Diagnostic significance of petiole anatomy in the identification of *Bauhinia* species (Fabaceae)

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Abstract: Petiole anatomical studies were conducted on six species of Bauhinia viz., B.blakeana Dunn., B.malabarica Roxb., B.purpurea1 L., B.purpurea2 L., B.racemosa Lam. and B.tomentosa L. belonging to family Leguminosae (Fabaceae) in search of useful and stable taxonomic characters for the identification of species. Petiole showed diagnostic values that are of taxonomic importance. The result showed substantial variation in the petiole outline, thickness in cuticle, cortex main vascular regions and presence of cell inclusions. Petiole outline in B .racemosa, B. blakeana and B.purpurea1 was planoconvex, while B.malabarica, B.tomentosa and B.purpurea 2 had circular. In the main vascular region B.malabarica, B.tomentosa, B. racemosa, B.purpurea1 had two inverted medullary bundle, while B.blakeana had one inverted medullary bundle and no medullary bundle was observed in B.purpurea2. Cell inclusions were in the form of rhomboidal crystals observed in B.blakeana, B.racemosa, B.purpurea1 and spheraphides observed in B.malabarica, B.tomentosa and B.purpurea 2. Cluster analysis was done for the petiole anatomical characters of all six species of Bauhinia, in which three major clusters were observed linked to each other. A diagnostic key was made which suggests that the study provides referential anatomical information for the correct identification of the species.

Key words: Anatomical, Bauhinia, Petiole, species

Introduction

The classification of plants is mainly based on morphological and anatomical concepts. The application of anatomical characters in plant classification dates back to Bureau (1864), who used anatomical features for the determination of taxa with Bignoniaceae for the first time. The role of anatomical data in traditional taxonomy has been recognized since the variations within the species, genera or family is usually reflected in anatomical features. Anatomical data have been used to good effect at all levels of the taxonomic hierarchy, as well as for identification and assessment of the taxonomic relationship among taxa of the flowering plants (Stuessy, 1990). Metcalfe and Chalk (1979) noted that anatomical studies have great value in establishing the identities of herbaria specimens, especially with sterile material.

Anatomical data have been shown to lend additional support to systematics in many taxonomic groups, acting as important criteria for interspecific and infrageneric delimitations in *Solanum* L. (Araújo et al., 2010, Nurit-Silva et al., 2012, Sampaio et al., 2014), *Ficus* L. (Araújo et al., 2014), and *Bauhinia* (Duarte and Debur, 2003, Lusa and Bona, 2009, Albert and Sharma, 2013), and also can contribute to the quality control of medicinal plants (Araújo et al., 2014, Porto et al., 2016). Ingole and Patil (2003) studied the pattern of some Verbenaceae family member's vasculature where they found petiolar anatomy to be taxon specific and useful in identification of different taxa. Petiolar anatomy of *Cinnamomum* species has been used as an aid for taxonomic discrimination (Baruah, 2007).

Bauhinia L. is an extremely variable genus of shrubs, medium sized trees or large trees of more than 200 species, in the subfamily Caesalpinoideae of the large flowering family Leguminosae (Fabaceae) with a pantropical distribution. The genus named after the twin Bauhin brothers, is characterized by the bilobed leaves with a cleft at the apex, forming two rounded lobes. From the base, the veins spread out fan-wise and the leaf is more or less folded along the centre rib. *Bauhinia* is also known as Mountain Ebony or simply Orchid tree, and 'Kachnar' in India and Pakistan. *Bauhinia* trees typically reach a height of 6-12 m and their branches spread 3-6 m outwards. The lobed leaves usually are 10-15 cm across. The five-petaled flowers are 7.5-12.5 cm diameter, generally in shades of red, pink, purple, orange, or yellow, and are often fragrant. The tree begins flowering in late winter and often continues to flower into early summer (Cooke, 1903).

