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Research Article

Pharmacognostic Studies of the Leaves of *Stachytarpheta jamaicensis* Linn. (Vahl) (Verbenaceae)

Udodeme H O*, Odoh U E, Ugwu P N, Diovu E O, Okonta E O, Onyekere P F, Ezugwu C O

Department of Pharmacognosy and Environmental Medicines, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

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ABSTRACT

The pharmacognostic standards of fresh, powdered and transverse sections of *Stachytarpheta jamaicensis* (SJ) leaf was carried out to determine its macroscopical, microscopical (both qualitative and quantitative), analytical standards and phytochemical profile. The macroscopy revealed a simple, glabrous, relatively thick and slightly fleshy leaf that is obovate with symmetrical base, bluntly acute apex, with a serrate-dentate margin. The qualitative microscopy of the powdered leaf shows xylem vessels with phloem parenchyma cells, epidermal cells with diacytic type of stomata, epidermal cells with anticlinal sinuous wall, palisade cells attached to epidermal cells, multicellular uniseirate trichomes, irregular shaped prism calcium oxalate, and small bundle of fibre. The quantitative microscopy of the leaf of SJ showed the values of palisade ratio, stomatal number (upper and lower epidermis), stomatal index (upper and lower surface), vein-islet number and vein termination number to be 4.42 ± 2.53, (105.67 ± 2.73, 277 ± 17.08), (28.00 ± 2.31, 21.00 ± 2.51), 15.67 ± 0.66 and 3.50 ± 0.00 respectively. For the analytical standards; 11.85 ±0.06, 2.17 ± 0.00, 8.80 ± 0.14, 2.04 ± 0.02, 2.51 ± 0.15, 4.85 ±0.22 and 4.30 ± 0.02 were obtained for total ash, water soluble ash, sulphated ash, acid insoluble ash, alcohol soluble extractive value, water soluble extractive value and moisture content respectively. The qualitative phytochemical analysis of SJ leaves showed the presence of carbohydrates, reducing sugars, alkaloids, glycosides, saponins, tannins, flavonoids, resins, proteins, steroids and terpenoids. These specific standards obtained through experimentation are of importance in the establishment of diagnostic indices for the identification, standardization and preparation of monograph on the plant.

Keywords: *Stachytarpheta jamaicensis L. (Vahl),* Pharmacognostic Standards, Macroscopy, Microscopy, Analytical Standards, Phytochemical analysis.

INTRODUCTION

Stachytarpheta jamaicensis (SJ) originates from the New World tropics and at present has a pantropical distribution[1]. It is now a pantropical weed present in east and West Africa, Madagascar, the Ryukyu Islands of Japan, Taiwan, the Indian Subcontinent, Australia, Indonesia, Malaysia and on many pacific Islands[2]. It is commonly known as Blue porterweed and locally known in Hausa as *Tsarkiyar Kuusuu*, in Yoruba as *Agogo Igun* and in Efik as *Aran-umon.* In African ethnomedicinal practices, most plant extracts are generally used in the form of infusion or decoction. SJ is used in ethnomedicine to treat numerous ailments. The root decoctions are abortive. Decoctions of leaves are vermifuge to children[3]. Triturated fresh leaves have been used on ulcers. It is also used as maturative cataplasm for boils. Bruised leaves have been rubbed on sprains and bruises[2]. In Brazil, it is used for cough, fever, to expel worms and promote menstruation; as a diuretic and laxative. It is also used for rheumatism[3]. In phytomedicine, the leaves of *Stachytarpheta jamaicensis* are used for birth control, abortion, treatment of menstrual disorders and as a galactogogue[4]. A flavonoid, scuttelarain has been isolated, with cardioprotective, anti-inflammatory and antiviral actions[3]. Hopidulin, another flavonioids, is reported to be bronchodilator, antispasmodic and anti-asthmatic[3]. Study of leaves isolated a new lanostane triterpenoid 16β-(β-D-glycopyramosyl-3-8, dihydroxylanstan-5, 22-diene-11-methroxy-1β-yl-6-0-(2, 3-dimethoxybenzoyl)-β-d-glycopyranoside[4]. The methanol extract of *Stachytarpheta jamaicensis* leaves showed significant antidiarrheal activity and moderate inhibitory activity against *E. coli*, *Staph.* epidermis and *P. aeruginosa*[5]. Crude aqueous extract showed activity against *B. subtilis*, *E. coli*, *C. albicans*, *S. aureus*, *P. aeruginosa*, *P. valgaris*, and *P. mirabilis*.[6]. Study showed more antimicrobial activity with the chloroform extract against gram positive organisms like *S. aureus, E. faecalis, and B. subtilis*. The chloroform and alcohol extracts showed antifungal activity against *C. albicans* and *Saccharomyces cere viseae*[7]. Inhibitory effect of leaf extracts of *Stachytarpheta jamaicensis* (*Verbenanceae*) was done on the respiratory burst of rat macrophages. Extract showed potent O_2-scavening activity. Study suggests SJ may have potential pharmaceutical value for immunologic disease related to oxidative stress[8]. The ethanol extract of *Stachytarpheta jamaicensis* exhibited significant schizonticidal activity comparable to that of the standard drug, chloroquine. The

