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ANATOMICAL AND MICROMORPHOLOGICAL STUDIES ON SEVEN SPECIES OF *HELIOTROPIUM* L (BORAGINACEAE JUSS.) IN SOUTH OF SAUDI ARABIA

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ABSTRACT: *The genus Heliotropium L. (Heliotropiaceae) in south Saudi Arabia has been studied taxonomically; seven species were collected, recognized, typed and nomenclatured. The most valuable taxonomic characters were those of distinct micromorphological data such as leave venation, stomata, hairs, pollen grains and stem anatomy. Light microscopies have been used in these investigations. Trichomes and pollen grain data in addition stem anatomy indicated a good taxonomic tool in differentiation between species of this genus. The resulted data have been treated numerically using the cluster analysis method of unweighed pair group (UPGMA). Traditional measurements also helpful for the discrimination of species strikingly; the electrophoregram gives H. longiflorum in a taxonomic separated level which in turn i suggest this species may be treated as separated species. H. longiflorum characterized with prominent anatomical information, P/E (1.8µm) in addition to presence of spindle hair form. On the other hand, each studied species are appeared at separated taxonomical levels which support to the idea that genus Heliotropium characterized with a distinct data which in turn his special recipes in family Boraginaceae.*

KEYWORDS: *Heliotropium* L., Leaf venation, Stomata, Trichomes, Pollen grain, Stem anatomy.

INTRODUCTION

Heliotropium L. related to Heliotropiaceae Schrad. which included in Boraginaceae Juss. It considered paraphyletic taxon (Chase., 1993 and Ferguson, 1999)). Now Heliotropiaceae deal as a separate family (Nadia Diane, *et al*., 2002). *Heliotropium* is selected for its importance and their strong resemblances on the morphological characters. The infrageneric classification of *Heliotropium* into sections has been a debatable matter; early DeCandolle (1845) divided Heliotropium into four sections and excluded the genus *Heliophytum*. Recently Asmaa Olwey (2014) mention this genus divided to Section Heliotropium, Section Orthostachys, Section Pleurolasia, Section Pseudocoelomae, Section Pterotropium, Section Ruditotheca and Section Zeylanica. In Saudi Arabia, the genus *Heliotropium* comprises 10 species distributed Alfarhan *et al*. 2005 and Masrahi (2012). Distribution and forms of trichomes of important qualities are used to differentiate between the different genera and species of plant (Metcalfe and Chalk, 1950). Leaf epidermal characters are useful in systematic and phylogeny of several plant taxa and can be employed as useful taxonomic characters in segregating the major groups of plants (Mbagwu *et al.*, 2007; Ayodele and Zhou, 2008 and Albert and Sherma, 2013). Leaf anatomy and distribution of foliar trichrome of four *Heliotropium* species have been investigated in Saudi Arabia by Mona Alwahibi and Najat Bukhary (2013). A comparison between the anatomical leaves and stems structure of five species of genus *Heliotropium* in Sudan were carried out by Hoyam Osman and Maha Kordofani (2012). Erdtman (1952) reported the occurrence of tricolpate pollen grain in *H. vellosum* and *H. indicum.* The aim of this study is to

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evaluation the systematic relationships of their similarity and dissimilarity compared to the modern findings of taxonomic relationships of species within in the genus.

MATERIAL AND METHODS

Fresh samples of the seven species of the genus *Heliotropium* were collected from different localities of South of Saudi Arabia. The plant specimens identified according to Alfarhan *et al*. (2005) and Masrahi (2012). Ten sample epidermal strips of desired lengths were removed from lower surface of the leaves; these were fixed in 20% glycerine. The slides were examined under the light microscope at 10x and 40x.Various anatomical features, such as number and type of stomata, length and width of stomata, trichomes form and types were studied. The stomata size and hairs were recorded with the help of a calibrated eyepiece. The stomatal index (S.I.) was calculated using the formula adopted from Salisbury (1972) that is:

$$SI = \frac{S}{S + E} \times 100$$

Where S donates the number of stomata per unit area and E the number of epidermal cells in the same unit area. Pollen grains from fresh plants were collected on a slide and acetolyzed according to the method of Erdtman (1960). Acetolyzed pollen grains are photographed by light microscope in Jazan university laboratory. For anatomical studies, five stem cross sections were done using freshly-collected material or material fixed in ethanol, this material was cut freehand or in a semirotative microtome to make semi-permanent and permanent slides for the microscopic slides, carried out according to the usual Johansen techniques (1940).

