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EREMOMASTAX POLYSPERMA (BENTH.) DANDY (ACANTHACEAE): A PHYTOPHARMACOGNOSTIC STUDY

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ABSTRACT

The quality control parameters of various medicinal plants used in indigenous system of medicine are becoming more relevant today and are important tools in the formulation of high quality herbal products. The aim of this study was to employ the quality control parameters in the evaluation of the leaf and stem of *Eremomastax polysperma*. The plant leaves and stems were collected, air dried, pulverized and stored in a clean glass container. Standard procedures were carried out to obtain the microscopic features of the fresh and powdered sample, micromeritic, chemomicroscopy, fluorescence properties, soluble extractive values, moisture contents and ash values. Results of the microscopic study using the fresh and powdered leaf samples revealed the presence of diallelocytic, pericytic and copericytic stomata, on both abaxial and adaxial surfaces and multicellular trichromes with mean length of 68.30±3.81µm and 53.77±6.06. Results of the micromeritic properties of the powdered samples showed bulk volumes of 50.00±0.17cm and 42.50±0.24cm, tapped volume of 35.17±0.14cm and 28.67±0.27cm, bulk density of 0.20±0.00g/ml and 0.24±0.33g/ml, tapped density of 0.29±0.00g/ml and 0.35±0.01g/ml, flow rate of 0.46±0.03g/s and 0.33±0.00g/s, angle of repose of 37.20±0.44

and 37.89±0.44 degrees, Carr's index of 31.03±0.82% and 31.43±0.82%, Hausner's ratio of 1.45±0.02 and 1.46±0.02, true density of 1.68±0.02 and 2.10±0.07, parking fraction of 0.12±0.00 and 0.11±0.01, P^H of 6.55 and 6.68 when cold, 6.39 and 6.40 when hot for the powdered leaf and stem respectively. Chemomicroscopy study revealed the presence of lignin, mucilage, calcium oxalate crystals, protein and oil in both leaf and stem, while starch was present in leaf only. Calcium carbonate was absent in both leaf and stem samples. Results for the ethanol-soluble extractive values were 3.00±0.00%$^w/_w$ and 2.50±0.00%$^w/_w$, water-soluble extractive values were 5.00±0.00%$^w/_w$ and 3.50±0.00%$^w/_w$, methanol-soluble extractive values were 4.00±0.04%$^w/_w$ and 2.50±0.00%$^w/_w$ for the powdered leaf and stem respectively. Results for the moisture contents were 10.50±0.01%$^w/_w$ and 9.50±0.00%$^w/_w$, total ash values were 20.00±0.00%$^w/_w$ and 15.50±0.00%$^w/_w$, acid-insoluble ash values were 2.00±0.00%$^w/_w$ and 1.00±0.00% $^w/_w$, water-soluble ash values were 4.50±0.00%$^w/_w$ and 3.50±0.00%$^w/_w$, sulfated ash values were 29.50±0.01%$^w/_w$ and 24.50±0.02$^w/_w$. In conclusion, the above evaluation methods and parameters therein could be used to identify and authenticate both the fresh and powdered crude drug products of *Eremomastax polysperma*.

KEYWORDS: Chemomicroscopy, *Erimomastax polysperma*, Micromeritic, Phytopharmacognostic, Phytotherapeutics

INTRODUCTION

The use of herbs as medicine is the oldest form of healthcare known to humanity and has been used in all cultures throughout history.[1] Plants have universal role in the treatment of diseases in the major systems of medicine. In recent era, there has been great demand for plant-derived products in developed countries. These products are increasingly being sought out as medicinal products, nutraceuticals, and cosmetics. Today, with the present surge of interest in phytotherapeutics, the availability of genuine plant material is becoming scarce.[2] Since crude drugs form the basis for the manufacture of numerous medicinal preparations, accurate determination of drug identity forms an essential part of the study of plants.[3] It becomes extremely important to make an effort towards standardization of the plant materials as medicine.[4]