**Author for Correspondence*

Table 1: Results of Phytochemical Analysis

S. No	Constituents	Inference				
		CME	HF	EF	BF	WF
1.	Carbohydrates	+	-	-	+	+
2.	Reducing sugars	+	-	-	+	+
3.	Alkaloids	+	+	+	+	+
4.	Glycosides	+	-	+	+	+
5.	Saponins	+	-	+	+	+
6.	Tannins	+	-	+	+	+
7.	Flavonoids	+	-	+	+	-
8.	Resins	+	+	+	-	-
9.	Proteins	+	-	+	+	+
10.	Oils	+	+	-	-	-
11.	Steroids	+	+	+	+	+
12.	Terpenoids	+	+	-	-	-

CME = Crude Methanol extract, HF = N-Hexane fraction, EF = Ethyl acetate fraction, BF = N-Butanol fraction, WF = Water fraction.
- = not present, + = present.

Table 2: Observations from Macroscopic observations of whole leaf.

S. No	Parameters	Observations
1.	Colour	Green and often have a slight bluish or grayish tinge
2.	Margin	Serrate-dentate
3.	Apex	Bluntly acute/obtuse to slightly acute
4.	Composition of lamina	Simple
5.	Shape of lamina	Obovate
6.	Midrib	Raised at the lower surface but flat on the upper surface
7.	Venation	Reticulate, Pinnate
8.	Base	Symmetrical
9.	Size	2-12 cm long and 1-5 cm wide are borne on stalks
10.	Texture	Relatively thick and slightly fresh
11.	Surface	Hairless (Glabrous) or have a few hairs along the veins on the underside (i.e. sparsely strigose)
12.	Odour	Characteristic
13.	Taste	Tasteless

Table 3: Results of Quantitative Microscopy.

Parameters		Values
Palisade ratio		4.42 ± 2.55
Stomatal number:	upper epidermis	105.67 ± 2.73
	Lower epidermis	277.00 ± 17.08
Stomatal index:	upper surface	28.00 ± 2.31
	Lower surface	21.00 ± 2.51
Vein-islet number		15.67 ± 0.66
Veinlet termination number		3.50 ± 0.00

Values shown are Mean ± SEM, n = 3

Table 4: Results of Analytical standards.

Parameters	% composition
Total ash	11.85 ± 0.06
Water soluble ash	2.17 ± 0.00
Sulphated ash	8.80 ± 0.14
Acid insoluble ash	2.04 ± 0.02
Alcohol soluble extractive value	2.51 ± 0.15
Water soluble extractive value	4.85 ± 0.22
Moisture content	4.30 ± 0.02

