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PHARMACOGNOSTIC EVALUATION OF THE LEAVES AND STEMS OF *JUSTICIA SECUNDA* VAHL. (ACANTHACEAE)

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ABSTRACT

Justicia secunda Vahl. (Acanthaceae) is commonly known as "bloodroot" and "sanguinaria" in Barbados and Venezuela respectively. In South-Eastern Nigeria, it is locally called "obarabundu". The Ogbiapeople of Otuoke-Otuaba, Bayelsa, Niger-Delta region of Nigeria calls it "asindiri" or "ohowaazara". The leaves are used for treatment of wound, anaemia, and pain within the abdominal region**.** The leaf decoction is consumed in some parts of Nigeria, Cote-d'Ivoire, and Congo for the purpose of improving haematocrit count. The leaves have been demonstrated to possess anti-sickling, antimicrobial, antihypertensive and haematinic activities. The aim of this study was to evaluate pharmacognostic parameters of *Justicia secunda* such as microscopy, micromeritics, chemomicroscopy, fluorescence, extractive values, moisture content and ash values. The leaves and stems were collected, identified, air-dried, weighed and subjected to the above evaluation parameters using standard procedures. In microscopy, the fresh leaf, stem and their powders were subjected to clearing, staining and examination under microscope. The micromeritics properties were determined using powdered leaf and stem. For chemomicroscopy, their powders were treated with different chemicals/ reagents and viewed under microscope. For the fluorescence properties the powders were macerated in different solvents and their extracts viewed at day

light, the lower and higher wavelength of the ultraviolet lights. For extractive values, cold maceration with ethanol, methanol, and distilled water were used as the solvents. The moisture content was determined using loss on dry method, where crucibles and their contents were weighed and heated in an oven at 105^0C until completely dried, then the moisture contents were determined and their percentages calculated. These crucibles were transferred into the furnace and heated at 450^0C for 8 hours and the total ash values were obtained. The acid-insoluble ash and water-soluble ash values were obtained by washing the ashes with dilute hydrochloric acid and distilled water respectively. It was passed through the ashless filter paper, dried and then incinerated in the furnace at 450^0C and their ash values were calculated. For sulfated ash, the powders were mixed with concentrated sulphuric acid and incinerated at 800^0C for 8 hours. The results obtained from microscopy, the leaf has amphistomatic stomata, multicellular covering trichomes on the adaxial surface and none on abaxial surface. The epidermal cell wall pattern was undulated both on the adaxial and abaxial surfaces. Stomatal index was 33.30% on abaxial surface and 2.53% on adaxial surface. The micromeritics analysis of the leaf and stem powders reveal good flow and fair flow respectively. The results of chemomicroscopy for leaf and stem revealed the presence of mucilage, calcium oxalate crystals and oil except lignin in stem and starch in the leaf. For water-soluble extractive values the results of leaf and stem were 26.00%$^w/_w$ and 12.00%$^w/_w$, methanol-soluble extractive value 12.00%$^w/_w$ and 4.50%$^w/_w$, ethanol-soluble extractive value 14.3%$^w/_w$ and 4.80%$^w/_w$, moisture content 9%$^w/_w$ and 9%$^w/_w$, total ash values were 16%$^w/_w$ and 7%$^w/_w$, acid-insoluble ash values 2% $^w/_w$ and 1%$^w/_w$, water-soluble ash values 4% $^w/_w$ and 4%$^w/_w$, sulfated-ash values 21.50%$^w/_w$ and 7%$^w/_w$ respectively. In conclusion, the results obtained from the pharmacognostic studies will provide information about the identity, quality and purity of *Justicia secunda*.

KEYWORDS: Haematinic, Haematocrit, *Justicia secunda*, Micromeritic, Microscopy, Pharmacognostic, Therapeutic.

