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Macro and micromorphological properties of genus *Tephrosia* Pers. (Fabaceae) and their taxonomical significance, at Aden governorate, Yemen

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Abstract

The morphological and anatomical characters of two wild *Tephrosia* species grown in Aden were investigated. The leaf; the shape, apex, margin, base, texture, size and venation of lamina were studied. The epidermal characters including the properties of epidermal cells (Shape, size & frequency), stomata properties (Type of stomata complex, size, frequency, index and ratio) stomata properties of (Adaxial & Abaxial) and type and size of trichomes and the anatomical characters (Thickness of Mesophyll and number of abaxial collenchyma layers in the midrib) and type of trichomes were determined.

The stem and petiole characters and main midrib were investigated. The macro and micromorphological characters show a high significant in taxonomic value for the separation between the two studied *Tephrosia* species.

They were 4 species in 1992 and in this study only 2 were found, that due to the human influence and heavily urban raise at every habitat in Aden.

Keywords: *Tephrosia* Pers., morphology, anatomy Aden, Yemen

1. Introduction

The *Tephrosia* generic name is derived from the Greek word (Tephros), meaning "ash-colored" [15] and in Arabic we call It (غبيراء) Gubera, which mean ash-colored too.. The genus *Tephrosia* Pers. is related to family Papilionaceae, and tribe Millettieae the genus *Tephrosia* Pers. comprises about 300 to 400 species of annual and perennial woody herb, distributed in tropical and subtropical regions of the world [19, 20]. In Yemen four species were mentioned from south Yemen by [10], eleven species of *Tephrosia* were described briefly from North Yemen [5], therefore 18 species and seven subspecies belonging to *Tephrosia*. Four of them are endemic motioned from united Yemen as follow: *Tephrosia apollinea* (Del.) DC. Found in Harib, Soqotra. subsp. *longistipulata* Vierh. In Hadhramaut, Ataq. *T. elata* Deflers. Found in Ibb, Saadah, J. Ashsharq, J. Rayma, J. Milhan, Haraz. *T. desertorum* Scheele. in Bayt al Faqih. *T. dura* Baker in J. Gedu: Shabwa, Hadhramaut. *T. hadramautica* Thulin * in Hadhramaut. *T. mahrana* Thulin * in W of Al Ghaydah. *T. nubica* (Boiss.) Baker. subsp. *arabica* (Boiss.) Gillett. in Harib, Al Jawf, Hadhramaut. subsp. *nubica*. In Tihama & foothills, J. Buraa, Aden, Abyen. *T. odorata* Balf. f. * Socotra. *T. pentaphylla* (Roxb.) G. Don. Rare: in J. Buraa, J. Rayma, al Udayn, al Mahabisha. *T. pumila* (Lam.) Pers. Widespread: Adhabab, J. Marran, Khawlan Ashahm. *T. purpurea* (L.) Pers. subsp. aff. *dunensis* Brumm. In Tihama. subsp. *leptostachya* (DC.) Brumm. Found in Baytal Faqih, al Manssuriyyah, Aden, Tihama, Abyen, Hadhramaut, Soqotra, *T. quartiniana* Cufod. In Bayt al Faqih, Abyen, Al Mahara, *T. schweinfurthii* Defl. Widespread in Taiz, At Turbah, Saadah, al Qa'idah, Huth, Shabwa, Hadhramaut. *T. socotrana* Thulin * from Socotra. *T. strigosa* (Dalz.) Santa. & Mahes. From Socotra. *T. subtriflora* Hochst. ex Baker. Found in Tihama, al Hujariyah, S. of Zabid, Hadhramaut, Socotra. *T. uniflora* Pers. subsp. *petrosa* Gillett & Ali. From Aden, Hadiyah. subsp. *uniflora*. From Tihama, Taiz-Hagdah, Aden, Haraz, Socotra. *T. villosa* (L.) Pers. subsp. *ehrenbergiana* (Schweinf.) Brumm. From J. Marran in Khawlan Ashsham. [1], out of them only four species belonging to the genus found in Aden [3] they were 4 species in 1992 and in this study only 2 were found, that due to the human influence and heavy urban raise at every habitat in Aden. In Saudi Arabia, there are about 8 species of *Tephrosia*, which are mainly distributed in the northwestern, western, south and southwestern regions [4].

