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Leaf Anatomical Study on some *Cassia sensu lato* species from Sudan

W. E. Abdalla[1*], H. E. Khalid[2], A. N. Guma'a[3], G. E. B. El Ghazali[4]

Abstract

Leaflet anatomy of fifteen *Cassia* L. *sensu lato* species from Sudan was studied including epidermal peels and sections through midrib regions in order to elucidate its importance in solving the problem of either amalgamate or segregate the genus into the three allied genera, namely: *Senna* Miller, *Chamaecrista* Moench, and *Cassia* L. *sensu stricto*. One type of cell shape (polygonal) as well as one type of stomata (paracytic) were shown from the epidermii of the leaflets of all species. Trichomes, when present, were always simple, unicellular, mostly thick-walled, with acute or rounded apices, sometimes with bulbous bases, straight or curved. The stomatal index and sections through midrib regions for the studied species did not support the segregation or amalgamation of the related genera, however, it proved to be useful in species identification.

Keywords: Leaf anatomy; Plant taxonomy; *Senna*; *Cassia*; *Chamaecrista*; Sudan.

Introduction

The genus *Cassia* L. (Family: Fabaceae/Leguminosae, subfamily: Caesalpinioideae) is considered as one of the largest genera of angiosperms, it is found naturally in tropical and subtropical regions worldwide [1, 2, 3]. Its numerous economic applications, particularly in herbal medicine, account for its significance. Numerous species in the genus have laxative activities [4, 5, 6, 7, 8] and are listed in different international pharmacopoeias, such as Indian Herbal Pharmacopoeia [9], Potter's New Cyclopaedia of Botanical Drugs and Preparations [10] and British Herbal Pharmacopoeia [11]. From a taxonomic point of view, it has been discovered that the existence of morphological complexity makes certain species challenging to identify. Consequently, several taxonomists divided this genus into three related genera: *Senna* Miller, *Chamaecrista* Moench, and *Cassia* L. *sensu stricto*. Some writers, however, continue to group all of the species under a single genus, *Cassia* L. *sensu lato* [3]. There are 23 species of *Cassia* L. *sensu lato* in Sudan, including 13 species of *Senna*, 7 species of *Cassia sensu stricto* and 3 species of *Chamaecrista* [12, 13, 14]. It is agreed that the data dealing with the histological structure of the vegetative organs of flowering plants can usefully be employed for many purposes such as: the identification of fragmentary material - especially for quality control of natural drugs to determine the purity or the presence of adulterations in the drug, as an aid towards establishing the interrelationships of taxa at/ and above the species level, and also it could help in identifying some herbarium specimens [15, 16, 17]. Numerous researchers have emphasized the importance of leaf anatomy as a tool for plant taxonomy [18, 19, 20, 21]. Identification of the genus and the species will be aided by the leaves' intricate anatomical characteristics. Leaf's diagnostic characteristics can be used to identify pollutants and

Affiliation:
[1]Department of Biology, College of Science, Qassim University, Buraydah 51452, Saudi Arabia

[2]Department of Pharmacognosy, Faculty of Pharmacy, University of Khartoum, Sudan

[3]Department of Biology, Faculty of Education, University of Khartoum, Sudan

[4]Department of Plant Taxonomy & Phytochemistry, Medicinal and Aromatic Plants and Traditional Medicine Research Institute, National Centre for Research, Sudan

***Corresponding author:**
W. E. Abdalla, Department of Biology, College of Science, Qassim University, Buraydah 51452, Saudi Arabia.

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adulterations. For instance, the form, type, distribution and number of the stomata; the size, shape, and structure of the epidermal cell walls; and the abundance, distribution and form of the epidermal trichomes are all important diagnostic traits [16, 22].

In Leguminosae, considerable use of epidermal characteristics as taxonomic aids has been made [23]. While actinocytic stomata are primarily found in certain species of *Senna* Mill, researchers such as [24, 25, 26] conducted epidermal investigations, particularly stomatal studies, on several species of the subtribe Cassiinae and discovered that the stomata were primarily of paracytic type with mesogenic ontogeny. According to [27], cuticular investigations of certain species of *Cassia sensu lato*, revealed that the species vary in the size and shape of their epidermal cells, the kind of their anticlinal wall, the shape and ornamentation of their non-glandular hairs, and occurrence of stomata on one or both surfaces of leaflets. From all of the above and due to its importance as a taxonomic tool, leaf (leaflet) anatomy including epidermal characters and transverse sections through lamina in the midrib region, was selected to be studied amongst other anatomical characters for elucidating interrelationships amongst different *Cassia sensu lato* species in Sudan.

