Comparative Morphological and Anatomical Studies of *Hymenocrater bituminosus* Fisch. & C.A.Mey. (Lamiaceae) in Turkey

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Abstract: *Hymenocrater bituminosus* Fisch. & C.A.Mey. is the only species of the genus *Hymenocrater* Fisch. & C.A.Mey. in Turkey. In this study, the morphological features of the species, such as stem, leaf, flower, and nutlet, are described in detail. The morphological results were compared to the *Flora of Turkey*, *Flora Iranica*, and *Flora of the USSR*. As a result of this study, the description of this species has been expanded, contributing to the knowledge of the flora of Turkey. In anatomical studies, transverse sections of stems and leaves were examined and are supported by illustrations and photographs. Furthermore, trichomes in stems, leaves, and calyces were investigated. Anatomical characters of the species were observed to be similar to the usual features of *Lamiaceae* anatomy.

Key Words: Anatomy, Hymenocrater, Lamiaceae, morphology

Türkiye'deki *Hymenocrater bituminosus* Fisch. & C.A.Mey. (Lamiaceae) Üzerinde Karşılaştırmalı Morfolojik ve Anatomik Çalışmalar

Özet:*Hymenocrater bituminosus* Fisch. & C.A.Mey., Türkiye'deki *Hymenocrater* Fisch. & C.A.Mey. cinsinin tek türdür. Bu çalışmada, türün gövde, yaprak, çiçek ve tohumunun morfolojik özellikleri detaylı bir şekilde incelenmiştir. Morfolojik sonuçlar, Türkiye Florası, İran Florası ve Rus Florası ile karşılaştırılmıştır. Çalışma sonucunda türün deskripsiyonu genişletilerek Türkiye Florası'na katkılar sağlanmıştır. Anatomik çalışmalarda, gövde ve yapraktan alınan enine kesitler incelenerek çizim ve fotoğraflarla desteklenmiştir. Ayrıca, gövde, yaprak ve kaliksteki tüy örtüsü incelenmiştir. Türün anatomik özelliklerinin, *Lamiaceae* familyasının genel anatomik yapısına benzer olduğu gözlenmiştir.

Anahtar Sözcükler: Anatomi, Hymenocrater, Lamiaceae, morfoloji

Introduction

The family Lamiaceae has an important role as a source of medicinal and aromatic plants of commercial importance. The genus *Hymenocrater* Fisch. & C.A.Mey., which belongs to the family Lamiaceae, is represented in Turkey by only one species, *H. bituminosus* Fisch. & C.A.Mey. This plant is distributed in East Anatolia, Transcaucasia, north-west and central Iran, and Khorassan. It is an Irano-Turanian element and grows at high altitudes (1900-2100 m) in East Anatolia (Mill, 1982). The genus *Hymenocrater* is represented by 11 species in *Flora Iranica* and 2 species in *Flora of the USSR* (Gorshkova, 1976; Rechinger, 1982).

There are few studies on the essential oil of *Hymenocrater* species in Russia, Iran, and Turkey. It is reported in the *Flora of the USSR* that *H. bituminosus* has commercial value due to a lemon aroma (Gorshkova, 1976). The essential oil of *H. incanus* Bunge, which is endemic to Iran, was analysed by Mirza et al. (2001). Composition of the essential oil of *H. calycinus* (Boiss.) Benth. was investigated by Firouznia et al. (2005) and the essential oil of *H. bituminosus* in Turkey was analysed by Kürkçüoğlu et al. (2005).

There are many studies of the anatomical and morphological characteristics of some species belonging to the family Lamiaceae in Turkey (Gönüz & Özörgücü, 1999; Başer et al., 2000; Kaya et al., 2000, 2002; Satıl et al., 2002; Kandemir, 2003); however, there has been no investigation of *Hymenocrater* species as yet.

Herein, the morphological and anatomical features of H. *bituminosus* were studied in order to provide more detailed descriptions of the species.

Materials and Methods

Plant material

Specimens were collected from 2 different regions of Turkey (Figure 1): A9 Kars: 16 km from Kağızman to Kars, 13 vi 2002, T. Dirmenci (1908!) VANF; B9 Van: 25 km from Gürpınar to Güzelsu, edge of Zernek Dam, 13 vi 2002, 1800-1900 m, F. Satıl (1035!) & M. Ünal (VANF).

Voucher specimens were deposited at the Herbarium of the Science and Arts Faculty of Yüzüncü Yıl University (VANF) and the Herbarium of the Science and Arts Faculty of Balıkesir University, Turkey.

