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Pharmacognostic evaluation of the root *Cassia sieberiana* D C: A promising ethnomedicinal plant

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Abstract
Cassia sieberiana D.C. (Fabaceae) though widely used in traditional medicine as an analgesic in dysmenorrhoea, ulcer and general body pains; there is no published report on it standardization. Pharmacognostic evaluation of the root will therefore assist in standardization for quality, purity and sample identification of the plant drug. Evaluation of fresh and powdered root of this plant was carried out to determine its pharmacognostical profile, including macroscopic, microscopic, histochemical and quantitative parameters. Chemical profile of the root was also determined using thin layer chromatography (TLC). The root was found to be cylindrical in shape, brown in colour, with characteristics odour and very bitter taste when chewed. When the root was cut transversely it revealed the presence of cork cells, narrow cortex, beneath which is a layer of parenchyma cells, prominent vessels, biserate medullary rays and small pith. Cellulose, hemicellulose and lignified cell wall were present. Calcium oxalate crystals are prisms in shape, measuring 19.29 ± 1.4 µm. Starch grains are oval in shape measuring 10.64 ± 0.82 µm. Moisture content was found to be 6.2 ± 0.3 (%w/w), total ash was 5.8 ± 0.43 (%w/w), acid-insoluble ash was 1.0 ± 0.24 (%w/w), water-soluble ash was 3.5 ± 0.24 (%w/w), alcohol soluble extractive was 12.0 ± 0.47 (%w/w), water soluble extractive was 6.0 ± 0.47 (%w/w), Swelling index was 3.5 ± 0.00 (%w/w), foaming index was less than 100, tannins content, was 39 (%w/w), Bitterness value, was 8400 unit/g. Phytochemical constituents include anthraquinones, flavonoid, saponins, steroid/terpenoids, tannins, and cardiac glycoside. These findings will be useful towards establishing pharmacognostic standards and preparation of monograph of the root of *C. sieberiana*.

Keywords: Fabaceae; standardization; quantitative parameters

1. Introduction
Cassia sieberiana D.C. (*Cassia kotchiyana* Oliv) belongs to the family Fabaceae (Leguminosae – Caesalpinioideae). The plant is widely distributed in the Southern Sahel and Sudan Savanna from Senegal to Cameroon, Gambia East to DR. Congo and Uganda [1, 2]. *C. sieberiana is* widely used for the treatment and prevention of various diseases. The phytochemical analysis of the roots had shown the presence of flavonoids, anthracenic derivates and non- hydrolysable tannins [3]. Previous studies showed that ethanolic root extract of *C. sieberiana* had an anti-parasitic effect, myorelaxant and anti-spasmodic activity [4]. It was also shown that *C. sieberiana* extracts had anti-microbial activity against *Neisseria gonorrheal, Herpes simplex* virus type 1 and African swine fever virus [5]. Analgesic and anti-inflammatory activity of aqueous root extract of *C. sieberiana* was also investigated [6]. The flavones from leaf extract of *C. sieberiana* cause diuresis and have anti-bacterial and anti-inflammatory activity. In-vitro test only showed low activity of the extracts against trypanosomes leaf extracts were found to be active against *Staphylococcus lutea, Mycobacterium phlei, Bacillus subtilis* and *Proteus sp*. but not against *Staphylococcus albus, Pseudomonas acruginosa* or *Escherichia coli* [7]. Though the plant, *C. sieberiana* is used extensively in traditional medicine, the detail pharmacognostic standards for quality control profile including bitterness value, swelling index, foaming index, tannin content are still lacking. The present work has been undertaken to establish the various pharmacognostical parameters, which could serve as a measure of authentication and quality control of the crude drug.

2. Materials and Methods
2.1 Solutions, Chemicals and Reagent
Freshly prepared solutions and analytical grade chemicals were used in all the experiments.

