International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 16-05-2021, Accepted: 31-05-2021, Published: 15-06-2021
Volume 6, Issue 3, 2021, Page No. 693-699

Pharmacognostic and physicochemical evaluation of *Phyllocephalum rangacharii* (Gamble) Narayana: An ethnomedicinal plant

Aswathy V Nair[1*], SR Suja[2], R Prakashkumar[3]

[1] Research scholar, Ethnomedicine and Ethnopharmacology Division, KSCSTE-JNTBGRI, Palode, Thiruvananthapuram, Kerala, India

[2] Senior Scientist and HOD, Ethnomedicine and Ethnopharmacology Division, KSCSTE- JNTBGRI, Palode, Thiruvananthapuram, Kerala, India

[3] Director, KSCSTE-JNTBGRI, Palode, Thiruvananthapuram, Kerala, India

Abstract

Crude herbal medicinal preparations have been used to treat skin wounds and related inflammation and pain since the ancient time. The plant *Phyllocephalum rangacharii* (asteraceae) is used by Cholanaikkan tribal group of Kerala to treat wounds, inflammations and other related conditions. The present investigation is focused on establishing pharmacognostic and physicochemical parameters for the identification and authentication of the plant drug. The aim of the work is to evaluate the pharmacognostic and physicochemical characters of *Phyllocephalum rangacharii.*

The pharmacognostic and physicochemical characteristics obtained through the present investigation is useful in the standardization of the plant sample and prevention of adulteration in drug industries.

Keywords: pharmacognosy, physicochemical evaluation, traditional knowledge, organoleptic

Introduction

As a biodiversity rich country India has an immense wealth of traditional knowledge on medicinal plants. Traditional knowledge is a valuable system which is continuously developed over generations by tribal and rural communities in different parts of the world and transmitted from one generation to the next in oral form (Kannaiyan, 2007) [11]. The indigenous knowledge of ethnic groups about the plants plays an important role in the biodiversity conservation through the conservation of wild areas, including natural ecosystems such as 'sacred groves'. The tribal healers have the knowledge on rare medicinal plants and their use in indigenous health practices. Industrialization and habitat destruction are the real dangers that unfavorably affect the actual knowledge holders and leads to the depletion and disappearance of the ethnic knowledge. Well documented ethnic knowledge and empirical evidence based tribal health practices can act as a starter in the modern drug research and discovery.

The standardization of quality control parameters is an important aspect in the authentification of herbal drugs. Pharmacognostic evaluation of drugs has assumed immense significance in the realm of pharmaceutical industry due to the realization that reproducibility and quality of herbal drugs are directly related to the authenticity of the plant material (Vilash *et al*, 2016). The evaluation of unique pharmacognostical characters of herbal drugs help in the identification and standardization of drug samples in drug industry. These studies lay down different parameters to authenticate the identity of plant material and also for recognizing any kind of adulteration.

The present study is focusing on the detailed pharmacognostical evaluation of an ethnomedicinal plant in Kerala, *Phyllocephalum rangacharii* (Gamble) Narayana. *Phyllocephalum rangacharii* belonging to the family asteraceae, is an ethnomedcinal herb with wound healing and anti-inflammatory properties. The plant is traditionally used by Cholanaikkan tribal group of Kerala for curing wound, inflammation and other related problems. The plant locally known as "Garumath" and it is strictly endemic to Western Ghats regions. The present investigation is taken up to establish certain chemical and botanical standards which would help in crude drug identification as well as in checking adulteration (Prasanth and Lekshmana ravu, 2018) [3].

Materials and Methods

Collection of plant material

Fresh plant material were collected from its natural habitat and authenticated by the plant taxonomist of the Institute. Voucher specimen was deposited and preserved for future identification at Jawaharlal Nehru Tropical Botanic Garden and Research Institute herbarium (TBGT 38010)

Standardization of Parameters

Macroscopic and organoleptic evaluation

The macroscopical and organoleptic evaluation of the plant parts were carried out by observing the shape, colour, size, odor, taste, surface, surface fracture, texture, apex, phyllotaxy type, venation, leaf margin etc

Microscopic Study

Study of foliar micromorphology

Leaf samples were treated with 2-5% sodium hydroxide solution for 12 to 24 hours at room temperature until the leaf become transparent. The treated leaf samples were washed repeatedly in water, stained with safranin and observed under microscope.