Material and Methods

Collection and identification of plant material: Plant material were collected fresh from Khartoum State and Nahr El Neel State and identified by the authors using identification keys available from Sudanese Flora [13, 14] and further authenticated with herbarium specimens deposited at: Herbarium of the Botany Department, Khartoum University; Herbarium of the Forestry Department, Institute of Forests´ Research, and Herbarium of the Medicinal and Aromatic Plants and Traditional Medicine Research Institute, Khartoum, Sudan. All species names were updated using the: Plants of the World Online, of the Royal Botanic Gardens, Kew https://powo.science.kew.org/

Preparation of temporary slides for epidermal tissue systems

Adaxial and abaxial surfaces of young leaflets were painted with transparent fixative and left for 1-2 min. A transparent tape was fixed over the fixative and left for 1-2 min., and then removed and mounted over a slide. The slides were immediately examined under optical microscope (OLYMPUS BX51), then pictured using a digital camera of the type (OLYMPUS DP20). Figures (4,5,6) show an example of the epidermal tissue view for some of the species under study. Using the formula (S/E+S) X 100, where S is the number of stomata in an area and E is the number of epidermal cells in the same area, the stomatal index was determined as described by [20].

Preparation of permanent slides

Sass techniques [28] (with some modification) was used for preparing permanent slides from leaflet tissues as follows:

Initial preparation of plant material

Using FAA fixative (Formaldehyde: Glacial acetic acid: 70% Ethyl alcohol (5: 5: 90 v/v)), the sample tissues were segmented and fixed for a minimum of 72 hours. Following a distilled water wash, the fixed segments were serially dehydrated using ethyl alcohol increasing concentrations of 50%, 70%, 90%, 95%, and 100%, respectively. Each concentration held the segments for a day. The segments were cleared by transferring them every three hours from a 1:1 cedar wood oil: absolute alcohol mixture to pure cedar wood oil, then to a mixture of xylene and cedar wood oil, and lastly to pure xylene overnight. The plant segments were moved every 40 minutes from a mixture of xylene and wax to pure wax during the wax embedding process, which was done in an oven set to 60˚C. After cooling in water, the melted wax which contain the plant segments was poured into a mold and cut.

Sectioning

Using a rotatory microtome of the type (Leitz 1512, West Germany) set at 4–7 microns, the sample tissues were sectioned. Section ribbons were gathered with a brush over clean glass slides that had been moistened with distilled water to allow the ribbons to swell. The slides were then placed on a hot plate for 30 minutes so as to allow the material to expand as much as possible and remove any remaining water.

Staining

The slides containing the tissue slices were submerged in pure xylene for approximately one minute in order to dewax the tissues. The sections were then transferred into successive decreasing concentrations of ethyl alcohol, which were 100%, 95%, 90%, 70%, and 50%, respectively, in order to dehydrate them. Sections were stained by saturating them with safranin stain that had been dissolved in 50% ethyl alcohol. The sections were then dehydrated back into increasing concentrations of ethyl alcohol as 50%, 70%, 90%, 95%, and 100%, respectively. They were then cleaned in clove oil, mounted in a drop of Canada balsam, and covered with a cover slip after being dyed with quick green stain that had been dissolved in absolute ethyl alcohol.

Drying

The formerly prepared slides were left to dry in an electric oven set at 60˚C for at least 72 hours.

Microscopic examination and photography

A digital camera of the type (OLYMPUS DP20) connected to the same light microscope (OLYMPUS BX51) was used for taking images of the slides. Figures (1,2,3)

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shows transverse sections through midrib region for some of the species under study.

Results

Leaf epidermal peels revealed the following results:

i- One type of cell shape which was more or less polygonal was shown from the epidermii of the leaflets of all species;

ii- One type of stomata was observed in leaflets of all species (on both abaxial and adaxial surfaces) which was paracytic, with one subsidiary cell smaller than the other;

iii- Trichomes, when present, were always simple, unicellular, mostly thick-walled, with acute or rounded apices, sometimes with bulbous bases, straight or curved;

iv- The stomatal index for some studied species did not support the segregation or amalgamation of the related genera (*Cassia sensu stricto*, *Senna* and *Chamaecrista*). Table (1) summarizes these findings.

Table 1: Stomatal index for leaflets of some *Cassia sensu lato* species in the Sudan.

