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PHARMACOGNOSTIC EVALUATION AND PHYTOCHEMICAL SCREENING OF THE LEAF EXTRACTS OF *SENNA SINGUEANA* (Delile) Lock (FABACEAE)

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ABSTRACT

Introduction: *Senna singueana* (fabaceae) is a shrub used in traditional medicine to treat various illnesses.

Aim: The aim of the present research is to evaluate pharmacognostic parameters and detect the phytochemical constituents in *Senna singueana* leaves crude extract.

Study design: Macroscopical, Microscopical, Physicochemical, Chemomicroscopical evaluations and phytochemical screening using standard methods.

Place and duration of study: This study was conducted in the Department of Pharmacognosy and Drug development, faculty of Pharmaceutical sciences, Ahmadu Bello University, Zaria-Kaduna state between 2016 – 2017.

Methodology: The plant was identified, aunthenticated, collected and dried. This study evaluated the plant for its pharmacognostic and qualitative phytochemical profile using standard methods.

Results: Macroscopic examination carried out revealed fresh leaf of sizes, 2.48,2.50, 2.52 cm length by 1.63, 1.64, 1.65 cm width and are green in color, the taste is characteristics and texture, smooth. The leaves are elliptical in shape, oblong at apex and have compound venation. Microscopic examination of the transverse section through the midrib revealed covering trichomes, spongy parenchyma, palisade cells, lower and upper epidermis, vascular bundles consisting of xylem and phloem tissues, parenchyma cells. Chemomicroscopic characters present are tannins, starch, lignin, mucilage, suberin, aleurone grains and cellulose. The physicochemical evaluation indicated the average moisture content of 6.03%, total ash value 7.33%, Acid insoluble ash value 1.5%, water soluble ash value 4.50% while the alcohol and water extractives values are 36.66%and 31.33% respectively. The preliminary phytochemical screening revealed the present of triterpenes/steroids, flavonoids, alkaloids, saponins, cardiac glycosides, carbohydrates and proteins.

Conclusion: The identification and standardization profile of *Senna singueana* are deduced for compilation of a monograph. The present of some pharmacologically active phyto-constituents are established.

Key words: *Senna singueana*, pharmacognostic, phytochemical, physicochemical, macroscopic, microscopic, chemomicroscopic

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INTRODUCTION

Medicinal plants have been used in traditional medicine since prehistoric time to treat various forms of illnesses [1]. The World health organization [2] has estimated that 80% of the world's population depends on medicinal plants. However, reports on the adulteration or absence of the actual known herbs or plant responsible for the required activity in some herbal preparations are on the increase [3]. As a result of this, the patient/customer's health could be in danger and may lead to serious health problems in the patients. Hence, it has become very pertinent to make an effort towards improving the quality and reducing adulteration of crude drugs. [4]. *S. singueana,* family fabaceae is a shrub or small tree, scaly fissured and thick bark, with deciduous and open crown [5]. The tree grows up to 2 to 6 m tall and brings forth it flowers before the onset of rains (one of its unique characteristics) [6, 7)]. Common name is winter cassia, scrambled eggs, locally it is called 'Rumfu' in Hausa [8], Shadarat al bashime in *Arabic (*Niger*) [9]*. It is a common medicinal plant which is widely distributed in the wild in Africa including Nigeria, Namibia, Zambia, Malawi, Cote d'ivoire, Congo and Mozambique. Traditionally, the leaves are eating as vegetable, aids in ripening of bananas and as an infusion, it is used to treat venereal disease, malaria, convulsions, epilepsy, coughs, intestinal worms, constipation, heartburn and stomach-ache [10]. The leaves, either as a decoction or infusion, or as a dried powder, are applied to wounds caused by leprosy and syphilis. An infusion of the leaves is applied as eye drops to cure conjunctivitis [11]. Several studies have been carried out on the plant *S. singueana* root and stem bark [12, 13, 14] but only few and incomplete pharmacognostic and phytochemical evaluation on the leaves of this plant have been reported (10). Although *S. singueana* has numerous medicinal uses, researchers have been more focus on the root bark [7]. The leaves are a more sustainable source of medicine than root or stem bark [7, 11]. A number of phytochemicals have been detected in the plant *S. singuean*a especially from the root and bark [7, 11]. The present study reports the pharmacognostic evaluation, and phytochemical constituents of the leaves of *S. singueana* leaves. These parameters will be useful in authentication, standardization of the crude drug.