Eremomastax polysperma (Benth.) Dandy is an erect or somewhat scrambling perennial herb growing up to 1. 3 – 2 m tall. It is erect, stems are quadrangular and glabrous to sericeous-puberulent when young.[5] Leaves are ovate, green surface with purple underside. Margin is sub-entire to grossly crenate-dentate, usually becoming less tooted upwards. Apex is

acuminate to cuspidate; base is truncate to shallowly cordate.[6] The plant is widespread from West Africa through Central African Republic and North Congo Kinshasa to South Sudan and South-west Ethiopia and Madagascar. The leaves of *Eremomastax polysperma* was reported to contain alkaloids, tannins, saponins, terpenes, flavonoids, anthraquinones, phenol and cardiac glycosides, protein, fat, carbohydrate, vitamin A, vitamin B_1, vitamin B_{12}, vitamin C, potassium, calcium, phosphorus, iron, magnesium, and zinc.[7] It has been used in the management of diabetes, sickle cell disease, treatment of anaemia and infertility.[6] It is also reported to possess analgesic and anti-inflammatory properties and can act as immune booster.[8]

Phylogeny of *Eremomastax polysperma* (Scientific Classification) According to Angiosperm Phylogeny Group (APG) System.[9]

Kingdom – Plantae

***Clade* -** Angiosperm

Clade – Eudicot

Clade – Astride

Order – Lamiales

Family – Acanthaceae

Genus – Eremomastax

Species – polysperma

Figure 1: Eremomastax polysperma (BENTH.) DANDY in its Natural Habitat.

Common name: African blood tonic plant, golden seal, blood plant.

Local name: Igbo: Ukwuanyi, Yoruba: Acoyun, oyun, Hausa: Esinyin, Ibibio: Edemididuot.

MATERIALS AND METHODS

Collection and Identification of Plant

The leaves and stems of the plant were collected from Ikot Akpe, Uyo Local Government Area, Akwa Ibom state, Nigeria in may 2018. The plant was identified and authenticated in the Faculty of Pharmacy, University of Uyo herbarium with the identification number: UUPH NO. 1(i)

METHODOLOGY

Preparation of the Collected Plant

The fresh plant materials (5.0 kg weight) were separately air dried, pulverized and packed in dry containers, well labelled and used when needed.

Anatomical Studies

Microscopic Evaluation of Leaf

For the purpose of anatomical studies, the standard median portion of the well expanded matured leaf was obtained. Epidermal peels of both abaxial and adaxial surfaces were made by placing the leaf on a clean glass slide with the surface to be studied facing down. The specimens were irrigated with water holding it downward from one end and then the epidermis above the desired surface was scrapped off carefully with sharp razor blade. The loose cells were then washed with water and stained in 1% aqueous solution of safranin-O for 4-8 minutes and washed again in water to remove excess stain and mounted in 10% glycerol on a glass slide and covered with a glass cover slip and then viewed using an Olympus CX21 binocular microscope. Photomicrographs were taken from good preparations using the Olympus CX21 binocular microscope fitted with an MD500 amscope microscope eyepiece camera.[10]

Quantitative Leaf Microscopy

Quantitative microscopy parameters such as leaf constant studies which include 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 ten (10) microscopic fields chosen at random were used and data presented as mean ± SEM.

The stomatal index (S.I) was determined according to Metcalfe and Chalk[11] using the formula:

Stomatal Index (SI) = S/E +S x 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[12] 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 (Vb). The cylinder was gently tapped repeatedly to obtain a constant volume noted as the tapped volume (Vt). Bulk density was calculated using the formula below;

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

Where;

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

Where $B\rho$= Bulk density

M = Mass of powder

Vb= Bulk volume of powder

$T\rho$ = Tapped density

Vt= tapped volume

Interparticulate porosity is 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 is calculated using the formula

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

While Carr's Index is measured as

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

Where; $T\rho$ = Tapped density

$B\rho$ = Bulk density.

