RJB Research Journal of Botany

OPEN ACCESS

# Comparative Microscopic Analysis of Leaf Petiole Anatomy in Ten Leguminous Species from Central Sudan

*Marwa EL-Haj Moemin and Hatil Hashim EL-Kamali*

*Department of Botany, Faculty of Science and Technology, Omdurman Islamic University, Omdurman, Sudan*

## ABSTRACT

**Background and Objective:** Petiole anatomy plays a crucial role in plant taxonomy, yet limited data exists on its significance in leguminous species. This study investigates the anatomical structure of petioles in ten leguminous species from Central Sudan, spanning the sub-families Caesalpinioideae, Faboideae and Mimosoideae to explore and assess the utility of petiole anatomical features in the taxonomy of selected leguminous species, providing insights into species-level classification. **Materials and Methods:** Transverse sections of petioles from ten leguminous species (*Caesalpinia pulcherrima*, *Cassia italica*, *Cassia obtusifolia*, *Cassia siamea*, *Cassia sieberiana*, *Tephrosia apollinea*, *Vigna unguiculata*, *Crotalaria saltiana*, *Cajanus cajan* and *Leucaena leucocephala*) were examined using light microscopy. The anatomical features, including epidermal cell shape, hypodermal layers, vascular bundle arrangement and trichome types, were analyzed. A common significant level used in biological studies is $p<0.05$. **Results:** The six distinct petiole anatomical patterns were identified across the species. Differences were noted in epidermal cell shapes, hypodermal layer structures, vascular bundle arrangements and trichome types. Two main anatomical patterns emerged: One characterized by a small pith and abundant sclerenchyma and another with a large pith and limited sclerenchyma. **Conclusion:** Petiole anatomy provides valuable insights for species-level taxonomy and the definition of smaller natural groups within leguminous species. However, it is less effective for broader taxonomic classifications.

## KEYWORDS

Petiole structure, vascular bundles, tissue composition, petiole trichome types, leguminous species



## INTRODUCTION

Petioles are critical structures in plants, serving as the essential link between the stem and the leaf blade, which is the primary site of photosynthesis[1,2]. The primary role of the petiole is to provide mechanical support, allowing the leaf to adjust its orientation towards the sun, thereby optimizing light capture for photosynthesis.

The study of leaf petiole anatomy is essential for understanding plant diversity, evolution, physiology and ecological interactions. Its significance spans from taxonomic and phylogenetic applications to physiological and ecological research, making it a vital area of investigation in plant biology.

ISSN: 2152-0461 (Online)
ISSN: 1816-4919 (Print)
https://doi.org/10.3923/rjb.2024.49.58

Received: 23 Aug. 2024
Accepted: 23 Oct. 2024
Published: 24 Oct. 2024

Several scientists have examined petiole vascular bundles, emphasizing the importance of petiole shape, epidermal cell features, fibres, crystal types, secretion elements, hairs and vascular bundle anatomy. Taxonomic significance of petiole anatomy, proposing that petiole characters can be used to identify families, genera and even species in some cases[3].

The taxonomy of the Cassia group is still puzzling because of the extreme morphological variability and ambiguous boundaries between taxa. Anatomical features provide characters to supplement the macro-morphological characters of plant species. Foliar anatomical characters such as stomata and trichomes have been found instrumental in solving taxonomic problems in the case of *Senna*[4]. A transverse section taken from the petiole of *Vigna* sp. showed the petiole has an irregular shape and consists of epidermal cells is also uniseriate with rectangular-shaped cells and covered with simple, unicellular and unbranched trichomes. One layer of circular collenchyma cells is located under the epidermis. The cortex consists of orbicular parenchymatous cells. The stele is divided into large two adaxial bundles and smaller three abaxial bundles forming the main trace, above which lie laterally a pair of secondary bundles. Pericyclic fibers two layers are present as a separate layer above the phloem of each bundle of the main foliar trace only (adaxial and abaxial bundles) while each secondary bundle has its separate fiber cap. Petiole vascular bundles are collateral types such as stem. The secretory cells are close to the phloem. The pith is composed of polygonal parenchymatous cells with intercellular space[5].