MATERIALS AND METHODS

Plant collection and preparation

The fresh plant material (the leaves, fruits and flower) was collected by a local plant collector Mallam Ibrahim of the department of Pharmacognosy, Ahmadu Bello University Zaria. The collected parts for authentication and identification by a taxonomist at the herbarium section of the Department of Botany, Ahmadu Bello University, Zaria, in August, 2016. A Voucher number was given. Sufficient quantity of the leaves was collected, dried at room temperature, powdered and stored at room temperature in a close container for further uses.

Pharmacognostics Evaluations

Macroscopic Examination

The leaf of the plant was evaluated for macroscopic characters using organoleptic parameters such as colour, odour, taste,

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texture, shape and fracture as described by Brain and Turner, 1975 [17].

Microscopical examination

Anatomical sections of the leaf sample were examined under the microscope and features were described using the terms according to Dutta, 2003 [15] and Evans, 2009[17]. The transverse section across the midrib of the fresh leaf of *S. singueana* was prepared using a blade. Leaf epidermises (upper and lower) were peeled. The prepared sections were cleared using 70% chloral hydrate solution and boiled on a water-bath for thirty minutes to remove obscuring materials. The cleared samples were mounted on a separate microscope slides, using dilute glycerol. They were then observed under the microscope and appropriate images of the diagnostic features were taken and documented.

Chemo-microscopic Studies

Small amount of the finely ground powdered leaves was cleared in a test-tube containing 70% chloral hydrate solution. The cleared sample was mounted on a microscope slide, using dilute glycerol. Using various detecting reagents, the presence of some cell inclusions and cell wall materials were detected in accordance with [16, 17].

Physicochemical Constants

The quantitative physicochemical parameters (solvent extractive values, ash values, moisture content determined as described in the British Pharmacopoeia [2012].

Extraction of the leaf powder

Extraction of the plant material was done using the method described by Kokate *et al.*, [19]. Briefly, five hundred grams (500 g) of the pulverized plant sample was extracted with n-hexane, ethyl acetate and methanol successively in a soxhlet apparatus. The extracts were concentrated to dryness in a water bath and kept in a desiccator for further uses.

Preliminary Phytochemical Screening

Preliminary phytochemical tests were carried out on each of the extracts i.e hexane (HE), ethyl acetate (EE) and methanol (ME) extracts according to standard procedures. [16, 18].

RESULTS

Plant Collection and Identification

The plant was identified as *Senna singueana* (fabaceae) with a specimen voucher number (6863) given and deposited in the Herbarium unit, Department of Botany, Ahmadu Bello University, Zaria in August, 2016.

Pharmacognostic Evaluation

Macroscopic Characteristics of *S. singueana*

The morphological characters of *senna singueana* leaves are summarized in Table 1 below. The fresh leaves are green in colour, smooth, has a characteristic odour with a distinct taste. The sizes of the leaf ranges from 2.48, 2.50, 2.52 cm by 1.63, 1.64, 1.65 cm, elliptic in shape and acute at apex with compound venation. The leaves are arranged in whorls of 3 at each node or rarely opposite. There surface is pubescent and measured up to 8 cm in length and 2.5 cm in breath (Table 1).

