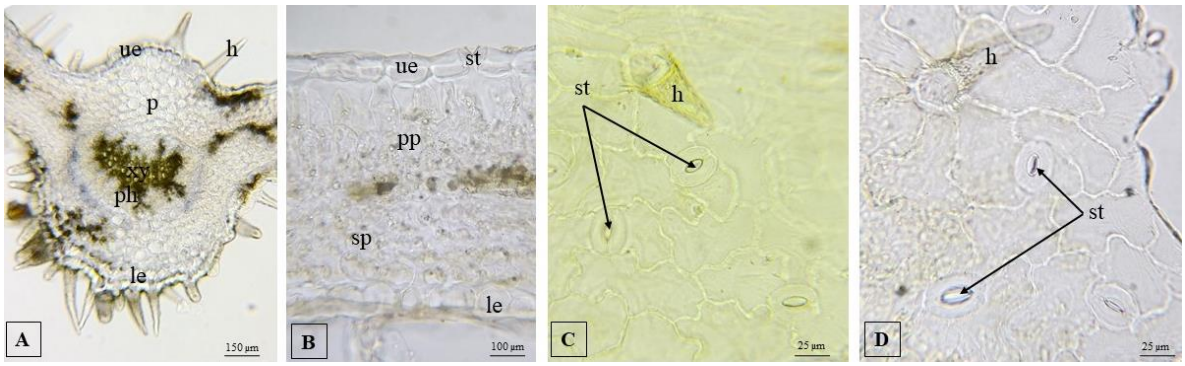

Figure 2. Basal leaf anatomical features of *A. limonifolium* subsp. *limonifolium* (A: Midrib cross section, B: Lamina cross section, C: Lamina upper surface section, D: Lamina lower surface section); h: unicellular hair, le: lower epidermis, p: parenchyma, pp: palisade parenchyma, ph: phloem, sp: spongy parenchyma, st: stomata, ue: upper epidermis, xy: xylem

The cauline leaf is bifacial (Figure 3). The upper and lower epidermis of the midrib consists of square-rectangular cells. The midrib protrudes outward on both surfaces, but it is more on the abaxial surface. The vascular bundle is embedded in thin-walled parenchymatous cells in the midrib, and the phloem is located on the abaxial side of xylem. The mesophyll has 1-2 rows of palisade parenchyma. The unicellular non-glandular hairs and anomocytic stomata with 3-5 subsidiary cells were seen in the both surface sections of the cauline leaf.

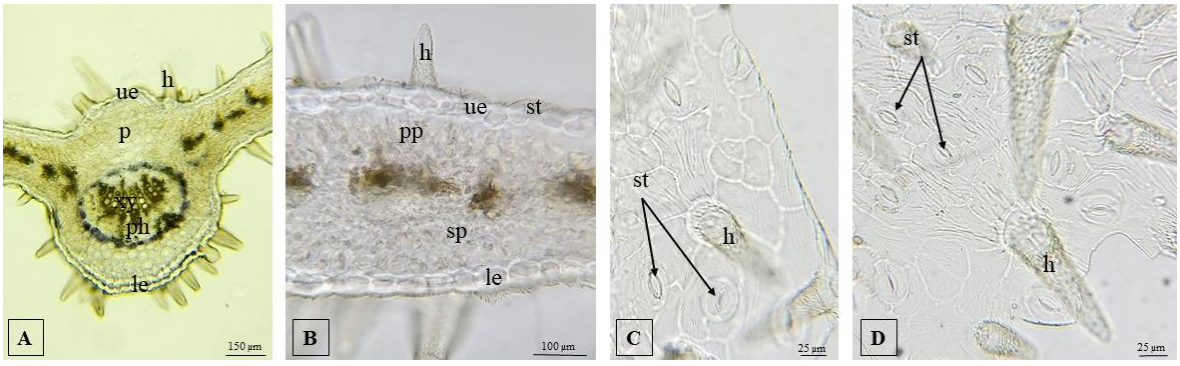

Figure 3. Cauline leaf anatomical features of *A. limonifolium* subsp. *limonifolium* (A: Midrib cross section, B: Lamina cross section, C: Lamina upper surface section, D: Lamina lower surface section); h: unicellular hair, le: lower epidermis, p: parenchyma, pp: palisade parenchyma, ph: phloem, sp: spongy parenchyma, st: stomata, ue: upper epidermis, xy: xylem

Asyneuma limonifolium* subsp. *pestalozzae

The basal leaf is bifacial (Figure 4). The midrib is domed outward in both sides. It is more protruding in the abaxial side. The cuticle is thick on both epidermal surfaces. In the midrib, the phloem is arc-shaped and surrounds the xylem from the abaxial side. The epidermal layer is composed of square-rectangular cells. Palisade parenchyma has 1-2 rows. The epidermis layer contains unicellular non-glandular hairs and stomata. The stomata are anomosytic type and have 3-5 subsidiary cells.

