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PHARMACOGNOSTIC STUDIES ON THE LEAF OF *BASILICUM POLYSTACHYON* LINN MOENCH (LAMIACEAE)

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ABSTRACT

Basilicum polystachyon, commonly called Musk basil and locally "*kimbar Rafi"* in Hausa, Nwansinwansi in Ghana. This study was carried out to establish a comprehensive report on the quality control and standardization of *B. polystachyon*. The preparation of the fresh and powdered samples of leaf was carried out according to WHO guideline. The result of macroscopic evaluation of the leaf of *B. polystachyon* revealed simple leaf type, opposite leaf arrangement, acuminate leaf apex, acute leaf base, reticulate venation, crenate leaf margin, ovate leaf shape, glabrous surfaces, green leaf colour, papery, tasteless and pleasant aroma. The result of the microscopy of the abaxial and adaxial surf ace of the leaf revealed diacytic type of stomata, multicellular and glandular trichomes, vessels and fibres. Chemomicroscopy of the pulverized leaves revealed presence of cellulose cell wall, gum, mucilage, cutin and lignin as cell wall materials while the cell inclusions present were protein in form of aleurone grains, calcium carbonate, calcium oxalate crystals, inulin and tannins. The quantitative leaf microscopy on the average gave the stomatal number (12.60-11.75±0.85-10.90), stomatal index (22.68-18.89±3.79-15.10), palisade ratio (6.56-6.25±0.31-5.94), vein islets (47.31-44.75±2.56-42.19) and vein-let termination number (23.94-20.75±3.19-17.56). The physicochemical parameters of the pulverized leaves revealed moisture content (12.85-11.58±1.27-10.31), total ash (14.06-11.75±2.31-9.44), acid insoluble ash (5.22-4.13±1.09-3.04), water soluble ash (6.31-5.88±0.43-5.45), alcohol extractive value (3.80-3.73±0.07-3.66), and water extractive value (3.14-3.07±0.07-3.00). Findings from this study have provided pharmacognostic standards for the quality control, identification and standerdisation of *B. polystachyon.*

Key Words: *Basilicum polystachyon,* Macroscopy, Microscopy Chemo-microscopy, Physico-chemical parameters, Standardization.

INTRODUCTION

Medicinal plant is said to be any plant in which in one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (WHO, 2011).

The purpose of this study was to provide pharmacognostic standards for the quality control of *B. polystachyon* and established scientific evidence for proper identification and standardization.

Basilicum polystachyon, commonly called Musk basil and locally "*kimbar Rafi"* in Hausa and Nwansinwansi in Ghana. It is an important ethnomedicinal herb belonging to the family Lamiaceae. It is widely distributed in tropical areas, in east hemisphere, including Asia, Africa and Australia. It is an annual or perennial herbaceous plant, and it is a very rare species in the lamiaceae

(Labiatae) family. It is found growing in moist shady places. The plant is an aromatic plant (Singh, *et al.,* 2018). It has much branched 4- angled, erect stem, leaves ovate, acuminate, obtuse or subacute, apiculate irregularly crenate, serrate, glabrous, base rounded or truncate, narrowed into a slender petiole as long as the blade, flowers pale pink or creamy- white, shortly pedicellate in numerous closely placed whorls forming slender shortly pedunculate spicate paniculate racemes, corolla bilabiate nutlets, broadly elipsoid, compressed, smooth, brown. Leaves are dorsiventral, greenish, simple, eliptic oblong, petiolate, margins toothed, membranous, minutely pubuscent 4-8 × 1.75-4 cm shrivelled or shrunken when dry. Petiole is slender, minutely pubuscent it has no characteristics taste but possesses a pleasant aroma (Madhavan, *et al.,* 2013) The seed is the dispersal unit of plant and an important stage in the higher plant lifecycle with respect to its survival as a species. Germination incorporates those events that commence with the uptake of water by the quiescent dry seed and terminates with the elongation of the embryonic axis (Bewley and Black, 1994).

The leaves of *B. polystachyon* are used internally as tea, and externally as a lotion. The crushed leaves are used in Indonesia as a sedative, and to relieve painful sprains and limbs. Decoction of leaves is taken as sedative, for epilepsy, palpitation of heart, neuralgia nervous headaches, nervousness after childbirth, rheumatism and convulsion. In East Africa, fresh roots are chewed against cough, or cooked with food to reduce flatulence. In Kenya, an infusion of the fruit is taken for parturition in the case of delayed birth. Normads in Kenya burn the plant inside milking pots to give a pleasant smell to the milk. In East Africa, the plant is burnt indoors as a mosquito and snake repellent. In west tropical Africa, the juice of the plant is used to cure headache in children by squeezing a drop of the juice of the leaf in the nostrils. In Nigeria, the leaves are used to flavour food and as a sedative (Madhavan, *et al.,* 2013).