Numerical Analysis

All the examined specimens were used as operational taxonomic units OTU's and analyzed by means of Hierarchical Cluster analysis using Euclidean distance measuring similarity and dissimilarity percent. The relationships between the species of *Heliotropium* are illustrated as dendrogram STATISCA 6.0 program.

RESULTS AND DISCUSSION

Leaf and Inflorescence Morphology

Some distinct characters such as inflorescence length, Leaf shape and venation have been studied and summarized in Table 1.

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Table 1. Leaf and inflorescence measurements the studied taxa

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.longiflorium***	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

According to the terminology of Hickey and Wolf (1975) and Ash *et al*. (1999), two types of venation are found; Hyphodromous and Brochidodromous.

Stomatal behavior

The qualitative and quantitative leaf epidermal features of the taxa and their frequency of and stomata indexes are presented below in Table 2 and Fig. 1. Amphistomatic stomata are common in all the studied taxa. Their rananculous distributed on the adaxial and the abaxial surface of the leaves. The st length, width and and frequency also varied. The maximum stomatal length and pore size was appear in *H. petrocarpum* (19mm) followed by *H.strigosium being* 17mm and the minimum stomata length was noticed in *H. zeylanicum* (7 mm) followed by *H. longiflorium* (9 mm).

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.longiflorium	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

Three types of stomata are recognized; anomocytic type is common in the tree species; *H.petrocarpum*, *H.strigosium* and *H.zeylanicum.* paracytic type found only in *H. arbainense*. An Anisocytic type was noticed in *H.longiflorium* and *H.lasiocarpum.* Stomatal index differed between the taxa, where the highest one of 44.87 % was calculated in *H.jizanense* while the lowest index (28.57 %) was noticed in *H.longiflorium*.

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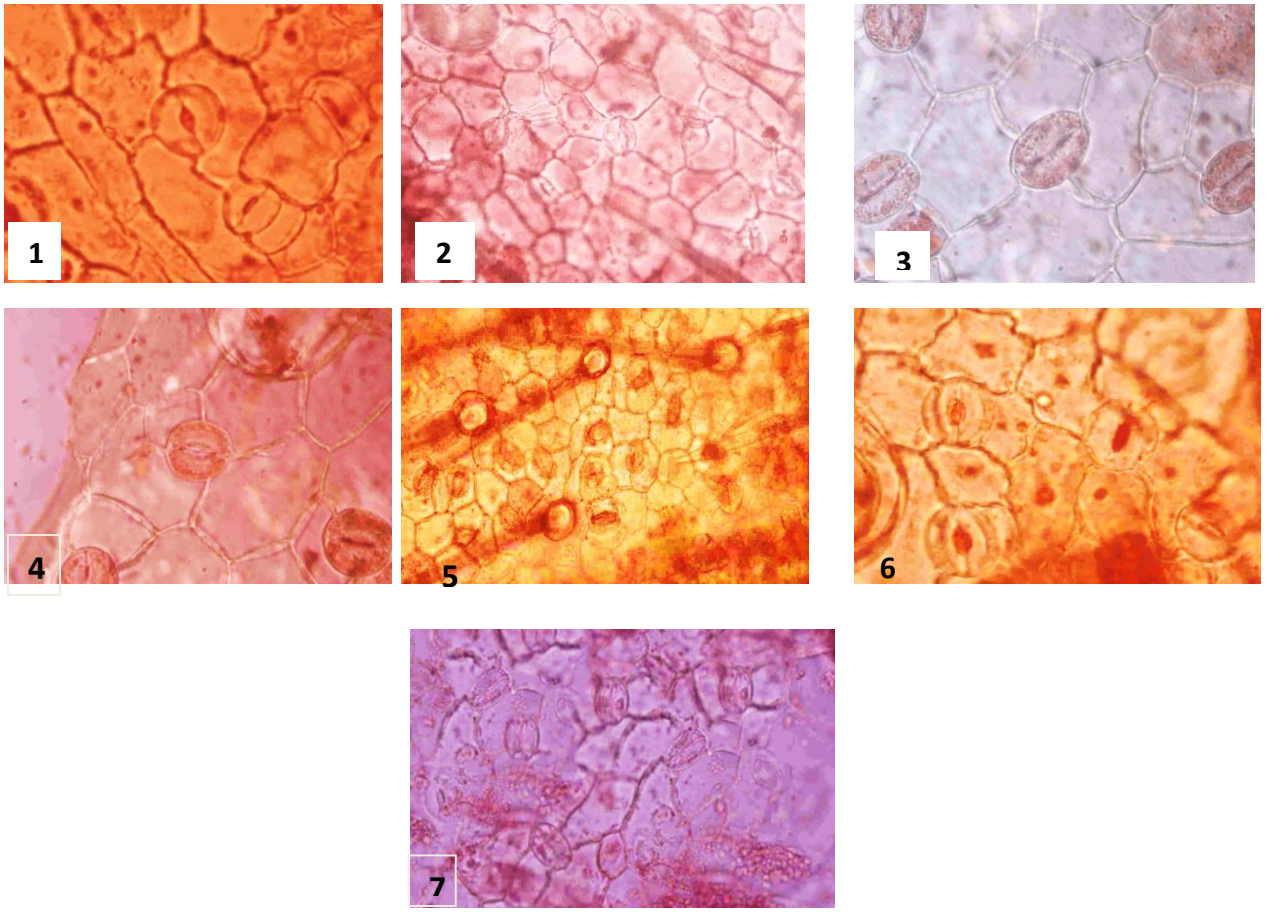