Morphological and Anatomical study

Morphological characteristics were determined from fresh and herbarium materials. The taxonomic description of the plant was carried out according to *Flora of Turkey and the East Aegean Islands* (Mill, 1982) and was also confirmed by the herbarium samples of deposited species in VANF. An Olympus SZX12 stereomicroscope with a drawing tube was used for morphological study. Anatomical studies were conducted on fully flowered fresh plants and herbarium material. Whole plants or portions of them were stored in 70% ethanol. Developed middle cauline leaves and stems from fully flowered plants were used for anatomical study. Transverse sections of leaves and stems were made manually.

Tissues were stained with Sartur reagent and embedded in glycerine jelly (Baytop, 1972). An Olympus BX50 phase contrast binocular microscope with a drawing tube was used for anatomical studies and drawings.

Results

Morphology

Perennial herbs or subshrubs, flowering stem 30-60 cm. Stem and branches rarely eglandular and glandular hairs. Leaves ovate or ovate-oblong, $10-30 \times 6-24$ mm, obtuse or subacute, cordate to truncate, crenate to dentate; petiole 1.5-11 mm. Bracts similar to leaves, but slightly smaller, oblong-lanceolate, $5-10 \times 2.5-3$ mm, sessile or shortly petiolate, 1-2 mm. Verticillasters 2-3flowered, pedicel 0.5-1 mm. Calyx 10-20 mm, 5-lobed, pale green, papery, pubescent, densely glandular, calyx lobes $6-16 \times 3-10$ mm, obtuse or mucronate, calyx tube 3-5 mm, pubescent, glandular. Corolla 12-21 mm, violet, resupinate, corolla tube 12-18 mm, upper lip 4-5 mm and 2-lobed. Lower lips 3-lobed, lateral lobe similar to upper lips, the middle lobe is longer than the laterals. Stamens 4, 4-5 mm, exserted from lower lip. Style 1.5 mm, exserted from lower lip. Nutlets ovoid, $2.5-3 \times 1.4$ -2 mm, brown, minutely verrucose (Figure 2).



Figure 1. Distribution of *H. bituminosus* in Turkey (n).



Figure 2. H. bituminosus (FS 1035). A: habit; B: leaves; C: bract; D: flower; E: nutlet.

Flowering period: May-June

Ecology: *H. bituminosus* grows on bare shaly slopes, limestone scree, and on steppes, 1800-3000 m. It shares its habitat with *Alopecurus arudinaceus* Poiret, *Sedum album* L., *Marrubium vanense* Hub.-Mor., *Dianthus crinitus* Sm. subsp. *crinitus*, *Arabis caucasica* Willd subsp. *caucasica*, *Salvia kronenburgii* Rech.f., and *Artemisia taurica* Willd.

Anatomy

Stem: The epidermis is composed of a single layer. The upper and lower walls of the epidermis are covered with a thick cuticle and they are thicker than the lateral walls (Figures 3,4). Eglandular and glandular hairs are rare. Eglandular trichomes are generally 2-3-celled with cuticular micropapillae, unbranched. Glandular hairs are

quite simple in morphology. They have a short unicellular stalk and head (Figure 5).

The collenchyma tissue, located immediately under the epidermis, is 6-8-layered on the corners and 2-4-layered between the corners. They are irregular and fairly thick-walled. Parenchyma tissue is usually composed of squashed cells. This tissue is 3-4-layered on the corners and 1-2-layered between the corners. The sclerenchymatic tissue, located under the parenchyma, is 3-6-layered on the corners and 1-2-layered between the corners. Endodermis is indistinguishable.

The phloem is 3-5-layered and consists of irregular or rectangular cells. The cambium is not observed. The secondary xylem forms a large ring that comprises the trachea and tracheids. Trachea cells are round or ovoid, while tracheids are polyhedral. Rays are usually



Figure 3. *H. bituminosus.* A-B: Cross-section of stem. ep: epidermis; co: collenchyma; pa: parenchyma; sc: sclerenchyma; ph: phloem; xy: xylem; pi: pith.



Figure 4. *H. bituminosus.* Cross-section of stem. ep: epidermis; co: collenchyma; pa: parenchyma; sc: sclerenchyma; ph: phloem; xy: xylem; pi: pith. Scale bar: 100 μm.



Figure 5. Eglandular and glandular trichomes in *H. bituminosus*. A: in leaf; B: in stem; C: in calyx.

uniseriate. The pith consists of large hexagonal or polyhedral parenchymatous cells (Figures 3,4).

Leaf: The epidermis is composed of a single layer of cells, and the cells are rectangular or oval. Size of upper and lower epidermis is similar. Epidermal cells are covered with a thick cuticle. Stomata type is diacytic (Figure 6) and occurs on both surfaces (amphistomatic leaves). They are located almost on the same level as epidermis cells.

Eglandular trichomes are 2-4-celled with cuticular micropapillae, unbranched, and consist of elongated cells. Glandular trichomes are generally stalked and their head is unicellular (Figure 5). Trichomes are located on both surfaces of the leaf.

