Research Article

Pharmacognostic and Physicochemical Evaluation of *Bacolepis nervosa* Stem and Leaf

Jenecius A[1], Mohan V. R.[2*]
[1]Department of Botany, St. Mary's College (Autonomous), Thoothukudi, Tamil Nadu.
[2]Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Thoothukudi, Tamil Nadu, India.
***Corresponding author's E-mail:** vrmohanvoc@gmail.com

Received: 09-11-2016; **Revised:** 02-01-2017; **Accepted:** 10-01-2017.

ABSTRACT

In the present study, an attempt is made to study the pharmacognostic features of stem and leaf of *Bacolepis nervosa*. Macroscopic and microscopic evaluations were performed to establish the qualitative diagnostic features. Various physicochemical parameters (loss on drying, ash values, extractive values) were also determined for the effective standardization of the medicinal plant material. Besides all the above mentioned conventional methods of standardization, the drug powder was also subjected to powder analysis with different chemical reagents and for fluorescent drug analysis on exposure to different wavelength of ultraviolet light. The result of these studies could be useful for current identification and detection of adulterants of the plant material.

Keywords: *Bacolepis nervosa*, physicochemical, pharmacognostic**,** Nilgiri Biosphere Reserve.

INTRODUCTION

Human existence on the Earth is made possible by the vital role played by plant kingdom. Nature has provided many things for humankind over the years, including tools for the first attempt at therapeutic intervention.[1] Traditional system of medicines are prepared from a single plant or combinations of more than one plant. Medicinal plants have a long standing history in many indigenous communities and continue to provide useful tools for treating various diseases. The practice of traditional medicine is based on hundreds of years of belief and observations, which predate the development and spread of modern medicine.[2] Today, there is a widespread interest in the usage of herbal drugs. This interest is mainly from the belief that herbal medicines are safe, inexpensive and have no adverse effects.[3]

Medicinal plants are moving from the fringes to the main stream with a greater number of people seeking remedies and health care from these practices.[4] It is no wonder that the world's one-fourth population i.e. 1.42 billion people are dependent on traditional medicines for the treatment of various ailments.[5]However, a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is due to the lack of documentation and stringent quality control. There is a need for documentation of research work carried out on traditional medicines.

With this backdrop, it becomes extremely important to make an effort towards standardization of the selected plant material to be used as a medicine. The process of standardization can be achieved by stepwise pharmacognostic and phytochemical studies. These studies help in identification and standardization of the plant material. Correct identification and quality assurance of the plant material are essential prerequisites to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy.[6]

Bacolepis nervosa (Wight & Arn.) Decne. ex Moq. (Periplocaceae) is an endemic plant to Nilgiri Biosphere Reserve, Western Ghats, Tamil Nadu, and India. Literature does not record any pharmacognostical and physicochemical works that has been carried out on this plant. This research paper describes some pharmacognostical and physicochemical characteristics studied.

MATERIALS AND METHODS

Collection of Plant Material

The aerial parts (stem and leaf) of *Bacolepis nervosa* (Wight & Arn.) Decne. ex Moq. were collected from Kothagiri, Nilgiri Biosphere Reserve, Western Ghats, Tamil Nadu, India. The plant sample was identified with the help of local flora and authenticated by Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen of collected plants was deposited in the Ethnopharmacology Unit, PG & Research Department of Botany, V.O. Chidambaram College, Thoothukudi District, Tamil Nadu, India.

Microscopic Studies

Care was taken to select healthy plants. The required samples of different organs were cut and removed from the plants and fixed in FAA solution (Formalin - 10 ml, Acetic acid - 5 ml and 70% Ethyl alcohol - 85 ml). After 24 hours of fixing, the specimens were dehydrated with graded series of Tertiary Butyl Alcohol (TBA) as per the schedule given by Sass (1940).[7] Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58° - 60°C) until TBA solution attained

super saturation. The plant materials were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the section was 10 to 12 µm. Dewaxing of the section was done by customary procedure.[8] The sections were stained with Toluidine blue as per the method published by O' Brien *et al*. (1964).[9] Since Toluidine blue is a polychromatic stain, the staining results were remarkably good. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. Wherever necessary, sections were also stained with Safranin and Fast - green.

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid[7] were prepared. Glycerin mounted temporary preparations were made for macerated / cleared materials.

Photomicrographs

Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Labphot 2 microscopic unit. For normal observations bright field was used, for the study of crystals polarized light was employed. Since these structures have briefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard books.[10]

Preparation of Powder

The stem and leaf of *B. nervosa* (BNS and BNL) were cut into small fragments and shade dried until the fracture was uniform and smooth. The dried plant materials were powdered separately by using a blender and sieved to get uniform particles by using sieve No. 60. The final uniform powder of stem and leaf was used for various experimental studies.

Physicochemical Characteristics

The percentage of loss of weight on drying, total ash, acid insoluble ash, water soluble ash, sulphated ash and extractive values in various solvents was obtained by employing standard method of analysis described in Pharmacopoeia of India.[11]

Fluorescence Analysis

The drug powder was treated with acids like 1N HCl, conc. HCl, 50% H_2SO_4, conc. H_2SO_4, 50% HNO_3, conc. HNO_3, acetic acid and conc. HNO_3+NH_3; alkaline solutions like aqueous sodium hydroxide, 40% NaOH + 10% lead acetate and alcoholic sodium hydroxide; solvents like acetone, benzene, chloroform, petroleum ether, methanol and ethanol; other chemical reagents like ferric chloride and ammonia. They were subjected to fluorescence analysis in daylight and in short UV light (254 nm) and long UV light (365 nm). The fluorescence analysis was carried out as per the standard procedures.[12]

RESULTS

Pharmacognostical Studies

Plant Name : *Bacolepis nervosa* (Wight & Arn.) Decne. Ex.Moq.

