

MICROMORPHOLOGICAL AND PHARMACOGNOSTIC STUDIES OF LEAF AND STEM OF *Solenostemon monostachyus* P. Beauv (LAMIACEAE)

ABSTRACT

Solenostemon monostachyus P. Beauv (Lamiaceae), its ethnomedical uses include anti-plasmodial, anti-pyretic, antiulcerogenic, antioxidant, anti-inflammatory and anti-nociceptive activities. The aim of this study was to employ the quality control parameters in the evaluation of the leaf and stem of *S. monostachyus*. The plant leaves and stems were collected, identified, air-dried, pulverized and stored in separate glass containers. Standard procedures were employed to obtain the microscopic features of the fresh and powdered samples, micromeritic, chemomicroscopy, fluorescence properties, moisture contents, ash values and soluble extractive values. The results of the microscopic studies using fresh and powdered leaf samples revealed the presence of diacytic stomata on both the abaxial and adaxial surfaces (amphistomatic), with stomatal index of 27.9% and 14.8% respectively. The result of the micromeritics properties of the powdered leaf and stem samples showed angles of repose of 38.0° and 46.0°, Carr's index of 23.7% and 32.5% and Hausner's ratios of 1.3 and 1.5 respectively. Results for the moisture content, total, acid-insoluble and water-soluble ash values were 11.7%^{w/w}, 13.7%^{w/w}, 1.8%^{w/w} and 9.4%^{w/w} for the leaf and 13.3%^{w/w}, 17.3%^{w/w}, 1.8%^{w/w} and 9.2%^{w/w} for the stem respectively. Extractive values for water-soluble, methanol-soluble and ethanol-soluble were 26.5%^{w/w}, 32.3%^{w/w}, 14.5%^{w/w} and 15.5%^{w/w}, 15.8%^{w/w} and 14.5%^{w/w} for the leaf and stem respectively. Chemomicroscopy indicated the presence lignin, calcium oxalate crystals, protein and oil in the leaf and not in the stem. The results obtained therefore could be used to establish pharmacopoeial standard for the fresh and powdered drug product of *S. monostachyus*, thus preventing adulteration.

KEYWORDS: Amphistomatic, Carr's index, chemomicroscopy, Hausner's ratio, micromorphology, micromeritics, pharmacognostic, *Solenostemon monostachyus*

INTRODUCTION

Solenostemon Monostachyus from the family Lamiaceae is an annual weed in anthropogenic habitats and rocky savannahs [1]. The plant is erect with a long inflorescence of blue or violet flowers. It is slightly succulent, aromatic herb up to 100 cm tall, branched; stem erect or decumbent, 4-angled, shortly pubescent. Leaves opposite, simple; stipules absent; petiole 1.5–4 cm long; blade ovate, 5–9 cm × 3–6 cm, cuneate at base, obtuse to acute at apex, margin crenate, puberulous and gland-dotted below, distinctly veined. It is widespread in African countries particularly West and Central Africa which include Ghana, Sierra Leone, Nigeria, Cameroon, Gabon. The plant

tends to survive well in rocky savannahs as a perennial herb or even as a subshrub, in grassland areas with slightly acidic soil and also during rainy seasons. Thus, it is found in waterlogged areas, close to water bodies and dies off during dry seasons [2]. The aerial parts of the plant are used in various decoctions traditionally by the Ibibios of the Niger Delta region of Nigeria to treat stomach ulcer, fever and malaria [3], [4], hemorrhoid and other inflammatory diseases. The decoction of the plant is also used to treat hypertension and as a diuretic [5]. The leaf sap is considered sedative and stomachic and is applied internally to treat colic, convulsions, fever, headache and cough especially in children and externally against eyesight troubles. Moreover, leaves are used to treat dysmenorrhea, hematuria, female sterility, rheumatism, foot infections and snakebites. The roots are used to treat onchocerciasis (river-blindness) and craw-craw. The plant has many ritual uses, especially related to pregnancy [6]. The plant has been reported to possess antioxidant [7], [8] antihypertensive [9] and antimicrobial activities [10], antiulcerogenic [11] and antidiabetic and hypolipidemic activity [12]. Isolated compounds from *S. monostachyus* includes, Rosmarinic acid, methyl rosmarinate, Caffeic acid, Methyl Caffeate, Apigenin, Luteolin, Apigenin glucoronide, Quercetin [13].