2.2 Collection and Identification of Plant sample.

Fresh *C. sieberiana* root was collected in Giwa town, Giwa Local Government Area of Kaduna State, in April, 2010. The plant was taxonomically authenticated by Mal Umar Shehu Gallah with Voucher specimen number 900202 deposited at the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

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2.3 Preparation of Plant Materials

The roots, was removed from the plant and washed in clean water to removed sand, sliced into pieces and air dried for two weeks. It was then pulverized into coarse powder with mortar and pestle and stored in cellophane bag at room temperature until required for experiment. Sampling of plant material was done by passing the coarse powder through wire sieves used to sieve powdered medicinal plant materials with nominal aperture size expressed in µm. A 250µm sieve size powder was used to test for degree of fragmentation (sieve test) [8].

2.4 Macroscopic Examinations

The macroscopic characters of the samples were carried out based on the method described by [8].

2.5 Microscopic Examinations

Transverse section of the root was prepared as described by [9]. The roots were fixed in formalin: acetic acid: alcohol: 1: 1: 18 upon collection from the field, following the fixation, the plant specimen was dehydrated in graded solution of alcohol and cleared using a graded series of alcohol-chloroform solution. Paraffin wax was added slowly to infiltrate the tissue and then placed in an embedding oven and maintained at 60^0 C until it melted. Block of paraffin was made using an L- shaped mold. After the paraffin wax had hardened, it was mounted on a microtome, at 12 µm thicknesses. Ribbons obtained were mounted on glass slides and dewaxed in xylene, then hydrated in ethanol, the tissues were then stained and differentiated in Safranin and Countered stained in fast green, dehydrated and cleared in xylene. The slides were Covered slips and dried over a slide dryer at 40^0 C. the slides were examined under the microscope and microphotographs were taken and recorded.

The powdered root was also treated separately with appropriate chemical reagents on microscopic slide and observed under the microscope for the presence of chemical substances such as cellulose, hemicelluloses, lignin, calcium oxalate crystals, calcium carbonate, starch, mucilage, tannin, fixed oils and fats [8].

2.6 Quantitative evaluations

Quantitative analysis was carried out on powdered drug using the modified methods described by [8].

2.7 Extraction and Preliminary Fractionation of Powdered Roots of *C. sieberiana*

The extract was prepared according to the method described by [10]. The coarse powdered root sample (2.5kg) was extracted exhaustively with absolute ethanol (maceration) three times (w/v 1:3) at room temperature for a week. The ethanol crude extract was combined and concentrated on a Büchi rotary evaporator at 450°C giving the crude ethanol extract (80g) it was then stored in sterile container for further used. Sixty grams (60g) of crude ethanolic extract was suspended in distilled water (500ml). The aqueous portion was extracted with ethyl acetate (6x 200 ml) and *n*-butanol (5 x 200 ml). Fractionation of plant sample was adapted from [11].

2.8 Preliminary Phytochemical Screening of Extract of *C. sieberiana*

Phytochemical screening of the crude ethanol and fractionated extracts were performed for the presence of secondary metabolites, using standard phytochemical methods as described by [12, 13].

Thin layer chromatography of various class of compounds previously screened were then conducted according to [12]

3. Results

3.1 Macroscopic Studies

Roots cylindrical, branched, thickness varying with age with characteristic odour and very bitter taste.

Plate I: *Cassia sieberiana* plant showing the leaves in its natural habitat.

Plate II: *Cassia sieberiana* root

Table 1: Macroscopic Characters of *Cassia sieberiana* Roots

	Observations	
Characters	**Fresh**	**Powdered**
Shape	Cylindrical	
Size	5-20 mm in diameter	
Surface characteristics	Smooth	Coarse
Texture	Hard	Rough
Odour	Characteristics	Choking
Taste	Very bitter	Very bitter
Colour	Brown	Creamy

