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Full Length Research Paper

Pharmacognostic evaluation of the leaves and roots of *Cassia sieberiana* DC.

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***Cassia sieberiana* DC. (Leguminosae - Caesalpinioideae), commonly known as drumstick and 'aridantooro' in Yoruba, is a perennial tree native to Africa. It is used in ethno-medicine to manage arthritis and rheumatism. Pharmacological activities such as myorelaxant, antispasmodic, anti-inflammatory, and antimicrobial have been reported in literature. Pharmacognostic investigation including microscopy, chemomicroscopy, physicochemical analysis and phytochemical investigations including thin layer chromatographic finger printing were conducted on fresh and powdered leaf and root samples of this plant. The macro and microscopic studies revealed the leaves to be simple, petiolated, glabrous and pinnately veinnated. The lower epidermal surface is characterized by abundant anomocytic stomata, polygonal epidermal cells and numerous uniseriate, unicellular trichomes. Quantitative leaf analysis revealed the following: stomatal number (163.8), stomatal index (19.04), palisade ratio (17.01), vein islet number (56.45) and vein termination number (61.45). Chemomicroscopic characters present include lignins, tannins, mucilage, starch, oils and calcium oxalate crystals. The physicochemical parameters evaluated are moisture content of 6.3%, total ash of 4.2%, acid-insoluble ash of 3.4%, sulphated ash of 11.0%, water-soluble ash of 0.8%, alcohol-soluble extractive of 21.3%, and water-soluble extractive of 16.7%. Chromatographic fingerprints of ethanol 70% extracts show major spots at R_f = 0.18 daylight (brown), UV_{366} (deep brown), spray reagent at 100°C (brown); R_f = 0.57 daylight (brown), UV_{366} (deep brown), spray reagent at 100°C (brown); R_f = 0.89 daylight (green), UV_{366} (red), spray reagent at 100°C (brown). The pharmacognostic evaluation of the leaves of *C. sieberiana* is reported here for the first time. The results of this research provide information which can be included in official monograph of the plant for its proper identification and quality control.**

Key words: *Cassia sieberiana*, pharmacognostic studies, physicochemical studies, chemomicroscopy.

INTRODUCTION

Cassia sieberiana DC. (Leguminosae -Caesalpinioideae) is a shrub or small tree, 15 to 20 m tall; bole short, twisted; bark fissured, grey to brown, with blackish stripes and young branches densely hairy. It is wide spread in India and tropical Africa including northern and southern Nigeria, especially in cultivated or old clearings by the

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road side and open grassy areas (Dalziel, 1956; Irvine, 1961). It is commonly called Drumstick tree (English), Gama Fada, Marga (Hausa), Margaje (Fulani), Kiskatigrai (Kanuri), Apagban (Edo), Kuhuwa (Tiv), Efo, Ifo, Aridantooro (Yoruba) (Keay, 1964).

In different parts of Nigeria, various extracts of *C. sieberiana* have been used in ethnomedicine to manage tooth ache, jaundice, inflammatory conditions, tiredness and joint pains (Madusolumuo et al., 1999); fever, diarrhoea, leprosy, bilharzia, stomach pains, ulcers, infections, haemorrhoids, pleurisy or burns, and elephantiasis (Tamboura et al., 2005). Pharmacological studies have shown that various extracts have antimicrobial and antifungal activities (Asase et al., 2008); analgesic, anti-inflammatory, antiparasitic, antimalarial, myorelaxant, antispasmodic, and antisickling activities (Duwiejua et al., 2008; Sy et al., 2009; Fatokun et al., 2015).

The major secondary metabolites present in *C. sieberiana* are phenols, anthraquinones, alkaloids, glycosides, flavonoids and saponins (Hafiza et al., 2002). The anthraquinone glycosides are responsible for many of the medicinal properties observed in the plant, although anthraquinones have not been isolated from the plant (Akomolafe et al., 2003).