Anatomical studies of the leaf, stem and root

Free hand transverse sections of leaf through midrib, T.S of young stem and root were prepared and stained with safranin. Stained specimens were mounted on glass slides using glycerin and cover slip. The prepared sections observed under light microscope with camera attachment and photomicrographs were taken.

Quantitative leaf microscopy

Stomatal number and stomatal index

A small piece of leaf was cleared by boiling with sodium hydroxide solution. The upper and lower epidermis was peeled separately. The leaf of *P.rangacharii* is too difficult to peel and separate, because of the presence of dense trichomes. The peeled epidermis was stained and placed on slide and mounted with glycerine water. Average number of stomata per mm2 of the epidermis of the leaf (stomatal number) was calculated from the microphotographs taken with camera attached microscope. Ten values were determined separately using following equation.

Stomatal index (SI) = S × 100 /E+S.

Where, S= the number of stomata per unit area
E = the number of epidermal cells in the same unit area of leaf.

Determination of vein-islet number and vein-let termination number

The vein-islet number is the average number of vein-islets per mm ^{2} of a leaf surface midway between midrib and margin and the average number of terminated vein-let islets per mm^2 of a leaf was taken as vein-let termination.

Powder analysis

The fresh plant material was (whole plant) thoroughly washed, shade dried and powdered. The powder is boiled with chloral hydrate for 5-10 min, and then stained with 1% safranin and mounted on a glass slide using glycerin and observed under microscope to determine the presence of various structures and components of plant tissue.

Physicochemical evaluation

The physicochemical constants such as pH, and loss on drying were performed according to the official methods prescribed in Indian pharmacopeia. Swelling index, foaming index, ash values, extractive values were performed as per quality control methods for medicinal plants material by WHO guidelines.

pH of 1% solution

1g of the plant powder was dissolved in 100ml of distilled water, filtered and pH of the solution was determined using a digital pH meter.

Loss on drying

About 2-3 g of plant powder is accurately weighed in a China dish and kept in a hot air oven maintained at 105˚C for 5 h. After 5 hours the powder was cooled in a desiccator and the loss in weight was recorded. This procedure was repeated till constant weight obtained.

Loss in weight Loss on drying (%), LOD= $\frac{loss\ in\ weight}{weight\ of\ drug\ in\ gram}$*100

Swelling index

1g of plant powder was accurately measured and poured into a 25ml measuring cylinder. 25 ml of water added to it and shaken the mixture thoroughly every 10 minutes for 1 hour and allowed to stand for 3 hours at room temperature. The volume (ml) occupied by the plant material, including any sticky mucilage was measured and calculated the mean value for the individual determinations related to 1g plant powder.

Foaming index

1g of plant material was accurately measured and transferred to a conical flask containing 100 ml distilled water and maintained at moderate boiling for 30 minutes. The solution was cooled and filtered into 100ml volumetric flask and added sufficient amount of water to make up the volume. The prepared decoction was poured into 10 test tubes as successive portions of 1ml, 2ml, 3ml … to 10ml and adjusted the volume of the liquid in each tube to 10ml with water. The tubes were shaken in a length wise manner for 15 sec (two shakes per second) and allowed to stand for 15 min. The foam height in each tube was measured.

Foaming index was calculated using the following formula =$\frac{1000}{a}$

a- Volume of plant decoction (ml) used for preparing the dilution in the tube where foaming to a height of 1cm was observed.

Determination of ash

About 2g of plant material is placed in a previously ignited silica crucible and incinerated by gradually increasing the heat until the material is free from carbon. The residue is allowed to cool in a suitable desiccator and weighed. Then the content of total ash in mg per g of plant material was calculated.

Acid insoluble ash

To the crucible containing total ash added 25 ml of Hydrochloric acid and gently boiled for 5 minutes. The insoluble matter was collected in a pre-weighted Gooch crucible, and allowed the residue to cool in a desiccator and weighed. The content of acid insoluble ash in mg per g of plant material was calculated

Water soluble ash

To the crucible containing total ash added 25 ml of water and gently boiled for 5 minutes. The insoluble matter was collected in a pre-weighted Gooch crucible, and allowed the residue to cool in a desiccator and weighed. The content of water soluble ash in mg per g of plant material was calculated by subtracting the weight of the residue.