No.	Species name	Stomatal Index for leaflet	
		Adaxial surface	Abaxial surface
1	*Senna italica*	22.2 (16.1-28.2)	28.6 (22.5-32.4)
2	*S. alexandrina*	29.9	30.2
3	*S. occidentalis*	12.8 (11.1-14.6)	21.0 (15.0-27.3)
4	*S.* sp. aff. *occidentalis*	18.5 (13.3-25.0)	23.4 (19.1-28.9)
5	*S. alata*	10.2 (8.0-13.3)	18.6 (17.9-19.0)
6	*S. obtusifolia*	28.0 (22.7-31.1)	29.4 (25.0-40.0)
7	*S. auriculata*	9.2 (6.6-11.1)	10.2 (7.4-14.8)
8	*S. siamea*	2.4 (0-6.3)	10.8 (6.7-17.5)
9	*S. surattensis*	14.3 (11.4-16.7)	15.6 (11.1-19.2)
10	*Cassia javanica* subsp. *nodosa*	Not clear	22.2 (17.9-26.9)
11	*Chamaecrista nigricans*	33.3	38.6

An artificial key for identifying the studied species was constructed as shown in table (2).

Table 2: Identification key for fifteen *Cassia sensu lato* species in the Sudan using leaflet anatomy.

A.	Mesophyll of the lamina is of isobilateral structure:					
	B.	Trichomal cells thicker than epidermal cells; collenchyma cells beneath the epidermis in the midrib region in 2-3 layers …….				*Senna alexandrina* Mill.
	BB.	Trichomal cells thinner than epidermal cells; collenchyma cells beneath the epidermis in the midrib region in 4-5 layers ……..				*S. italic*a Mill.
AA.	Mesophyll of the lamina is of dorsiventral structure:					
	C.	Papillae present on the lower epidermis:				
		D.	Upper and lower epidermii glabrous ……..			*Cassia sieberiana* DC.
		DD.	Upper and lower epidermii hairy:			
			E.	Trichomes acute at apices; upper palisade single-layered …….....		*S. alata* (L.) Roxb.
			EE.	Trichomes rounded at apices; upper palisade double-layered ……..…		*C. grandis* L.f.
	CC.	Papillae absent from the lower epidermis:				
		F.	Main vascular bundle accompanied by another one on its upper side within the midrib region ……			*C. fistula* L.
		FF.	Main vascular bundle not accompanied by another one on its upper side within the midrib region:			
			G.	Multicellular glandular trichomes present on the lower epidermis ……..		*S.* sp. aff. *occidentalis* (L.) Link
			GG.	Multicellular glandular trichomes absent from the lower epidermis:		
				H.	Upper palisade consists of more than one layer ……..……..	*C. javanica* subsp. *nodosa* (Buch.-Ham. ex Roxb.) K.Larsen & S.S.Larsen
				HH.	Upper palisade consists of a single layer:	
					I.	Upper and lower epidermii glabrous:

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						J.	Phloem tissue in the main vascular bundle larger than the xylem tissue ……..			*C. arereh* Delile
						JJ.	Phloem tissue in the main vascular bundle smaller than the xylem tissue:			
							K.	Collenchyma cells are accumulating above the main vascular bundle …		*S. obtusifolia* (L.) H.S.IRWIN & BARNEBY
							KK.	Collenchyma cells not accumulating above the main vascular bundle …		*S. occidentalis* (L.) Link
					II.	Upper and lower epidermii hairy:				
						L.	Upper palisade layer filling more than 2/3 of the mesophyll ……			*Chamaecrista nigricans* (Vahl) Greene
						LL.	Upper palisade layer filling less than 2/3 of the mesophyll:			
							M.	Papillae present on the lower epidermis ……		*S. siamea* (Lam.) H.S.Irwin & Barneby
							MM.	Papillae absent from the lower epidermis:		
								N.	Paranechyma cells present in 7 to 8 layers below the main vascular bundle ……	*S. surattensis* (Burm.f.) H.S.Irwin & Barneby
								NN.	Paranechyma cells present in 3 to 4 layers below the main vascular bundle ……	*S. auriculata* (L.) Roxb.