Some of the *Bauhinia* species have a long history of traditional and medicinal applications. Whole plant of *B. purpurea* L. has been used in dropsy, rheumatism, convulsions, delirium, septicaemia (Asolker, et al., 2000). The young pods and mature seeds of kachnar are known to be cooked and eaten by tribles such as the Kathkors and Gondas of India (Rajaram and Janardhanan, 1991). Species of *Bauhinia* are rich in polyphenolics and are known for its medicinal properties (Patil, 2003). *B. purpurea* L. possesses potential antiproliferative and antioxidant activities (Zakaria et al., 2011). Various extracts of leaves of *B. racemosa* L. has been studied to develop a new pharmaceutical drug for prevention of enteric infection (Dahikar et al., 2011). Stem bark of *B. racemosa* L. is astringent and used in the treatment of headache, fever, skin diseases, tumour, blood diseases, dysentery and diarrhoea (Prakash and Khosa,1976). Studies confirms the antidiabetic potential of roots (Saravanan, 2019), stem barks (Kumar et al., 2012) and leaves (Filho, 2009) of *Bauhinia* species. *B.variegata* and its major constituent, roseoside, have demonstrated enhanced insulin release from the beta-cell lines INS-1(Frankish, 2010).

Bauhinia is well known for its ornamental shrubs and trees, such as *B. blakeana* Dunn being first chosen as the city flower of Hong Kong, China in 1965. In addition, some species, e.g., *B. championii* (Benth.) Benth., *B. purpurea* L., *B. tomentosa* L., have local pharmacological uses (Anju, 2011; Kumar 2011). Numerous phytochemical studies have been done on the species of *Bauhinia* which proves its importance, but the anatomical studies needs to be undertaken to understand the differences and variation among the species.

Some earlier anatomical studies on *Bauhinia* species have recorded anomalous structures especially in lianas. Approximately 150 species of *Bauhinia* has been subjects for the investigation of anomalous stem structure (Wagner, 1946). Schenck (1893) has listed three types of anomalous secondary growth in *Bauhinia*: the formation of a cleft xylem mass, the formation of successively younger zones of xylem and phloem and winging and waving of the stem. The present study targets the anatomical details, their variation and anomalous structures observed in six *Bauhinia* species.

Material and methods

The material for the present study was obtained from the orchard of Junagadh Agriculture University, Gujarat. (India). Leaf petioles were fixed in formalin (40%): acetic acid: ethyl alcohol (70%) 5:5:90 (v/v) FAA fixatives. Fixed samples were dehydrated in graded series (20, 35, 55, 75, and 95,100%) of TBA (tertiary-butyl-alcohol) and embedded in paraffin wax (Johansen, 1940). Sectioning was done through the central portion of the petiole using a rotary microtome; stained with Safranin and fast green, dehydrated in an ethanol series (70% to absolute), mounted in DPX and dried in the oven at 50˚C. Serial sections of the petiole were observed and micro photographed to visualize the cuticle, resin canals, arrangement of vascular strands and other anatomical features.

Results

Petiole anatomical characteristics observed in the present study were petiole outline, epidermis, cuticle, petiole vascular bundle arrangement and cell inclusions. Petiole outline was circular in all the species with slight variation on the adaxial side. In species *B .racemosa, B. malabarica B.purpurea 1*, planoconvex condition was observed while it was circular in *B.blakeana, B.tomentosa, B.purpurea 2* adaxially. The adaxial and abaxial epidermis was covered with thick film of epicuticular waxes.

Epidermis was single layered with epidermal walls straight, wavy or sinuate, had varying cuticle thickness in all the species (fig 1a-d). *B .racemosa* had the highest cuticle thickness (fig 1e). Cuticle was confined to the epidermal wall in *B.malabarica, B.blakeana, B.tomentosa* and *B.purpurea2*. Cuticle in *B.racemosa* and *B.purpurea1* was penetrating the radial wall of epidermis (fig 1e & 1c). The median portion of the petiole was arc shaped in cross section in all *Bauhinia* species. The cortex of all the species was made of parenchyma, with patches of tannin filled cells and idioblasts (fig 1 a-e).

The vascular system was surrounded by a few layers of Sclerenchyma. In *B.blakeana*, oblong collateral bundle, two small lateral cortical bundles seen in lateral projection (fig 2a), inverted medullary bundle where the phloem was facing the epidermis while xylem was found towards the pith (fig 3a) *B.malabarica* had oblong collateral bundle, two small lateral cortical bundles seen in lateral projection, tannin cells prominent in phloem region, inverted medullary bundles arranged in a ring form appearing like apmhivasal vascular bundles (fig 2b & 3b). *B.purpurea1* had oblong collateral bundle, two small lateral cortical bundles at lateral projection, two inverted medullary bundles placed opposite to each other (fig 2c & 3c). In *B. purpurea 2* oblong collateral bundles, arc shaped vasculature, small cortical bundles (fig 2d& 3d), Phloem with large number of sphaeraphides were observed. *B. racemosa* had crescent shaped collateral vascular bundles, no cortical bundles; two inverted medullary bundles arranged opposite to each other (fig 2e & 3e). In *B.tomentosa* crescent shaped collateral vascular bundles were observed with two bicollateral small mdeullary bundles and lot of tannin filled cells were seen. (fig 3f& 3f).