The study is necessary because there is limited published data on petiole anatomy and its taxonomic implications, particularly for leguminous species. Understanding the anatomical structures of petioles can provide valuable insights into species-level taxonomy and help differentiate between closely related species. The Leguminosae family is economically and ecologically important, improved taxonomic resolution within this family is essential for accurate identification, classification and utilization of these plants. This research work aimed to explore and assess the utility of petiole anatomical features in the taxonomy of selected leguminous species, providing insights into species-level classification.

## MATERIALS AND METHODS

**Study area:** This study was designed and carried out in the Department of Botany, Faculty of Science and Technology, Omdurman Islamic University, Omdurman, Sudan, between June, 2020 and July, 2020.

**Plant material:** The plant specimens were gathered on 15 June 2020 from the Shambat area within the Faculty of Agriculture, Khartoum University, located in Khartoum State.

**Methods:** The leaf petioles underwent fixation in formic acid and acetic acid (F.A.A), were then preserved in 70% alcohol. Both fresh and preserved materials were utilized. Freehand sections from the various portions of each petiole were stained with 1% safranin and 1% fast green. The prepared slides were examined using an Olympus research microscope (Olympus Corporation, in Tokyo, Japan). The permanent slides are currently housed in the Department of Botany, Faculty of Science and Technology, Omdurman Islamic University.

**Statistical analysis:** The Chi-square test was studied for categorical data (presence/absence of certain petiole anatomy across the species (petiole shape, trichome type across species and vascular bundle counts across species). This can determine whether there is a significant association between species and the occurrence of certain features. A common significant level used in biological studies is $p<0.05$. This indicates that there is a 95% confidence that the observed differences in petiole anatomy across the species are statistically significant and not due to random chance[6].

## RESULTS AND DISCUSSION

In this study, the petiole anatomy of ten leguminous plant species belonging to the sub-families Caesalpinioideae, Faboideae and Mimosoideae were compared. Petiole anatomical structures hold significant importance in the legume family (Leguminosae).

Several scientists, including Heneidak and Shaheen[7] and Kocsis *et al.*[8], have examined petiole vascular bundles, emphasizing the importance of petiole shape, epidermal cell features, fibers, crystal types, secretion elements, hairs and vascular bundle anatomy. The taxonomic significance of petiole anatomy characters can be used to identify families, genera and even species in some cases.

A study on *Clematis* taxa in South Korea on petiole shape and taxonomic relevance explored petiole micromorphology and anatomical structures, highlighting their value for species identification. Petiole shape variation has been emphasized as a key trait for delimiting closely related species, which might be relevant to analysis of diverse species based on petiole morphology[9].

Adaptive functions of trichomes in plant species under stress conditions. Studies on trichomes emphasize their function in species' defense mechanisms, offering a comparison of glandular and non-glandular trichomes across different species and their ecological roles[10]. A recent study by Karaismailoğlu[11] focused on the petiole anatomy of 21 representatives of the Alysseae tribe in Turkey. It examined the petiole shape and vascular bundle arrangements, providing significant insights into taxonomic distinctions among species. This study emphasizes how petiole shape can be a key taxonomic marker across different species[11].

The previous studies[9-11] can offer both confirming and contrasting perspectives related to our traits of interest, supporting or questioning the role of these characteristics in species differentiation and adaptation.

### Sub-family: Caesalpinioideae

***Caesalpinia pulcherrima*:** The transectional outline through the central (middle) region appears roundish. Epidermal cells exhibit a squarish shape. The hypodermis comprises 2-3 layers. Vascular bundles form a completely closed cylinder, while vascular bundles per wing are absent as shown in Fig. 1.

***Cassia italica*:** The outline is irregularly oval. One large abaxial and three adaxial bundles are discernible, with pericyclic fibers present as separate layers above the phloem of each bundle. Cortical sclerenchyma is absent and dense unicellular trichomes are present (Fig. 2a-b).

***Cassia obtusifolia*:** The petiole displays a crescent shape, with epidermal cells covered in simple, unicellular and unbranched trichomes. The cortex consists of parenchymatous cells. The stele is divided into two large adaxial bundles and three smaller abaxial bundles, with pericyclic fibers present outside the phloem of each bundle. Cortical sclerenchyma and crystals are absent and unicellular trichomes are sparse (Fig. 3).

