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Macro and Micromorphological Studies on Seven Species of *Heliotropium* L. (Boraginaceae Juss.) in South West of Saudi Arabia

Wael Taha Kasem[1,2]

[1]Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt
[2]Biology Department, Faculty of Science, Jazan University, Jazan, KSA
Email: wael_kasm@yahoo.com

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Abstract

Genus *Heliotropium* L. (Heliotropiaceae) in south west of Saudi Arabia has been studied; seven species of *H. arbainense* Fresen, *H. longiflorum* (D.C.) Jauber & Spach, *H. petrocarpum* Hockst & Steud, *H. strigosium* Willd, *H. zeylanicum* Lam., *H. jizanense* Al-Turki and *H. lasiocarpum* Fisch were collected, recognized, typed and nomenclatured. The most valuable characters were those of macro and distinct micromorphological data such as stomata, hairs, pollen grains and stem anatomy. Light microscopy has been used in these studies. From the obtained results, trichomes and pollen grains data in addition to stem anatomy indicated good taxonomic tools to differentiate between species of this genus. Different traditional measurements were strikingly also helpful for the discrimination of species. *H. longiflorum* was characterized by distinctive data such as prominent anatomical information, P/E (1.8 µm) in addition to presence of spindle hair which in turn can be separated as a taxonomic level. Also, the results revealed a complete affinity between the two species of *H. jizanense* and *H. lasiocarpum*. An artificial key is provided for the differentiation between the studied species.

Keywords

***Heliotropium* L., Leaf Venation, Stomata, Trichomes, Pollen Grains, Stem Anatomy**

1. Introduction

Heliotropium L. related to Heliotropiaceae Schrad., which was included in Boraginaceae Juss. It considered pa-

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raphyletic taxon [1] [2]. Now Heliotropiaceae deals as a separate family [3]. *Heliotropium* is selected for its importance and strong resemblances on the morphological characters. The infrageneric classification of *Heliotropium* into sections has been a debatable matter. Recently, Olwey [4] mentioned this genus divided into Section Heliotropium, Section Orthostachys, Section Pleurolasia, Section Pseudocoelomae, Section Pterotropium, Section Ruditotheca and Section Zeylanica. There are 10 species of genus *Heliotropium* distributed in Jazan of Saudi Arabia [5] [6]. Distribution and forms of trichomes are important qualities to be used to differentiate between the different genera and species of plant [7]. Leaf epidermal characters are useful in systematic phylogeny of several plant taxa and can be employed as useful taxonomic characters in segregating the major groups of plants [8]-[10]. [11] investigated leaf anatomy and distribution of foliar trichrome of four *Heliotropium* species which have been investigated in Saudi Arabia. On the other hand, [12] carried out a comparison between the anatomical leaves and stems structure of five species of genus *Heliotropium* in Sudan. [13] reported the occurrence of tricolpate pollen grain in *H. vellosum* and *H. indicum*. Genus *Heliotropium* is characterized by its striking similarity on morphological characters, so this study aims to evaluate the systematic relationships of their similarity and dissimilarity compared to the modern findings of taxonomic relationships of species within the genus.

2. Materials and Methods

Fresh samples of the seven species of the genus *Heliotropium* were collected from different localities of Jazan. The plant specimens were identified according to [5] [6] Ten sample epidermal strips were removed from lower surface of the leaves were; these were fixed in 20% glycerine. The slides were examined under the light microscope at 40×. Various anatomical features, such as number and type of stomata, length and width of stomata, trichomes form and types were studied. The size of stomata and hair were recorded with the help of a calibrated eyepiece. The stomatal index (S.I.) was calculated using the formula adopted from [14] that is:

$$SI = S/S + E \times 100$$

where S is the number of stomata per unit of area, and E the number of epidermal cells in the same area. Pollen grains from fresh plants were collected on a slide and acetolyzed according to the method of [13]. Acetolyzed pollen grains were photographed by light microscope. For anatomical studies, stem cross sections were done using freshly-collected material or material fixed in ethanol, this material was cut free hand or in a semi rotative microtome to make semi-permanent and permanent slides for the microscopic slides, carried out according to the usual Johansen's methods [15].