Kingdom - Plantae

Clade - Tracheophytes

Clade - Angiosperms

Clade - Eudicots

Order - Asterids

Family - Lamiaceae

Genus - *Solenostemon*

Species - *Solenostemon monostachyus* P. Beauv [14].

Common name: Monkey's Potato

Local name: Ibibio- Ntorikwot; Yoruba- Olojogbodu or Ewe rinrin



Figure 1: *Solenostemon monostachyus*, in its natural habitat

MATERIALS AND METHOD

Identification, Collection and Preparation of the Plant Material

Plant sample was collected from the field around Faculty of Pharmacy, University of Uyo in March, 2021. The plant was identified by Prof. (Mrs) Margaret E. Bassey of Botany and Ecological studies, Faculty of Sciences, University of Uyo with herbarium identification number: UUPH No. 38(b). The fresh plant materials were air-dried, pulverized and packed in a dry container, well labelled and used when needed.

Anatomical Studies

Microscopic Evaluation of Leaf

The standard median portion of the well expanded matured leaf was obtained. Microscopical examinations of the Epidermis of both adaxial and abaxial surfaces were made by placing the leaf on a glass slide. The sample was irrigated with water and scraped gently with a sharp razor blade till loose cells from the epidermis were washed away with water and the desired epidermis was reached. The epidermal peels were further cleared with sodium hypochlorite and rinsed gently with water. The epidermal peels were stained with aqueous solution of safranin-O for (five) 5 minutes and 10% glycerol. The stained samples were mounted on a binocular microscope. Photomicrographs were taken from good preparations using the Olympus CX21 binocular microscope fitted with an MD500 microscope eyepiece camera. Measurements were done at $\times 10$ while $\times 40$ for photomicrographs [15].

Quantitative Microscopy of the Leaf

Quantitative microscopy parameters such as leaf constant studies namely stomatal length and width, guard cell length and width, stomatal number, stomatal index, epidermal cell length and width, epidermal cell number, epidermal cell thickness were carried out using standard procedures.

All measurements were made using a calibrated ocular micrometer and 10 microscopic fields chosen at random were used and data presented as mean \pm Standard Error of Mean (SEM).

Stomatal Index Determination

The stomatal index (S.I) was determined according to Metcalfe and Chalk [16], [17].

using the formula:

The sample (quantitative microscopy) was placed under the microscope and the stomatal index was determined using the formula;

$$S.I = \frac{S}{E+S} \times 100$$

Where S = Number of stomata per unit area
 E = Number of epidermal cells in the same area

Micromeritics

The flow property was determined using standard methods [18], [19] which constitutes;

Bulk Density and Tapped Density

The weight of 10 g of dried powdered leaf was weighed into 100 ml measuring cylinder and the volume occupied was noted as the bulk volume (V_b). The cylinder was gently tapped repeatedly to obtain a constant volume noted as the tapped volume (V_t). Bulk density was calculated using the formula below;

$$B\rho = \frac{M}{V_b}$$

Where;

$$T\rho = \frac{M}{V_t}$$

Where $B\rho$ = Bulk density
 M = Mass of powder
 V_b = Bulk volume of powder
 $T\rho$ = Tapped density
 V_t = tapped volume

Interparticulate porosity was also calculated using the formula below;

$$IP = \frac{\rho_T - \rho_B}{\rho_T * \rho_B}$$

Hausner's Ratio and Carr's index

Hausner's ratio a function of interparticle friction was calculated using the formula

$$\text{Hausner's ratio} = \frac{T\rho}{B\rho}$$

While Carr's Index is measured as

$$\text{Carr's index} = \frac{T\rho - B\rho}{T\rho} \times 100$$

Where; $T\rho$ = Tapped density
 $B\rho$ = Bulk density.