***Cassia siamea*:** The petiole exhibits a nearly circular to broadly oval outline in the transverse section. The outermost layer consists of a single layer of barrel-shaped epidermal cells. The hypodermis is multi-layered, composed of collenchyma cells beneath the epidermis. Ground tissue, just below the hypodermis, comprises thin-walled parenchymatous cells. Vascular bundles contain a xylem towards the upper side and a phloem towards the lower side as shown in Fig. 4.

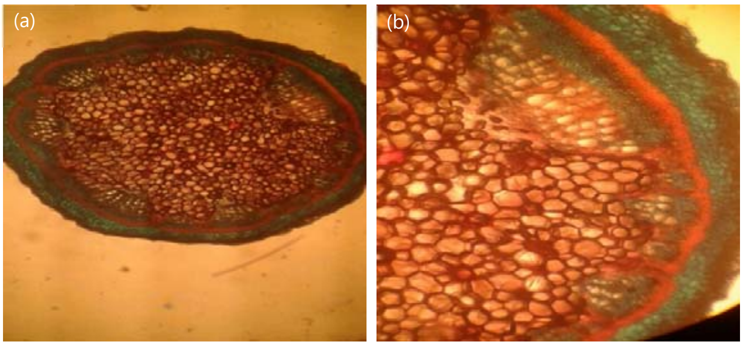


Fig. 1: TS in petiole of *Caesalpinia pulcherrima* (Central region)

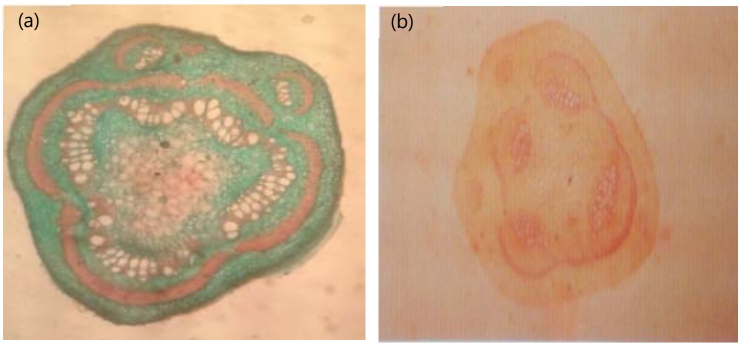


Fig. 2(a-b): TS of petiole in *Cassia italica* (Distal region)

Fig. 3: TS of petiole in *Cassia obtusifolia* (Distal region)

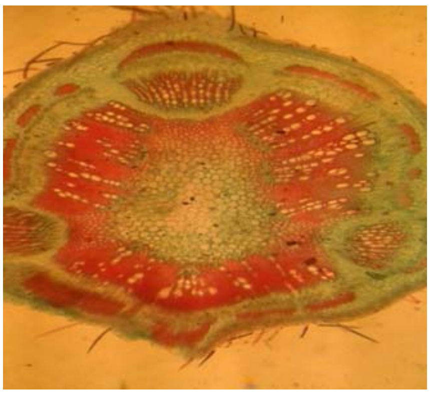

Fig. 4: TS of petiole in *Cassia siamea* (Distal region)

***Cassia sieberiana*:** In the proximal region, the leaf trace exhibits an irregular shape, with the adaxial and abaxial bundles merging into a nearly complete vascular cylinder, surrounded by a continuous layer of pericyclic fibers. There is an absence of secondary bundles and cortical sclerenchyma, while dense unicellular and multicellular trichomes are present (Fig. 5a-c).

**Sub-family: Faboideae**

***Tephrosia apollinea*:** The petiole exhibits a cordate shape and is divided into two adaxial bundles and one large abaxial bundle. Laterally positioned are pairs of secondary bundles, with pericyclic fibers present separately above the phloem of each bundle. Each secondary bundle is capped with its own set of fibers and dense unicellular trichomes adorn the surface as shown in Fig. 6.

***Vigna unguiculata*:** The petiole has a nearly terete outline, with two adaxial and three abaxial bundles. It possesses a large pith composed of parenchymatous cells, with minimal sclerenchyma surrounding the vascular bundle (Fig. 7).

***Crotalaria saltiana*:** In cross-section, the petiole of *Crotalaria saltiana* appears cordate. The stele is distinctly divided into two adaxial bundles and one large abaxial bundle. Epidermal cells are covered with simple, unicellular trichomes, while the cortex comprises parenchymatous cells. A large pith consisting of parenchymatous cells is present (Fig. 8).