INTRODUCTION

The traditional use of medicinal plant can lead to the discovery of new potent botanical agent in the treatment of several diseases. The global market value of medicinal plant products exceeds 100 billion per annum.[1] Medicinal herbs or plants have been known to be an important potential source of therapeutics or curative aids. This involves the use of medicinal plant not only for the treatment of disease but also as potential material for maintaining good

health and condition.[2] Many countries in the world depend on herbal medicine for primary healthcare, because of their better cultural acceptability, better compatibility and adaptability with the human body and poses lesser side effect. Standardization of herbal medicine is the process of prescribing a set of standard or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is the process of developing and agreeing upon technical standards.[3]

Justicia secunda belongs to the family of Acanthaceae, commonly known as "bloodroot" and "sanguinaria" in Barbados and Venezuela respectively.[4,5] In South-Eastern Nigeria, it is locally called "obarabundu". The Ogbia people of Otuoke-Otuaba, Bayelsa, Niger-Delta region of Nigeria calls it "asindiri" or "ohowaazara". *Justicia secunda* grows in humid soil around rivers or creeks and can be located in tropical and pantropical regions of the world.[6] Phytochemical evaluation of *J. secunda* leaves revealed the presence of alkaloids, polyphenols, flavonoids, tannins, leucoanthocyanins, quinones and anthocyanins.[7] It also contains quindoline, luteolin, auranamide, secundarellone A, B and C, aurantamide acetate, and pyrrolidone derivatives have been documented for *J. secunda* leaves.[8] In folklore medicine, *J. secunda* leaves are used for treatment of wound, anaemia, and pain within the abdominal region.[9] The leaf decoction of *J. secunda* is consumed in some parts of Nigeria, Cote-d'Ivoire, and Congo for the purpose of improving haematocrit count.[7] The leaves have been demonstrated to possess anti-sickling, antimicrobial, antihypertensive and haematinic activities.[10] It has anti-inflammatory potential. Onoja *et al.*[5]

Scientific Classification

Scientific classification based on angiosperm phylogeny group system (APG).[11]

Kingdom: Plantae

Clade: Angiosperm

Clade:	**Eudicot**
Clade:	**Asterids**
Order:	**Lamiales**
Family:	**Acanthaceae**
Subfamily:	**Acanthoidae**
Genus:	***Justicia***
Species*:*	***secunda***

Local name Ibibio: **Ibok iyip**

Botanical Name: ***Justicia secunda* VAHL**

Figure 1: *Justicia secunda* VAHL. in its natural habitat.

MATERIALS AND METHODS

Collection and Identification of Plant

The leaves and stem of stem of plant evaluated were collected from Ikot Akpe, Off Abak Road, Uyo Akwa Ibom State, May 2018. The plant *Justicia secunda* was identified and the herbarium number was **UUPH 1(e).**

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.

Macro-morphological Evaluation of Leaf

Organoleptic (sensory) parameters of fresh leaf as well as their powders such as colour, odour, taste and texture were evaluated by the sense organs and documented.

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.[12]

Quantitative Leaf Microscopy

Quantitative microscopy parameters such as leaf constant studies viz. stomatal length and width, guard cell length and width, stomatal number, stomatal index, epidermal cell length and width, epidermal cell number, epidermal cell thickness was 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[13], 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[14], 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 viz. mucilage, lignin, starch, oils, calcium carbonate and calcium oxalate crystals.[15]

Fluorescence Analysis of Leaf and Stem Powders

The fluorescent analysis of dried leaf powder was carried out using standard method.[16]

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, sulfated ash), soluble extractive values such as ethanol, methanol and water were performed according to the official method prescribed and the WHO guidelines on quality control methods for medicinal plant materials.[17]

