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PHYTOPHARMACEUTICAL STANDARDIZATION OFTHE LEAVES AND STEMS OF *JUSTICIA INSULARIS* T.ANDERS (ACANTHACEAE)

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ABSTRACT

In recent times, there has been an increase in consciousness of the need for standardization of medicinal plants with potential therapeutic uses. *Justicia insularis* is used in the treatment of chest and heart problems, labour pains and dysmenorrhoea. This study is aimed at evaluating the pharmacognostic parameters of leaves and stems of *J. insularis*. These include microscopy, micromeritics, chemomicroscopy, moisture contents, ash values, soluble extractive values, fluorescence properties using standard procedures. The results obtained from microscopy were amphistomatic distribution of stomata, sinuous cell wall pattern, multicellular and glandular trichomes, diacytic and copericytic types of stomata on the abaxial and adaxial surfaces of the leaf, glandular trichomes on the transverse section of the stem. Stomatal numbers were 1.32 ± 0.25µm, 0.51 ± 0.09µm and stomatal index of 21.4%, 10.51% on abaxial and adaxial surfaces respectively. The result of the micromeritics properties of the powdered leaf and stem showed poor flow characteristic with the angle of repose of 37.60 ±0.38 and 42.00 ±0.54 degrees, Hausner's ratio of 1.45 ±0.00 and 1.41±0.01 and Carr's index of 31.00±0.00% and 30.00 ±0.65% respectively. Moisture contents for the leaf and stem were13.5 and 12.5%$^{w}/_{w}$, total ash 16.5 and 11.0%$^{w}/_{w}$, acid-insoluble ash value 2 and 1%$^{w}/_{w}$, water-soluble ash value 6 and 5%$^{w}/_{w}$, sulfated ash values 19 and 13%$^{w}/_{w}$, water-soluble extractive values23 and 22%$^{w}/_{w}$, ethanol-

soluble extractive values 11 and 15%$^{w}/_{w}$ and methanol-soluble extractive values 1 and 14%$^{w}/_{w}$ respectively. In conclusion, the results obtained from the studies will provide useful information about the identity, purity, quality of *J. insularis* and will also adequately contribute to the formation of monograph of the plant.

KEYWORDS: Chemomicroscopy, *Justicia insularis*, Micromeritic, Phytopharmaceutical, Standardization, Therapeutic.

INTRODUCTION

Mostly, all medicines whether synthetic or of plant origin, should fulfil the basic requirements of quality, safety and efficacy.[1] Reproducibility of quality, safety and efficacy are achieved by comprehensive processes and procedures of standardization. Standardization of herbal medicines is therefore the process of achieving, establishing and prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that exhibit an assurance of quality, efficacy, safety and reproducibility.[2] It is the process of developing and recommending technical standards of identity for medicinal plants. A collection of such authentic values or data called standards, constitutes the monograph of such plant which can be incorporated into any herbal pharmacopoeia. *Justicia insularis* T. Anders is an herbaceous and perennial plant of 30-75cm high with opposite ascending branches. Its leaves are simple, opposite and the flower, white, pink or purple.[3] Studies revealed the presence of alkaloids, flavonoids and glycosides in the leaves. It is used in the treatment of labour pains, dysmenorrhoea and infertility. Aqueous extract mixture has also been proven, in a series of studies to induce ovarian steroidogenesis and folliculogenesis in female.[4]

Phylogeny of *Justicia insularis* (Scientific Classification) According to Angiosperm Phylogeny Group (APG) System IV, 2016[5]

Kingdom: Plantae
Clade: Angiosperms
Clade: Eudicots
Clade: Asterids
Order: Lamiales
Family: Acanthaceae
Genus- *Justicia*
Species- *insularis*

Common Name: Hunter's Weed

Ibibio Name: *Mmeme*, Yoruba Name (Owo): *Isepe – akere*, Yoruba (Ondo) – *Esisi*.

