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Morphological and Phytochemical Characterization of Cassiinae in Nigeria

[1]Okpara Onyinyechi and [2]Abiodun Ayodele
[1]Department of Botany, Joseph Sarwuan Tarka University, Makurdi, Nigeria
[2]University of Ibadan, Oyo State, Nigeria

ABSTRACT

Background and Objective: Cassiinae is the largest subtribe that constitutes the tribe Cassieae (Caesalpiniodeae) in the Fabaceae family. The autonomy and classification of the generic categories (Cassia, Senna and Chamaecrista) in the subtribe were established in this work using multiple character variations obtained from anatomical, morphological and phytochemical evidence to determine the taxonomic relationship among 10 studied taxa. This has led to the production of a reliable identification key. **Materials and Methods:** A total of 45 vegetative and reproductive characters, 13 anatomical and 13 phytochemical characters were qualitatively and quantitatively evaluated. The characters obtained were subjected to numerical and principal component analysis following Sokal and Sneath principles. The phenetic relationship among the groups was determined by hierarchical cluster analysis using the R programme. **Results:** A dendrogram containing three groups and a few subgroups representing the 10 taxa was produced. Cluster 1 comprises *Cassia fistula* and *Cassia sieberiana*. Cluster 2 contains only *Chamaecrista rotundifolia* while cluster 3 includes *Senna alata*, *Senna hirsuta*, *Senna occidentalis*, *Senna siamea*, *Senna polyphylla*, *Senna obtusifolia* and *Senna surattensis*. The resultant delimitation into 3 groups is a result of the combination of OTU of gross morphological and phytochemical characters during numerical analysis. The two-dimensional model of principal component analysis used highlighted shared characters of taxonomic significance that support delimitation of members of the group. **Conclusion:** This study affirms that morphological features are not sufficient enough but indispensable in the identification and classification of a clade.

KEYWORDS

Cassiinae, Cassieae, principal component analysis, morphological character, anatomical character, phytochemical character, cluster

INTRODUCTION

Cassiinae is one of the five subtribes in tribe Cassieae (Carotoniinae, Dialiinae, Duparquetiinae, Cassiinae and Labicheinae) and is notably the largest in terms of number of species consisting of about 740 species. The subtribe is categorized into 3 genera namely: *Cassia*, *Senna* and *Chamaecrista* and belongs to the subfamily Caesalpinoideae in the family Fabaceae. They are mostly cultivated for their economic, medicinal and ecological benefits as they promote food security, sustainable agricultural practices, sustenance of biodiversity and climate change[1]. Members of the subtribe are rich in phytochemical compounds useful

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in traditional medicine and the pharmacological industry. They are usually recognized by their showy flowers; thus, they are aesthetically relevant[2]. The sub-tribe are distributed in both wet and dry regions with a few in the temperate regions of the world[3]. The systematic study of chemical variation in plant taxa is useful in the classification of plants based on their specific secondary metabolites and biosynthetics[4]. There are three broad categories of compounds used in the classification of plants namely; primary metabolites, secondary metabolites and semantides[4]. It has been proven that certain compounds and related substances may be characteristic of certain taxonomic groups and evidently, the pathway of chemical evolution can be established since chemical substances are retained by metabolic processes[5]. Compounds retained by groups or ancestors, may lead to the understanding of relationships within and between groups[6,7]. Information obtained from this method is best used in conjunction with other sources of taxonomic evidence such as morphology, anatomy, cytological and molecular data to establish a system of classification that reflects natural relationships as accurately as possible. Based on the critical comparative study carried out in this work variations and similarities range from the gross morphological, anatomy and phytochemical characters obtained to serve as a tool for finding distinctive diagnostic characters that complemented and enhanced the delimitation and classification of members of the group. Thus, the combination of gross morphological and phytochemical characters in this study provided taxonomic evidence useful to trace evolutionary relationships within the taxa using the natural system of classification.

This study aimed to examine the taxonomic relationships among selected species in the subtribe Cassiinae. The specific objectives of this study were to:

- Determine the gross morphological and phytochemical character variations among studied taxa
- Determine the phenetic relationships among taxa using gross morphological and phytochemical evidences
- Provide a good and reliable taxonomic key for the recognition of members of the subtribe Cassiinae

MATERIALS AND METHODS

Plant collection: This study was carried out from May, 2021 to January, 2022. Ten species representing three genera in the sub-tribe Cassiinae (Fabaceae: Caesalpinoideae) in Nigeria were collected and determined into species based on the keys to species and classified into genus according to Irwin and Barneby[8]. Herbarium species housed at the University of Ibadan Herbarium and Forest Research Institute of Nigeria (FRIN) were used to confirm specimens by comparison with fresh specimens and used for morphometric studies. The specimen collected includes *Chamaecrista rotundifolia* (Pers.) Greene, *Senna hirsuta* (L.) H.S. Irwin & Barneby, *Senna alata* (L.) Roxb, *Senna occidentalis* (L.) Link, *Senna siamea* (Lam.) H.S. Irwin & Barneby, *Cassia fistula* L., *Cassia sieberiana* DC, *Senna obtusifolia* (L.) H.S. Irwin & Barneby, *Senna polyphylla* (Jacq.) H.S. Irwin & Barneby and *Senna surattensis* (Burm.f.) H.S. Irwin & Barneby.

Leaf morphometrics: Vegetative and floral macro-morphological characters of species in the Cassiinae subtribes were examined in this study. The quantitative characters determined include leaflet width and length, petiolule length, blade/rachis length ratio and leaf length/width ratio. The qualitative characteristics examined were the type of inflorescence and position, leaf arrangement, venation, shape, margin, surface beneath and above, texture, apex, base, fruit shape and color. The margin, length and width of specimens under study were measured using a twine and meter rule.

Anatomical studies

Preparation of leaf epidermal peels: Fresh leaves of each species were preserved in 50% ethanol and rinsed in ordinary water. About 1 cm^2 cut from the standard median portion of the leaf of each specimen was made. Each specimen was put in a glass petri dish and concentrated nitric acid was added so that the

leaf was immersed in acid. These were left in the sun outside the laboratory to hasten the action of the acid. The formation of air bubbles in the leaves indicated the separation of the upper and the lower epidermis from the mesophyll. The specimens were then transferred into a new Petri-dish and rinsed several times in distilled water. The epidermis for each specimen was separated with a pair of forceps and cleaned with a camel hair brush by removing the residual mesophyll layer[9]. Specimens were counter-stained with Alcian blue for another 5 min. Stained sections were mounted on microscope slides with 2 drops of 25% glycerol added and covered with a cover slip.

Preparation of petiolule section: Petiotule sections were made using a rotary microtome '820'. Sectioned specimens were cleared with 5% sodium hypochlorite to remove pigments and then rinsed in distilled water. The very thin sections were collected and stained in safranin for 5 min. The specimens were counter-stained with Alcian blue for another 5 min. Stained sections were mounted on clear microscope slides with 2 drops of 25% glycerol added and covered with a cover slip. The slides were previewed under the microscope (Olumpus corperation, Shinjuku Monolith, 3-1, Nishi Shinjuku 2-chrome, Shinjuku-Ku, Tokyo, Japan) and the edges were carefully sealed with transparent nail polish. Photomicrographs of the slides were captured using an Olympus CX 31 photomicroscope with a built-in digital camera[10].

Phytochemical analysis: The qualitative and quantitative phytochemical characters were analyzed and determined using fresh leaves of the ten taxa identified in the herbarium were air-dried at a temperature between 36-38°C for two weeks. The dried samples were ground to powder and tested for the presence of phytochemical constituents following the following standard methods described as follows.

Alkaloid determination: Standard measurement procedures were used to weigh 5 g of fine powder of samples, weighed 250 mL of 10% acetic acid into ethanol and added to a covered beaker and allow to stand for 4 hrs. The filtered extract concentrates were made to pass through a water bath at 55°C to one-quarter of the original volume. The concentrated ammonium hydroxide was added to the extract in drops until the precipitation was complete. Then carefully collected and washed in dilute ammonium hydroxide and filtered. The residue obtained was dried and weighed. The weighed residue becomes the alkaloids[11].

Determination of saponin content: Grounded samples of fine powder weighing 20 g were put into a conical flask and 100 cm^3 of 20% aqueous ethanol was added and heated over a hot water bath at 55°C for 4 hrs with continuous stirring. The mixture was filtered and the residue was re-extracted with another 200 mL of 20% ethanol. The combined extracts were reduced to 40 mL over water bath at 90°C. The concentrate was then transferred into a 250 mL separatory funnel and 20 mL of diethyl ether was added and continuously shaken. The aqueous layer was retained while the ether layer was disposed of. The purification was repeated. This time about 60 mL of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The retained solution was heated in a water bath. After evaporation, the samples were dried in an oven to a constant weight. The dried samples are the saponins and is calculated as percentage[12].

Determination of total phenolic content: The concentration of phenolic content in dried samples of Cassiinae extracts was evaluated using standard spectrophotometric method of Folin-Ciocalteu assay. The reaction mixture consisting of 1 mL of extract and 9 mL of distilled water was taken into a volumetric flask measured at 25 mL, 1 mL of Folin-Ciocalteu phenol reagent was treated to the mixture. The volume was set at 25 mL. Standard solutions of gallic acid measuring (20, 40, 60, 80 and 100 µg/mL) were prepared and incubated for 90 min at room temperature and the absorbance for tests and standard solutions determine against the reagent blank at 550 nm with Infitek UV-visible. spectrophotometer SP-MUV6000 (Jinan, Shandong, China). The total phenolic content was expressed as mg of GAE/g of extract.

