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Pharmacognostic evaluation of the leaves of *Sida acuta* Burm.F. (Malvaceae)

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Abstract

Sida acuta Burm F. (Malvaceae) is an erect, branched small perennial herb or small shrub growing abundantly in Nigeria. In the Southern part of Nigeria, the plant is used to hasten delivery. In Nicaragua, the decoction of the entire plant is taken orally for asthma, fever, aches and pains, ulcers and for venereal diseases. The Pharmacognostic profiles such as phytochemical, macroscopical, microscopical, chemomicroscopical, and quantitative evaluations were carried out on the powdered leaves and anatomical sections of the fresh plant. The phytochemical analysis of *Sida acuta* powdered leaves revealed the presence of cardiac and saponin glycosides, flavonoids and alkaloids. The leaves are simple and alternate in arrangement, green in colour, and pubescent. Each leaf is shortly petiolate possessing a lanceolate lamina with a serrated margin and an acute apex. The powdered drug was subjected to microscopical examination. The stomata are anisocytic, epidermal cells are wavy, numerous unicellular non-glandular trichomes are present, and there are prismatic crystals of calcium oxalate, fragments of lignified fibres and simple starch grains. Chemomicroscopical tests on the powder showed the presence of lignin, starch, calcium oxalate and mucilage. This investigation also reports the palisade ratio (10.00 -11.80 -15.0 ± 0.49), the stomata number (9.00-17.20-31.00 ± 2.14) for the lower epidermis and 3.00-7.10-12.00 ± 0.94) for the upper epidermis, the stomata index (18.37-31.00-32.00 ± 2.88 for the lower epidermis and 8.00-15.80-23.01 ± 1.90 for the upper epidermis), the vein islet number (36.00-38.10-42.00 ± 1.10), the vein termination number (14.00-19.10-24.00 ± 1.03), the total ash values (8.63 ± 0.07 % w/w), the acid insoluble ash values (0.65 ± 0.06 % w/w),the moisture content (9.47 ± 0.18 % w/w),the water soluble extractive values (2.93 ± 0.46 % w/w) and the alcohol soluble extractive value (1.35 ± 0.08 % w/w) for the leaves of *Sida acuta*. This study thus provides a monograph on the plant for its proper identification and detection of adulteration /substitution.

Key words: *Sida acuta* leaves, Pharmacognostic profile.

Introduction

Sida acuta Burm F. (Malvaceae) is an erect, branched small perennial herb or small shrub up to 1 m high which grows abundantly on cultivated fields, waste areas, roadsides and open clearing in Nigeria (1). The plant is commonly known as "Udo"

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or "ire-agwo" in Igbo, "Osanpotu" in Yoruba, "Riegueyoto" in Bini and "Itseketu" in Ora.
The plant has a variety of local uses. The leaf juice is used in India for vomiting and gastric disorders (2). In Nigeria, the infusion of the leaves is given to women in labour; the decoction of the leaves is prescribed during malaria fever; the root is a bitter tonic, astringent and antipyretic (3). In Nicaragua, the decoction of the entire plant is taken orally for asthma, fever, aches and pains, ulcers, anthelmintic medication as well as for venereal diseases (4, 5).
In previous studies we reported the analgesic, anti-inflammatory, anti-ulcer activities of *Sida acuta* (6), and the uterine muscle activities of the aerial parts of the plant (7). Having thus established the scientific rationale for the ethnomedical application of this plant, we then carried out further studies to establish the pharmacognostic characteristics of the plant. The characteristics examined were the macroscopy, microscopy, chemomicroscopy and evaluative parameters of the leaves of *Sida acuta* which could be useful for the proper identification and standardization of the herbal medicinal material.

Materials and methods

Plant material
Sida acuta plants were collected in June 2006, at Ikpoba hill Benin City. Nigeria. The plant was authenticated at the Forestry Research Institute of Nigeria, Ibadan, where a herbarium voucher specimen (FHI No.107151) was deposited. A second specimen was deposited in the herbarium of the Department of Pharmacognosy, faculty of Pharmacy, University of Benin.
Fresh leaves of the plant were used for the macroscopical and microscopical studies. The fresh leaves were air dried on the laboratory bench for 5 days and then ground to coarse powder using an electric mill model. The powdered leaves were used for the Pharmacognostic studies which were phytochemical screening, macroscopy, microscopy and the quantitative analysis of the powdered plant material.