3. Results and Discussion

3.1. Leaf and Inflorescence Morphology

Some distinctive characters such as inflorescence length, leaf shape and venation have been studied and summarized in **Table 1**.

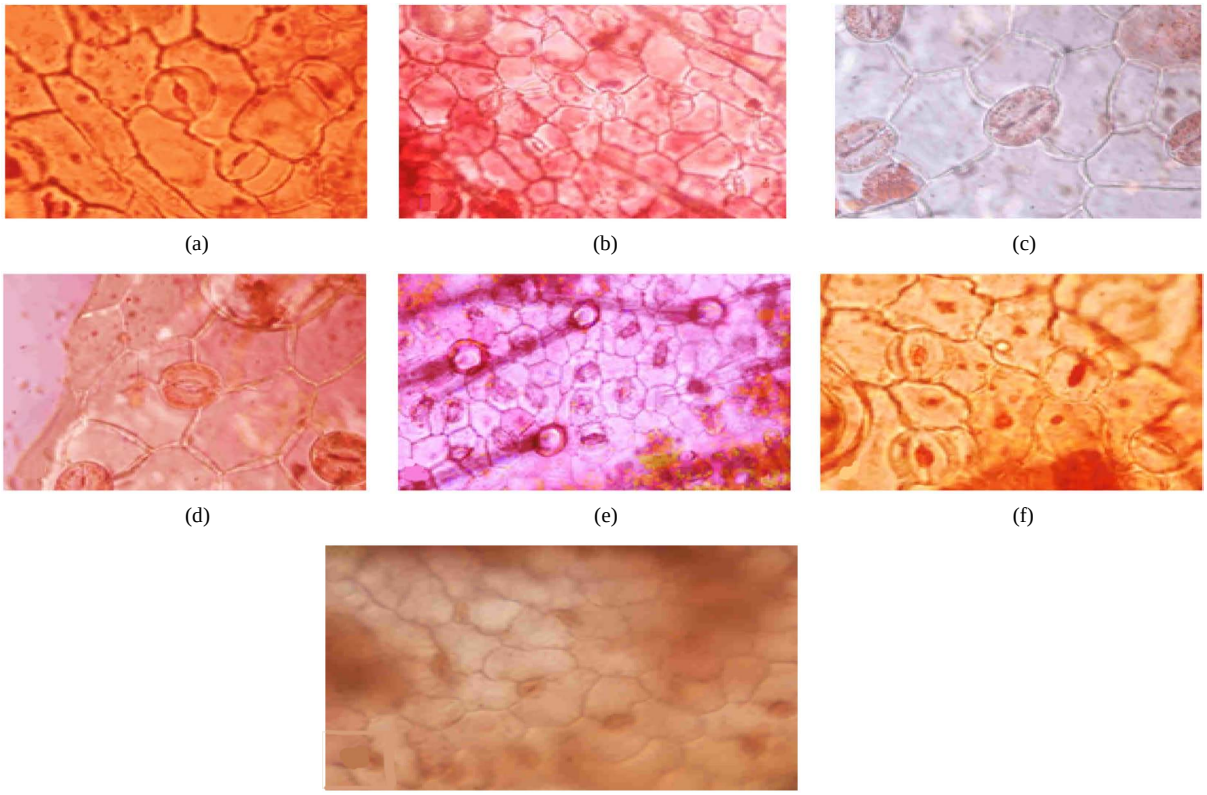
According to the terminology of [16] [17], there are two types of venation found: Hyphodromous and Brochidodromous.

3.2. Stomatal Behavior

The qualitative and quantitative leaf epidermal features of the taxa and their frequency of stomatal indexes are presented in **Table 2** and **Figure 1**. Amphistomatic stomata are common in all the studied taxa. Their ranunculus distributed on the adaxial and the abaxial surface of the leaves. The stomatal length, width and frequency also varied. The maximum stomatal length and pore size were observed in *H. petrocarpum* (19 mm) followed by *H. strigosium* being 17 mm and the minimum stomata length was found in *H. zeylanicum* (7 mm) followed by *H. longiflorium* (9 mm). Three types of stomata were recognized; anomocytic type is common in the tree species: *H. petrocarpum*, *H. strigosium* and *H. zeylanicum*. Paracytic type was found only in *H. arbainense*. Anisocytic types were noticed in *H. longiflorum* and *H. lasiocarpum*. Stomatal index differed between the taxa, where the highest value of 44.87% was calculated for *H. jizanense* while the lowest index (28.57%) was noticed in *H. longiflorum*.