The evaluation of pharmacognostic and proximate parameters is very essential in establishing the quality, identity and purity of crude drugs. Pharmacognostic standards must be set for every crude drug to be included in a herbal pharmacopoeia. Moisture content is among the most essential and commonly used measurements in the processing, preservation and storage of medicinal plants (African Pharmacopoeia, 1986).

Ash values and extractive values are reliable tools in detecting adulteration and also help in establishing the purity of crude drugs. Ash from medicinal plants is the total sum of the residue remaining after all moisture has been removed as well as the organic material (such as fat, protein, carbohydrates, vitamins and organic acid) have been incinerated at a temperature of about 500°C. Extractive values are primarily useful for the determination and evaluation of the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in particular solvents (African Pharmacopoeia, 1986). Similar studies have been carried out on root samples collected outside Nigeria (Sam et al., 2013).

MATERIALS AND METHODS

Collection

Leaves and roots of *C. sieberiana* were collected from the Suleja Local Government Area of Niger State, Nigeria in January, 2016. The plant specimens were authenticated and a herbarium specimen was deposited at NIPRD Herbarium with Voucher number NIPRD/H/6736.

Chemicals, reagents and solvents

All chemicals, reagents and solvents used during the experimentation were of analytical grade.

Morphological evaluation

Leaf and root samples of *C. sieberiana* were subjected to macroscopic analysis, viz., organoleptic characteristics such as appearance, taste, colour, odour, shape, texture, fracture, etc., of the drug. These parameters are considered to be quite useful in quality control of the crude drug and were evaluated as specified by WHO guidelines (African Pharmacopoeia, 1986; WHO, 1992).

Microscopy

Microscopic analysis was carried out on the pulverized root, the adaxial and abaxial epidermal surfaces of leaves and the pulverized leaf samples. A quantity of each pulverized sample was cleared in chloral hydrate, mounted in glycerin-water (1:1) and viewed under the microscope at different magnifications. The method of Ugbabe and Ayodele (2008) was used to prepare epidermal surfaces of leaves. About 5 mm^2 to 1 cm^2 leaf fragments were obtained from the standard median portion of leaves and macerated in concentrated nitric acid in Petri-dish for 18 to 24 h. The appearance of air bubbles indicated the readiness of the epidermises to be separated. The fragments were transferred into water in a Petri-dish with a pair of forceps. The upper, lower epidermises and mesophyll were separated and cleaned using forceps and carmel hair brush. Each surface was transferred into 50% ethanol to harden and later stained with safranin O for 5 min. The excess stain was washed off in water and the epidermal peel was mounted on a slide with glycerin.

Chemomicroscopic studies

Chemomicroscopic studies of the pulverized leaf and root samples were carried out using reagents and stains like iodine, concentrated sulphuric acid, concentrated hydrochloric acid, ferric chloride, Sudan III, ruthenium red and phloroglucinol with concentrated HCl (1:1) to test for the presence of various metabolites (African Pharmacopoeia, 1986; Ugbabe and Ayodele, 2008).

Quantitative microscopy

The quantitative examinations of leaf samples such as vein islet number, vein termination number, palisade ratio, stomatal number and stomatal index were carried out using standard methods (African Pharmacopoeia, 1986; Ugbabe and Ayodele, 2008).

Physicochemical evaluation

Various physicochemical parameters such as moisture content, total and sulphated ash values, acid-insoluble and water-soluble ash values and water and alcohol extractive values were determined following WHO guidelines (African Pharmacopoeia, 1986; WHO, 1992).

Chromatographic fingerprinting

Analytical thin layer chromatography (TLC) was done on silica gel G60 F_{254}, 0.2 mm layer and KC18 silica gel 60 Å, 200 µm. The

Table 1. Macroscopic and organoleptic characteristics of *C. sieberiana* leaf and root.