Figure 1: *Justicia insularis* in its habitat.

MATERIAL AND METHODS

Collection and Identification of Plant Material

The plant *Justicia insularis* T. Anders from the family Acanthaceae was collected in the month of November, 2017 from a location in Itiam/Ewet Housing Estate, Uyo Local Government Area of AkwaIbom State, Nigeria. It was identified by Professor (Mrs) U. A. Essiett, a taxonomist in the Department of Botany and Ecological Studies, Faculty of Science, University of Uyo. The Herbarium Specimen is with the Voucher Number UUPH1C(i) was deposited in the Herbarium, Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo. The leaves were cleaned, some air-dried and pulverized.

METHODOLOGY

Preparation of Surface Specimens of Leaves

The fresh plant materials of *J. insularis* collected in the field were immediately taken to the laboratory for the purpose of anatomical studies; small portions were obtained from the standard median part of the leaf of mature and well expanded leaf epidermal peels of both abaxial and adaxial surfaces were made by placing the leaf blade taken from a standard median portion of the leaf on a clean glass slide, with the surfaces to be studied facing downward. 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 washed away from the epidermal peels with the aid of soft carmel hair brush and water until the desired epidermis below was reached. The epidermal peels were stained in 1% aqueous solution of safranin-O for 4-8 minutes, rinsed carefully in water to remove excess stain and mounted in 10% glycerol. Mounted specimens were then viewed

digitally on an Amscope MD500 mounted on Olympus CX21 microscope and photomicrographs taken.[6]

Quantitative Leaf Microscopy

Quantitative microscopy was done to determine stomatal number and stomatal index. The upper and lower epidermis were carefully exposed using sharp edge of razor blade and further cleared with sodium hypochlorite and washed thoroughly with water. They were placed on a slide, mounted in 10% glycerol, placed on the stage photomicrograph and viewed under the microscope and the stomatal number and epidermal cells per unit area were counted using a graduated amscope equipment in10 different fields.[7] The stomatal index was determined using the formula below:

$$S.I = \frac{S}{E+S} \times \frac{100}{1}$$

Where S = Number of stomata per unit area

E = Number of epidermal cell in the same area

Preparation of Sections

Thin cut of the cross or transverse sections of the leaf and stem were prepared by cutting with a sharp razor blade at a right angle to longitudinal axis of the leaf. Longitudinal axis, either in the radial direction (radial section) or in the tangential direction (tangential section). Each sample was placed on a slide, stained with safranin- O and mounted with glycerol, covered with cover-slip and viewed under the digital photomicroscope and the photograph of the image taken.

Preparation of Microscopical Specimen of Powdered samples

A little quantity of the powdered sample was cleared using sodium hypochlorite, then a small quantity transferred on to a slide, stained with safranin- O, mounted with glycerol and a cover-slip, slightly pressed to remove excess fluid from the margin of the cover-slip with a strip of filter-paper, then view under a microscope and the photograph of the image was taken.[8]

MICROMERITIC PROPERTIES OF *J. insularis* POWDER

The flow property was determined using standard methods.[9,10] 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 was 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 was 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.[11]

Fluorescence Analysis of Leaf and Stem Powders

Fluorescent analysis of dried leaf powder was carried out using standard method.[12]

Physico-chemical Evaluation of Leaf and Stem Powders

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 prescribed and the WHO guidelines on quality control methods for medicinal plant materials.[8,13]

RESULTS

Result for the leaf epidermal characters is as shown in Table 1 which analysed the anatomical features in the abaxial and adaxial surfaces and dimensions of the parameters.