Extractive Values Determination and characteristics of fractions

5 gram of the powdered drug was dissolved in 100ml of respective solvents and keep in air tighten bottles for 6 hrs with intermittent shaking. Each extract was filtered into a pre-weighted flask. The solvents were then evaporated and the flasks were again weighted to know weight of the

extract. The solvent used for extractive values were hexane, chloroform, acetone, methanol, ethanol and distilled water. Determination of percentage extractive values carried out by the following formula.

$$\text{Percentage extractive value (w/w)} = \frac{weight\ of\ the\ extract}{weight\ of\ the\ plant\ drug} * 100$$

Results

Macroscopic and Organoleptic evaluation

The whole plant was observed on the spot at the time of collection for its morphological characteristics (table.no 1)). The plant is herb with slightly aromatic odor, of about ± 30cm, sometimes up to 1m.It is commonly found in grassland habitats. The flowering season of the plant usually occur in between October to February.

Leaves: Leaves are elliptic to lanceolate in shape and shows alternate arrangement. They are shortly petiolated or sometimes sessile with densely hirsute upper surface and white tomentose lower surface. The leaf margin is crenate – serrate. Venation is reticulate.

Stem: Stem is cylindrical, villous with purple colour. Mature stem is dark purple colour.

Root: Tap root system with a characteristic aromatic odor, mature root shows secondary thickening.

Inflorescence: Inflorescence is capitula, auxillary or terminal in position. Usually pedunculate and subtended by lanceolate bracts. Receptacles are flat; corolla is infundibulam and purple in colour. The corolla tube is 2-3 mm long. Anthers are linear and acute at apex. Ovary is oblong and glabrous, it is 10 ribbed and pappus hairs are absent:

Seed: Achenes are cylindric, 2-3 mm long, pale brown in colour and 10 ribbed.

Table 1: Macroscopic and organoleptic characters of *P.rangacharii*

No	Part of plant	Colour	Taste	Odour
1	Leaves	Light green (upper) Whitish green (lower)	Bitter	A characteristic aromatic odor
2	Stem	Purple	No characteristic taste	Similar to leaves
4	Root	Brown	No characteristic taste	A strong aromatic smell
4	Seed	Brown	No characteristic taste	No characteristic smell
5	Plant powder	Olive green	Bitter	A strong aromatic odor

Microscopic Study

Study of foliar micromorphology

Cleared Leaf samples were stained using 1% safranin, and observed under microscope. The following are the observations (Fig 2).

Epidermis: epidermal cells are irregular in shape and the outline of cell wall is wavy in both the surfaces. Shape of epidermal cells became elongated near the primary veins.

Stomata: lower surface of the leaf consists of stomata. Stomata are anomocytic type. The stomata and guard cells are surrounded by a limited number of subsidiary cells which are quite alike the remaining epidermal cells. Subsidiary cells are 3-5 in number.

Trichome: Epidermal trichomes are present on both sides of the leaves. Three types of trichomes are present. Upper epidermis is covered with non-glandular, multicellular, unbranched trichomes.Lower epidermis is tomentose which is covered with whitish, long wooly entangled hairs. They are also non glandular in nature. Additionally lower epidermis contains peltate trichomes also.

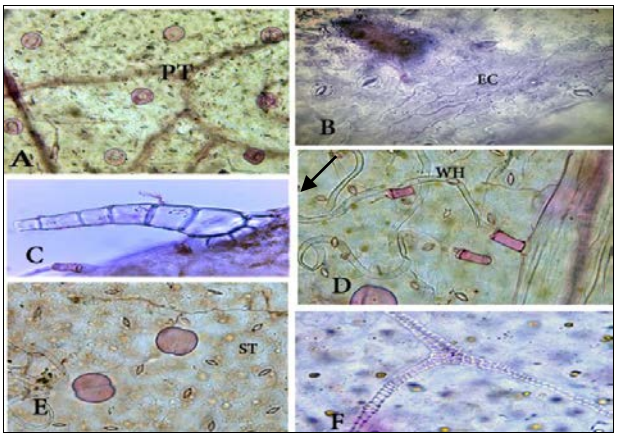

Fig 1: Foliar leaf micromorphology of *P.rangacharii*

A-Microscopic view of cleared leaf surface showing peltate trichomes and stomata (10x). B–Cleared leaf surface showing slightly elongated epidermal cells near primary veins. C–Multicellular non–glandular trichome on leaf surface. D. leaf surface showing tomentose hair originated from the epidermal cells. E–enlarged view of peltate trichomes and stomata (20x). F–spiral vessels (ST–stomata, EC-epidermal cells, PT-Peltate trichome, WH–wooly hair/tomentose hair)