Standardization is a system that ensures a predefined amount of quantity, quality and therapeutic effect of ingredients in each dose of crude drug. Herbal product cannot be considered scientifically valid if the drug tested has not been authenticated and characterized in order to ensure reproducibility in the manufacturing of the product (Sekham, 2011). Herbal drugs or its standardized extracts or pure active compound needs analytical techniques to confirm its identity, quality, purity, potency, safety and efficacy of the plant (Zafar, 2005).

Phytochemicals are biologically active naturally chemical compounds found in plants, which provides health benefits to humans further than those artributed to macronutrients and micronutrients. They protect plants from diseases and damage and contribute to the plant's color, aroma and flavour. In general, they protect plants cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack (Mathai, 2000).

Thin layer chromatography is one of the most popular and simple chromatographic technique used for separation of compounds. In the phytochemical evaluation of herbal drugs, TLC is being employed extensively to enable rapid analysis of herbal extracts with minimum sample clean-up requirement, to provide qualitative and quantitative information of the resolved compounds and to enable the quantification of chemical constituents (Pravin, *et al.,* 2012).

The objective of the study was to evaluate the pharmacognostic features of the leaf of *B. polystachyon*, to determine the preliminary phytochemical constituents and develop the

thin layer chromatographic profile of methanol leaf extract of *B. polystachyon.*

METHODS

Collection, Identification and Preparation of the Plant Material

The fresh, matured plant material of *Basilicum polystachyon* comprising leaves, stem and flowers was collected from Kofan Gayan Zaria Local Government Area, Zaria in the month of August 2019 and was identified and authenticated in the Herbarium unit, Department of Botany, Ahmadu Bello University, Zaria by comparison with existing voucher specimen (VSN: 70750). The leaves were dried, powdered and stored in air tight container for further use.

Pharmacognostic evaluation of the leaf of *Basilicum polystachyon*

Pharmacognostic studies of the leaf of *B. polystachyon* were carried out and macroscopic, organoleptic, quantitative and qualitative microscopic, chemomicroscopic studies and physicochemical constant were all carried out according to (WHO, 2011; Evans, 2009).

Determination of Macroscopic and Organoleptic Evaluation on the leaf of *Basilicum polystachyon*

The features of the leaf of *B. polystachyon* evaluated for macroscopic and organoleptic studies were; Average length of the leaf margin, Average length of petiole, Average width of the leaf, Leaf apex, Leaf arrangement, Leaf base, Leaf margin, Leaf shape, Leaf surface, Leaf venation, Leaf type, Major veins radiating from the leaf base, colour, taste, texture (WHO, 2011 (Table I and Plate I).

Microscopic evaluation of the leaf of *Basilicum polystachyon*

Quantitative microscopy of the leaf powder was performed to determine the size and dimensions of calcium oxalate and fibre (Evans, 2009). Section of the fresh leaf sample was cleared in chloral hydrate solution in a test tube using water bath. The cleared sample was mounted using glycerol on a slide using cover slip and viewed under microscope (Evans, 2009). The parameter, stomata number, stomata index, vein islet number and veinlet termination number were determined as described by Brain and Turner (1975) and Evans (2009). Micrometric evaluation of the powdered leaf of *B. polystachyon* was performed to determine the size and dimensions of some diagnostic features namely; calcium oxalate crystals, stomata and trichomes (Evans, 2002) and results summarized in table II and III respectively.

Qualitative microscopic evaluation on the leaf of *Basilcum polystachyon*

Anatomical sections were cut using a microt ome, powered sample of the fresh leaf of *B. polystachyon* were examined under a compo und microscope and features were described according to (Evans, 2009; WHO, 2011).

The transverse section of the midrib, powdered sample and surface preparation of the leaf of *B. polystachyon* were all prepared. Sections were cut, cleared using chloral hydrate solution and mounted using dilute glycerol on separate slides. The anatomical features observed were; the nature of epidermal cells, types of stomata, calcium oxalate crystals, trichomes as well as the presenceand arrangement of other anatomical features of the leaf of *B. polystachyon* was observed. (Plate II).

Chemo-microscopic evaluation of the leaf of *Basilcum polystachyon*

The micro-chemical detection of cell walls and cell contents of the plant were carried out following the methods outlined as described in WHO guidelines on quality control methods for medicinal plant materials

(Evans, 2009; WHO, 2011). Small amount of the pulverized powder of the leaf of *B. polystachyon* was cleared in a test-tube containing 70% chloral hydrate solution. It was boiled on a water-bath for about thirty minutes to remove obscuring materials. The cleared sample was mounted on a microscope slide, using dilute glycerol. Using various detecting reagents, the presence of some cell inclusions and cell wall materials. Table IV, Plate VI VII and VIII respectively.