Angle of repose

$$\theta = \text{Tan}^{-1}\left(\frac{\text{Heap height of powder}}{\text{Radius of heap base}}\right)$$

Chemomicroscopic Analysis of Leaf Powder

Powdered leaf was examined for its chemomicroscopic properties namely mucilage, lignin, starch, oils, calcium carbonate and calcium oxalate crystals using standard procedures [20].

Fluorescence Analysis of Leaf Powders

The fluorescent analysis of dried leaf powder was carried out using standard method [21].

Physico-chemical Evaluation of Leaf Powders

The physicochemical parameters such as moisture content, ash values (total ash, acid insoluble ash, water soluble ash, sulfated ash), soluble extractive values such as ethanol, methanol and water-soluble extractive values were performed according to the official method prescribed by the WHO guidelines on quality control methods for medicinal plant materials [17,22,23]

Table 1: Results for the microscopic features of *S. monostachyus*

Parameters	Abaxial	Adaxial
Stomatal type	Diacytic stomata	Diacytic stomata
Stomatal length (µm)	17.5 (21.7±0.88) 25.6	21.4 (24.9±0.81) 29.7
Stomatal width (µm)	13.3 (16.7±0.69) 19.6	16.2 (17.7±0.46) 20.9
Stomatal number (Per area view)	53 (48.5±0.96) 44	23 (28.4±1.06) 33
Stomatal pore width(µm)	2.5 (4.7±0.54) 7.7	4.7(7.53±0.61) 10.2
Stomatal pore length(µm)	7.5 (11.84±1.09) 18.6	10.5 (13.3±0.60) 16.2
Stomatal index	27.9%	14.8%
Epidermal wall shape	Irregular	Irregular
Epidermal wall pattern	Sinuous	Undulate
Epidermal cell length (µm)	42.3 (49.4±1.6) 56.5	35.4 (43.13±2.38) 56.8
Epidermal cell width (µm)	19.0 (22.9±1.23) 30.1	16.1 (22.1±1.06) 26.6
Epidermal layer number	117 (125.5±2.0) 137	158 (163.7±2.38) 169
Thickness of epidermis (µm)	1.2 (2.0±0.23) 2.9	1.5 (3.0±0.24) 3.9
Trichome type	Collapsed and multicellular	Multicellular
Trichome length (µm)	10.1 (55.5±9.78) 114.4	36.8 (109.44±20.74) 200.8
Trichome width (µm)	9.8 (13.1±1.12) 21.0	16.5 (23.5±1.58) 29.7

Data was presented in Mean ±SEM (Mean±Standard Error of Mean of 10 determinations).