Fig 1. Photographical images of epidermal cells showing the different types of stomata (x=400)

Epidermal Trichromes

Unicellular epidermal trichromes have been watched on both the surface of stem and leaves. Also, glandular hairs found in all the seven studied taxa. Basal hair form and vestibule differed between the taxa where appeared hollow in *H. arbainense*, *H. peterocarpum* and *H. jizanense*. Spindle hair form found only on *H. longicarpum*, density, type, basal cell and their walls are presented below in Table 3 and photographed in Fig.2.

Table 3. Hair Leaves measurements for the seven studied species

Data / Species	Glandular hairs	Unicellular hairs	Hair Density	Hair wall	Hair Vestibule	Basal form
H. arbainense	present	present	dense	smooth	hollow	oval
H.longiflorium	present	present	dense	rough	absent	rounded
H.petrocarpum	present	present	low	smooth	hollow	oval
H.strigosium	present	present	dense	rough	filled	oval
H.zeylanicum	present	present	low	rough	filled	globose
H.jizanense	present	present	low	smooth	hollow	circular
H.lasiocarpum	present	present	low	rough	filled	absent

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

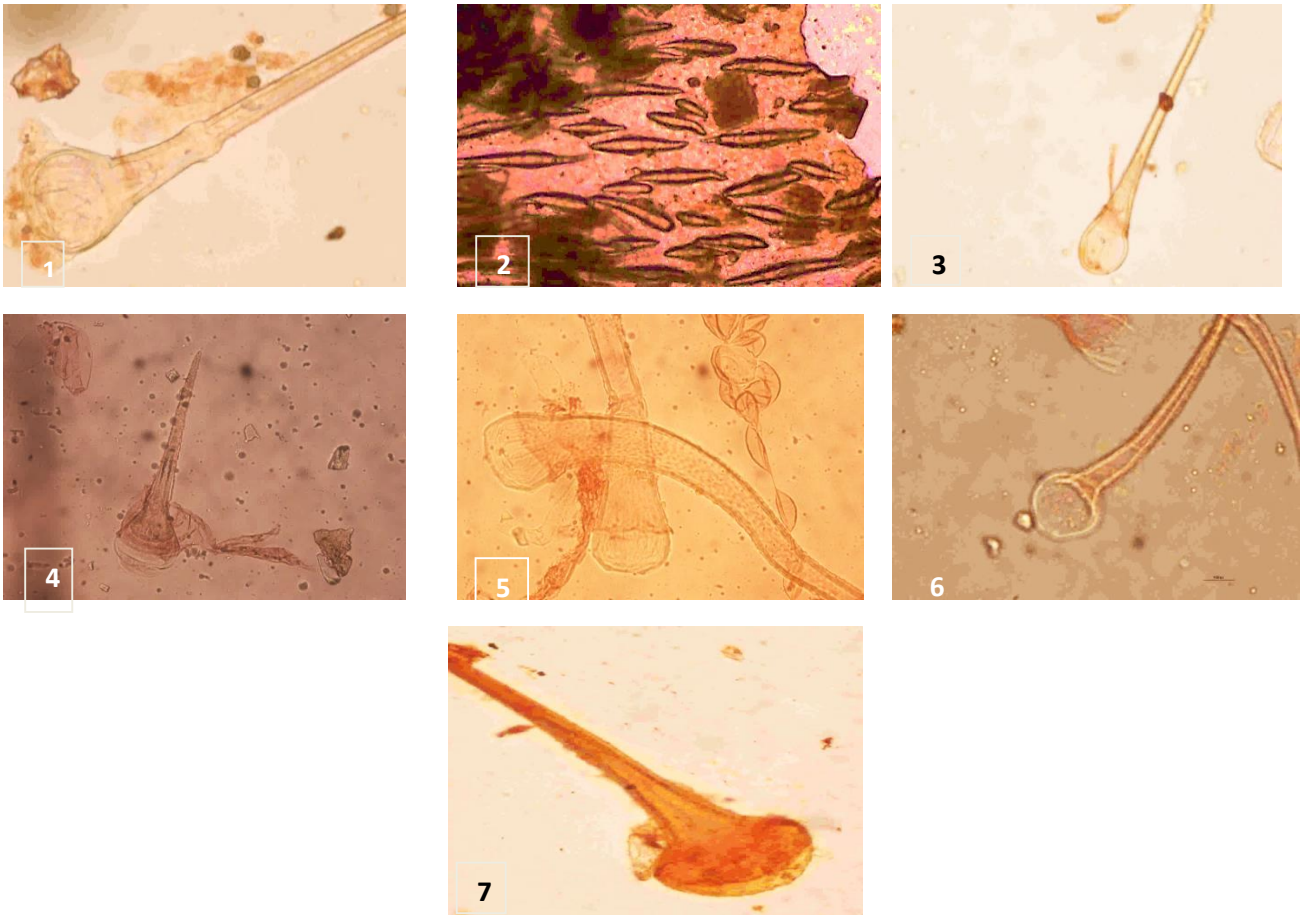

Fig 2. Photographical image of different types of Trichome (x=100)

