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Pharmacognostic studies of the leaves and seeds of *Cassia occidentalis* (Linn.) (Leguminosae)

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Abstract
Cassia occidentalis is used in Ghana mainly for the management of hypertension. The aim of the present studies was to establish standardization parameters for the authentication and quality control of the leaf and seeds of *Cassia occidentalis*. The macromorphological, qualitative and quantitative features, physicochemical, phytochemical and some spectroscopic features of the medicinally used parts were evaluated using standard methods. The plant was found to be a glaborous herb with compound pinnate leaves. It has 6-8 pairs of leaflets. The leaflets are broadly lanceolate to ovate, with an acute apex and possessing a gland near the base of the leaf rachis. The seeds are ovoid about 4 mm long flattened with a smooth and hard testa. It is amphistomatic with anomocytic stomata. The quantitative indices of the leaf and physicochemical parameters have also been established. A 0.5% ethanol extract of the leaves and seeds showed characteristic UV maxima which may be invaluable in its identification. The pharmacognostic features established in this study may be used as part of a pharmacopoeial standard for the correct identification and quality control of *Cassia occidentalis*.

Keywords: Cassia occidentalis, fluorescence analysis, quantitative microscopy, standardization, Thin layer chromatography, UV spectra

1. Introduction
Nature has served as a source of medicinal agents for thousands of years. An impressive number of modern drugs have been isolated from natural sources [1]. Awareness and general acceptability of the use of herbal drugs in today's medical practice is increasing. According to the World Health Organization, 80% of the world's population uses plant-based remedies as their primary form of healthcare [2]. This surge in the use of herbal product has also given way to various forms of adulteration leading to consumers' and manufacturers' dissatisfaction and in some instances fatal consequences. The challenge is innumerable and enormous, making the global herbal market unsafe [3].

Cassia occidentalis is commonly known in Ghana as 'mmofraborodee' (Twi) and 'Devidevikpelimumu' (Ewe). It is a pantropical plant that grows on wastelands in villages and towns and on roadsides. The seeds are the primary material of interest though the leaf and roots are also used. The seeds are roasted and used as a coffee substitute. The plant's tissues contain a host of phytoactive chemicals that may support its numerous applications in folk medicine [4]. The whole plant is useful as a purgative and as a tonic. The seeds and leaves are used as cure for cutaneous diseases [5]. The roasted seeds are used to manage hypertension in Ghana. It is used for fever, menstrual problems, tuberculosis, diuretic anaemia, liver complaints, and as a tonic for general weakness and illness [6]. Leaves of *C. occidentalis* are externally applied for wound healing, itching, bone fracture, ringworm, skin diseases and throat infection. The plant is reported to cure leprosy. An infusion of the bark is used in folklore medicine for diabetes [7].

The plant has been reported to have considerable antimicrobial, antioxidant, hepatoprotective, antimalarial, larvicidal, anti-inflammatory, antidiabetic, antianxiety, antidepressant, analgesic, antipyretic and immunosuppressive activities [8].

Proper use of the plant depends on correct identification and appropriate methods of extraction or processing [9]. Hence in the present study, the pharmacognostic profile of the leaves and seeds of *Cassia occidentalis (L)* was carried out to assist in the standardization of the plant and guarantee quality, purity and to obtain a monograph for the correct identification of the plant.

2. Materials and Method

2.1 Chemicals

All the chemicals and reagents used in this study were obtained from BDH chemicals (BDH Ltd, Poole, England).

2.2 Plant collection and processing

The leaves and seeds of *Cassia occidentalis* (Fig 1) were harvested from the physic garden of the Kwame Nkrumah University of Science and Technology (KNUST) in the month of October, 2014. The leaves were air dried for 48 hours and then milled to coarse powder. The seeds were air dried for 5 days, milled and sieved to remove the hard seed coat. The powders were then kept in paper bags and stored at ambient temperature until ready for use.