Evaluation of the physicochemical constant of the leaf of *Basilicum Polystachyon*

The physicochemical examination of the powdered leaf of *B. polystachyon* was determined according to the method outlined by (WHO, 2011; Evans, 2009). Moisture content (loss on drying), total ash, water soluble ash, acid insoluble ash values, extractive values (water and alcohol) of the leaf of *B. Polystachyon* were determined (Table V).

Preliminary phytochemical screening of *Basilicum polystachyon* leaf

The 70% methanol extract of *B. Polystachyon* was subjected to preliminary phytochemical screening using standard methods, as outlined for detection of phytochemicals as reported by Brain and Turner, (1975); Sofowora, (1993); Harbourne, (2007); Evans, (2009) and WHO, (2011) and the results summarizes in table VI below.

Thin layer chromatographic profile of the leaf of *Basilicum polystachyon*

Thin Layer Chromatographic (TLC) plates of 20 × 20 cm coated with silica gel 60 F_{254} were used and one-way ascending technique was employed for the analysis. The 70% methanol extract was dissolved in the initial extraction solvent for TLC profiling. The plates were cut into size of 5×10 cm and spots were applied manually on the plates using capillary tube after which plates were dried and developed in chromatographic tank using various solvent system based on preliminary studies. The plates were viewed under UV light or iodine vapour. Developed plates were sprayed using general detecting reagent (*p*-Anisaldehyde) and specific detecting reagents (Ferric chloride for phenolic compound, Liebermann-Buchard's for steroids/triterpenes, Dragenddorff for Alkaloid and Aluminium chloride for flavonoids) and heated at 105ºC where applicable. Number of spots, colours and retardation factors (R_f values) for each of the spots were determined and recorded (Gennaro, 2000; Stahl, 2005). The best solvent systems were developed after several trials and errors by combination of different solvents with different polarities. The solvent systems that give reasonable components of the extracts were documented (Table VII). The R_f value of each spot were calculated using the formula below;

$$\text{Retardation factor } (R_f) = \frac{\text{Distance moved by the solute}}{\text{Distance moved by the solvent}}$$

RESULTS

Macroscopic features of the leaf of *B. polystachyon*

The average length of the leaf margin was found to be 4.42 ± 0.18 while leaf apex was acuminate in nature (Table 1).

Plate I*:* Upper (A) and Lower (B) Surfaces of the Leaf of *Basilicum polystachyon* and *Basilicum polystachyon* in its natural habitat at Kofan Gayan Zaria Local Government Area, Kaduna State.

Table I: Macroscopic features of the leaf of *B. polystachyon*

S/N	Macroscopic Parameters	Inference (±SEM)
1	Average length of the leaf margin	4.60-4.42±0.18-4.24
2	Average width of the leaf	2.45-2.34±0.11-2.23
3	Average length of petiole	1.78-1.64±0.14-1.50
4	Leaf apex	Acuminate
5	Leaf arrangement	Opposite
6	Leaf base	Acute
7	Leaf margin	Crenate
8	Leaf shape	Ovate
9	Leaf surface	Glabrous
10	Leaf venation	Reticulate
11	Leaf type	Simple
12	Colour	Green
13	Taste	Tasteless
14	Texture	Papery
15	Odour	Pleasant aroma

KEY: SEM = Standard Error of Mean

Table II: Quantitative Microscopic Features of the Leaf of *B. polystachyon*

S/N	Parameter	Value (Mean ± SEM)
1	Stomatal number	12.60-11.75±0.85-10.90
2	Stomatal index	22.68-18.89±3.79-15.10
3	Palisade ratio	6.56-6.25±0.31-5.94
4	Vein-let termination number	23.94-20.75±3.19-17.56
5	Vein islet number	47.31-44.75±2.56-42.19

KEY: Mean Value of 5 counts; SEM = Standard Error of Mean

Table III: Micrometric Evaluation of some Diagnostic Features of *B. polystachyon* leaf

Character	Dimension	
	(µm)Length	(µm)Width
	Mean ±SEM	Mean ±SEM
Upper epidermis	234.21-227.87± 6.34-221.53	149.02-143.49± 5.53-137.96
Lower epidermis	223.93-215.92± 8.01-207.91	149.09-142.43± 6.66-135.77
Trichomes	145.86-138.59± 7.27-131.32	50.29-47.32± 2.97-44.35
Calcium oxalate crystals	28.06-26.25± 1.81-24.44	19.36-19.11 ± 0.25-18.86
Fibre	1084.63-993.68±90.95-902.73	112.45-112.13± 0.32-111.81