Table 1: Showing the Epidermal, Stomatal and Trichome Characteristics of *J. insularis.*

Leaf Surface	Abaxial Surface	Adaxial Surface
Epidermal Cell Wall	Sinuous	Sinuous
Distribution of Stomata	Amphistomatic	Amphistomatic
Morphological Type of Stomata	Diacytic and Copericytic	Diacytic and Copericytic
Stomatal Length (µm)	6.18 (7.75±0.27) 8.90	6.88(7.98±0.29)9.55
Stomatal Width (µm)	3.94(4.49±0.10)4.90	4.38(5.59±0.35)3.94
Stomatal Pore Length (µm)	3.46(3.91±0.15)4.84	3.63(4.31±0.27)5.97
Stomatal Pore Width (µm)	1.00(1.27±0.07)1.53	1.03(1.48±0.11)2.00
Stomatal Number	0.50(1.32±0.25) 2.70	0.10(0.51±0.09) 0.7
Epidermal cell Number	4.00(4.85±0.21)6.10	3.40(4.36±0.20)5.30
Stomatal Index	21.4 %	10.5 %
Type of Trichome	Multicellular covering and Glandular trichomes	Multicellular covering trichomes, Glandular trichomes
Length of Trichome (µm)	11.49(69.56±13.63)122.79	12.04(53.36±7.09)88.30
Width of Trichome (µm)	3.88(5.75±0.39)7.45	4.88(6.22±0.35)8.04
Length of Epidermal Layer (µm)	17.47(24.06±1.11)28.50	14.85(19.77±1.26)26.91
Width of Epidermal Layer (µm)	2.70(4.69±0.74)9.95	4.23(6.02±0.52)8.76