Anatomical studies of the leaf, stem and root

Leaf

The T.S of leaf (Fig 3) showed the outer most single layer of epidermal cells on both the surfaces. Multicellular, unbranched trichomes of epidermal origin are present. Epidermis is followed by a single layer of green tubular palisade cells. This layer is followed by 2-3 layers of spongy parenchyma. Lower epidermis consists of long wooly entangled hairs. Crystal inclusions are involved in palisade and spongy parenchyma. The druse type calcium oxalate crystals present in these cells are smaller than crystals present in other parts. Outer most layer of the midrib (Fig 4) is epidermis and it is covered with cuticle. Two -three layers collenchymatous hypodermis present below the epidermis. Three vascular bundles are embedded in the parenchymatous ground tissue. The parenchyma cells of pith also show druse type crystal inclusions (1/cell). Vascular bundles are conjoint collateral.

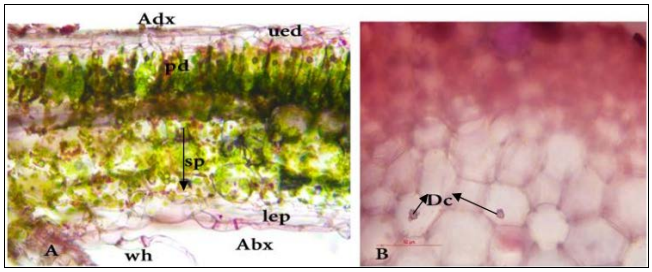

Fig 2: Anatomy of leaf lamina of *P.rangacharii*

A- Anatomy of leaf lamina**,** B-parenchyma cells of mid rib ground tissue showing druse type calcium oxalate crystals. (Adx- adaxial surface, Abx- abaxial surface,Pd- palisade parenchyma,Sp –spongy parenchyma,Ued- upper epidermis, lep- lower epidermis, Wh- wooly hairs, Dc- druse calcium oxalate crystals)

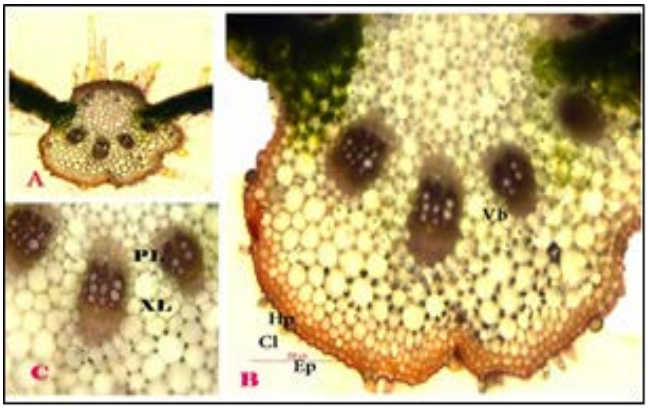

Fig 3: Anatomy of leaf midrib of *P.rangacharii*

A-T.S of midrib, B- enlarged view of midrib, C- Vascular bundles of midrib (Vb - Vascular bundles, Cl – cuticle, Ep-epidermis, hp–hypodermis, pl-phloem, xl-xylem)

Stem

The T.S of stem showed a single layer of epidermis with numerous non-glandular, multi-cellular trichomes. The epidermis is followed by 1-3 layers of collenchyma cells. This collenchymatous band is meant for giving mechanical support to the growing stem, and this layer is called hypodermis. Next to the hypodermis few layers of thin walled parenchyma (cortex) occur. The last layer of cortex is a wavy band made of parenchyma cells (starch sheath). This is the limiting layer of extrastelar ground tissue. Stele – vascular bundles are arranged in the form of a ring (9-17). They are typically collateral and open with xylem and phloem on the same radius. Xylem is internal and phloem is external. Cambial strip is present between xylem and phloem. Against every vascular bundle there is a patch of sclerenchyma tissue forming a cap like structure called pericycle or bundle cap. Xylem parenchyma is found near the xylem. Pith is parenchymatous with druse type calcium oxalate crystal inclusions.

