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PHARMACOGNOSTIC PROFILE OF LEAVES OF *SESBANIA BISPINOSA* (JACQ.) W. F. WIGHT FOR THE ESTABLISHMENT OF QUALITY PARAMETERS

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ABSTRACT

The present study was aimed at pharmacognostic evaluation of leaves of *Sesbania bispinosa* (Jacq.) W. F. Wight. This study is important and lays down parameters for standardization and authentication of medicinal plants with the help of which adulteration and substitution can be prevented. The pharmacognostic investigations were carried out in terms of morphological, organoleptic, microscopic, physico-chemical studies and behaviour of leaf powder with different chemical reagents to identify the diagnostic features of the leaves. The detailed pharmacognostic studies have given a clear idea regarding the different cell characters and various constants. Determination of physical constant values involving moisture content, ash values and extractive values, qualitative chemical and quantitative leaf constants have given standard numerical values for comparison and detection of adulterants. The important diagnostic features of the leaf include anomocytic stomata, cystoliths in palisade layer, annular and spiral vessels, prismatic calcium oxalate crystals, starch grains and pericyclic fibres. These results would also be useful for compilation of a suitable monograph for a plant under study. As no reports are available on pharmacognostic studies of leaves of *Sesbania bispinosa* (Jacq.) and in order to ensure the use of only genuine and uniform material in preparation of herbal formulation, present work on standardization paramaters was carried out. Our findings will serve towards establishing pharmacognostic standards on identification, purity, quality and classification of the plant, which is gaining significance in plant drug research.

KEYWORDS: Leaf constants, Microscopic, Organoleptic, Pharmacognostic, Physico-chemical, *Sesbania bispinosa*.

INTRODUCTION

In recent times, there is a renewed interest in drugs of natural origin simply because they are considered as green medicine and green medicine is always supposed to be the safe. The advantage of natural drugs is their easy availability, economic and less or no side effects but the disadvantage is that they are the victims of adulteration. The more effective the natural drug, more is its demand and the chances of non-availability increases. To meet the growing demand, the natural drug is easily adulterated with low grade material.[1] Pharmacognosy is the study of medicines derived from natural sources, mainly from plants. It basically deals with standardization, authentication and study of natural drugs. Pharmacognostic study includes parameters which help in identifying adulteration in dry powder form also.[2] This is again necessary because once the plant is dried and made into powder form, it loses its morphological identity and becomes easily prone to adulteration. Such studies will help in authentication of the plants and ensure reproducible quality of herbal products which will lead to safety and efficacy of natural products.[3]

Quality and quantity of chemical constituents play very significant role in therapeutic efficacy of medicinal plants.[4] The misuse of herbal medicine or natural products starts with wrong identification. The most common error is one common vernacular name given to two or more entirely different species.[5] All these problems can be solved by pharmacognostic studies of medicinal plants.

Sesbania bispinosa (Jacq.) W. F. Wight (Dhaincha) is a crop generally cultivated for its nutritive value to soil.[6] It is cultivated in monsoon season almost throughout India. It is an ideal green manure crop as it is quick-growing, easily decomposable with low moisture requirements and produces maximum amount of organic matter and nitrogen in the soil.[7] The plant is used in Ayurveda and Sidha and its decoction as antacid and febrifuge. Bark and seeds are astringent, for diarrhea. It was reported that around Las Bela, it is used for wounds and powdered roots are administered to snakebite victims, inducing emesis and perhaps a cure.[8] The fiber and seeds of *Sesbania bispinosa* yield gums such as galactomannans, lignins and cellulose.[9] Leaf paste applied to small babies all over the body and also given bath against whooping cough. Ayurvedics regard the root as alexiteric, anthelmintic, diuretic, and galactagogue and used to treat eye diseases. Seeds are stimulant, emmenagogue and

astringent.[10] It possesses several value added medicinal properties.[11] Medicinally, seeds are mixed with flour and applied to ringworm, other skin diseases, and wounds.[12]

Systematic studies have not been reported for Pharmacognosy of leaf of *Sesbania bispinosa* (Jacq.) W. F. Wight. Hence, this work is an effort to establish the Pharmacognostic and chemical standardization of leaves of *Sesbania bispinosa* (Jacq.). However, no scientific standard parameters are available to determine the quality and genuineness of the drug. In the present study an attempt was made to standardize the drug using pharmacognostic studies and behaviour of leaf powder with various chemical reagents, which can further lead to provide an authentic information towards the quality and also standardization of the drug.