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

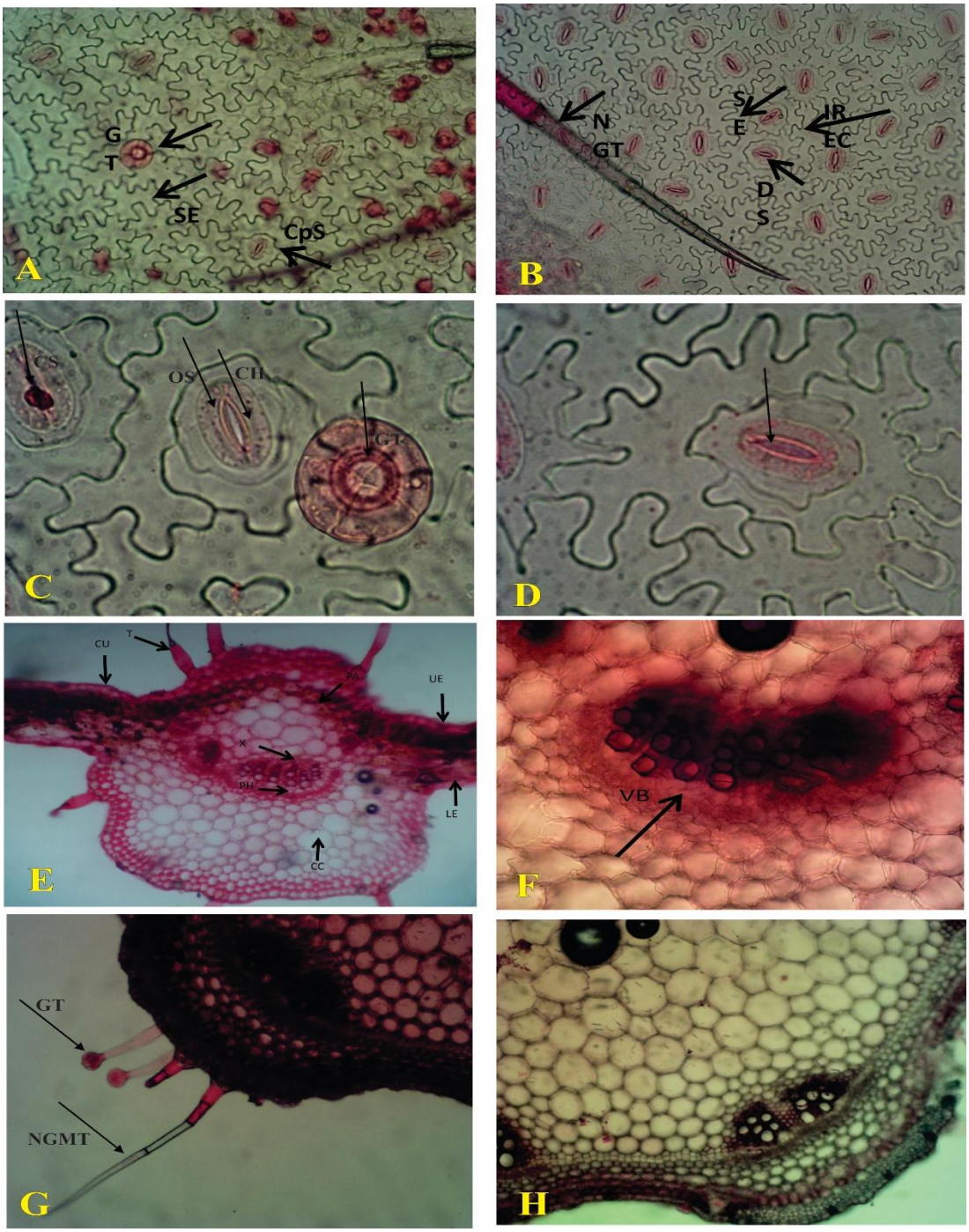

Figure 2 2A Adaxial surface: (GT- Glandular trichome; CpST- Co-pericytic stomata; SE- Sinuous Epidermal Cell) ×100: 2B Abaxial surface (NGT-Non-glandular trichome; DS-Diacytic stomata; IREC-Irregular epidermal cell) ×1002C: Abaxial Surface (×400) Showing Closed and Opened Copericytic Stomata with Chloroplast embedded in the Guard Cells and Glandular Trichome. 2D: Abaxial surface (×400)showing Diacytic Stoma. 2E:Transverse Section (T-Trichome, CU- Cuticle, PA- Parenchyma, CC-Collenchyma, X- Xylem, PH- Phloem, UE- Upper epidermis and LE- Lower epidermis) ×400:2F VB-Vascular bundle: 2G: Transverse Section of the Stem: Epidermis,

Hypodermis, parenchyma, schlerenchyma, vascular bundles and crystal idioblasts ×400: 2H:Transverse Section of the Stem: GT- Glandular trichome; NGMT-Non-glandular Multicellular Covering Trichomes×400.