Family : Perplocaceae

Exomorphic Features (Plate 1)

Distribution : Southern Western Ghats of Tamil Nadu.

Status : rare and endemic

Habit : climbing wiry shrub; branchlets pubescent.

Leaves : elliptic-lanceolate, 4-10 x 2-5 cm thick, acute at base, entire or slightly revolute at margin, acute-short acuminate at apex, lateral nerves 7-11 pairs, prominent; petiole ca. 1.2 cm long.

Flowers : terminal and axillary, dichotomous cymes, densely villous; peduncle ca. 5 cm long, densely villous; pedicel ca. 1.2 cm long, densely villous.

Calyx : deeply 5-lobed with ovate glands between the lobes; lobes ovate, ca. 1.5 mm long.

Corolla : rotate, 5-lobed; corona of five, broad membranous scales.

Stamens : 5, inserted on the corolla throat; filaments very short and broad; anthers attached to the style apex and inflexed; pollen masses in pairs in each cell.

Ovary : 2, ovate, ca. 1 mm long; style -flat at apex.

Fruit : 2, divaricate, smooth, follicular mericarps, ca. 10 cm long; seeds ovate-oblong, ridged ventrally, tipped with a long white coma.

Plate 1

Entire plant of *Bacolepis nervosa* (Wight & Arn.) Decne. ex. Moq.

Microscopic Studies

Anatomy of the Stem

The young stem is circular in cross sectional outline, measuring 800 µm in radius. It consists of dark and thick crushed epidermal layer and one or two superficial layers of periderm. The cortical zone is wide measuring 100 µm thick. The cortex is parenchymatous and homogeneous. An endodermoid layer is slightly visible at the inner boundary of the cortex. The endodermoid cells are spindle shaped and thin walled. Inner to the endodermoid layer occur several discrete masses of fibres arranged in a ring **(Plate 2a)**. The secondary phloem is wide and forms a continuous sheath along the outer boundary of the xylem cylinder. There are small masses of internal phloem or medullary phloem occurring in a ring along the inner boundary of the xylem cylinder **(Plate 2b)**.

The xylem cylinder consists of outer thick portion of secondary xylem elements and inner cylinder of primary xylem elements. The outer secondary xylem consists of compact, radial, long lines of xylem fibres and several small clusters of vessels distributed with wide gaps in between the clusters. The inner part of the xylem cylinder represents primary xylem which includes several long, radial lines of xylem elements which are circular and wide. The outer part of the secondary xylem is somewhat unequal in distribution due to localization of wide vessels **(Plate 2a & b)**.

An old stem is 1.45 mm thick in radius. It is circular in sectional view with smooth outline. The epidermis and subepidermal layer are compressed into thick dark cylinder **(Plate 2c)**. There are two or three layers of superficial periderm which is located inside the dark cylinder **(Plate 2d)**. The cortical zone consists of tangentially elliptical, thin walled, compact parenchyma cells. Endodermoid layer, sclerenchyma masses are fairly distinct in the old stem.

The vascular cylinder consists of outer continuous zone of secondary phloem and inner thick cylinder of xylem with wavy outer contour **(Plate 2d)**. The phloem cylinder includes small clusters of sieve elements which occur in alternate masses of parenchyma cells. The vascular cylinder including phloem and xylem is 450 µm thick. The xylem includes outer portion of secondary xylem and inner portion of primary xylem. The secondary xylem consists of inner zone of mostly xylem fibres which occur in compact parallel lines with rectangular cells and thick walls. There are also xylem rays which are thin with single row of cells.

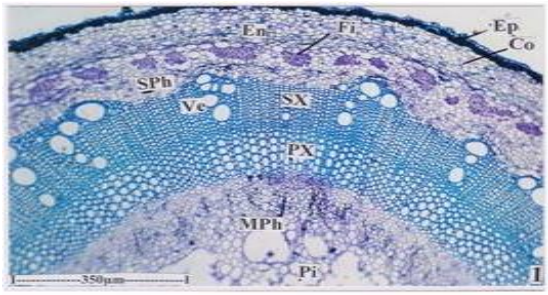
In the outer part of the secondary xylem occur large wide circular thick walled vessel elements which are 60 µm in diameter. The presence of many wide vessels in the outer boundary indicates the initiation of the secondary growth**.** The inner zone of the xylem cylinder consists of long narrow primary xylem elements **(Plate 2d)**. The primary xylem consists of wide, circular, thick walled metaxylem cells which are wider in the outer part and becomes gradually narrow, collapsed and crushed protoxylem elements. The medullary phloem or inner phloem includes large clusters of phloem elements which are wide and angular. These medullary phloem elements are in isolated strands forming an inner circle of medullary phloem.

Anatomy of the Leaf

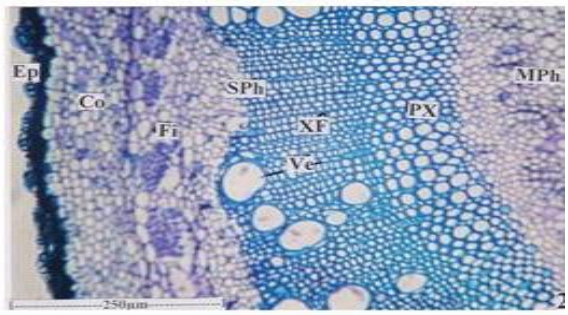
In cross sectional view, the leaf exhibits thick midrib and uniformly thin and even lamina **(Plate 3a)**.