Determination of tanin content: The tannins were determined following standard methods by Folin-Ciocalteu. About 0.1 mL of the grounded plant extract was added to a volumetric flask (10 mL) containing 7.5 mL of distilled water and 0.5 mL of Folin-Ciocalteu, phenol reagent, 1 mL of 35% Na_2CO_3 solution and diluted to 10 mL with distilled water. The mixture was shaken vigorously and kept at room temperature of about 37-38°C for 30 min. Standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/mL) were prepared in the same manner as previous procedures explained earlier. The absorbance test and standard solutions weres measured against the blank at 75 nm with Infitek UV-visible spectrophotometer SP-MUV6000 (Jinan, Shandong, China). The tannin content measurements were expressed in terms of mg of GAE/g of extract[13].

Determination of total flavonoid content: The total flavonoid content was determined using the aluminium chloride colorimeter assay. The reaction mixture is made up of 1 mL of extract and 4 mL of distilled water was taken in a 10 mL volumetric flask. In the flask, 0.30 mL of 5% sodium nitrite was treated and after 5 min, 0.3 mL of 10% aluminium chloride was mixed. After 5 min, 2 mL of 1 M sodium hydroxide was treated and diluted to 10 mL with distilled water. Standard solutions of quercetin (20, 40, 60, 80 and 100 µg/mL) were prepared. The absorbance for test and standard solutions was determined against the reagent blank at 510 nm with Infitek UV-visible spectrophotometer SP-MUV6000 (Jinan, Shandong, China). The total flavonoid content was expressed as mg of QE/g of extract[14].

Determination of glycoside content: The total glycoside content was determined by adding 2.00 g of grounded sample to 20 cm^3 of water and heated for 5 min in a water bath, then, filtered using the Gem filter paper (12.5 cm). The following tests were carried out on the filtrate:

- Add 0.2 cm^3 of Fehling's solutions A and B were mixed with (5 cm^3) of the filtrate until it became alkaline (tested with litmus paper). A brick-red coloration upon heating showed presence of glycoside
- About 15 cm^3 of 1.0 M sulphuric acid was used to repeat the test in (a) and the variation in quantity of precipitate was evaluated and compared with that of test (a). High precipitate content in test B indicates the presence of glycoside[15]

Determination of terpenoid content: Measurement of the terpenoid content was investigated using the analytical method. Each powdered sample about (0.30 g) was weighed into a beaker and extracted for 2 hrs. A mixture of chloroform (2 cm^3) and concentrated tetra-oxo sulphate (VI) acid (3 cm^3) was added to 5 cm^3 of each extract to form a layer. The presence of a reddish-brown coloration at the interface indicates positive results for the presence of terpenoids[16].

Determination of steroids and phyto-steroids content: Glycoside is dissolved in 1% ferric sulphate solution in (5%) glacial acid. One or two drops of concentrated sulphuric acid are added. A blue colour develops due to the presence of deoxy sugar[17].

Determination of cardiac glycosides (Keller Killiani test): To 1 mL of plant extract equal volume of chloroform is added and subjected to 2-3 drops of concentrated sulphuric acid. The appearance of a brown ring indicates the presence of steroids and the appearance of bluish brown ring indicates the presence phyto-steroid[17].

Determination of anthraquinone: To determine the presence of anthraquinone content about 5.0 g of each sample extract were shaken with 10 mL of benzene in a test tube and filtered. As 5 mL of 10% ammonia was added to the filtrate. The mixture was shaken vigorously to obtain. pink, red or violet colour in the lower portion of the ammonia. This is an indication of the presence of free hydroxyl-anthraquinone[17].

Determination of quinones content: To 1 mL of plant extract, 1 mL of concentrated sulphuric acid was added. The formation of red color indicates the presence of quinones[17].

Determination of coumarin content: To 1 mL of plant extract, 1 mL of 10% NaOH was added. The formation of yellow colour indicates the presence of coumarins[13].

Data analysis: The data generated from the morphological and phytochemical characters were analyzed using descriptive statistics. Hierarchical cluster and principal component analysis were carried out to show the phenetic relationships among the taxa. Pair group clustering was measured using the R software version 4.4.0 for statistical computing and the graphics[13].

Principal component analysis (PCA): Principal component analysis (PCA), was used to examine relationships among several quantitative variables:

$$\text{PCA method used } Y = XB+E$$

where, Y is matrix comprising of 10 species from Cassiinae and 70 characters, X is matrix scores and B is matrix of Eigen vectors (latent vectors) which are themselves calculated from the data matrix of the character scores. The minimum Eigen criterion was used to select how many factors can best explain the matrix[18].

RESULTS

Macro-morphological studies: Vegetal and floral morphological characteristics were examined in ten species belonging to the subtribe Cassinae in Nigeria. The locality, collectors' name, voucher number and date of collection of specimens were inventoried from two herbaria in Ibadan, Oyo State (Forest Institute of Nigeria and University of Herbarium) as shown in Table 1. Results from Table 1, were used to produce a species distribution map illustrated in Fig. 1. All species investigated were found dominant in the Southwestern Region of Nigeria. *Senna obtusifolia* is dominant in the North Eastern region. While *Senna alata*, *Senna obtusifolia* and *Cassia sieberiana* are prominent in the North Western part. *Chamaecrista rotundifolia* and *Senna occidentalis* are the species commonly distributed in the North Central Guinea Savanna Region. Figure 2a-j were showing photograph of Cassiinae species in their natural habitat. Table 2 shows the localities of operational taxonomic units (OTUs) used for micro-morphological and phytochemical studies were all within the Ibadan metropolis as inventoried. Table 3 shows the qualitative macro-morphological characteristic features determined for the studied specimen. A total of 38 qualitative characters involving both vegetal and floral characters were examined. Varying features such as habit type, leaflet arrangement, venation, presence or absence of stipule, leaflet shape margin, apex and base, fruit color and seed shape, seeds per lobe number and inflorescence position were evaluated. Leaves of members of Cassiinae are characteristically alternately arranged, venation pinnate with presence or absence of stipule. Leaflet shapes are asymmetrical, sub-rotund or broadly obovate. Leaflet margins are majorly entire with apex round, acute or acuminate. The leaflet base is mostly rounded or cuneate. The fruit and seed color are brown at maturity, with several seeds per lobe ranging from 20-100 in number. The inflorescence is mostly racemose and scarcely paniculate, positioned either as terminal or axillary. The seed shapes of species vary as either flattened, obovate, or rhomboid. Table 4 shows results from measurements on the quantitative macro-morphological characters considered. Several observed variations such as leaflet length and width ranging from 0.62-29.62 and 0.34-9.86 cm, rachis (2.40-38.50 cm), petiolule (0.10-0.50 cm) and blade lengths (1.54-51.48 cm) were evaluated in each species and their mean values and standard error recorded.