Phytochemical screening
Phytochemical tests were carried out on the powdered plant material employing standard phytochemical procedures to establish the presence or otherwise of secondary metabolites such as alkaloids, steroids, flavonoids, tannins and saponin glycosides in the crude drug. (8, 9).

Macroscopical examinations
The following macroscopic characters were noted for the fresh leaves of *Sida acuta*. Physical characters such as colour, condition, composition /arrangement of leaf,

shape,size, apex, base, venation, margin, attachment of the leaf and sensory characters such as texture, odour and taste (9).

Microscopical examinations

Epidermal characters: Fragments of the leaf were mounted on a clean slide with dilute glycerol after clearing the specimen with chloral hydrate. The leaf fragments were covered with clean cover slip and observed under a compound microscopy. The presence/absence of the following was observed and appropriate drawings made by means of the Abbé camera Lucida: Epidermal cells, stomata (type and distribution), and epidermal hairs (types of trichomes and distribution).

A small quantity of the powdered leaf was also cleared, mounted, and observed for any cell inclusions: calcium oxalate crystals, oil globules, and starch grains. The transverse sections of the fresh leaves through the lamina and the midrib were prepared using cassava pith, by placing the leaves in between the cut surface of the cassava pith and carefully cutting the sections. The cut sections were cleared by boiling in chloral hydrate solution, mounted in dilute glycerine solution and observed under the microscope. Appropriate drawings were then made by means of the Abbé camera lucida.

Chemomicroscopical examinations

Examinations of the powder was carried out to detect the presence of various chemical constituents such as starch, mucilage, calcium oxalate, lignin, hemicellulose and cellulose using the methods described in the literature (9,10).

Quantitative evaluation

Quantitative evaluation was carried out on the powdered plant material to determine the moisture content, total ash value, acid-insoluble ash value, water soluble ash value and the extractive values. Other parameters evaluated included the palisade ratio, stomata number, stomata index, vein islet number and vein termination number. All studies were carried out employing standard procedures (9,10,11). Each determination was carried out ten times and the results expressed as the mean $\pm$ SEM values for the moisture content, total ash value, acid-insoluble ash value, water soluble ash value and the extractive values. The results for the palisade ratio, stomata number, stomata index, vein islet number and vein termination number were expressed as a range of the lowest value, the mean value and the highest value $\pm$ SEM.

Results

Phytochemical tests

The phytochemical analysis of *Sida acuta* powder revealed the presence of cardiac

and saponin glycosides, flavonoids and alkaloids (Table 1).

Table 1: Results of phytochemical tests

S/n	Test	Observation	Inference
1.	Molischs test for carbohydrates	Deep violet ring was observed at the interface	Carbohydrates present
2.	Fehling s solution test for reducing sugar	A brick-red precipitate was observed	Glycoside present
3.	Frothing test for saponin glycosides	Persistent frothing was observed	Saponin glycoside present
4.	Blood haemolysis test for saponin glycoside	Clear zones of haemolysis was observed	Saponin glycoside confirmed
5.	Borntragers test for anthraquinone glycosides	No pink colouration was observed	Anthraquinone glycoside absent
6.	Test for cyanogenetic glycosides	Yellow colour of sodium picrate paper retained.	Cyanogenetic glycoside absent
7.	Keller-killiani test for deoxy-sugar	A brown ring was observed at the interface	Deoxy-sugar present in cardiac glycosides
8.	Keddes test for lactone ring	A violet colour that faded gradually with the deposition of whitish crystalline so lid was observed	Lactone ring present in cardiac glycosides
9.	Liebermans test for steroidal ring	A colour change from violet to blue to green was observed	Steroidal ring present in cardiac glycosides
10.	Salkowskis test for steroidal ring	A reddish-brown colour was observed at the interface	Steroidal ring present in cardiac glycosides
11.	Tests for flavonoids	A yellow colour which turned to colourless was observed	Flavonoids present
12.	Aqueous ferric chloride test for Tannins	No blue black, green or blue green precipitate or colouration observed	Tannins absent
13.	Test for Phlobatannins	No red precipitate was observed	Phlobatanins absent
14.	Test for alkaloids using water, methanol and chloroform as extracting solvents	Wagners, Hagers and Dragendorffs reagent gave characteristic precipitates with methanol and chloroform extracts	Alkaloidal base present

Macroscopical features of the leaves of Sida acuta

The leaves are simple and alternate in arrangement. Green in colour on both surfaces, the upper side being somewhat darker than the lower and they are pubescent. Each leaf has a short petiole with a lanceolate lamina between 3-5 cm long and 1-1.5 cm wide. It has a serrated margin and an acute apex (Figure 1).The leaves have a slight odour and a slightly bitter taste.