Including, *T. apollina*, *T. leptostachya* and *T. pubescens* which treated as subspecies or varieties of *T. purpurea*, in addition to *T. quartiniana* and other species recorded by [4]. The morphological diversity of some *Tephrosia* species (Fabaceae) in Saudi Arabia were carried out by [2]. Plant morphology has served largely for systematics, using morphological characteristics to carve up diversity into its systematic subunits [12]. The stomata ratio (SR) was helpful in defining the type of leaf [16]. Our aim is to investigate the macro and micromorphological characters of two species of *Tephrosia*, which hope to be more reliable the diversity of these taxa.

2. Materials and Methods

Fresh samples of *Tephrosia* were collected from different localities from Aden of Yemen during the period May 2014 to February 2015 and identified according to [4, 6]. For leaf morphology at least five to seven matured and well investigated to record the leaf architecture characters of each species which were based on the terminology of [7]. To study the epidermal characters fresh matured and well expanded leaves of the *Tephrosia* taxa were soaked in concentrated Nitric acid for 0.5 to 4 hrs. to dissecting the upper (Adaxial) and lower (Abaxial) epidermis [11]. The two epidermal layers were stripped and stained with Safranin, excess stain was rinsed off with clean water and mounted in glycerol on clean slides then covers by cover slide [14]. The slides were observed by using Leica (ATC 2000) microscope. The characters determined were stomata complex features (stomata type, size & frequency); epidermal cell features (shape, size & frequency) and Trichomes features (Type & frequency). Photographs of lamina epidermis (Adaxial & Abaxial) characters were taken by Canon (IXUS255 HS) digital camera. The stomata index (SI) was calculated using the formula of Salisbury [16]; $SI = [S \setminus (S+E)] \times 100$, where S= No. of stomata in an area of $625\mu m^2$ & E= No. of epidermal cells in an area of $625\mu m^2$. It is the ratio of the number of stomata on the abaxial epidermis to the number of stomata on adaxial epidermis, if $SR > 1$ the leaves are classified as amphistomatic, if $0.1 < SR < 1$ as hypoamphistomatic and if $SR < 0.1$ as hypostomatic [17]. The stomata size (length x width);

epidermal cell size (length x width) and guard cells area (length x width x Franc's constant which is 0.78525) were based on average obtained from observation of 40 individual, by the help of ocular micrometer calibrated with stage micrometer (value of $400\times$ Iocular small division = $0.25\mu m$) and Image j program. For the anatomical studies leaf lamina were cut to small samples each sample were fixed in formalin acetic acid alcohol (FAA) solution for two days. After removing the fricative by distilled water, they were dehydrated with ethyl alcohol solution of 30%, 50%, 60%, 70%, 85%, 90%, and 100% before being embedded into paraffin and sectioned by using a rotary microtome. The sections were stained in a Safranin O/Fast Green combination. The anatomical characters were examined by using Leica (ATC 2000) microscope and by utilizing the available anatomical literatures of [9, 8]. Photographs of the leaf sections were taken by Canon (IXUS255 HS) digital camera.

3. Results and Discussion

The observed morphological characters of *T. nubica* and *T. purpurea* were listed in Tables 1 and 2 and were illustrated in figures 1-8. Generally, the most important descriptions were discussed as follows:

3.1 Morphological characteristic of *T. nubica*

The plant is subshrubs or perennial herbs about 100 cm in height, and the stem is erect and woody with gray color with hairy texture. The leaves are alternate (12-45mm tall) with small leafy stipules about 1.5-2.7mm length with light green color, the leaf base is pulvinate. Leaflet texture is hairy and ranged from 1-3 pairs about 3-13mm length and is 5-12mm width, oblong shape with emarginated apex. The Inflorescence is raceme 104mm length, heavy trichomes. The flower is hermaphrodite and zygomorphic type; the calyx is tubular, with 5 free sepals, Corolla is imbricate with 5 free petals Papilionaceous type, with valvate aestivation. The Androecium has 10 stamens united in one bundle or two, one stamen become free and the other stamens are united. The Gynoecium consist from one carpel and the placentation is marginal type. The fruit is small pod, hairy is about 15mm length x 10mm width (Fig. 1, a-f).