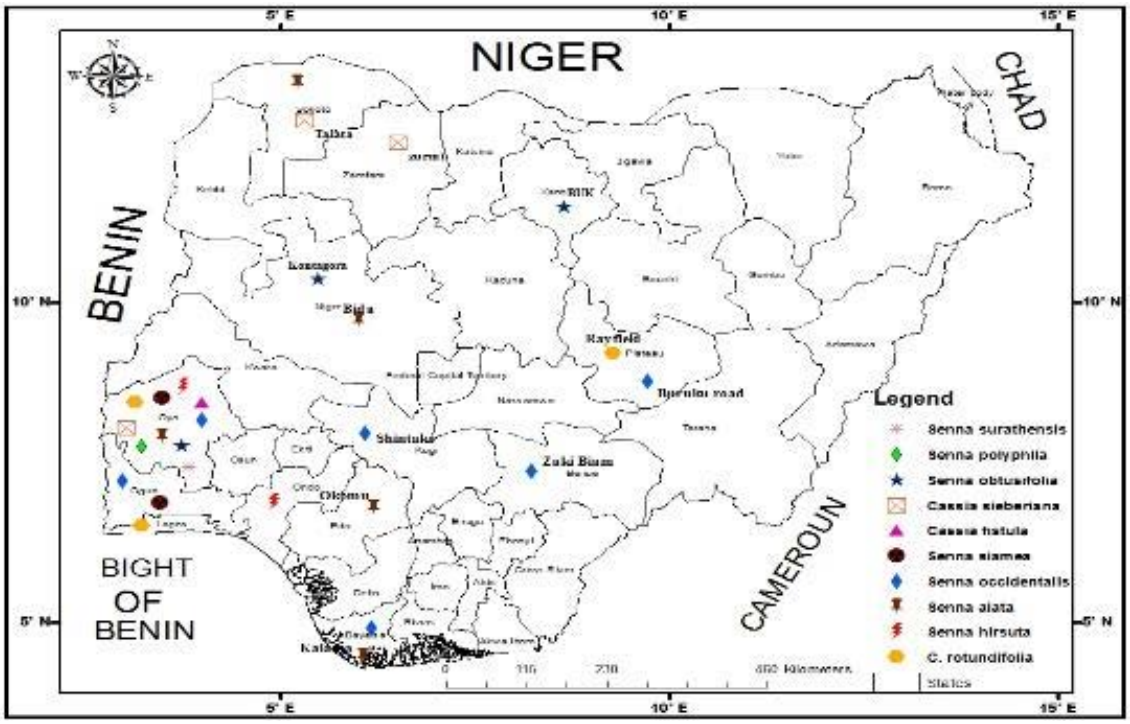

Fig. 1: Map showing the distribution of Cassiinae species in Nigeria

Anatomical studies: Figure 3a-e show the photomicrograph of epidermal leaf surfaces (adaxial and abaxial), main vein, lamina and petiolule of studied specimens. Figure 3 showed a summary of all the micro-morphological features examined for each OTUs studied. Table 5 describes the qualitative micro-morphological characters examined. Descriptive features such as cell shape, stomata shape, anticlinal wall pattern, cell wall striation, presence or absence of trichomes and presence or absence of crystals were investigated. The stomatal cells of examined specimens were mostly anisocytic and anomocytic. Cell shapes varied as either irregular or polygonal. Trichomes varied on either adaxial and abaxial surfaces of the epidermal cells, interestingly, trichomes were absent in *Cassia fistula*, *Cassia sieberiana* and *Senna polyphylla*. Crystalic structures were observed in *Senna siamea*, *Cassia fistula* and *Senna surattensis*. Table 6 shows the quantitative micro-morphological characters evaluated. The highest stomata index value was recorded in *Senna alata* (91.9%) and lowest in *Senna surattensis* (7.7%). The stomata density ranged from (0.00-102.5 m/sec^2). The highest stomatal length was evaluated in *Chamaecrista rotundifolia* (10.63 µm) and the lowest in *Senna obtusifolia* (0.75 µm). Variations in the transverse section of the lamina showed double-layer thick cells in all species examined except in *Chamaecrista rotundifolia*. Cell shapes in studied species were mostly cuboidal except in *C. rotundifolia* which had an elongated type. The outlines in the main vein were mostly protruding and shaped and were either elongated or cuboidal in most cases. Epidermis in the main vein was mainly collateral in all with exception of *Senna alata* which differed as bicollateral. Parenchymatous cortex in the main vein ranges from crescent to heart-shape with unique circular types found only in *Senna hirsuta*. The micro characters of the petiolule examined in all specimen indicated varying outline shapes such as U-shaped in OTU 1, 2, 3,4 5, 6, 7, 8, 9 and 10, undulated outline in *S. hirsuta* and *C. Sieberiana* and flat outline surfaces in *S. siamea*, *S. obtusifolia* and *S. surattensis*. Also, the sclerenchyma cells in the petiolule were mostly thin and scarcely thick with majority having small pith except in *Chamaecrista rotundifolia*. Delimitation within species using the petiolule characters were not holistic because of the absence of a well-defined petiolule structure in *Senna polyphylla*.

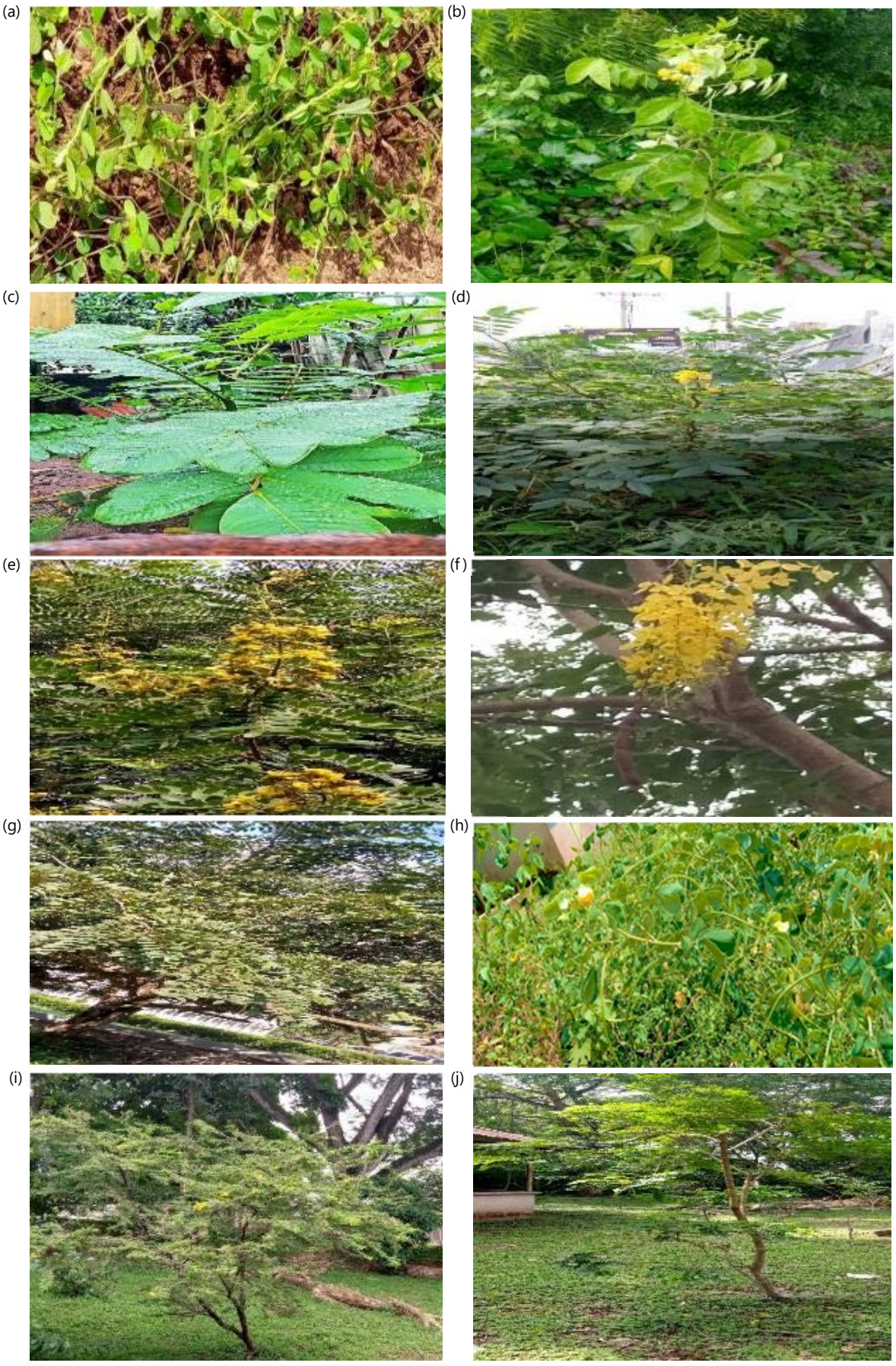

Fig. 2(a-j): Cassiinae species in their natural habitat, (a) *Chamaecrista rotundifolia*, (b) *Senna hirsute*, (c) *Senna alata*, (d) *Senna occidentalis*, (e) *Senna siamea*, (f) *Cassia fistula*, (g) *Cassia sieberiana*, (h) *Senna obtusifolia*, (i) *Senna polyphylla* and (j) *Senna surattensis*