Angle of repose

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

pH

A pH meter (Jenway, Stafford Shire, UK) was used to determine the pH of both hot and cold extract of the leaf.

Chemomicroscopic Analysis of Leaf and Stem Powders

Powdered leaf was examined for its chemomicroscopic properties using standard procedures.[13]

Fluorescence Analysis of Leaf and Stem Powders

The fluorescent analysis of dried leaf powder was carried out using standard method.[14]

Physico-chemical Evaluation of Leaf and Stem Powders

The physicochemical parameters such as moisture content, ash values (total ash, acid insoluble ash, water soluble ash, sulphated ash), soluble extractive values viz. ethanol, methanol and water were performed according to the official method.[15]

RESULTS

Table 1: Results for the Microscopic Features of *Eremomastax Polysperma* and Standard Error ff Mean (SEM).

Leaf Surface	Abaxial Surface	Adaxial Surface
Stomatal type	Diallelocytic, Pericytic, Copericytic	Diallelocytic, Pericytic Copericytic -
Stomatal length (µm)	4.80(6.08±0.34)8.11	5.31(6.59±0.28)8.48
Stomatal pore width(µm)	2.63(3.71±0.27)5.78	3.85(4.99±0.18)5.75
Stomatal pore length(µm)	1.69(4.40±0.39)6.10	2.53(3.10±0.12)3.84
Stomatal pore width(µm)	0.39(0.64±0.13)0.87	0.32(0.77±0.08)1.13
Stomatal number	5.00(8.20±0.87)11.0	5.00(9.70±1.34)21.00
Epidermal cell number	32.00(39.60±1.78)52.00	60.00(90.70±4.53)109.00
Stomatal index	13.33(17.15±1.18)20.93	5.88(9.66±1.19)19.09
Epidermal cell wall pattern(µm)	Sinuous	Sinuous
Epidermal cell length (µm)	11.70(13.82±0.98)17.30	9.36(15.47±1.06)20.80
Epidermal cell width (µm) Trichome type	4.45(5.47±0.27)7.5 Multicellular	3.13(4.56±0.36)7.16 Multicellular
Trichome length(µm)	54.96(68.30±3.81)92.35	29.36(53.77±6.06)79.99
Trichome width(µm)	6.01(7.84±0.36)9.91	10.55(6.98±0.74)10.55