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Table 1: Organoleptic Features of *S. singueana* Leaf

S/N	Morphological Characters	Observations
1	Colour	Green
2	Odour	Characteristics
3	Taste	Distinct
4	Texture	Smooth
5	Shape	Elliptical
6	Apex	Obtuse
7	Venation	Compound
8	Size	2.48,2.50, 2.52 cm by 1.63, 1.64, 1.65 cm

Microscopic Examination

The transverse (TS) section of the leaf through the midrib (Plate I) shows trichomes, spongy parenchyma, palisade cells, lower and upper epidermis, vascular bundles consisting xylem and phloem tissues, parenchyma cells. The lower epidermis (plate III) shows paracytic type of stomata as the stoma appears to be embedded in one or two subsidiary cells parallel to the guard cells while the upper epidermis (Plate II) showed unicellelualr trichomes and epidermal cells.

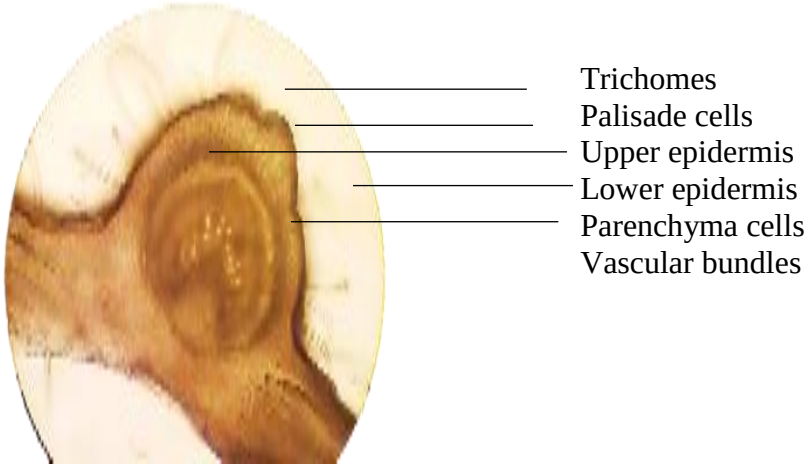

Plate I: Photomicrograph of the transverse section of *S. singueana* leaf through the midrib

Mag x 100

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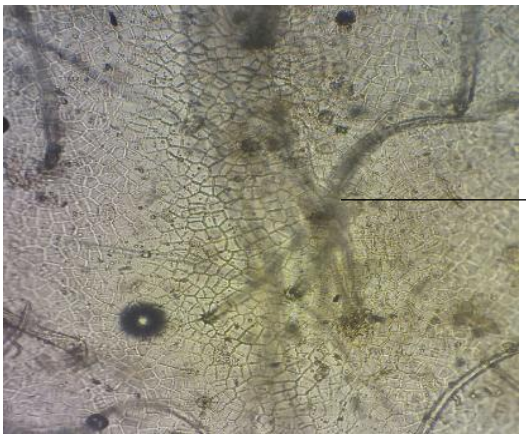

Plate II: photomicrograph of Upper Epidermis of *S. singueana* leaves.

Mag x 10

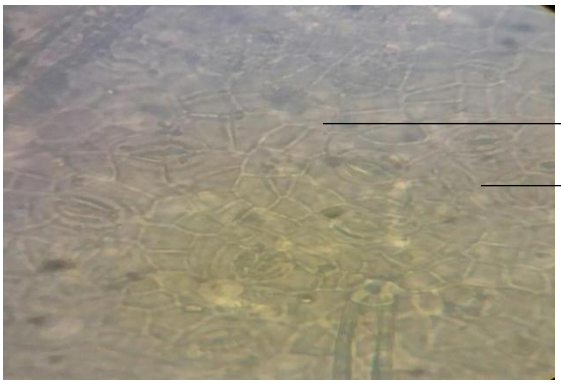

Plate III: Photomicrograph of the lower Epidermis of *S. singueana* leaf showing some features

Chemomicroscopical Studies of *S. Singueana* Powdered Leaves

Chemomicropical examination of the powdered leaves revealed the presence of tannins, starch, and lignin. The cellulose cell wall result is shown in Table 2.