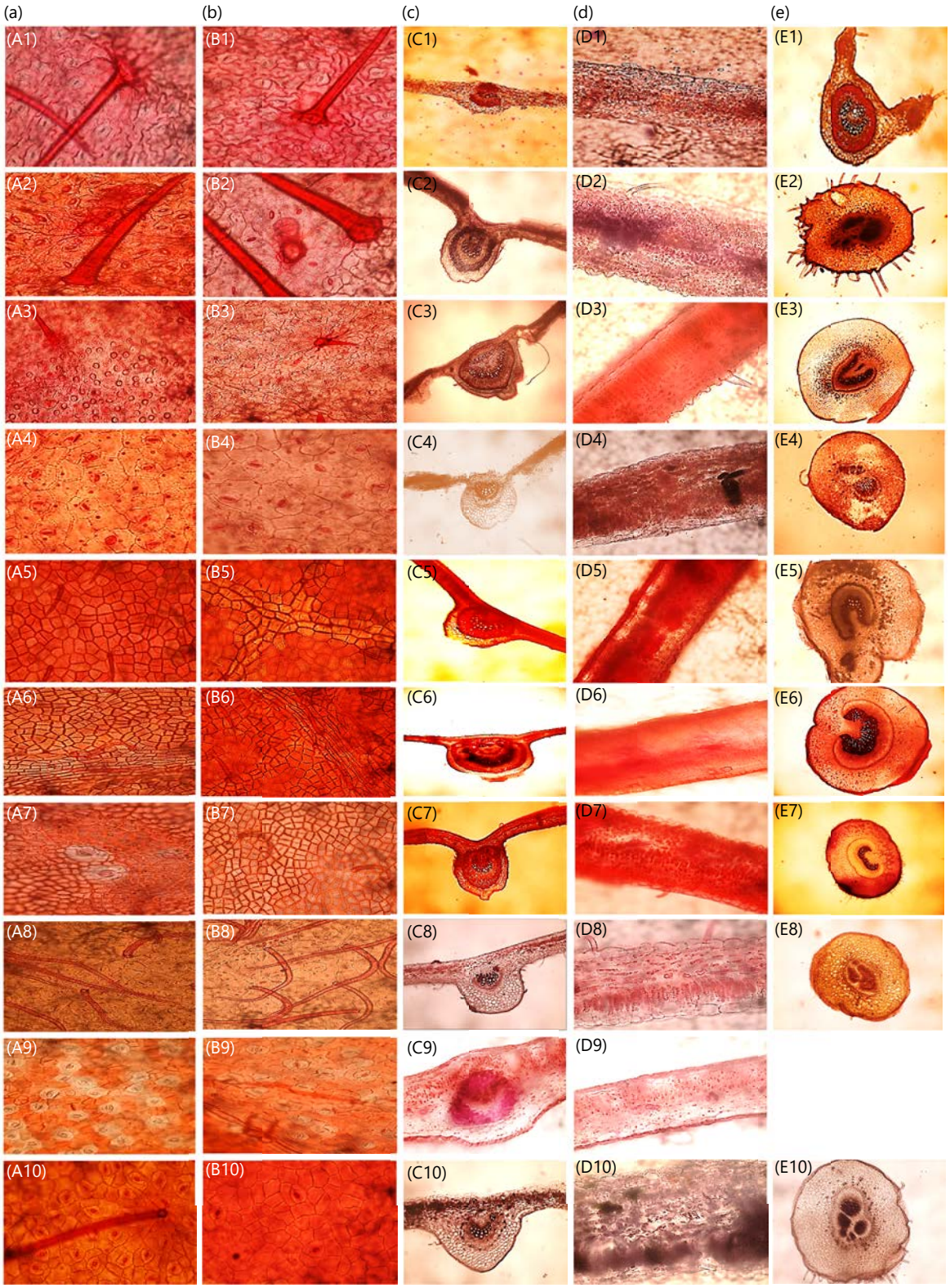

Fig. 3(a-e): (a) A1, A2, A3, A4, A5, A6, A7, A8, A9 and A10 photomicrograph of the leaf epidermal surfaces (abaxial) of ten Cassiinae species, (b) B1, B2, B3, B4, B5, B6, B7, B8, B9 and B10 photomicrograph of the leaf epidermal surfaces (adaxial) of ten Cassiinae species, (c) C1, C2, C3, C4, C5, C6, C7, C8, C9 and C10 photomicrographs of the transverse sections of the main vein (midrib) of Cassiinae species, (d) D1, D2, D3, D4, D5, D6, D7, D8, D9 and D10 photomicrographs of the transverse sections of the lamina of Cassiinae species and (e) E1, E2, E3, E4, E5, E6, E7, E8, E9 and E10 photomicrographs of the transverse sections of the petiolule of Cassiinae species

Table 1: List of herbarium specimens used for macro-morphological studies

Taxa	Locality	Collector's name	Collector(s)/voucher number	Date
Chamaecrista rotundifolia (Pers.) Greene	Bridge Embarkment Waterworks Ogbomosho Oyo	Dr. J. Lowe 799	UCI 2019	17/12/1965
	University of Ibadan Campus	Dr. J. Lowe 1017	UIH 2020	14/10/1966
	Roadside, Appleton Road, University of Ibadan	P.W. Richards	UIH 19181	03/11/1980
	Jos	G.S. Puri	UIH 2014	14/10/66
	Between Olobeweji and Ado Awaye	P.W. Richards	UIH 2013	3/11/1980
	Oyo Iseyin-Ado Road	A.A. Lady	UIH 2012	31/10/1962
	Ilora Oyo	G.W. Iveus	UIH 13316	12/4/1972
	Rayfield Jos Division	W.J. Howard	UIH 16448	19/08/1973
	Benue-Plateau State	Dr. G.S Puri.	UIH 2015	1/12/1962
	Gambari Forest Reserve	Sharma	UIH 19099	12/08/1971
	Lagos Waste Ground	Dr. J. Lowe 485	UIH 2018	24/11/1964
Senna hirsuta (L.) H.S. Irwin & Barneby	17 Miles Ore-Benin Province, Ondo District	J.F. Barker	UIH 1988	17/09/65
	Barthroad Student Village, UI	L.O. Ogunniran	UIH 20111	10/1/1983
	UI Second Gate by Road Side	A.O. Chivinge	UIH 19905	13/10/1978
	Student Village UI	K.T. Kumi	UIH 20106	10/01/1983
	UI Campus near Fish Pond	Aghachi	UIH 18159	6/01/1976
	Behind Botany Nursery	E.J. Enamuotor	UIH 18176	28/1/1974
	Behind Zoological Garden	A.M. Salaam	UIH 19919	13/10/1978
Senna alata (L.) Roxb.	Adeagba A.T. Apete Ibadan	E.A. Olayinola	UIH 23005	11/05/2021
	Okomu Forest National Park	M.K. Ayodele	UIH 22671	04/04/2018
	Sokoto	E. Willie	UIH 14265	29/10/1971
	Botany Department, UI	O.B, Oluwatobi	UIH 22376	13/10/2013
	Department of Botany	Allsworth Jones and Rosen	UIH 20072	13/02/83
	Kaiama Town, Kolokumi Area, Yenagoa Division	K.R.M. Williamson	UIH 15344	27/10/1973
	Pici Village 8 km West of Bida	R.M. Blench	UIH 19635	28/01/1982
	Botany Department	A.A. Ogunshe	UIH 22291	2008
Senna occidentalis (L.) Link	Ibadan	O.O. Omotayo	UIH 22503	22/03/2016
	University of Ibadan	C.I. Ogbonna	UIH 20144	1983
	Ibadan	K. Williamson	UIH 12968	17/06/1971
	Shintako Kabba	J.F. Redhead	UIH 21008	21/08/1964
	Near Kaiama in Town, Kolokuma Area (Yenagoa)	K.R.M. Williamson	UIH 15342	27/10/1973
	Ijebu Ode	J.A. Okusi	UIH 2005	28/08/1935
	Eleiyele Ibadan	Bamiyo	UIH 13433	10/07/1963
	Zaki, Biam, Benue Province	D. Vermeer	UIH 16731	October 1975
	Jos Bukuru Road	Dr. G.S. Puri	UIH 2007	25/07/1963

Table 1: Continue

Taxa	Locality	Collector's name	Collector(s)/voucher number	Date
Senna siamea (Lam.) H.S. Irwin & Barneby	Ejioku, North East Ibadan	A.B. Oyelami	UIH 21700	08/12/1991
	University of Ibadan Campus	J. Lowe	UIH 20053	02/02/1983
	Ijebu Ode	J.A. Okusi	UIH 2021	30/08/1937
Cassia fistula L.	BOT/MIC Department UI	J.B. Macjob	UIH 21615	23/05/1991
	Botany Department UI	Ezomoe Evelyn	UIH 21587	02/05/1991
Cassia sieberiana DC.	Behind Trenchard Hall, University of Ibadan	J. Lowe	UIH 20325	29/02/1984
	Aworo F.R. in High Forest in Family Open Place on Well Drained Gradualslope, Growing among *Dialium celtis* and *Antiaris*	B.O. Davimica	FHI 0027923	24/10/1968
	LAUTECH Premises Open Space	R.W.J. Keay and Forest Students	FHI 0109967	24/04/2014
	Zurmi Katsina Motor Road about 2 Miles from Zurmi	of Ibadan Forest school	FHI 0016135	12/04/1946
	Talata Mafora by Zaria-Sokoto Road	R.W.J. Keay	FHI 007555-2	26/06/1944
	Forest Hill, in Rest House Compound	J. Olorunfemi	FHI 0031502	13/101/1958
	Arboretum Forest Research Ibadan	H. Donyeachum	FHI 0054745	07/01/1965
	Forest Research Headquarters' compound, Ibadan.	M.G. Latilo	FHI 043899-1	15/03/1960
Senna obtusifolia (L.) H.S, Irwin & Barneby	Zaria Railway Side	G. Jochson	UIH 14264	30/11/1972
	Near Pond on New Site Bayero University, Kano	J. Lowe	UIH 20470	02/12/1984
	Salka, West of Kontagora.			
	Back of Physics	R.M. Blench	UIH 19595	November 1981
	Department, UI	P.P. Adeonipekim	UIH 21713	14/12/1991
	Kusoji Mokwa-Kontagora Road	Sharma	UIH 20978	19/09/1977
	Zaria-Kano Road	Sharma	UIH 20952	04/09/1977
Senna polyphylla (Jacq.) H.S. Irwin & Barneby	In front of main library, Botany University of Ibadan	J.O. Inegbedion	UIH 6630	09/11/1977
	Garden of British Deputy High Commissioner, Bodija, Ibadan	J. Lowe	UIH 18573	10/04/1979
	Akwanga Keffi Road	S.O. Oyewole	UIH 19050	November 1975
Senna surathensis (Brum. F.). Irwin & Barneby	Gambari Forest Reserve, Teak Plantation Area	Mr A. Adebambo	FHI 0108182	14/12/2007
	University of Ibadan, Botanical Gardens	M.G latilo	FHI 0043497	23/10/1959
	Ibadan University Medical College (U.M.C) Compound Tree	R.W.J. Keay	FHI 0025685	14/03/1950

Table 2: Operational taxonomic units (OTUs) and locality used for morphological and phytochemical studies