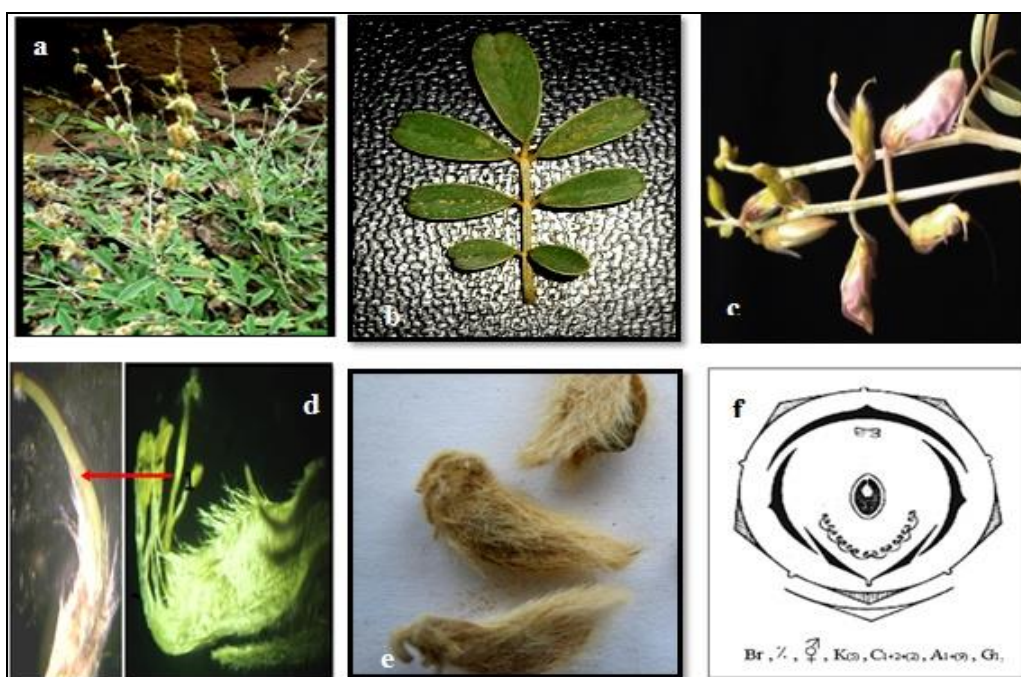


Fig 1: *T. nubica*; (a) whole plant, (b) Plant leaf (c) Inflorescence (d) sexual organs (d) fruits and (f) floral diagram of ($\times=100$).

3.2 Anatomical characters of *T. nubica*

3.2.1 Stem anatomy

Stem cross section was showed row rectangular epidermal cells with large number and long non glandular unicellular trichomes (1 μ m). Cortex consist from 6-8 cellular layers with 4-6 rows from Collenchyma cells. Endodermis with free of starch grains, only has a few prismatic crystals as (Fig. 2 a). Vascular tissues consisting of 16 open primary vascular bundles and 7 secondary bundles. Sclerenchyma tissue composed of fibers located in the outer part of the cortex in the form of intermittent ribbons above each vascular bundle. Stem Nodes consists of the same tissue (epidermis, cortex, vascular cylinder and marrow). The difference is only in the presence of pluripotent nodules beyond the vascular cylinder of the stem representing the trace of the leaf as (Fig. 2b). Node of vascular tissue consists of 11 basic vascular bundles and 9 secondary vascular bundles, varying in size and arranged in an unrelated cylinder consisting of the tissues of the sclerenchyma, the phloem, the xylem and the pith. These bundles are arranged within the stem cylinder, of 3 large circular vascular bundles slightly out of the stem cylinder bundles. This pattern, formed by the three vascular bundles of the stem, reflects the close structural and causal relationship between the stem and its lateral strands (leaves). The extensions of the vascular system in the stem are called the leaves trace. The leaf extends the impact of its connection beam stem - stem bundle package to a level that enters the vascular tissues of the leaf, the leaf node contains on amount of secretory material.