MATERIALS AND METHODS

Sample collection

The leaves of *Sesbania bispinosa* (Jacq.) W. F. Wight belonging to family Fabaceae were collected in the month of August - September from Smt. C. H. M College campus in Ulhasnagar of Thane district. The plant was identified at Blatter herbarium, St. Xavier's College, Mumbai. The voucher specimens were deposited in St. Xavier's College Herbarium and Botany department of Smt. C. H. M College for further reference. The accession number for *Sesbania bispinosa* (Jacq.) W. F. Wight is SYJ1.

Sample preparation

The leaflets were separated and surface contaminants were removed by washing with de-ionized water. They were shade dried for ten days and then subjected to grinding in a mixer for powder formation to 60 # mesh size. The powder was stored in air tight glass containers with silica gel and used for further analysis.

Pharmacognostic studies of plant material

After confirmation of its botanical identity the leaves were subjected for morphological, organoleptic, microscopical, physicochemical and chemical reagent studies.

Morphological evaluation

Morphological characters of the leaves like leaf shape, size, colour, texture, margin type, apex, base, stipules and petiole etc were observed. Measurements were carried out using line ruler.[13]

Macroscopic or organoleptic evaluation

Evaluation of powdered leaves of *Sesbania bispinosa* (Jacq.) by color, taste, odour, texture etc. was carried out. The conclusions are drawn from studies resulted due to impression on organ of senses.

Microscopic evaluation

Microscopic study of sections of fresh leaflet and dried leaf powder was carried out to establish the quality parameters of *Sesbania bispinosa* (Jacq.) under study. Microscopic analysis was carried out on epidermal layers and transverse sections of leaflet. This method is used to identify the organized drugs by their known histological characters. [14-15] Microscope by virtue of its property to magnify permits the minute structure under study to be enlarged and can be seen to confirm the structural details of the drugs from plant origin.

A. Epidermal layers of leaflet: Leaflets of the specimens were cut at the median portions. These were soaked in concentrated nitric acid for about 24 hours. The appearance of air bubbles indicated the readiness of the epidermii to be separated. The samples were then transferred to petri dishes containing water and with the use of fine forceps and dissecting needle; the upper and lower epidermii were separated and mounted on a slide with glycerol.[16-17]

B. Transverse section of leaflet: For the anatomical analysis, thin transverse hand sections of the fresh leaflets were manually obtained by sectioning with razor blade and prepared by conventional micro techniques.[18] The sections were cleared for few minutes in chloral hydrate solution. They were washed in water and then stained with Saffranin. These were mounted on a slide with glycerol. Glycerin and iodine solution were used to determine calcium oxalate crystal and starch grains respectively. All slides were observed under a light microscope and photographs were taken using Sony digital camera (10x optical zoom; optical steady shot; 16.1 mega pixels) through eyepiece.

C. Quantitative microscopical parameters of leaflet : As a part of quantitative microscopy, stomatal number, stomatal index, palisade ratio and vein islet number were determined by using fresh leaves, camera lucida and stage micrometer.[19-20]

D. Powder Microscopy: Leaf powder was cleared, stained and mounted as per the standard method and studied.[21]

Physicochemical parameters

Physicochemical analysis such as loss on drying, ash value, extractive values, etc. were carried out on the powdered leaf sample following standard methods.[22-23] The aim of ashing was to remove all traces of organic matter.[24] These parameters are used as quality control parameters.

Behaviour with chemical reagents

The behaviour of the leaf powder of *Sesbania bispinosa* (Jacq.) with different chemical reagents was studied. Color change was observed under day light using reported method.[25-26]

RESULTS

Morphological studies of leaf and leaflet of *Sesbania bispinosa* (Jacq.) W. F. Wight