OTUs	Species	Locality
1	*Chamaecrista rotundifolia* (Pers.) Greene	Behind Botanical Garden, University of Ibadan, Ibadan
2	*Senna hirsuta* (L.) H.S. Irwin & Barneby	Agric Farm, University of Ibadan
3	*Senna alata* (L.) Roxb.	Abadina, shoe maker junction and front of Zik Hall, both inside University of Ibadan Campus
4	*Senna occidentalis* (L.) Link	Barika, Agbowo, Opposite University of Ibadan second gate
5	*Senna siamea* (Lam.) H.S. Irwin & Barneby	Besides Agric Farm University of Ibadan
6	*Cassia fistula* L.	Department of Botany, University of Ibadan
7	*Cassia sieberiana* DC.	Department of Agronomy, University of Ibadan
8	*Senna obtusifolia* (L.) H.S. Irwin & Barneby	Bank of Ajibode River, Behind Botanical Garden, University of Ibadan
9	*Senna polyphylla* (Jacq.) H.S. Irwin & Barneby	Department of Botany, University of Ibadan
10	*Senna surattensis* (Burm. F.). Irwin & Barneby	Botanical Garden, University of Ibadan

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Table 3: Qualitative macro-morphological characters of species in Cassiinae

Character	*Chamarcrista rotundifolia*	*Senna hirsuta*	*Senna alata*	*Senna occidentalis*	*Senna siamea*	*Cassia fistula*	*Cassia sieberiana*	*Senna obtusifolia*	*Senna polyphylla*	*Senna surattensis*
Habit	Herbaceous	Herb	Shrub	Herb	Tree	Tree	Tree	Herb/Undershrub	Shrub	Shrub
Leaflet shape	Asymmetrically sub-rotund to broad obovate	Lanceolate	Oblong-obovate	Ovate to ovate-oblong	Oblong to ovate-oblong	Ovate	Elliptic-oblong	Obovate	Elliptic-oblong	Ovate to ovate-oblong
Leaflet margin	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Leaflet apex	Round/mucronate	Acutely acuminate	Rounded	Aute or acuminate	Rounded	Acute	Subacute	Acute	Rounded	Bluntly emarginate
Leaflet base	Asymmetrical	Acute or round	Obliquely truncated or semi-cordate	Obtuse	Rounded to cuneate	Cuneate	Rounded or broadly cuneate	Cuneate to acute	Rounded to cuneate	Cuneate to rounded
Leaflet surface above	Ciliate	Villous	Glabrous	Glabrous	Glabrous	Glabrous and shining	Paler and rarely pubescent	Glabrous	Glabrous	Glabrous
Leaflet surface beneath	Pubescent	Villous	Carinate	Dull glabrous	Rough to delicately pubescent	Glabrous	Glabrous	Pubscent	Glabrous	Appressed pubscent
Leaflet texture	Membranous	Chartaceous	Chartaceous	Membranous	Sub coriaceous	Sub coriaceous	Sub coriaceous	Membranous	Chartaceous to membranous	Chartaceous to membranous
Leaflet venation	Pinnate	Pinnate	Pinnate	Pinnate	Pinnate	Pinnate	Pinnate	Pinnate	Pinnate	Pinnate
Leaflet arrangement	Alternate	Alternate	Alternate	Alternate	Alternate	Alternate	Alternate	Alternate	Alternate	Alternate
Leaf forms	Pinnately compound	Pari pinnately compound	Pari pinnately compound	Pari pinnately compound	Pari pinnately compound	Pari pinnately compound	Pari pinnately compound	Pari pinnately compound	Even pinnately compound	Pari pinnately compound
Petiole	Round	Stout	Terete	Subterete	Slightly grooved	Round	Elliptic,ovate, obovate-elliptic	Round	Pubscent	Sturdy
Petiole surface	N/A	Villous	Pubscent	Glabrous	Glabrous	Glabrous	Finely pubescent	Glabrous	Pubscent	Glabrous
Stipule shape	Lanceolate -cordate	Triangular	Asymmetrically triangular	Lanceolate	Subulate	Deltoid	Narrowly triangular	Linear	Subulate	Linear falcate
Stipule surface	Fallacious	Densely pubescent	Glabrous	Glabrous	Pubscent	Glabrous	Caducous	Membranous	Glabrous	Pubscent
Floral gland	Present	Present	Present	Present	Absent	Absent	Absent	Present	Present	Present
Bract	Persistent	Dischitous	Obovate	Lanceolate	Obovate	Ovate	Linear lanceolate	Linear acute	Ovate acute	Ovate acute
Bracteole	Present	Present	Absent	Absent	Absent	Present	Present	Absent	Absent	Present
Sepal shape	Lanceolate	Obovate/rounded	Obovate	Ovate	Rounded-ovate	Ovate, blunt	Elliptical	Ovate	Ovate	Ovate to orbicular
Sepal surface	Ciliate	Membranous-glabrous	Membranous	Membranous	Pubscent	Glabrous	Slightly pubescent	Membranous	Membranous	Glabrous
Petal shape	Obovate	Obovate	Obovate to orbicular	Obovate to spathulate	Orbicular to obovate	Obovate	Elliptical or ovate	Ovate, obtuse or round	Ovate	Ovate to oblong
Petal surface	Glabrous	Membranous to glabrous	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous to pubescent	Pubscent

Table 3: Continue

Character	*Chamarcrista rotundifolia*	*Senna hirsuta*	*Senna alata*	*Senna occidentalis*	*Senna siamea*	*Cassia fistula*	*Cassia sieberiana*	*Senna obtusifolia*	*Senna polyphylla*	*Senna surattensis*
Stigma type	Lateral	Terminal	Involute	Lateral	Subtermin, punctiform	Punctiform	Terminal/ subterminal	Terminal	Subterminal	Lateral
Style apex	Glabrous	Short	Pruinose	Glabrous	Stout	Sturdy	Slender	Glabrous	Glabrous	Glabrous
Anther shape	Linear oblong	Curved/beaked	Curved, swollen and long	Curved	Long and nearly straight	Long (longitudinal rim)	Dorsifixed	Base knob like attachment	Lateral ridged	Equal and straight with lateral longitudinal rim
Filament	Very short	Flat winged	Long	Long	Long	Long (towards the middle)	Longer exceeding	Slender and glabrous the petal	Curved	Thick and glabrous
Stamen	Unequal	Erect	Broadly elliptical	Erect	Glabrous	Curved	Unequal	Beaked and same size	Curved	Thick glabrous
Pedicel	Filiform	Pubscent	Sparely pubescent	Pubscent	Sturdy	Elongated	Deflexed lower	Thinly pubescent	Long	Sparsely pubscent
Ovary	Ovary	Tomentose	Grooved	Appressed	Stipitate	Thinly appressed	Stipitate	Strigulate	Stipitate	Curved
Fruit colour	Blackish brown	Brown	Dark brown	Dark brown	Brown	Dark brown	Dark brown -black	Brown	Brown	Brown
Fruit shape	Linear oblong	Flat and angular (subterete)	Winged and subterete	Subterete and sickle shaped	Flat	Terete	Straight and terete	Terete	Flat	Flat
Fruit surface	Glabrous	Ribbed hirsute	Chartaceous	Densely hirsute	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous
Fruit dehiscence	Elastically dehiscent valve coiling	Dehiscent	Dehiscent	Dehiscent	Indehiscent	Indehiscent	Indehiscent	Dehiscent	Dehiscent	Dehiscent
Inflorescence type	Racemose	Paniculate	Racemose	Paniculate	Paniculate	Racemose	Racemose	Racemose	Paniculate	Racemose
Inflorescence position	Axillary	Axillary or rarely terminal	Axillary	Axillary and terminal	Dense corymb	Axillary/terminal	Axillary	Axillary/ solitary	Axillary	Axillary
Seed colour	Pale brown	Dark olive	Brown	Brown	Light brown	Glossy brown	Brown	Glossy dark brown	Dark brown	Blackish smooth
Seed shape	Rhomboid to rectangular	Ovate	Obovate	Oval	Flat or oval	Flatten/transverse, ovate	Ellipsoid	Septate/rhomboid	Oval	Flattened
Number of seeds per lobe	Small seeds	50-80	20-35	25-35	20-30	40-100	Many	20-50	10-20	20-35

Table 4: Quantitative macro-morphological characters of selected species in Cassiinae

Taxa	Leaflet length	Leaflet width	Rachis length	Petiolule length	Blade length	Length/width ratio	Blade/rachis ratio
Chamaecrista rotundifolia	1.42±0.09	0.96±0.07	0.00±0.00	0.34±0.02	4.02±0.24	1:1	N/A
Senna hirsuta	5.01±0.38	2.28±0.14	12.04±0.79	0.11±0.01	10.98±0.69	3:2	6:7
Senna alata	13.62±2.66	6.61±0.80	25.53±2.52	0.18±0.03	30.37±3.75	7:4	16:13
Senna occidentalis	4.96±0.27	1.93±0.11	11.60±0.61	0.17±0.02	11.49±0.51	3:2	1:1
Senna siamea	4.67±0.89	1.79±0.13	15.89±5.20	0.23±0.03	11.13±1.76	3:1	3:4
Cassia fistula	10.13±0.31	5.13±0.13	24.97±6.23	0.53±0.01	24.68±0.78	2:1	1:1
Cassia sieberiana	4.89±0.59	2.39±0.28	15.77±2.51	0.41±0.04	11.93±1.18	3:2	3:4
Senna obtusifolia	2.33±0.63	1.36±0.40	4.52±0.75	0.18±0.03	5.49±1.77	2:1	1:1
Senna polyphylla	0.67±0.04	0.37±0.02	3.76±0.38	0.00 ±0.00	1.57±0.03	1:1	1:2
Senna surattensis	5.25±0.80	2.57±0.36	12.95±0.15	0.25±0.05	12.75±1.20	3:2	1:1