Plate 2

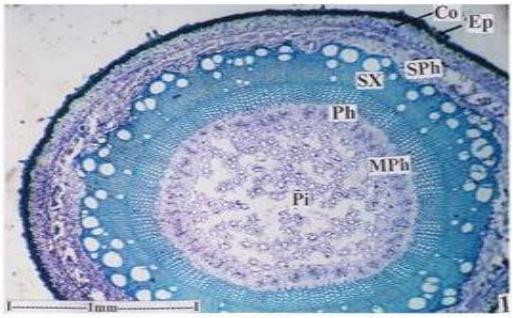
Microscopic characters of stem of *Bacolepis nervosa*



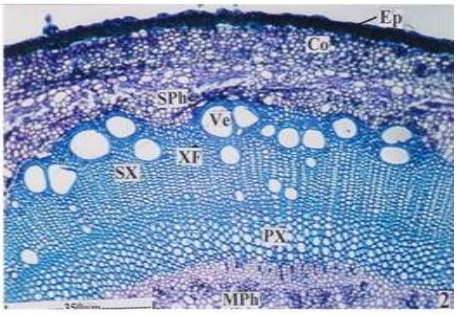
a) T.S. of young stem half sector



b) A sector enlarged of T.S. of young stem



c) T.S. of old stem



d) A sector of T.S. of old stem

Co-Cortex, En- Endodermis, Ep- Epidermis, Fi- Fibre, MPh- Medullary Phloem, Pi- Pith, Ph- Phloem, PX- Primary Xylem, SPh- Secondary Phloem, SX- Secondary Xylem, Ve- Vessels, XF- Xylem fibre

The midrib consists of thick adaxial cone and wide semicircular abaxial part. The midrib is 1.2 mm thick; the adaxial cone is 250 µm thick and the abaxial part is 1.1 mm wide. The epidermal layer of the midrib consists of small squarish thin walled cells with prominent cuticle **(Plate 3b)**. The ground tissue includes circular and angular compact parenchyma cells. The vascular strand is broadly arc shaped and bicollateral. The vascular arc is 800 µm wide and 200 µm thick. It consists of several, radial compact lines of xylem elements. The number of elements in each line is 4 to 6 cells. The cells are angular, wide and thick walled. Phloem elements occur both on the adaxial concavity as well as along the abaxial convex part of the strand. The phloem strands are in discontinuous small units comprising small thick walled angular sieve elements **(Plate 3b)**.

Lamina

The lamina is 200 µm thick. The adaxial epidermal layer consists of rectangular or squarish thin walled cells with smooth prominent cuticular layer. The cells are 20 µm thick. The abaxial epidermis is similar to those of the adaxial epidermis. The mesophyll tissue consists of narrow adaxial band of palisade tissue and much thicker abaxial zone of spongy parenchyma. The palisade cells are single layered, short, thin and less compact. The cells are 30 µm in height. The spongy parenchyma cells are many layered. The cells are spherical or lobed. They are loosely arranged forming wide air chambers divided by network of partition filaments formed by spongy parenchyma **(Plate 3c)**. Calcium oxalate druses are sparsely seen in the mesophyll tissue. The druses are isolated and are found to occur in the intercellular spaces of the mesophyll tissue **(Plate 3d)**.