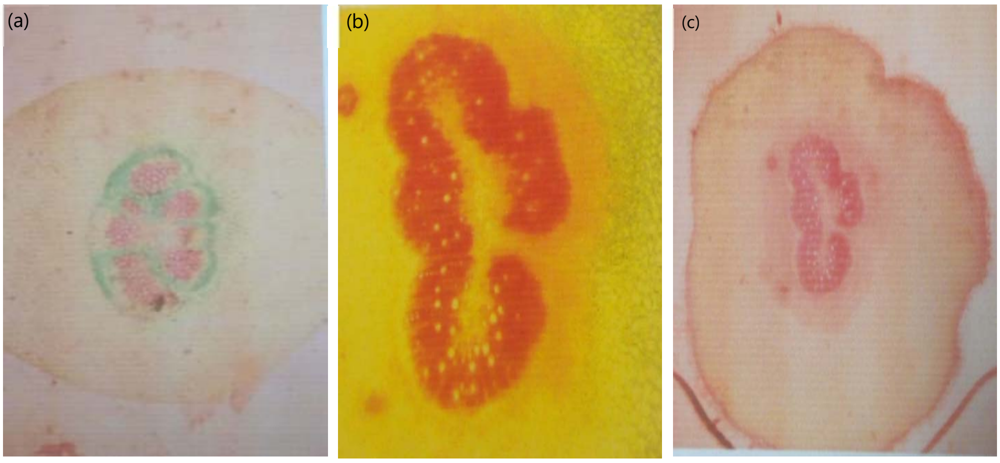


Fig. 5(a-c): TS of petiole in *Cassia sieberiana* (Proximal region)

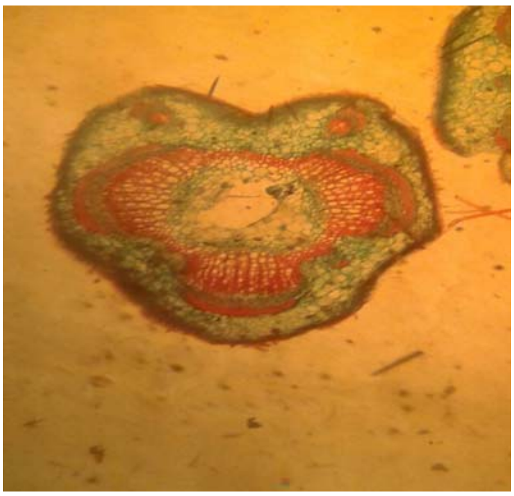

Fig. 6: TS of petiole in *Tephrosia apollinea* (Distal region)

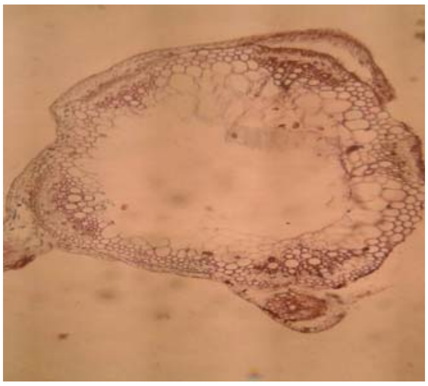

Fig. 7: TS of petiole in *Vigna unguiculata*

Fig. 8: TS of petiole in *Crotalaria saltiana*

Fig. 9: TS of petiole in *Cajanus cajan* (Distal region)

***Cajanus cajan*:** The petiole assumes an arc-shaped form, with two adaxial bundles and three abaxial bundles. It features a small pith made up of thick-walled parenchymatous cells, surrounded by ample sclerenchyma around the vascular bundle. The surface is adorned with numerous dense unicellular trichomes (Fig. 9).

**Sub-family: Mimosoideae**

***Leucaena leucocephala* (Syn. = *Lindera glauca*):** The petiole anatomy displays an arc-shaped pattern, characterized by a large pith composed of parenchymatous cells, with minimal sclerenchyma around the vascular bundles. It is distinctly divided into one large abaxial and eight adaxial bundles, with lateral pairs of secondary bundles (Fig. 10a-b).