Microscopical features of the leaves of Sida acuta

The epidermal cells contain numerous prismatic crystals of calcium oxalate. Anisocytic stomata are present mostly on the lower surface. The epidermis possesses numerous unicellular non-glanduler trichomes (Figure 2).

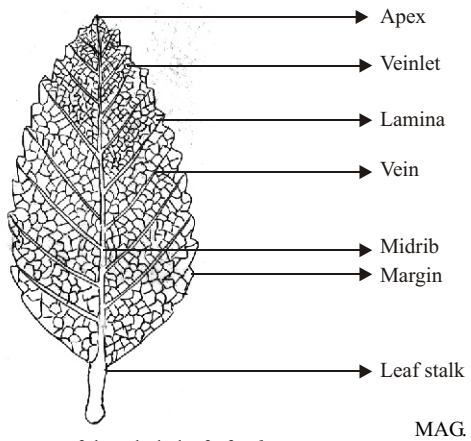


Fig.1. Macroscopy of the whole leaf of *sida acuta*.

A transverse section of the leaf of *Sida acuta* through the mid rib shows a dorsiventral structure. The epidermal cells have wavy walls and a straight cuticle. The midrib is partly surrounded by an arc of pericyclic fibres, above and below which, is a considerable amount of collenchyma. The xylem fibres occur in an arc

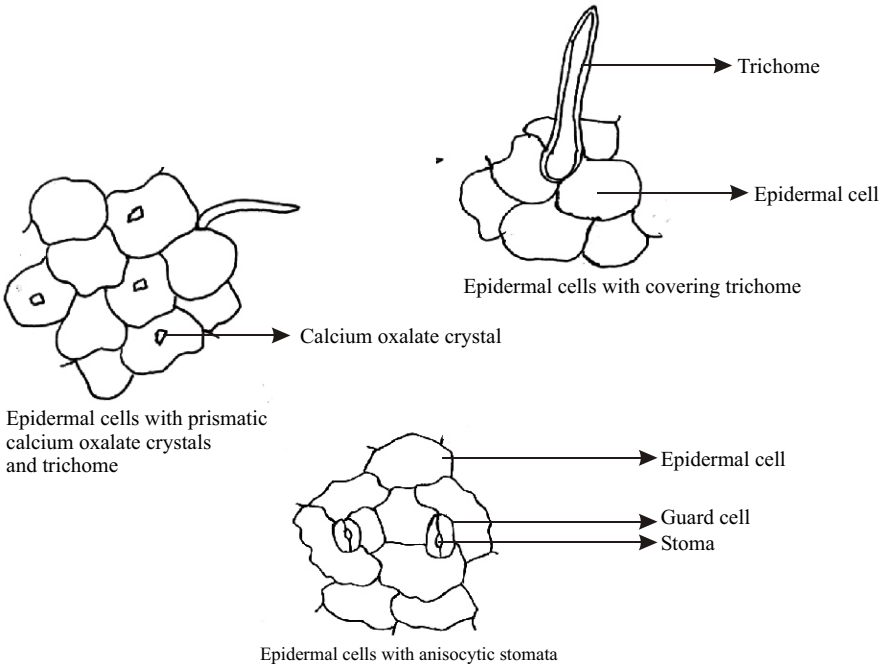
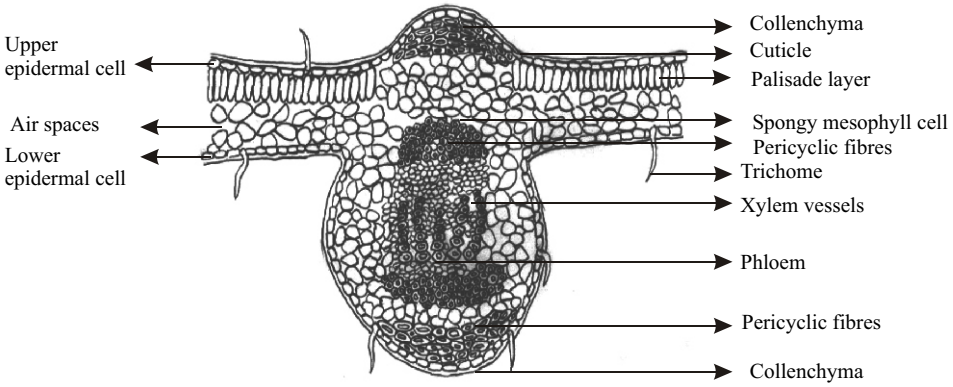
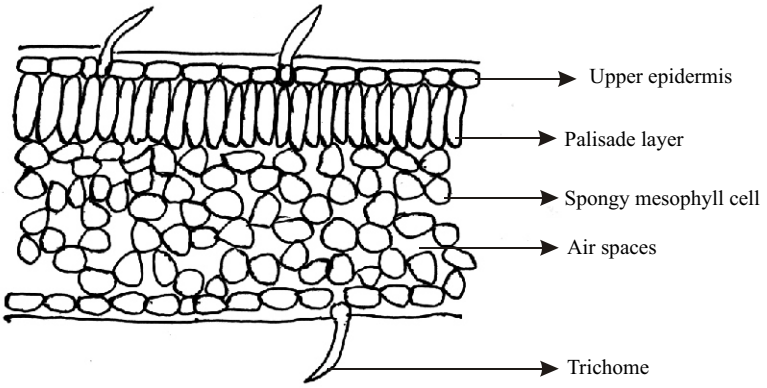