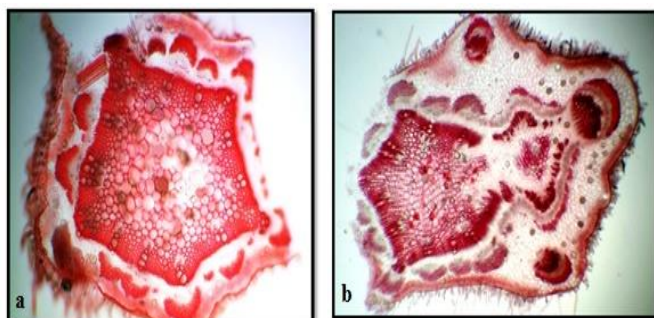


Fig 2: showing (a) T.S. of a. young stem and (b) stem nodule showing vascular bundles including leaf trace($\times=100$).

3.2.2 Leaf anatomy

Leaflet upper epidermis consists of a single row of thin round-walled parenchyma cells with a dense number of unicellular trichomes shorter in size about 75 μ m and has thick cellulose walls (Fig. 3a). Lower epidermis: consists of one row of parenchyma cells that differ from upper skin cells in their sizes and shapes has large number of unicellular longitudinal hairs up to 85 μ m, Palisade have three rows of cells in the Upper and two rows of cells in lower small and rectangular contain many green plastids, and contain very few prismatic crystals and may be non-existent. Spongy tissue consists of 1-2 rows of irregular shaped bronchial cells with few green plastids and wide visible distances as in (Fig. 3b). The primary vascular bundle found in the convex of the lower epidermis leaf middle rib is pear and larger than the secondary bundle. Xylem tissue of main vascular are oriented to the upper surface of the leaf. Secondary bundles is small size, consisting of xylem and Phloem distributed at one level in the area where the cells of the upper palisade tissue of the leaf connect with the spongy tissue. Leaf base cross-section revealed the cutinized epidermis (1.5 μ m thick) with long,

thick non glandular single hair as well as multicellular glandular (Fig. 3c). Cortex composed of a row of collenchyma cells. The inner part consists of 10-12 layers of large parenchyma cells, in which a few prismatic crystals are scattered. Three vascular cylinders were found. Pith composed of very narrow and composed of very small thin walls parenchymal cells. The leaf petiole is concave consists of a single row of polygonal cells surrounded by a thick cuticle layer up to 1.5 μ m thick, non-adipose with multicellular hair follicles (Fig. 3d). Petiole cortex consists of three types of external collenchyma cells and the inner cells of the cortex are 4-8 layers of parenchyma cells, it is the vascular tissue is made up of 8 vascular bundles, Pith is broad and consists of parenchyma cells differed in size as they move towards the center of the section and with star crystals.

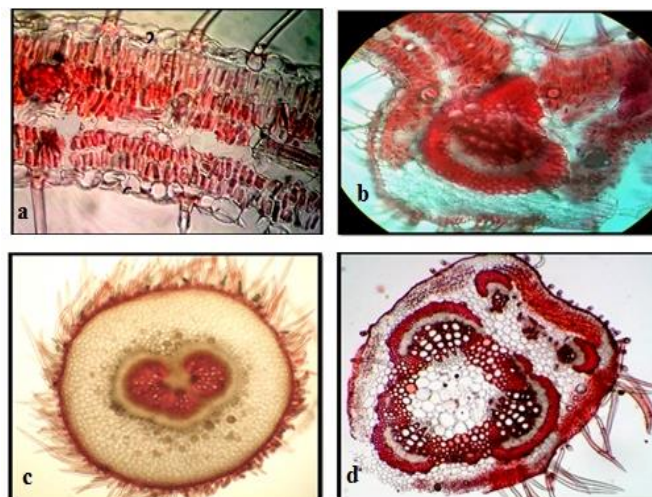


Fig 3: *T. nubica* (a) T.S. of leaflet wing with midrib, (b) T.S. of leaflet, (c) leaf base, (d) leaf petiole ($\times=100$).