3.3. Epidermal Trichromes

Unicellular epidermal trichromes have been watched on both the surface of stem and leaves. Also, glandular



(a) (b) (c)

(d) (e) (f)

(g)

Figure 1. Photographical images of epidermal cells showing the stomata types (x = 400). (a) *H. arbainense* (anisocytic); (b) *H. longiflorium* (paracytic); (c) *H. petrocarpum* (anomocytic); (d) *H. strigosium* (anomocytic); (e) *H. zeylanicum* (*anomocytic*); (f) *H. jizanense* (*anisocytic*); (g) *H. lasiocarpum* (*anisocytic*).

Table 1. Leaf and inflorescence measurements between the studied species.

No.	Data / Species	Leaf venation	leaf measurements			Inflorescence measurements	
			Length (cm)	Width (cm)	Leaf form	Length	Color
1	***H. arbainense***	Brochidodromous	2.0 - 3.0	2.0 - 3.0	ovate	0.8 - 1.0	Yellow
2	***H. longiflorum***	Hyphodromous	2.5 - 4.5	1.0 - 2.5	linear	2.0 - 4.0	White
3	***H. petrocarpum***	Hyphodromous	2.0 - 4.0	2.0 - 3.2	lanceolate	1.0 - 2.0	White
4	***H. strigosium***	Hyphodromous	1.9 - 2.9	0.8 - 1.2	linear	1.0 - 2.0	White
5	***H. zeylanicum***	Hyphodromous	3.0 - 4.0	0.5 - 2.5	lanceolate	4.0 - 6.0	White
6	***H. jizanense***	Hyphodromous	1.5 - 2.6	0.8 - 1.1	elliptic	2.0 - 3.0	White
7	***H. lasiocarpum***	Brochidodromous	2.0 - 2.1	0.3 - 0.5	ovate	1.0 - 2.0	White

Table 2. Stomata measurements and stomata indices for the species.

Data / Species	Epidermal No	Stomata No.	Stomata types	Subsidiary cells	Stomata length (mm)	Stomata width (mm)	Stomata index (%)
H. arbainense	20	12	Paracytic	Teteacytic	10	5	28.57
H. longiflorum	35	14	Anisocytic	Actinocytic	9	6	36.00
H. petrocarpum	32	18	Anomocytic	Teteacytic	19	17	41.66
H. strigosium	32	22	Anomocytic	Teteacytic	17	13	40.70
H. zeylanicum	28	20	Anomocytic	Tetracytic	7	5	37,50
H. jizanense	43	35	Anisocytic	Teteacytic	13	11	39.09
H. lasiocarpum	53	34	Anisocytic	Teteacytic	15	12	44.87

hairs were found in all the seven studied taxa. Basal hair form and vestibule differed between the taxa where hollow appeared in *H. arbainense*, *H. peterocarpum* and *H. jizanense*. Spindle hair form was found only in *H. longicarpum*; density, type, basal cell and their walls are presented in **Table 3** and photographed in **Figure 2**.

Densely hairy with filled basal cells was found in *H. lasiocarpum*, *H. strigosum* and *H. zeylanicum* hairs with rough walls.