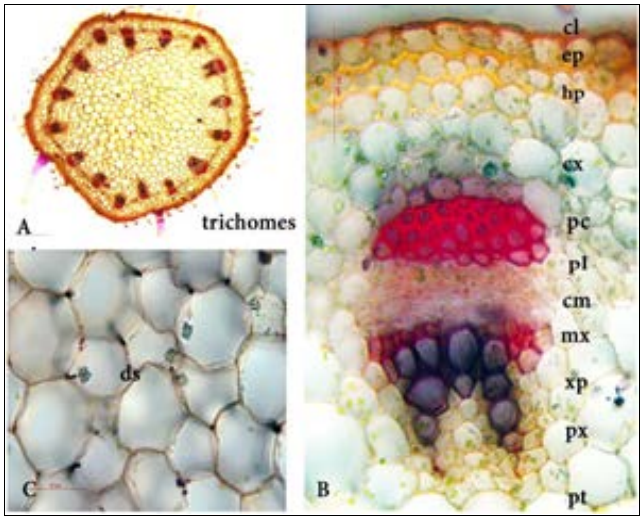

Fig 4: Stem Anatomy of *P.rangacharii*

A-Stem anatomy (ground plan) B-T.S of stem showing a portion enlarged. C-pith cells of stem showing druse type calcium oxalate crystals.

Cl – cuticle, Ep- epidermis, hp –hypodermis, cx- cortex, pc–pericycle, pl-phloem, cm –cambium, mx- metaxylem, xp-xylem parenchyma, px –protoxylem, pt-pith Dc- druse calcium oxalate crystals)

Root

In transverse section, the periderm layer on the outermost surface is thick and multilayered. Cortex is 6-8 layered and consists of parenchyma cells. Brown coloured prismatic crystals are present in the cortex of root, commonly in mature stem. Starch grains are also present in the cortex cells of mature stem. Cortex is followed by endodermis. Endodermis is the innermost layer of cortex made up of compactly arranged barrel shaped cells. It is followed by pericycle.

Stele: xylem is exarch in nature. The secondary vascular tissues along with medullary rays form a continuous cylinder (fig 6 & 7)

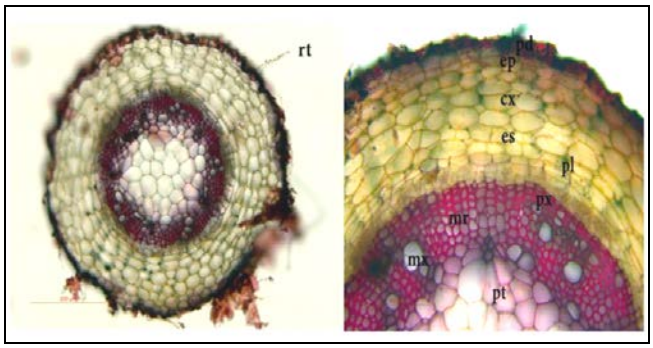

Fig 5: Root Anatomy of *P.rangacharii*

A-Root anatomy (ground plan) B-T.S of root showing a portion enlarged. Cl–cuticle, Pd-periderm, Ep-epidermis, Cx-cortex, Es–endodermis Pl-phloem, Mr-medullary rays, Mx-metaxylem, Px–protoxylem, Pt-pith, Rt-root hair

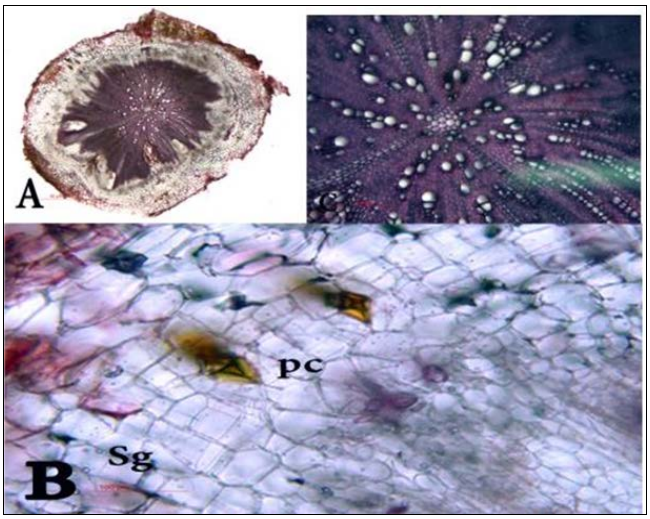

Fig 6: Root Anatomy of *P.rangacharii*

T.s of root with secondary thickening. B. Cortex of mature root (with secondary thickening). C–enlarged portion of root showing secondary xylem and medullary rays. (Pc–Prismatic Crystals, Sg-Starch Grains)