RESULTS

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

Leaf Surfaces	Abaxial Surface	Adaxial Surface
Epidermal Cell Wall pattern	Undulated	Undulated
Distribution of Stomata	Amphistomatic	Amphistomatic
Morphological Type of Stomata	Amphidiacytic and dialelocytic	Amphidiacytic and diallelocytic
Stomatal Length (µm)	4.62(5.72±0.25)7.52	4.41(5.84±0.22)6.58
Stomatal Width (µm)	3.03(3.91±0.27)5.88	2.74(3.86±0.27)5.20
Stomatal Pore Length (µm)	2.72(3.73±0.23)5.28	2.17(2.97±0.26)4.56
Stomatal Pore Width (µm)	0.37(0.76±0.08)1.16	0.37(0.57±0.04)0.77
Stomatal Number	28.00(34.70±1.61)44.00	0.00(3..10±0.91)10.00
Epidermal cell Number	62.00(69.49±1.75)78.00	81.00(119.20±10.56)179.00
Stomatal Index(%)	33.30	2.53
Type of Trichome	Nil	Multicellular
Length of Trichome (µm)	Nil	30.13(48.13±4.85)75.93
Width of Trichome (µm)	Nil	3.87(4.53±0.20)5.65
Length of Epidermal Layer (µm)	13.40(17.19±1.06)22.84	9.68(13.78±0.79)17.52
Width of Epidermal Layer (µm)	3.50(4.95±0.31)7.16	3.38(5.44±0.47)8.27