There are also two types of stomata complexities in both epidermis the density of which is high for the total density of the two surfaces to 95.89%. Parasitic and Anisocytic are the two types found in *T. nubica*. The upper and lower epidermis contains several stomata, the density of stomata in the upper epidermis is lower than that of the lower epidermis, with a density of 14.35% and 14.70% for upper and lower epidermis, respectively (Fig 4a-d).

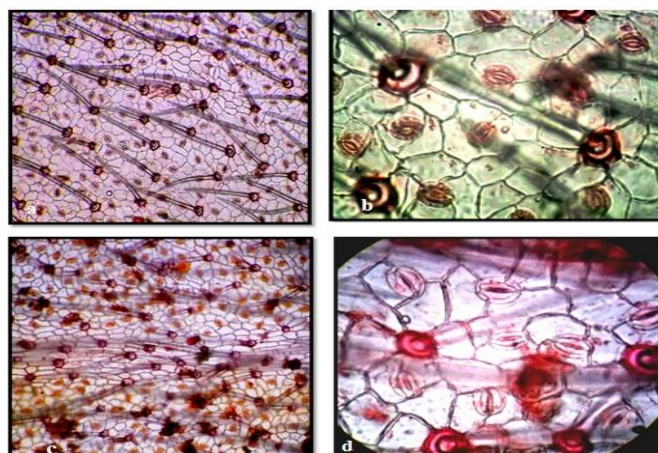


Fig 4: showing upper epidermal leaflet in which (a). whole epidermis with long unicellular hairs, (b). paracytic and anisocytic stomata, (c & d) lower epidermal leaflet in which c. whole epidermis with long dense unicellular hairs, d. anisocytic stomata($\times=100$).

3.3.1 Morphological description *Tephrosia purpurea* (L.) Pers.

The plant is herb up to 80-100 cm tall, the stem is plump, green in the young, The leaves are alternate, fed with small leafy stipules of a length of 2.5-4.5 mm. Their base is pulvinate, light green, with a neck length about 10-21 mm. The leaves are swinging from 2-6 pairs (3-13 leaflet), dark green, fluffy texture, rectangular, apex is knotted or hollow, The Inflorescence is raceme, hairy up to 3 cm long, the flower is hermaphrodite and irregular type hairy (Fig. 5a & b). The calyx composed of five united sepals and valvate, the corolla

is consisting of five petals of papilionaceous with usually five petals that include a large upper petal. The Androecium contains 10 stamens, the gynoecium consist from one carpel and the placentation is marginal type, no hairs on the stigma and ovary. The fruit is small long pod pappus and small; length is 33mm, while the width is 10mm. Fruit is rectangular pod, Pappus, small size with a length of up to 3.3 cm (Fig. 5 c & d). the floral formula