Character	Observations			
	Leaf		Root	
	Fresh	Powder	Fresh	Powder
Colour	Dark green	Brownish green	Dark brown	Brownish yellow
Odour	Characteristic smell	Characteristic smell	Aromatic (Weak)	Aromatic (Strong)
Texture	Papery	-	Hard	-
Type	Simple	-	-	-
Taste	Bitter	Bitter	Tasteless	Tasteless
Apex	Acuminate	-	-	-
Shape	Ovate	-	Cylindrical	-
Surface	Smooth	-	Smooth	-
Base	Cordate	-	-	-
Venation	Pinnate	-	-	-
Size Length	5.8 - 6.5 cm	-	-	-
Width	2.7 - 3.8 cm			

Figure 1. (A) Leaf; (B) Fruit, (C) Root, and (D) Bark of *Cassia sieberiana*.

plates were developed, after spotting the ethanol (70%) extract at the origin, using solvent system dichloromethane: methanol (7:3). Detection was done in daylight, under UV_{366} and with 10% aqueous H_2SO_4 spray reagent. Plates were dried at 100°C after spraying. Retardation factor (R_f) of each spot was calculated (Nigerian Herbal Pharmacopoeia, 2008).

Photomicrography

Photomicrographs of different sections were taken at different magnifications (×100 and 400) using a Leica CM E microscope with a Digital Microscope Eyepiece attachment and Photo Explorer 8.0 SE Basic software.

Statistical analysis

The data obtained were expressed as mean ± standard error of mean (SEM), and n represents the number of replicates in an experiment.

RESULTS AND DISCUSSION

Macroscopic and microscopic methods are central to the identification of different parts of medicinal plants. Different morphological characters were observed on macroscopic examination of the leaf and root samples (Table 1).

The transverse section (TS), of the leaf showed the presence of covering trichomes on the upper epidermis; well-developed collenchyma cells were seen below the lower epidermis. The mid-region showed the vascular bundles (phloem and xylem) separated by the cambium, and also the pith parenchymatous cells (Figures 1 and 2).

Microscopy of *C. sieberiana* upper leaf surface showed polygonal epidermal walls and a few trichomes but no stomata. The lower epidermal surface was characterized by abundant anomocytic stomata, polygonal epidermal cells and numerous uniseriate, unicellular trichomes with large base and tapering ends. The presence of numerous stomata on just one surface of the leaf (hypostomatic) implies that transpiration takes place on the abaxial (lower) surface for photosynthesis and water loss. The leaf surface also showed the presence of palisade cells, vein- islets and vein-terminations (Figure 3). Leaf constants such as stomatal number, stomatal index, palisade ratio, vein-islet number and veinlet termination number were measured. These parameters, especially stomatal index, are important in the identification of different plants as they vary from plant to plant (Table 3). Chemomicroscopic evaluation of the comminuted leaf

Figure 2. Transverse section (×400) of *C. sieberiana* leaf showing: trichomes (t); epidermis(e); collenchyma (c); - xylem (x); pith parenchyma (p); phloem (ph); cambium (ca).

