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Pharmacognostic studies and elemental analysis of the leaves of *Cassia obtusifolia* Linn. (Family Fabaceae)

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Abstract
The plant *Cassia obtusifolia*, family Fabaceae, found in northern Nigeria and in most tropical and sub-tropical countries, is eaten as food as well as employed ethno-medically in the treatment of various ailments. These include treatment of furuncles and ulcers, and externally for conjunctivitis. The aim of this study is to determine some of the pharmacognostic characters of the leaves of *C. obtusifolia.* The macroscopic, microscopic, organoleptic and chemomicroscopic characters as well as the numerical standards, preliminary phytochemical screening and elemental analysis of the leaves were determined. The microscopical studies showed unicellular covering trichome that is conical in shape, with thick and distinctly warty walls. Scattered paracytic stomata, appearing both in the upper and lower surfaces. The results of the numerical standards showed the moisture content, total ash, acid insoluble ash, water and alcohol extractive values of 17.5, 12.0, 3.0, 16.0 and 8.0 respectively. Preliminary phytochemical screening confirmed the presence of tannins, coumarin derivatives and saponins while the elemental analysis shows the presence of important metals such as potassium (7680±1150), calcium (14900±1070) and chromium (143 +_LDL). The present study will be of benefits in developing a standardized profile of the leaves of *C. obtusifolia*, which would be of immense use to identify and establish the authenticity of the plant.

Keywords: *Cassia obtusifolia, Fabaceae, Pharmacognostic, Tafasa, Elemental analysis*

INTRODUCTION

Cassia obtusifolia belongs to the family Fabaceae. It is an erect species, branched, bushy annual or perennial up to 90 cm high and reproduces from seeds. It is a pan-tropic weed of roadside grassland and cultivated fields, common near settlements and widespread in northern Nigeria. The dried entire plant is applied as paste in the treatment of skin diseases [1]. In Nigeria and India, the fresh leaf is used as food [2]. Senegalese use the hot water extract of the leaf orally in the treatment of furuncles and ulcers and externally for conjunctivitis [3].

Reported phytochemical constituents in the plant include alkaloids, flavonoids, tannins, vitamins and proteins. The reported biological activity of the plant includes the use of methanolic extract of the leaf at a dose of 500mgkg^{-1} as an antihepatotoxic on rats [4] and 400mgkg^{-1} of the same extract using the same animal showed alkaline phosphatase inhibition and anti-inflammatory activities [5]. In view of the common uses of this plant as food and as medicine, this work was

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carried out to study some of the pharmacognostic characters of the plant.

EXPERIMENTAL

The chemicals used during the study were of analytical grade. The instruments were well calibrated before use.

Plant material. Fresh leaves of *Cassia obtusifolia* were collected in Zaria, Kaduna State, Nigeria. The plant was identified at the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, where a voucher specimen (No.1370) was deposited. The leaves were air-dried, powdered and the powder was stored in an airtight container prior to use.

Macroscopic/Microscopic studies. Leaves of *C. obtusifolia* were subjected to morphological examination. Microscopic evaluation of the leaf was carried out by taking the transverse section using standard procedures and then subjecting them to microscopic examination. The powdered samples were also subjected to histological examinations using standard procedures and the diagnostic features were identified, recorded and observed under the microscope. Various leaf constants e.g. stomatal number, stomatal index, palisade ratio, vein islet number and veinlet termination number were also determined [6,7].

Physicochemical evaluation of the leaf of *C. obtusifolia.* The fresh and dried powdered leaf material was used for the determination of numerical standards e.g. water soluble extractive value, alcohol soluble extractive value and loss on drying. These will be determined in accordance with the WHO [7] guidelines.

Preliminary phytochemical screening. Phytochemical screening was carried out in accordance with the WHO [7] guidelines. Qualitative chemical tests to determine the presence of various phytoconstituents e.g. alkaloids, glycosides, phenolics e.g. tannins, saponins and phytosterols were carried out.

Elemental analysis. This was done by using Energy Dispersive X-ray Fluorescence (EDXRF) transmission emission technique at the Centre for Energy Research and Training, Ahmadu Bello University, Zaria using a method outlined by Idris [8].