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

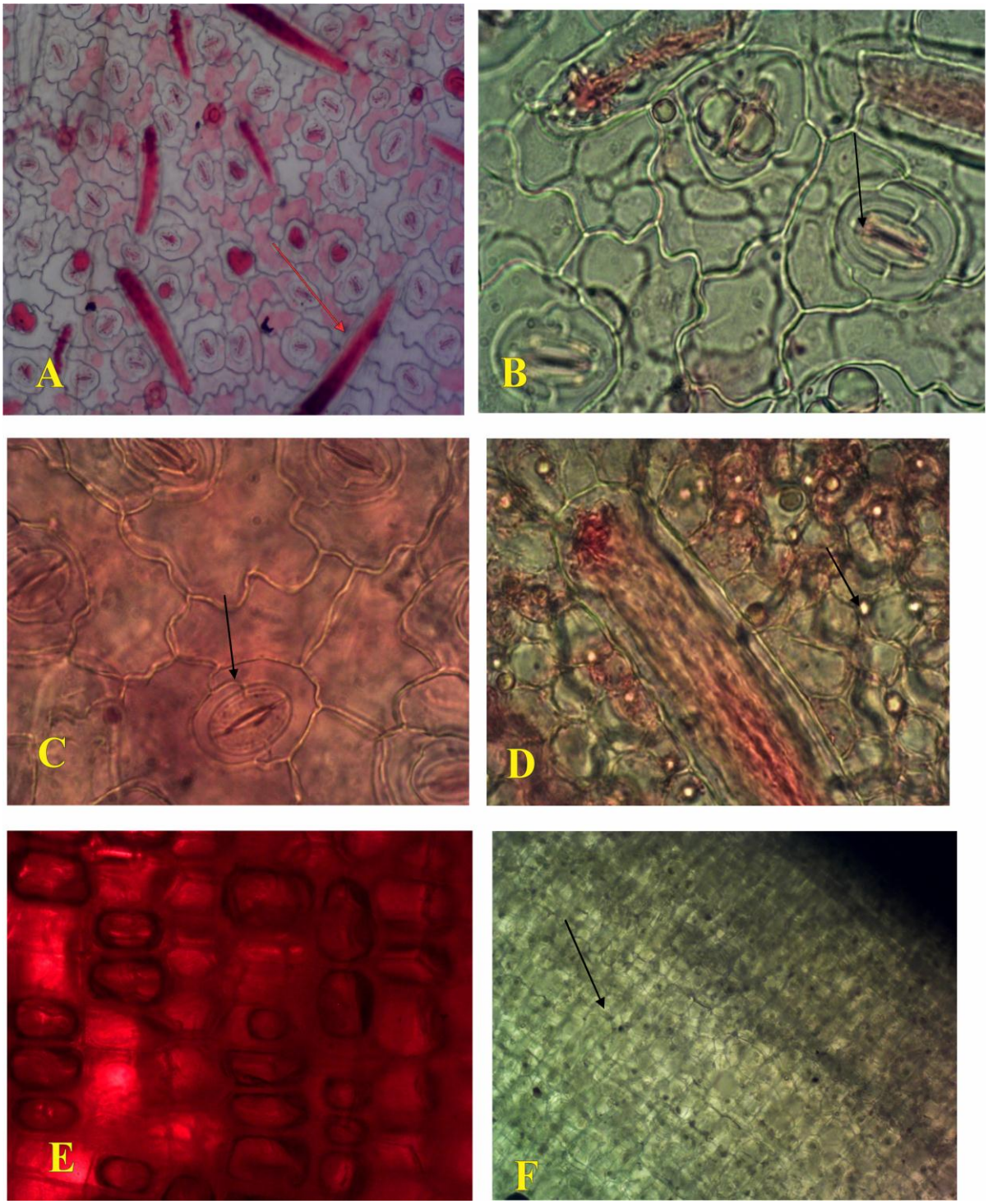

Fig 1: Microscopy of *J. secunda.*

A: Abaxial Surface Showing Trichome X 400

B: Abaxial Surface Showing Diallelocytic Stomata X 400

C: Adaxial Surface Showing Diallelocytic Stomata X 400

D: Adaxial Surface Showing Druce Crystals X 400

E: L/S of Mature Stem Showing Phloem Parenchyma X 400

F: L/S of Stem showing sieve tube X 100

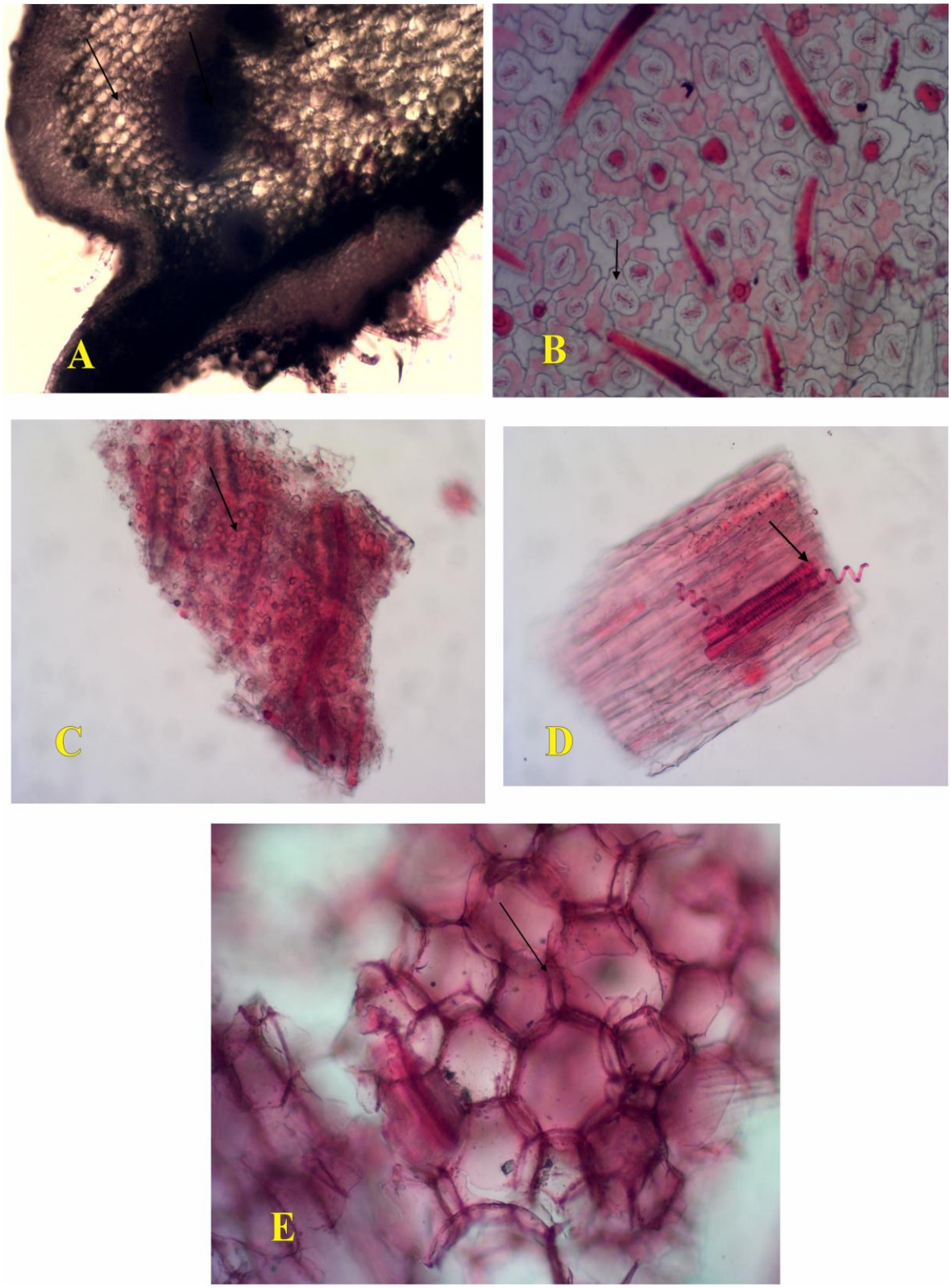

Fig 2: A: T/S of Leaf Through Midrib Showing Collenchyma And Cortex X 400

B: Analysis of powder leaf Showing Stomata Ontogeny X 100

C: Powdered analysis of leaf showing tracheids X100

D: Microscopy of Powder Stem Showing Conducting Strands of Xylem X 100

E: Analysis of Powdered Stem Showing Collenchyma x 400

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

Micromeritic Parameters	Leaf Powder	Stem Powder
Bulk Volume (mL)	32.83±0.17	62.50±0.33
Tapped Volume (mL)	22.17±0.00	44.33±1.64
Bulk Density (g/mL)	0.30±0.03	0.15±0.00
Tapped Density (g/mL)	0.40±0.00	0.23±0.00
True density(g/mL)	1.32±0.01	1.36±0.01
Hausner Ratio	1.30±0.00	1.41±0.05
Carr's Index (%)	23.36±0.46	29.10±2.40
Diameter of Heap (cm)	6.79±0.06	7.99±0.08