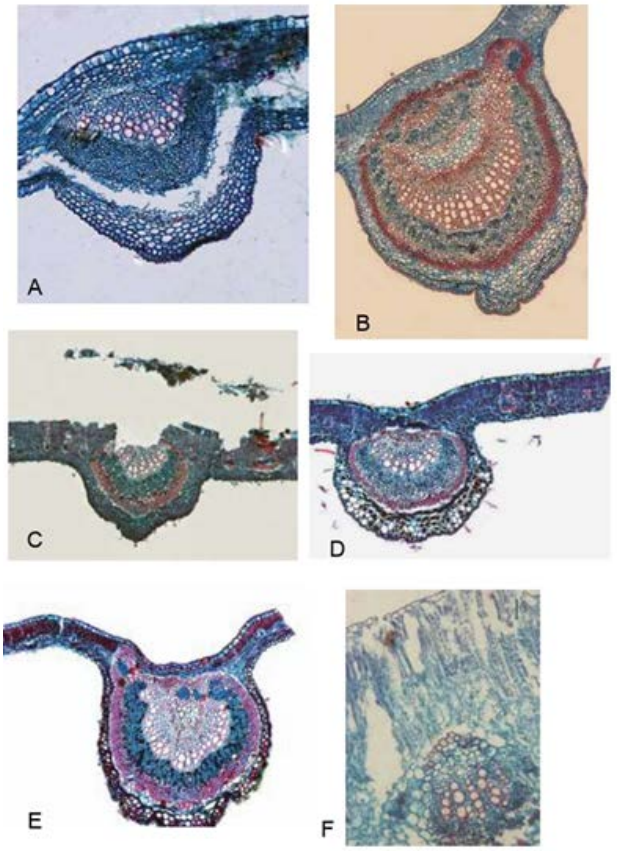

Figure 1: T.S. of leaflet through midrib region, **A:** *Cassia arereh*; **B:** *C. fistula*; **C:** *C. grandis*; **D:** *C. javanica* subsp. *nodosa*; **E:** *C. sieberiana*; **F:** *Chamaecrista nigricans.*

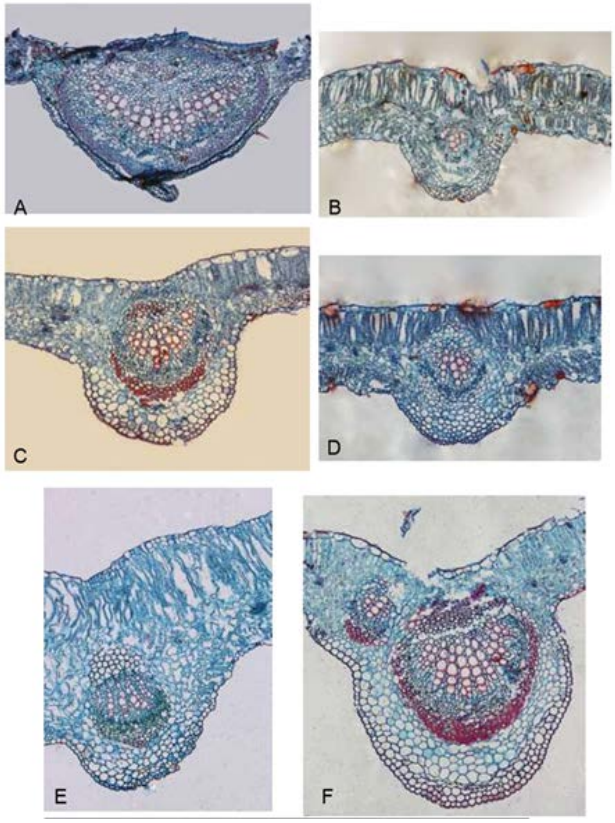

Figure 2: T.S. of leaflet through midrib region, **A:** *Senna alata*; **B:** *S. alexandrina*; **C:** *S. auriculata*; **D:** *S. italica*; **E:** *S. obtusifolia*; **F:** *S. occidentalis*.

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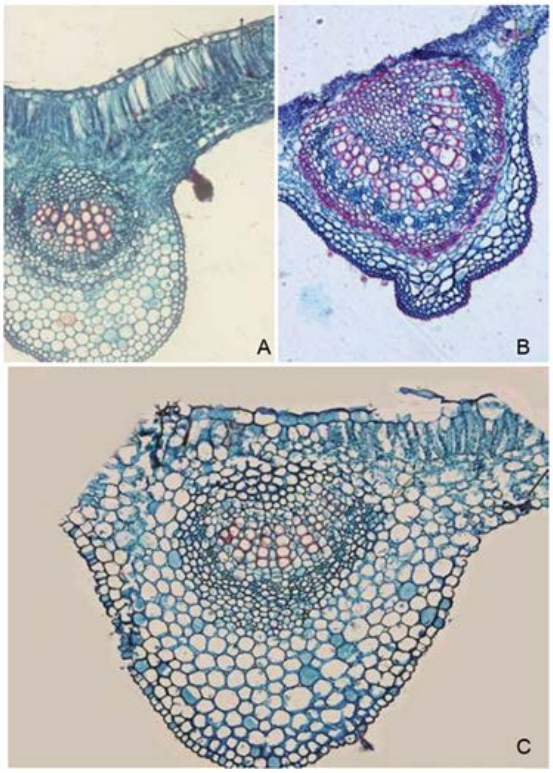