Table 2: Chemomicroscopical Studies of *S. Singueana* Powdered Leaves

Constituents	Detecting agent	Inference
Starch	N/50 iodine	Present
Lignin	Phloroglucinol	Present
Tannin	5 % Fe Cl	Present
Mucilage	ruthenium red	Present
Calcium carbonate	HCl	Absent
Calcium oxalate	HCl	Present

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Cellulose	chlor zinc iodine	Present
Suberin	sudan red	Present
Aleurone grains	iodine in ethanol	Present

Physicochemical studies

Result of the physicochemical analysis of the *S. singueana* leaf is shown in Table 3. The physicochemical analysis of *S. singueana* powdered leaf reveals the parameters such as moisture content, total ash values, acid insoluble ash values, water soluble ash values, alcohol soluble extractive value and water - soluble extractive values.

Extraction of the Powdered Leaf

The dried powdered leaf of *S. singueana* (600 g) was extracted successively using soxhlet apparatus with sufficient quantity of n-hexane, ethyl acetate and methanol to obtain 13.06 g, 44.22 g and 85.90 g of n-hexane, ethyl acetate and methanol extracts respectively as shown in Table 4 below.

Table 3: Physicochemical Constants of *S. singueana* Leaves

Parameters	Values(%W/*W) *
Moisture content	6.03 ± 0.87
Total Ash value	7.33 ± 1.30
Acid insoluble ash value	1.50 ± 0.02
Water soluble ash value	4.50 ± 0.00
Alcohol extractive value	36.67 ± 0.88
Water extractive value	31.33 ± 0.88

* Mean of 5 counts

Table 4: Weight, Percentage yields of *S. singueana* Extracts

Extract	Weight (g)	% Yield (W/W)
n- hexane	13.06	2.61
Ethyl acetate	44.22	8.84
Methanol	85.90	17.18

Qualitative Phytochemical Screening

Result of preliminary phytochemical screening of the leaves of *S. singueana* is shown in Table 5 below:

Table 5. Preliminary Phytochemical Screenings

Phytochemical	n- hexane extract	Ethylacetate extract	Methanol extract
Alkaloid	Present	Present	Present
carbohydrate	present	Present	Present
Tannins	Absent	Absent	Present
Triterpenes/steriod	Present	Present	Present
Flavonoid	Absent	Present	Present
Anthraquinones	Absent	Absent	Present
Cardiac glycoside	Absent	Present	Present
Protein	Present	Present	Present
Saponins	Present	Present	Present

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DISCUSSION

The plant used for this study, was identified as *Senna singueana* from the fabacea family and a voucher specimen (6863) deposited in the herbarium. Some studies lack voucher specimens**,** which serve as permanent records of scientific investigations [20]. This omission makes positive identification impossible and hinders reproducibility. The need to recognize and describe plants has always been especially important because of their use for food and medicinal purposes. Plant classification is constantly changing. Shifts in species alignments and groupings are made as new evidence comes to light hence a need for voucher herbarium specimen for future references [21].

Table 1 showed the macroscopica parametes of the plant *S. singueana.* Macroscopical evaluation provides the simplest and quickest means to establish the identity and purity of medicinal plant in order to ensure the quality of a particular drug [22] Also, in establishing a monograph of the crude drug, detailed information of the morphological characters of the plant is needed Reports of herbal remedies devoid of the active constituents and crude drugs adulteration makes such an evaluation very necessary. Microscopically, Transvers section (Plate 1) of the plant showed some features which can also be used to confirm or establish the identity of this plant because, structural similarities in plants parts is an important means by which plants are categorised, grouped and hence, identified. Different plants have specific type of xylem vessels according to lignin present on its wall. One can easily check the purity of drug powder in microscopy by pattern of xylem vessel in comparison to standard literature. The upper epidermis (plate II) has numerous unicellular covering trichomes but has no stomata. These are features similar to results obtained from the findings on *Senna siamea* (Lam.) leaf by Esievo *et al.*, 2016 [23]. Unicellular trichrome (plate II) revealed in the upper epidermis is one of the unique features of senna leaves, an important tool to check for adulteration. The lower epidermal surface of *S. singueana* leaf (plate III) showed the presence of abundant paracytic type stomata as indicated by their unique features of two subsidiary cells at the opposite pole surrounding the stomata and numerous epidermal cells. The stomata are one of the best standard to identify a plant microscopically.