Pollen Grain Characters

Pollen grains types form and different measurements are recorded below in Table 4. Tricolpate pollen grains type was noticed in all the studied taxa (Fig. 3). Oval form found in *H.longiflorium.* circular found in *H. arbainense* and *H.petrocarpum.* elliptical form was noticed in *H. strigosium,* rounded form was recorded in *H. zeylanicum* also, triangular was noticed in *H.jizanense*. On the other hand, the pollen length, aperture size also varied between the taxa. The maximum pollen length and width (P⁄E) of 1.81 µm was recorded in *H.longiflorium* followed by 1.68 found in *H.strigosium.* The lowest P⁄E was noticed in *H. arbainense* (0.70 µm).

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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 apertures (µm)	Pollen Form	Pollen Types
H. arbainense	28	20	0.8	0.70	0.8	circular	Tricolpate
H.longiflorium	20	11	0.7	1.81	0.7	circular	Tricolpate
H.petrocarpum	25	22	0.5	1.13	0.5	circular	Tricolpate
H.strigosium	27	16	0.3	1.68	0.3	elliptical	Tricolpate
H.zeylanicum	32	28	0.4	1.14	0.4	rounded	Tricolpate
H.jizanense	41	35	0.9	1.17	0.9	triangular	Tricolpate
H.lasiocarpum	38	31	0,8	1.22	0,8	oval	Tricolpate