Height of Heap (cm)	2.17±0.09	3.27±0.07
Flow Time (sec)	7.81±0.12	33.33±2.96
Flow Rate (g/sec)	1.28±0.02	0.30±0.03
Packing Fraction	0.23±0.00	0.12±0.00
Angle of Repose (^{0})	32.6^0	39.25±0.30
pH		
Cold	6.60	6.60
Hot	6.35	5.63
Interparticulate porosity	1.81±0.14	0.76±0.02

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

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

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

+ = Present, - = Absent

Table 4: Showing Fluorescence analysis of the Leaf and Stem of *J. secunda.*

Extracts	Ordinary light		UV-365ηm		UV-253.7ηm	
	Leaf	Stem	Leaf	Stem	Leaf	Stem
Water	Purple	Light purple	Light brown	Colourless	Grey	Grey
Methanol	Green	Yellow	Brown	Purple	Grey	Grey
Ethanol	Green	Yellow	Brown	Purple	Grey	Grey
Dichloromethane	Green	Light brown	Brown	Purple	Grey	Grey
N-Hexane	Yellow	Colourless	Pink	Pink	Colourless	Colourless

Table 5: Physicochemical constants of leaf and stem of *Justicia secunda.*

Parameters	Leaf (%w/w)	Stem (%w/w)
Moisture content	9	9
Total ash	16	7
Water-soluble ash	4	4
Acid-insoluble	2	1
Sulphated ash	21.5	7
Extractive value (%w/w)		
Water-soluble	26	12
Ethanol	14.3	4.8
Methanol	12	4.5

