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Pharmacognostic Studies of the Leaf of *Senna siamea* (lam.) Irwin & Barneby Family: Caesalpiniaceae

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Authors' contributions

This work was carried out in collaboration between all authors. Authors KBE and OTF designed and carried out all the study, wrote the protocol and wrote the first draft of the manuscript. Author JAI was involved in microscopic description and author OFK was involved in the supervision of the work and editing of the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To establish pharmacognostic standard for *S. siamea* and provide chromatographic fingerprints with a view to assisting in the quality control of medicinal products from the plant.
Study Design: Pharmacognostic evaluation of the leaf of *Senna siamea*.
Place and Duration of Study: Pharmacognosy laboratory, department of medicinal plant research and traditional medicine, national institute for pharmaceutical research and development (NIPRD), Abuja, Nigeria.
Methodology: Chemomicroscopic evaluation and determination of physicochemical properties (moisture content, ash values, extractive values) of the powdered leaves, macroscopy and microscopy of anatomical sections of the leaf were carried out using standard procedures.
Results: Evaluation of the macro and microscopic characters showed that the leaves are dark green, simple and entire. The leaf epidermis is polygonal, has paracytic stomata on the lower surface and uniseriate trichomes. Quantitative leaf analysis revealed stomatal number (464.7),

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stomatal index (38.23), palisade ratio (4.48), vein islet number (21.71) and vein termination number (20.71). Chemomicroscopic characters present include lignins, tannins, mucilage and oils. The physicochemical parameters evaluated are: Moisture content 7.33%, total ash 6.46%, acid-insoluble ash 5.32%, sulphated ash 8.47%, water-soluble ash 1.87%, alcohol-soluble extractive 5.12% and water-soluble extractive 16.71%. Chromatographic fingerprints of ethanol (70%) extract showed major spots at R_f = 0.74 daylight (yellow), UV_{366} (fluorescent), spray reagent (brown); R_f = 0.91 daylight (green), UV_{366} (red), spray reagent (brown).

Conclusion: The results from this study have provided information on the morphological, anatomical features and physicochemical parameters of the leaf of *Senna siamea*. The findings from this study will be useful towards establishing standards which can be included in official monograph of the plant for its proper identification and quality control.

Keywords: Senna siamea; pharmacognostic standards; microscopy; physicochemical studies.

1. INTRODUCTION

Plants are used as medicine to maintain human health from ages [1]. In Nigeria and many other African countries, application of medicinal plants especially in traditional medicine is currently well acknowledged and established [2]. However, the challenges of standardization of herbal medicinal products threaten its wide acceptance. The misuse of herbal medicines or natural products starts with wrong identification. The most common error is that one vernacular name is given to two or more entirely different species [3] hence collectors from the wild must be careful to authenticate the plant collected and not rely on the vernacular names from the locality. It is essential to lay down pharmacognostic specifications for each medicinal plant to aid in the authentication and ultimately ensure the quality and purity of final herbal products. The use of up to date techniques and already laid down standards by herbal pharmacopoeias is employed [4-6].

Senna siamea (Pheasant Wood or Kassod Tree) is a tropical tree, native to Southeast Asia, India, and Sri Lanka. This plant is a shrub, 10-12 m tall, occasionally reaching 20 m and is widely grown throughout tropical Africa. It belongs to the Caesalpiniaceae Family [7,8].

Senna siamea has been used traditionally in the management of constipation, diabetes, insomnia [9], hypertension, asthma, typhoid fever, and diuresis [10]. Leaves and bark of the plant were used locally as antimalarial medication [11,12]. The leaf is also used in the treatment of anaemia and fever [13]. Pharmacologically, the plant has been reported to have antimalarial, antidiabetic, [14], antitumor or anticancer [15,16], laxative [17] anti-inflammatory, [16], analgesic, antipyretic, anxiolytic, antidepressant, and sedative [18] properties. The following chemical constituents have been isolated from *S. siamea* in various parts of the world: β–sitosterol, chrysophanol, p–coumaric acid, apigenin–7–O–galactoside, 2–methyl–5–acetonyl–7–hydroxy–chromone, imeric anthraquinones, cassiamin A, B and C, cassianin, siameanin and siameadin, the isoquinoline alkaloid siamin and the dioxaphenalene derivative barakol [19]. The present study is aimed at investigating the macromorphology and pharmacognostic evaluation of the leaves of *Senna siamea* towards its standardization and monograph development.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

S. siamea was collected from Suleja, Niger State, Nigeria in the month of January 2016. The plant was authenticated and herbarium specimen was deposited at NIPRD Herbarium with Voucher no NIPRD/H/ 6737.