Average value of five determinations; n = 5, SEM=Standard Error of Mean, µm = Micrometer

Qualitative microscopic features of the leaf of *Basilicum polystachyon*

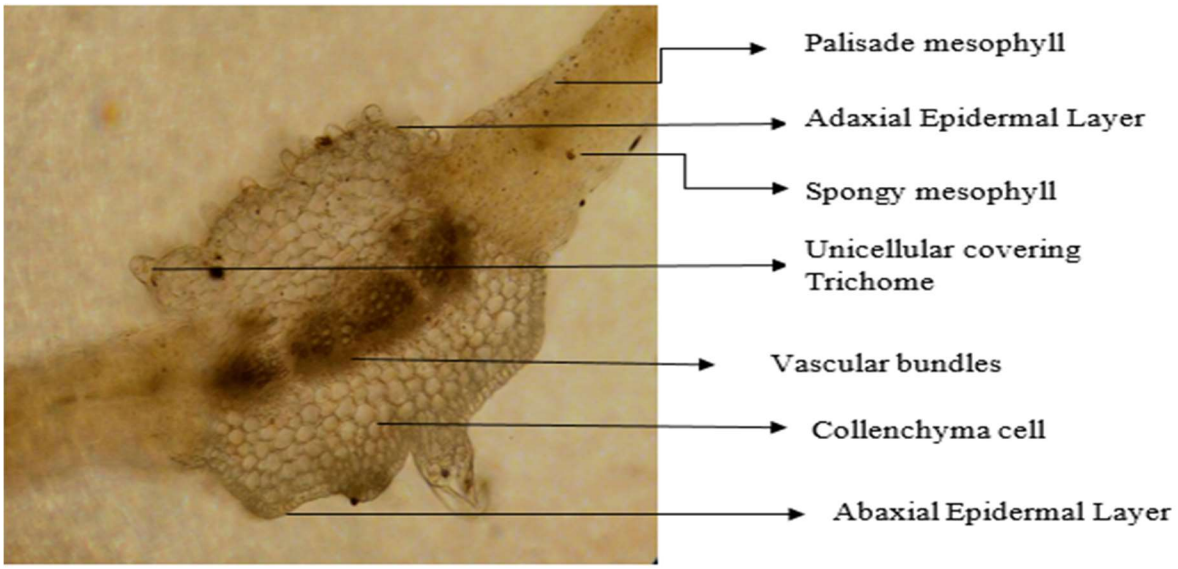

Plate II: Photomicrograph of the Transverse Section of the Midrib of the Leaf of *Basilicum polystachyon* (Magnification X 400)

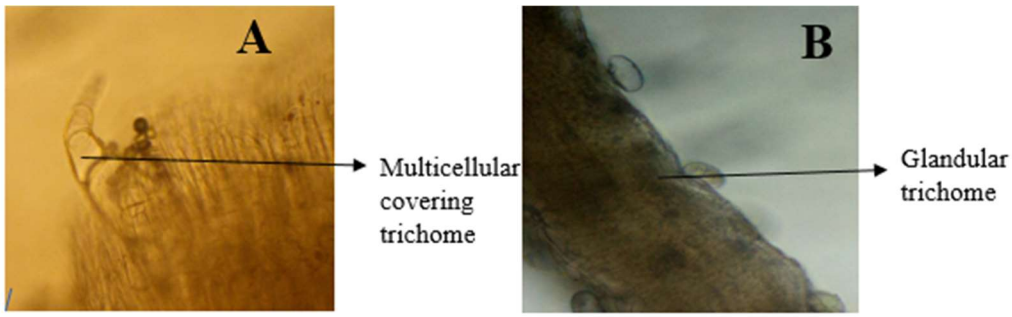

Plate III: Photomicrograph of (A) Multicellular covering trichome and (B) Glandular trichomes on the Leaf of *Basilicum polystachyon* (Magnification X400).

