Pharmacognostic Studies and Standardization of *Cassia Sieberiana* Roots

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**ABSTRACT**

*Cassia sieberiana* D.C. [Caesalpiniaceae] though widely used in traditional medicine as an analgesic in dysmenorrhoea, ulcer, general body pains and in veterinary medicine, there is no report on its standardization. **Objective:** To establish standards for a partial monograph preparation. **Materials and methods:** Standardization followed British Pharmacopoeia Standardization methods. Plant materials were collected and air dried for five days and then oven dried at 52°C for one hour and milled. A quantity of *C. sieberiana* powdered root was mounted separately in each of 10% iodine, chloral hydrate, phloroglucinol in conc HCl and Sudan III and examined under the microscope to identify the cell types and cell inclusions present. Mounted slides were photographed using digital camera Olympus OIL-8CC. Total ash value, acid insoluble ash, solvent extractives, pH and phytochemical analysis of extracts followed methods as in British Pharmacopoeia (BP) 1990. **Results:** Transverse section (TS) of the root bark shows outermost layer of cork, beneath which is a layer of parenchyma cells and a ring of stone cells. The longitudinal section (LS) of the root bark conforms to the same tissue arrangement. Calcium oxalate crystals are pyramidal shaped with base diagonals measuring 40 to 160µ. Starch grains are oval; length ranging from 60 to 180µ and diameter from 40 to 120µ. Total ash; 7.43-8.37, Acid insoluble ash; 4.80-5.50% w/w. Ethanol (70%) extractive; 14.28-14.30% w/w; Chloroform-water extractive; 15.99-19.98%w/w, pH (1% w/v at 28°C); 4.88. Constituents: Anthraquinones, Saponins, Sterols, Steroidal glycosides, Tannins, Triterpenes. **Conclusion:** Established standards can form the basis for monograph compilation **Keywords:** Cassia sieberiana, micromorphology, root, standardization.

**INTRODUCTION**

Plants have been used for various purposes since prehistoric times[1],[3],[5] *Cassia sieberiana* root bark and rootlets are chewed to treat abdominal pains,[8] as diuretic, for tooth ache, skin diseases, as antihelminthic, haemorrhoids, leprosy,[5],[6] and also widely used in veterinary medicine in Burkina Faso.[7] The plant belongs to the family Caesalpiniaceae R.Br[8] and grows in savannah forests, thickets and coastal scrubs.[4] The first report of the chemical constituents of *C. sieberiana* is probably that by Tambora and co workers in Burkina Faso.[7] The analgesic property of the root has also been reported.[5],[9] The root powder is reported to competitively antagonize acetylcholine and histamine in an isolated guinea pig ileum.[10] These findings lend credence to the use of the plant as an analgesic. With the current level of usage of *C. sieberiana* root it will be a great help if the root of the plant used as the drug is standardized. This work therefore aimed at conducting scientific study to standardize *Cassia sieberiana* root.

Standardization is the process of developing and agreeing upon technical specifications associated with a functional product. A document that establishes the technical specifications, criteria, methods, processes, or practices forms the standard.[11] In this work British Pharmacopoeia and other established methods were followed in the standardization studies.

**Partial monograph of Cassia sieberiana**

**Definition**

_Sieberiana_ root consists of the dried root bark and rootlets of *Cassia sieberiana* D.C. (pseudonym: *Cassia kotschyana* Oliv; _Senna sieberiana_ DC. [Caesalpiniaceae]

**Botanical name:** *Cassia sieberiana*
**Family:** Caesalpiniaceae

**Common English name:** West African laburnum, drumstick tree.

**Vernacular name** Akuko bewu, pirto rodom (Twi in Ghana)

**Plant material of interest:** Dried root bark and rootlets

**Macroscopical characteristics** The outer surface of mature root bark is brown and inner, buff in colour. The dried bark is curved with convex outer surface

**Materials and Methods**

Plant materials were collected from Mamfe–Akwapem in the Eastern Region of Ghana in August, 2009 and authenticated by Mr. Ofori–Lartey, a horticulturist in charge of the Herbarium at the Centre for Scientific Research into Plant Medicine, Mampong Akwapem, Ghana. A voucher specimen number KNUST/HM1/09/L 027 was deposited in the herbarium of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi. The root bark was cut into small pieces and air dried for five days and then oven dried at 52°C for one hour after which they were milled. The powder was put into a plastic container and stored in the project laboratory in the Department of Pharmacognosy, Kwame Nkrumah University of Science and Technology, Kumasi till they were needed

**Reagents and chemicals**
Except where otherwise stated all chemicals and reagents were purchased from Sigma Company, USA. Pre-coated silica gel chromatographic plates were obtained from Merck, Germany.

**Micromorphological studies of root bark**

**Transverse section (TS) of root bark**

Fresh root bark was peeled and air-dried for five days to stabilise constituents and soaked in chloroform water overnight to soften. A segment from the bark measuring about 5 mm × 3 mm was cut out and sectioned (free hand) with a razor blade. The sections were mounted in chloral hydrate overnight to clear and examined under the light microscope, Olympus-AX7320.

The best sections were mounted in phloroglucinol in conc. HCl. Another set of sections were mounted separately in 10% iodine, Sudan III and sodium picrate to characterise constituents of various tissues in the root bark.