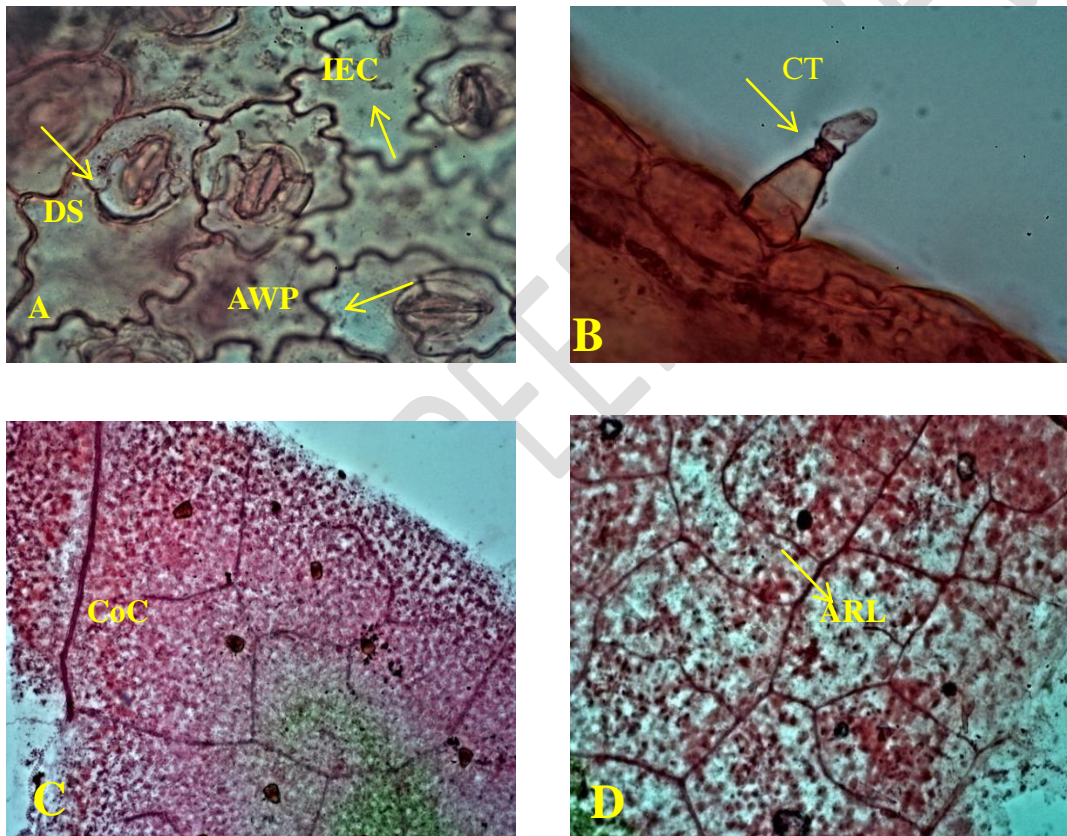


Figure 2: Abaxial Epidermal layer showing epidermal cell shape and anti-clinal cell wall A: Irregular epidermal cell (IEC) $\times 40$; Undulate-Sinuuous Anticlinal Wall Pattern (AWP); Diacytic stomata (DS) B: Collapsed trichomes (CT) C: Druse calcium oxalate crystals. D: Quadrangularr and Polygonal areole (ARL) and linear nerve endings.

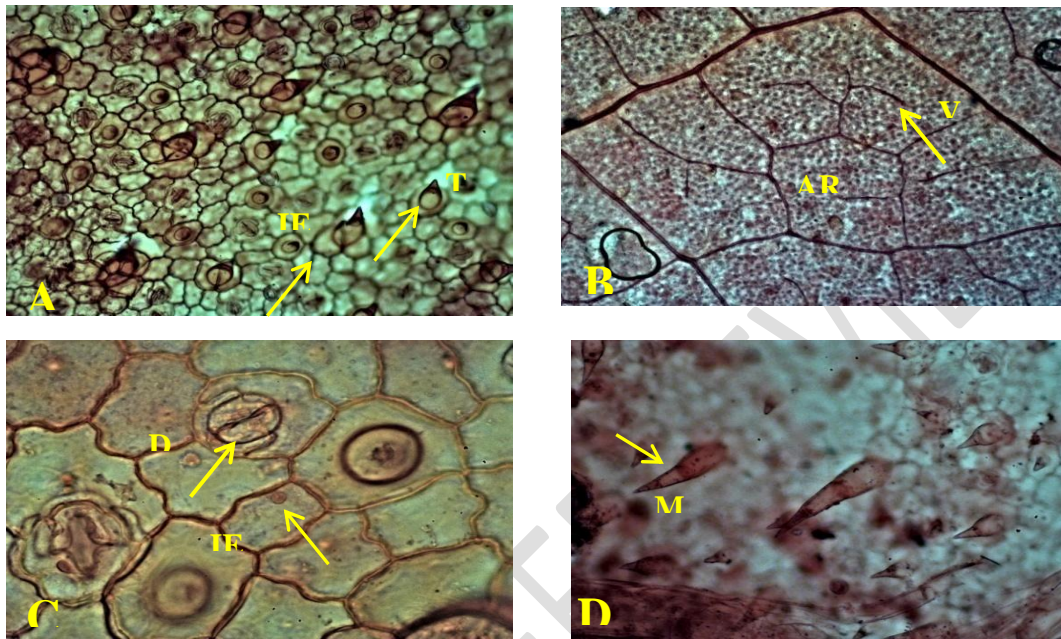


Figure 3: Adaxial Epidermal layer showing epidermal cell shape and anti-clinal cell wall A: Irregular epidermal cell (IEC) $\times 10$; Trichome base (TB). B: Areole (AR) Quadrangular and polygonal, Vein termination (VT) linear $\times 4$. C: Diacytic stoma (DS) $\times 40$. D: Multicellular trichome (MT) $\times 10$.