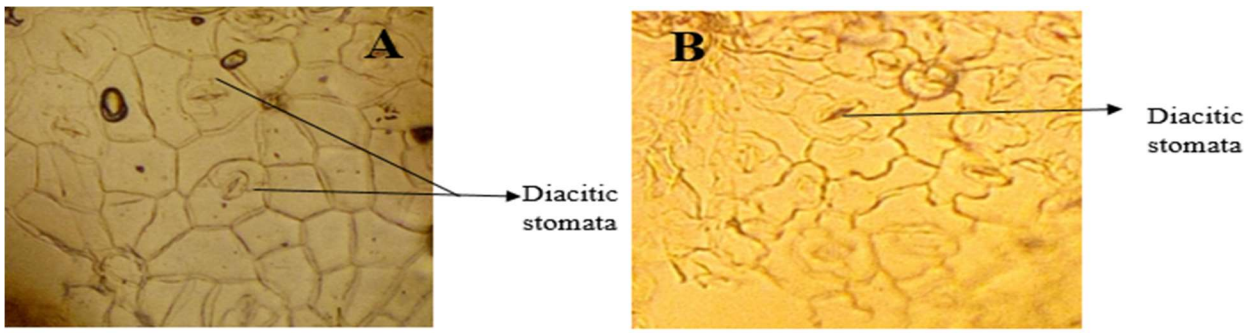

Plate IV: Photomicrograph showing Diacytic Stomata on (A) Upper Epidermal Layer;(B) Lower Epidermal Layer on the Leaf of *Basilicum polystachyon* (Magnification X 400).

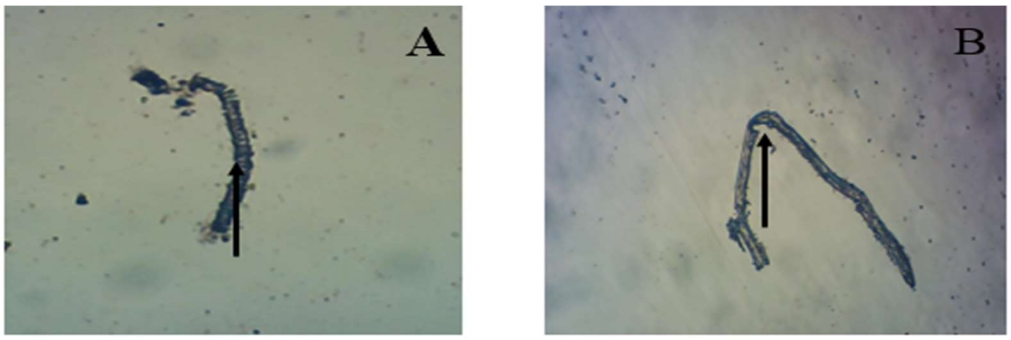

Plate V: Photomicrograph of (A) Vessel and (B) Fibre in the Leaf of *Basilicum polystachyon* (Magnification X 400).

Table IV: Chemo-microscopic features of the leaf of *B. polystachyon*

Constituent Tested	Reagent	Inference
Cell Wall Material		
Cellulose	Iodinated Zinc Chloride	Cellulose Present
Lignin	Phloroglucinol and Concentrated HCl	Lignin Present
Suberin- / Cutin	Sudan red	Cutin Present
Gum and mucilage	Ruthenium red	Gums and Mucilage Present
Cell Inclusion		
Calcium carbonate	Concentrated Hydrochloric acid	Calcium carbonate Present
Calcium oxalate Crystals	Concentrated Hydrochloric acid	Calcium oxalate crystals Present
Starch grains	N/50 Iodine	Starch grains Present
Tannins	Conc. Ferric Chloride	Tannins Present
Aleurone grains	Iodine in Ethanol	Aleurone grains Present
Inulin	Spherical Aggregation of Crystals	Inulin Present