The transverse section of the root is circular in outline (plate III). It outer layer is composed of a lignified cork with several layer of thick walled, flat, polygonal cells with reddish brown content, which are impregnated with suberin. This is followed by thin walled epidermal cells. The cortex portion of the root

is composed of horizontally elongated parenchyma cells, containing starch grains, which are abundant, simple, mostly spherical, reniform – oval with central hilum. The phloem is a continuous ring consisting of sieve tubes, fibres are aseptate with pointed end. Medullary rays are well developed and are biseriate. The xylem occupies the entire central portion with prominent vessels, Pith is present as also showed in plate IV. This anatomical study showed diagnostic features that revealed characteristic pattern of arrangement of the cellular components of root of *C. sieberiana.*

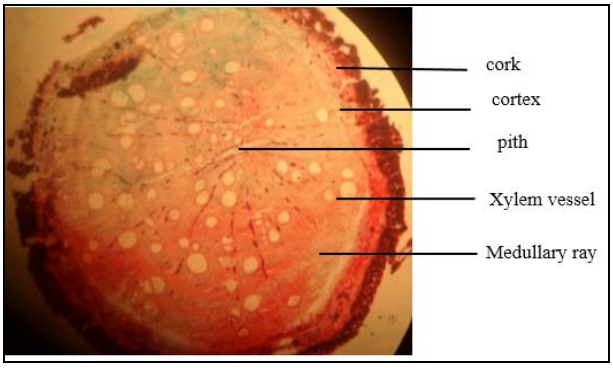

Plate III: Transverse section of *Cassia sieberiana* root. Showing the whole section (MG X40)

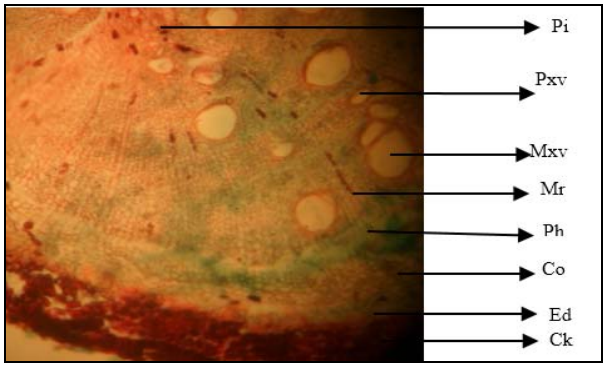

Plate IV: Transverse Section of *Cassia sieberiana* root. showing the central portion. *Where, Ck- Cork, Ed- Epidermis, Co- Cortex, Ph- Phloem, Mr- Medullary ray, Pxv- Protoxylem vessel, Mxv- Metaxylem vessel, Pi- Pith* (MGX100)

3.2 Histochemical Studies on Powdered roots

The length of fibre is 74.48 µm. The starch grains are simple and oval with diameter 10.64µm, calcium oxalate crystals are prisms in shaped with base diagonal measuring 19.29µm.powdered microscopy are seen in plate V-XII below.

Plate V: Lignified cells

Plate VI: Calcium oxalate crystal

Plate VII: pericycle Fibre

Plate VIII: Boardered pithed xylem vessels

Plate IX: Collenchyma cells

Plate X: wood element containing starch grains

Plate XI: Cork cells,

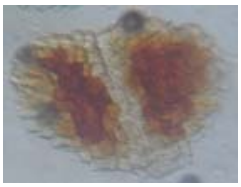

Plate XII: Cork cells with cell inclusion

Chemo microscopic studies of the root revealed the presence of starch, calcium oxalate, tannins cellulose, hemicellulose, lignin, suberin and mucilage. As given in table 2 below

Table 2: Histochemical detection of cell and cell wall material of powdered root.