Plate 1: Portion of a Plant

Plate 2: Entire Leaves

Plate 3: Dried Leaflets

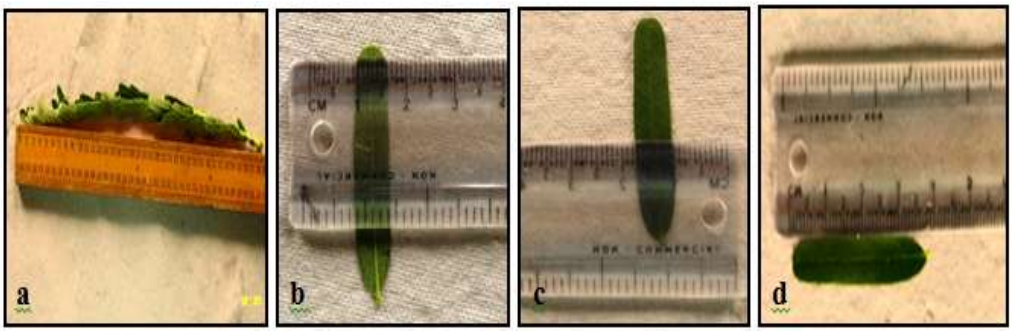

Plate 4 (a–d): Dimensions of entire leaf and leaflets

Morphology

Leaves of *Sesbania bispinosa* (Jacq.) are long, bipinnately compound with axis usually aculeate, to about (min. 5.5) 9.5-29.5 (max. 35) cm long, 20-80 foliate. Texture smooth, alternate arrangement. Stipules linear lanceolate, 5-10 mm long, adaxially pubescent, late caducous; petiole 2-20 mm long. Leaflets are opposite, oblong to linear, 0.75-2 (max. 2.6) cm

in length x 1.5-3 (max.5) mm in width, mucronate, dark green on upper surface and pale green on lower surface, 20-50 (55 max) pairs; base and apex obtuse. The midrib is prominent on lower surface.

Table 1: Organoleptic Characters of leaf powder of *Sesbania bispinosa* (Jacq.) W. F. Wight.

Sr. No.	Parameter	Observation
1.	Colour	Green
2.	Odour	Bitter
3.	Taste	Characteristic
4.	Texture	Fine
5.	Colour of Ash	White

Microscopical studies of fresh leaflet of *Sesbania bispinosa* (Jacq.) W. F. Wight