Values of percentage composition shown are Mean ± SEM, n = 3

antiplasmodial activity confirms its folkloric use in the treatment of malaria[9]. The effects of *Stachytarpheta jamaicensis* tea on plasma lipid profile and atherogenic indices were studied in rabbits. Treatment caused significant decrease in plasma total cholesterol, LDL, VLDL and triglycerides with significant decrease in atherogenic indices. The result suggests the use of SJ tea in the management of primary and secondary dyslipidemia[10]. Study evaluated the analgesic activity of various extract of dried leaves on acetic induced writing responses in Swiss albino mice. Result showed significant analgesic effect[11]. Study evaluated the wound healing effect of a hydroalcoholic leaf extract of *S. jamaicensis* on streptozotocin induced diabetic rats. Results showed significant dose-dependent wound healing potential with a significant increase in percentage wound closure, tensile strength, hydroxyproline, Hexosamine, DNA and total protein content together with decrease in period of epithelization and blood sugar levels[12]. The aim of this study, therefore, seeks to investigate the pharmacognostic standards of this plant so as to obtain its diagnostic

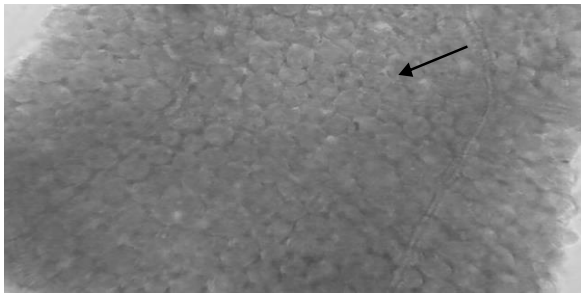

Figure 1: Photomicrograph of powdered leaf of SJ showing clustered palisade cells.

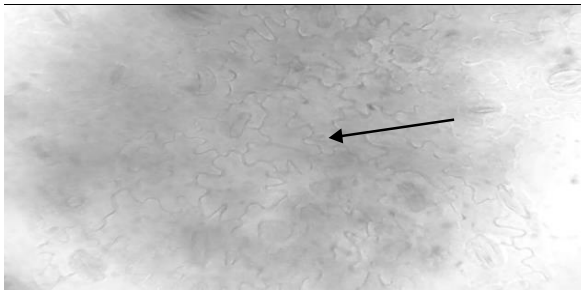

Figure 2: Photomicrograph of powdered leaf of SJ showing epidermal cells with anticlinal sinuous wall.

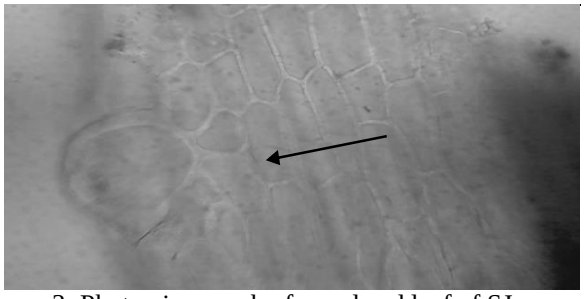

Figure 3: Photomicrograph of powdered leaf of SJ showing large phloem parenchymal cells.

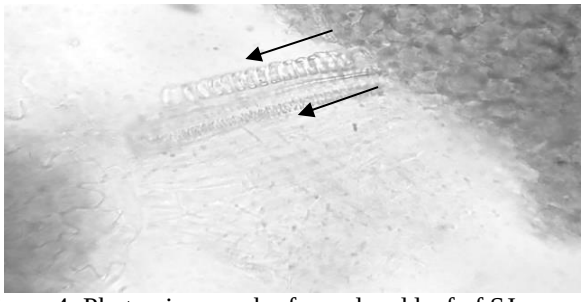

Figure 4: Photomicrograph of powdered leaf of SJ showing spiral and annular xylem vessel.

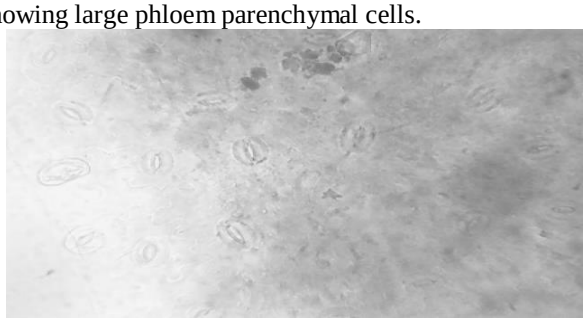

Figure 5: Photomicrograph of the sectional view of TS of SJ showing stomata in the lamina of transverse section.