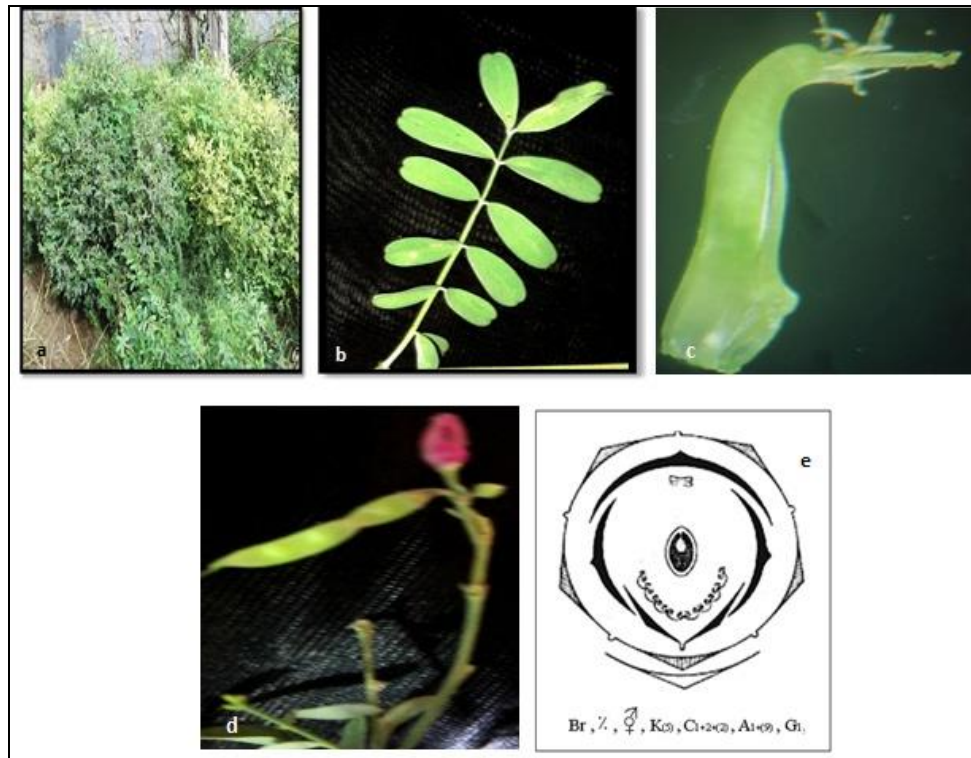
$$\text{Br}, \frac{1}{2}, \frac{\text{♂}}{\text{♀}}, \text{K}(5), \text{C}_{1+2+(2)}, \text{A}_{1+(9)}, \text{G}_1,$$


Fig 5: *T. purpurea*; (a) whole plant, (b) Plant leaf, (c)sexual organs in plant, (d) fruit with inflorescence and (e) floral diagram(x=100).

Table 1: Macromorphological characters of the studied *Tephrosia* species

Character	<i>T. nubica</i>	<i>T. purpurea</i>
plant height (cm)	90 -100	40-60
stem color	green to gray	Green
leaf length (mm)	12-45	32-80
length of leaf petiole (mm)	5-15	10-21
number of leaflets pairs	1-3	2-5
shape of leaflet blade	Rectangular	Rectangular
leaflet length	16-46	9-33
leaflet width	5-12	5-14
leaflet texture	Hairy	Hairy
leaf base shape	Pulvinate	Normal
stipules length/mm	1.5- 2.7	2- 4.5
inflorescence length/mm	104	30
hairs in flower	heavy in ovary and stigma	Absence
Calyx	5 sepals united-valvate	5 sepals united-valvate
Corolla	5 petals papilionaceous	5 petals papilionaceous
pod shape(mm)	small pappus pod	long pappus pod
pod length (mm)	15	33
fruit width(mm)	10	5

3.3.2 Anatomical description

Table 2& Figure 6-8 illustrated the main micromorphological characters of the *T. purpurea* can be summarized as follows:

3.3.2.1 Stem anatomy

Stem cross section was showed one row of polygonal

parenchyma cells with unicellular non glandular (1µm) hair follicles. Stem cortex composed of 8-10 layers (1-6 layers of collenchyma cells with a few prismatic crystals. Vascular cylinder consists of six open vascular bundles and 10 secondary bundles different in shape and size, sclerenchyma tissues have collenchyma thick cells with malignant walls

made up of fibers located in the outer part of the cortex. A complex fabric that connects water and salts. (Fig. 6 a) Pith: Broad containing a thin parenchyma cells with thin walls growing as they move toward the center of the stem, including free spaces without crystals. Stem nodes differed than stem structure in the presence of extrusion of vascular in the node beyond the vascular cylinder (10 primary vascular bundles and 6 secondary vascular bundles) represents the effects of the leaf trace (Fig. 6 b).