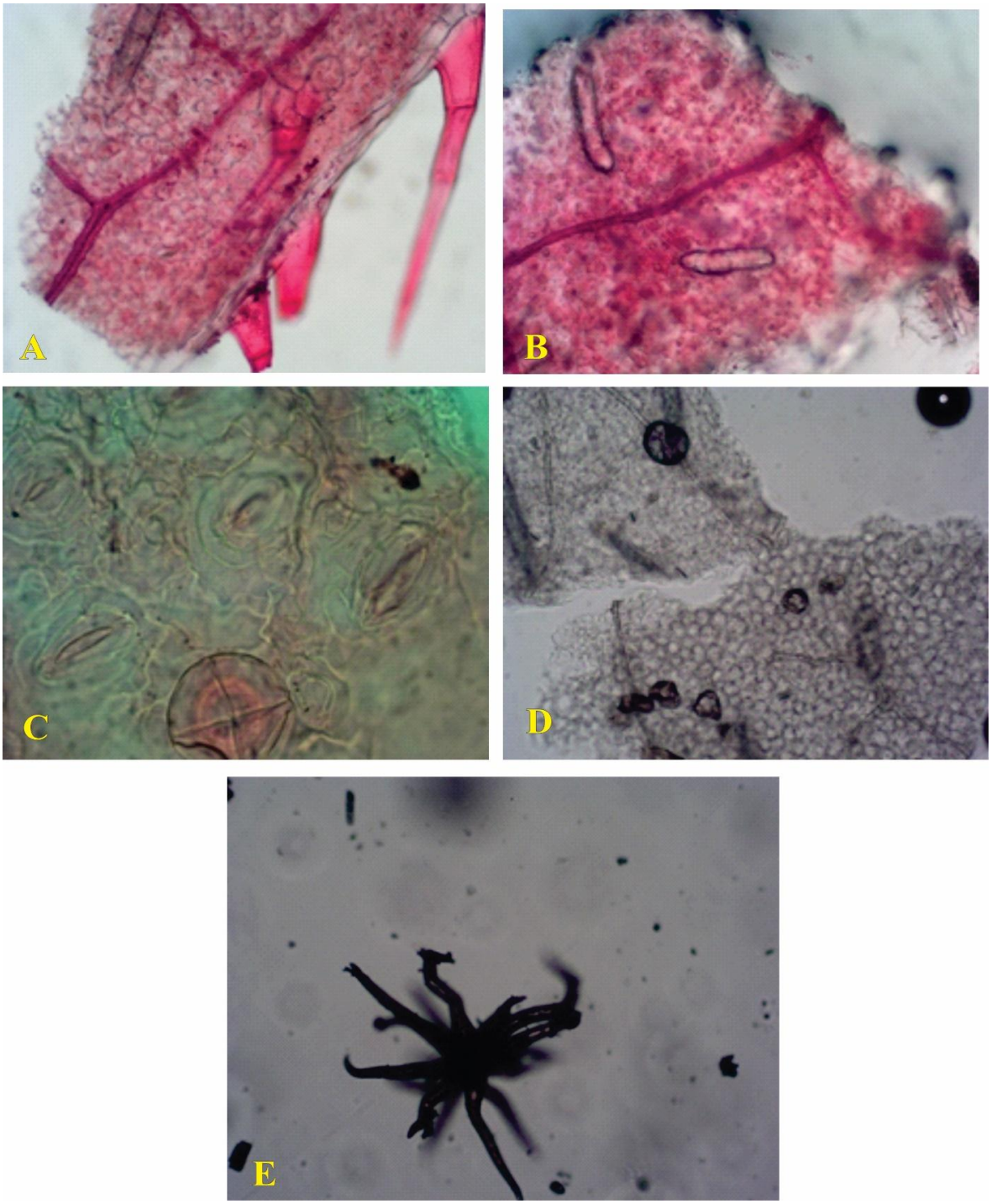

Figure 3 Showing Powdered *J.insularis* Leaf with Non-glandular trichome (A) Trachieds (B)Glandular Trichome (C) Calcium Oxalate Crystals (D) and Stellate Trichomes (E) ×100.