Fig 1: Whole plant (a) and harvested leaves and seeds (b) of *C. occidentalis*

2.3 Organoleptic evaluation

Organoleptic evaluation was done by observing the leaves with the unaided eye and taking note of the colour, size and other diagnostic parameters. The taste and odour of the leaves and seeds were further noted.

2.4 Macromorphological studies

Different macroscopic parameters of the leaf and seeds were noted. Evaluation of the leaves included the type of leaf, shape, arrangement, apex margin, venation, base, texture and colour.

2.5 Microscopic and histologic techniques

2.5.1 Study of transverse sections

For qualitative microscopic analysis, free hand (razor blade) transverse sections of the midrib and petiole of the leaf were made. Lignified, cellulosic and other identifying features were studied staining with phloroglucinol in concentrated HCl and N/50 iodine. Microscopic evaluation of the tissues was supplemented with photomicrography of different magnifications taken with Leica ICC50 HD (Jos Hansen and Soehne Gmh Germany). Standard procedures were followed according to Kokate *et al* [10]

2.5.2 Powder microscopy

The coarsely powdered leaves and seeds of *C. occidentalis* were studied under the microscope. Small quantities of the leaves and seeds were mounted on a slide using chloral hydrate, phloroglucinol in concentrated HCl and iodine solution. Photomicrographs of the different cellular structures and inclusions were taken.

2.5.3 Quantitative microscopy

Microscopic features not easily characterized by general microscopy were studied. This included stomatal number, stomatal index, palisade ratio, vein islet and vein termination numbers. They were evaluated according to the methods described by [11].

2.6 Physicochemical parameters

Physicochemical analysis such as total ash, water soluble ash, acid insoluble ash, petroleum ether, alcohol and water soluble extractives as well as loss on drying of various plant parts were determined according to standard methods [12].

2.6.3 pH of extracts

The pH of a 1% aqueous and hydro-alcoholic extracts of the leaves and seeds was determined using a pH meter.

2.7 Fluorescence analysis

The water, petroleum ether and ethanol extractives were observed for characteristic fluorescent colours under visible light, short (254 nm) and long (365 nm) UV length regions.

2.8 Phytochemical screening

The presence of secondary metabolites such as tannins, alkaloids, glycosides, terpenoids and phytosterols were determined according to standard methods.

2.9 Thin layer chromatography (TLC)

About 5 g each of the powdered leaves and seeds were cold macerated using 80 mLs of chloroform for 24 hours. The filtrate was concentrated to 5 mL at room temperature and used for the analysis. Analytical TLC was done on silica gel G60 F_{254}, 0.25 mm layer developing with chloroform/Pet. ether [9:1]. Separated compounds were detected with anisaldehyde H_2SO_4.

3. Results and Discussion

Crude drugs are usually obtained from wild sources and are mostly collected by illiterate and unskilled people unaware of their botanical information, authentication and standardization parameters. This usually affects the safety of the final product. For safe and efficacious herbal medicine production, appropriate control of starting material is extremely crucial. Pharmacognostical evaluation of different parameters is the vital etiquette for standardization of herbals [13].

It has been suggested that *C. occidentalis* seeds can serve as a cheap source of protein, energy, as well as antioxidant micronutrient supplements in both man and animal [14]. *Cassia occidentalis* is a plant with potentially limitless uses and is of importance to properly establish a partial monograph for its correct identification.

3.1 Organoleptic and macromorphological features

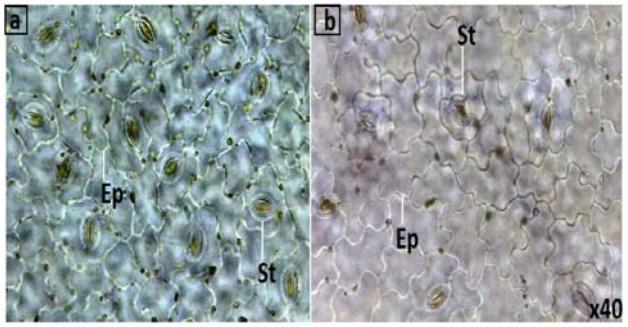
Cassia occidentalis is a semi-woody herb. The leaf is green on the adaxial surface and light green on the abaxial surface. It has a characteristic smell. The leaf powder has a bland taste. The lamina is broadly lanceolate to ovate with an acute apex. It is 4-9 cm long and 1.5 – 3 cm broad with an entire margin and an obtuse base bearing a gland at the base of the leaf rachis. The venation is reticulate. The leaf is a compound

pinnate with 4-5 pairs of leaves. The leaf has a glabrous surface (Fig 1a).