2.2 Chemicals, Reagents and Solvents

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

2.3 Macroscopic and Microscopic Analyses

The leaf of *S. siamea* was subjected to macroscopic studies using the methods of African pharmacopoeia [20-23] which comprised of organoleptic characteristics *viz*. colour, odour, appearance, taste, shape, texture etc. of the leaf. These parameters are considered useful in quality control of crude drugs [21,22].

Microscopic analysis was carried out on epidermal layers of the leaf. Leaves of the plant were cut at the median part. These were soaked in concentrated nitric acid for about 24 hrs [24].

The appearance of air bubbles indicated the readiness of the epidermises to be separated. Each piece was transferred to a petri dish containing distilled water with the use of fine forceps and dissecting needle; the upper and lower epidermises were separated, stained with safranin and later mounted on a slide with glycerol.

2.4 Chemomicroscopic Studies

Chemomicroscopic studies were carried out on the powdered plant material [25]. Reagents and stains like iodine, concentrated sulphuric acid, concentrated hydrochloric acid, ferric chloride, Sudan III, ruthenium red and phloroglucinol with conc. HCl (1:1) were used for the study.

2.5 Quantitative Microscopy

The quantitative microscopy was done using [25] Vein islet number, vein termination number, palisade ratio, stomatal number stomatal index and were determined.

2.6 Microphotography

Photomicrographs of the sections were taken using Leica CM E microscope with Digital Microscope Eyepiece attachment and Photo Explorer 8.0 SE Basic software at different magnifications (x100 and x400).

2.7 Physicochemical Evaluations

Physicochemical parameters of *S. siamea* leaf powder were determined [20,23,25]. Total ash, water-soluble ash, acid-insoluble ash, and sulphated ash values were determined. Alcohol and water-soluble extractive values were also determined.

2.8 Chromatographic Fingerprinting

Analytical TLC was done on silica gel G60 F_{254}, 0.2 mm layer using the method specified in the Nigerian herbal pharmacopoeia [26] Development of the plate was done using CH_2Cl_2: MeOH. Detection was in daylight, as well as under UV_{366} and also 10% aqueous H_2SO_4 spray reagent. The retardation factors (R_f) of each spot were calculated.

2.9 Statistical Analysis

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

3. RESULTS

3.1 Macroscopic and Organoleptic Features of the Leaf of *S. siamea*

Senna siamea is a shrub, 10-12 m tall, occasionally reaching 20 m. Leaves are simple, dark green in colour with papery texture, lanceolate shaped, apex emarginated and base cordate, margin entire, surface glaborous, venation pinnate and average leaf size 3.1 ± 0.02 (width) and 6.25 ± 0.05 (length).

3.2 Microscopic Description

This involves the description of different microscopic character of the plant such as leaf content, stomata type, trichomes etc. quantitative leaf constant such as stomatal number, stomatal index, palisade ratio, vein islet and vein termination number were evaluated.

3.3 Chemo Microscopic Features

This involves the determination of chemical constituents in the plant using specific reagents.

3.4 Determination of Physicochemical Values

The following results were obtained from the physicochemical evaluation.

3.5 Chromatographic Fingerprinting

Analytical TLC was done on silica gel G60 F_{254}, 0.2 mm layer. Detection was in daylight, UV_{366} and 10% aqueous H_2SO_4 spray reagent. Six (6) major spots were obtained at R_f = 0.10 daylight (colourless), UV_{366} (fluorescent), spray reagent (brown); R_f = 0.63 daylight (colourless), UV_{366} (fluorescent), spray reagent (brown); R_f = 0.74 daylight (yellow), UV_{366} (fluorescent), spray reagent (brown); R_f = 0.79 daylight (colourless), UV_{366} (red), spray reagent (colourless); R_f = 0.85 daylight (colourless), UV_{366} (fluorescent), spray reagent (colourless); R_f = 0.91 daylight (green), UV_{366} (red), spray reagent (brown).

4. DISCUSSION

There is an increasing interest in herbal medicines which may be due to the belief that herbal medicines are safe, cheap and have no adverse effects [27], coupled with an increase in scientific justification for many ethnomedicinal claims. However, there are still some challenges facing complete acceptance of herbal alternative medicines which may be due to lack of proper documentation as well as appropriate standardization and quality control processes. It is a fact that the therapeutic efficacy of medicinal plants depends on the quality and quantity of its chemical constituents and that the misuse of medicine or natural products in general started with wrong identification [27]. Therefore, for the preparation of herbal medicines, there is need for proper identification of the plant materials to ensure some level of standards for such products. Proper identification can be achieved through pharmacognostic and phytochemical studies. Pharmacognostic studies are reliable and affordable tools in the quality control of crude drugs [28]. Hence, it is very essential to lay down pharmacognostic specifications for medicinal plants being used as drugs [27].