Text	Observations	Inference	Histological zones
Starch	Blue-black color	Present	Epidermis, Parenchyma,
Calcium oxalate	Crystals disappear without effervescence	Present	Parenchyma, Medurally rays
Tannin	Greenish black color	Present	Endodermis, Cork cells
Fixed oil & fat,	No observation	Absent	
Protein	Yellow stain	Present	Epidermis, Cork cells, pith
Cellulose	Bluish color	Present	Cell wall
Hemicellulose	Blue color	Present	Cell wall
Lignin	Cherry red	Present	Cell wall
Suberin	Orange red	Present	Endoderms
Mucilage	Pink color	Present	Simple tissues
Aleurine grains	Brown	Present	Parenchyma

3.4 Quantitative Parameters

A moisture content of 6.2 ± 0.3% was observed for the powdered root. The total ash was 5.8 ± 0.43% while acid insoluble ash and water soluble ash were found to be 1.0 ± 0.24 and 3.5 ± 0.24% respectively. Alcohol soluble extractive was found to be 12 ± 0.47% and water soluble extractive 6 ± 0.47%., foaming index (<100), swelling index (3.5 ml) tannins content (39%), the bitterness value (8400 unit/g). As indicated in Table 3.

Table 3: Quantitative Parameters of *C sieberiana*

Parameter	Results*
Moisture content	6.2 ± 0.30 (%w/w)
Total ash	5.8 ± 0.43 (%w/w)
Acid insoluble ash	1.0 ± 0.24 (%w/w)
Water soluble ash	3.5 ±0.24 (%w/w)
Alcohol soluble extractive	12 ± 0.47 (%w/w)
Water soluble extractive	6 ± 0.47 (%w/w)
Foaming index	Less than 100
Swelling index	3.5 ml
Tannins content	39 (%w/w)
Bitterness value	8400 units/g

N = 3

3.5 Extraction of Plant Material

C. sieberiana crude extract is dark brown in colour with a Pleasant smell and a pasty consistency. The total solid recovered from ethanol extraction was 80g having percentage yield of 3.2%. The partitioning of the fractions weighed 14.01 g, 9.98 g and 7.80 g for Ethyl acetate, n-butanol, and Aqueous respectively. The percentage yields for the fractions are 0.6%, 0.4%, and 0.3% for Ethyl acetate, n- butanol and Aqueous respectively as indicated in Table 4. Ethyl acetate fraction was found to be higher than the other fraction.

Table 4: Percentage yield of the Extract and Fractions

Extract/ fractions	Colour	Mass (g)	Percentage Yield (%)
Ethanol Extract	Dark brown	80.00	3.2
Ethyl acetate	Light brown	14.01	0.6
n-Butanol	Deep brown	9.98	0.4
Aqueous	Pale brown	7.80	0.3

Phytochemical parameters

Phytochemical constituents are; Anthraquinones, saponin, sterols, tannins, triterpenes, flavonoid, glycoside. Ethylacetate fraction revealed the presence of anthraquinones, flavonoid, tannins, glycoside and steroid/terpenoids. The n-butanol fractions showed the presences of tannins, and saponin. While the aqueous fractions show the presence of saponin. These secondary plant metabolites are known to possess various pharmacological effects and may be responsible for various action of *cassia sieberiana* as indicated in Table 5

Table 5: phytochemical screening of the crude extract, Ethylacetate, butanol and Aqueous fraction of *C. sieberiana* root.

Constituent	Crude extract	Ethyl acetate	butanol	Aqueous
Anthraquinones	Present	Present	Absent	Absent
Tannins	Present	Present	Present	Present
Flavonoids	Present	Present	Absent	Present
Saponins	Present	Absent	Present	Present
Terpernoids/Steroid	Present	Present	Present	Absent
Cardiac Glycoside	Present	Present	Absent	Absent
Alkaloid	Absent	Absent	Absent	Absent

Spraying the TLC plates with specific spraying reagents confirmed one spot and yellow colour for flavonoids (Plate), tannins has two spots and they are blue black in colour at ethyl acetate and n – butanol fractions portion (Plate). Terpenoids, cardiac glycoside, anthraquinone has one spot each at ethyl acetate fraction portion. (Table 6).