3.4. Pollen Grain Characters

Pollen grains types form and different measurements are recorded in **Table 4**. Tricolpate pollen grains types were noticed in all the species (**Figure 3**). Oval form was found in *H. longiflorum*. Circular was found in

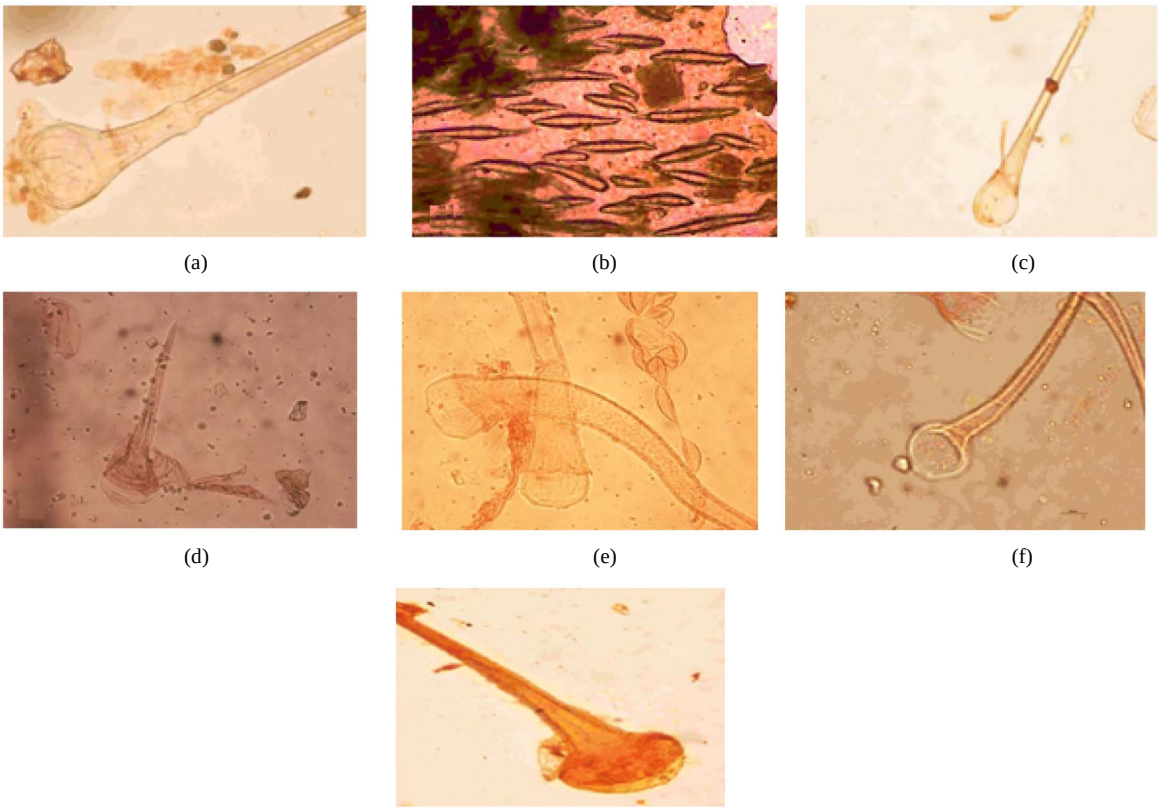


Figure 2. Light microscopic images (X = 100) showing hollow and filled vestibule basal hair in the studied species. (a) *H. arbainense*; (b) *H. longiflorium* (paracytic); (c) *H. petrocarpum*; (d) *H. strigosium*; (e) *H. zeylanicum*; (f) *H. jizanense*; (g) *H. lasiocarpum*.

Table 3. Hair leaves measurements for the seven studied species.

Species \ Data	Hair density	Hair wall	Hair vestibule	Basal form
H. arbainense	Dense	Smooth	Hollow	Oval
H. longiflorum	Dense	Rough	Absent	Rounded
H. petrocarpum	Low	Smooth	Hollow	Oval
H. strigosium	Dense	Rough	Filled	Oval
H. zeylanicum	Low	Rough	Filled	Globose
H. jizanense	Low	Smooth	Hollow	Circular
H. lasiocarpum	Low	Rough	Filled	Absent