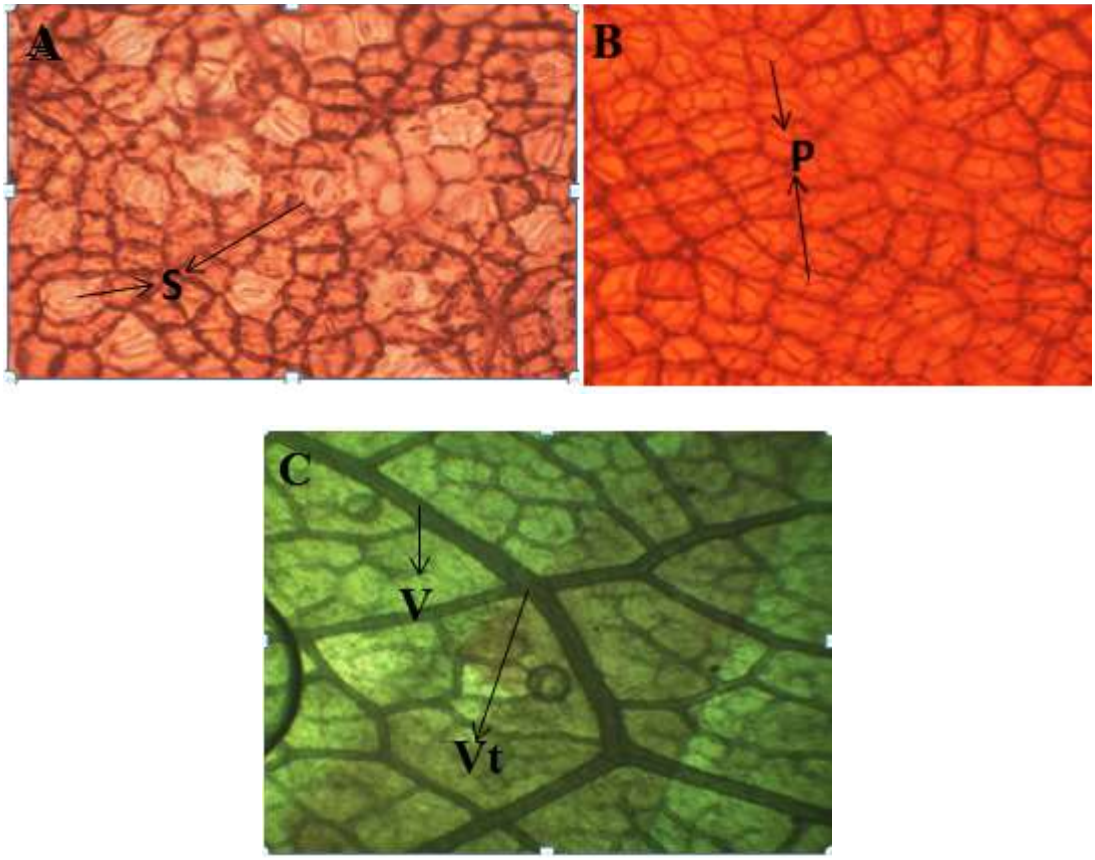

Figure 3. Microscopy (×400) of epidermis of *C. sieberiana* leaf showing: (A) anomocytic stomata (s) on abaxial surface; (B) palisade cells (p); (C) vein islet (v) and vein terminations (Vt).

and root of *C. sieberiana* indicated the presence of lignin, tannins, cellulose, starch and oils. Protein was absent in the leaves but present in the roots (Table 4).

Microscopy of comminuted leaf and root samples indicated the presence of tetragonal prism/rosette types of calcium oxalate crystals in leaf and prism type in root samples, respectively (Figures 4 and 5). Rosette crystals are formed from tetragonal calcium oxalate crystals (Trease and Evans, 2003). Other characteristic features of the comminuted roots are starch grains, pericyclic fibres reticulate xylem vessels and cork cells (Figure 6).

Moisture content obtained for *C. sieberiana* leaf and root were 6.34 and 4.54%, respectively. This suggests a low moisture content as it is lower than the limit for water content (8 to 14%) for vegetable drugs (African Pharmacopoeia, 1986).

Table 2. Physicochemical evaluation of *C. sieberiana* leaf and root (dry matter).

Parameter	Leaf (% w/w)	Root (% w/w)
Moisture content (n=6)	6.3±0.004	4.5±0.001
Total Ash (n=2)	4.2±0.0	7.4±0.3
Acid –insoluble Ash (n=2)	3.4±0.0	1.5±0.1
Water –soluble Ash (n=2)	0.8±0.0	2.6±0.1
Sulphated Ash (n=2)	11.0±0.0	8.3±0.3
Alcohol-soluble extractive (n=3)	21.3±0.3	19.4±0.5
Water -soluble extractive (n=3)	26.5±0.3	2.7±0.4

Table 3. Quantitative microscopy of *C. sieberiana* leaf.

