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**Research Article**

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# Leaf and Stem Anatomy of five species from the genus *Heliotropium* L. (Boraginaceae) in Sudan

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**ABASTRACT**

*In this study a comparison between the anatomical structure of the leaves and stems of five species from the genus Heliotropium (H. ovalifolium, H. bacciferum, H. strigosum, H. supinum and H. sudanicum) in Sudan were carried out to outline the diagnostic characters; thus helping to identify them, to classify them using the anatomical characters. It had been found that all the species have barrel-shaped epidermis of one layer. Hairs were found in two groups:basal cells in one row with smooth wall which include (H. bacciferum and H. supinum) ,basal cells in many rows and the wall is glandular (H. ovalifolium,H. strigosum and H. sudanicum). The hypodermis consist of collenchymatous cells except H. bacciferum which have paranchymatous cells cells. Whereas the general cortex composed of paranchymatous cells. Pholem element and xylem are noted. The transverse sections of the leaves showed that the mesophyle consist of 2-5 layers of spongi cells, palisade layers varied from (1–4) layers, the main vascular bundle was one in all studied species, lateral bundles was absent in H. ovalifolium and it differs from 2 in (H.strigosum ,H. bacciferum and H. supinum) to 4 in (H. sudanicum). The stomata length and width were measured, moreover stomata indices were calculated and it was found to be similar within the genus Heliotropium.*

**Key words:** *Heliotropium*, Boraginaceae, Stem, Leaf, Epidermis, Stomata.

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## INTRODUCTION

This study is aimed to provide valuable and reliable illustrated anatomical descriptions of the leaves and stems of five species of the genus *Heliotropium* L. This genus was selected for its strong resemblances on morphological characters. The literature survey revealed that no microscopial studies were carried on it in the Sudan. Boraginacea Juss. the Borage or Forget-me-not family includes a variety of shrubs, trees, and herbs, totaling about 2,000 species in 146 genera found worldwide. Economically the family is of considerable importance; fruits of the Boraginaceae species are edible but not very tasty, a tea is made from the dried leaves, stalks and berries of *Ehretia rigida* subsp. *nervifolia*. Dried, ground root powder of *Trichodesma angustifolia;* mixed with cold water is used for diarrhea [9]. According to [5] the family was divided into 6 tribes: *Boraginoideae ,Heliotropioideae, Cordioideae, Ehretioideae, Hydrophylloideae and Lennooideae.Heliotropium* is a genus of about 14 species in Sudan .They are known for having often spiral leaves , hairs and helicoid cymose inflorescences. The anatomy of *Heliotropium* was studied by [4], [8] and[2].

## EXPERIMENTAL SECTION

Fresh specimens of the five species of the genus *Heliotropium* were collected from river Nile bank and Shambat, Faculty of Forestry, University of Khartoum.

1. **Preparation of Permanent Slides:**

The permanent slides were prepared from the leaves and stems [6]. They were segmented and fixed in formalin: glacial acetic acid: 70% (5:5:90 v/v ).Then the fixed parts of the plants were washed with distilled water and dehydrated by serial of ethyl alcohol (50% ,70%,90%and 100% respectively).The plant organs were transferred to mixture of 1:1 Cedar wood oil absolute ethyl alcohol and then in to pure cedar wood oil ,followed by a mixture of cedar wood oil and xylene ,and finally left over night in pure xylene.wax embed in was carried out in an oven adjusted at 60c where the plant organs were transferred from mixture of wax and xylene into pure wax, each change took about 20 minutes and finally into another container of pure wax. The melted wax containing the plant organs were poured into a mold cooled in water and trimmed. The wax were stuck to the wooden blocks before sectioning with a rotary microtome (Lei12 1512-west Germany), adjusted at 10-20 microns for transverse sections of stems and leaves using a brush. The ribbons of sections were collected on glass slides and cover with egg albumin to keep attached to the slides. They were left 15 minutes on a hot plate at temperature below 60ºc which is the milting point of embedding wax; to give maximum expansion of the tissue on to slides. Then the slides were left for an overnight to ensure complete dryness before staining, the staining required successive process of dewaxing, rehydration then staining and dehydration.The processes were carried out by passing the slides through a series of coupling jars containing xylene, absolute ethanol 95%,90%,70%,50% ethanol, safranine stain, 50%, 70%, 50%,95%, absolute ethanol, fast green stain, xylene at them, a drop of D.P.X (mountant ) was added to the slide before placing the cover slips. [7].The prepared slides were left to dry in an oven adjusted at 60$^o$c for the least three days.