Figure 3: T.S. of leaflet through midrib region, **A:** *Senna* near *occidentalis*; **B:** *S. siamea*; **C:** *S. surattensis*.

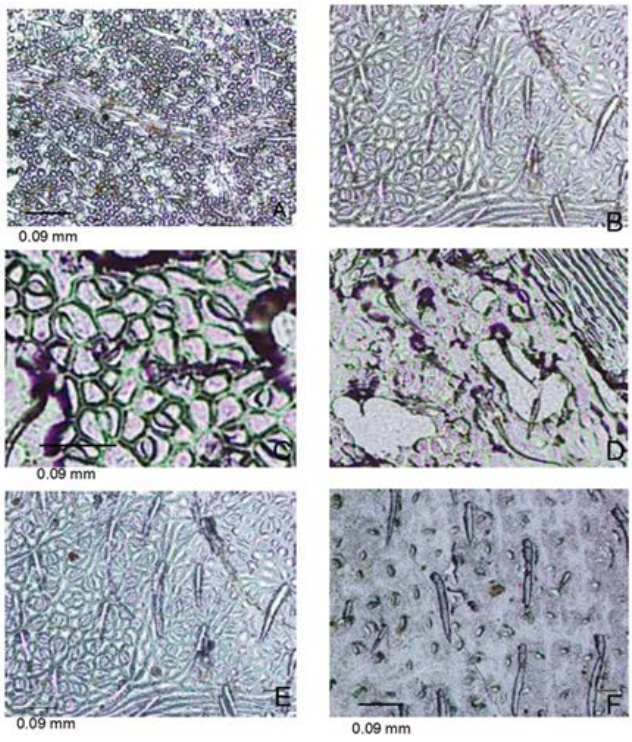

Figure 4: Leaflet epidermis showing trichomes and stomata for: **A:** *Cassia fistula*; **B:** *C. javanica* subsp. *nodosa*; **C & D:** *C. nigricans*; **E:** *Senna alata*; **F:** *S. alexandrina*.

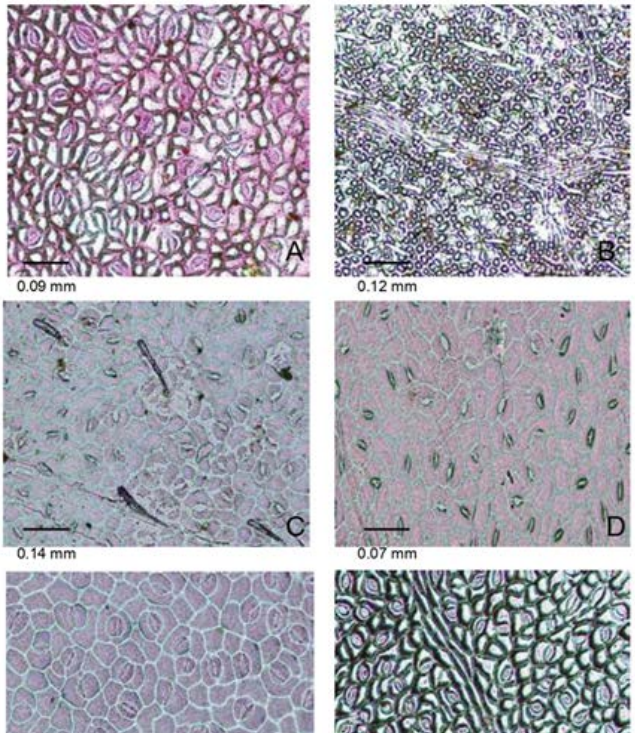

Figure 5: Leaflet epidermis showing trichomes and stomata for: **A & B:** *Senna auriculata*; **C:** *S. italica*; **D:** *S. obtusifolia*; **E:** *S. occidentalis*; **F:** *S.* near *occidentalis*.