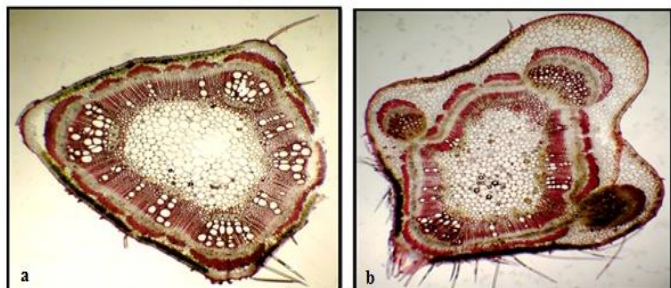


Fig 6: showing (a) T.S. of young stem (b). stem nodule showing vascular bundles including leaf trace of *T. purpurea* (X=100).

3.3.2.2 Leaf Anatomy

A cross section of the blade showed a single row of thin rectangular parenchyma cells in the Upper epidermis with many single unicellular hairs, higher than those found in the lower epidermis. Also, a single row of branched cells found in Lower epidermis with many unicellular hairs, single and longitudinal, long, the length of the length of the length of hair is 52 μm . The density is more than that of the upper epidermis, up to 43.42%. (Fig. 7 a). Mesophyll tissue consists of three layers of tissue namely Upper Palisade which contain four rows of rectangular cells about 11 μm contain very few prismatic crystals and may be non-existent. Lower palisade composed of three rectangular cell layers up to 7 μm long, shorter than the top palisade. Spongy tissue: It consists of 1-2 of irregularly shaped parenchyma cells containing few numbers of green plastids with wide visible distances (Fig. 7b a). The vascular tissue consists of primary bundle found in the leaf middle rib, secondary bundles are smaller in size, consisting of xylem and Phloem, distributed at one level in the area where the cells of the upper palisade tissue of the leaf connect with the spongy tissue (Fig. 7 c). The leaf base in which a row of rectangular cells found in the epidermis surrounded by a thick layer of chitin up to 1.5 μm thick with single long non glandular unicellular hair and multicellular

glandular hairs. Cortex consist of two-cell cell composed of 3 4 cells of the collenchyma cells. The inner part consists of 10-12 layers of large parenchyma cells, in which a few prismatic crystals are scattered. Vascular tissue consists of 3 primary vascular bundles. Pith is narrow and composed of large-sized parenchyma cells, including intercellular spaces. Leaf petiole is concave from the upper part in which the epidermis consists of a single row of closely polygonal cells surrounded by a thick cuticle layer up to 1.5 μm thick non-glandular hairs, and glandular multicellular hair. The cortex consists of three types of external cells, including 5-6 layers of non-linked collenchyma cells in which the internal cells of the crust are 3-5 layers of parenchyma cells and exist 3-4 layers collenchyma top core bundle. The vascular tissue is made up of 10 vascular bundles. The Pith is broad and consists of polygonal parenchyma cells. (Fig. 7e).

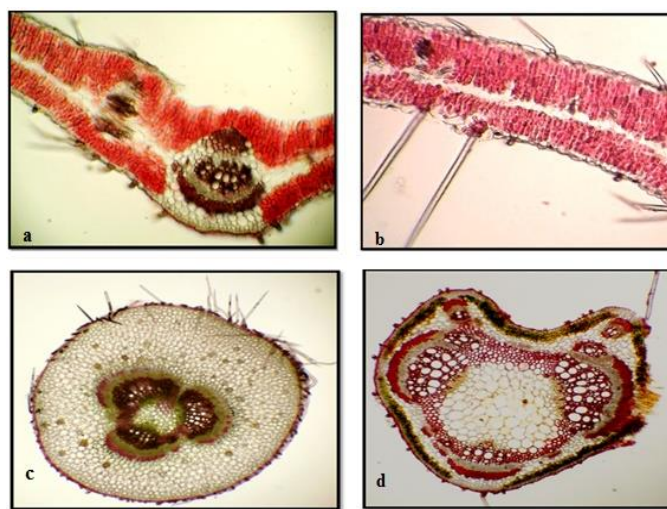


Fig 7: showing (a) T.S. showing a leaflet with midrib, (b) leaflet, (c) leaf base, (d) leaf petiole