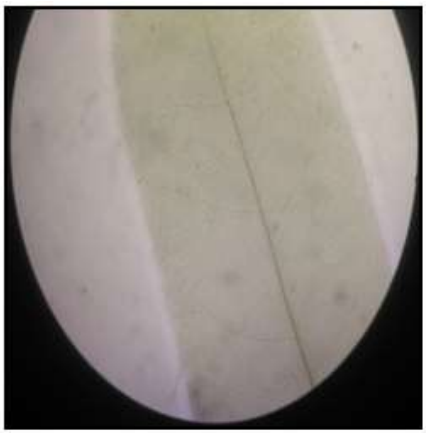

Plate 5: Adaxial surface with veinislet

Plate 6:Abaxial surface with veinislet

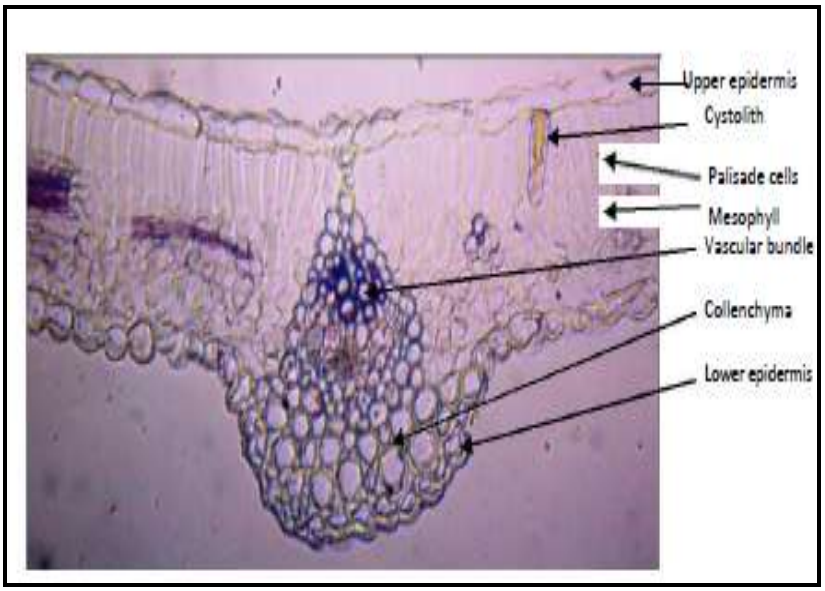

Plate 7: T. S. of leaflet passing through midrib

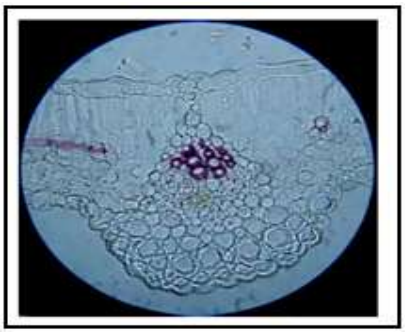

Plate 8: Enlarged portion of midrib

Plate 9: Part of lamina with vascular bundle

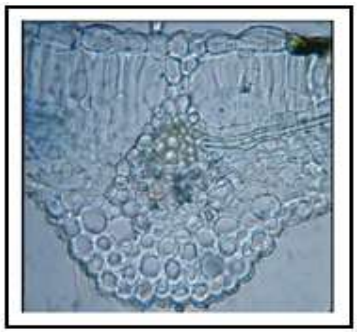

Plate 10: Prisms of Ca-oxalate crystals in VB

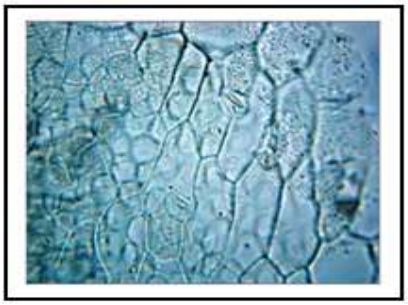

Plate 11: Upper epidermis with stomata &underlying palisade cells

Plate 12: Lower epidermis with stomata

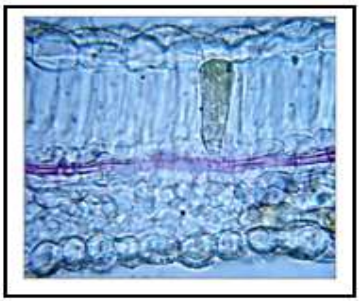

Plate 13: Lamina showing vessels, & palisade layer with cystolith and epidermis

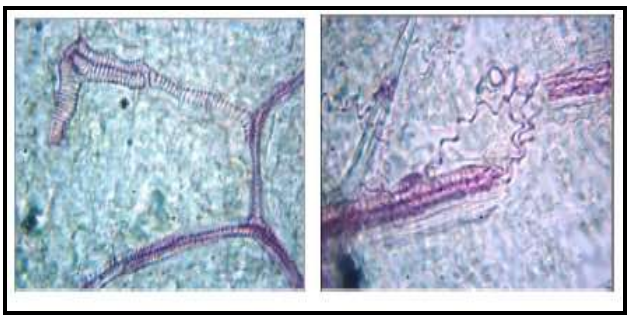

Plate 14: Annular and Spiral vessels

Microscopy

T.S. of Leaflet

It shows a strip of upper and lower epidermis with a wavy layer of cutin deposition. Below the upper epidermis of lamina lie a row of the distinct palisade layer showing cystoliths at intervals and a wide zone of spongy parenchyma. In surface view, it contains many anomocytic stomata, more abundant on lower surface. Thick Midrib protrudes out abaxially (lower surface). 2-3 rows of collenchymatous tissue lies beneath the epidermis of the midrib. Midrib shows distinct vascular bundle made up of stele with proto and metaxylem showing exarch type and prismatic crystals of calcium oxalate surrounding the vascular bundle in

collenchymatous tissue. The mesophyll has two rows of palisade tissue under the upper epidermis with the remaining spongy tissue made up of loosely arranged spherical cells with intercellular spaces. Xylem vessels with annular and spiral thickenings are seen from midrib throughout the lamina.

Table 2: Dimensions of various structures as observed in Leaf Microscopy

Parameters	Mean Values
Calcium oxalate prisms surrounding vascular bundle in midrib	5-15µ long
Xylem vessels	15µ in diameter
Stomata (guard cells)	30µ long
Lamina	180µ broad
Midrib	315µ broad

Table 3: Quantitative Microscopy of fresh leaflet of *Sesbania bispinosa* (Jacq.) W. F. Wight

Leaf Constants	Mean Values
Stomatal number (upper surface)	250
Stomatal number (lower surface)	276
Stomatal index(upper surface)	11.5-13.0 per sq. mm.
Stomatal index(lower surface)	13.0 -14.5 per sq. mm.
Palisade ratio	7-11 per sq. mm.
Vein islet number	7-13 per sq. mm.