The leaf is isolateral. The mesophyll is differentiated into 2-seriate palisade and 1-4-seriate spongy parenchyma. The palisade tissue is under the upper and lower epidermis. The shape of the palisade parenchyma in transverse section is cylindrical. The spongy parenchyma cells, circular or ovoid, are located between the palisade tissues.

The midrib region is well developed and forms a projecting part towards the outside. Vascular bundles are collateral. The xylem faces towards the upper surface, while the phloem faces the lower epidermis. The upper and lower vascular bundles are covered with sclerenchymatous cells. There are collenchymatous cells, 2-3-layered, under the upper and lower epidermis in the midrib (Figures 6,7).



Figure 6. *H. bituminosus*. A-B: cross-section of leaf; C: surface view of upper epidermis; D: surface view of lower epidermis: ue: upper epidermis; pp: palisade parenchyma; sp: spongy parenchyma; xy: xylem; le: lower epidermis; ph: phloem; sc: sclerenchyma.



Figure 7. *H. bituminosus.* Cross-section of leaf midrib. ue: upper epidermis; pp: palisade parenchyma; xy: xylem; ph: phloem; sc: sclerenchyma. Scale bar: 100 μm.

Discussion

Hymenocrater bituminosus is a unique species in Turkey, which belongs to the genus *Hymenocrater*. It grows in Kars and Van provinces in East Anatolia. There have been no previous morphological or anatomical studies of the *Hymenocrater* species.

In this paper, we report the findings of a morphological study of *H. bituminosus* so as to improve the present knowledge of morphology for systematic purposes.

Our morphological results have been compared to those published in the *Flora of Turkey* (Mill, 1982), *Flora Iranica* (Rechinger, 1982), and *Flora of the USSR* (Gorshkova, 1976). The comparison of morphological characters is shown in Table 1.

The description of specimens in our study is different from the descriptions in the *Flora of Turkey* and the flora of neighbouring countries, in terms of some characteristics.

Petiole, bracts, sepal, calyx tube, trichomes in calyx, stamen, style, and nutlet sizes have been reported here for the first time. Some morphological variations have been observed in the indumentum and bract length characteristics (Table 1). The stem has simple hairs with sparsely glandular hairs. The indumentum in the calyx is

Characters	Results of this study	Flora of Turkey	Flora of the USSR	Flora Iranica
Flowering stem	30-60 cm	30-60 cm	30-100 cm	40-50 cm
Trichomes in stem	rare pubescent and glandular	glabrous and branches tuberculate	subglabrous	subglabrous
Leaves	10-30 × 6-24 mm	10-30 × 8-20 mm	15-25(30) × 12-20 mm	20-25(-35) × 10-15(-20) mm
Petiole	1.5-11 mm	-	3 mm	5-10 mm
Bracts	5-10 × 2.5-3 mm	-	(2.5)-5 × 1 mm	6-7 mm
Verticillasters	2-3-flowered	2-3-flowered	5-7-flowered	3-8-flowered
Calyx	10-20 mm	10-20 mm	14-16 mm	17-23 mm
Sepal	6-16 × 3-10 mm	-	10-12 × 6-10 mm	11-17 × 7-10 mm
Calyx tube	3-5 mm	-	4-4.5 mm	4.5-5 mm
Trichomes in calyx	hairy	-	glabrous	glabrous
Corolla	12-21 mm	15-21 mm	15-17(20) mm	± 20 mm
Stamen	4-5 mm	-	-	-
Style	1.5 mm	-	1.6 mm	_
Nutlets	2.5-3 × 1.4-2 mm	-	3-3.5 × 1.8-2 mm	± 3-1.8 mm

Table 1. Morphological characteristics of *H. bituminosus* in Turkey compared to *H. bituminosus* in other Flora.

denser than the stem's (Figure 5). Eglandular trichomes in the calyx are 1-4-celled and with cuticular micropapillae. In addition, there are glandular trichomes in the calyx. They are short, unicellular stalked, rarely bicellular, and head.

H. bituminosus is the most similar species to *H. elegans* Bunge, which is distributed in north-east Persia and Turkmenia. Both species are differentiated by their corolla colours; *H. bituminosus* has a reddish corolla, whereas *H. elegans* has a blue-whitish corolla.

Our research results are consistent with the representative taxa reported by Satil et al. (2002) and Kaya et al. (2002). The usual features of Lamiaceae

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anatomy (Metcalfe & Chalk, 1950) were observed in anatomical studies of the species. The stem is square in transverse section with well-defined groups of collenchyma in the corners. The midrib area is well developed in the leaf anatomy. The vascular bundle is covered with sclerenchymatous cells in the upper and lower sides, as is *Sideritis gülendamiae* (Kaya et al., 2002).

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