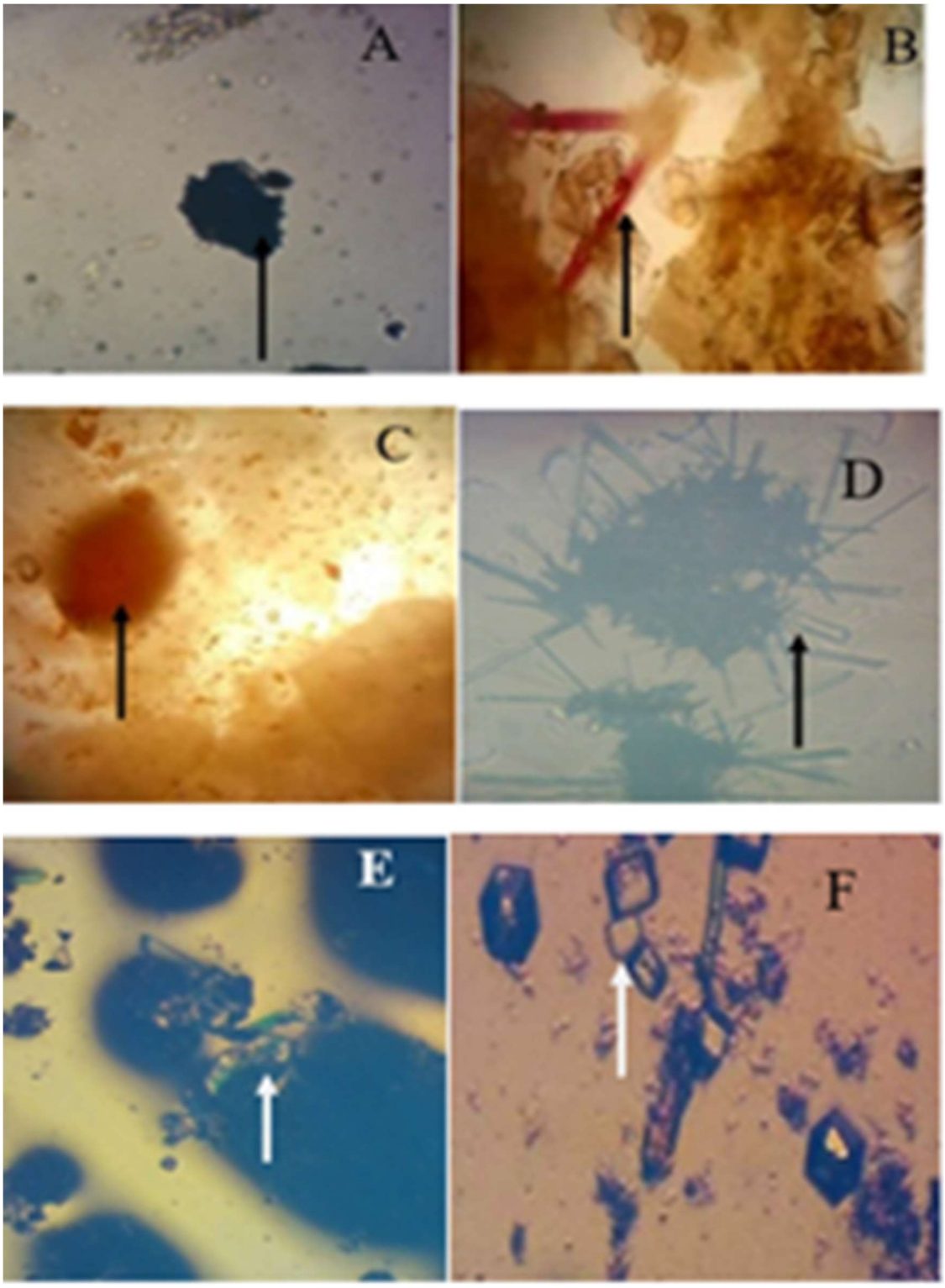

Plate VI, VII and VIII: Photomicrograph of (A) Cellulose Cell Wall (B)Lignin(C)Cutin, (D)Inulin(E) Tannin and (F) prismatic Calcium Oxalate Crystals in powdered leaf of *Basilicum polystachyon.*

Table V: Physicochemical constant of the leaf of *B. polystachyon*

S/N	Parameter	Value (%w/w) Mean ±SEM
1	Moisture content value	12.85-11.58±1.27-10.31
2	Total ash value	14.06-11.75±2.31-9.44
3	Water Soluble ash value	6.31-5.88±0.43-5.45
4	Acid Insoluble ash value	5.22-4.13±1.09-3.04
5	Water Extractive value	3.14-3.07±0.07-3.00
6	Alcohol Extractive value	3.80-3.73±0.07-3.66

KEY: Average values of five determinations; **SEM** = Standard Error of Mean.

Table VI: Preliminary phytochemical profiles of the methanol leaf extract of *B.polystachyon*

Constituent	Test	Observation	Inference
Carbohydrates	Molisch's test	Reddish coloured ring at the interface	Present
	Fehling test	Brick red precipitate	Present
Saponins	Frothing test	Honey comb froth that persisted after 15mins	Present
	Haemolysis test	Heamolysis in test tube B containing extract	Present
Cardiac Glycosides	Keller–Kiliani's test For deoxy sugar	Reddish brown colour at the interface	Present
	Kedde's test for cardenolides	Purple blue colour	Present
Flavonoids	Shinoda's test	Pink or red colour	Present
	Sodium Hydroxide test	Yellow colour	Present
Anthraquinones	Borntrager's test	Pink or cherry red colour	Present
	Modified Borntrager's Test	Pink or red colour in the ammonia layer	Present
Steroids and Triterpenes	Liebermann–Burchard's test	Brown colour	Present
	Salkwoski's test	Red colour	Present
Tannins	Ferric Chloride test	Greenish colour	Present
	Lead Sub-acetate test	Whitish yellow precipitate	Present
	Bromine water test	Buff coloured precipitate	Present
Alkaloids	Dragendorff's test	Reddish brown colour	Present
	Mayer's test		Present
	Wagner's test		Present