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

DISCUSSION

Adulteration by illegal addition of pharmaceutical substance or their analogs and misidentification of crude drugs pose serious health problems and that an effective control by regulatory authorities is needed to safeguard the consumers. The result obtained from microscopy of *Justicia secunda* in table 1 was found to be amphistomatic stomata on both surfaces, multicellular covering trichomes on the abaxial surface and none on adaxial surface, The epidermal cell wall pattern was undulate both on the adaxial and abaxial surfaces. Stomatal index was relatively constant and is not much affected by factors such as age of plant, size of leaf, environmental conditions. Stomatal index of abaxial surface of *Justicia secunda* was 33.3% and that of adaxial was 2.53%. Every plant possesses characteristic tissue features which can be identified by microscopy of leaf powder analysis which are used in identification and detection of adultrant. The microscopic study also showed the presence of amphidiacytic/diallelocytic stomata on both surfaces. The micromeritics properties like bulk density, tapped density, angle of repose, Hausner's ratio and Carr's index indicated the flow properties as well as interparticulate resistance between powders. This information predicts the stability and solubility of crude drug. Angle of repose of the leaf powder which is 1.28^0 indicated a free flowing material and the stem which is 39.25^0 indicates fair flow. The micromeritics properties in table 2 help to characterize and standardize the pre-formulation properties of herbal drug powder, in order to determine its suitability for formulation into solid dosage forms. Chemomicroscopy analysis in Table 3 of the powdered leaf of the plant recorded the presence of mucilage, calcium oxalate, starch and oil while that of the stem recorded the presence of mucilage, lignin, calcium oxalate and oil. In fluorescence analysis in Table 4 the powdered drug treated with water, methanol, ethanol, dichloromethane and n-hexane were observed under ordinary light, short wavelength of uv light and long wavelength

of uv light. The colour changes for leaf and stem powders were distinctive and reproducible revealing the solvent properties of the phytoconstituents.

From result in Table 5, Water extractive value showed the highest value, which was found to be 26.00%$^{w}/_{w}$. and 12.00%$^{w}/_{w}$ for leaf and stem respectively compared to that of ethanol of 14.3%$^{w}/_{w}$ and 4.8%$^{w}/_{w}$, methanol which were 12%$^{w}/_{w}$ and 4.5%$^{w}/_{w}$ respectively. This may be due to the presence of high amount of water soluble compounds in the leaves and stem of *J. secunda.* Water permeates the cells of the leaf and stem and thus a better solvent for extraction of *J. secunda.* The moisture content of *J. secunda* leaf powder (9%$^{w}/_{w}$) and stem powder (9%$^{w}/_{w}$) which are within the recommended range of 8-14%$^{w}/_{w}$, for vegetable drug according to African pharmacopoeia, 1986 is an indication that the plant can be stored for a long period of time with less probability of microbial attack. Ash value are useful indicator of the purity of any drug and give information relative to its adulteration/ contamination with inorganic matter. Total ash value of *J. secunda* leaf was 16%$^{w}/_{w}$. which is above the limit and the stem is 7%$^{w}/_{w}$ which is within the limit as indicated in European pharmacopoeia, 2007[18] that the limit of total ash value for crude vegetable drug range should not exceed 14%$^{w}/_{w}$, total ash content which is the total amount of material remaining after incineration is not sufficient to reflect the quality of leaves since the plant material often contain calcium oxalate crystals in particular. Acid-insoluble ash gives more consistent value than total ash value. Acid-insolube ash value for leaf of *J. secunda* 2%$^{w}/_{w}$ and 1%$^{w}/_{w}$ for stem which are within European pharmacopoeial limit (not exceed 2%$^{w}/_{w}$.).[18] Water-soluble ash value represents the water soluble portion of total ash and it is 4%$^{w}/_{w}$. for leaf and 4%$^{w}/_{w}$ for stem. Sulfated ash value for leaf and stem were 21.50%$^{w}/_{w}$ and 7%$^{w}/_{w}$ respectively which indicates that the residual substance not volatilized when the sample was incinerated with concentrated sulphuric acid and is a method intended for determining the amount of inorganic substances contained as impurities in an organic substance, but occasionally for determination of the amount of inorganic substances contained as component of an organic substance.

CONCLUSION

The result obtained from the pharmacognostic studies will provide information about the identity, quality and purity of *Justicia secunda*. The result collectively might be useful to supplement information for further studies on *Justicia secunda* leaf and stem.

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