Results presented as Mean±SEM of Ten (10) Replicates

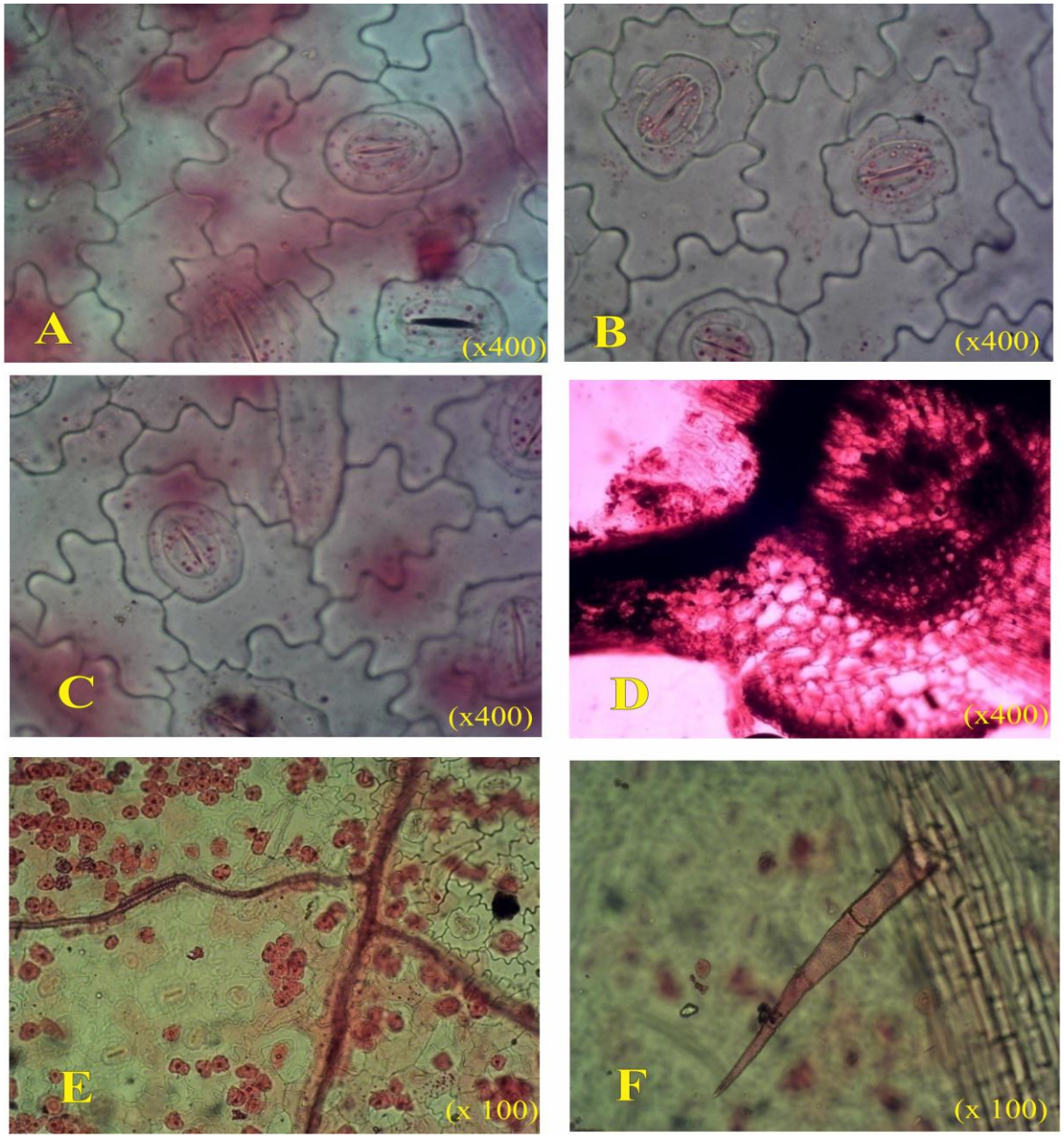

Figure 2: Microscopic features of fresh leaf of *Eremomastax polysperma* (a) Copericytic stomata (b) Pericytic stomata (c) Diallelocytic stomata (d) midrib of the leaf showing vascular bundles (e) calcium oxalate crystals (f) Multicellular trichome (Metcalf and Chalk, 1979., Stace, 1980).