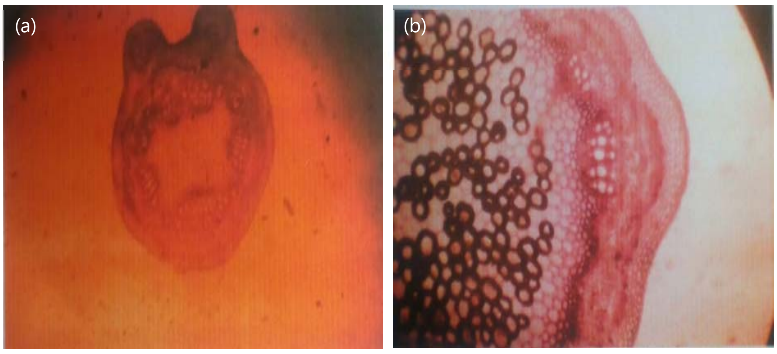


Fig. 10(a-b): TS of petiole in *Leucaena leucocephala* (Distal region)

The petiole outlines of the studied leguminosae species reveal six distinct patterns: (i) Crescent-shaped in *Cassia obtusifolia*, (ii) Terete in *Vigna unguiculata*, (iii) Nearly circular to broadly oval in *Cassia siamea*, (iv) Oval in *Cassia italica*, (v) Arc-shaped with wings in *Cajanus cajan* and *Leucaena leucocephala* and (vi) Cordate in *Crotalaria saltiana* and *Tephrosia apollinea*.

Epidermal cells generally exhibit a barrel-shaped or rounded morphology, although in some instances, such as *Caesalpinia pulcherrima*, they appear squarish. The hypodermis typically consists of collenchymatous cells. In *Cassia obtusifolia* and *Vigna unguiculata*, it is 2-3 layered, while in *Cassia italica, Tephrosia apollinea* and *Crotalaria saltiana*, it is 3-4 layered. In *Caesalpinia pulcherrima*, *Cajanus cajan* and *Leucaena leucocephala*, it ranges from 4-5 layers and in *Cassia sieberiana*, it is 6-9 layered.

In *Cassia italica*, there is one large abaxial and three adaxial bundles. *Cassia obtusifolia, Vigna unguiculata* and *Cajanus cajan* possess two large adaxial and three abaxial bundles. *Tephrosia apollinea* and *Crotalaria saltiana* have two adaxial and one abaxial bundle. *Leucaena leucocephala* exhibits one abaxial and eight adaxial bundles. *Cassia sieberiana* displays a horse-shoe-shaped arc of vascular tissue in the center, while *Caesalpinia pulcherrima* features a ring of 13 vascular bundles. The number of vascular bundles varies among species: 13 in *Caesalpinia pulcherrima*, 9 in *Leucaena leucocephala*, 7 in *Cassia sieberiana*, 5 in *C. obtusifolia*, *Vigna unguiculata* and *Cajanus cajan*, 4 in *Cassia italica*, 3 in *Cassia siamea*, *Tephrosia apollinea* and *Crotalaria saltiana*.

The trichomes vary among species, unicellular in *Vigna unguiculata* and *Cajanus cajan* while multicellular in *Crotalaria saltiana*. The presence of both unicellular and multicellular was in *Tephrosia apollinea*. The trichomes were non-glandular in *Tephrosia apollinea*, *Crotalaria saltiana* while grandular in *Vigna unguiculata* and *Cajanus cajan*.

The 2 distinct patterns in petiole anatomy were noticed in this study. The first pattern features a small pith with thick-walled parenchymatous cells and abundant sclerenchyma surrounding the vascular bundle, observed in species like *Caesalpinia pulcherrima* and various *Cassia* species. The second pattern is characterized by a larger pith composed of thin-walled parenchymatous cells and minimal sclerenchyma, seen in plants such as *Vigna unguiculata* and *Cajanus cajan*. These findings suggest that petiole anatomy can be a valuable tool for species-level taxonomic differentiation and identifying certain natural groups, though it is less effective for defining broader taxonomic sections.

Table 1 shows six distinct petiole shapes (crescent, terete, oval, arc-shaped with wings, cordate and nearly circular to broadly oval) across various species. Each species exhibits different adaptive strategies potentially related to leaf support and physiological function.