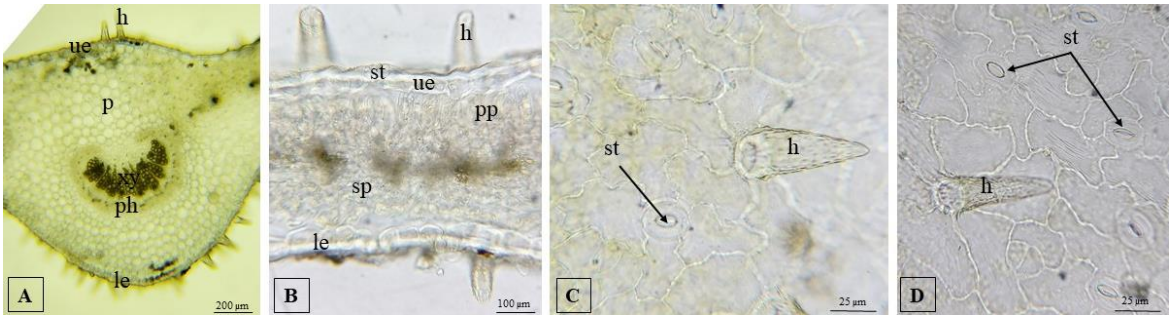

Figure 4. Basal leaf anatomical features of *A. limonifolium* subsp. *pestalozzae* (A: Midrib cross section, B: Lamina cross section, C: Lamina upper surface section, D: Lamina lower surface section); h: unicellular hair, le: lower epidermis, p: parenchyma, pp: palisade parenchyma, ph: phloem, sp: spongy parenchyma, st: stomata, ue: upper epidermis, xy: xylem

The cauline leaf is bifacial (Figure 5). The midrib is slightly domed in the adaxial side and extremely in the abaxial side. The main vein is embedded in thin-walled parenchymatous cells and the phloem is surrounded the abaxial side of xylem. Unicellular, non-glandular hairs are found more densely on the lower surface of the cauline leaf. The anomocytic stomata are located on the both epidermis surfaces, with 3-5 subsidiary cells. The upper epidermal cells of the cauline leaf lamina are similar to the lower epidermal cells, square-rectangular in shape and larger than the lower epidermal cells.

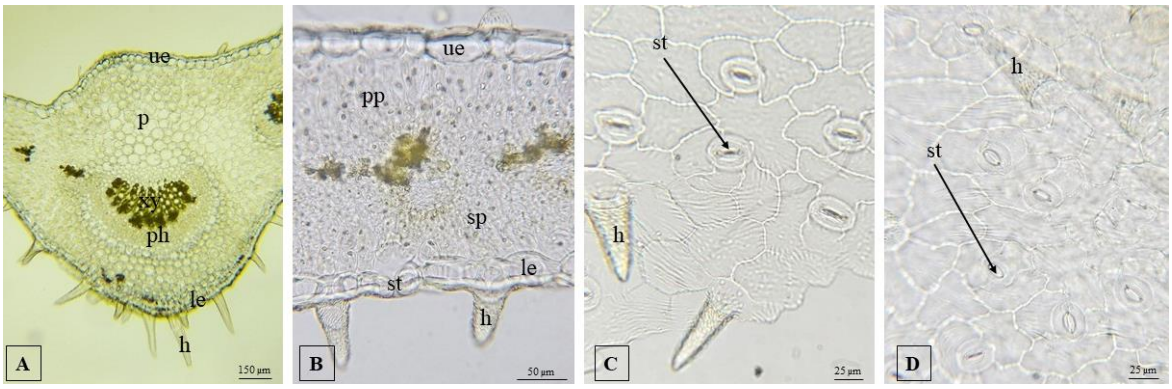

Figure 5. Cauline leaf anatomical features of *A. limonifolium* subsp. *pestalozzae* (A: Midrib cross section, B: Lamina cross section, C: Lamina upper surface section, D: Lamina lower surface section); h: unicellular hair, le: lower epidermis, p: parenchyma, pp: palisade parenchyma, ph: phloem, sp: spongy parenchyma, st: stomata, ue: upper epidermis, xy: xylem

Asyneuma linifolium subsp. *linifolium*

The leaf is monofacial (Figure 6). The palisade parenchyma has 1-2 rows in the mesophyll. The spongy parenchyma cells are located between the two palisade parenchyma. The upper epidermal cells are larger than the lower epidermal cells and are square or sometimes oval in shape. Unicellular non-glandular hairs are observed sparsely only in the upper epidermis. The anomocytic stomata are located on the both epidermis surfaces, with 3-5 subsidiary cells.