Table 6: Chromatography separations of phytochemicals of various extract by TLC using chloroform: ethylacetate : methanol : water (15:8:4:1)

Phytochemicals	Spot	Colour	R_f	Extract portion
Flavonoid	1	Yellow	.23	EAA
Tannins	2	Blue black	.13, 04	EAA, B
Terpenoid	1	Brown	.4	EAA
Glycoside	1	Purple	.5	EAA
Anthraquinones	1	Red	.3	EAA

R_F – Refractive index, EAA-Ethylacetate, B-Butanol

4. Discussion

The primary steps for establishing the quality control profile of any plant drug is the macroscopic and microscopic evaluation

and according to [8], botanical standards should be proposed as a protocol for the diagnosis of the herbal drug. Macroscopically, *Cassia sieberiana* roots are cylindrical in shape, brown in color, bitter in taste and have no characteristic odour.as shown in Table1. Microscopically, The T.S of the root showed circular outline (Plate III). The outer layer is followed by lignified cork with several layers of thin walled, flat, polygonal cells with reddish brown content, impregnated with suberin. The cortex is composed of horizontally elongated parenchyma cells, containing starch grains, abundant, simple, mostly spherical, reniform – oval with central hilum; phloem is a continuous ring consist of sieve tubes, fibres aseptate with pointed end, companion cells and phloem parenchyma. Medullary rays are well developed and are biseriate. Xylem occupies preliminary idea about the type of compounds and their accumulation in the plant tissues. The result of this study is supported by the report of [14]. Histochemical studies of *C. sieberiana* is of great interest for quality control in basic research and drug production the entire central portion with prominent vessels, pith is present, the powdered roots microscopy as showed in Figure 2 gives a, especially for imported items and for raw material sold by traditional herbalists. These parameters are unique to the plant and are required in its standardization.

Physicochemical evaluation of powdered root *C. sieberiana* is useful towards establishing pharmacognostic standards on identification, purity, quality and classification of this plant material in future investigations or applications, which is gaining relevance in plant drug research [8]. The total ash determined was 5.8 ± 0.43 (% w/w) for *C. sieberiana* root in contrast with [15] determination having 2.1 ±0.07 total ash this variation is due to the geographical locations attributed to possible varying mineral contents in the soil. The loss on drying was 6.2 ± 0.3 (% w/w), the less the value of moisture content, the lesser it prevent bacterial, fungal or yeast growth. An excess of water in medicinal plant materials will encourage microbial growth, the presence of fungi or insects, and deterioration following hydrolysis [8]. Water soluble and alcohol (70% methanol) soluble extractive values were found to be 6.0 ± 0.47 (% w/w) and 12.0 ± 0.47 (% w/w) respectively in contrast to the findings of [14] who showed that ethanol (70%) extractive 14.28% w/w was lower than chloroform - water extractive 15.99% w/w, this may be due to different strength of alcohol used and also differences in geographical locations. Swelling index 3.5 ± 0.00 ml, this showed that the root has swelling ability which is due to an appreciable amount of mucilage and hemicelluloses present. The persistent foam that was observed when an aqueous decoction of the root was shaken shows that the plant contains little saponins. The tannins content was 39% w/w which shows that *C. sieberiana* root has astringency. The plant roots possess a strong bitter taste 8400 unit/g indicating that it stimulates the secretion of gastro intestinal tract gastric juices [8].

the phytochemicals confirm in this study is in contrast to the study of [10, 14], This difference may be explained by the fact that variation may sometimes occur in bioactive compound of different part of the same plant and even in same plant parts found in different environment [16]. The phyto constituents, as showed in Table 4 which implies the definite presence of certain constituents in the sample. It also revealed clues for identifying and distinguishing the plant from other species.

5. Conclusions

C. sieberiana is currently being used in treatment of various disease conditions without standardization or concerns as to its level of quality. The results of this investigation could, therefore, serve as a basis for proper identification, collection and investigation of the plant. The present study may be useful to supplement the information with regard to its standardization and identification and in carrying out further research and its use in traditional system of medicine.

6. Acknowledgements

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7. Consent

It is not applicable.

8. Ethical Approval

It is not applicable.

9. Competing Interests

Authors have declared that no competing interests exist.

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