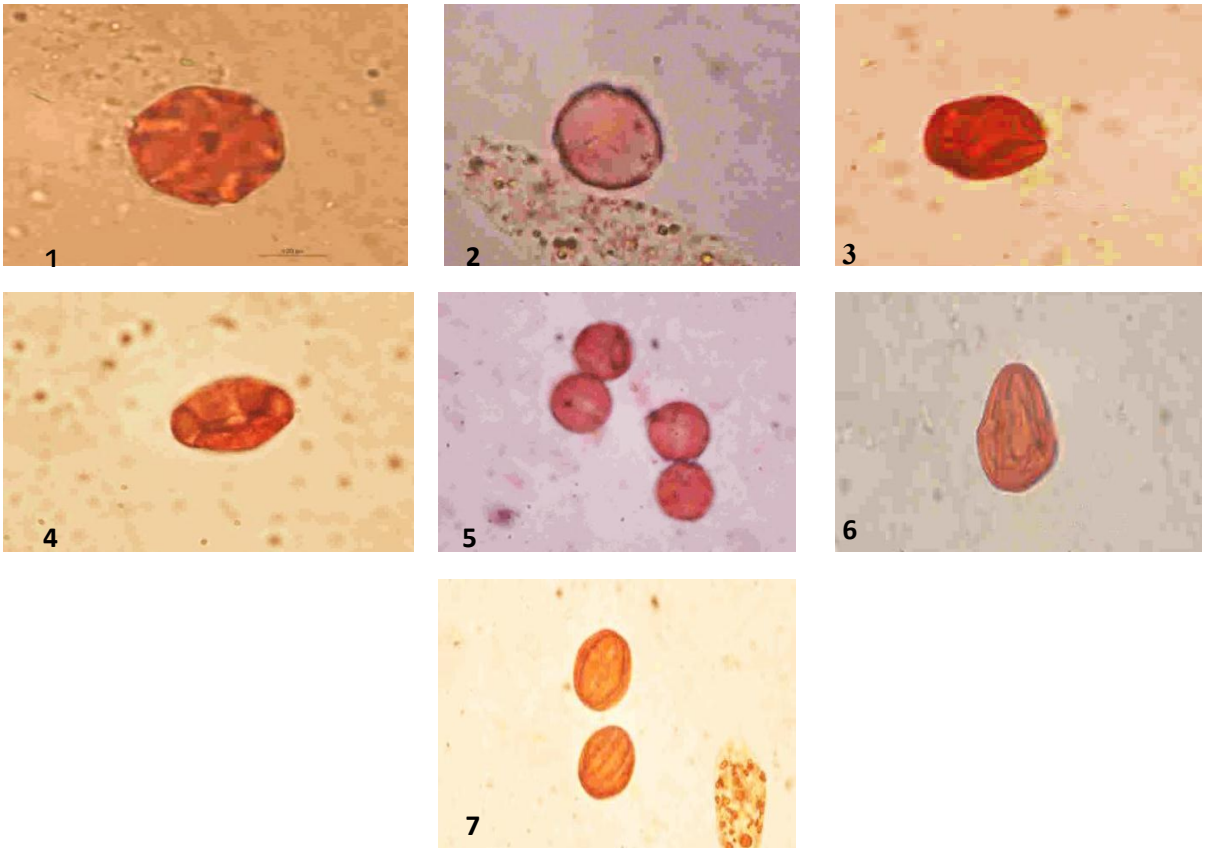

Fig 3: Photographical image of different types of pollen grains (x=1000)

Stem Anatomy

Anatomical characters and different measurements between the taxa are recorded below in Table 5. Epidermis in all taxa is consists of single layer in all taxa except *H.pterocarpum* has two layers of cubical or rounded cells covering with trichromes. Hairs occurrence and other epidermal outgrowths have been noticed in all species; the hypodermis consist of one elongated chlorenchyma cells in *H. arbainense*, *H. pterocarpum* and *H.zeylanicum*. Two layers of chloranchyma cells are in the species H. *longiflorium* and *H. strigosium.* Three layers of parenchyma cells found only *H. jizanense.* The cortex was found in angular with 4-5 layers of

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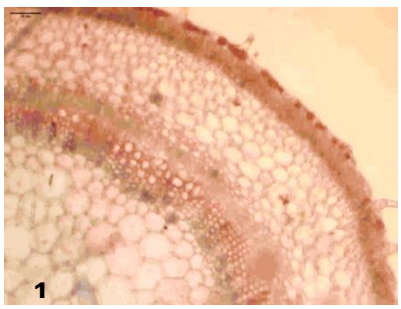
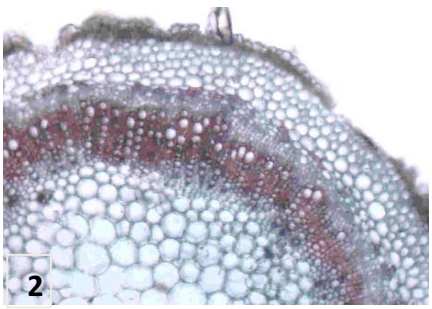
collenchymacells are present in *H. arbainense* and *H. jizanense.* 5-6 layers of lacunar collenchyma cells are found *H. strigosium.* A normal collenchyma cell was found in *H.longiflorium, H.petrocarpum* and *H.zeylanicum.*