RESULTS AND DISCUSSION

The pharmacognostic studies carried out on the leaf of *C. obtusifolia* Linn. include: macroscopic studies (Table 1) which examined the macromorphological feature of the leaves and organoleptic properties; microscopic studies, which is divided into qualitative microscopical studies (Table 5), chemo–microscopical studies (Table 2) and quantitative microscopical studies (Table 3). Also under pharmacognostic studies, other physical constants (Table 4) e.g. extractive values, moisture contents, total ash values, acid insoluble ash and water-soluble ash values were determined. Pharmacognostic study is usually the initial step to confirm the identity and to assess the quality and purity of the crude drug. Quality control of crude drugs is very challenging task because of complex nature of chemical constituents. Fig. 1 shows the Microscopical features from the tranverse section and powdered leaves of *C. obtusifolia Linn.* Microscopical evaluation is the simplest and reliable tool for correct identification of herbs as well as small fragment of crude drugs or powdered drugs and detection of adulterants and substituents [9,10]. Plate I and Table 1 show the leaf of *C. obtusifolia* in its natural environment and Macromorphological features of the leaf. It has alternate arrangement of leaves and petiolate. It has a compound lamina with pinnate venation. The leaf has an entire margin with minutely mucronate apex and a rounded/ asymmetrical base.

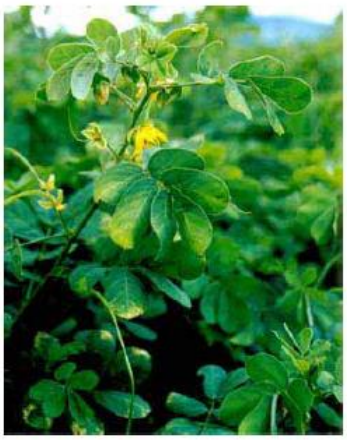
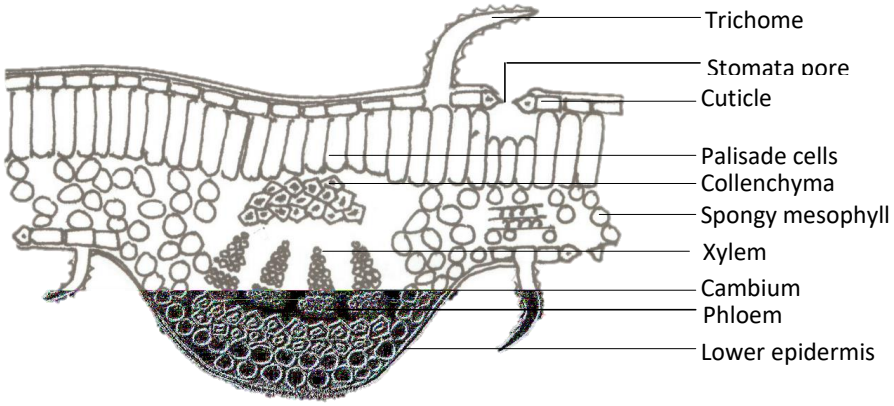