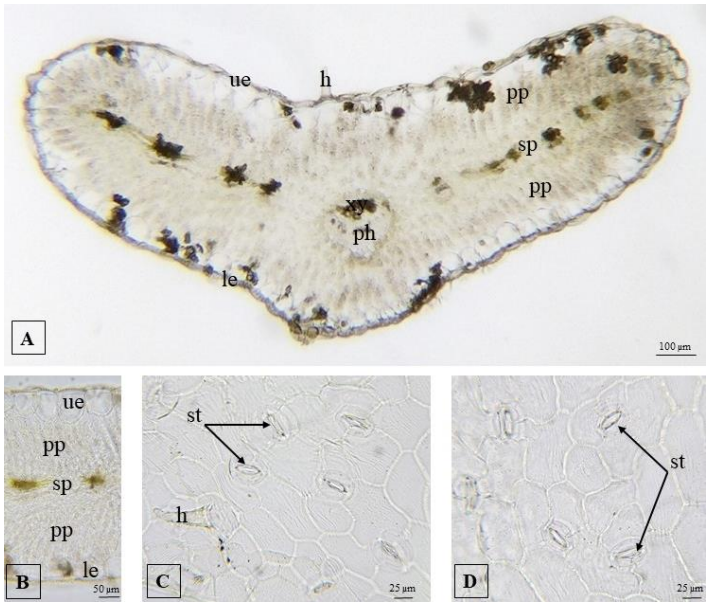

Figure 6. The leaf anatomical features of *A. linifolium subsp. linifolium* (A: Leaf cross section, B: Lamina cross section, C: Lamina upper surface section, D: Lamina lower surface section); h: unicellular hair, le: lower epidermis, pp: palisade parenchyma, ph: phloem, sp: spongy parenchyma, st: stomata, ue: upper epidermis, xy: xylem

Asyneuma linifolium subsp. *nallihanicum*

The leaf is bifacial (Figure 7). The upper and lower epidermal cells are the same size, mostly oval, rarely square. The spongy parenchyma is located under the 1-2 row palisade parenchyma in the lamina. The phloem accompanies to the xylem on the abaxial side in the main vascular bundle. Hairs are absent on the both surfaces. The stomata are anomocytic type and have 3-5 subsidiary cells and are observed in the both epidermis.

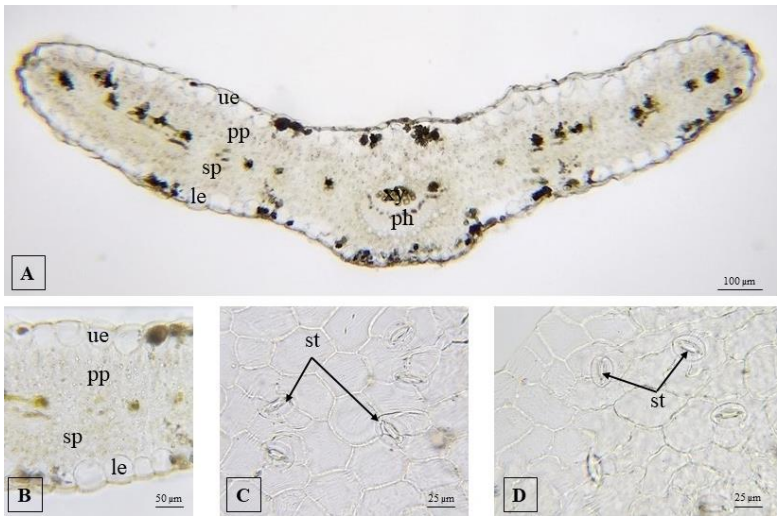

Figure 7. The leaf anatomical features of *A. linifolium subsp. nallihanicum* (A: Leaf cross section, B: Lamina cross section, C: Lamina upper surface section, D: Lamina lower surface section); le: lower epidermis, pp: palisade parenchyma, ph: phloem, sp: spongy parenchyma, st: stomata, ue: upper epidermis, xy: xylem

The results showed that, the anatomical structures of the basal and cauline leaves of *Asyneuma limonifolium* subsp. *limonifolium* and *A. limonifolium* subsp. *pestalozzae* were similar. The leaves are bifacial, the palisade parenchyma 1-2 rows. In addition, the unicellular, non-glandular hairs are located on the both epidermal layers. The anomocytic type stomata are located on the both surfaces. The leaf of *A. linifolium* subsp. *linifolium* is monofacial and the leaf of *A. linifolium* subsp. *nallihanicum* is bifacial. Also, on epidermal layer of *A. linifolium* subsp. *linifolium* unicellular hairs present, on epidermal layer of *A. linifolium* subsp. *nallihanicum* absent. The anomocytic stomata were found on the upper and lower leaf surfaces of the both subspecies. Previous studies have reported the presence of dorsiventral leaf, 1-2 rows of palisade, ranunculaceae type stomata on the both surfaces, and bundle sheath [40-41]. These data are consistent with our results. In addition, Metcalfe and Chalk (1965) reported the presence of epidermal cells with sinuous anticlinal walls, also acicular crystals and yellowish sphaerocrystals for the Campanulaceae family anatomical structures. In addition, the existence of unicellular, non-glandular hairs with papillae-like swelling is known [41-42].

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AUTHOR CONTRIBUTIONS

Concept: Ş.Y., M.M.H.; Design: Ş.Y., M.M.H.; Control: Ş.Y., M.M.H.; Sources: Ş.Y., M.M.H.; Materials: Ş.Y., M.M.H.; Data Collection and/or Processing: Ş.Y., M.M.H.; Analysis and/or Interpretation: Ş.Y., M.M.H.; Literature Review: Ş.Y.; Manuscript Writing: Ş.Y.; Critical Review: Ş.Y., M.M.H.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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