Cell inclusions like rhomboidal crystals, sphaeraphides and tannin filled cells were observed in the anatomical details of petiole. The six species either had rhomboidal crystals or sphaeraphides but not both. Rhomboidal crystals were found in parenchyma cell of cortical region in *B.blakeana, B.purpurea1, B.racemosa* (fig 4a, 4c, 4e) while sphaeraphides were present in the stelar region inside the phloem cells observed in *B.malabarica, B.purpurea2, B.tomentosa* (fig 4b, 4d, 4f). In *B.blackeana, B.purpurea1* and *B.tomentosa*, tannin filled cells were found scattered in the cortical and stelar region (fig 2a, 2c, 2f) while in *B.malabarica, B.purpurea2* and *B. racemosa*, the tannin filled cells formed a continuous layer in the phloem cells of the stelar regions and also surrounding the medullary bundles (fig 2b, 2d, 2e). *B.purpurea2* and *B. racemosa* had a heavy deposition of tannin filled cells in the cortical region in addition to the stelar region.

In order to study the affiliation between the species, the petiole anatomical characters were analysed. A cluster analysis was applied based on overall similarities of the individual species of *Bauhinia*. Three main clusters resulted from the cluster analysis (fig 5). *B. racemosa, B.blackeana* and *B.purpurea1*formed a cluster, *B.malabarica, B. tomentosa* formed other cluster and *B.purpurea2* solely formed last cluster. Out of these three clusters, *B.blackeana* and *B.purpurea1* have clustered very closely and show highest similarity value~60 while *B.malabarica* and *B. tomentosa* have clustered with a similarity value~50. Further *B.racemosa* clustered with *B.blackeana and B.purpurea1*at similarity value~33.33. First and second cluster of *B. racemosa, B.blackeana, B.purpurea1* and *B.malabarica, B. tomentosa* were linked with a similarity value~12 and the third cluster consisting solely of *B.purpurea2* was linked with other two clusters with least similarity value (fig 5).

Based on all the studied parameters and observations in the petiole anatomical investigation, it was possible to build an indented diagnostic key for six species of *Bauhinia*.

Diagnostic key for the identification of six species of *Bauhinia*

1. Outline of petiole-Planoconvex----------------*B. blackeana, B. purpurea1, B. racemosa*

1.1 Oblong collateral vascular bundle--------------------------------- *B. blackeana, B. purpurea1*

  1.1.1 Single inverted medullary bundle--------------------------------------- ***B. blackeana***

  1.1.2 Two inverted medullary bundle placed opposite to each other----- ***B. purpurea1***

1.2 Crescent collateral vascular bundle-- ***B. racemosa***

2. Outline of petiole-Circular-----------------*B. malabarica, B. purpurea2, B. tomentosa*

2.1 Oblong collateral vascular bundle------------------------------- *B. malabarica, B. purpurea2*

  2.1.1 Absence of medullary bundle-- ***B. purpurea2***

  2.1.2 Presence of inverted medullary bundles in a ring form-------------- ***B. malabarica***

2.2 Crescent collateral vascular bundle-- ***B. tomentosa***

Discussion

Petiole plays a vital role in the transport of nutrients and water to the leaves and provides support to the leaf lamina as well as protruding leaves to solar radiation for photosynthesis. Petiolar vascular systems are generally complex and provide a range of structures which can prove to be of considerable diagnostic value (Solereder, 1908, Metcalfe and Chalk, 1979, Ashton, 1982, Rojo, 1987, Pardi et al., 1991). Hare (1942) described the various arrangements of vascular bundles in the petiole and stated that the petiole anatomical characters are diagnostic and possibly of value for the purpose of classification. Metcalfe and Chalk (1979) recommended that the centre portion of the petiole is the largely reliable position for comparative purposes. Howard (1974) considered the vascular structure of the petiole as a taxonomic character and most useful at the generic levels.