Plate 1: *Cassia obtusifolia* leaf in its natural environment

Microscopical studies of *C. obtusifolia* Linn. leaf



(i) Transverse section *of Cassia obtusifolia* Linn. leaf

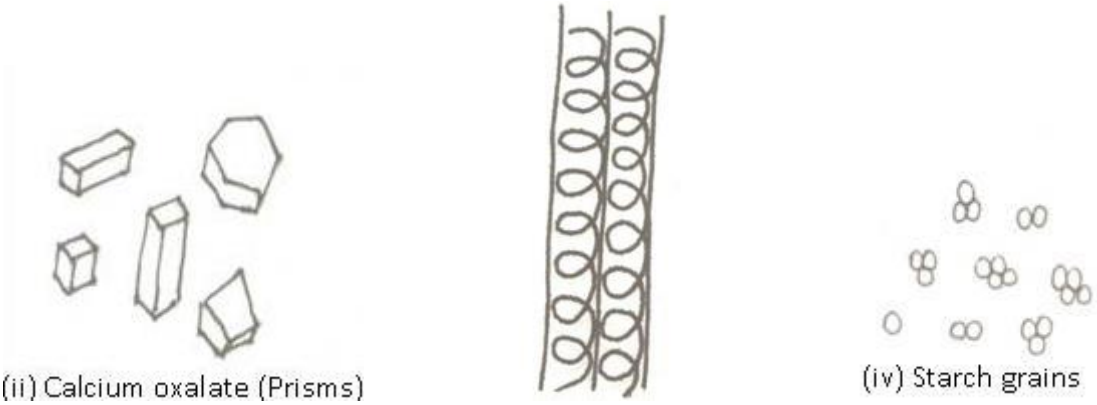
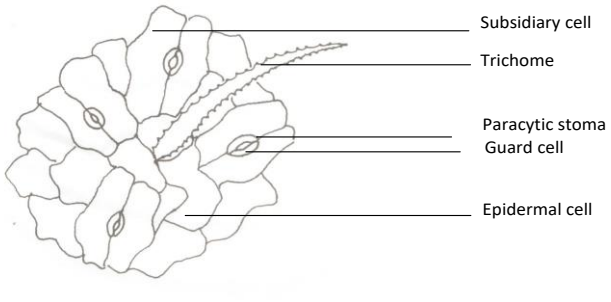
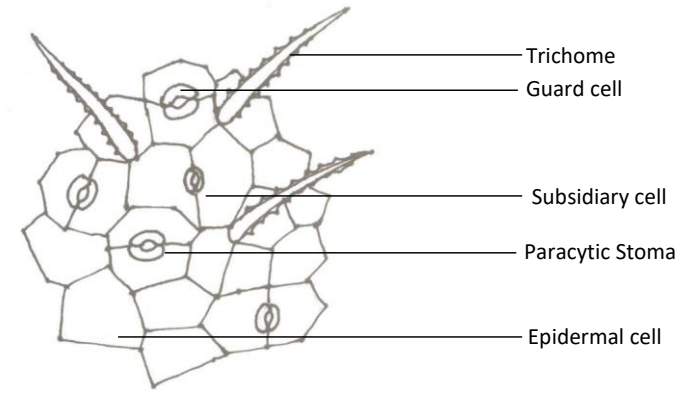


Fig. 1. Microscopical features from the transverse section and powdered leaves of *C. obtusifolia Linn.* Mag. X400.



(i) Upper epidermis



(ii) Lower epidermis

Fig. 2: Microscopical surface preparation from the leaf of *Cassia obtusifolia* Linn. Mag. X400

Table 1: Macromorphological Features of the Leaf of *C. obtusifolia* Linn.

Features		Description
Duration		Annual
Arrangement		Alternate
Petiole		Present (1.8 to 3.5cm)
Lamina	(i) Composition	Compound
	(ii) Venation	Pinnate
	(iii) Margin	Entire
	(iv) Apex	Minutely mucronate
	(v) Base	Rounded / asymmetrical
	(vi) Surface	Upper Glabrous Lower pubescent
	(vii) Texture	Smooth

Organoleptic Properties: (i) Colour – Green (ii) Odour – Strong (iii) Taste – Characteristic

Table 2: Chemo-microscopical Features of the Leaf of *Cassia obtusifolia* Linn.

Constituents	Observation
Cellulose	+
Lignin	+
Tannins	+
Mucilage	+
Starch	+
Calcium oxalate crystals	+

Key: + (Present)

Table 3: Quantitative Microscopical Characters of the Leaf of *C. obtusifolia* Linn.

Parameters Evaluated		Mean Value		
Stomatal Number	*Upper epidermis*	300	325	350
	Lower epidermis	209	227	245
Stomatal Index	*Upper epidermis*	39.0	40.9	42.8
	Lower epidermis	34.8	38.6	42.4
	Palisade Ratio	4.6	5.8	7.0
	Vein Islet Number	12	14	16
	Vein Termination Number	29	33	37

Table 4: Physicochemical parameters of *C. obtusifolia* Linn.

Parameters (%)	*C. obtusifolia*
Moisture Content	17.5
Total Ash Values	12.0
Acid Insoluble Ash Values	3.0
Alcohol Soluble Extractive Values	8.0
Water Soluble Extractive Values	16.0

Table 5: Phytochemical constituents from the leaf of *C. obtusifolia* Linn.

Constituents	*C. obtusifolia*
Alkaloids	+
Tannins	+
Reducing Compounds	+
Anthracenoside	-
Coumarin Derivatives	+
Saponins	+
Steroidal Glycosides	+
Flavonoids	+
Polyuronides	+
Carotenoids	+

Key: + Present - Absent

Table 6: Elemental analysis of the leaves of *C. obtusifolia* Linn.