Quantitative leaf microscopy

The Quantitative leaf characteristics were observed and the results were depicted below. (table.no 2 and fig no.7)

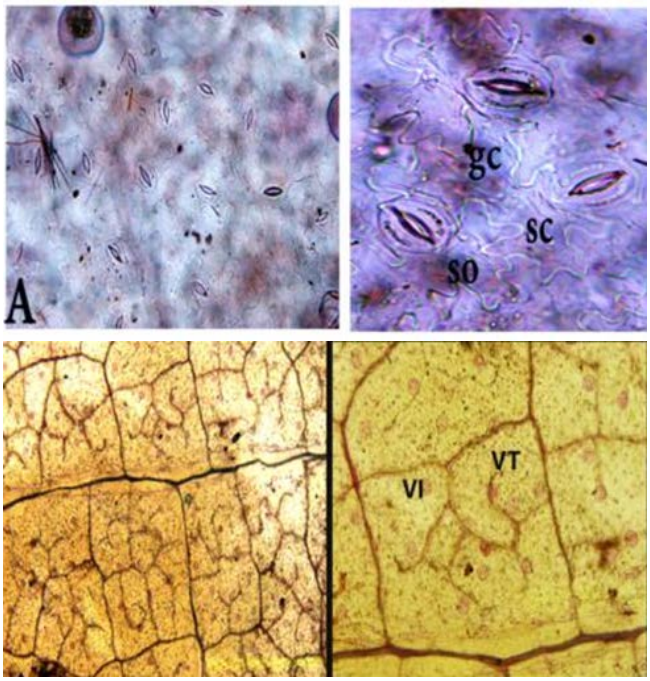

Fig 7: Quantitative leaf microscopy of *P.rangacharii*

A-Cleared leaf surface showing stomata, B-Cleared leaf surface showing veinlets and vein iselets. (So-Stomatal opening, Gc-Guard cells, Sc-Subsidiary cells, Vi-Vein islets, Vt-Veinlet termination)

Table 2 and 3: Quantitative leaf microscopy of *P.rangacharii*

No.	Parameters	Mean±SD(µM)
1	Stomatal length	268.9±9.35
2	Stomatal width	195.34±3.2
3	Length of peltate trichome	494±17.3
4	Width of peltate trichome	384±11.9

Table 3

No.	Parameters	Mean ±SD
1	Stomatal number-upper surface(per field)	**0**
2	Stomatal number-lower surface(per field)	45.2±5.40
3	Stomatal index –upper surface	17.52±2.9
4	Vein islet number	10±4
5	Veinlet termination number	7.6±2.1

(Values are expressed as mean ± SD of ten values)

Powder analysis

The plant powder were analyzed and it revealed the presence of multicellular trichomes, tomentose hair, anomocytic stomata, epidermal cells, calcium oxalate crystals (druse type and prismatic) spiral vessels, peltate trichomes etc (Fig 8)