Anatomical features are widely used in plant taxonomy for infra-generic classification of Rubiaceae (Martinez-Cabrera et al., 2009), Lamiaceae (Akcin et al., 2011) and Rutaceae (Vun et al., 2015), also it has been used to delineate the genus at species level for *Mormordica* (Aguoru and Okoli, 2012) and *Mangifera indica* varieties (Sharma et al., 2012) when the flowers and fruits are unavailable. Ruzi et al. (2009) studied the systematic significance of the types of petiole vascular bundles of Dipterocarpus Gaertn. (Dipterocarpaceae), and the study showed that the type of vascular bundles can be used for grouping species in Dipterocarpus and for species identification. In the present study the petiole outline could be categorised into two major categories circular and planoconvex. Further categorisation could be based on the pattern of arrangement of the vascular bundles and the type of medullary bundles if present.

Dubey et.al (1989) reported that the abaxial arc becomes deeper and then forms a complete ring of collateral vasculature, adaxial bundles lies in the ring and is inverted in *B.purpurea*. The collateral condition was similar in all the studied species of *Bauhinia* and the inverted bundles were observed in all species except for *B.purpurea 2*. *B.blakeana* had a single inverted medullary bundle, while *B.purpurea1* and *B. racemosa* had two inverted medullary bundles placed opposite to each other. In *B.malabarica*, the medullary bundle forms a complete ring which is inverted appearing as amphivasal vascular bundle. As compared to all the species, *B.tomentosa* had a different condition where the vascular arc of collateral bundles does not form a ring and remains as such. The two medullary bundles are embedded in the arc and were found surrounded by phloem on all sides. According to Dubey et.al (1989), petiole vasculature consists of two abaxial bundles and a few smaller bundles in addition to the inner and outer adaxial bundles, ultimately forming four lateral arcs, two outer facing each other and two inner facing the outer ones, which was also recorded in the present study.

In the cortical regions and phloem, presence of cell inclusion were recorded either in the form of tannin, rhomboidal crystals or sphaeraphides. Metclafe and Chalk (1950) recognized the crystal types such as crystal-sand, raphides, clustered crystals and shpero-crystals. Welle et al (1983) reported rhombic crystals in ray and parenchyma cells woods of the tribe Guettardeae. Seven kinds of crystals were isolated from the root of *Morinda officinalis* by Houz eg al (1986). Earlier studies (Tarsil et al , 2009; Dessein et al 2001;Rarhna Kumari et al., 2002) indicated the taxonomic significance of crystals within species, genera and varieties. Their total presence or absence in the plant body is taxon valuable (Patil & Patil 2011). Tannin filled cells were observed in the parenchymatous cortex and phloem cell. Tannins are the most abundant secondary metabolites made by plants and they can defend leaves against insect herbivores by deterrence and/or toxicity (Barbehenn, 2011).

The anatomy of petiole was diagnostic for separating the species of Bauhinia. This is a good example of the usefulness of cuticle deposition, vascular bundle patterns, cell inclusion for species classification and identification.

Conclusion

The combination of petiole outline with the type of vascular bundle arrangement and the type of inclusions and its position in all the species examined in the present study appeared to have considerable potential for identification of the different species of *Bauhina*. To conclude it is clearly depicted that petiole anatomical features showed interspecific variation and are potential parameters which help in identifying the different species of a genera. Each of the six studied species was observed to acquire unique features which could be differentiated under anatomical investigations.

