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Anatomical and Pollen Ornamentation Study on *Hymenocrater* species in North East of Iran

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Abstract: The present study tends to investigate the anatomy and palynology of *Hymenocrater* species in Northeast of Iran. To conduct the comparative study of anatomy characters, sections from stem and leaf were prepared using microtome and differential staining. In this part of investigation, arrangement of vessel in stem, stoma type and arrangement of mesophyll in leaf were studied. For the palynology study, too a comparative investigation on the species showed, the pollen was prolate spheroidal, hexacolpate, bireticulate and semi-tectate. Finally a variation between the shape of lumina in eu-reticulate and supra reticulate of pollen was observed.

Key words: Anatomy, Lamiaceae, palynology, pollen, *Hymenocrater*, Iran

INTRODUCTION

Hymenocrater Fisch and Mey. from Lamiaceae family, Stachyioideae subfamily, having numerous variety of species, it is expanded from Iran to Iraq, Pakistan, Afghanistan (Parsa, 1949; Groshkova, 1954; Jalas, 1972; Boissier, 1975; Davis, 1982; Rechinger, 1982; Hedge, 1990). *Hymenocrater* has nine species in Iran (Rechinger, 1982), about four of which are found in north-east of Iran. About 55% species of *Hymenocrater* is endemic for Iran (Naghbi *et al.*, 2005). This genus has aromatic essential oils and antimicrobial effects (Groshkova, 1954; Firoznia *et al.*, 2005; Zadidi and Crow, 2005). The purpose of present study was to investigate variation of internal structure and identification of *Hymenocrater* species on the basis of micromorphology and internal structure because morphologically identification of this species is difficult. This respect we collected fresh plants from their localities. Then we prepared some cross section of stem and leaf of *H. platystegius*, *H. calycinus*, *H. elegans* and *H. bituminosus*. In terms of comparative anatomic studies on *Hymenocrater*, there has been a report about *H. bituminosus* (Satil *et al.*, 2007). The other reports are about *Salvia* and *Cyclotrichum* from Lamiaceae (Metcalf and Chalk, 1983; Kaya *et al.*, 2000). In palynological studies, the comparison were made among the pollen grains of *Hymenocrater*. In this part of study, pollen grains were extracted and acetolysed, to study shape and ornamentation of the pollen through SEM and LM. The previous palynology investigation had been done in *Salvia*, *Origanum* and *Lycopus* (Moon and Hong, 2003; Akyalcin, 2003). So, comparative palynology of *Hymenocrater* carried out for first time in Iran.

MATERIALS AND METHODS

As for the anatomic study, the examined species were collected from the localities in north-east of Iran during May- June 2005-2006 (Table 1). For preparing of cross section of stem and leaf, base of stem and basal leaves were selected from 8-9 specimens. The fresh specimens were fixed in FAA then, dehydrated with ethanol and later, some slices prepared with microtome. The section-12 micron thick-stained with Safranin and Fast-green (Johnson, 1940; Chamberlain, 1990). For the stem maceration, this organ was placed in Jeffery solution for four hours to soften.

Table 1: The localities of studied *Hymenocrater* species

Species	Localities
<i>H. platystegius</i> Rech. F.	Mashhad, Mayan, 1753 m, Jafarzadeh and Morshedi, 146. Mashhad to Torghabeh, Asghad, 1400 m, Jafarzadeh and Sabbagian, 1115. Shandiz, 1700 m, Jafarzadeh and Sabbagian, 107.
<i>H. calycinus</i> (Boiss) Benth.	Chenaran, Golmakan, Ghaleh payeh, 1200 m, Jafarzadeh, 125. Dargaz to Chehelmir, 2240 m, Jafarzadeh and Faiz, 108.
<i>H. bituminosus</i> Fisch and Mey.	Dargaz to Tivan valley, 1500 m, Jafarzadeh and Faiz, 200. 7 km Mashhad to Shandiz, 1700 m, Jafarzadeh and Bagheri, 103. Mashhad, Asghad, 1450 m, Jafarzadeh, 110.
<i>H. elegans</i> Bunge.	Dargaz to Tivan, 2040 m, Jafarzadeh and Faiz, 207. Golmakan, Ghaleh payeh, 1200 m, Jafarzadeh, 122. Dargaz, Tandoureh protected area, 2050 m, Jafarzadeh and Faiz, 214.