Table 1: Variation in petiole shape among studied legume species

| Species | Crescent-shaped | Terete | Oval | Arc-shaped with wings | Cordate | Nearly circular to broadly oval |
|---|---|---|---|---|---|---|
| *Cassia obtusifolia* | 1 | 0 | 0 | 0 | 0 | 0 |
| *Vigna unguiculata* | 0 | 1 | 0 | 0 | 0 | 0 |
| *Cassia italica* | 0 | 0 | 1 | 0 | 0 | 0 |
| *Cajanus cajan* | 0 | 0 | 0 | 1 | 0 | 0 |
| *Leucaena leucocephala* | 0 | 0 | 0 | 1 | 0 | 0 |
| *Crotalaria saltiana* | 0 | 0 | 0 | 0 | 1 | 0 |
| *Tephrosia apollinea* | 0 | 0 | 0 | 0 | 1 | 0 |
| *Cassia siamea* | 0 | 0 | 0 | 0 | 0 | 1 |

Table 2: Variation in petiole trichome-types among selected legume species

| Species | Unicellular | Multicellular | Both types | Glandular | Non-glandular |
|---|---|---|---|---|---|
| *Vigna unguiculata* | 1 | 0 | 0 | 1 | 0 |
| *Cajanus cajan* | 1 | 0 | 0 | 1 | 0 |
| *Crotalaria saltiana* | 0 | 1 | 0 | 0 | 1 |
| *Tephrosia apollinea* | 0 | 0 | 1 | 0 | 1 |

Table 3: Variation in vascular bundle counts among studied legume species

| Species | 3 Bundles | 4 Bundles | 5 Bundles | 7 Bundles | 9 Bundles | 3 Bundles |
|---|---|---|---|---|---|---|
| *Caesalpinia pulcherrima* | 0 | 0 | 0 | 0 | 0 | 1 |
| *Leucaena leucocephala* | 0 | 0 | 0 | 0 | 1 | 0 |
| *Cassia obtusifolia* | 0 | 0 | 1 | 0 | 0 | 0 |
| *Vigna unguiculata* | 0 | 0 | 1 | 0 | 0 | 0 |
| *Cajanus cajan* | 0 | 0 | 1 | 0 | 0 | 0 |
| *Cassia italica* | 0 | 1 | 0 | 0 | 0 | 0 |
| *Cassia sieberiana* | 0 | 0 | 0 | 1 | 0 | 0 |
| *Cassia siamea* | 1 | 0 | 0 | 0 | 0 | 0 |

In Table 1 petiole shapes were observed across various species. The table displays the presence (1) or absence (0) of different petiole shapes, such as crescent-shaped, terete, oval, arc-shaped with wings, cordate and nearly circular to broadly oval, for each species.

Table 2 categorized the species based on the presence or absence of unicellular, multicellular, glandular and non-glandular trichomes. The variation in trichome type across species suggests evolutionary adaptations for protection, water retention, or defense against herbivores. Table 3 highlighted the variation in the number of vascular bundles (3, 4, 5, 7 and 9) among species. The variation in bundle count is likely linked to differences in water and nutrient transport efficiency, affecting the plant's adaptation to diverse environments. These tables and traits provide the basis for a detailed Chi-square analysis, where the association between the observed traits and species distribution is statistically tested.

The Chi-square test results for petiole shape, trichome type and vascular bundle counts across the studied legume species were performed. The results suggest that petiole shape and trichome types do not significantly vary among the species in a way that would indicate a systematic anatomical difference. The variation in petiole shapes and trichome types observed across the legume species could be due to random chance, rather than being a consistent trait that differentiates the species. The variation observed in vascular bundle counts among the studied legume species is likely due to random rather than a significant anatomical difference. Therefore, the anatomical traits-petiole shapes, trichome types and vascular bundle counts, appear to show no significant statistical differences among the studied species[11]. By using Chi-square analysis, statistically determine if the observed traits in these tables are significantly different from expected values, providing insights into evolutionary patterns, functional morphology or species-specific adaptations.

Table 2 displayed the presence (1) or absence (0) of various trichome types, including unicellular, multicellular, both types, glandular and non-glandular trichomes in different plant species.

Table 4: Results of Chi-square test for anatomical traits across the species

| Anatomical trait | Chi-square statistic | d.f | p-value | Results |
|---|---|---|---|---|
| Petiole shapes | 40.0 | 35 | 0.257 | No statistically significant difference between the observed and expected distributions of petiole shapes across the species |
| Trichome types | 6.25 | 6 | 0.396 | No statistically significant difference in the distribution of trichome types across the species |
| Vascular bundle count | 32.0 | 28 | 0.275 | p-value was greater than 0.05 and failed to reject the null hypothesis<br>This suggests that the variation in vascular bundle counts across species was not statistically significant |

Table 3 displayed the distribution of vascular bundle numbers (3, 4, 5, 7 and 9 bundles) for each species, indicating presence with a 1 and absence with a 0.