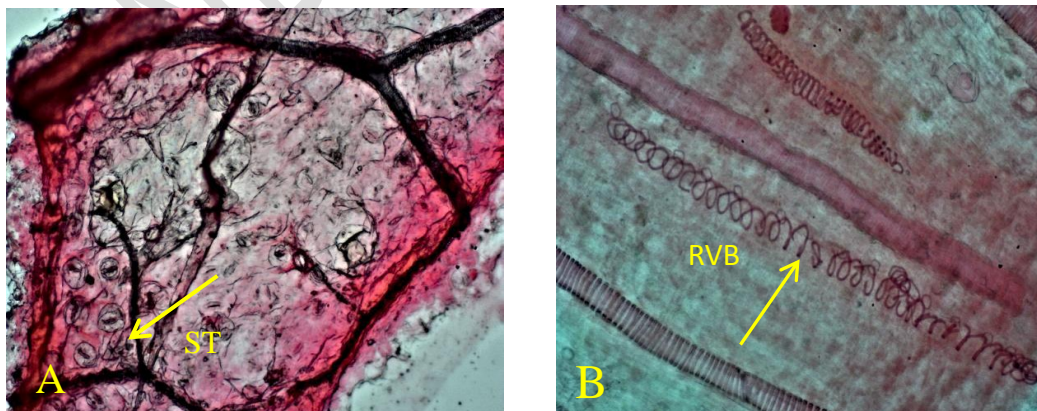


Figure 4: Powder analysis A: Stomata (ST) ×10, B: Ring of vascular bundles (RVB) ×10. C: Multicellular trichome (MT) ×10.

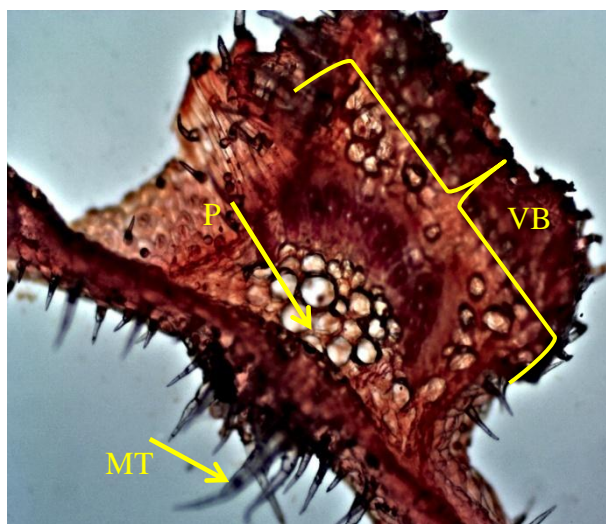


Figure 5: Petiole analysis VB: Vascular bundles, P: Parenchyma cells ×10, Multicellular trichome (MT) ×10.

Table 2: Results for Micromeritic properties of *S.monostachyus* leaf and stem powders

Micromeritic Parameters	Leaf Powder	Stem Powder
Bulk volume(ml)	41.0±0.71	40.0±0.71
Tapped volume(ml)	31.30±0.41	27.0±0.71
Bulk density(g/ml)	0.24±0.00	0.25±0.00
Tapped density(g/ml)	0.32±0.00	0.37±0.01
Flow rate(g/s)	0.90±0.04	0.11±0.01
Angle of repose(degrees)	38.0±0.16	44.6±1.89
Carr's Index (%)	23.7±1.63	32.5±2.59
Hausner's Ratio	1.3±0.03	1.5±0.05

Result presented as Mean ± SEM (Standard Error of Mean of three (3) replicate).