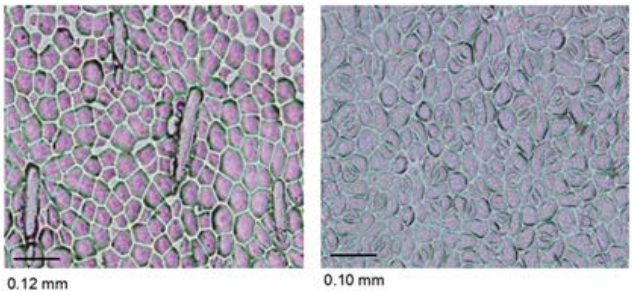

Figure 6: Leaflet epidermis showing trichomes and stomata for: **A:** *Senna siamea*; **B:** *S. surattensis.*

Discussion

Plant anatomical characters have limited value for differentiating species or groups of species under a particular rank, according to [29, 20] because the differences between them are typically quantitative rather than qualitative and frequently require statistical treatment based on more material than is commonly available. They went on to say that anatomy can occasionally be highly useful for identifying certain plant species. For instance, microscopical techniques are very useful for determining the botanical identity of commercial samples of fibers, timbers, medicinal plants, etc., or for identifying herbarium specimens that do not have flowers or fruits. They can also be used to prevent fraud, adulteration, and substitution, and they have occasionally been crucial in determining the guilt or innocence of

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suspected criminals. Their suggestions could interpret that some anatomical findings of this study did not clearly show taxonomic interrelations between the species under study, however, it could be useful in examination of commercial samples of these medicinal plants. Leaflet anatomy of fifteen species from the genus *Cassia sensu lato* present in Sudan was studied using optical microscope. The findings did not support the amalgamation or separation of the studied species into the three relevant genera (*Senna*, *Cassia sensu stricto* and *Chamaecrista*), although they proved to be useful in species identification. [16] and [29] suggested that leaf architectural design aid in taxonomy, and for closely allied species, characters such as stomatal index could be useful. However, when examining leaf epidermal characters in this study for some *Cassia sensu lato* species, the stomatal index did not support the amalgamation or separation of the relevant genera (*Senna*, *Cassia sensu stricto* and *Chamaecrista*) (Table 1). This might be attributed to many factors such as ecological variation, plant age variation, or other conditions. Besides that, no such evidence was previously recorded for *Cassia sensu lato* species. One of the characters which have received different and opposing opinions about its taxonomic weight is the stomatal index. [30, 31] said that the variation in stomatal index value is due to internal factors, mainly humidity and nutritional conditions. [32], working on *Cassia acutifolia* Del. and *C. angustifolia* Vahl (now *Senna alexandrina* Mill., Arabian and Indian varieties, respectively), also demonstrated that the differences in stomatal index values were statistically significant. On the other hand, he noted that the stomatal index varied considerably for each species and that this was of little value as a differential character. He also investigated the possibility that the stomatal index might vary significantly in different parts of the same leaflet. Some researchers, e.g. [31, 33, 34] have found that the stomatal number per unit area is highest near the leaf apex and decreasing towards the base, and also greater at the leaf margins than near the midrib. In contrast, other researchers, e.g. [35, 36, 37] found stomata to be most numerous at the base of leaves and adjacent to the midrib. From all of the above, it is concluded that quantitative characters such as stomatal index may be treated controversially, and even if it is considered to be taxonomically valid, a great amount of material must be tested to minimize the degree of error. Out of the fifteen *Cassia sensu lato* species studied, only *Senna alexandrina* and *S. italica* showed isobilateral mesophyll of lamina; only *Cassia grandis* and *C. javanica* subsp. *nodosa* showed multilayered upper palisade; only *S.* sp. aff. *occidentalis* showed multicellular glandular trichomes on the lower epidermis; only *Cassia fistula* showed another vascular bundle accompanying the main vascular bundle in the midrib region. These characters showed interspecific variation although it may reflect some degrees of affinities between the species under study.

Conclusion

Leaf anatomical characters such as shape of lamina, number of palisade layers, shape of trichomes, number of vascular bundles in the midrib region, stomatal index and epidermal cell shapes, were found to be useful characters for species identification, however it did not prove to be useful traits for amalgamating or segregating the genus *Cassia sensu lato* into the three allied genera *Senna*, *Cassia sensu stricto* and *Chamaecrista*.

Declaration of Competing Interest

The authors declare no conflict of interest.

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