**Microscopical Examinations**:

The prepared permanent slides were examined under the microscope (Azeiss microscope ) at X4, X10, X50, X100 magnification. From each sample 3-5 sections were examined. Photomicrographs were taken by microscope equipped with a 35 mm automatic camera. The prepared slides were used to study and to compare different anatomical structure of stems and leaves.

**2. Preparation of Temporary Slides:**

The epidermal strips of desired lengths were removed from lower surface of the leaves by conventional method, 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 ,types of trichomes and their length and width were studied .The size of the stomata and hairs were recorded with the help of a calibrated eyepiece. Ten different observations were made for each species and their mean was calculated. The stomatal index (S.I.) was calculated using the formula adopted from [3], as under: SI=X/X+Yx100 ,where's X=Number of stomata per unit leaf area, Y=Number of epidermal cells per unit leaf area.

## RESULTS

**1. Transverse sections of stem :**

The upper epidermis was a single layer of barrel-shaped dense cytoplasm and with conspicuous nuclei, it is was found as one layer in all the species. The presence of hairs and other epidermal outgrowths have been noted in all species Plates (1.1 - 1.4). The hypodermis consist of 2 layers of parenchymatous cells in the species *H .bacciferum*. In the other species it is 2-3 layers of collenchymatous cells. The cells of the general cortex consist of parenchymatous cells in 5 layers in (*H. ovalifolium* and *H. supinum*), 4 layer in *(H. bacciferum* and *H. sudanicum*), and there are 6 layers in *H. strigosum*.in which there is a procambium ring at the second internodes. The phloem elements and xylem are noted. These vascular bundle are separated by ordinary paranchymatous cells which are, smaller in size than those composing the cortex or the pith, plates (1.5-1.9).

**2. Transverse sections of leaves:**

The epidermal cells are rectangular and show the presence of an external cuticular layer .The upper epidermal cells are somewhat larger than the lower. Unicellular epidermal trichomes have been noted on both surfaces .Stomata is of anomocytic type and are distributed on the upper and lower epidermis of the eaves. Their frequency of distribution and the stomatal indices are presented in table (1). Palisade layers range from 2 to 4 in all studied

species; except *H. ovalifolium* which had 1 layer. The main vascular bundle is 1 in all studied *Heliotropium* species. The lateral vascular bundle or the lateral venis are: 1in ***(****H. ovalifolium*), 2 in ***(****H. bacciferum****,*** *H. strigossum****,*** *H. supinum*), and 4 in (*H. sudanicum*), Plates (2.1-2.5).

**Table (1). Stomata measurements and stomata indices for the seven studied species**

| Species name | Nuumber of Epiderms cell in microscope felid | No of Stomata in microscope felid | Stomata index (mm) | Stomata length (mm) | Stomata width(mm) |
|---|---|---|---|---|---|
| *H.ovalifolium* | 0.045 | 0.03 | 250.0 | 0.023 | 0.011 |
| *H.bacciferum* | 0.105 | 0.048 | 318.75 | 0.016 | 0.008 |
| *H. strigosum* | 0.06 | 0.015 | 500.0 | 0.021 | 0.019 |
| *H. supinum* | 0.066 | 0.075 | 188.0 | 0.029 | 0.012 |
| *H. sudanicum* | 0.078 | 0.045 | 273.33 | 0.015 | 0.010 |