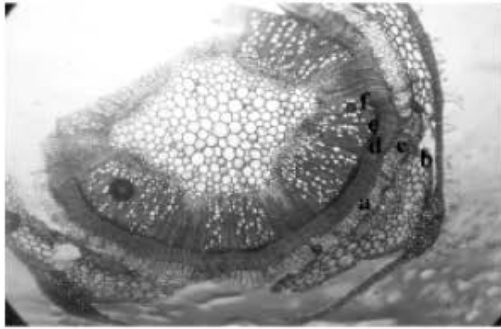


Fig. 1: Stem cross section of *H. elegans*; a: epidermis layer, b: collenchyma layer, c: parenchyma layer, d: sclerenchyma layer, e: suber layer and f: phloem tissue

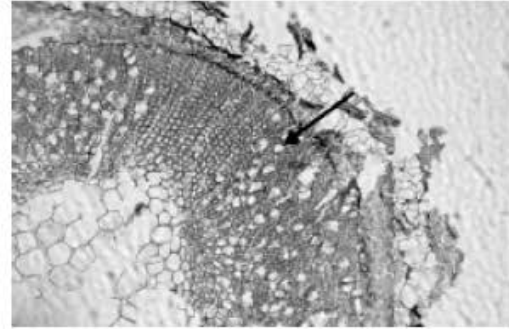


Fig. 4: Stem cross section of *H. bituminosus*. The arrow showing solitary and cluster (x 416)

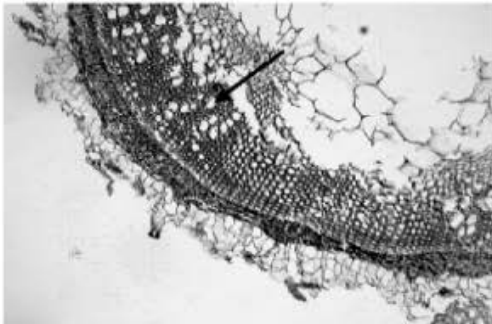


Fig. 2: Stem cross section of *H. platystegius*. The arrow showing solitary vessel (x 416)

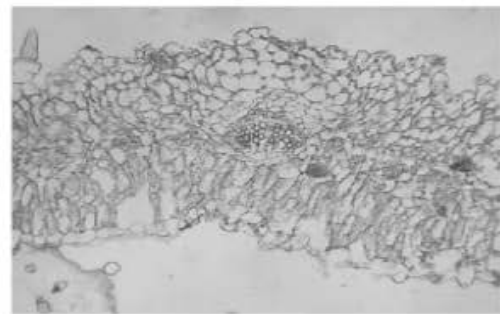


Fig. 5: Dorsi-ventral mesophyll in leaf cross section of *H. bituminosus* (x 940)

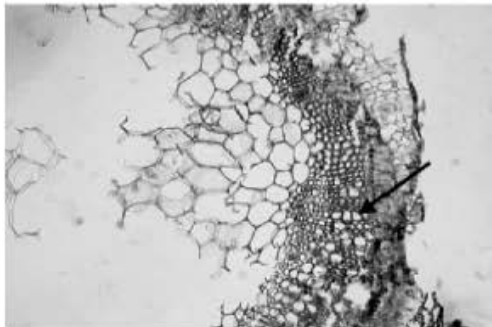


Fig. 3: Stem cross section of *H. cabycinus*. The arrow showing radial chain pore (x 416)

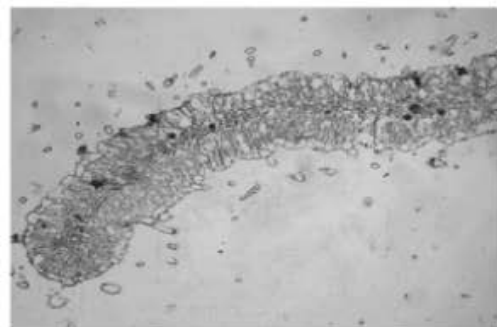


Fig. 6: Isolateral mesophyll in leaf cross section of *H. cabycinus* (x 416)

In the palynological study, the pollen was extracted from the anther and dehydrated by glacial acetic acid, then acetolised, coated with sputter finally studied by LM Olympus and SEM LEO1450VP. (Erdtman, 1971; Moore *et al.*, 1991). The pollen terminology was adapted from Punt *et al.* (1994). Finally, clustering analysis carried out with using JUMP.4 software for identifying close species.

RESULTS

Anatomic results: The results from the anatomic studies demonstrated the stem with following tissues:

- Some angular collenchymatous layers with small cells under the epidermis.