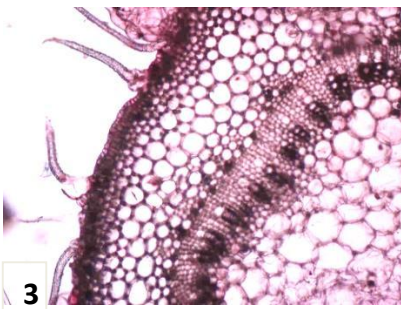
Vascular strands in all the taxa studied are separated by parenchymatous cells which are smaller in size those than cortex or pith. Vessels are scattered and clustered into two groups in *H.arbainense* and *H.petrocarpum* or three groups in *H. longiflorum, H. strigosium, H. zeylanicum* and *jizanense.* In the old stem sections continuous ring of fascicular and iterfascecular cambium has been observed in all the studied taxa which in turn gives a few vessels but mostly xylem fibers and uniseriate medulla. Primary phloem becomes appear as dark staining mass in *H.longiflorium* and *H.strigosium*. Vessels are scattered in wood and clustered into two groups in *H.arbainense* and *H.petrocarpum* or three groups in *H.longiflorium, H.zeylanicum* and *H.jizanense* (Fig. 5).

Table 5: Anatomical measurements of the different studied taxa

Data / Species	Epidermal layers	Hypoderm layers	fascicular and intra fascicular cambium	Cortex cells	Vessels clusters	Wood porous	Pith cells
H.arbainense	one	One, elongated	present	Angular	Two groups	Diffuse	Rounded
H.longiflorium	one	Two, elongated	present	Normal	Three groups	Diffuse	Rounded
H.petrocarpum	two	One, elongated	present	Normal	two groups	Diffuse	Rounded
H.strigosium	one	Two, elongated	present	Lacunar	Three groups	Diffuse	oval
H.zeylanicum	one	Three, rounded	present	Normal	Three groups	Diffuse	Rounded
H.jizanense	one	One, elongated	present	Angular	Three group	Diffuse	oval
H.lasiocarpum	one	One, elongated	present	circular	Three group	Diffuse	rounded







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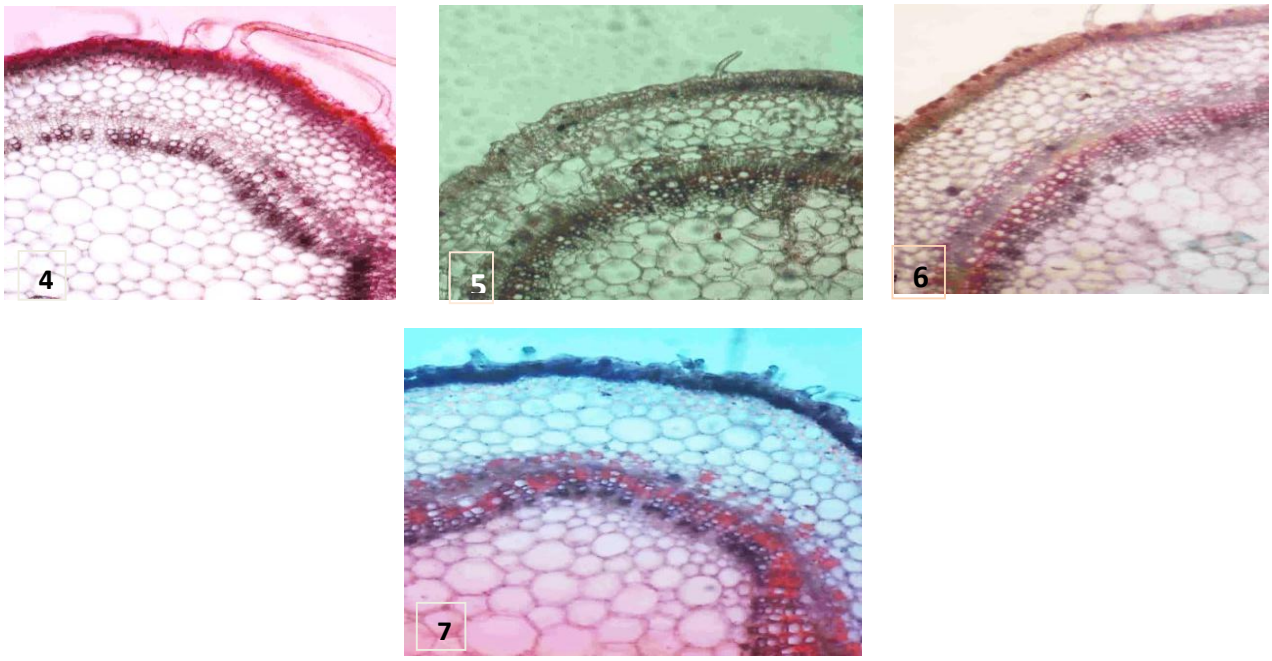