Parameter	(Range) Mean ± SEM
Stomatal number: abaxial surface*	(149 -180) 163.8 ± 3.2
Stomatal number: adaxial surface	-
Stomatal index: abaxial surface*	19.0 ± 0.0
Vein-islet number+	(43-61) 56.5 ± 1.7
Vein-termination number+	(43-80) 61.5 ± 3.7
Palisade ratio+	17.1

*n= 10; +n = 4.

Table 4. Chemomicroscopic evaluation of *C. sieberiana* leaf and root.

Parameter	Leaf sample	Root sample
Lignin	+	+
Mucilage	+	+
Cellulose	+	+
Tannins	+	+
Starch	+	+
Calcium oxalate crystals	+	+
Oils	+	+
Proteins	-	+

Results indicate a high shelf life of the fresh plant. Results for ash analysis on dry matter of the leaves showed that total, acid-insoluble, water-soluble and sulphated ash were 4.17, 3.36, 0.85 and 11%, respectively while for values for the roots were: 7.4, 1.47, 2.6 and 8.9%, respectively (Table 2). Results are indicative of low inorganic contents though the values are subject primarily to the soil type/mineral composition of soil used to cultivate the plant. The extractive values obtained for the roots indicated that constituents were more efficiently extracted into 70% ethanol than aqueous solvent whilst the reverse was obtained for the leaves as shown in Table 2. It can be said that there are more constituents soluble in alcohol in roots than in leaves (Table 5). Results obtained for the physicochemical properties of roots vary from that reported by Ajayi et al. (2015), who reported a higher moisture content, 9.5% and much lower total, acid-insoluble and water-soluble ash values of 2.2, 0.4 and 0.5%, respectively. The results also vary slightly with those reported by Bello et al. (2016), who reported a higher moisture content, 6.2% and lower total, acid- insoluble and water soluble ash values of 5.8, 1.0 and 3.5%, respectively. This could be due to differences in geographical location (samples were collected from Jos, Plateau State and Giwa, Kaduna State, Nigeria, respectively), time of collection and varying mineral contents in the soil.

Result of chromatographic fingerprinting

Detection was in daylight, UV_{366nm} and 10% v/v aqueous H_2SO_4 spray reagent plates were dried at 100°C after spraying. Major spots were obtained as shown in Table 5. Results obtained for the physicochemical properties of roots vary from that reported by Ajayi et al. (2015), who reported a higher moisture content, 9.5% and much lower