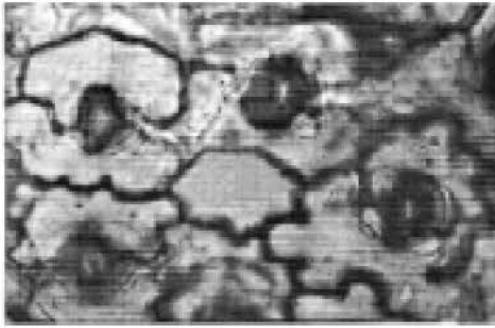


Fig. 7: Diacytic stoma type in *H. bituminosus*

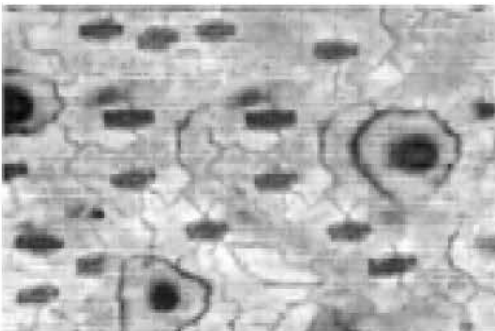


Fig. 8: Anemocytic stoma type in *H. platystegius*



Fig. 9: Anisocytic stoma type in *H. calycinus*

- Some parenchymatous layers with large cells
- Some sclerenchymatous layers with small cells.
- Suber layer under the sclerenchymatous layers. (Fig. 1).
- Vascular bundles which the arrangement of vessel was variable for example solitary in *H. platystegius* (Fig. 2), radial chain pore in *H. calycinus* (Fig. 3), solitary and cluster in *H. bituminosus* (Fig. 4) and cluster- radial chain pore in *H. elegans*. In all of studied species, sclerid and fiber observed. The results from stem maceration confirmed the presence of vessel with simple perforation plate.

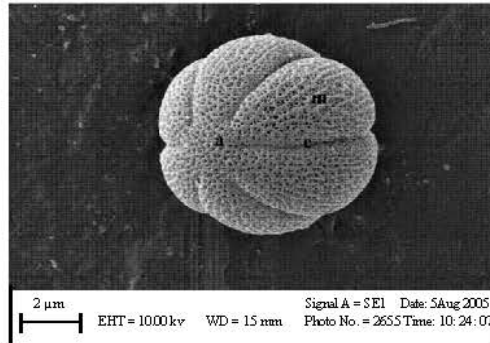


Fig. 10: The pollen of *H. elegans*: (a) apocolpium (m) mesocolpium (c) colpium (x 5000)

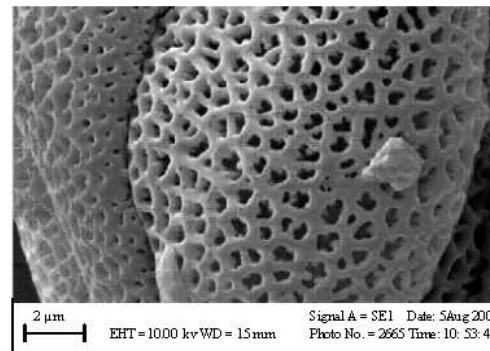


Fig. 11: The pollen of *H. elegans* (x 20000)

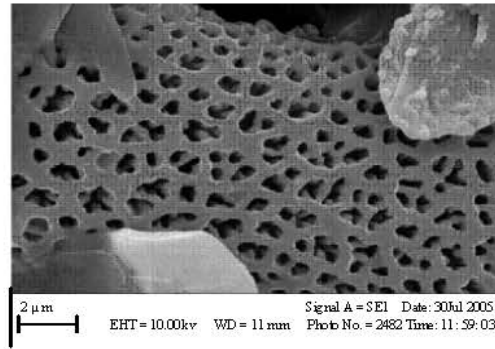


Fig. 12: The pollen of *H. platystegius* (x 2000)

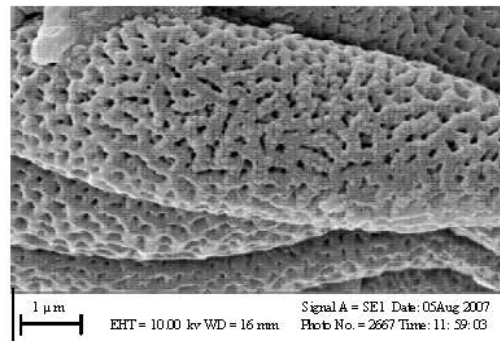


Fig. 13: The pollen of *H. calycinus* (x 2000)