**Plate (1.1):stem hairs of *H.ovalifolium***

**Plate (1.2):stem hairs of** *H.strigosum.*

**Plate (1.3):stem hairs of *H. supinum***

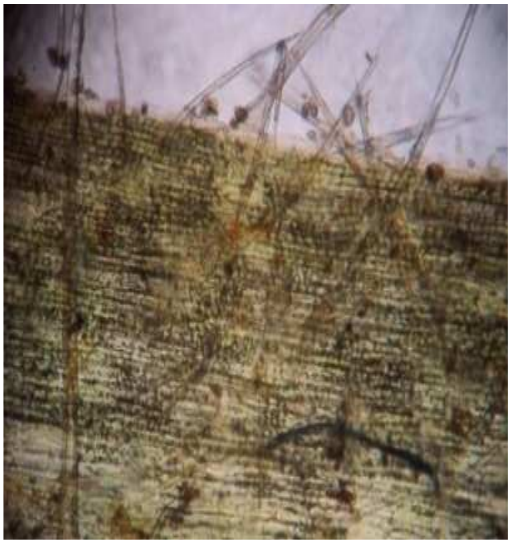


**Plate (1.4):stem hairs of *H. sudanicum*.**

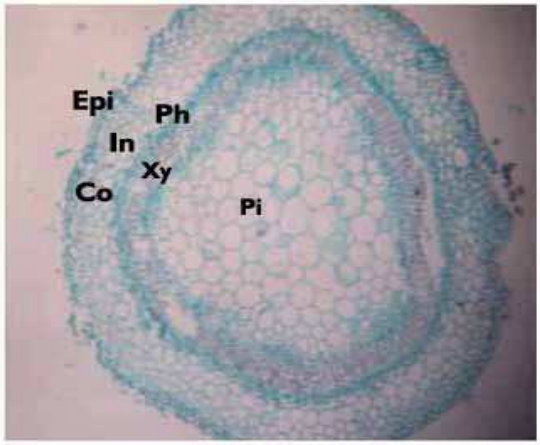



**Plate (1.5):T.S of stem of *H. ovalifolium*.**

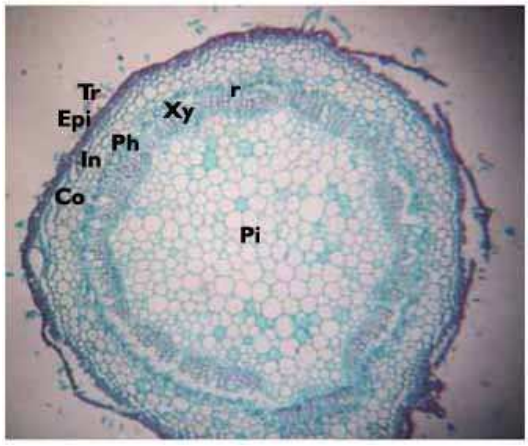



**Plate (1.6):T.S of stem of *H. bacciferum***

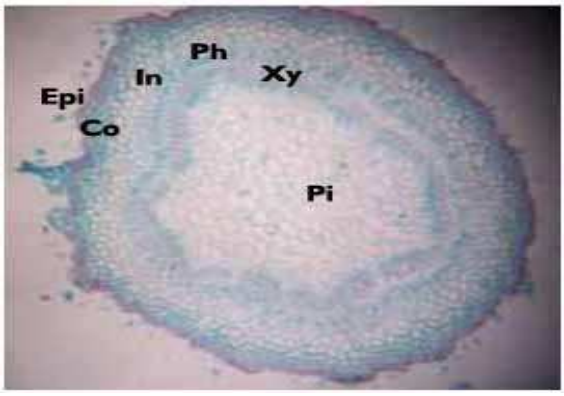



**plate (1.7):T.S of stem of *H. strigosum***

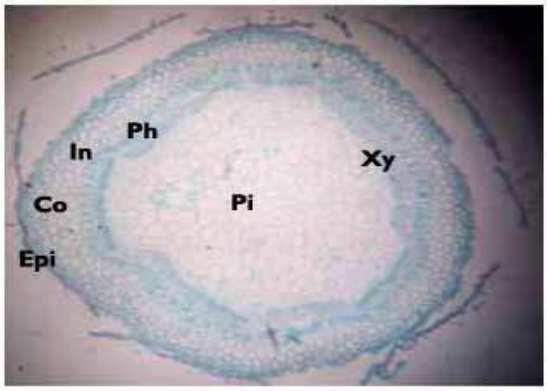



**Plate (1.8):T.S of stem of *H. supinum***

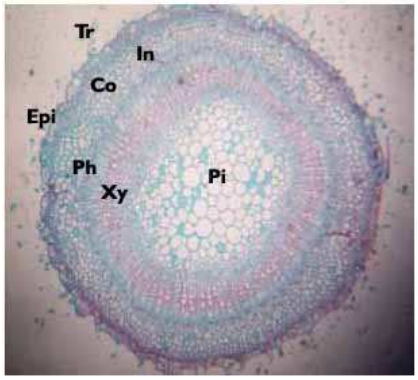



**Plate (1.9):T.S of stem of *H. sudanicum.***

*tr = trichome; epi = co = cortex; in = indodermis; ph = phloem; xy = xylem; r = rays, pi= pith.*