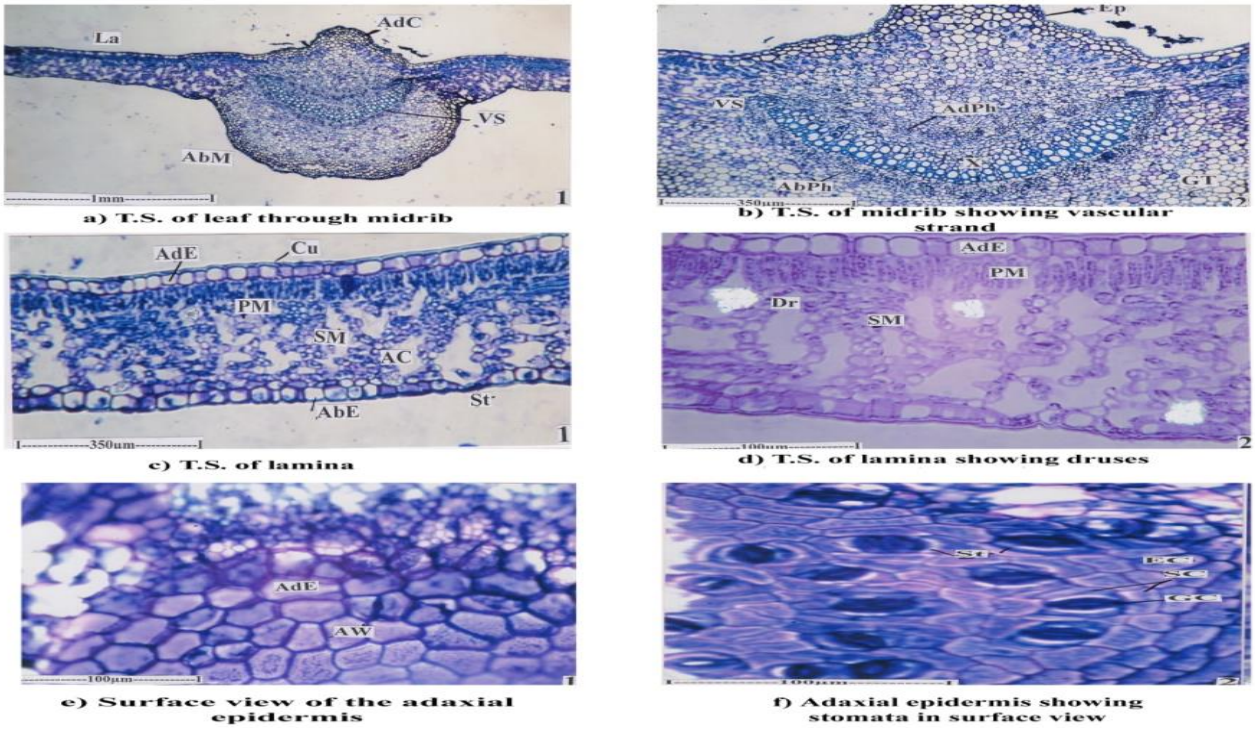
Epidermal cells and stomata

The epidermal tissue was studied from surface view of the paradermal sections. The adaxial epidermis consists of small, polyhedral parenchyma cells with thick straight anticlinal walls **(Plate 3e)**. The adaxial epidermis is apostomatic. The abaxial epidermis is stomatiferous. The epidermal cells are rectangular, thin walled and the anticlinal walls are straight. The stomata are aggregated into clusters comprising 10 to 15 stomata in a cluster **(Plate 3f)**. The stomata are tetracytic comprising a pair of lateral subsidiary cells and another pair of polar subsidiary cells**.** The guard cells are broadly elliptical with narrow stomatal pore. The guard cells are 30 × 20 µm in size.

Venation pattern

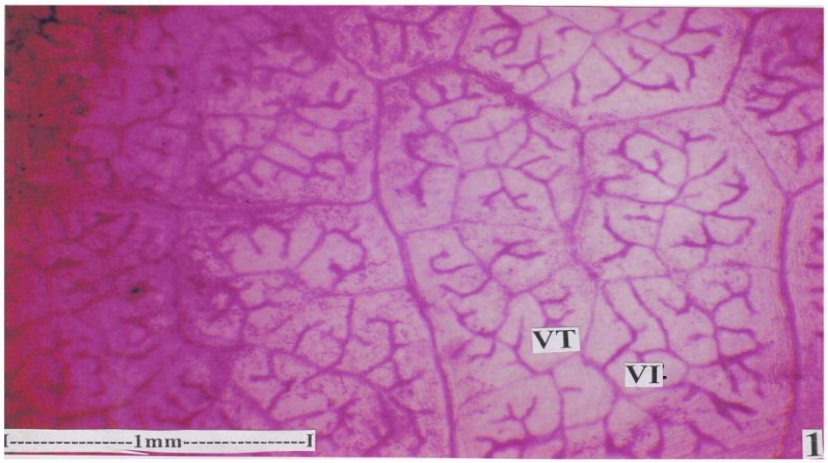
The venation is densely reticulate. The major secondary veins are thin and less prominent **(Plate 4a)**. The secondary lateral veins are uniformly thick and repeatedly branched and dense. The vein islets are wide and variously shaped. Within the islets occur either simple (unbranched) terminations or branched once or many times forming dendroid outline. Ends of the terminations are thicker due to terminal accumulation of short tracheids **(Plate 4b)**.

Plate 3
Microscopic characters of leaf of *Bacolepis nervosa*

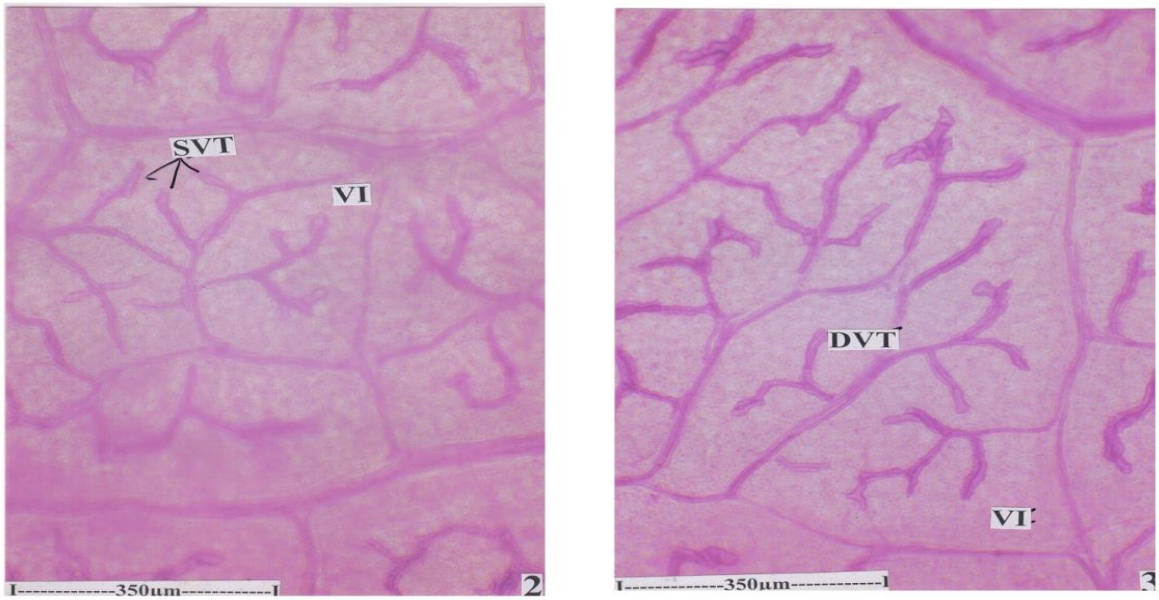


AbM-Abaxial Midrib, AbPh- Abaxial Phloem, AdC- Adaxial Cone, AdPh- Adaxial Phloem, Ep-Epidermis, La- Lamina, VS-Vascular Strand, AbE- Abaxial Epidermis, AC- Air Chamber, AdE- Adaxial Epidermis, Cu- Cuticle, Dr- Druses, PM- Palisade Mesophyll, SM- Spongy Mesophyll, St- Stomata, AW- Anticlinal Wall, EC- Epidermal Cells, GC- Guard Cells, SC- Subsidiary Cells