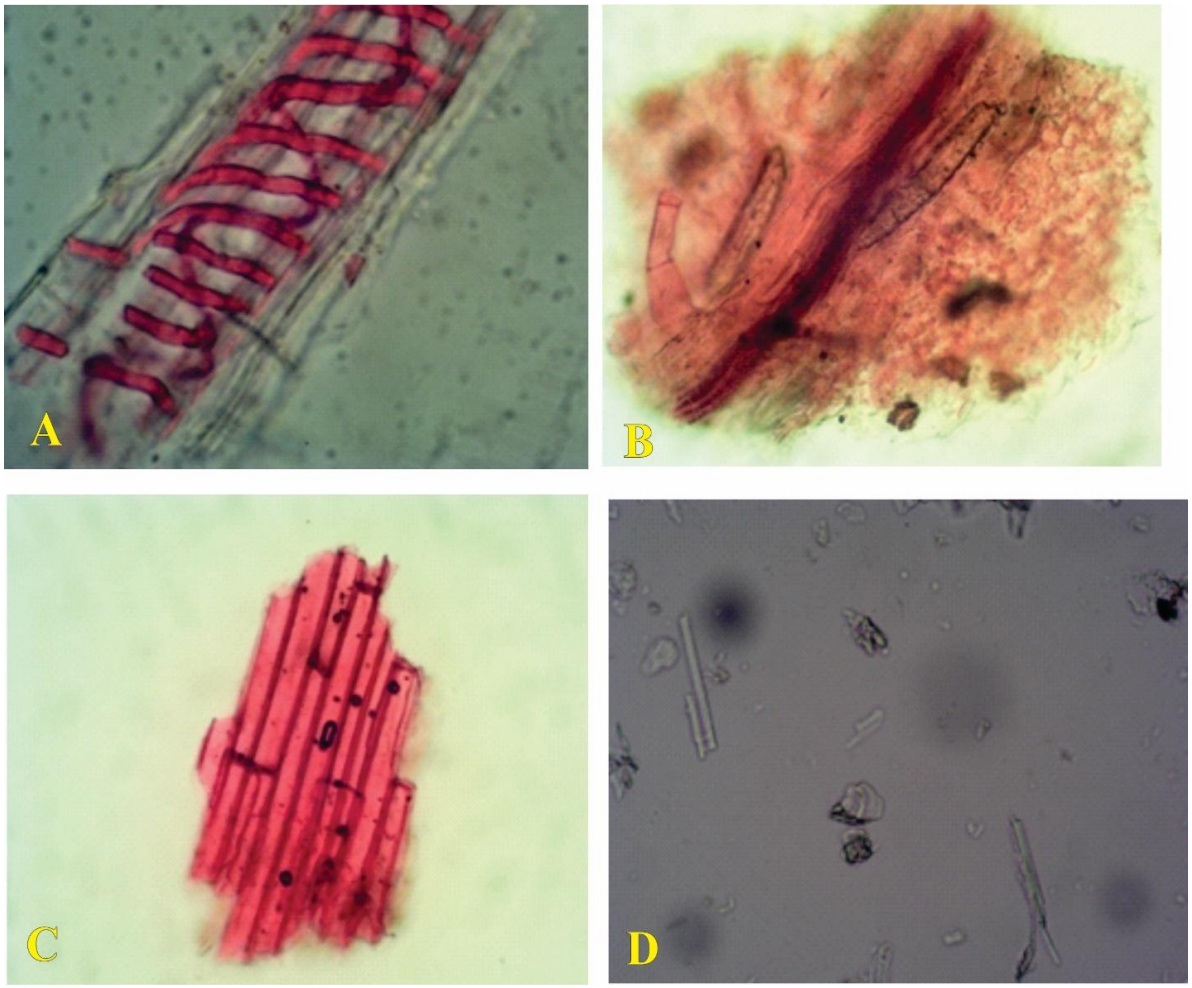

Figure 4: Showing Powdered *J. insularis* stem with Rings of xylem vessle (A), Trichome and Tracheids (B), Fibre (C) and Calcium Oxalate Crystals (D) ×100.

Table 2: Showing Micromeritic evaluation of *J. insularis* Powdered Leaf and Stem.

Micromeritic Parameters	Leaf Powder	Stem Powder
Bulk Volume (mL)	49±0.00	38.67±0.33
Tapped Volume (mL)	34±0.00	27.33±0.33
Bulk Density (g/mL)	0.20±0.00	0.26±0.00
Tapped Density (g/mL)	0.29±0.00	0.37±0.00
Hausner's ratio	1.45±0.00	1.41±0.01
Carr's Index (%)	31±0.00	30.00±0.65
Diameter of Heap (cm)	7.57±0.12	6.97±0.30
Height of Heap (cm)	2.92±0.06	3.13±0.03
Flow Time (sec)	14±0.58	32.33±1.33
Flow Rate (g/sec)	0.72±0.03	0.31±0.01
Packing Fraction	0.16	0.18
Angle of Repose (^{0})	37.60±0.38	42.00±0.54
Ph Cold Hot	 6.32 6.06	 5.76 5.72

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

Table 3: Showing Chemomicroscopic Evaluation of Leaf and Stem of *J. Insularis* Powder.