The legumes (pods) are brown, flat, slightly curved and contain brown to light brown, ovoid seeds. The seeds are flattened with a smooth and hard testa. The length of the pods was found to be in the range of 8-10 cm containing 59±2 seeds per pod. The length of the seeds was found to be between 3-5 mm. The weight of 50 seeds of *C. occidentalis* averaged 22.28±0.27 mg.

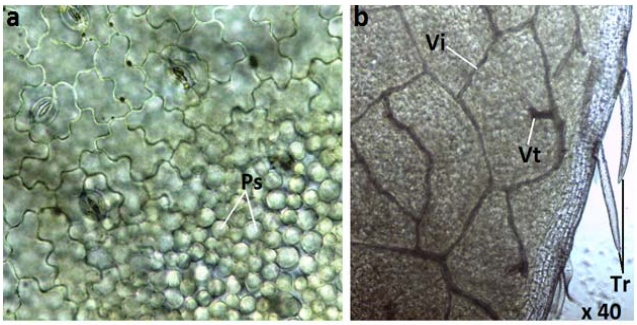
3.2 Leaf anatomy and microscopy

The leaf is dorsiventral with a flat lamina. It is amphistomatic with ranunculaceous stomata on both abaxial and adaxial surfaces (Fig 2a & 2b). The lateral leaf surface displayed unicellular clothing trichomes (124.0 -333.3µm) in length. (Fig 3b). The stomatal number and index were 104 -130 -156 and 13 -14 -16% for the upper and 247 -273 -312 and 26-27% for the lower surfaces respectively. The vein islet number and veinlet terminations (Fig 3b) ranged 8-11 and 3-5 respectively. The palisade cells were conspicuous (Figure 3a) with a palisade ratio of 6-8-11.



St=stomata, Ep= epidermal cells

Fig 2: Stomatal cells on abaxial (a) and adaxial surfaces (b)



Tr= Trichomes containing granules, Ps=palisade cells, Vi- Vein islets, Vt = vein termination

Fig. 3: Trichomes and palisade cells of *C. occidentalis*

T/S of the midrib showed a bulging ventral surface with a slight depression on the dorsal surface. The dorsal and ventral surfaces were lined with rectangular shaped epidermal cells with elongated palisade cells lying orthogonal to it (Fig 4). The epidermal cells were covered by a thin cuticle. The collateral vascular bundle was chordate shaped.

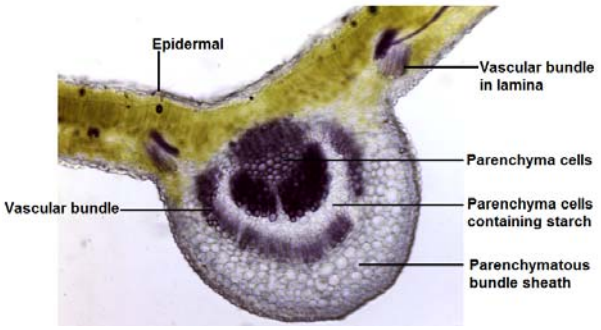
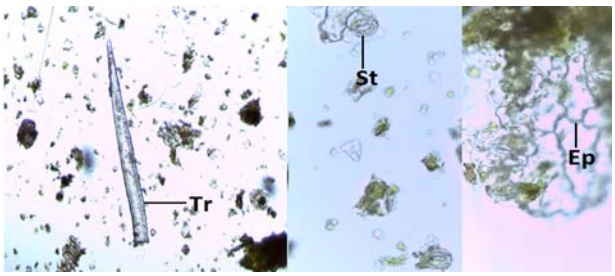


Fig 4: T/S of the midrib of *C. occidentalis*

3.3 Powder microscopy of the leaves

Powder microscopy of the leaves revealed the presence of broken trichomes, anomocytic stomata and wavy epidermal cells (Fig. 5).