Plate 4
Microscopic characters of leaf of *Bacolepis nervosa*



a) Venation pattern of the lamina



b) Vein islets and vein terminations enlarged

DVT- Dendroid Vein Termination, SVT- Simple Vein Termination, VI- Vein Islet, VT- Vein Termination

Powder Microscopy

The powder preparation of the sample exhibits the following elements:

Epidermal Cells and Stomata

A small piece of epidermal peeling of the leaf was seen in the powder. The peeling showed epidermal cells and stomatal type. The epidermal cells are polygonal, small with straight thin anticlinal cells **(Plate 5a**). Stomata are diffusely distributed in the epidermis. The stomata are tetracytic or cyclocytic type. Each stoma is surrounded by 6 subsidiary cells which are rectangular and thin walled. The guard cells are circular and they are 20 µm in diameter.

Laticifer

Long, narrow, cylindrical, thin walled laticifers are seen in the powder. Laticifers are articulated non anastomosing type. Dark granular particles are

abundant in the lumen of the laticifers. A segment of laticifer is 480 µm long and 25 µm thick **(Plate 5b)**.

Fibres

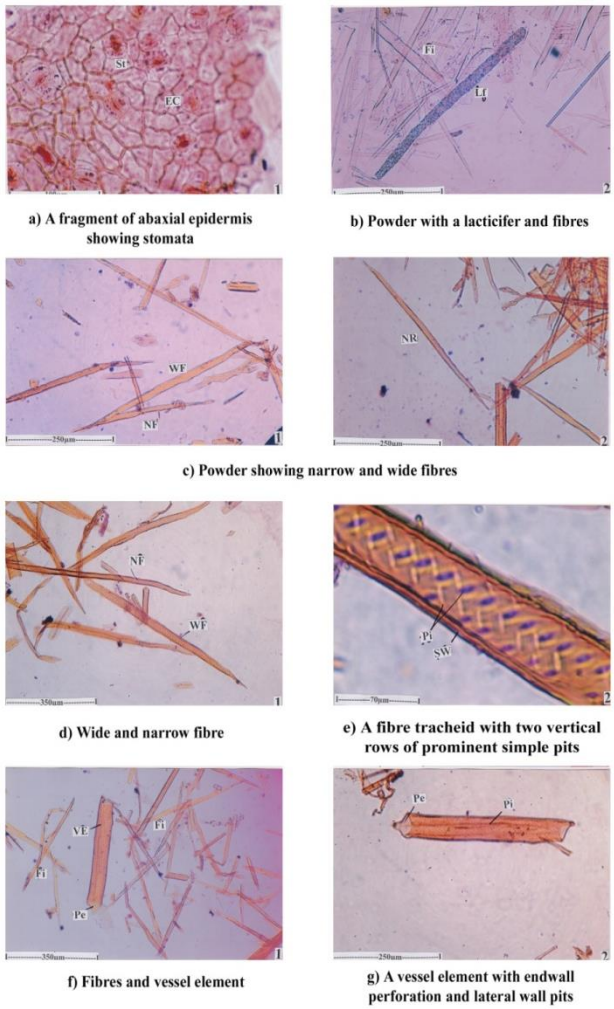
Xylem fibres are abundant in the powder **(Plate 5c & d)**. The fibres may be narrow or wide. The narrow fibres are long, thick walled, tapering at the ends and the lumen is narrow. The narrow fibre is 450 µm long and 10 µm thick**.** The wide fibre has thin wall, wide lumen and the ends are less tapering. The wide fibres are 500 µm long and 15 µm thick. Some of the fibres have elliptical slit like simple pits which occur in crisscross manner. These fibres are called fibre tracheids **(Plate 5e)**. The pits are multiseriate and they are obliquely oriented**.** The fibre tracheids are 700 µm long and 25 µm wide.

Vessel Elements

Vessel elements are less frequent in the powder. They are long, narrow and cylindrical. Some of them have short tails or tailless. On the lateral wall of the vessel element occur circular, multiseriate bordered pits. The end wall perforation is wide, circular and horizontal or slightly oblique. The vessel elements are upto 450 µm long and 50 µm wide **(Plate 5f & g)**.

Plate 5

Powder microscopy of *Bacolepis nervosa*



a) A fragment of abaxial epidermis showing stomata

b) Powder with a lacticifer and fibres

c) Powder showing narrow and wide fibres

d) Wide and narrow fibre

e) A fibre tracheid with two vertical rows of prominent simple pits

f) Fibres and vessel element

g) A vessel element with endwall perforation and lateral wall pits

EC- Epidermal Cells, Fi- Fibres, Lf- Laticifers, St- Stomata, Fi- Fibre, NF- Narrow Fibre, WF- Wide Fibre, Pi- Pits, SW- Secondary Wall, Pe- Perforation, VE- Vessel Elements

Physicochemical Parameters

The physicochemical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The moisture content, ash values and extractive values of stem and leaf of *Bacolepis nervosa* were determined. The results are depicted in **Table 1**.

Moisture content

Percentage of loss on drying or moisture content of the stem and leaf was found to be 70% and 78% respectively which signifies that the drug is properly dried and stored **(Table 1)**.