The lower epidermal surface of *S. siamea* leaf indicated the presence of polygonal epidermal cells, abundant paracytic type stomata (Fig. 2) and numerous uniseriate, unicellular covering trichomes. The upper epidermis has covering unicellular trichomes, polygonal epidermal cells and no stomata. Microscopy of the leaf powder showed fragment of epidermal cell showing trichome base, trichome and palisade cells (Fig. 3). The transverse section of the lamina consists of epidermis, collenchymatous and parenchymatous cells. There were trichomes covering the upper epidermis. Vascular bundles were prominent, well-differentiated and consisted of xylem and phloem cells. Collenchyma cells were mostly found below the epidermis (Fig. 1).

Chemomicroscopic evaluation of the leaf powder indicates the presence of lignin, tannins and oils. Calcium oxalate crystals, cellulose, starch and proteins were absent (Table 3).

The moisture content of the leaf powder was 7.33%. This value falls within the limits for water content (8-14%) for vegetable drugs [30]. High water content promotes the growth of microorganisms leading to degradation and spoilage [29]. Ash values are used to determine the quality and purity of crude drugs [27]. They also determine the level of inorganic composition and other impurities present in the drug [31]. The total ash, acid-insoluble ash, water-soluble ash and sulphated ash values for *S. siamea* were 6.46%, 5.32%, 1.87% and 8.47% respectively (Table 1). These values suggest minimal contamination in the plant part. They indicate minimal amounts of silica especially sand as well as siliceous earth present in the sample investigated [29]. Alli 2009 [32] reported that the moisture and ash contents of *S. siamea* collected from Ado-Ekiti, Ekiti State (South Western, Nigeria) were 46.01% and 12.93% respectively. The disparities in the values are possibly due to collection from different geographical regions i.e. South West and North Central Nigeria, different soil types and different collection times.

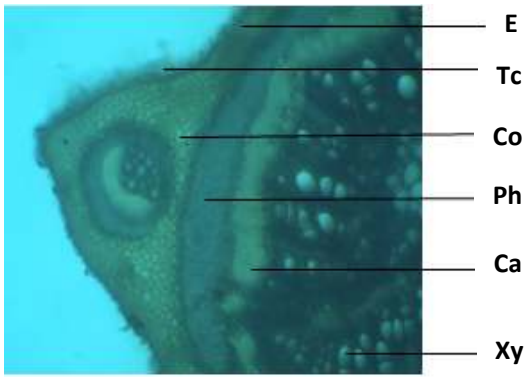

Fig. 1. Transverse section of the leaf of *S. siamea*. (Magnification x 400)

Tc = trichomes (covering hairs), E = epidermis, Co - collenchyma cells, Ph = phloem, Ca = cambium, Xy = xylem

Table 1. Physicochemical evaluation of the leaf of *S. siamea*

Parameters	Result (% w/w)
Moisture content	7.33
Total ash	6.46
Acid–insoluble ash	5.32
Water–soluble ash	1.87
Sulphated ash	8.47
Alcohol-soluble extractive	5.12
Water-soluble extractive	16.71

Extractive values are primarily useful in the detection of adulterated drugs. Estimation of extractive values determines the amount of the extractable constituents in a given amount of plant material when extracted with given solvents. The composition of these active constituents depends on the nature of the drug and the solvent used for extraction [33,27]. The alcohol-soluble and water-soluble extractive values of *S. siamea* were 5.12% and 16.71%

respectively (Table 1). A greater yield was obtained using the aqueous medium than the organic medium. It can be said that there are more polar compounds in the spp.

Although reports of various constituents isolated from *S. siamea* from other parts of the world are available, further studies on the compounds present in Nigerian *S. siamea* are needed as