Mean value±Standard Error of the Mean

Table 5: Qualitative leaf micro-morphological characters of species in the Cassiinae

	Cell shape		Stomata type		Anticlinal wall pattern		Cell wall striation		Trichome		Crystal	
Taxa	AD	AB	AD	AB	AD	AB	AD	AB	AD	AB	AD	AB
Chamaecrista rotundifolia	Irregular	Irregular	Anomocytic	Anomocytic	Undulate	Undulate	-	-	+	+	-	-
Senna hirsuta	Irregular	Irregular	Anomocytic	Anisocytic	Straight and curved	Undulate	+	-	+	+	-	-
Senna alata	Irregular	Irregular	Anisocytic	Anomocytic	Undulate	Curved	-	+	+	-	-	-
Senna occidentalis	Polygonal	Polygonal	Anisocytic	Anisocytic	Straight and curved	Straight and curved	+	-	-	+	-	-
Senna siamea	Polygonal	Polygonal	Paracytic and anisocytic	Anisocytic	Straight	Straight	+	-	+	+	+	-
Cassia fistula	Polygonal	Polygonal	Absent	Absent	Straight	Straight	-	+	-	-	+	-
Cassia sieberiana	Polygonal	Polygonal	Absent	Anisocytic	Straight	Straight	-	-	-	-	-	-
Senna obtusifolia	Irregular	Irregular and polygonal	Anisocytic	Anisocytic	Curved	Curved	-	-	+		-	-
Senna polyphylla	Irregular	Polygonal	Anisocytic and anomocytic	Anisocytic and anomocytic	Undulate	Straight	+	-	-	-	-	-
Senna surattensis	Polygonal	Irregular	anomocytic	Anisocytic	Straight	Straight and curved	-	-	+	+	+	-

AB: Abaxial, AD: Adaxial, +: Present and -: Absent

Table 6: Quantitative leaf micro characters of species in Cassiinae

	Number of cells/×400 area of view		Epidermal cell length (µm)		Epidermal cell width (µm)		Stomatal density per ×400 area of view		Stomatal length (µm)		Stomatal width (µm)		S.I (%)	
OTUs	AD	AB	AD	AB	AD	AB	AD	AB	AD	AB	AD	AB	AD	AB
1	10.75±3.99	125.25±9.08	28.93±1.90	31.25±1.72	23.13±0.82	25.10±0.53	53.25±5.18	45.25±4.43	1.70±0.05	4.94±0.66	1.33±0.05	1.28±0.03	81.31	66.17
2	140.25±11.76	222.75±8.44	29.75±1.56	30.00±1.86	25.75±1.29	22.75±1.02	39.00±3.34	126.00±7.91	2.48±0.04	2.40±0.08	1.68±0.11	1.88±0.16	55.91	90.14
3	191.75±6.71	291.25±9.62	23.25±1.97	16.00±1.19	22.25±1.84	14.00±0.41	79.00±3.60	172.00±12.44	2.20±0.06	2.18±0.15	1.40±0.18	1.20±0.06	75.49	91.88
4	164.50±8.10	218.00±9.85	29.75±1.60	31.25±1.41	26.50±1.07	30.25±1.26	67.75±4.75	119.75±5.87	2.08±0.13	2.13±0.10	1.35±0.06	1.20±0.05	72.86	88.76
5	222.25±14.12	298.00±50.65	28.25±1.35	26.25±2.30	21.25±1.76	19.25±2.08	19.50±6.13	45.50+8.69	2.03±0.10	2.18±0.09	1.3±0.06	1.33±0.07	20.42	40.94
6	340.0±23.31	314.75±9.14	23.00±1.33	24.25±1.58	13.25±0.38	15.25±1.20	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
7	338.25±17.45	381.00±17.87	13.00±0.73	18.75±2.02	12.00±0.33	11.25±0.42	Absent	12.00±1.66	Absent	2.45±0.09	Absent	1.10±0.06	Absent	7.68
8	156.25±14.21	191.50±15.18	30.75±1.45	4.89±1.55	19.75±1.15	24.00±1.00	55.25±4.87	42.00±7.91	1.23±0.08	1.80±0.10	0.35±0.04	0.17±0.06	66.51	43.19
9	256.25±0.31	248.50±0.20	25.25±0.06	24.75±0.16	10.00±0.09	23.75±0.14	66.75±0.22	79.75±0.39	2.35±0.15	2.13±0.19	1.1±0.13	1.23±0.14	52.6	61.27
10	192.25±13.65	280.75±12.55	28.75±1.08	30.25±2.05	25.25±0.73	27.50±1.55	9.25±2.47	118.50±4.65	2.73±0.08	2.50±0.14	2.13±0.09	1.40±0.06	12.02	74.85

AB: Abaxial, AD: Adaxial, S.I (%): Stomatal index percentage and OTUs: Operational taxonomic units represents each species studied

Table 7: Qualitative phytochemical characters of species in the Cassiinae

Taxa	SAP	ALK	FLA	TAN	COM	STD	TPD	CARD	PHL	QUN	GLY	PSD	ANTH
Chamaecrista rotundifolia	+	+	+	+	+	+	+	-	+	+	-	+	-
Senna hirsuta	+	+	+	+	+	+	+	-	+	+	-	+	-
Senna alata	+	+	+	+	+	+	+	-	+	+	+	+	-
Senna occidentalis	+	+	+	+	+	+	+	-	+	+	-	+	-
Senna siamea	+	+	+	+	+	+	+	+	+	+	-	+	-
Cassia fistula	+	+	+	+	+	+	+	-	+	+	-	+	-
Cassia sieberiana	+	+	+	+	+	+	+	-	+	+	-	+	-
Senna obtusifolia	+	+	+	+	+	+	+	-	+	+	-	+	-
Senna polyphylla	+	+	+	+	+	+	+	+	+	+	+	+	-
Senna surattensis	+	+	+	+	+	+	+	+	+	+	+	+	-

SAP: Saponins, ALK: Alkaloids, FLA: Flavonoids, TAN: Tannins, COM: Coumarins, STD: Steroids, TPD: Terpenoids, CARD: Cardiac glycosides, PHL: Phenols, QUN: Quinones, GLY: Glycosides, PSD: Phyto-steroids, ANTH: Anthraquinones, -: Absent and +: Present

Table 8: Quantitative phytochemical characteristics of species in Cassiinae

Taxa	Saponins (%)	Flavonoids (mg/g)	Alkaloids (%)	Tannins (mg/g)	Phenols (mg/g)
Chamaecrista rotundifolia	2.57	26.78±0.38	17.05	3.20±0.07	6.76±0.04
Senna hirsuta	1.09	20.23±0.31	18.10	2.61±0.03	2.99±0.06
Senna alata	2.19	42.31±0.44	8.62	5.46±0.02	11.97±0.07
Senna occidentalis	1.55	34.05±0.38	10.95	4.32±0.03	4.99±0.04
Senna siamea	1.10	32.83±0.26	27.70	2.35±0.02	4.84±0.06
Cassia fistula	1.95	30.91±0.38	25.40	2.67±0.03	6.36±0.06
Cassia sieberiana	5.75	46.87±0.31	0.70	11.69±0.00	24.38±0.04
Senna obtusifolia	1.50	26.21±0.62	14.05	3.79±0.03	7.01±0.04
Senna polyphylla	3.25	32.19±0.38	10.45	7.13±0.04	14.84±0.03
Senna surattensis	4.90	34.47±0.31	21.40	10.40±0.00	19.26±0.04

Phytochemical analysis: Table 7 and 8 show the qualitative and quantitative phytochemical characters evaluated for species in the subtribe Cassinae. Results from qualitative analysis indicated the presence of phytochemical metabolites such as saponins, alkaloids, flavonoids, tannins, coumarins, steroids, terpenoids, phenols, quinones and phyto-steroids in all ten species studied. Other organic metabolites such as glycosides were present in *Senna alata*, *Senna polyphylla* and *Senna surattensis*. While cardiac glycosides were present in *Senna Siamea, Senna polyphylla* and *Senna surattensis*. The quantity of phytochemical metabolites examined ranges from highest to lowest in the following phytochemical compounds; saponins (5.75-1.09%)], alkaloids (25.40-0.70%), flavonoids (47.44-26.07 mg/g), tannins (11.69-2.31 mg/g) and phenols (4.74-24.44 mg/g).