Ash values

The analytical results for total ash of stem and leaf were found to be 7.0% and 7.7% respectively. The amount of acid insoluble ash present in stem and leaf was 1.3% and 1.7% respectively. The water soluble ash of stem and leaf was found to be 4.2% and 4.0% respectively. The amount of sulphated ash present in stem and leaf were 6.2% and 4.8% respectively.

Extractive values

Percentage of the extractive values of various extracts is given in **Table 1**. The results showed that various extracts of leaf contain greater proportion by mass of the extractive values than various extracts of stem. Petroleum ether, benzene, chloroform, ethyl acetate, methanol, ethanol and aqueous soluble extractive values of stem were 4.6%, 7.6%, 8.6%, 7.4%, 9.2%, 11.7% and 15.3% respectively and leaf were 6.2%, 6.8%, 9.2%, 8.2%, 15.2%, 16.2% and 16.1% respectively **(Table 1)**. In stem and of leaf extracts, water soluble extractive values were higher followed by alcohol and chloroform soluble extractive values while the least amount of extractive value was observed in petroleum ether extract.

Fluorescence Analysis

Fluorescence analysis of stem and leaf powder was studied at day light and UV light (245 nm and 365 nm) and the observations are presented in **Tables 2 and 3**. Fluorescence studies of stem powder revealed the presence of fluorescent green with 1N aqueous NaOH, 1N alcoholic NaOH, 1N HCl, Conc. HCl, Conc. H_2SO_4, 50% H_2SO_4, Conc. HNO_3 and benzene under UV light of shorter wavelength. The leaf powder treated with 1N aqueous NaOH, 1N alcoholic NaOH, Conc. HCl, Conc. HNO_3 and ethanol revealed the presence of fluorescent green under UV light of shorter wavelength.

Table 1: Physicochemical characters of the stem and leaf of *Bacolepis nervosa*

S. No.	Parameters	Values (%)	
		Stem	Leaf
1.	Moisture content	70 ± 8.11	78 ± 9.21
2.	**Ash values**		
	Total ash	7.0 ± 0.11	7.7 ± 0.10
	Acid insoluble ash	1.3 ± 0.01	1.7 ± 0.01
	Water soluble ash	4.2 ± 0.05	4.0 ± 0.03
	Water insoluble ash	2.8 ± 0.03	3.7 ± 0.02
	Sulphated ash	6.2 ± 0.07	4.8 ± 0.03
3.	**Extractive values**		
	Petroleum ether	4.6 ± 0.03	6.2 ± 0.05
	Benzene	6.8 ± 0.03	7.6 ± 0.05
	Chloroform	8.6 ± 0.13	9.2 ± 0.02
	Ethyl acetate	7.4 ± 0.05	8.2 ± 0.14
	Methanol	9.2 ± 0.07	15.2 ± 0.13
	Ethanol	11.7 ± 0.15	16.2 ± 0.11
	Water	15.3 ± 0.16	16.1 ± 0.17

Table 2: Fluorescence analysis of the stem powder of *Bacolepis nervosa*

S. No.	Experiments	Visible light	UV light	
			245 nm	365 nm
1	Powder as such	Brown	Dark brown	Black
2	Powder + 1N NaOH (aqueous)	Dark brown	Fluorescent green	Black
3	Powder + 1N NaOH (alcohol)	Brown	Fluorescent green	Black
4	Powder + 1N HCl	Pale brown	Fluorescent green	Dark blue
5	Powder + Conc.HCl	Pale brown	Fluorescent green	Violet
6	Powder + Conc.H_2SO_4	Brown	Fluorescent green	Violet
7	Powder +50%H_2SO_4	Green	Fluorescent green	Blue
8	Powder + Conc. HNO_3	Brick red	Fluorescent green	Violet
9	Powder +50% HNO_3	Orange	Dark green	Black
10	Powder + 40%NaOH +10% lead acetate	Brown	Pale green	Violet
11	Powder + Acetic acid	Pale brown	Pale green	Dark blue
12	Powder + Ferric chloride	Green	Dark green	Dark violet
13	Powder + Chloroform	Pale brown	Green	Violet
14	Powder + Benzene	Pale brown	Fluorescent Green	Blue
15	Powder + Petroleum ether	Pale brown	Dark Green	Dark Blue
16	Powder + Ethanol	Pale green	Green	Blue
17	Powder +Acetone	Brown	Pale green	Violet
18	Powder + Methanol	Pale brown	Pale green	Blue
19	Powder + NH_3	Brown	Black	Blue
20	Powder + NH_3 + HNO_3	Brown	Pale green	Blue

DISCUSSION

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. In the developed and developing countries, as people are becoming aware of the potency and side effects of synthetic drugs, there is an increasing interest in the natural product remedies with a basic approach towards the nature. Throughout the history of mankind, many infectious diseases have been treated with herbals. Herbal preparations called “Phytopharmaceuticals” are

preparations made from different parts of plants. They come in different formulations and dosage forms including tablets, capsules, powder, extract, tincture and cream.[13] The misuse of herbal medicine or natural products starts with wrong identification. Hence, standardization of herbal raw material is very important today before subjecting the plant material to biological screening.