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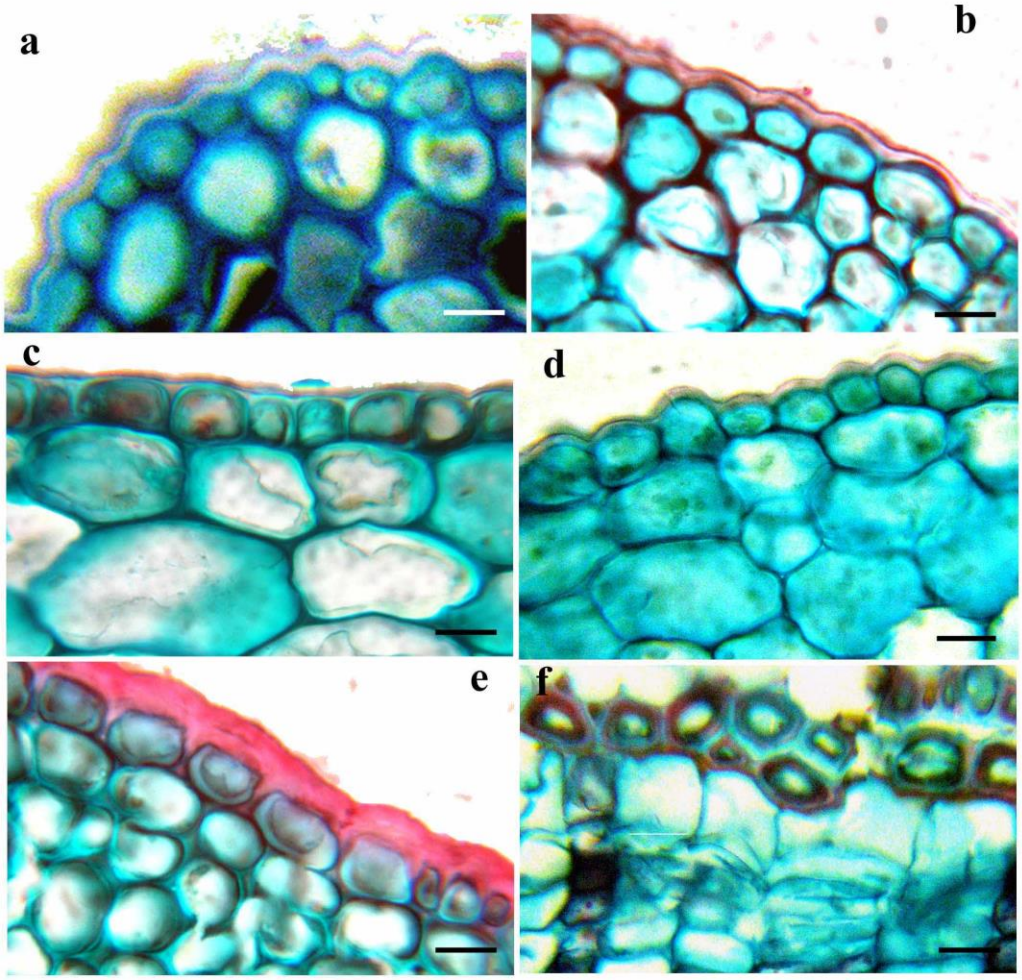

figure-1

fi

Epidermal and sub-epidermal regions of the petiole (Scale bars = 50µm)

a. *B. blakeana*
b. *B. malabarica*
c. *B .purpurea 1*
d. *B. purpurea 2*
e. *B. racemosa*
f. *B. tomentosa*