Microscopic characters of leaf powder of *Sesbania bispinosa* (Jacq.) W. F. Wight

Plate15: Stomata

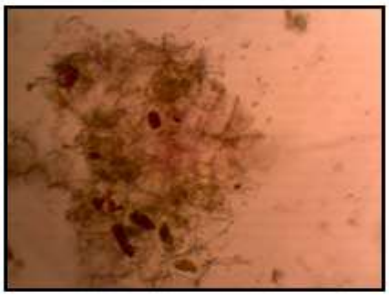

Plate16: Epidermal Cells

Plate 17: Starch Grain

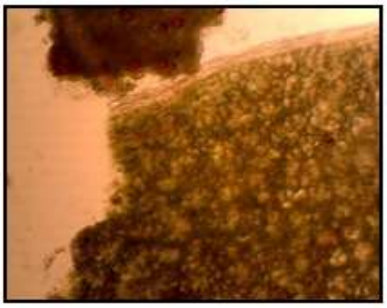

Plate18: Fragment of Lamina

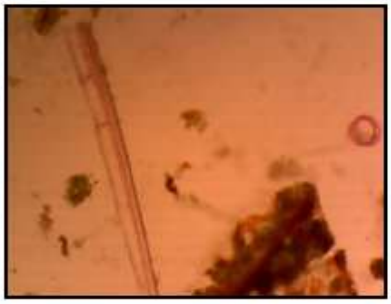

Plate19: Fibres

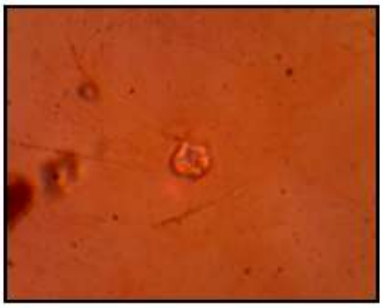

Plate20: Calcium Oxalate Crystal

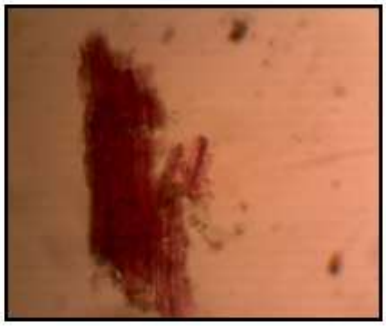

Plate21: Xylem Tracheids

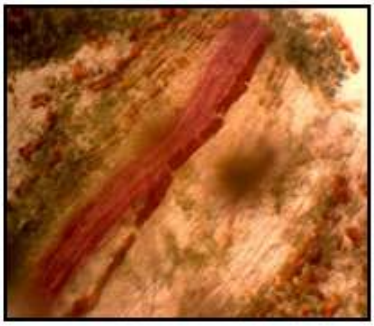

Plate22: Xylem Vessels

Plate23: Xylem with starch grains

Powder Microscopy

Microscopy of leaf powder of *Sesbania bispinosa* (Jacq.) shows fragments of lamina with parenchyma containing prismatic crystals of calcium oxalate; Vessels with adjacent tracheids show annular and spiral thickenings and fibres; fragments of upper epidermis and lower epidermis contains anomocytic stomata. Starch grains are seen scattered.

Table 4: Physico-chemical parameters of leaf of *Sesbania bispinosa* (Jacq.) W. F. Wight

Sr.No.	Parameter	Content (% w/w) Mean Values
1.	Foreign organic matter	0.31
2.	Moisture content (LOD)	3.46
Ash values		
3.	Total Ash	10.38
4.	Acid Insoluble Ash	3.2
5.	Water Soluble Ash	2.65
6.	Sulphated Ash	2.78
Extractive Values		
7.	Petroleum Ether	6.5
8.	Benzene	7.4
9.	Methylene Chloride	10.4
10.	Chloroform	6.4
11.	Diethyl ether	4.2
12.	Ethyl Acetate	9.6
13.	Acetone	3.1
14.	Ethanol	8.8
15.	Methanol	11.8
16.	Water	13.5

Table 5: Behaviour of leaf powder of *Sesbania bispinosa* (Jacq.) W. F. Wight with chemical reagents in Visible Day Light.