Herbal remedies used as medicine is often available in fragmentary form, and identification relies on microscopical rather than macroscopical evaluation [24]. Complete and accurate characterization of herbal plant materials must involve microscopic analysis in order to ensures it quality and purity and of course, safety of the crude drug. Macroscopic and microscopic evaluation have been proposed for herbal standardization in most regulatory procedure and pharmacopoeias. The leaf powder indicated the presence of calcium oxalate crystals, lignin, tannins, mucilage, cellulose, suberin and aleurone grains while calcium carbonate were absent (Table 2). These parameters are also vital for identification and quality control of crude drugs.

The physical constants values are similar to that of *Senna siamea* by Esievo *et.al*, 2016 [23]. The moisture content of the leaf powder was found to be 6.03 %. This value falls within the limits for water content (8 -14%) for vegetable drugs [25]. High water content promotes the growth of microoganisms leading to degradation and spoilage. Ash values are also criteria to detect the purity and

quality of crude drugs as they determine the level of inorganic composition and other impurities present in the drug [26]. The total ashes, acid- insoluble ash, water - soluble ash, are 5.00%, 1.50% and 4.50% respectively (Table 4.3). These values suggest minimal contamination in the leaf. Extractives values are another criterion used to judge the purity of crude drug and to determine adulteration. These values estimate the extractable constituents in a giving amount of plants material when extracted with a giving solvent. The compositions of these constituents depend on the nature of the drug and the solvent used for the extraction. The alcohol soluble and water - extractive values of *S. singueana* leaf were 36.66% and 31.33% respectively (Table 4). The constituents are more soluble in alcohol than aqueous solution. This is also seen in Table 5 when the powdered drug was extracted with methanol, it produced a higher percentage yield of 17.8%W/W. This is because, highly polar solvent has the ability to extract more of the phytochemical constituents. Preliminary phytochemical analysis of the leaves extract of *S.singueana* revealed the presence of some secondary metabolites which includes alkaloids, carbohydrates, tannins, flavonoid, anthraquinones, cardiac glycosides and triterpenes (Table 5). Phenolic compounds which are known to have antibacterial activity were revealed in the leaves extract. Flavonoid was present in both ethyl acetate and methanol extracts. The presence of flavonoids in the leaves extract of *S. singueana* plant indicates that it might have antioxidant properties thereby helping the body to fight against diseases [27]. Saponins and Tannins were present in the methanol extracts of *S. Singueana* leaves. Plants containing saponins are believed to have antioxidant, antiviral, anti-inflammatory anticancer properties [28]. The above result of phytochemical screenings can be related with the result obtained from the preliminary phytochemical screening from leaf and seed extracts of *Senna alata* L. Roxb [29] and also from *Cassia siberiana (*Fabacea) leaves [30].

CONCLUSION AND RECOMMENDATION

The result of this study provides some pharmacognostic standards of the plant *S. singueana* and also provides supporting evidence for it use in traditional medicine. The pharmacognostic standards could be used to prepare a monograph and prevent adulteration. Due to the growing interest in drugs from natural sources, it is recommended that, further study should be carried out on this plant in terms of isolation and characterisation of the phyto constituents and development of new drug from this plant.

Competing Interests

Authors have declared that no competing interest exist.

Authors Contribution

Oduma S.E; designed the study, funding, performed the laboratory analysis, managed literature searches and write up. Danmalam H. U; laboratory supervision, reading and editing. Abah Johnson O; literature review, abstract editing, proofreading. All authors read, edit and approved the final manuscript.

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