Fig 4: Photographical image different transvers sections of stem of (x=100)

Numerical Analysis

Table 6. Summarized morphological, stomatal, pollen grain, trichomes and stem anatomy between the studied taxa

Species / Used Data	*H. arba*	*H. long.*	*H. ptero.*	*H. strig.*	*H. zeyla*	*H. jizan*	*H. lasio*
I- Morphological Characters							
Leaf shape							
1-Oval, 2- lanceolate, 3- cordate	1	3	2	3	3	3	1
Leaf venation							
1-Hyphodromous, 2- Brochidodromous	1	1	1	1	1	1	2
Inflorescence Length (cm)							
1-Length≤ 0.4 cm, 2- ≥ Length 0.4	1	1	2	1	2	2	1
Flower Color							
1- White, 2- yellow	2	1	1	1	1	1	1
II- Stomatal Characters							
Types							
1-Anomocytic, 2-Anisocytic,3-Paracytic	3	2	1	1	1	1	1
Density	2	1	1	1	1	1	2
1-densely, 2- low densely							
Subsidiary cells	1	1	1	1	2	1	1
1-Tetracytic,2-Actinocytic							
Stomata measurements							
1-Stomata length range from 7-10 mm, 2-Length range from 11-19 mm	2	1	2	2	1	2	2
Anticlinal cells							

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1-wavey, 2-straight, 3-curved	1	1	2	3	1	2	2
Stomatal index (SI)							
1-ranged from 28.0% - 37.0%, 2- ranged from 38.0% - 47.0%	2	1	2	2	1	2	2
III-Trichromes characters							
Glandular hair							
1-present, 2-absent	1	1	1	1	1	1	1
Spindle hair							
1-present, 2-absent	2	1	2	2	2	2	2
Unicellular hair							
1-present, 2-absent	2	1	1	1	1	2	2
Hair density							
1-densely, 2- low densely	1	2	1	2	1	2	2
Hair Wall							
1-smooth, 2-rough	1	1	1	1	1	1	2
Hair Base							
1-Present, 2-absent	1	2	1	1	4	3	5
Hair Base form							
1-oval,2-rounded,3-circular,4-globose,	1	2	1	2	2	1	3
Base vestibule							
1-Hollow, 2-filled, 3-not found	1	3	1	2	1	1	2
IV- Pollen grain Characters							
Pollen Type							
1-colporate, 2-Non-colporate	1	2	1	1	1	1	1
Pollen Form							
1-ovate,2-circular,3-triangular,4-elliptic	2	2	2	3	2	3	1
Aperature measurements							
P/E range from 0.3-0.7µm	2	1	2	1	1	2	2
1-present,2-absent							
P/E range from 0.8-1.7µm	2	2	1	2	1	2	1
1-present,2-absent							
P/E range from $\geq$ 1.7µm							
1-present,2-absent	2	1	2	2	2	2	2
V- Anatomical characters							
Epidermal cell layer							
1-single,2- multiple	1	1	1	1	1	2	1
Hypodermal layer							
1-single,2- two, 3- three layers	1	2	3	2	1	1	1
Fascicular cambium							
1-present, 2-absent	1	1	1	1	1	1	1
Intrafascicular cambium							
1-present, 2-absent	1	1	1	1	1	1	1
Lacunar cortex cell							
1-present, 2-absent	2	2	1	2	1	2	2
Angular cortex cell	2	1	2	1	2	2	2
1-present, 2-absent							
Vessels cluster	2	2	2	2	2	1	2
1-two,2- more than	1	1	1	1	1	1	1