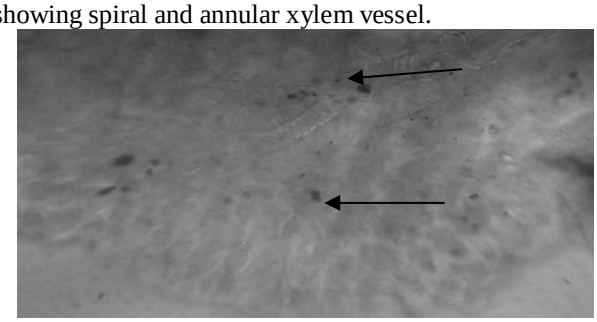

Figure 6: Photomicrograph of the sectional view of TS of SJ showing collenchyma cells and annular xylem vessels

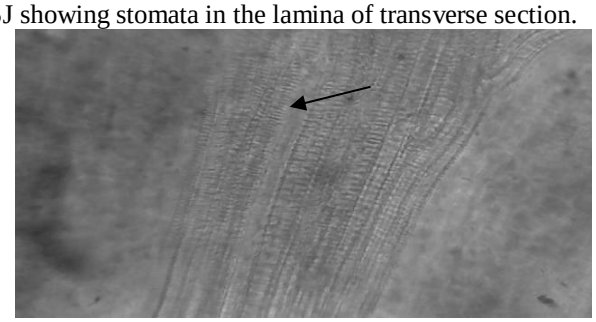

Figure 7: Photomicrograph of the sectional view of TS of SJ showing bundle of scalar form-like xylem vessels.

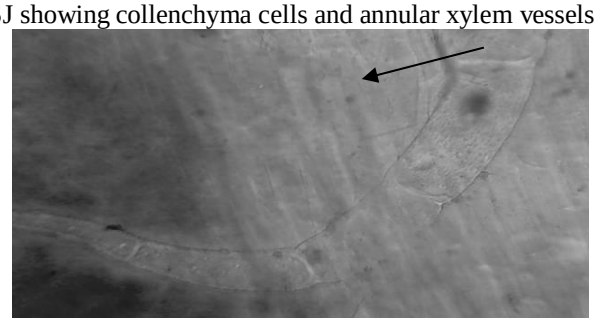

Figure 8: Photomicrograph of TS of the lower epidermis of SJ showing multicellular uniseirate trichome and phloem parenchyma cells.

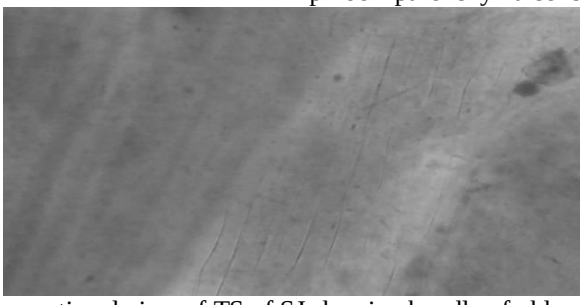

Figure 9: Photomicrograph of the sectional view of TS of SJ showing bundle of phloem cells adjacent to xylem vessel

characters as well as establish diagnostic indices which can be compiled into a monograph.

MATERIALS AND METHODS

Collection, Identification and Preparation of Plant materials

The samples of SJ leaves were collected in Udenu Local Government in Enugu State of Nigeria in June, 2014. The plant was identified and authenticated by Mr. A.O. Ozioko of the International Centre for Ethnomedicine and Drug Development (Inter CEDD), Nsukka, Enugu-State, Nigeria. The voucher specimen was deposited at the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka. SJ fresh leaves were air-dried in shade, pulverized and the leaf powder was used for Pharmacognostic Standardization.

Phytochemical Analysis

Chemical tests were performed on the powdered leaf in order to detect the presence or absence of major secondary plant metabolites of pharmacognostic importance using standard methods[13,14].

Pharmacognostic Standardization

Macroscopic Examination of the leaves

The fresh leaves of SJ were virtually examined using the methods described by Evans[15]. The macroscopic features of the leaves which include type of margin, veination, size, shape, base, apex, mid-rib, surface character and texture were observed and noted. The organoleptic properties such as colour, odour and taste of the plant materials were also observed and noted.