Elements	Conc. in ppm)	Elements	Conc. in ppm)
Potassium	7680±1150	Arsenic	18 .2 ± LDL
Calcium	14900±1070	Lead	26.9 ± LDL
Titanium	460 ±LDL	Boron	9.25 ± LDL
Vanadium	447 ±LDL	Rubidium	13 .9 ± LDL
Chromium	143 +_LDL	Strontium	59.9 ± 4.60
Manganese	128 ± LDL	Thorium	12. 6± LDL
Iron	191 ± 32.1	Yttrium	7. 18 ± LDL
Nickel	38.4± LDL	Uranium	10. 8 ± LDL
Copper	28.5 ± LDL	Zirconium	5.96 ± LDL
Zinc	19.0 ± LDL	Niobium	5.12 ± LDL
Gallium	16 .2 ±LDL		

LDL = Least detectable limit ppm = parts per million

The upper surface of the leaf is glabrous while the lower pubescent/hairy. Organoleptically, the leaves were greenish in colour having a strong odour and a taste that is characteristic. Fig. 2 shows the microscopical surface preparation from the leaf of *Cassia obtusifolia* Linn. Diagnostic features identified from the leaf of *C. obtusifolia* include; unicellular covering trichome that is conical in shape, with thick

and distinctly warted walls. Trichomes are epidermal outgrowths of considerable value for taxonomic purposes for some plants. These outgrowths play a role in plant defense especially with regard to phytophagous insects [11]. They may also be involved in the regulation of temperature and water repellency as well [12]. Scattered paracytic stomata, appearing both in the upper and lower surfaces. The presence of stomata promotes heat dissipation by water loss, maximizing the control of water loss by leaf and increases photosynthetic potential [13], these features are essential for the plant as the leaf is involved in photosynthesis. Numerous calcium oxalate crystals (prism) are important parameters for identification and standardization of *C. obtusifolia* and it is a clear indication that the plant is rich in oxalic acid with which higher plants syntheses the crystals and deposit them in specialized organ or tissue [14,15]. Oval shaped starch grains (Fig. 1), xylem and phloem fibres were observed. Transverse section of the leaf showed dorsiventral arrangement of the cells, with elongated palisade cells in the upper epidermis and the rounded spongy mesophyll cells in the lower epidermis with large intercellular spaces. In addition, collenchymatous cells, cuticular cells, spiral xylem vessels and phloem with a distinct cambium were observed. Table 2 show the Chemo-microscopical Features of the Leaf of *Cassia obtusifolia* Linn. Features identified to be present during chemo-microscopical studies include, starch and calcium oxalate crystals (cell inclusions), tannins (cell constituents), and cellulose, lignin and mucilage (cell wall materials).

Table 6 show the elemental analysis of the leaves of *C. obtusifolia.* Some of the essential micronutrients found in the plants includes; copper, chromium, iron, manganese, potassium, calcium and zinc. On the other hand, heavy metal like, nickel, lead, and arsenic were also found in the plants. Also, found in this plant are radioactive elements, these include yttrium, uranium, and niobium. Concentrations of minerals in *C. obtusifolia* determined by this study include iron (191 ± 32.1 ppm), manganese (128 ± LDL) and nickel (38.4± LDL) as against the permissible limit set by FAO/WHO [16] for edible plants. However, for medicinal plants, the WHO [17] limits has not yet been established for Fe, Mn and Ni, The literature, Sheded *et al.* [18] shows similarity in Fe content (between 261 and 1239 ppm), and wide differences in Mn (44.6 and 339 ppm) content in selective medicinal plants of Egypt. Trace elements concentration in *C. obtusifolia* include Zn (19.0 ± LDL), Cu (28.5 ± LDL) and Pb (26.9 ± LDL) which is above the permissible limit, Cu (3.00 ppm) as set by FAO/WHO [16] for edible plants. However, these results are within the permissible limits for Zn set by China and Singapore as 150 ppm and above the limit for lead (10 ppm) as set by China, Malaysia, and Thailand [17]. The overall results indicated clearly the contents of the essential metals such as iron, manganese and nickel were within acceptable limits of the toxic metals such as lead are within safe limit. Therefore, *C. obtusifolia* can also be beneficial sources of appropriate and essential trace elements.

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