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

+ = Present, - = Absent

Table 4: Physicochemical constants of leaf and stem of *Justicia insularis.*

Parameters	Leaf (%w/w)	Stem (%w/w)
Moisture content	13.5	12.5
Total ash	16.5	11.0
Water-soluble ash	6	5
Water-insoluble	11	6
Acid-insoluble	2	1
Acid-soluble	14.5	10.5
Sulfated ash	19	13
Extractive value (%w/w)		
Water-soluble	23	22
Ethanol	11	15
Methanol	1	14

Data are represented as mean ± standard error in triplicate

Table 5: Showing Evaluation of florescence properties of *J .insularis* leaf and stem extracts in various solvents under ultraviolet (UV) lights.

Extract	Sample	Physical observation Colour	UV-365 nm Colour	UV-253.7 nm Colour
Hexane	Leaf	Yellow	Milky	Light grey
	Stem	Light yellow	Light pink	Light grey
Dichloromethane	Leaf	Green	Maroun	Brown
	Stem	Brown	Orange	Light brown
Ethanol	Leaf	Green	Orange	Brown
	Stem	Yellowish green	Red	Grey
Methanol	Leaf	Light brown	Maroun	Grey
	Stem	Light brown	Light blue	Grey
Water	Leaf	Green	Orange	Brown
	Stem	Brownish green	Red	Grey
Ethylacetate	Leaf	Green	Pink	Colourless
	Stem	Light green	Pink	Colourless

DISCUSSION

The quality control of vegetable crude drugs is paramount importance under current European Union (EU) regulations: herbal products can only be manufactured under license in uniformity with the rules and guidance for pharmaceutical manufacturers and distributors.[14]

The results obtained for the abaxial and adaxial surfaces were sinuous epidermal cell wall pattern, amphistomatic distribution of stomata, diacytic and copericytic types of stomata, multicellular covering non-glandular and glandular trichomes were present. Also, the average stomatal frequency (stomatal number) were more on the abaxial surface (1.32 ± 0.25) than adaxial surface (0.51 ± 0.09), stomatal index were 21.4% on abaxial and 10.5% on the adaxial surfaces as shown in Table1.

The micromeritic evaluation of powdered vegetable drugs which include bulk and tapped volumes, bulk and tapped densities of the leaves and stems of *J.insularis* were determined to be 49.00 ± 0.00 and 34.00 ± 0.00 mL, 38.67 ± 0.33 and 27.33 ± 0.33 mL., 0.20 0.00 and 0.29 ± 0.00 mg/mL, 0.26 ± 0.00 and 0.37 ± 0.00 mg/mL respectively as shown in Table 2. Subsequently, the Hausner's ratio and Carr's index for the leaves and stems were 1.45 ± 0.00 and 31.04 ± 0.00 %., 1.41 ± 0.01 and 29.08 ± 0.65 % respectively as presented in Table 2.The Hausner's ratio and Carr's index are parameters that are used to determine the powder flow property and powder characteristics. Hausner's ratio values less than 1.25 indicate good flow while those greater than 1.25 indicates poor flow. From the experiment, Carr's index and Hausner's ratio of both the leaf and stem were greater than 25 % and 1.25 respectively and these indicate that the powder has a poor flow property as shown in Table 2. This is as a result of some factors that affect a powder's flowability hence affecting the powder characteristics. The factors include: moisture content, temperature, particle size, particle shape (texture) and time of storage at rest. The moisture content affects the powder flow because typically as the powder moisture content increases, so does it cohesive strength and forces increase and then subsequently the flowability of the powder is significantly affected. Temperature also affects the powder flow because some powders are sensitive to increase in temperature and others are sensitive to constant temperature. As particle size of a powder becomes finer, so does the cohesive force increases and subsequently it becomes difficult to handle. The time of storage at rest in a container affects the flowability of a powder because the powder remains at rest, it becomes more cohesive and hence difficult to flow.[15] 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.[16]

Chemomicroscopy study revealed the presence of mucilage, cellulose and oil in the leaf and stem, calcium oxalate crystals and lignin in the stem and leaf while Protein and Starch were present in the leaf only as shown in Table 3.