Microscopic Examination of Powdered leaves

Qualitative Microscopy

A sample of the leaf powder was placed on the slide, two drops of chloral hydrate solution was added to moisten the powder and also act as a clearing agent. The slide was passed across the flame of a bursen burner repeatedly until bubbles occurred. It was allowed to cool, the slide was covered with glycerin followed with cover slip and was viewed under the photomicroscope (magnification x 100). The microscopic characters were observed and noted.

Quantitative Microscopy

This was determined following standard method[15]. A constant range of values for palisade ratio, stomatal number, stomatal index, vein-islet number and veinlet termination number were obtained.

Microscopic examination of transverse section

An anatomical section of the fresh leaf was prepared for the microscopic studies. The staining was done using standard laboratory methods[15,16]. The transverse section was done by sectioning of the specimen using a sledge micrometer. It was transferred into a staining jar and stained in safranin for 5 minutes. The section was washed with distilled water, followed with alcohol, and thereafter stained again with 1 % fast green for 5 minuted and washed with absolute alcohol. It was transferred into a jar containing 50/50 alcohol/xylene and washed until they became clear. The section was cleared with chloral hydrate solution and mounted on a slide with dilute glycerine.

Determination of Analytical Standards

The qualitative and quantitative analysis of the chief chemical constituents of the crude drugs, determination of the various ash values as well as solvent extractive values followed the specification as described in the British Pharmacopoeia[17].

RESULTS

Phytochemical analysis

The results of the phytochemical screening of SJ leaf are shown in Table 1.

Macroscopic examination of the whole leaf

The macroscopic examination of the whole leaf of *Stachytarpheta jamaicensis* are shown in Table 2.

Microscopic examination

Microscopy of the Leaf Powder

The photomicroscopy of the powdered leaf of *Stachytarpheta jamaicensis* showed a number of features as shown in

Transverse Section of the Leaf.

The transverse section of the leaf of *Stachytarpheta jamaicensis* showed the outline of the microscopical characters present in the powdered leaf with a magnification of x200 as shown in Fig 10

Quantitative Microscopy of the Leaf

The quantitative microscopy of the leaf of *Stachytarpheta jamaicensis* gave values for palisade ratio, stomata number, stomatal index, vein-islet number and veinlet termination number as presented in Table 3.

Analytical standards of the leaf

The analytical standards of the leaf of *Stachytarpheta jamaicensis* showed the percentage composition of the total ash, water soluble ash, sulphated ash, acid insoluble ash, alcohol soluble extractive value, water soluble extractive value and moisture content as presented in Table 4.

DISCUSSION

Phytochemical analysis of the extract and fractions revealed the presence of biologically active constituents such as alkaloids, carbohydrates, reducing sugars, flavonoids, steroids, saponins, terpenoids, tannins, glycosides, resins, proteins and oils (Table 1). The presence of these phyto-chemical constituents has been reported in *S. jamaicensis*[6]. Pharmacognostical standardization of *Stachytarpheta jamaicensis* (SJ) showed that it has a set of peculiar identities, specific characteristics which are generally unique and of unshared qualities[18]. With the macroscopical standards, the macroscopic characters of the leaves such as margin, venation, base, apex, midrib etc. were seen to be serrate-dentate, reticulate/pinnate, symmetrical, bluntly acute and raised at the lower surface but blunt at the upper surface respectively. These morphological features of *S. jamaicensis* leaves observed were consistent with the descriptions reported by Akobundu and Agyakwa[19]. The organoleptic properties such as colour, odour and taste of the plant material were also seen to be green, characteristic and tasteless respectively. Qualitative microscopical examination revealed the presence of epidermal cells with anti-clinal sinuous walls, stomata (diacytic type), annular