Table 3: Fluorescence analysis of the leaf powder of *Bacolepis nervosa*

S. No	Experiments	Visible light	UV light	
			245 nm	365 nm
1	Powder as such	Green	Brown	Black
2	Powder + 1N NaOH (aqueous)	Dark brown	Fluorescent green	Blue
3	Powder + 1N NaOH (alcohol)	Brown	Fluorescent green	Dark blue
4	Powder + 1N HCl	Pale brown	Pale green	Violet
5	Powder + Conc. HCl	Pale brown	Fluorescent green	Blue
6	Powder + Conc. H_2SO_4	Dark brown	Dark green	Black
7	Powder +50% H_2SO_4	Dark green	Dark green	Black
8	Powder + Conc.HNO_3	Orange	Fluorescent green	Black
9	Powder +50% HNO_3	Orange	Pale green	Violet
10	Powder + 40%NaOH +10% lead acetate	Brown	Pale green	Dark blue
11	Powder + Acetic acid	Pale brown	Pale green	Pale blue
12	Powder + Ferric chloride	Green	Dark green	Violet
13	Powder + Chloroform	Pale brown	Pale green	Blue
14	Powder + Benzene	Dark brown	Dark green	Violet
15	Powder + Petroleum ether	Pale brown	Pale brown	Black
16	Powder + Methanol	Pale brown	Dark green	Blue
17	Powder +Ethanol	Pale green	Fluorescent green	Violet
18	Powder + Acetone	Brown	Pale green	Black
19	Powder + NH_3	Brown	Blue	Fluorescent green
20	Powder + HNO_3 +NH_3	Brown	Pale green	Black

Pharmacognostic study is the initial step to confirm the identity and assess the quality and purity of the crude drug. Pharmacognostic techniques used in plant standardization include microscopical and physicochemical parameters.[14] According to World Health Organization (WHO), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken.[15]

It is globally accepted that herbal based drugs have many advantages over the synthetic drugs. However, one of the major problems in utilization of phytodrugs is correct diagnosis of the medicinal plants that are used either in the traditional systems or modern systems of preparation of the drugs. It is regrettable to note that most of the people involved in the manufacture or preparation of herbal drugs lack the basic background of botanical knowledge of the drugs. Consequently adulteration or substitutions of plants in the place of original ones permeate the pharmaceutical industries, rendering the herbal drugs undependable and invalid. This will lead unpopularity of phytodrugs among the people. So, it is most essential that a medicinal plant, high pharmacological potentials, should be subjected to thorough botanical standardization so that these wouldn't cause any ambiguity with respect to botanical identity of the plants. Identifications of plants involve the study of the external features of vegetative and floral parts. This study must be complimented with anatomical parameters which are very often useful to identify the fragmentary plant specimens. Raw drugs pose problem of identifications and to establish their genuineness when they lack any external diagnostic features or organoleptic clues. During such situation, the microscopic analyses of the specimen will offer a helping hand to establish and identify the phytodrugs. Since, literature dealing with the anatomy of *Bacolepis nervosa* is lacking, the present study may be claimed as the first comprehensive investigation of the stem and leaf of *Bacolepis nervosa*. The present investigation has laid down a set of anatomical features of stem and leaf, which can be employed for botanical diagnosis. The following are the salient features of identification of stem and leaf of *Bacolepis nervosa*.

Salient Anatomical Features of *B. nervosa*

- Young stem consists of crushed epidermal layer and one or two superficial layers of periderm, parenchymatous cortex, distinct endodermoid layer, discrete circular masses of fibres, secondary xylem with outer and medullary phloem.
- Old stem is basically similar to the young stem excepting thick cylinder of secondary xylem where wide, thick walled vessels are diffusely distributed.
- The leaf consists of prominent adaxial cone and thick, wide abaxial midrib.
- The vascular strand is wide and deep cup shaped with bicollateral vascular elements.
- The lamina is bifacet. It consists of large vertically oblong cells with thick cuticle in the epidermal layers. The mesophyll includes narrow palisade zone and wide, many layered spongy parenchyma.

- Adaxial epidermis is apostomatic. The epidermal cells have thick and straight anticlinal walls.
- The stomata are tetracytic type.
- The venation includes wide vein islets and branched dendroid type of vein terminations.
- The powder preparation shows wide and narrow fibres, tracheids, long, narrow vessel elements with simple, horizontal end wall perforations and epidermal peeling with stomata of tetracytic type.
- Non anastomosing, non articulated long cylindrical laticifers with granular inclusions are occasionally seen in the powder.

Microscopical evaluation is the simplest and reliable tool for correct identification of herbs as well as small fragment of crude drugs or powdered drugs and detection of adulterants and substituents.[16, 17]

Physicochemical standardization is a prerequisite in quality control of herbal drugs. The efficacy of herbal drug mainly depends upon its physical and chemical properties. Therefore, the determination of physicochemical characters for the authenticity of the drug is necessary before being subjected to pharmacological activities. The qualitative and quantitative analysis of major bioactive chemical components of crude drug constitute important and reliable part of quality control protocol as any change in the quality of the drug directly affects the constituents.[18]

Evaluation of ash and extractive values of crude drugs help in the identification and determination of its purity and quality.[18] Loss on drying of plant materials should be determined and the water content should be controlled. This is especially important for materials that absorb moisture easily or deteriorate quickly in the presence of water. The test for loss on drying determines both water and volatile matter.[15] The commonly applied parameter for the detection of impurities and adulteration of drug is the estimation of ash value, which establishes the quality and the purity of drug. Ash value can also detect the nature of the material added to the drug for the purpose of adulteration. In the present study, the total ash value is more for the leaf than the stem of *B. nervosa*. Both the samples have more water soluble ash than acid insoluble ash. These ash values are generally considered the index of the purity as well as identity of the drug.