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Diffuse wood porous 1-present, 2-absent	1	1	1	1	1	1	1
Vessels arrange 1-present, 2-absent	1	2	2	2	1	1	2
Pith cells types 1-Oval, 2-rounded							

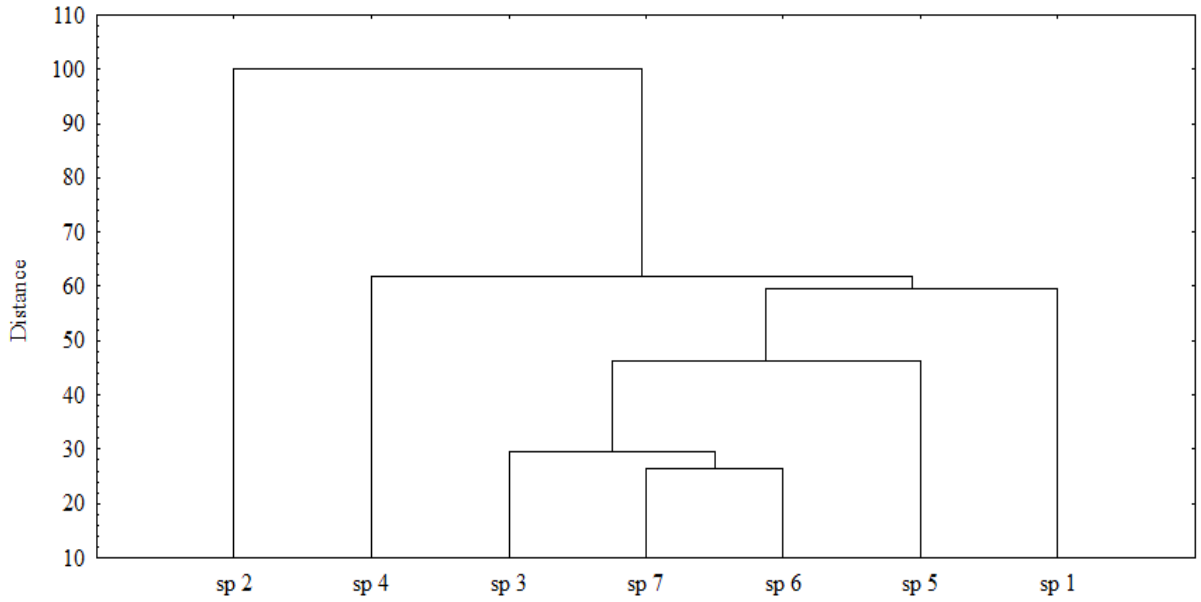

Fig 5. UPGMA- phenogram based on 34 taxonomic characters obtained from different studies for different taxa.

All previous studied characters are treated by the use of Hierarchical cluster analysis (Table 6). The dendrogram help the say that genus *Heliotropium* must be deal as a separate genus in family Boraginaceae. The dendrogram resulting from analysis divided the studied taxa into two clusters. The first included *H. longiflorum*. The remainder divided into two sub clusters, *H.strigosium* which split off other species at the taxonomic distance (dissimilarity percentage) of about 60 %. The second sub cluster included a complete affinity between *H. jizanense* and *H. lasiocarpum* which coupled with *H.pterocarpum.* On the other hand, *H. arbainense* and *zeylanicum* are treated as a separate level at taxonomic distance of 55% and 39%, respectively. In general, leaf venation varied between the taxa brochidodromous noticed in *H. arbainense and H. lasiocarpum* while the remainders have hyphodromous type. Such results similar to other reports of Diane *et al*., 2002. Although a stomatal index varied between the taxa but indicates a little significance as a taxonomic character within the species and that confirm the results of Altaf, *et al*., 2003. 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 *jizanense*. Spindle shape found only in *H.longiflorium* with rough hair wall which differed in the previous studies. On the other hand, pollen grains indicated a good taxonomic data where are differed in form and pollen apertures. Such results are in agreement with Qureshi 1985. Also, the pollen length, apertures size also varied between the taxa studied. *H. longiflorum* has

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a distinct anatomical data which support data obtained 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 in having 2 layers of chlorenchymatous cells whereas the other have 1-2 layers of parenchyma cells. Vessel forms, number and wood porous differed between the taxa.

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