Table VII: Thin Layer Chromatographic Profile of Methanol Leaf Extract of *Basilicum polystachyon* Sprayed with General and Specific Detecting Reagents

Detecting reagents	Number of spots	Colour of spots	R_f values
***P*-anisaldehyde**	7	Green	0.21
			0.39
		Green	0.59
		Blue	0.67
		Yellow	0.73
		Blue	0.83
		Purple	0.89
		Black	
Ultraviolet (UV) light	2		0.92
		Pink	0.97
		Pink	
Ferric chloride ($FeCl_3$)	3	Black	0.48
		Green	0.88
		Green	0.97
Aluminium Chloride ($AlCl_3$)	3	Blue	0.48
		Pink	0.87
		Pink	0.97
Liebermann-Buchard's	1	Brown	0.95
Dragendorff's	1	Orange	0.65
Bontrager's	1	Yellow	0.62

DISCUSSION

Basilicum polystachyon is traditionally used for the management of various ailments including insomnia. Thus, there is no report on the standardization of its crude drug (Madhavan, *et al.,* 2013). This research work is aimed at providing standards in terms of identity, purity and quality of the crude drug and also to provide a scientific basis for it use traditionally as a sedative.

Macroscopically, the leaf of *B. polystachyon* has 4.42±0.18, 2.34±0.11 and 1.64±0.14 as it averages length of leaf, average width of leaf and average length of petiole of the leaf respectively. The leaf has a crenate margin, glabrous surfaces, acuminate apex, base acute, opposite leaf arrangement, ovate shape, reticulate venation, simple type of leaf, this description is in conformity with previous findings made by (Madhavan, *et al.,* 2013) on *B.polystachyon* as seen on Table I and plate I. *B. polystachyon* leaf was found to be tasteless, green in colour and has a pleasant aroma. Both macroscopic and organoleptic features are in agreement with the findings of (Madhavan, *et al.,* 2013) in *B. polystachyon* which also revealed the same macroscopic and organoleptic features. The sensory perception (odour, colour, texture and taste) often provides the simplest and quickest means of identification of crude drug (African Pharmacopoeia, 1985).

Morphological and organoleptic evaluation showed some important diagnostic features which were peculiar to *B. polystachyon* leaves when compared with other members of the family Lamiaceae. These morphological features could be of immense significance in its proper identification and differentiation from similar plant species (Ghani, 1990). The transverse section of the leaf showed scattered vascular bundles which is characteristic of monocots. Anatomical features of the internal structures of plant drugs provides important diagnostic features for the identification of both entire and powdered crude drugs and detection of adulterants in plant materials as seen in plate II (Ghani, 1990). Microscopic examination of *B. polystachyon* leaf showed numerous diacytic stomata on both abaxial and adaxial surfaces and vessel. These concurred with the findings of Madhavan *et al*. (2013) on the leaf *B. polystachyon*. Furthermore, previous work carried out by Venkateshapa and co-workers showed that *Orthosiphon rubicundus, Ocimum basilicum* and *Coleus forskohlii* all in the Lamiaceae family reported diacytic type of stomata (Venkateshapa *et al.,* 2013). Although Venkateshapa and co-workers reported anomocytic stomata type in *Leucas cephalotes* of Lamiaceae family, this suggests the possibility of different species within the family possessing different stomata types peculiar to the family (Venkateshapa *et al*., 2013). The differences may also be attributed to different geographical distributions and wide evolutionary taxonomical relationship in the family between the species. *B. polystachyon* also showed multicellular covering trichomes and glandular trichomes which are in agreement with the findings of Mohammad Abd El-Aziz and co-workers. *Ocimum basilicum* of the Lamiaceae family which also showed multicellular and glandular trichomes are of diagnostic features of basil leaf epidermis (Mohammad Abd El-Aziz *et al.,* 2014).

Quantitative microscopy was used to study microscopic characters not easily characterized by general microscopy; stomata number, stomata index, palisade ratio, vein islet number and veinlet termination number were all investigated and reported from the leaf. Stoma (plural-stomata) is a minute epidermal opening covered by two kidney-shaped guard cells in dicotyledonous leaves (Kokate *et al.,* 2009). The guard cells, in turn, are surrounded by epidermal (subsidiary) cells. Stomatal number is the average number of stomata per square millimeter of the epidermis of the leaf (kokate *et al.,* 2009). The stomata number may vary from the leaves of the same plant grown in different environment or under different climatic conditions (kokate *et al.,* 2009).