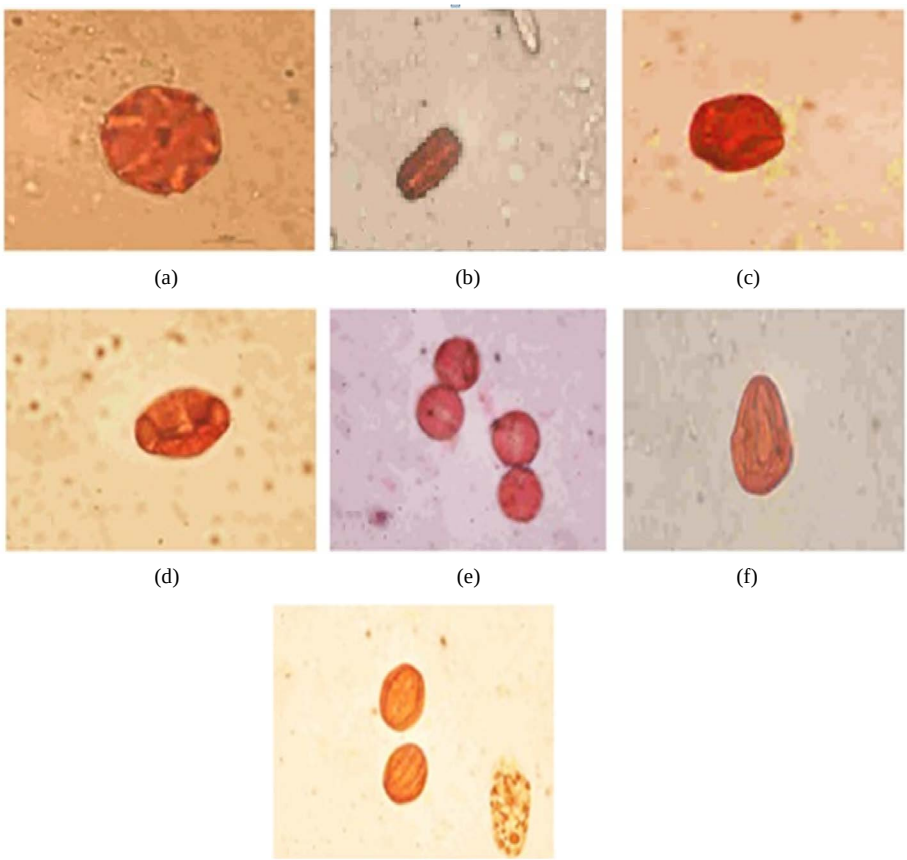


Figure 3. Equatorial view by light microscopic images (X = 1000) of oval form in *H. longiflorum*, rectangula in *H. arbainense* and *H. petrocarpum*, elliptical in *H. strigosium*, rounded in *H. zeylanicum* and triangular in *H. jizanense*

Table 4. Pollen grain measurements for the seven studied species.

Data / Species	Pollen length (µm)	Pollen width (µm)	Pollen apertures (µm)	P/E ratio (µm)	Pollen form
H. arbainense	28	20	0.8	0.70	Circular
H. longiflorum	20	11	0.7	1.81	Circular
H. petrocarpum	25	22	0.5	1.13	Circular
H. strigosium	27	16	0.3	1.68	Elliptical
H. zeylanicum	32	28	0.4	1.14	Rounded
H. jizanense	41	35	0.9	1.17	Triangular
H. lasiocarpum	38	31	0.8	1.22	Oval

H. arbainense and *H. petrocarpum.* Elliptical form was noticed in *H. strigosium*. Rounded form was recorded in *H. zeylanicum*, and also triangular was noticed in *H. jizanense*. The pollen length and aperture size also varied between the taxa. The maximum pollen length and width (P/E) of 1.81 µm were recorded in *H. longiflorum* followed by 1.68 found in *H. strigosium.* The lowest P/E was noticed in *H. arbainense* (0.70 µm).