Tr=Trichome, St= Stomata, Ep=epidermal cell

Fig 5: Powder microscopy of *C. occidentalis* showing a broken trichome, wavy epidermal cells and stomata

3.4 Physicochemical parameters

The physicochemical parameters of the leaf and seed of *C. occidentalis* are shown in Table 1. The water soluble extractives of both the leaf and seed were higher than that of the ethanol and petroleum ether soluble extractives respectively. Thus water and ethanol are better solvents for the extraction of the medicinal principles of *C. occidentalis*. These values may be useful for the detection of exhausted materials and possible adulteration.

The water and ethanol extractives were weakly acid to neutral (pH= 5.9-6.6). Thus traditional decoctions and bitters of *C. occidentalis* may not cause any gastrointestinal irritation. The different solvent extractives also showed characteristic UV fluorescence at 254 and 365 nm (Table 2-3). These characteristic fluorescence colours may serve as useful parameters for the identification and subsequent quality control of *C. occidentalis*.

Table 1: Physicochemical parameters of *C. occidentalis*

Physical Parameter	Leaves	Seeds
Water soluble extractive (mg/g)	300.8±9.6	196±12
Alcohol soluble extractive (mg/g)	180.8±20	92±2.0
Pet-ether soluble extractive (mg/g)	76.0±0.8	10.0±2.0
Total Ash (%$^{w}/_{w}$)	15.42	12.02
Acid -Soluble Ash (%$^{w}/_{w}$)	5.59	7.49
Water soluble Ash (%$^{w}/_{w}$)	5.29	3.23
Organic carbon (%$^{w}/_{w}$)	42.29	43.99
Moisture content (%$^{w}/_{w}$)	69.81±0.31	6.82±0.31

Table 2: Fluorescence characteristics of leaf extractives

Plant extract (leaf)	Visible light	Long wavelength	Short wavelength
Pet-ether	Yellowish green	Red	Dark
Ethanol	Black	Red	Black
Water	Yellow	Light green	Yellow

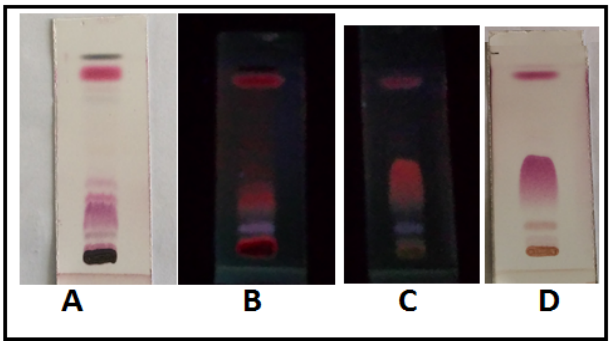
Table-3: Fluorescence characteristics of seed extractives

Plant extract (seed)	Visible light	Long wavelength (365 nm)	Short wavelength (254 nm)
Pet-ether	Yellowish green	Yellow	Olive yellow
Ethanol	Reddish brown	Light green	Dark green
Water	Light yellow	Yellowish green	Light yellow

3.5 Phytochemical and UV spectroscopic properties

Phytochemical screening of the plant parts revealed the presence of tannins, flavonoids, saponins, terpenoids, phytosterols and glycosides. These secondary metabolites may be responsible for its pharmacological activities such as anti-epileptic [14], hepatoprotective activity against paracetamol induced liver damage in rats [15] anti-inflammatory effects of the seeds [16], antibacterial [18] and antifungal [19] properties.