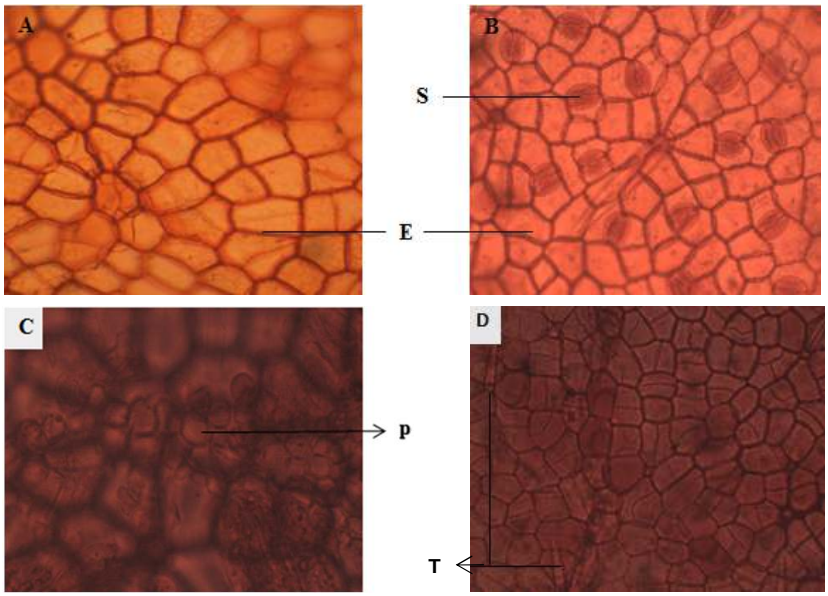

Fig. 2. Microscopic features of (Abaxial and Adaxial) epidermal surfaces of *S. siamea* leaf (Magnification x 400)

(A) Polygonal epidermal cells on adaxial surface (B) paracytic stomata on the abaxial surface (C) palisade cells on the adaxial epidermis (D) trichomes on adaxial surface. P-palisade cells; S-stoma; E-epidermal cells; T- trichomes

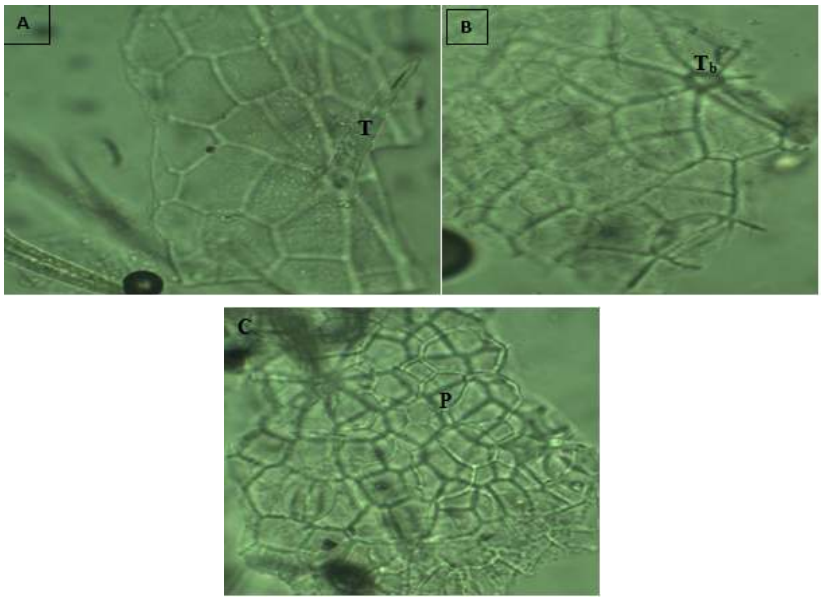

Fig. 3. Microscopic features of leaf powder of *S. siamea* (Magnification x 400)

(A) Polygonal epidermal cells with trichomes {T}; (B) polygonal epidermal cells showing trichome base {T_b}; (C) palisade cells {P}

seasonal, climatic and geographical factors could lead to difference in the class of secondary metabolites present or a total absence of a particular class of secondary metabolites.

Table 2. Quantitative leaf microscopy of *S. siamea*

Parameters	Range (per $mm^{2)}$	Mean ± SEM
Stomatal number abaxial surface*	437 - 484	464.7 ± 5.1
Stomatal number adaxial surface	absent	absent
Stomatal index abaxial*	-	38.2 ± 0.0
Vein islet number+	17 - 30	21.8 ± 1.5
Vein termination number+	16 - 28	20.7 ± 1.6
Palisade ratio+	3.75 - 5.25	4.48 ± 0.3

**n= 10, + n = 4*

Table 3. Chemomicroscopic evaluation of the leaf of *S. siamea*

Parameters	Result
Lignin	+
Cellulose	-
Tannins	+
Mucilage	+
Starch	-
Calcium oxalate crystal	-
Oils	+
Proteins	-

5. CONCLUSION

The results from this study have provided information on the morphological, anatomical features and physicochemical parameters of the leaf of *Senna siamea*. These parameters can be used for identification and quality control of the plant and plant drug and provide information which may be incorporated into the second edition of the Nigeria Herbal Pharmacopoeia (NHP) and the West African Herbal Pharmacopoeia (WAHP).

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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