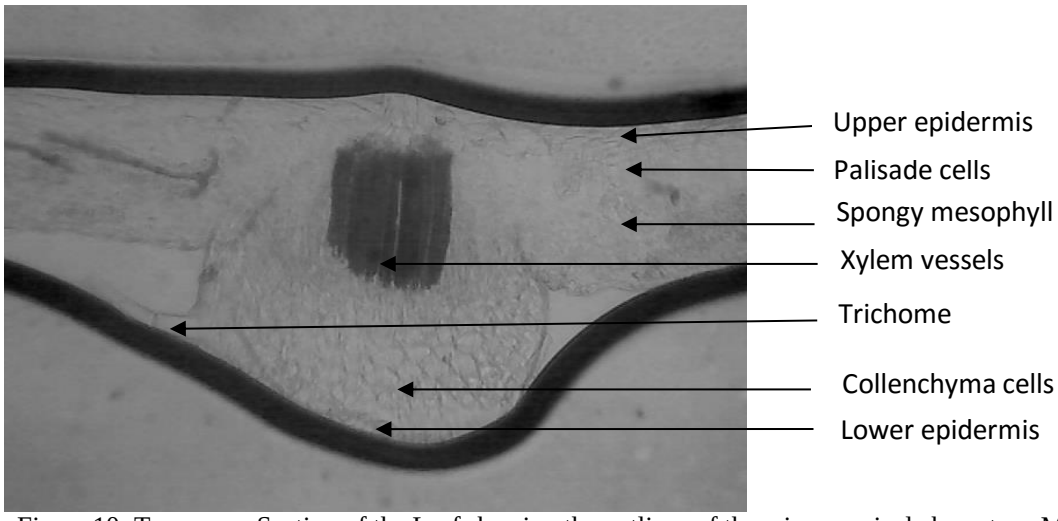

Figure 10: Transverse Section of the Leaf showing the outlines of the microscopical characters. Magnification x 200.

and spiral xylem vessels, typical phloem cells, both unicellular and multicellular uniseriate trichomes, bundle of fibres, palisade cells and irregular/prism calcium oxalate crystals (Figs 1-9). Quantitive microscopy revealed the presence of numerous stomata on the lower epidermis of *S. jamaicensis* while the upper epidermis had few scattered stomata cells. This feature in association with observable characters aided in the determination of stomata number, stomata index, vein-islet number, vein-islet termination number and palisade ratio. From the results (Table 3), the stomata index for the upper epidermis gave a higher value than for the lower epidermis which is not correlating with recommended figures. This is due to the large nature of the epidermal cells which does not increase with corresponding increase in the number of stomata cells. The analytical standardization of *S. jamaicensis* powdered leaves were within pharmacopoeial standards and developed numerical standards which could be used as reference guides for their identification and assessment of their quality and purity. The total ash value was 11.80 ± 0.06 %. The total ash value is useful to exclude drugs which have been coated with chalk, lime or calcium sulphate to improve their appearance. The acid insoluble ash is more reliable than the total ash value. This is because the calcium oxide/carbonate yield by the incinerated oxalate being soluble in dilute hydrochloric acid is removed. The results showed that the *S. jamaicensis* leaf powder gave acid insoluble ash of 2.04 ± 0.02 %. Based on this result, it could be inferred that the drastic reduction from total ash value is because the calcium oxalates and carbonates were soluble in hydrochloric acid and were thus removed. The water soluble ash value gave 2.17 ± 0.00 %. The water soluble ash is used to detect the presence of materials exhausted by water. It is an important indication of the presence of exhausted material substituted for the genuine article[20]. The sulphated ash value also produces a more consistent ash than the total ash because all oxides and carbonates are converted to sulphates at the high temperatures used; this gave 8.80 ± 0.14. The extractive yields are used as means of evaluating crude drugs, the constituents of which are not readily estimated by other means. In some cases, the amount of a drug soluble in a given solvent is an index of its purity. The results showed that the constituents of *S. jamaicensis* leaves are very soluble in both alcohol (2.51 ± 0.15 %) and water (4.85 ± 0. 22 %). Moisture content gave 4.30 ± 0.02 % at room temperature; this shows possible hydrolysis of active components when exposed to air.

CONCLUSION

The results presented in this study could serve as diagnostic parameters for proper identification as well as preparation of a monograph on *Stachytarpheta jamaicensis* Linn. (Vahl).

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