3.5. Stem Anatomical Data

Stem anatomical characters and their measurements between the studied species are recorded in **Table 5** and stem photographs are listed in **Figure 4**. Epidermis in all species consists in a single layer. All taxa except *H. pterocarpum* have two layers of cubical or rounded cells covering with trichromes. Hairs and other epidermal outgrowths have been noticed in all species; the hypodermis consists of elongated chlorenchyma cells in *H.*

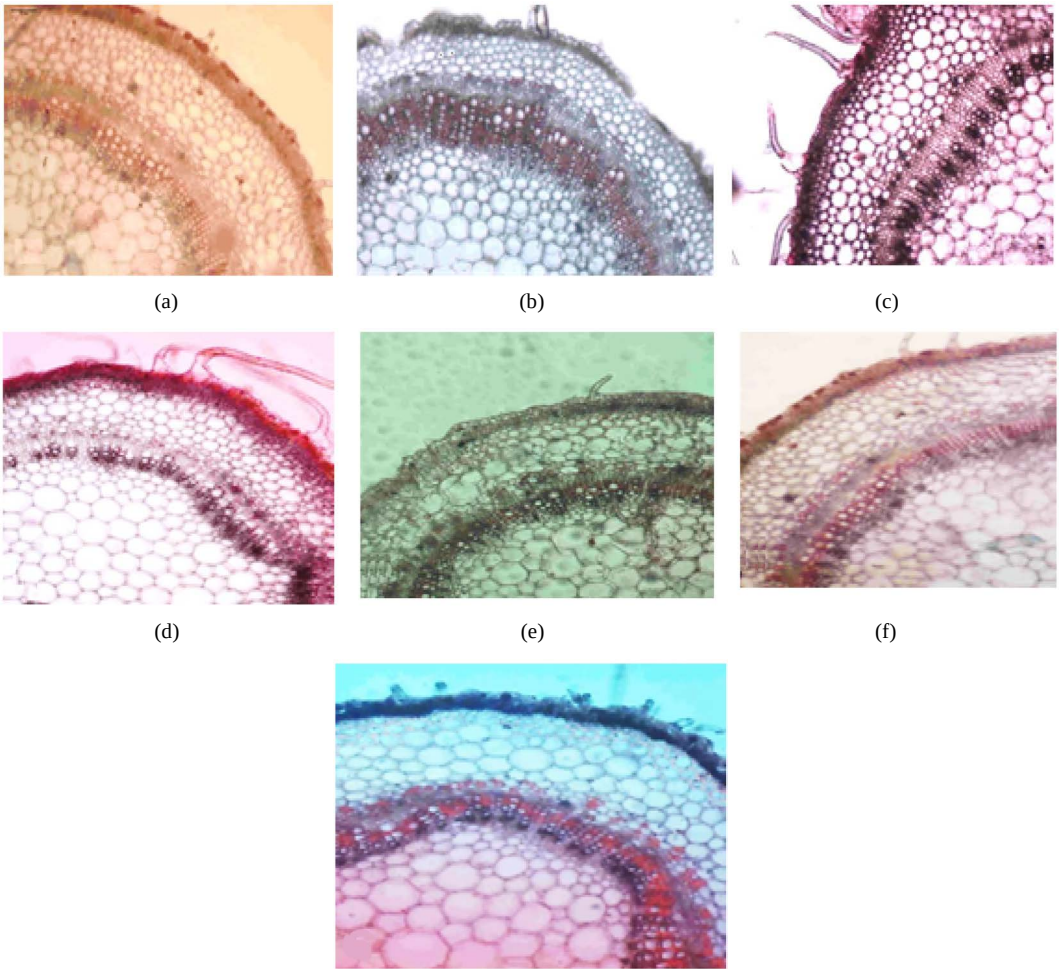


Figure 4. Light microscopic micrograph (X = 100). (a) *H. arbainense*; (b) *H. longiflorium*; (c) *H. petrocarpum*; (d) *H. strigosium*; (e) *H. zeylanicum*; (f) *H. jizanense*; (g) *H. lasiocarpum*.

Table 5. Anatomical measurements between the studied species.