Figure 2: Epidermal characters of the leaf of *Sida auta* Mag. X 400

arrangement. The cells are round and spirally arranged. The phloem also forms a continuous arc below the xylem fibres. The palisade cells are cylindrical in shape and present on the upper epidermis only. The spongy mesophyll shows thin walled irregular parenchymatous cells .The transverse section of the leaf of *Sida acuta* through the lamina shows a thin walled cuticle, cylindrical palisade cells and a spongy mesophyll with thin walled irregular parenchymatous cells (Figure 3).



A. Tranverse section through the Mid rib

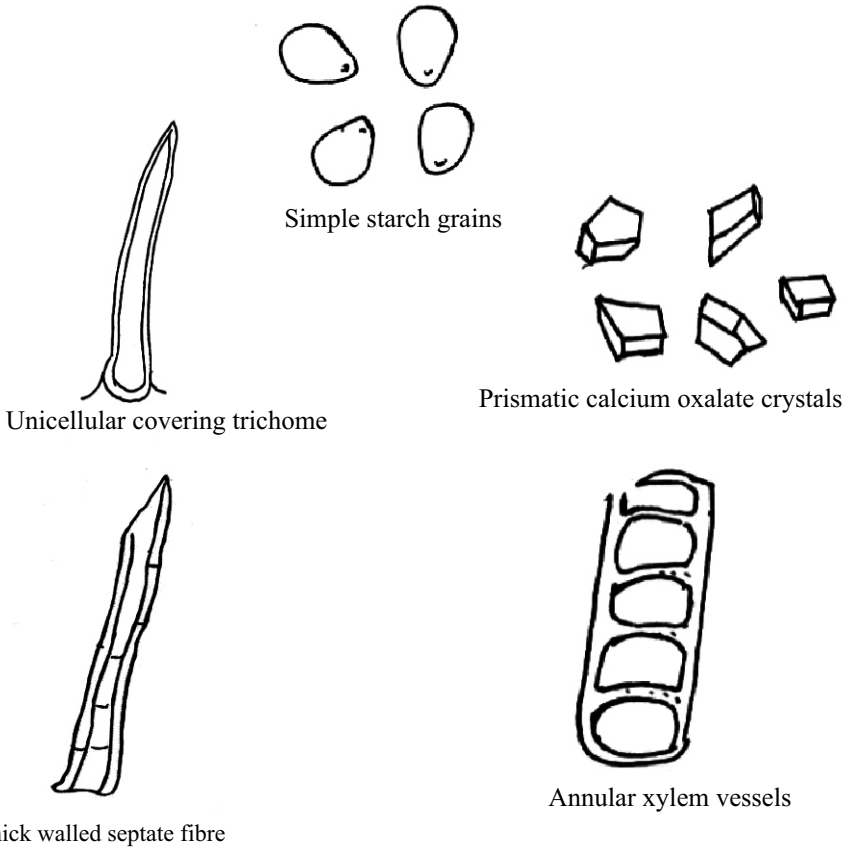


B. Tranverse section through the lamina.

Figure 3: Transverse section of the leaf of *Sida acuta* Mag. X400

Structures of diagnostic importance showed by the powdered *Sida acuta* leaves include the presence of wavy epidermal cells with anisocytic stomata, sharply pointed thick walled annular and reticulate vessels size, fragments of lignified fibres

of length 107.10 µm and width 28.56 µm with unicellular non-glanduler trichomes, simple starch grains of size 14.28-21.42 µm, which are oval in shape and prismatic crystals of calcium oxalate (Figure 4).