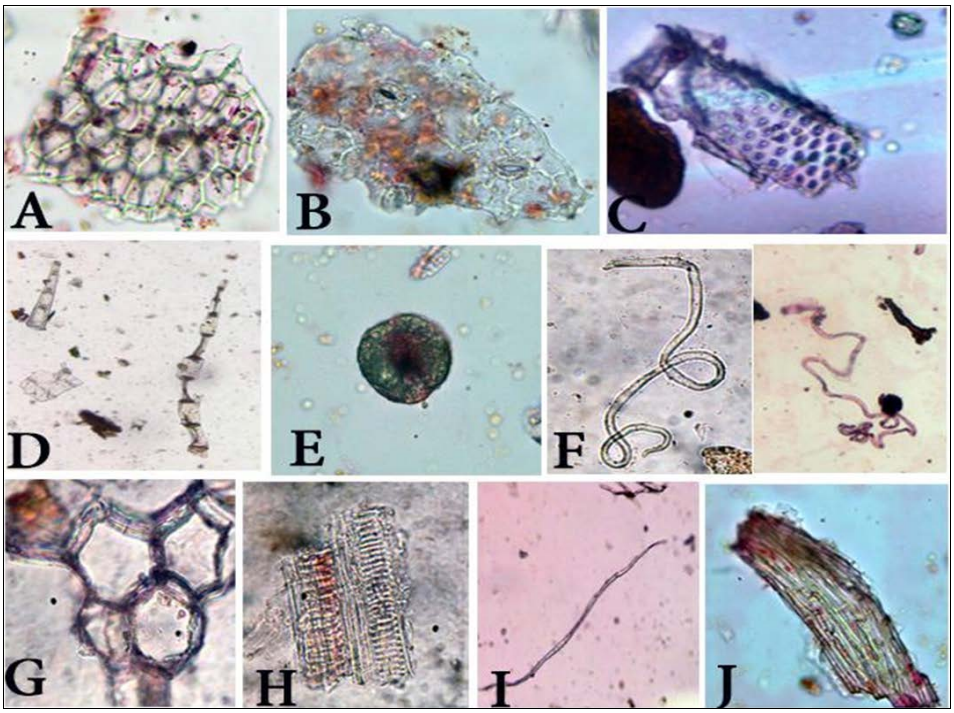

Fig 8: powder analysis of *p.rangacharii*

A-Sclerenchyma cells, B-Epidermal cells with stomata, C-Radial view of bordered pitted vessels, D-Multicellular trichome, E-Peltate trichome, F-tomentose hair, G-Parenchyma cells showing prismatic calcium oxalate crystal inclusions, H-Parts of trachieds, I-xylem fiber, J. Tangential view of vessel with bordered pits

Physicochemical evaluation

Table 4: Physicochemical parameters of *P.rangacharii*

No	Parameter	Results
1	Ph of water solution -1% w/v	6.38±1.34
2	Loss on drying	6.6% w/w
3	Swelling index	0
4	Foaming index	200
5	Total ash	10.37% w/w
6	Acid insoluble ash	0.605% w/w
7	Water soluble ash	4.705% w/w

Extractive value determination and characteristics of fractions

Table 5: Extractive value determination

No.	Name of extract	Colour	Consistency	Odour	Yield(g/100gof powder)
1	Hexane	Yellow	Solid	Characteristic	0.59
2	Chloroform	Green	Solid	Characteristic	3.04
3	Acetone	Light green	Solid	Characteristic	2.5
4	Methanol	Green	Solid	Characteristic	3.67
5	Ethanol	Dark green	Sticky solid	Characteristic	5.5
6	Water	Grayish brown	Solid	Characteristic	2.45

Discussion

The present study reveals that the macroscopic and organoleptic evaluation, microscopic and anatomical studies of leaf, stem and root, physicochemical evaluation and extractive value determination are of great importance in the proper identification and standardization of the investigated plant.

Morphologically white tomentose lower surface of leaf is a recognizable character which assists in quick identification of the fresh plant (Geethakumari *et al*, 2008) [9]. The size and shape of epidermal cells is sometimes one of the distinctive characters which help in the identification of plants. Here the epidermal cells are irregular in shape with wavy outline. The stomata are strictly anomocytic in nature. The characters like stomatal index, length and width of stomata are specific to a particular plant. In the present study these characters showed distinct values (table.no 2 &3). Vein islet number and veinlet termination number are also important characters. The type and features of trichomes are one of the predominant characters which will help in the easy identification of the plant. There are three type of trichomes found in the epidermis of leaf and stem. The presence of non-glandular, multicellular, unbranched trichomes on the upper epidermis, whitish, long wooly entangled tomentose hairs on the lower epidermis and peltate glandular trichomes on lower epidermis can be considered as t identification parameters. Peltate trichomes have short one-celled stalks and large flattened heads, which are formed by four or eight cells arranged in a simple disk or by 12–18 cells in two concentric circles. They are involved in the synthesis of secretory products (Shan–Shan Huang *et al*, 2007).The anatomical studies of the plant leaf, stem and root were carried out by taking transverse sections by hand. The observations obtained from the studies are also act as identification characters. The most distinct character obtained from the anatomical studies is the presence of druse type calcium oxalate crystals in the leaf, stem and root cells. The druses were made up of hundreds of microcrystals tightly packed together in a single macro structure (Paula and Enrique, 2001). The microscopic analysis of dried plant powder revealed the presence of multicellular non glandular trichomes, long wooly tomentose hairs, peltate trichomes, vessel elements, anomocytic stomata, irregularly shaped epidermal cells, abundant thin walled parenchymatous cells, xylem vessels with pitted thickenings, parts of fiber, calcium oxalate crystals etc.