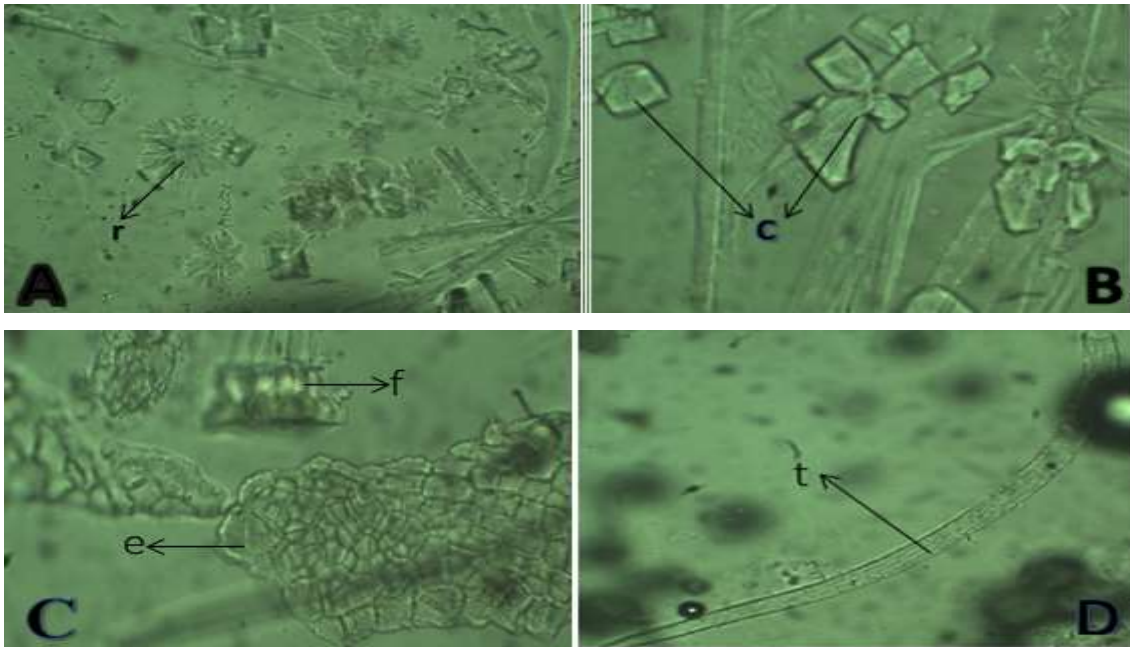

Figure 4. Microscopy of leaf powder of *C. sieberiana* (x400): A and B showing calcium oxalate crystals- rosette (r) and tetragonal crystals (c); C showing epidermal cells (e) and fibre (f) and D showing uniseriate trichome (t).

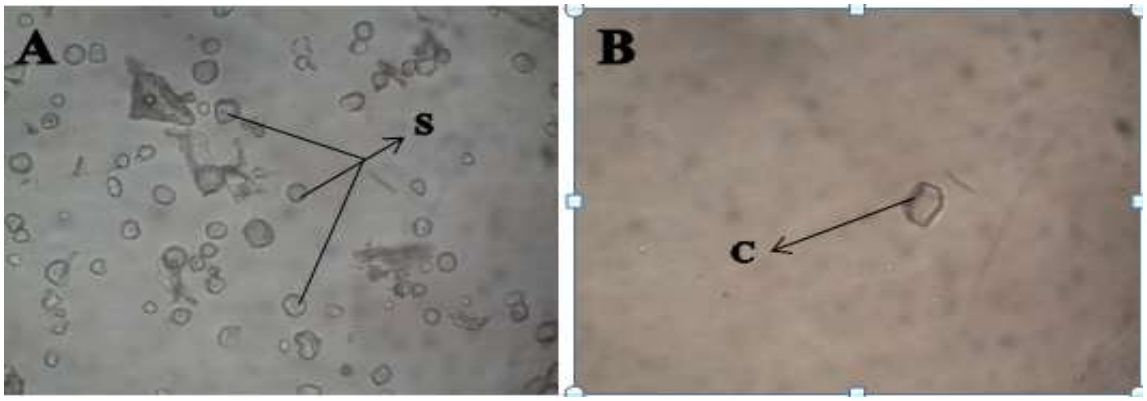

Figure 5. Microscopy of root powder of *C. sieberiana* (x400): A and B showing starch granules (S) and prism - shaped calcium oxalate crystal (C).

total, acid-insoluble and water-soluble ash values of 2.2, 0.4 and 0.5%, respectively. The results also vary slightly with those reported by Bello et al. (2016), who reported a higher moisture content, 6.2% and lower total, acid-insoluble and water soluble ash values of 5.8, 1.0 and 3.5%, respectively. This could be due to differences in geographical location (samples were collected from Jos, Plateau State and Giwa, Kaduna State, Nigeria, respectively), time of collection and varying mineral contents in the soil.

Conclusion

The pharmacognostic evaluation of *C. sieberiana* leaf is being reported for the first time and results from this study have provided information on the morphological and anatomical features and the physicochemical parameters of *C. sieberiana* leaf and root. These parameters can be used for identification and quality control of the plant drug and provide information which may be incorporated into the Nigeria Herbal Pharmacopoeia (NHP) and the West African Herbal Pharmacopoeia (WAHP).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