The extractive values in different organic solvents are based on the quantity, which are soluble in them. It makes a valuable test to check the quality of drug and any variation in the chemical constituents may cause a change in the extractive values. Thus, it helps in the determination of the adulteration and is an index of the purity of drug. The extractive values of stem and leaf of *B. nervosa* were determined by successive extraction in different solvents. Since, the extractive percentage of the drug has not been reported in the literature, it may be taken as an addition to the existing stock of knowledge. The variation in the extractive values may be possible due to the presence of specific compound, solubility, soil condition, atmospheric condition and water content of the same.[20]

Modern methods like powder analysis and fluorescence drug analysis are very useful in standardization of plant material. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescent green in the visible range in daylight. The ultraviolet light produces fluorescent green in many natural products which do not visibly fluoresce in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. The organic molecules absorb light over a specific range of wave length and re-emit radiations and hence it can be used for the identification of the powdered drug, extract or fractions of herbs.[21] Crude drugs are often assessed qualitatively in this way and it is an important parameter for pharmacognostic evaluation of crude drugs.[22]

CONCLUSION

The pharmacognostical characters reported in this work can serve as a valuable source of information and provide suitable diagnostic tool for the standardization as well as identification of adulterants in future investigation or application. It will also be immense using carrying out further research and revalidation of its use. The microscopic features could help in laying down micro and morphological standards as per WHO guidelines for authentication of the original drug. The other study viz. physical evaluation, preliminary phytochemical test and fluorescence analysis add to its quality control and quality assurance for proper identification.

REFERENCES

1. Farnsworth NR, Akerele O, Bingel AS, Soejarto DD and Guo Z. Medicinal plants in therapy, Bull. World Health Org, 63, 1985, 965-981.
2. Jeyaprakash K, Ayyanar M, Geetha KN and Sekar T. Traditional uses of medicinal plants among the tribal people in Theni district (Western Ghats), South India, Asian Pac. J. Trop. Biomed, 1, 2011, S20-S25.
3. Kaur K, Gupta AK, Ahmad S and Alam, P. Pharmacognostic studies on bark of *Murraya koenigii* Spreng. Int. J. Res. Pharmaceut. Biomed. Sci, 2, 2011, 1670-1677.
4. Saha D, Pahari SK, Maity T and Sur D. Pharmacognostic studies of the bark of *Parkinsonia aculeata*. Int. J. Pharm. Sci. Res, 1, 2010, 473-475.
5. Jena A, Saha D, Biswal B, Jana SB, Koley A, Sur, D and Battacharya A. Pharmacognostic studies of leaves of *Pterospermum suberifolium*. Int. J. Res. Pharmaceut. Biomed. Sci, 2, 2011, 252-255.

6. Kadam PV, Yadav KN, Narappanawar NS, Shivatare RS, Bhusnar, HU and Patil MJ.. Standardization of Gomutra Haritaki vati: An Ayurvedic formulation, Pharmacog. J, 3, 2011, 19-24.
7. Sass JE. Elements of Botanical Microtechnique. Mc Graw Hill Book Co; New York, 1940, pp. 222.
8. Johansen DA. Plant Microtechniqe, 1st edition, McGraw Hill Book Co. Inc., New York & London, 1940, P.104-106.
9. O'Brien TP, Feder N and McCull ME. Polychromatic staining of plant cell walls by toluidine blue-O, Protoplasma, 59, 1964, 368-373.
10. Esau K. Plant anatomy, John Wiley and Sons, New York, 1964, pp. 1-767.
11. Anonymous 1996. Indian Pharmacopoea, Vol. I & II. Government of India, Ministry of Health and Family Welfare. The Controller of Publications, Civil Lines, Delhi - 110 054.
12. Chase CR and Pratt R. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification, J. Am. Pharm. Assoc, 28, 1949, 324-331.
13. Samantha MK, Pulok K, Mukherjee Prasad MK and Suresh B. Development of Natural Products, the Eastern Pharmacist, August. 2000, pp. 23-27.
14. Ravichandra VD and Paarakh PM. Pharmacognostic and phytochemical investigation on leaves of *Ficus hispida*, Int. J. Pharm. Pharmaceut. Sci, 3, 2011, 131-134.
15. Anonymous. Quality Control methods for medicinal plant material. An authorized publication of WHO. First Edition, New Delhi, India. A.I.T.B.S. Publishers & Distributors, 2002, pp. 18-21.
16. Ahmad I, Aqil F and Owais M. Modern Phytomedicine: Turning Medicinal Plants into Drugs, John Wiley & Sons, 2007, p. 34.
17. Pratima H and Pratima M. Pharmacognostic evaluation and phytochemical analysis of leaves of *Cajanus cajan* L. J. Advan. Develop. Res, 2, 2011, 181-185.
18. Mukherjee PK, Kumar V, Satheesh kumar N and Heinrich M. The Ayurvedic medicine *Clitoria ternatea*-from traditional use to scientific assessment, J. Ethnopharm, 120, 2008, 291-301.
19. Kokate CK, Purohit AP and Gokhale SB. Pharmacognosy 45th Edition. Nirali prakashan, Mumbai India .2010.
20. Nasrin AB, Ma AN, Choo YM, Mohamad S, Rohaya MH, Azali A and Zainal Z. Oil palm biomass as potential substitution raw materials for commercial biomass briquettes production. Amer. J. App. Sci, 5, 2008, 179-183.
21. Rashida GJN, Venkatarathnakumar T, Aruna AD, Gowri R, Parameshwari R, Shanthi M and Raadhika K. Pharmacognostic and preliminary phytochemical evaluation of the leaves of *Dalbergia sissoo* Roxb, Asian J. Pharm. Clin. Res, 5, 2012, 115-119.
22. Zhao Z, Liang Z and Guo P. Macroscopic identification of Chinese medicinal materials: Traditional experiences and modern understanding, J. Ethnopharmacol, 131, 2011, 556-561.

Source of Support: Nil, **Conflict of Interest:** None.