Table 3: Results for Chemomicroscopy of *S. monostachyus* powdered leaf and stem

Constituents	Quantitative Test	Leaf	Stem
Lignin	Ruthenium red, viewed under microscope	+	+
	Phloroglucinol + Conc HCL viewed under microscope	+	+

Starch	N/50 iodine, viewed under microscope	+	+
Calcium oxalate crystals	Sample cleared, viewed under microscope	+	+
Cellulose	N/50 iodine + 66% sulphuric acid	+	+
Oils	Sudan IV, viewed under microscope	+	-
Proteins	1% picric acid, viewed under microscope	+	+

Table 4: Results for fluorescence properties of *S. monostachyus* powdered leaf and stem

Extract	Physical observation	UV-365nm	UV-254nm
	Colour	Colour	Colour
Water			
Leaf	Brown	Grey	Ash
Stem	Brown	Grey	Ash
Methanol			
Leaf	Green	Orange	Green
Stem	Brown	White	Grey
Ethanol			
Leaf	Pale green	Orange	Green
Stem	Yellow	White	Grey
DCM			
Leaf	Green	Red	Green
Stem	Lemon	Pale yellow	Yellow
Hexane			
Leaf	Green	Light pink	Yellow
Stem	Green	Pink	Green
Ethyl acetate			
Leaf	Lemon	Pink	Lemon
Stem	White	Cream	White

Table.5: Results for water-soluble extractive value, ethanol-soluble extractive value, methanol-soluble extractive value for leaf powder of *S.monostachyus*

Parameters	Weight(g)	Percentage by weight (%^w/_w)
Water-soluble extractive Value	0.265	26.5
Methanol-soluble extractive Value	0.145	14.5
Ethanol-soluble extractive Value	0.158	15.8

Table 6: Results for water-soluble extractive value, ethanol-soluble extractive value, methanol-soluble extractive value for stem powder of *S.monostachyus*

Parameters	Weight(g)	Percentage by weight (%^w/_w)
Water-soluble extractive Value	0.323	32.3
Methanol-soluble extractive Value	0.155	15.5
Ethanol-soluble extractive Value	0.145	14.5

Table 7: Results for moisture content, total ash value, acid-insoluble ash value, water-soluble ash value of *S. monostachyus* leaf

Parameters	Weight(g)	Percentage (%^w/_w)
Moisture content	0.35±0.007	11.7
Total ash value	0.41±0.003	13.7
Acid-insoluble ash value	0.053±0.014	1.8
Water-soluble ash value	0.283±0.003	9.4

Table 8: Results for moisture content, total ash value, acid-insoluble ash value, water-soluble value of *S. monostachyus* stem

Parameters	Weight(g)	Percentage(%^w/_w)
Moisture content	0.4±0.01	13.3
Total ash value	0.52±0.00	17.3
Acid-insoluble ash value	0.05±0.01	1.8
Water-soluble ash value	0.28±0.01	9.2

Result and Discussion

Plants have been seen as the source of medicinal agent for thousand years since the origin of man [24]. The qualitative microscopic studies of the epidermal layers of the *S. monostachyus*, revealed the presence of diacytic type of stomata (cross-celled stomata) in which the stoma remains surrounded by a pair of subsidiary or accessory cells and whose common wall is at right angles to the guard cells [16] present on both the abaxial and the adaxial surfaces as shown in Figures 2A and 3C respectively. Collapsed trichomes were visible on the abaxial surface as shown in Figure 2B and multicellular trichomes were visible on the adaxial surface as shown in Figure 3D. The epidermal cell layers of the leaf showed mean stomatal index of 14.8% on the adaxial surface and 27.9% on the abaxial surface (Table 1). A mean stomatal length of 21.7 μm , stomatal width of 16.7 μm and stomatal number of 48.5 (per area) were obtained on the abaxial surface; while on the adaxial surface; a mean stomatal length of 13.3 μm , stomatal width of 7.5 μm and stomatal number of 28.4 were obtained (Table 1).