The upper and lower epidermis contains several stomata, with the density of stomata in the upper epidermis of the leaf being higher than that of the lower epidermis, with a density of 18.36% and 10.77% for the upper and lower skin respectively. The upper and lower epidermis contains a number of stomata, with the density of stomata in the upper epidermis of the leaf being higher than that of the lower epidermis, with a density of 18.36% and 10.77% for the upper and lower skin respectively both paracytic (92.5%) and anisocytic (7.5%) were scattered on both surface of the leaflets (Fig. 8 a & b).

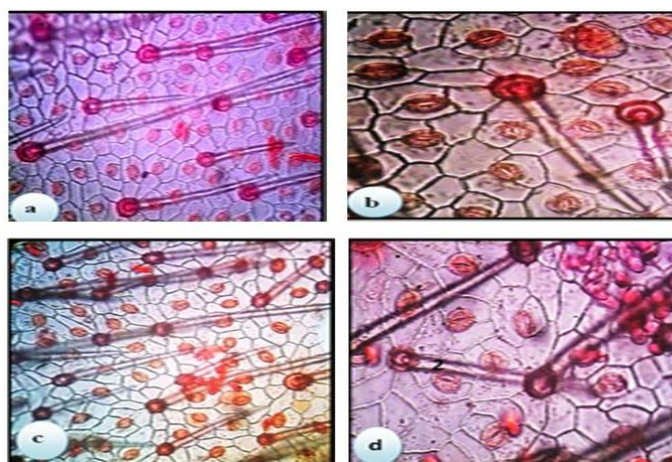


Fig 8: Showing upper epidermal leaflet in which (a). Whole epidermis with long unicellular hairs, (b). Paracytic and anisocytic stomata, (c & d) lower epidermal leaflet in which c. whole epidermis with long dense unicellular hairs, (d). anisocytic stomata (x=100).

Table 2: Micromorphological characters of the studied *Tephrosia* species.

a. stem anatomy	<i>T. nubica</i>	<i>T. purpurea</i>
Cuticle layer	1 µm	1 -1.5 µm
collenchyma layers	4-6	1-6
vascular bundles number	23	16
fiber form	separated	Separated
stem hairs	long and unicellular	short and unicellular
b. leaf anatomy		
b.1 upper epidermis		
thickness	1.5 µm	1 µm
trichome rate	9.17%	5.57%
length of hairs	75 µm	67 µm
stomata rate	14.35%	18.36%
b.2 lower epidermis		
Thickness	1.5 µm	1 µm
trichome rate	11.28%	43.42%
length of hairs	85 µm	52 µm
stomata rate	14.70 %	10.77%
parasitic type	89.95%	92.5 %
anisocytic type	10.05 %	7.5 %

The results proved that macro and micromorphological character are good taxonomic markers in plant identification and classification. The laminar shape, base, apex, margin and texture are the most useful morphological characters in separating the two studied species from each other. Epidermal characters have potential for taxonomic use as additional taxonomic characters [17]. The result of examining the epidermal cells shows that the length, size and frequency of epidermal cells vary from the leaf of one species to another among the genera. The results shows that size of stomata is taxonomically important and can be used in designation between the two studied taxa, on the other hand stomata complex type in the two *Tephrosia* species, was amonocytic and anisocytic; this is in agreement with observations recorded by [19]. Floral characters are the most important features that used in systematic of Papilionoidea [18] and agreement with Al-Ghamdi [2].

These anatomical studies for *Tephrosia nubica* consider the first ever done.

Key to the species

1. The pods are short and tomentose. The vascular bundles in stem are 23..... *T. nubica*.
2. The pods are longer and pubescent. The vascular bundles in the stem are 14.... *T. purpurea*.

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