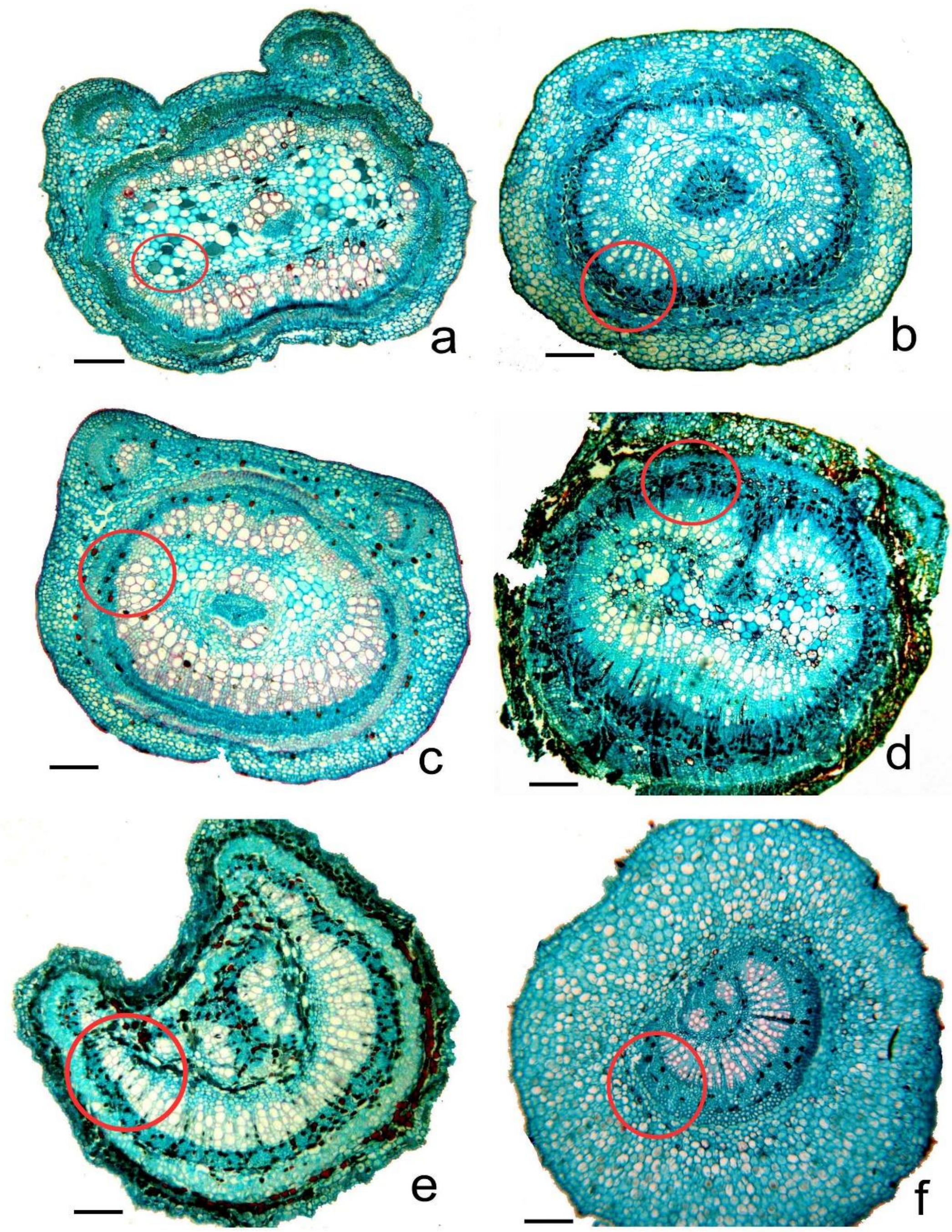

figure- 2

figure 2
Cross section of the petiole (Scale bars = 0.5 µm)

- *a.* *B. blakeana*, tannin cells (encircled)
- *b.* *B. malabarica*, tannin cells (encircled)
- *c.* *B .purpurea 1*, tannin cells (encircled)
- *d.* *B. purpurea 2*, tannin cells (encircled)
- *e.* *B. racemosa*, tannin cells (encircled)
- *f.* *B. tomentosa*, tannin cells (encircled)