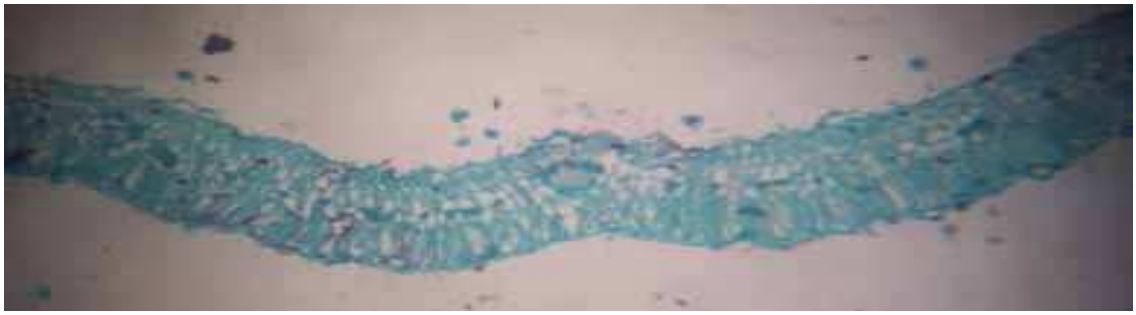


**Plate (2.1): T.S of *leaf of H. ovalifolium*.**

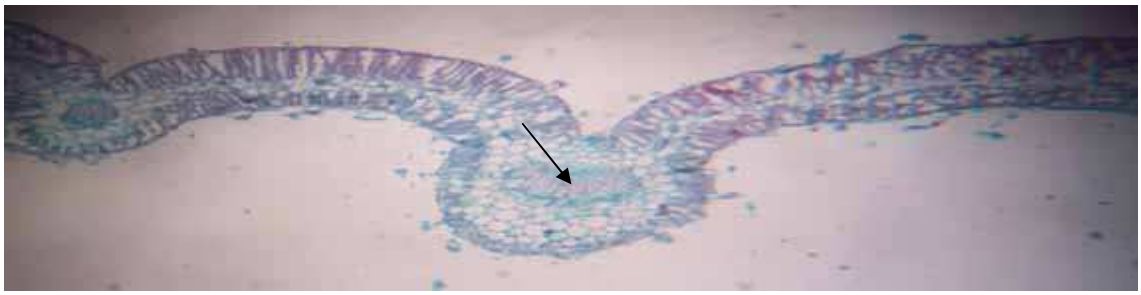


**Plate (2.2):T.S of leaf of *H. bacciferum*.**

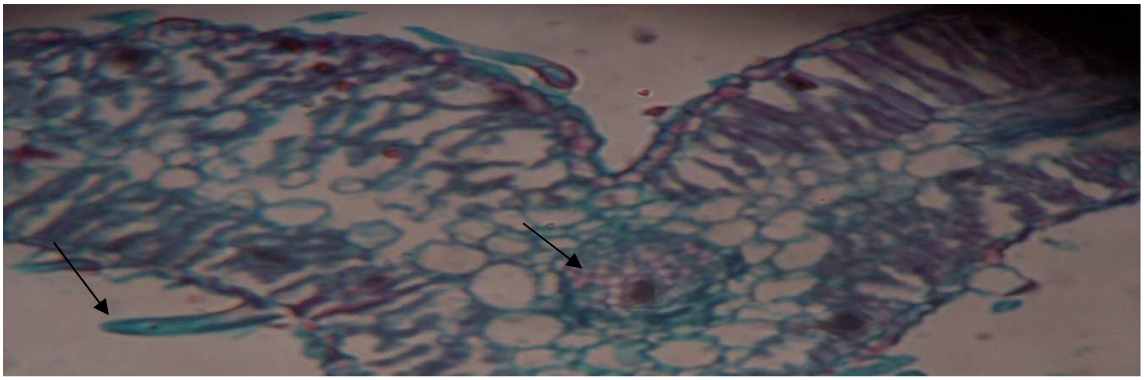
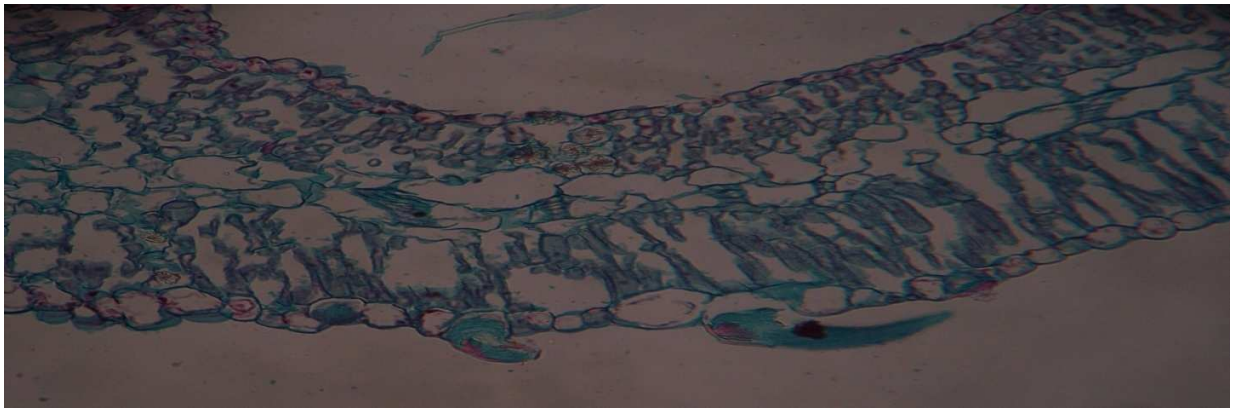