Sr. No.	Powder + Reagents	Colour / Precipitate
1.	Conc. Hydrochloric Acid	Fluorescent Green
2.	Conc. Nitric Acid	Dark Yellow
3.	Conc. Sulphuric Acid	Greenish Yellow
4.	Glacial Acetic Acid	Olive Green
5.	5% KOH	Greenish Yellow
6.	5% NaOH	Fluorescent Green
7.	5% FeCl3	Dark Green
8.	Copper Sulphate	Greenish Blue
9.	Iodine Solution	Green
10.	Picric Acid	Fluorescent Green
11.	Ammonia	Yellowish Green
12.	Water	Yellow
13.	Ethanol	Dark Green
14.	Methanol	Fluorescent Green
15.	Chloroform	Dark green
16.	Acetone	Fluorescent Dark green
17.	Ethyl Acetate	Dark Green
18.	Petroleum Ether(40^0-60^0)	Fluorescent Green
19.	Diethyl Ether	Dark Green
20.	Benzene	Fluorescent Green
21.	Toluene	Yellowish Green
22.	Pyridine	Olive Green
23.	n-Hexane	Pale Green
24.	n-Amyl Alcohol	Green
25.	n-Butyl Alcohol	Green
26.	n-Propyl Alcohol	Green

Note: Colour reactions are viewed under natural light by naked eye

DISCUSSION

The major problem faced in herbal formulation industry is the identification of an authenticated raw material and in absence of data, one can use any adulterant in the process of formulating the drug.[27] From the present study, important diagnostic characters that might be useful in determining authenticity and identifying adulteration of the crude drug are observed. Organoleptic and microscopic analysis along with physicochemical parameters are determined on plant samples in order to establish appropriate data that can be useful in identifying crude drugs, particularly those supplied in the powder form.[28] The results obtained for morphological studies reported in the present work establishes the macroscopic parameters (Plate 1-4) and organoleptic parameters (Table 1), that characterize the genuine plant drug *Sesbania bispinosa* (Jacq.) W. F. Wight., belonging to family Fabaceae.

In traditional medicine the healers use this plant against snake bite, as a poultice for wound healing activity. Scientific parameters are available for seed in ayurvedic pharmacopoeia but not for leaf, to identify the true plant material and to ensure its quality. The evaluation of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in herbal pharmacopoeia, pharmacognostical parameters and standards must be established. Therefore some diagnostic features have been evolved to identify and to differentiate the *Sesbania bispinosa* (Jacq.) leaf from other crude drugs and adulterants.

In this regard the important pharmacognostic features of the leaves of *Sesbania bispinosa* (Jacq.) have been documented here in our present study. The leaf sample examined was found to have alternate arrangement of bipinnately compound leaves with relatively long stalk. Leaflets are oblong to linear in pairs, mucronate, dark green upper surface and pale green lower surface; base and apex obtuse. The transverse section of leaf (Plate 5-14) showed epidermal strips of both upper and lower surfaces with wavy layer of cutin, interspaced with anomocytic stomata (more abundant on the lower surface). Palisade layer showed cystoliths at intervals. Veins and veinlets are made up of lignified annular and spiral vessels.

The microscopy of leaf powder (Plate 15-23) revealed the characteristic anomocytic stomata, fibres, epidermal cells, fragments of lamina, xylem tracheids and vessels. The presence of abundant prisms of calcium oxalate crystal which indicate the presence of the calcium salt of oxalic acid that is present usually about 1.0% in plants[29] and singly occurring starch grains can be used as identifying character of the plant. The dimensions of various structures as observed in leaf microscopy (Table 2) and leaf constant parameters determined in the quantitative microscopy (Table 3) are relatively constant for plants and can be used to differentiate closely related species.

The moisture content of the crude drug is not too high (falls within the limit of the general requirement of 8-14%), indicating less probability of microbial degradation. Excess moisture in the crude drug may lead to the breakdown of important constituents and growth of microorganisms during storage of drug.[30] The physiological ash value is high while the non-physiological ash values are low. The extractive values are moderate. The evaluation of total ash value can be used to detect the foreign organic matter and adulteration of sand and earth.[31] Studies on physico-chemical constants (Table 4) can serve as a valuable source of information and provide suitable standards to deterrmine the quality of this plant. The

behaviour of the leaf powder upon treatment with different chemical reagents were also observed and reported (Table 5).

CONCLUSION

The present work summarizes some important morphological, organoleptic, microscopic characters of the leaf and leaf powder of *Sesbania bispinosa* (Jacq.) W. F. Wight. Standardization is essential measure for quality, purity and sample identification. Morphology, macroscopy and microscopy along with the quantitative analytical microscopy is one of the simplest and cheapest methods for establishing the correct identity of the crude drug used as phytomedicine. Proximate and Chemical analysis of leaf powder confirm the quality and purity of plant and its identification. The result generated from this study would be useful in identification and standardization of the plant material towards quality assurance and also for preparation of a monograph on the plant.

These quality standards might be incorporated in quality control monographs for establishing the correct identity and quality of the crude drug.

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