The micromeritic studies of the leaf powders showed angle of repose of 38.0°, indicating a fair flow, Hausner's ratio, a passable flow and Carr's index of 23.7%, indicating a passable flow also. That of the stem showed angle of repose of 44.6° (passable flow), Hausner's ratio of 1.5 (very poor flow) and Carr's index of 32.5 (very poor flow) (Table 2). Micromeritics is an important consideration in the development of solid dosage formulation which is mostly used for physical, mechanical and chemical processes [25]. The micromeritic properties indicate flow properties as well as interparticulate resistance between these powders. This information predicts the stability and solubility of crude drugs. These studies influence a number of processing parameters in manufacturing pharmaceutical formulations. The knowledge and effect of particle size distribution of active pharmaceutical ingredient as well as excipients will be useful in solving the

difficulties in critical process parameters. In particular regards to tablet and capsule, controlling the particle size and particle size distribution is mainly important because they have a direct impact on the flowability, tableting, content uniformity, weight variation and dissolution rate which will inadvertently affect the bioavailability of the drug. Good flow properties of powders are essential for uniform filling into dies of tableting machines and for easy movement of materials around a production facility. Factors that affect the flow properties of powders include: moisture content, temperature, particle size, particle shape (texture), and time of storage. The angle of repose is considered to be the most classical technique used for characterizing the flow properties of powders. It is a characteristic related to interparticulate friction or resistance to movement between particles [26]. An alternative test is to determine Carr's index which relates the bulk density to the tapped density. From the results obtained, the powders were said to have passable to fair flow characteristics [18]

Chemomicroscopy study revealed the presence of mucilage, lignin, calcium oxalate crystals, protein and starch but absence of oil. Calcium oxalate crystals play a central role in a variety of important functions, including tissue calcium regulation, protection from herbivory and metal detoxification [27]. Thus, plants with calcium oxalate crystals shows good antioxidant properties when formulated into pharmaceuticals.

In soluble-extractive values determinations, water had the highest extractive value of 26.5%^{w/w} and methanol had the lowest of 14.5%^{w/w} for the leaf powders (Table 5). Water also had the highest extractive value while ethanol had the lowest extractive values of 32.3%^{w/w} and 14.5%^{w/w} respectively for the stem powders (Table 6). Extractive values helps to measure the amount of constituents which are extractable by the solvents under specified conditions. They are equally useful in estimating the specific constituent based on its solubility in a particular solvent used for its extraction.

The fluorescence property of the powdered sample for different solvent extracts revealed different colors indicating the presence of phytochemicals like: anthocyanins, phenols, tanins and flavonoids when viewed in daylight, lower and higher wavelengths of UV light. This property is useful in characterizing crude drugs, identifying authentic samples and recognizing adulterants. In a mixture of different drugs of two or more species, fluorescence studies help to identify a particular drug by the use of estimates of intensity of fluorescence [28].

The moisture content obtained was 11.7 %^{w/w} for the leaf powder and 13.3 %^{w/w} for stem powder which are within the African Pharmacopoeia, 1986 recommended range of 8-14 %^{w/w}. The obtained moisture content is indicative of the plant's shelf life as high moisture content is uneconomical and at the right temperature, could lead to enzymatic activation and hydrolytic reactions as well as proliferation of microbial growth which may ultimately lead to degradation of active constituents. Generally, the amount of ash contained in a crude vegetable drug must be low as it is a determinant of the purity and quality of crude drug. The total ash values for the leaf and stem of the plant were 13.7 %^{w/w} and 17.3 %^{w/w} respectively, that of the stem was above the recommended limit of 14 %^{w/w} according to the European Pharmacopoeia [29]. The acid-insoluble ash values of both the leaf and stem of the researched plant were each 1.8 %^{w/w}, falling within the recommended limit of 2 %^{w/w} according to the European Pharmacopoeia [29]. Acid-insoluble ash value gives an idea of the measured amount of silica especially sand and other siliceous material present in the drugs while total ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, magnesium and calcium giving an estimation about the purity and quality of the drug and the water soluble ash value gives an estimation of the inorganic contents inherent in the sample.

Conclusion

The data obtained from these studies will provide information about the identity, quality and purity of *Solenostemon monostachyus*. The result collectively might be useful to supplement information for further studies on leaf and stem of this plant.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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