MAG. X 400

Fig. 4. Microscopic characters of the the powdered leaves of *Sida acuta*

Chemomicroscopic

Red coloured fibres observed on addition of phloroglucinol and conc. HCL showed the presence of lignin in the powdered leaves. A blue-black colouration observed on mounting the powdered leaf sample in N/50 iodine was indicative of the presence of starch. The presence of calcium oxalate was shown by shiny crystals that disappeared on addition of 66 % sulphuric acid to the powdered sample mounted in iodine solution. Red colouration observed on addition of ruthenium red showed the

presence of mucilage in the powdered crude drug.

Quantitative evaluation

The results of the quantitative evaluation of the crude drugs are shown in Table 2. The results for the total ash, acid insoluble ash, alcohol soluble extractive value, water soluble extractive value, moisture content, palisade ratio, stomata number, stomata index, vein islet number and vein termination number were determined.

Table 2: Summary of the results of the quantitative determinations

Parameters	**Mean Values (% w/w) ± SEM**
Moisture content	9.47 ± 0.18
Total ash	8.63 ± 0.07
Acid insoluble ash	0.65 ± 0.06
Water soluble ash	2.09 ± 0.18
Alcohol soluble extractive	1.35 ± 0.08
Water soluble extractive	2.93 ± 0.46
Other Parameters	**Range values ± SEM**
Palisade ratio	10.00 -11.80 -15.0 ±0.49
Stomatal number :Lower epidermis	9.00-17.20-31.00 ± 2.14
:Upper epidermis	3.00- 7.10-12.00 ± 0.94
Stomatal index :Lower epidermis	18.37-31.00-32.00 ± 2.88
:Upper epidermis	8.00-15.80-23.01 ±1.90
Ve in islet number	36.00-38.10-42.00 ±1.10
Veinlet termination number	14.00-19.10-24.00 ± 1.03

Discussion

The plants *Sida acuta* contain bioactive constituents such as alkaloids, flavonoids, cardiac and saponin glycosides. These could be collectively or individually responsible for the activities of the plant as reported earlier. The plant was also subjected to Pharmacognostic study. The macroscopical and microscopical character obtained for the leaves of *Sida acuta* could serve as diagnostic parameters for the plant. The quantitative values determined for the plant could also serve in the identification and differentiation of *Sida acuta* from other plants. The moisture content obtained in the determination of quantitative standards meets the Pharmacopoeia limits of water content for vegetable drugs, which is between 8-14 % (12). The presence of excessive water greater than this value in any sample of this vegetable drug would promote the growth of microbes, fungi or insects and the hydrolysis of constituents leading to a deterioration of the drug. Such sample could be rejected.

The ash values obtained for the plant are important since ash may be derived from the plant tissue itself (physiological or natural ash) as well as from the extraneous matter, especially sand and soil, adhering to the surface of the drug (non-physiological ash). The determination of the physiological or natural ash and the non physiological ash together is called the total ash determination. Total ash may vary within wide limits for specimen of genuine drugs due to the variable natural or physiological ash, in such cases the ash obtained is treated with acid in which most of the natural ash is soluble leaving the silica as acid-insoluble ash which represents most of the ash from the contaminating soil (13, 14). Any significant deviation in the percentage of ash reported in this work may indicate adulteration or substitution of the drug. The ethanol and water soluble extractive values obtained are important because any addition of exhausted material to the crude drug would be indicated by a lowering of these extractive values.

Conclusion

This work has been able to establish the Pharmacognostic standards for a genuine sample of *Sida acuta* herb. Establishing standards is an integral part of establishing the correct identity and quality of a crude drug. Before any drug can be included in the Pharmacopoeia these standards must be established. A drug claimed to be similar to *Sida acuta*, but whose characters significantly deviate from these reported values should be regarded as either a substituted, contaminated or adulterated sample.

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