Table 2: The anatomic and pollen characters of *Hymenocrater* studies species

Species	The arrangement of vessel	The arrangement of mesophyll	Stoma type	The shape of lumina in apocolpium
<i>H. platystegius</i>	Solitary	Dorsi-ventral	Diacytic-Anemocytic	Circular
<i>H. calycinus</i>	Radial chain pore	Isolateral	Diacytic-Anemocytic-Anisocytic	Narrow-oblong
<i>H. bituminosus</i>	Solitary-cluster	Dorsi-ventral	Diacytic	Circular
<i>H. elegans</i>	Cluster-radial chain pore	Dorsi-ventral	Diacytic-Anisocytic	Circular

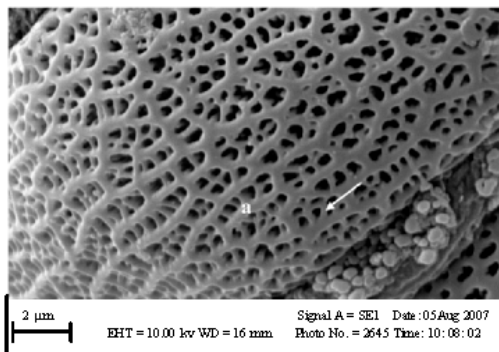


Fig. 14: The pollen of *H. bituminosus* (x 5000)

No. of clusters	Distance	Leader	Joiner
3	6.646913922	<i>H. platystegius</i>	<i>H. bituminosus</i>
2	7.516284269	<i>H. platystegius</i>	<i>H. elegans</i>
1	7.637015523	<i>H. platystegius</i>	<i>H. calycinus</i>

Dendrogram

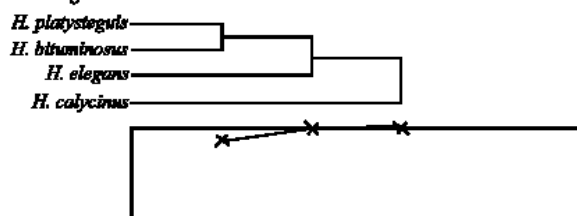


Fig. 15: Dendrogram on the basis of anatomy and palynology characters

The results from the anatomic study of the leaf showed, the arrangement of mesophyll was dorsi-ventral except *H. calycinus* (Fig. 5, 6). The stoma type was diacytic in *H. bituminosus*, diacytic with anemocytic in *H. platystegius*, diacytic with anemocytic and anisocytic in *H. calycinus* (Fig. 7-9).

Palynology analysis: The pollen was prolate spheroidal, bireticulate, hexacolpate, semi-tectate and agranulate (Fig. 10). The lumina of supra reticulate in *H. elegans* was uniseriate and oblong (Fig. 11), in *H. platystegius* rectangular (Fig. 12). The lumina of eu-reticulate was circular except *H. calycinus* which narrow and very oblong was observed (Fig. 13, 14). The shape of lumina in apocolpium was circular but in *H. elegans* was tetrahedral.

Finally using 46 character of anatomy and the pollen clustering analysis carried out and prepared dendrogram (Fig. 15, Table 2).

DISCUSSION

As the results from the anatomic studies about *Hymenocrater* suggest, we can state the anatomic structure of the stem in all species under the investigation, were similar. Also, their internal structures were similar to *Salvia* and *Cyclotrichum* (Metcalf and Chalk, 1983; Kaya *et al.*, 2000). Satil observed just radial chain pore the arrangement of vessel in *H. bituminosus* and he didn't report suber layer under the sclerenchymatous tissue. Also he identified only diacytic stoma type (Satil *et al.*, 2007) but present research results showed cluster- radial chain pore, suber layer, stoma type as diacytic and anemocytic. Also, only, the arrangement of mesophyll in *H. calycinus* was isolateral because, growing in stony mountains and rest of them were dorsiventral. The morphology of pollen in *Hymenocrater* was similar to *Lycopus* i.e., hexacolpate and bireticulate (Moon and Hong, 2003). Just differences observed among the species as size, shape of lumina.

Clustering analysis on the basis of anatomic and palynology results showed the closest species were *H. platystegius* and *H. bituminosus* because both of them were similar as arrangement of mesophyll, vessel and the shape of lumina. This results demonstrated, *H. calycinus* is different from the others, due having isolateral arrangement of mesophyll, compound stoma type, radial chain pore arrangement of vessel and circular in lumina at apocolpium. In conclusion using of micromorphology and anatomic characters can be useful for identifying of species.