Cluster and principal component analysis: Figure 4 is dendrogram showing the phenetic relationship among studied species using morphological characters along four groups were produced. Group 1 contains *Chamaecrista rotundifolia* only, group 2 contains *Senna polyphylla*, *S. obtusifolia* and *S. surattensis*. Group 3 contains *C. fistula*, *S. siamea* and *C. sieberiana*, while group 4 contains *S. hirsuta*, *S. alata* and *S. occidentalis*. However, Fig. 5 shows phenetic relationship among species using average linkage analysis of anatomical characters. the dendrogram for anatomical characters produced for groups. Group one contains *C. fistula*, *S. siamea* and *S. sieberiana*. Group two contains *S. Polyphylla* only, group three contains *S. occidentalis* and *S. surattensis*, group four contains only *C. rotundifolia* while group five contains *S. obtusifolia*, *S. hirsuta* and *S. alata*. Figure 6 shows phenetic relationship of phytochemical characters. The dendrogram produced contains four groups. Group 1 (*S. hirsuta*, *C. rotundifolia* and *S. obtusifolia*), group 2 (*S. siamea*, *S. occidentalis* and *C. fistula*), group 3 (*C. sieberiana*) and group 4 (*S. alata*, *S. polyphylla* and *S. surattensis*). The dendrograms produced in Fig. 7 illustrates the clustering of 10×96 data matrix of all data evaluated in the three data sets (morphology, anatomy and phytochemistry). Three clusters were formed. Cluster 1 contains *Cassia fistula* and *Cassia sieberiana*. Cluster 2 contains *Chamaecrista rotundifolia* alone. Cluster 3 contains *Senna hirsuta*, *Senna alata*, *Senna polyphylla*, *Senna occidentalis*, *Senna siamea*, *Senna obtusifolia* and *Senna surrattensis*. The three clusters formed represent the three genera (*Cassia*, *Senna* and *Chamaecrista*).

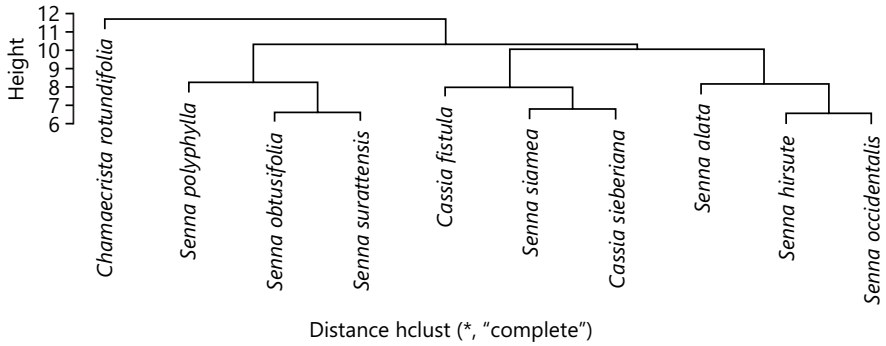

Fig. 4: Dendrogram showing phenetic relationship among species of the Cassiinae using average linkage analysis of morphological characters

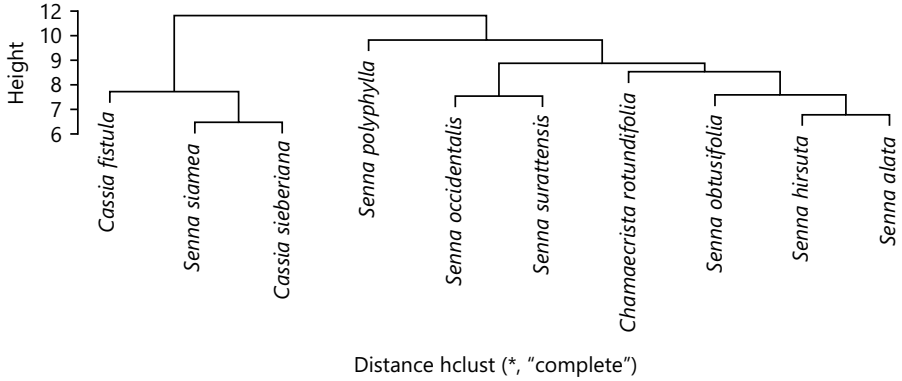

Fig. 5: Dendrogram showing phenetic relationship among species of the Cassiinae using average linkage analysis of anatomical characters

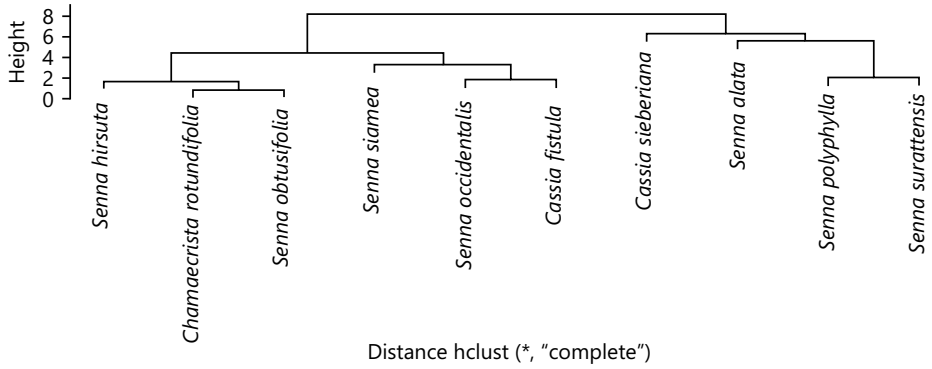

Fig. 6: Dendrogram showing phenetic relationship among species of the Cassiinae using average linkage analysis of morphology, anatomy and phytochemical characters

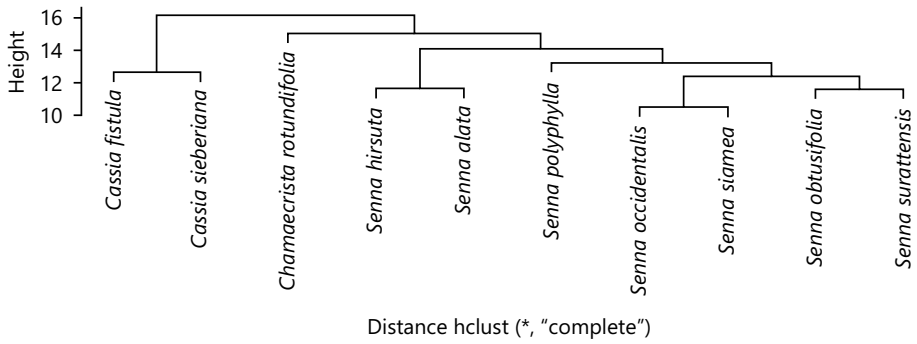

Fig. 7: Dendrogram showing phenetic relationship among species of the Cassiinae using average linkage analysis of morphology, anatomy and phytochemical character

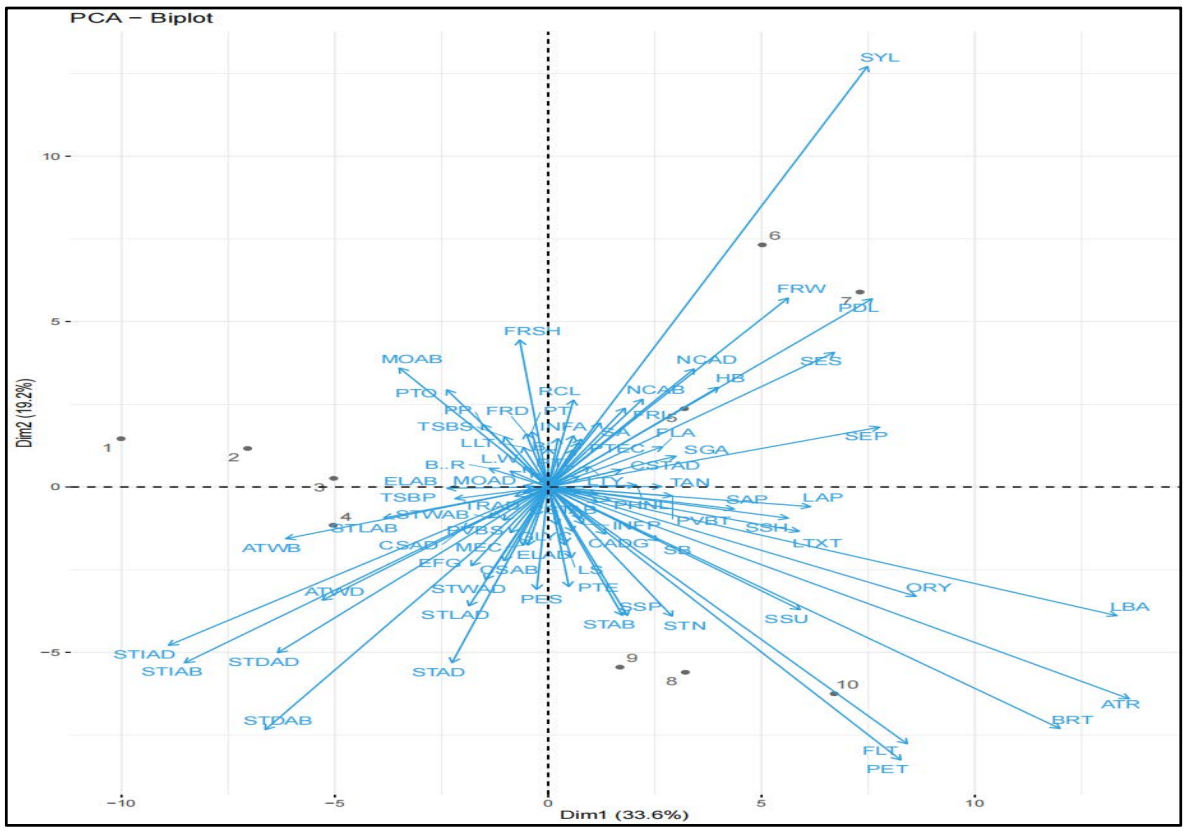

Fig. 8: Two-dimensional model of principal component analysis (PCA) obtained from the ordination of principal component axis of I, II, III and IV for 96×10 morphological, anatomical and phytochemical data matrix. Numbers 1 to 10 corresponds to OTUs as in Table 2