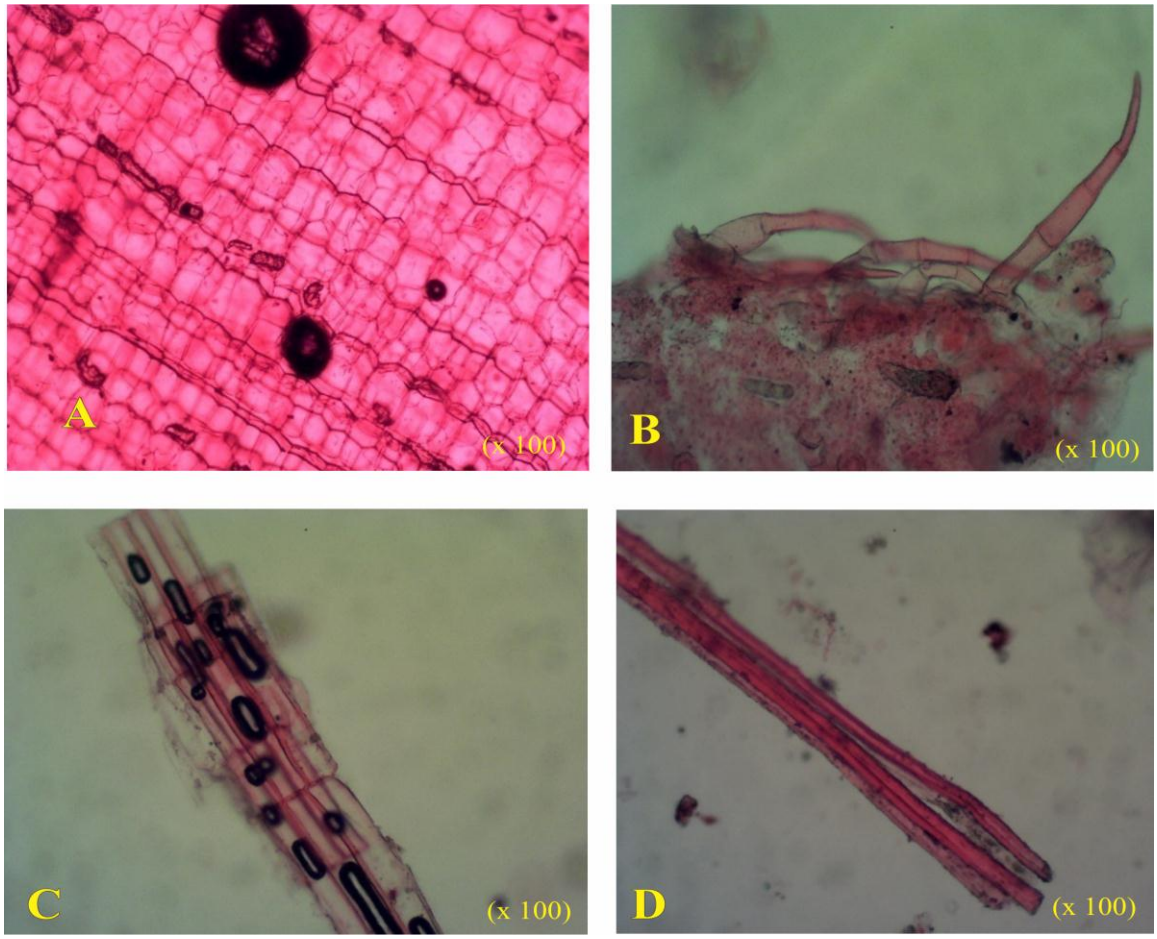

Figure 3: A: Microscopic features of fresh stem showing Sieve tube. B: Microscopic features of powdered leaf showing Multicellular trichome C: Microscopic features of powdered stem showing parenchymatous tissue D: Microscopic features of powdered stem showing Fibre.

Table 2: Results For Micromeritic Properties of *Eremomastax polysperma* And Standard Error of Mean (SEM).

Parameters	Leaf	Stem
Bulk Volume(cm)	50.00±0.17	42.50±0.24
Tapped Volume(cm)	35.17±0.14	28.67±0.27
Bulk Density(g/mL)	0.20±0.00	0.24±0.33
Tapped Density(g/mL)	0.29±0.00	0.35±0.01
Flow Rate(g/s)	0.46±0.03	0.33±0.00
pH cold	6.55	6.68
pH hot	6.39	6.40
Angle of repose(degrees)	37.20±0.44	37.89±0.44
Carr's index(%)	31.03±0.82	31.43±0.82
Hausner's ratio	1.45±0.02	1.46±0.02
True density(g/mL)	1.68±0.02	2.10±0.07
Packing fraction	0.12±0.00	0.11±0.01

Results presented as Mean±SEM of Three (3) Replicates

Table 3: Showing Chemomicroscopic Evaluation of Leaf and Stem of *Eremomastax Polysperma* Powder.