REFERENCES

Akyalcin, H., 2003. Pollen morphology of *Origanum* L. (Labiatae) taxon in Turkey. *Asian J. Plant Sci.*, 2: 28-41.

Boissier, E., 1975. *Flora Orientalis (Corolliflorae-Monocotyledoneae)*. Vol. 4. Asher and Co., B.V. Amsterdam, ISBN: 9061 23 3240, pp: 575-678.

Chamberlain, C.J., 1990. *Methods in Plant Histology*. 5th Edn., Arihant Publishers, Jaipu, India, pp: 112-132, 144-145.

Davis, P.H., 1982. *Flora of Turkey and East the Aegean Islands (Hymenocrater)*, Vol. 7. Edinburg University Press, Edinburg, ISBN: 0 85224 396 0, pp: 293- 294.

- Erdtman, G., 1971. Pollen Morphology and Plant Taxonomy Angiosperms. Vol. 2. Publishing Company, New York, USA
- Firoznia, A., A. Rustalyan, M. Nadimi, S. Masoudi and M. Bigdeli, 2005. Composition of the essential oil of *Hymenocrater calycinus* (Boiss) Benth. From Iran. J. Essential Oil Res., 17: 527-529.
- Groshkova, S.G., 1954. Flora of USSR (Labiatae). In: Izdatles Tvo Akademii Naus SSSR. Yuzopchuk, S.V. and B.K. Shishkin (Eds.). Vol. 20. Moskva-Leningrad, ISBN: 3-87429-240-1, pp: 328-330.
- Hedge, L., 1990. Labiatae. In: Flora of Pakistan (Labiatae), Ali, S.I. and Y.J. Nassir (Eds). Vol. 192. Printed at Bcc and Press, University of Karachi, pp: 131-132.
- Jalas, J., 1972. *Thymus* L. In: Flora Europaea, (Diapensiaceae-Myoporaceae). Tutin *et al.* (Eds.). Vol. 3. Cambridge University Press, Cambridge, ISBN: 521 08489XS, pp: 126.
- Johnson, G.A., 1940. Plant Microtechnique. 2nd Edn., McGraw Hill Company Inc., New York, pp: 126-180.
- Kaya, A., F. Satil, K.H.C. Bafler and G. Tumen, 2000. Morphological and anatomical study on *Cyclotrichum origani* (Labiatae). Turk. J. Bot., 24: 273-278.
- Metcalf, C.R. and L. Chal, 1983. Anatomy of the Dicotyledons. Vol. 2, Calderon Press, Oxford, ISBN: 0-19- 854559-2.
- Moon, H. and S. Hong, 2003. Pollen morphology of the genus *Lycopus* (Lamiaceae). Ann. Bot. Fennici., 40: 191-198.
- Moore, P.D., J.A. Webb and M.E. Collinson, 1991. Pollen Analysis. 2nd Edn., Black Well Scientific Publication Oxford, London, ISBN: 0 86542-895 6, pp: 39-82.
- Naghbi, F., M. Mosaddegh, S.M. Motamed and A. Ghorbani, 2005. Labiatae family in folk medicine in Iran, from ethnobotany to pharmacology. Iran. J. Pharmaceutical Res., 2: 63-79.
- Parsa, A., 1949. Flore de Iran. Vol. 4., Publication du Ministere De L Education: Museum D, Histoire Naturelle De Tehran, pp: 718-728 (In Latin).
- Punt, W., S. Blackmore, S. Nilsson and A. Le Thomas, 1994. Glossary of pollen and spore Terminology. Series. No. 1. Utrecht. LPP Foundation
- Rechinger, K.H. 1982. Flora Iranica (Labiatae). Vol. 150., Subtitle: Labiatae. Akademische Druck- u. Verlagsanstalt, Graz, Austria, ISBN: 3-201-00728-5, pp: 1-250 (In Latin).
- Satil, F., U. Murat and E. Hopa, 2007. Comparative morphological and anatomical studies of *Hymenocrater bitumonosus* Fisch and Mey (Lamiaceae) in Turkey. Turk. J. Bot., 31: 269-275.
- Zadidi, M.A. and S.A. Crow, 2005. Biologically active traditional medicinal herbs from Balochistan, Pakistan. Ethnol. Pharmacol., 96: 331-334.