The results of Chi-square test for anatomical traits (petiole shapes, trichome types and vascular bundle counts) across the species were shown in Table 4.

This study contributes to filling the knowledge gap on petiole anatomy in leguminous plants and its taxonomic relevance.

## CONCLUSION

This study highlights the taxonomic significance of petiole anatomy in distinguishing between ten leguminous species from the Caesalpinioideae, Faboideae and Mimosoideae subfamilies. Notable variations in vascular bundle arrangement and anatomical features underscore the importance of petiole structure in species identification within the Leguminosae family. Further research should focus on integrating petiole anatomical analysis with molecular data to enhance taxonomic classification. Expanding the study to include more species across different environments could provide deeper insights into the adaptive significance of petiole structures.

## SIGNIFICANCE STATEMENT

This research delivers important insights into the anatomical variation of leaf petioles among ten leguminous species from Central Sudan. Through detailed microscopic examination, it identifies six distinct anatomical patterns and two primary types of petiole structures. This study fills a significant gap in the literature concerning petiole anatomy within the Leguminosae family, facilitating better species-level identification and classification. The findings are crucial for advancing the understanding of plant adaptation, refining taxonomic classifications and supporting the effective conservation and utilization of medicinal plants in the region.

## REFERENCES


1. Faisal, T.R., E.M.K. Abad, N. Hristozov and D. Pasini, 2010. The impact of tissue morphology, cross-section and turgor pressure on the mechanical properties of the leaf petiole in plants. J. Bionic Eng., 7: S11-S23.
2. Levionnois, S., S. Coste, E. Nicolini, C. Stahl, H. Morel and P. Heuret, 2020. Scaling of petiole anatomies, mechanics and vasculatures with leaf size in the widespread Neotropical pioneer tree species *Cecropia obtusa* Trécul (Urticaceae). Tree Physiol., 40: 245-258.
3. Talip, N., D.F. Cutler, A.S. Ahmad, B.S. Ismail, A.R. Ruzi and A.A. Juhari, 2017. Diagnostic and systematic significance of petiole anatomy in the identification of *Hopea* species (Dipterocarpaceae). S. Afr. J. Bot., 111: 111-125.
4. Begum, A., M.O. Rahman and M. Begum, 2014. Stomatal and trichome diversity in *Senna* Mill. from Bangladesh. Bangladesh J. Plant Taxon., 21: 43-51.

5. Siahpoosh, A., M. Ghasemi, A. Majd, H.R. Memari and T. Nejadsattari, 2015. Vegetative and reproductive anatomy of *Vigna radiata* L. Trop. Plant Res., 2: 23-29.
6. Cochran, W.G., 1952. The $\chi^2$ test of goodness of fit. Ann. Math. Stat., 23: 315-345.
7. Heneidak, S. and A.S.M. Shaheen, 2007. Characteristics of the proximal to distal regions of the petioles to identify 15 tree species of Papilionoideae-Fabaceae. Bangladesh J. Plant Taxon., 14: 101-115.
8. Kocsis, M., J. Darók and A. Borhidi, 2004. Comparative leaf anatomy and morphology of some neotropical *Rondeletia* (Rubiaceae) species. Plant Syst. Evol., 248: 205-218.
9. Park, B.K., D.C. Son and B. Ghimire, 2021. Taxonomic relevance of petiole anatomical and micro-morphological characteristics of *Clematis* L. (Ranunculaceae) taxa from South Korea. PeerJ, Vol. 9. 10.7717/peerj.11669.
10. Gostin, I.N., 2023. Glandular and non-glandular trichomes from *Phlomis herba-venti* subsp. *pungens* leaves: Light, confocal, and scanning electron microscopy and histochemistry of the secretory products. Plants, Vol. 12. 10.3390/plants12132423.
11. Karaismailoğlu, C., 2020. Petiole anatomy of 21 representatives of tribe alysseae (Brassicaceae) from Turkey. J. Agric. Nat., 23: 1535-1544.