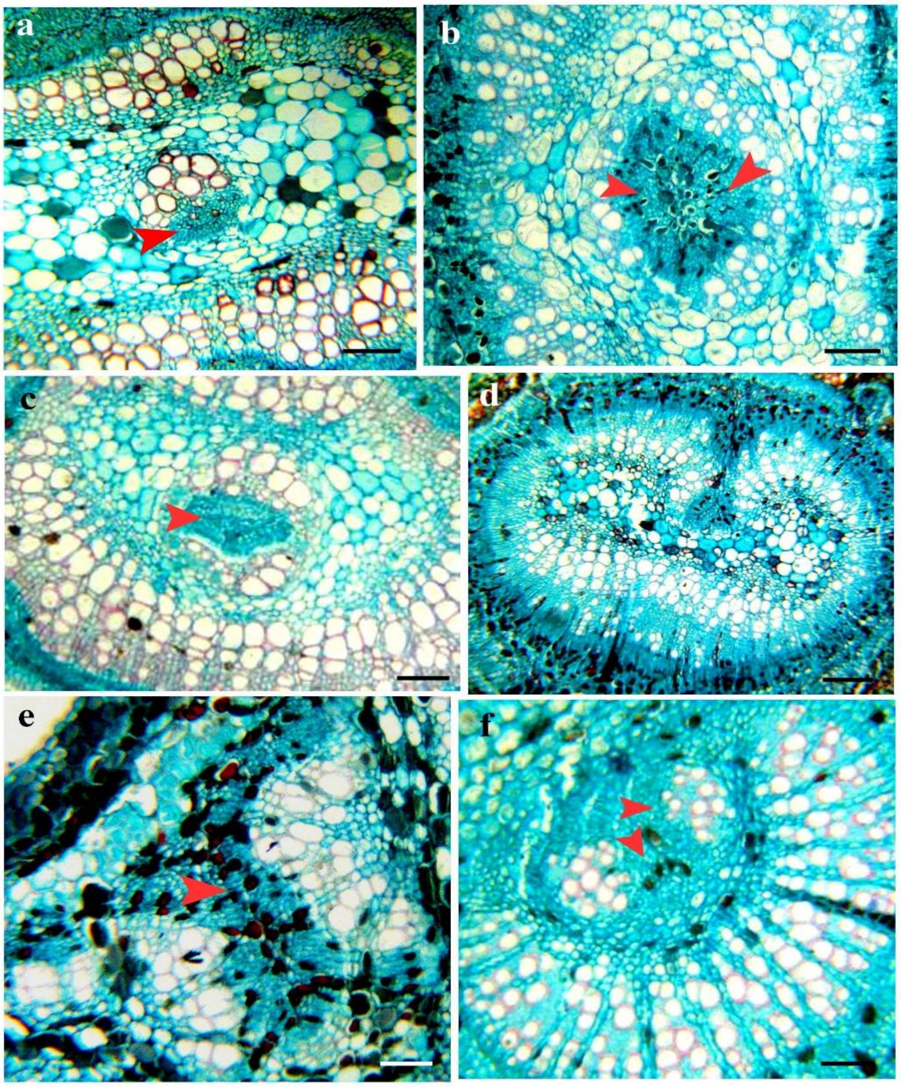

figure- 3

figure 3
Cross section of the petiole in the vascular regions (Scale bars = 0.5 µm)

a. *B. blakeana*, arrows-medullary bundles
b. *B. malabarica*, arrows-medullary bundles
c. *B .purpurea 1*, arrows-medullary bundles
d. *B. purpurea 2*
e. *B. racemosa*, arrows-medullary bundles
f. *B. tomentosa*, arrows-medullary bundles