The physicochemical evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. pH of 1% solution, ash value determination, swelling index, foaming index are particularly important in the evaluation of purity of drugs. The presence or absence of inorganic matter such as metallic salts and silica can be determined by performing the total ash. Water soluble ash is the water soluble portion of total ash. Acid insoluble ash indicates the non physiological ash due to the adherence of inorganic dust or dirt to the crude drug. The ash values of the crude drug signify the presence or absence of adulteration. (Kadam Prasad Vijay *et al*, 2013) [7]. The results of physicochemical evaluation of the dried plant powder are depicted in the table no.4

The extractive values are useful to evaluate the nature of chemical constituents present in crude drug and also to help in estimation of specific constituents soluble in a particular solvent (Kumar *et al*, 2011) [8].

Conclusion

Compilation of pharmacognostic standards for the purity and identity of crude drugs is an elementary process of drug development from medicinal plants. Microscopic and macroscopic standardization of crude drugs is one of the easiest and economical methods which can be used as the initial step towards establishing standards for correct identification of drugs. The present study established various organoleptic, macroscopic, microscopic and physicochemical parameters which may be useful for identifying the authenticity of plant material and thereby prevent adulterations. As a less studied ethnomedicinally important plant, the pharmacognostic data obtained through the study will be extremely useful in the future identification of the drug in drug industry.

Acknowledgement

The first author would like to thank the University Grants Commission for providing financial assistance (UGC-JRF). All the authors would like to acknowledge to KSCSTE-JNTBGRI for providing necessary laboratory facilities.

Declaration of Interest

The authors have declared that no competing interests exist.

References

1. African Pharmacopoeia,1986:1(2):123.
2. Anonymous. Indian Pharmacopoeia. New Delhi: Controller of Publication, Ministry of Health and Family Welfare, Govt. of India,1996:2:A-53-A-54.
3. DSNBK Prasanth and A Lakshmana Rao, Pharmacognostic Study of *Eranthemum nigrum* Stem, 2018. Curr Trends Biomedical Eng & Biosci, 2018,15(4).
4. Gupta. Pharmacognostic Standardization, Physico and Phytochemical Evaluation of Aerial Parts of *Mentha arvensis* Linn. International Journal of Pharmaceutical Sciences and Drug Research,2010:2(4):261-264.
5. Huang, Shan-Shan BK, Kirchoff, Jing-Ping Liao. The Capitate and Peltate Glandular Trichomes of *Lavandula pinnata* L. (Lamiaceae): Histochemistry, Ultrastructure and Secretion. Journal of the Torrey Botanical Society,2008:135:155-167.
6. Indian Pharmacopoeia,2018
7. Kadam Prasad vijay. Pharmacognostic Evaluation of fruits of Hleicteres isora L. (Sterculiacea). Journal of biological and scientific opinion,2013,1(1).

8. Kumar S, Kumar V, Prakash O. Pharmacognostic study and anti-inflammatory activity of *Callistemon lanceolatus* leaf. Asian Pac J Trop Biomed, 2011:1(3):177-81. http://dx.doi.org/10.1016/ S2221-1691(11)60022-1.
9. Geetha Kumary MP, Santhosh Kumar ES, Pandurangan AG. A new variety of *Phyllocephalum rangacharii* (Gamble) Narayana (Asteraceae) from Kerala,India. Indian Journal of Forestry,2008:31(1):119-120.
10. Paula V, Monje, Enrique J. Baran. Characterization of Calcium Oxalates Generated as Biominerals in Cacti. Plant Physiology,2002.
11. Prof S Kannaiyan. Biological diversity and traditional knowledge. Paper circulated for discussion at the National Consultation Workshop on Agro biodiversity hotspots and Access and benefit sharing held during July 19-20, 2007 at Annamalai University, Annamalai Nagar,2007.
12. Quality control methods for medicinal plant materials World Health Organization Geneva.