Stomatal index is said to be the percentage which the number of stomata form to the total number of epidermal cells, each stoma being counted as one cell (kokate *et al.,* 2009). The stomata index of *B. polystachyon* and stomata number varies considerably with the age of the leaf and due to changes in environmental conditions. Stomata index is relatively constant and is of diagnostic significance for a given species (kokate *et al.,* 2009). It is therefore, employed for differentiation of allied or closely related species of the same genus either in air-dried or in fresh conditions (kokate *et al.,* 2009). Vein-islet number is the number of vein-islet per square millimeter of the leaf surface midway between the midrib and the margin (kokate *et al.,* 2009). Vein-islet number is the number of vein-islet and is therefore, constant for a given species of the plant and is used as a characteristic for the identification of allied species. The number of vein-let termination per square millimeter of the leaf surface midway between the midrib and margin is said to be vein-let

termination number. The palisade ratio is the average number of palisade cells beneath the epidermal cell (kokate *et al.,* 2009). The transverse section of the leaf of *B. polystachyon* revealed some diagnostic features like the palisade mesophyll, adaxial and abaxial epidermal layers, spongy mesophyll, vascular bundles, and collenchyma cells. Furthermore, the microscopy of the powdered leaf revealed some prominent features like prism-type calcium oxalate crystals. The micrometric measurement of the leaf of *B. polystachyon* of some of the features were found to be upper epidermis, 227.87 ± 6.34 x 143.49 ± 5.53, lower epider mis, 215.92 ± 8.01 x 142.43 ± 6.66, trichomes, 138.59 ± 7.26 x 47.32 ± 2.97, cal cium oxalate crystals, 26.25 ± 1.81X 19.11± 0.25 and Fibre 993.68±90.95 x 112.13±.32respectively. (Tables II and III). The occurrence of the above-mentioned diagnostic features was observed among some members of Lamiaceae.

Chemo-microscopic features of pulverized leaves of *B. polystachyon* revealed the presence of cell wall materials such as cellulose, lignin, cutin, gums and mucilages, and cell inclusions such as calcium carbonate calcium oxalate crystals, starch, tannin, aleurone grains and inulin (Table IV plates VI, VII and VIII). All these microscopic structures are most valuable in the identification of powdered drugs as their identification is largely based on the form, presence or absence of certain cell types and cell inclusions (Eggeling *et al*., 2000).
The physicochemical parameters determined for the leaf of *B. polystachyon* which were to serve as reference standard for assessing its quality and purity include moisture content, total ash, water soluble ash acid insoluble ash, and water extractive and alcohol extractive values. The moisture content of the leaf of *B. polystachyon* was found to be 11.58±1.27 which is below the standard 14 that must not be exceeded as stated in British Herbal Pharmacopoeia (1990). The value was therefore not high to promote microbial growth that may subsequently degrade the phytoconstituents present in the leaf. The ash content indicates the amount of inorganic matter present in a sample (leaf of *B. polystachyon*). Total ash value was found to be 11.75±2.31, water soluble ash value was found to be5.88±0.43, acid insoluble ash value was4.13±1.09, water soluble extractive value was found to be 3.07±0.07while alcohol extractive value was found to be 3.73±0.07indicating that water has high extracting ability (Table V). Both extractive values were meant to determine exhausted and already used crude drug that may be fraudulently substituted as adulterants (Elujoba, 1999).

Preliminary phytochemical screening of the methanol leaf extract of *B. polystachyon* revealed the presence of carbohydrates, saponins, cardiac glycosides, flavonoids, anthraquinones, steroids and triterpenes, tannins and alkaloids (Table VI). The presence of similar phytochemicals in *B. polystachyon* has been reported in earlier studies (Madhavan*et al*., 2013). Result from the present investigation shows that *B. polystachyon* leaf is rich in phytochemicals which have several therapeutic applications (Madhavan *et al*., 2013). Phytochemical screening results could be useful as a guide for chemotaxonomic markers and could be used in the chemotaxonomic classification system of Lamiaceae family. Thus, these results may also serve as a guide for further phylogenetic studies in Lamiaceae family.

Similarly, the thin layer chromatographic profile of the methanol leaf extract of *B.polystachyon* confirmed the presence of alkaloids, flavonoids, cardiac glycosides, tannins, saponins, steroids and triterpenes and anthraquinones as observed from the preliminary phytochemical screening (Table

VII). This finding is in line with the work of Khair-ul-Bariya and colleagues who reported the presence of some of these phytochemicals in *Ocimum basilicum* (Khair-ul-Bariya *et al.,* 2012).

CONCLUSION

Identification and standardization of *B. polystachyon* was achieved using pharmacognostic evaluation.

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