**Plate (2.3 ):T.S of leaf of *H. strigosum* shows the main vascular bundle and unicellular hairs in upper and lower epidermis.**



**Plate(2.4):T.S. of *H.supinum* shows the uni and multicellular hairs.**

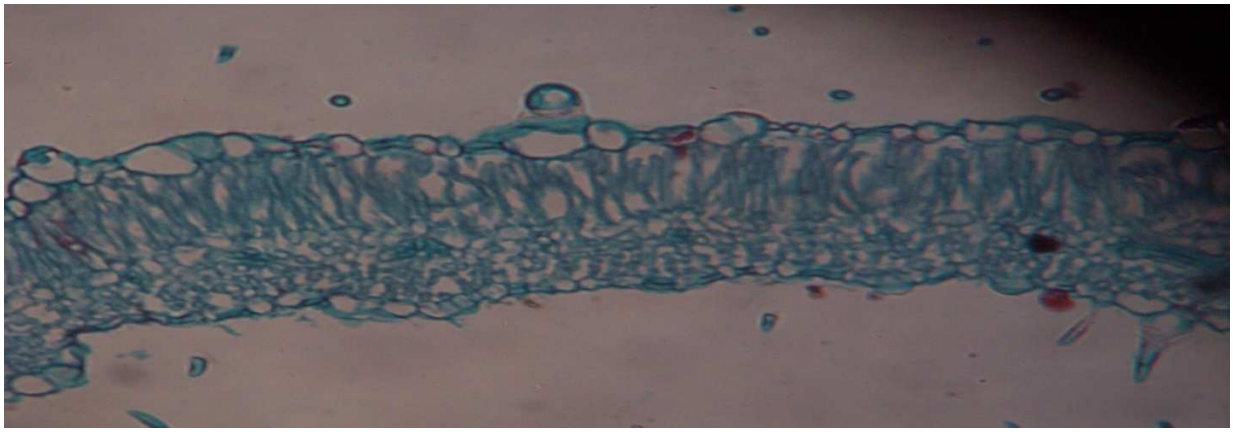


**Plate (2.5):T.S of leaf of *H.sudanicum* shows uni and multicellular hair(with upper gland).**

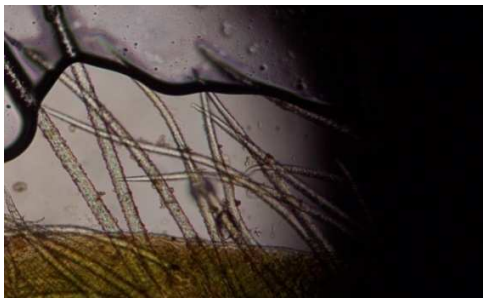


**Plate (2.6): Leaf Hairs of *H. ovalifolium***

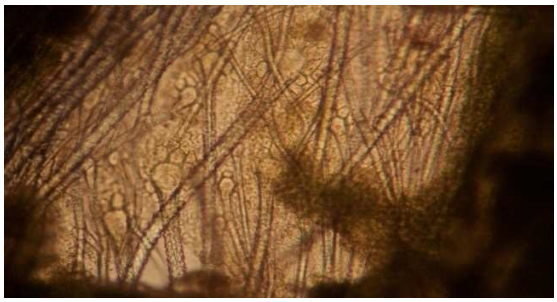


**Plate (2.7): Leaf Hairs of *H. bacciferum***

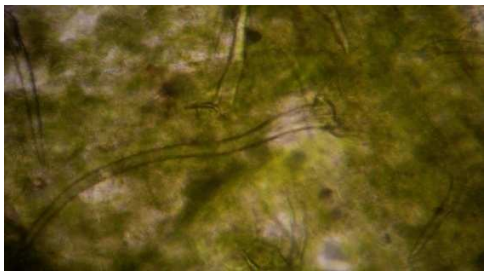


**Plate (2.8): Leaf hairs of *H. strigosum.***

**Plate (2.9): Leaf Hair of *H. supinum***

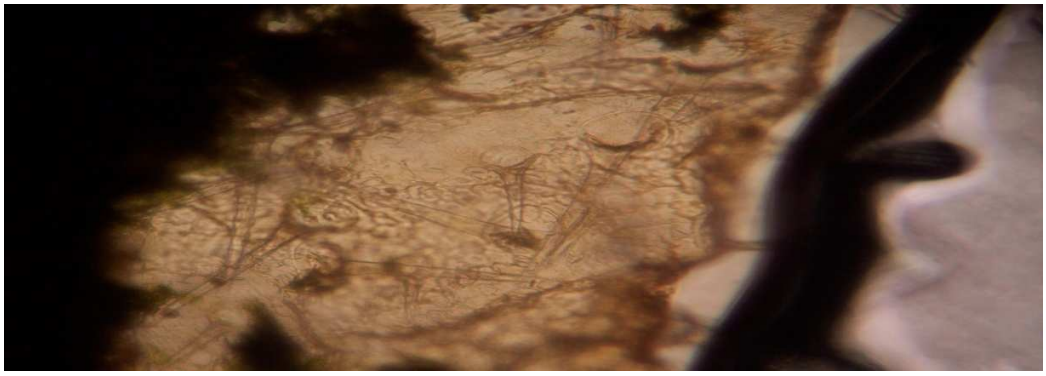


**Plate (2.10): Leaf Hairs of *H. sudanicum***

The characters studied include: Hair presence, Hair Density, Hair type, Basal cell and Hair wall. According to these five characters the studied species can be divided into two groups: Group (1): Densely hairy with basal cells in one row, hairs with smooth walls, this group include: *H. bacciferum* (plate 2.7) and *H. supinum* (plate2.9).Group (2):This group characterized by having densely hair or even woody leaves, with many rows basal cells and either scaly, granulate or scaly walls, are found in *H. strigosum* (plate 2.8), *H. sudanicum* (plate 2.10) and *H. ovalifolium* (plate 2.6).