Table 9: Squared Euclidean distance proximity matrix for 10×96 scored characters in ten species of Cassiinae

OTU	1	2	3	4	5	6	7	8	9
2	13.30792								
3	15.01758	11.96474							
4	12.56871	11.11334	12.02288						
5	14.32440	12.16883	12.96011	10.61969					
6	16.85361	15.97632	15.38459	14.23571	11.62061				
7	17.19873	15.07660	14.93372	14.46792	11.63000	13.11997			
8	13.62632	13.12539	13.03021	12.25797	12.12502	15.27391	14.71405		
9	15.05855	14.82971	14.38228	12.90279	12.97197	16.10664	14.65222	13.81268	
10	15.89423	14.22384	14.80636	12.85518	11.52693	15.06101	14.03293	11.93223	13.82951

Figure 8 showing two-dimensional model of Principal Component Analysis (PCA) obtained from the ordination of principal component axis of I, II, III, IV for 96×10 morphological, anatomical and phytochemical data matrix. Numbers 1 to 10 correspond to OTUs as in Table 2. The OTUs that occurred in each of the axes of the chart include; axis I contains OTUs 5, 6 and 7 (*Senna siamea*, *Cassia fistula* and *Cassia sieberiana*), axis II contains OTUs 8, 9 and 10 (*Senna obtusifolia*, *Senna polyphylla* and *Senna surattensis*), axis III contains OTUs 4 (*Senna occidentalis*) and axis IV contains OTUs 1, 2 and 3 (*Chamaecrista rotundifolia*, *Senna hirsuta* and *Senna alata*). Although almost all OTUs in each axis were not in clusters, they still appeared on the same axis or group. The squared euclidean distance proximity matrix for 10×96 characters in ten species in the Cassiinae was shown in Table 9. The Eigen values, variance and cumulative variance percentage for ten character matrix was shown in Table 10 using the combined data sets from anatomical, morphological and phytochemical features. Eigen value was highest in OTU 5 (9.31) and variance percentage was highest in OTU 4 (9.50%), followed by OTU 5 (8.10%).

Table 10: Eigen values, variance and cumulative variance percentage for ten (10) OTU character matrix

OTU	Eigen value	Variance (%)	Cumulative variance (%)
1	3.858952e+01	3.355286e+01	33.55286
2	2.210055e+01	1.921601e+01	52.76888
3	1.179316e+01	1.025393e+01	63.02281
4	1.092503e+01	9.499108e+00	72.52192
5	9.316319e+00	8.100364e+00	80.62228
6	7.363575e+00	6.402491e+00	87.02477
7	5.817797e+00	5.058465e+00	92.08324
8	4.968809e+00	4.320286e+00	96.40352
9	4.136348e+00	3.596477e+00	100.00000
10	2.527759e-30	2.197839e-30	100.00000

Table 11: Key to the species in the subtribe Cassiinae in Nigeria

1a	Herb shrub or tree with leaves pari pinnately compound, inflorescence raceme, venation pinnate, margin, entire, leaflet arrangement alternate.....1
2	Trees woody, with more than 10 m in height, bracteole present, leaf base rounded to broadly cuneate, leaf texture coriaceous, fruit indehiscent, leaf apex acute.....1. *Cassia fistula*
2	Leaf base rounded to cuneate, leaf texture coriaceous, fruit indehiscent leaf apex subacute .....2. *Cassia sieberiana*
3	Tree or shrub with less than 10 m in height with woody or herbaceous stem.....3
4	Bract shape obovate, bracteole absent, pedicel sparsely pubescent, cardiac glycosides present.....3. *Senna siamea*
4	Bract shape obovate, bracteole absent, pedicel sparsely pubescent cardiac glycosides absent .....4. *Senna alata*
5	Shrub with over 5 m in height.....5
6	Glycosides present, anticlinal wall pattern straight, inflorescence position fascicle .....5. *Senna polyphylla*
6	Glycosides present, anticlinal cell wall pattern straight and curved inflorescence position solitary.....6. *Senna surattensis*
7	Herb equal to 2 m in height herbaceous.....7
8	Stipule shape linear, stigma terminal, seed shape rhomboidal, epidermal cell shape irregular leaf surface above villous .....7. *Senna hirsuta*
8	Stipule shape linear, stigma terminal, seed shape rhomboidal, epidermal cell shape irregular, leaf surface above glabrous.....8. *Senna obtusifolia*
1b	Shrub or herb with leaves pinnately compound, inflorescence raceme, venation pinnate, margin entire, leaflet arrangement alternate.....9.
9	Shrub or herb equal or less than 2 m height 9
10	Petal shape obovate, dehiscent, fruits, pedicel pubescent, ...9. *Senna occidentalis*
10	Petal shape obovate, elastically dehiscent valve coiling fruit, pedicel filiform, ...10. *Chamaecrista rotundifolia*

The least value was obtained in OTU 3 as 1.03%. Cumulative variance was highest in OTUs 9 and 10, weighing a 100% each. Table 11 shows the key to the identification of species constructed based on characters of OTU evaluated in this work. It is important to create a very simple taxonomic key for identification and better understanding of shared or apomorphic characters.

DISCUSSION

The morphological, anatomical and phytochemical characters used for this study revealed the extent of variation and proved to be taxonomically relevant in the delimitation of selected taxa. The combination of characters from floral, vegetal, anatomy and phytochemical metabolites is employed to better understand the phenetic relationship among members of the subtribe Cassiinae. In Table 4-6 the dendrograms produced confirmed that taxonomic classification approaches do not rely on just morphological data but need to be used in combination with a wide variety of characters from different scientific fields to delimit and classify a particular taxon. Morphological data has been used successfully in combination with pollen characters to delimit the subtribe Cassinae[19]. The most informative morphological data sets identified in this work produced a great range of similarities. The PCA identified distinctiveness characters such as stomata index, stomata density, filament length, petal shape, bract

shape, nature of ovary, leaf base and anther contributing about 33.6% to the delimitation, while style apex, fruit width, pedicel, sepal surface and sepal shape contributed about 19.2%. The results in this work correspond with research on important morphological characters in several species of Cassiinae in South-Western Nigeria[20]. It is of interest to note that each data set analyzed separately which includes morphology, anatomy and phytochemical characters was not able to delimit the subtribe in isolation as shown in Fig. 4-6. In this study cluster analysis and principal component analysis are two component analytical tools used to trace relationships among members of the Cassiinae clade. The revelation of marked differences in the dendrogram produced for morphological, anatomical and phytochemical characters assessed is a strategic indication that one data character alone is insufficient to make a taxonomic conclusion that a particular group shares the same ancestral traits. The combination of multiple data characters was essential in the delimitation and classification of the groups. A similar approach was obtained in the numerical taxonomy of species of the subtribe Cassiinae reported by Kolawole *et al*.[21]. The difference in the clusters produced for phytochemical characters is a result of the different chemical affinity among studied taxa[22]. Results obtained from the combined data for morphology, anatomy and phytochemical characters produced a dendrogram that agreed with the taxonomic treatment of the subtribe by Irwin and Barneby[8] separating the species into distinct genera. This implies *Cassia* species, *Senna* species and *Chamaecrista* species are distinct genera and should be treated as such consequently supporting the polyphyletic nature of the subtribe. Principal Component Analysis (PCA) shows the following morphological characters contributed more to the closeness or affinity in the relationship among taxa petal shape, fruit length, bract, anther, leaf base, style and pedicel. The results also supported the report by the Legume Phylogeny Working Group on the Legume phylogeny and classification in the 21st century[23]. Hence qualitative floral morphological characters contributed more to delimitation and relationship among groups in this study. In the case of anatomical characters, the PCA analysis reveals that the anticlinal walls on both adaxial and abaxial surfaces, stomata density on adaxial and abaxial surfaces, stomata indices on adaxial and abaxial surfaces and petiolule vascular bundle shapes were a contributor to the variation among the taxa. The PCA analysis for phytochemical characters revealed that saponins were a major contributor to similarity in the group. Thus, phytochemical metabolic variations are significant characters in the classification of plants.

CONCLUSION

This research therefore has confirmed the autonomy and classifications of members of the subtribe Cassiinae into three generic categories using a combined data set which serves as a reliable database for natural classification of members of the group and suggests further findings by combining gross morphological and molecular data sets. This study has affirmed the importance of using multiple data sets to delimit members of a taxonomic clade. It is clear from the dendrogram produced for combined 10×96 data matrix for morphology, anatomy and phytochemical features that the systematic combination and analysis of data could serve as potentially significant criteria for identification but not a reliable delimitation tool when used in isolation for classification. The combination of the three data sets produced corresponding results similar to previous works on the subtribes and formed a reliable basis for an identification key.

SIGNIFICANCE STATEMENT

This study aimed to determine the taxonomic relationships among selected species in the subtribe cassiinae using the numerical taxonomic principles. The research objectives are focused on the evaluation of morphological and phytochemical character variations and determination of phenetic relationships among selected species in Cassiinae taxonomic key produced was relevant in the identification of the studied group. The findings in this work affirmed the importance of using multiple data sets to delimit members of a taxonomic clade. The combination of data sets from morphological, anatomical and phytochemical characters served as a reliable database for identification and natural classification. Further findings aimed to compliment and improve the traditional method of taxonomic delimitation used in this work is recommended.

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