Parameters	Leaf	Stem
Lignin	+	+
Starch	+	_
Oils	+	+
Calcium Carbonate	_	+
Calcium Oxalate crystal	+	+
Mucilage	+	+
Protein	+	+

+ = Present, - = Absent

Table 4: Results for Fluorescence Properties of *Eremomastax polysperma.*

		Physical observation	UV – 365 nm	UV – 253.7 nm
Extract	Sample	Color	Color	Color
Water	Leaf	Brown	Grey	Grey
	Stem	Brown	Grey	Grey
Methanol	Leaf	Green	Brown	Grey
	Stem	Greenish – yellow	Pink	Grey
Ethanol	Leaf	Green	Brown	Grey
	Stem	Greenish – yellow	Pink	Grey
DCM	Leaf	Green	Brown	Black
	Stem	Brown	Pink	Black
N- hexane	Leaf	Yellow	Pink	Grey
	Stem	Brown	Pink	Grey

Table 5: Physicochemical constants of leaf and stem of *Eremomastax Polysperma.*

Parameters	Leaf (%w/w)	Stem (%w/w)
Moisture content	10. 50±0.01	9. 50±0.00
Total ash	20.00±0.00	15. 50±0.00
Water-soluble ash	4. 50±0.00	3. 50±0.00
Acid-insoluble	2.00±0.00	1.00±0.00
Sulfated ash	29. 50±0.01	24. 50±0.02
Extractive value (%w/w)		
Water-soluble	5.00±0.00	3.5.00±0.00
Ethanol	3.00±0.00	2.50±0.00
Methanol	4.00±0.04	2.50±0.00

Results presented as Mean±SEM of Three (3) Replicates

DISCUSSION

Plants have been a source of medicinal agent for thousands of years since the origin of man.[1] Many of these plants have not been evaluated for proper identification despite the fact that the first step towards ensuring quality of any medicinal product is to ensure quality of its starting materials by proper authentication. In the recent times however, there has been an

increase in consciousness of the need for standardization of medicinal plants with potential therapeutic uses.[16] Despite modern techniques, identification of plant by pharmacognostic studies is more reliable.[17] Microscopic examination of section and powdered drugs, aided by stains, help in distinction of anatomy in adulterants. The qualitative microscopic studies of the epidermal layers of the researched plant, reveals the presence of diallelocytic, pericytic and copericytic types of stomata, present in both adaxial and abaxial surfaces (amphistomatic), and multicellular trichomes that are pointed and uniserrate, ranging from 54.96µm to 92.35µm in length. The epidermal cell wall pattern is prominent-sinuous in nature. The quantitative microscopic studies of the epidermal layers of the researched plant showed mean stomatal index of 17.15 and 9.66, mean stomatal length of 6.08µm and 6.59µm, mean stomatal number (per area) of 8.20 and 9.70 for the abaxial and adaxial surfaces respectively. Other microscopic features are presented in table 1. The Transverse Section of the midrib of the leaf revealed the presence of vascular bundle (xylem and phloem). The Longitudinal Section of the stem shows presence of conducting vessels.

The micromeritic studies showed angle of repose to be 37.20 and 37.89 degrees, Hausner's ratio were 1.45 and 1.46 and Carr's index were 31.03% and 31.43% for leaf and stem powders respectively. Micromeritics is an important consideration in the development of solid dosage formulations, which is mostly used for physical, mechanical and chemical processes.[18] It influences a number of process parameters in manufacturing pharmaceutical formulations. The knowledge and effect of particle size distribution of active pharmaceutical ingredient, as well as excipients, will be useful to solve the difficulties in critical process parameters. In particular regards to tablets and capsules, controlling the particle size and particle size distribution is mainly important because they have direct impact on the flowability, tableting, content uniformity, weight variation and dissolution rate which ultimately affect the bioavailability of 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. Angle of repose is a characteristic related to interparticulate friction or resistance to movement between particles.[19] An alternative test is to determine the Carr's index, which relates the bulk density of the material to the tapped density. From the results obtained, the powders can be said to have a fair to very poor flow characteristics.