Thin layer chromatogram of the chloroform extract of the leaf and seed of *C. occidentalis*, developed with pet-ether/Chloroform 1:9, is shown in Fig 6. The leaf extract displayed five prominent spots at R_f values of 0.35, 0.39, 0.48, 0.83 and 0.92 whereas the three prominent spots of the seed occurred at R_f's of 0.22, 0.51, 0.84. The compounds in the extracts also showed characteristic fluorescence at long wavelength (Fig 6 B and C). The TLC chromatogram showed a common prominent pink spot at $R_f \approx 0.8$ in both the seed and leaf. This claim is substantiated by the UV spectrum of a 0.5% ethanol extract of the plant parts scanned between 200 and 400 nm and 240 to 350 nm (Fig 8 and 9). The UV spectra of the seed showed two λ_{max} at 270 and 333 nm. Similarly the leaf displayed two λ_{ax} at 275 and 362 nm. These two wavelengths agreed with that published for anthraqinones (230-270 nm). Remarkable for both the seed and leaf extracts, was the presence of a chromophore with strong absorption between 206-250 nm for both plant parts (Fig 7a and 8a). Again this suggests the presence of metabolites, with extensive conjugated systems, which may be common to the leaf and seed of *C. occidentalis*.



A, D: sprayed with anisaldehyde sulphuric acid reagent; B, C: Observed under UV 365 nm

Fig 6: TLC chromatogram of the leaf (A and B) and seed (C and D) of *C. occidentalis*