The moisture content of *J. insularis* leaf and stem were 13.5 and 12.5% $^{w}/_{w}$ respectively as stated in Table 4. The African pharmacopoeia limit of moisture contents for vegetable crude drugs range is between 8-14% $^{w}/_{w}$. The moisture content for the leaf and stem fell within the limit. 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. The presence of excess moisture in a sample suggests that drug has been incorrectly prepared or inappropriately stored and hence it leads to the breakdown of important constituents by hydrolytic reactions or enzymatic activities and may encourage the growth of microorganisms (for example yeast and fungi) or insects during storage.[8]

Also from the result obtained, the total ash value for *J. insularis* leaf and stem were 16.5 and 11.0 $\%^{w}/_{w}$ respectively as shown in Table 4. The European pharmacopoeia limit of total ash value for crude vegetable drugs range should not exceed $14\%^{w}/_{w}$.[17] From the result of the study, the total ash value of the stem was found to be within acceptable limit of 11.0% $^{w}/_{w}$ as that of the leaf was not, with the value $16.5\%^{w}/_{w}$. The total ash value is a method used to measure the total amount of residual substances that is not volatilized when the drug sample is incinerated. Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium, etc. Ash may be derived from the plant itself and it is usually called the "physiological ash" or may come from the extraneous matter, especially sand and soil that adhere to the surface of the drug and it is usually called the "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.[8]

Also, from the result obtained, the acid-insoluble ash value of *J. insularis* leaf and stem were 2.0 ± 0.00 and 1.00 ± $0.00\%^{w}/_{w}$ respectively as shown in Table 4. The European pharmacopoeia limit of acid- insoluble ash value for crude vegetable drugs range should not exceed $2\%^{w}/_{w}$.[17] The determination of the acid- insoluble ash is a method that is intended to

measure the amount of silica, especially sand and siliceous material, present in the drug. The water-soluble ash value of the vegetable drug *J. insularis* leaf and stem were 6.00 and 5.00%$^{w}/_{w}$ as shown in table 4 from the experimental research carried out. The African pharmacopoeia limit of water-soluble ash value for crude vegetable drugs state that a lesser amount shows that there is less solubility of the ash in water while a higher value indicated a high solubility of the ash in water. The determination of the water- soluble ash value of a particular crude drug helps in the detection of the amount of the ash materials that are soluble in water.

The determination of sulfated ash generally is a method that determines the amount of inorganic substances contained as impurities in an organic substance but occasionally for the determination of the amount of inorganic substances contained as components in an organic substance, or the amount of impurities contained in a heat volatile organic substance. The sulfated ash is the residual substance that is not volatilized when the sample is ignited with concentration sulphuric acid. From the result of the study, the sulfated ash values for the leaf and stem of *J. insularis* were 19.00 and 13.00% w/w respectively as shown in Table 4. Moreover, metals thus remain as sulphates are more stable to heat. The determination of the extractive values helps to measure the amount of constituents which are extractable by the solvents under the specified conditions. They also give an idea about the nature of the chemical constituents present in a crude drug. From the experimental research carried out, the water-soluble extractive values for the leaf and stems of *J. insularis* were 23.00 and 22.00 %$^{w}/_{w}$ respectively, while that of the alcohol-soluble and methanol-soluble extractive values for the leaf and stem of *J. insularis* were 11.00 and 15.00%$^{w}/_{w}$, 1 and 14 % $^{w}/_{w}$ respectively as stated in Table 4. The result of the fluorescence properties of the plant extracts of different solvents spotted on a chromatographic plate showed different colours under ultraviolet lights of upper and lower wave lengths indicating different compounds present in the solvents as shown in Table 5.

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