Data / Species	Epidermal layers	Hypoderm layers	Cortex cells	Vessels clusters	Wood porous	Pith cells
H. arbainense	one	One, elongated	Angular	Two groups	Diffuse	Rounded
H. longiflorum	one	Two, elongated	Normal	Three groups	Diffuse	Rounded
H. petrocarpum	two	One, elongated	Normal	two groups	Diffuse	Rounded
H. strigosium	one	Two, elongated	Lacunar	Three groups	Un-diffuse	oval
H. zeylanicum	one	Three, rounded	Normal	Three groups	Diffuse	Rounded
H. jizanense	two	One, elongated	Angular	Three group	Diffuse	oval
H. lasiocarpum	one	One, elongated	circular	Three group	Diffuse	rounded

arbainense, *H. pterocarpum* and *H. zeylanicum*. Two layers of chlorenchyma cells were observed in *H. longiflorum* and *H. strigosium.* Three layers of parenchyma cells were found only in *H. jizanense.* The cortex was found in angular with 4 - 5 layers of collenchyma cells which were present in *H. arbainense* and *H. jizanense.* 5 - 6 layers of lacunar collenchyma cells were found in *H. strigosium.* A normal collenchyma cell was found in *H. longiflorum*, *H. petrocarpum* and *H. zeylanicum.* Vascular strands in all the taxa studied are separated by paren-

chymatous cells which are smaller in size than cortex or pith. Vessels are scattered and clustered into two groups in *H. arbainense* and *H. petrocarpum* or three groups in *H. longiflorum*, *H. strigosium*, *H. zeylanicum* and *H. jizanense*. In the old stem sections continuous ring of fascicular and iterfascicular cambium has been observed in all the studied species. Primary phloem appears as a dark staining mass in *H. longiflorium* and *H. strigosium*. Wood vessels are scattered and clustered into two groups in *H. arbainense* and *H. petrocarpum* or three groups in *H. longiflorum*, *H. zeylanicum* and *H. jizanense*. In general, leaf venation varied between the species, brochidodromous noticed in *H. arbainense* and *H. lasiocarpum* while the remainders have hyphodromous type. Such results are similar to report of [3]. It is obvious that, the stomatal index varied between the studied species in turn in accordance with the results of [18]. Apart from hairs, emergences and glandular hairs have been noted previously by different authors. Glandular hairs with multicellular stalk have been observed in all the seven species. Hairs vary from species to other which in turn indicated at significant taxonomic data; basal hair form appeared hollow in *H. arbainense*, *H. peterocarpum* and *H. jizanense*. Spindle shape was found only in *H. longiflorum* with rough hair wall which differed from the previous studies. Also, pollen grains indicated good taxonomic data which differed in form and pollen apertures. Such results are in agreement with [19]. Also, the pollen length and apertures size also varied between the taxa studied. *H. longiflorum* has distinct anatomical data which support data obtaining pollen grains in which colporate pollen aperture is P/E (1.8 µm) in addition to presence of hair spindle form. The hypodermis showed variation and accordingly *H. zeylanicum* was separated from the other studied species having 2 layers of chlorenchyma cells whereas the remainders species have 1 - 2 layers of parenchyma cells. Vessel forms, number and wood porous differed between the taxa. Wood porous found in all taxa except *H. strogosium* has unporous xylem form. Such results differed with results of [12]. On the other hand, *H. longiflorum* is characterized by distinctive data which in turn can be separated as a taxonomic level; the following key shows the possibility of using both the previous characters for identification of the studied *Heliotropium* species:

1a.	Flowers bracteates, weakly scorpoid form, with spindle hairs	5. *H. longiflorum*
1b.	Flowers bracteates, rarely scorpioid cymose, with elliptical pollen grains	*H. strigosum*
1c.	Flowers unbracteate	2
2a.	Calyx lobes confined, heavy hairs, 4 - 5 mm	*H. pterocarpum*
2b.	Calyx lobes free, have little hairs or not hairy	3
3a.	Plants annual, nutlets glabrous	5
3b.	Plants perennial, nutlets hairy	4
4a.	Nutlets not winged; stigma abruptly narrowed, filiform	*H. lasiocarpum*
4b.	Nutlets shortly winged; stigma conical	*H. jizanense*
5a.	Corolla green; inflorescence up to 15 - 20 cm long	1
5b.	Corolla yellow or white, inflorescence less than 12 cm long	6
6a.	Stigma sessile; flowers white, wood vessels clustered in two groups	*H. arbainense*
6b.	Stigma narrowly elongate; flowers yellow, wood vessels clustered in three groups	*H. zeylanicum*

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