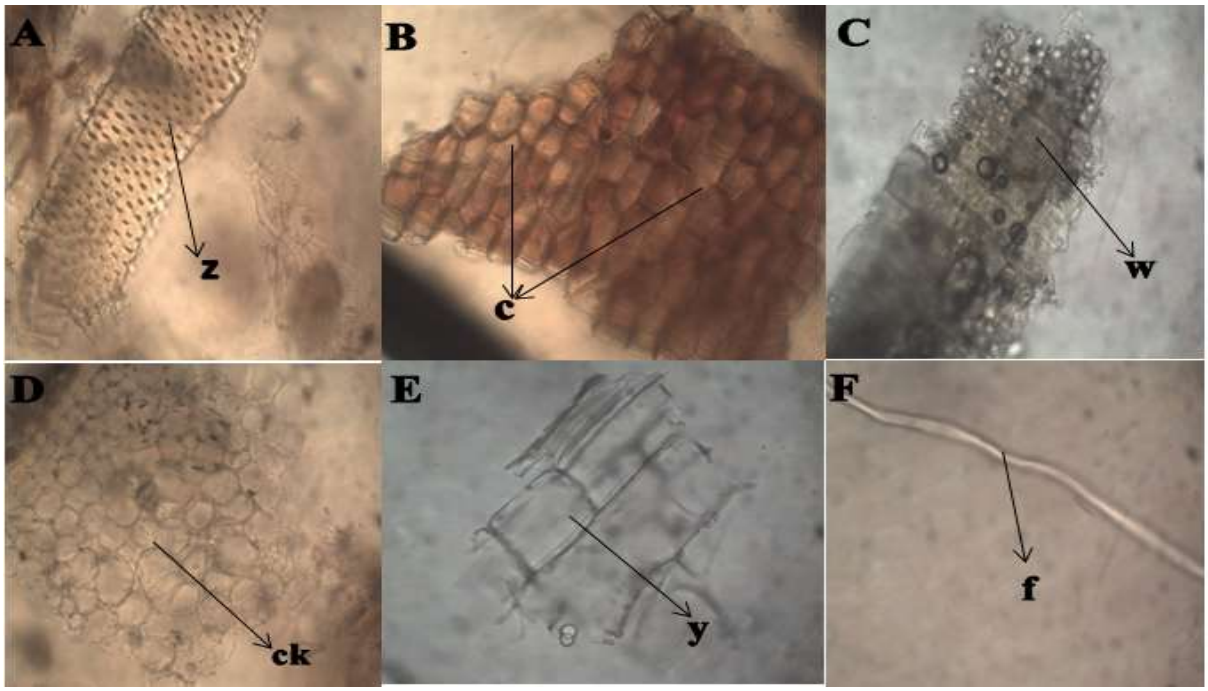

Figure 6. Microscopy of root powder of *C. sieberiana* (x400) showing: A, Reticulate/pitted xylem vessels (z); B, collenchyma cells (c); C, wood element containing starch granules (w);D, cork cells (ck); E, medullary rays (y); F, fibre (f).

Table 5. Chromatographic fingerprinting of *C. sieberiana* leaf and root powder.

Extract	R_f	Daylight	UV_{366}	10%v/v H_2SO_4
Leaf- normal phase T.L.C in CH_2Cl_2:CH_3OH (7:3)	0.18	Faint brown	Deep brown	Brown
	0.57	Faint brown	Deep brown	Brown
	0.89	Green	Red	Brown
Leaf –reverse phase T.L.C. in CH_2Cl_2:CH_3OH (7:3)	0.68	Light green	Deep red	Brown
	0.78	Greenish yellow	Light red	Brown
	0.89	Light brown	Deep red	Brown
	0.95	Light brown	Brown	Brown
Root -normal phase T.L.C in CH_2Cl_2:CH_3OH (7:3)	0.26	-	Red	Brown
	0.53	Green	Deep green	Brown
	0.66	Brown	Red	Brown
	0.74	Brown	Yellow	Brown
	0.90	-	Orange	Brown
Root–reverse- phase T.L.C. CH_2Cl_2:CH_3OH (7:3)	0.80	Brown	Deep brown	Brown
	0.90	Yellow	Light yellow	Brown
	0.93	Yellow	Yellow	Brown

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