**Longitudinal section (LS) of root bark**
The LS of the root bark was prepared in the same way as the TS

**Cell types in the powdered root**
A small quantity of *C. sieberiana* powdered root was mounted separately in each of 10% iodine, chloral hydrate, phloroglucinol in cone HCl and Sudan III and examined under the microscope to identify the cell types and cell inclusions present in the root powder. All specimens examined under the microscope were photographed using digital camera for microscopy, Olympus OIL-8CC

**Actions and uses** Anticholinergic and anti-histaminic. Strongly antispasmodic. Root used as an analgesic and worm expellant
Phisicochemical studies
Physicochemical determinations, total ash, solvent extractives, pH and phytochemical analysis of extracts followed methods as in British Pharmacoeopia (BP) 1990[12] and British Herbal Pharmacopoeia[13]

Thin layer chromatography (TLC) profile
*C. sieberiana* root powder (30 g) was soxhlet extracted with 300 ml of petroleum ether (40-60°C) for 18 hours. The marc was air dried and extracted with a total of 600 ml of methanol. Each extract was concentrated to a syrupy mass separately using rotary evaporator at 50°C. under reduced pressure and dried in a desiccator. The yields were: petroleum ether, 0.40 g (1.3% w/w) and methanol; 6.31 g (21.03% w/w)

The petroleum ether and the methanol extracts, 100 mg each were dissolved in 6 ml of chloroform and 8 ml methanol respectively. These were spotted on a pre-coated silica gel plate, Merck, -055540001 using a capillary tube and developed in a solvent system, chloroform-ethyl acetate (3:1) and ethyl acetate: methanol: water: petroleum ether (8:2:0.3:0.3) respectively.

The plates were dried in an oven and sprayed separately with alcoholic potassium hydroxide and anisaldehyde (AS) to detect the presence of anthraquinone and other non-anthraquinone compounds.

RESULTS

Macromorphology of root
*Cassia sieberiana* root is highly branched. The outer and inner surfaces are brown and buff in colour respectively.

The dried bark is curved with convex outer surface, figure 1.

Micromorphology
The transverse section (TS) of the root bark, figure 2 shows outermost layer of cork. There is a wide cortex made up of a ring of parenchyma cells, a continuous band of stone cells, stone cells in groups and fibres. The phloem shows large sieve tubes interspersed with phloem parenchyma and fibres.

The longitudinal section (LS) of the root bark, figure 3 shows the same tissue arrangement. The cell types and cell inclusions detected in the powdered root bark are given in figures 4, and 5.

Starch grains from *C. sieberiana* root powder are oval in shape with the length ranging from 60 to 180 μ (average 150 μ) and diameter ranging from 40 to 120 μ (average 50 μ)

Figure 1: Curved appearance of dried *C. sieberiana* root bark

Figure 2: Tissues of *C. sieberiana* root bark
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**Figure 3:** LS of *C. sieberiana* root bark

**Figure 4:** Some cell types from *C. sieberiana* root powder. a = unlignified fibres (×120), b = lignified fibres (×120), c = stone cells (×120), d = xylem vessels (×80), e = sieve tubes of phloem (×100), f = parenchyma (×100), a, b, c and e were mounted in phloroglucinol in conc HCl, f was mounted in N/10 iodine
The calcium oxalate crystals are pyramid shaped with base diagonals measuring 40 to 160µ (average = 60 µ), figure 5

Physicochemical studies
Results of the physicochemical studies are presented in Table 1.

Thin layer chromatography
The petroleum ether extract gave three anthraquinone compounds with Rf 0.69, 0.56 and 0.43 (Chloroform: Ethyl acetate (3:1), Temperature: 28-32°C.

DISCUSSION

*Cassia sieberiana* formulations are among the herbal preparations in popular traditional use as an analgesic due to its low or no observed side effects unlike that of allopathic pain killers: non-steroidal anti-inflammatory drugs (NSAIDs), that show a number of side effects including stomach ulcers, kidney failure and myocardial infarction especially in the elderly. The plant has the potential for development into a standard analgesic medicine for medical use. Three anthraquinones compounds whose Rf values were fairly constant in the given solvent systems were detected and therefore can serve as tlc finger prints. Tambora *et al* also reported of the detection of anthraquinones in the plant.[7] Further work being undertaken to unravel the chemical nature of the compounds responsible for the observed analgesic property will further enrich the monograph on the plant and can also lead to possible synthesis of the active compounds.

Conclusion The parameters established by this work, as summarised in the partial monograph could serve as the basis for *C. sieberiana* root standards.

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**Table 1: Results of physicochemical studies of *C. sieberiana* root**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>Total ash</td>
<td>7.43-8.37 (Average = 7.90% w/w)</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>4.80-5.50% w/w (Average = 5.15% w/w)</td>
</tr>
<tr>
<td>Ethanol (70%) extractive</td>
<td>14.28-14.30% w/w (Average = 14.38% w/w)</td>
</tr>
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<td>Chloroform-water extractive</td>
<td>15.99-19.98% w/w (Average = 17.99% w/w)</td>
</tr>
<tr>
<td>pH (1% w/v at 28°C)</td>
<td>4.88</td>
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<tr>
<td>Constituents detected in phytochemical screening</td>
<td>Anthraquinones, Saponin, Sterols, Steroidal glycosides, Tannins, Triterpenes</td>
</tr>
</tbody>
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**Figure 5:** Cell inclusions from *C. sieberiana* root powder. a = calcium oxalate crystals (×100), b = starch grains (×50) mounted in N/10 iodine
REFERENCES