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

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Abstract
Haplophyllum megalanthum Bornm. which has been included in the list of EN (Endangered) the IUCN threat category was investigated morphologically, anatomically and palynologically. 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 temperature 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; Parhoodeh 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 leave 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>
<thead>
<tr>
<th>Table 1. Biometric measurements of H. megalanthum</th>
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</tr>
<tr>
<td>Root</td>
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<tr>
<td>Peridermis cell</td>
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<td>Diameter of cortex cell</td>
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<td>Diameter of trache</td>
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<td>Stem</td>
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<td>Epidermis cell</td>
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<td>Diameter of cortex cell</td>
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<td>Diameter of trache</td>
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<td>Leaf</td>
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<td>Cuticle</td>
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<td>Upper epidermis cell</td>
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<td>Lower epidermis cell</td>
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<td>Palisade parenchyma cell</td>
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*Corresponding author
Fig. 1. Map showing the locality of *H. megalanthum*

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

<table>
<thead>
<tr>
<th>Pollen shape</th>
<th>W (μm)</th>
<th>SD (±)</th>
<th>E (μm)</th>
<th>SD (±)</th>
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<tbody>
<tr>
<td>P</td>
<td>32.00</td>
<td>1.88</td>
<td>32.25</td>
<td>1.28</td>
</tr>
<tr>
<td>E</td>
<td>32.33</td>
<td>4.43</td>
<td>32.91</td>
<td>3.91</td>
</tr>
<tr>
<td>Plg/Plt</td>
<td>8.00</td>
<td>1.77</td>
<td>7.80</td>
<td>1.81</td>
</tr>
<tr>
<td>clg</td>
<td>3.09</td>
<td>0.91</td>
<td>3.33</td>
<td>1.00</td>
</tr>
<tr>
<td>clt</td>
<td>19.07</td>
<td>5.67</td>
<td>22.93</td>
<td>5.84</td>
</tr>
<tr>
<td>Ex</td>
<td>1.19</td>
<td>0.31</td>
<td>1.65</td>
<td>0.51</td>
</tr>
<tr>
<td>Int</td>
<td>1.10</td>
<td>0.24</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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, standard deviation; Wodehouse, W.

Results

Morphological properties

The plant is 35–45 cm 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 6–27 mm in length and 3–12 mm in width. Inflorescence is compact and branches are densely patent-hairy. Sepals are elliptic–lanceolate, green, white–lanate. Its size is 2–3 × 4–7 mm. Petals are elliptic to oblong-ovate in shape, concave, entire and creamy-white. Its size is 4–7 × 7–16 mm. Filaments are narrow gradually attenuate from base to apex and filament is covered by villous in the lower half. They are 4–6 mm 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 × 0.25–1.5 mm. Species is distributed at the 400–2300 m 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 sclerenchymatical 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).
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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 32.32 μm (W), 32.25 μm (E); equatorial diameter 32.33 μm (W), 32.91 μm (E); Plg/Plt : 8.00 μm (W), 7.80 μm (E); colpus length 19.07 μm (W), 22.93 μm (E); exine 1.19 μm (W), 1.65 μm (E), intine 1.10 μm (W) thick. The *H. megalanthum* pollen morphological observations have given in Table 2.

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.

Fig. 9. Pollen grains of *H. megalanthum* (LM x100); 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 these 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 (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


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