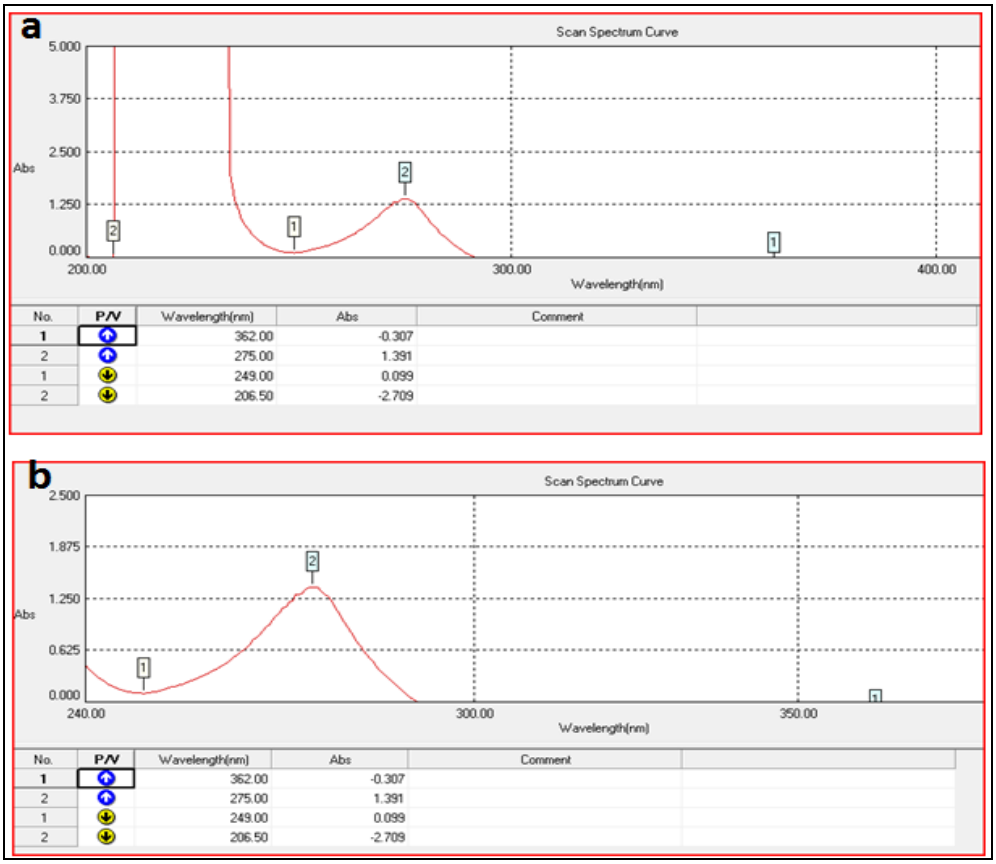


Fig 7: UV spectra of the ethanol extract of the seed of *C. occidentalis*

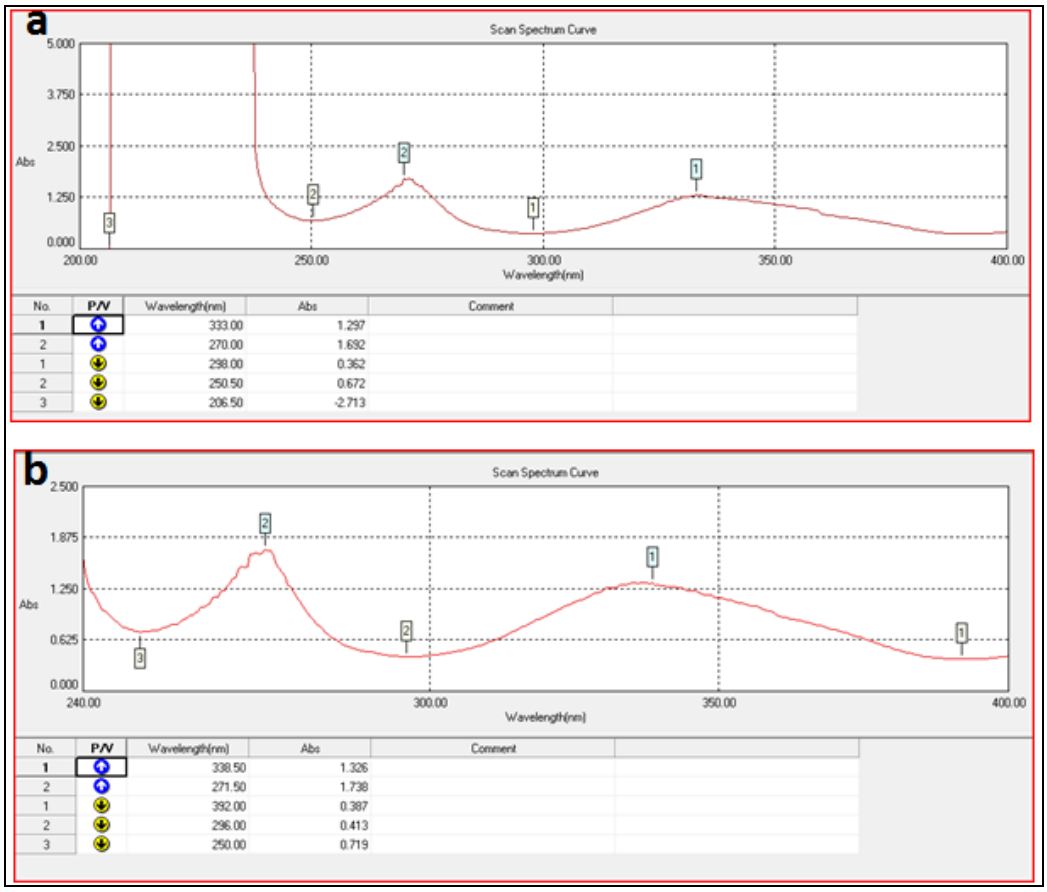


Fig 8: UV spectra of the ethanol extract of the leaves of *C. occidentalis*

4. Conclusion

The present studies have established organoleptic, macromorphological, microscopic and physicochemical properties which may be useful in the identification of *C. occidentalis*. The TLC chromatogram and phyto chemical analysis depicted the presence of similar compounds in both the seed and leaf. The characteristic UV spectra may be useful in the quality evaluation of *C. occidentalis* and herbal products containing its seeds or leaves.

5. Acknowledgment

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