## DISCUSSION

The anatomy of leaves and stems of five species of *Heliotropium* is studied in detail. The five studied species found to have one layer of epidermis, and hairs are present in all species. The hypodermis showed variation and accordingly *H. bacciferum* was separated from the other studied species in having 2 layers of parenchymatous cells whereas the other have 2-3 layers of collenchymatous cells. The cortex was found in 4 layers in *H. bacciferum + H. sudanicum*, 5 layers in *H. ovalifolium + H. supinum* and 6 layers in *H. strigousum.* Lateral bundle is absent In *H. ovalifolium*, 4 (*H. sudanicum*) .and 2 in *(H. bacciferum ,H. strigosum. H. supinum*.

Stomata width ranges from 0.008mm in (*H. bacciferum*) to 0.019 in (*H. strigosum*). Whereas the length of stomata was found to be 0.015 (*H. sudanicum*) mm to 0.029 mm (*H. supinum*). Stomatal indecies ranging from 273.33 to 500.0.This results indicated that the epidermal characters have very little significance as a taxonomic characters within the family and that confirm the results of [1]. Hairs vary from; with basal cell in one row; with smooth wall as in *H. bacciferum* and *H. supinum* to hairs with many basal cells in many rows as in *H. strigosum, H. sudanicum* and *H .ovalifolium*. Heliotropium bacciferum was separated from the other four species at the characters of having parenchymatous cell of the hypodermis, this confirm with its morphological characters.

So; The microscopical characters for the five studied species of *Heliotropium* leaves and stems could serve useful in the identification of this plant species.

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