Chemomicroscopy study revealed the presence of mucilage, lignin, calcium oxalate crystals, protein and oil. Starch was in the leaf only.

The water-soluble extractive values were 5.00%$^{w}/_{w}$ and 3.50%$^{w}/_{w}$, methanol-soluble extractive values were 4.00%$^{w}/_{w}$ and 2.50%$^{w}/_{w}$, ethanol-soluble extractive values were 3.00%$^{w}/_{w}$ and 2.50%$^{w}/_{w}$ for leaf and stem respectively as shown in table 5. Extractive values determines the amount of the active constituent in a given plant material when extracted with a particular solvent. The extractive values of a solvent with a crude drug are useful for their evaluation and also for the estimation of specific constituents soluble in that particular solvent used for extraction. They give idea about the nature of the chemical constituents present in a crude drug.

The moisture contents obtained were 10.50%$^{w}/_{w}$ and 9.50 %$^{w}/_{w}$ for the leaf and stem powders respectively, which were within the stated limit as shown in table 5. High moisture content is uneconomical, and in the presence of suitable 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. Excess moisture in a sample suggests that the drug has been incorrectly prepared or inappropriately stored. This is especially important for materials that absorb moisture easily or deteriorate quickly in the presence of water. Thus, moisture content limits are generally specified in pharmacopoeias. The African pharmacopoeia limit of moisture content for vegetable drug ranges from 8%w/w to 14%$^{w}/_{w}$ with few exceptions (e.g digitalis leaf, 6%$^{w}/_{w}$).

The total ash value were 20.00%$^{w}/_{w}$ and 15.50%$^{w}/_{w}$, acid- insoluble ash value were 2.00%$^{w}/_{w}$ and 1.00%$^{w}/_{w}$ (these shows low content of earthly materials), water- soluble ash value were 4.50%$^{w}/_{w}$ and 3.50%$^{w}/_{w}$ and sulfated ash values were 29.50%$^{w}/_{w}$ and 24.50%$^{w}/_{w}$ for the powdered leaf and stem respectively as shown in table 5. The Ash values are used to determine quality and purity of crude drug. It is a method to measure the amount of the residual substances not volatilized when the drug sample is incinerated in the furnace. Ash may be derived from the plant tissue itself and is usually called the “physiological ash”, or may come from extraneous matter, especially sand and soil adhering to the surface of the drug and is called “non-physiological ash”. Generally, the amount of ash contained in a crude vegetable must be low. It indicates to some extent the amount of care taken in the preparation of the drug.[20] It indicates also the presence of various impurities like carbonates, oxalates and silicates. The acid-insoluble ash value indicates contamination with earthly material.

Inorganic materials such as calcium oxalate and carbonate present in the total ash which can be removed by dilute hydrochloric acid leaving behind silica, such as sand and siliceous materials present in the crude drug. Thus, high acid- insoluble ash value indicates soil contaminant in the drug. Water- soluble ash value is used to estimate the amount of inorganic compound present in crude vegetable drugs. The sulfated ash is the residual substance not volatilized when the sample is incinerated with concentrated sulphuric acid. This method gives results which are more precise since inorganic substances contained in the plant sample remain as sulphates, and is thus used to determine the amount of inorganic substances contained as impurities in an organic substance, but occasionally for determining the amount of inorganic substances contained as components in an organic substance, or the amount of impurities contained in a heat volatile inorganic substance.

CONCLUSION

The data obtained in the pharmacognostic evaluation of *E. polysperma* can assist in the proper identification, collection and investigation of this plant as well as to establish the purity and adulteration of the drug preparation.

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