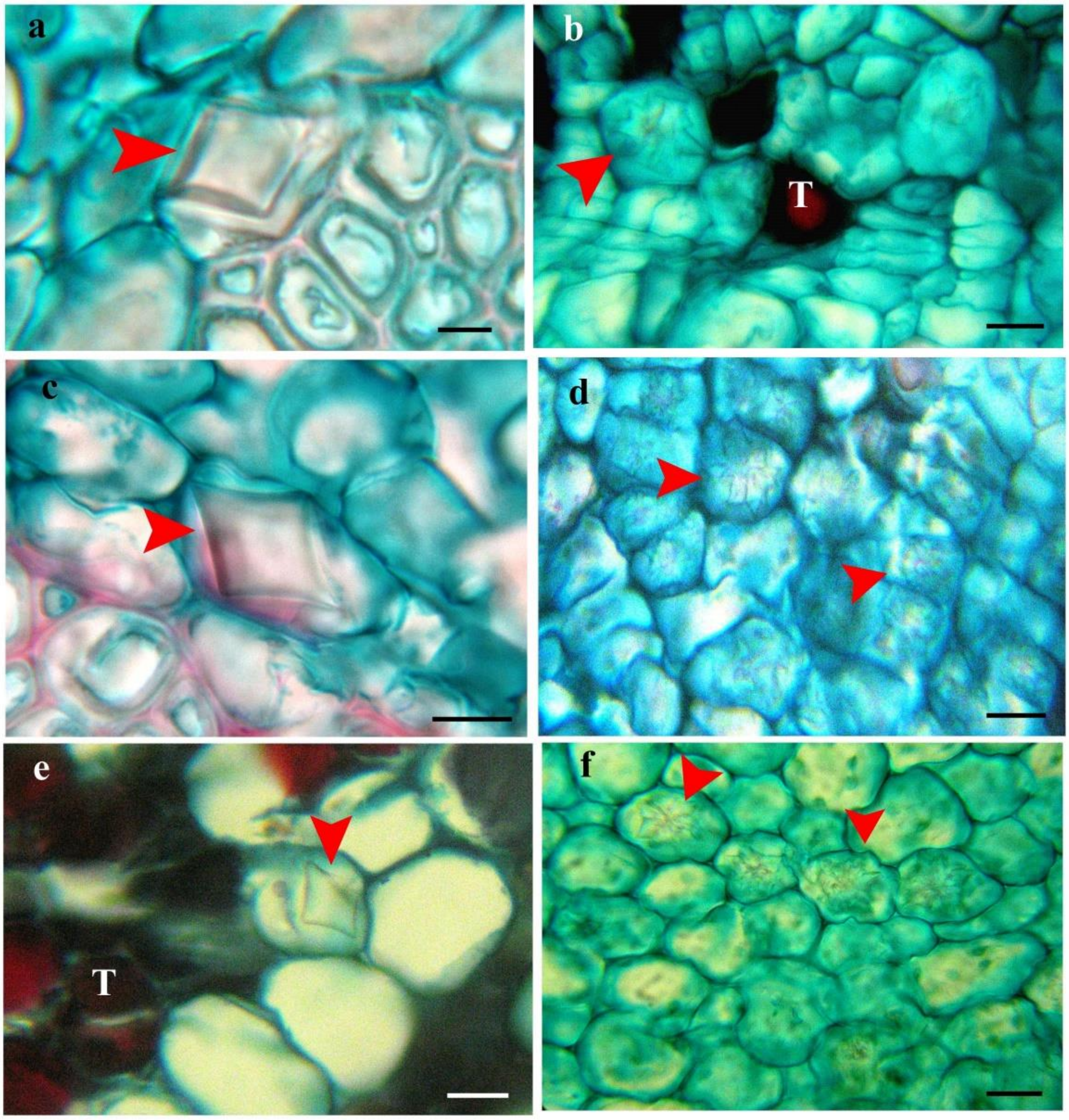

figure - 4

figure 4
Cortical and stelar regions showing Rhomboid cyrstal or sphaeraphides (Scale bars = 50µm)

a. *B. blakeana,* arrow- rhomboidal crystal
b. *B. malabarica,* arrow- sphaeraphides
c. *B .purpurea 1,* arrow- rhomboidal crystal
d. *B. purpurea 2,* arrow- sphaeraphides
e. *B. racemosa,* arrow- rhomboidal crystal
f. *B. tomentosa,* arrow- sphaeraphides

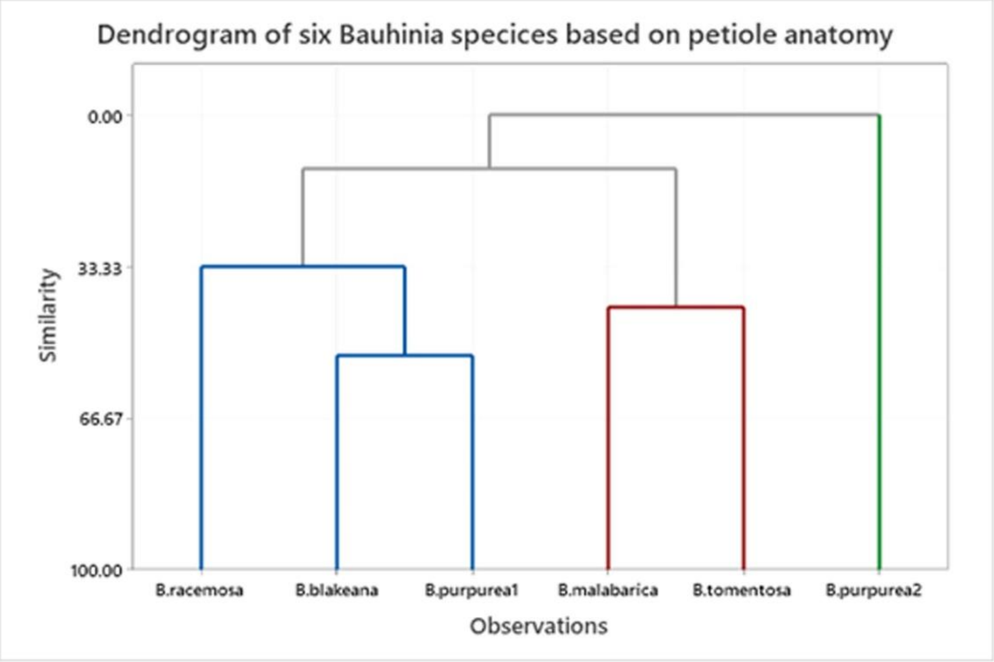

figure - 5

Cluster analyses of *Bauhinia* species based on petiole anatomical characters.