

Pharmacognostic, Phytochemical and Physicochemical Investigations of *Hypericum hookerianum* Wight & Arn. (*Hypericaceae*) of Palni Hills, India

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ABSTRACT

Introduction: *Hypericum hookerianum* Wight and Arn. (Hooker's wort) is a lesser known unfamiliar yet critically endangered native therapeutically active native medicinal plant. It is being characterized by the presence of various secretory glands. **Methodology:** In this present study, characterization of the plant in terms of morphology, anatomy and histochemistry of tissues and phytochemicals and antimicrobial potentials were made. **Results:** The epidermal layer contains oil cells while histochemistry showed the presence of the secondary metabolites. Qualitative analysis indicated presence of maximum phytochemicals in the high polar ethanolic extract; flavonoids, anthocyanin and phenol are prominently present and quantified. **Conclusion:** The results suggest that the less studied herb, *H. hookerianum* is a multifaceted high value species having a wide range of phytochemicals with abundant medicinal properties.

Key words: *Hypericum hookerianum*, Pharmacognosy, Physicochemistry, Palni Hills.

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INTRODUCTION

Hypericum is a large genus of herbs, shrubs and sometimes trees comprising 490 species of 36 sections.¹ They represent a large pool of natural resource for phytochemical, pharmacological and product development investigations as they accumulate pharmacologically important secondary metabolites. Many of the species contain structurally and functionally diverse phenolics, flavonoids and therapeutically important phenolic derivatives the dianthrone, hypericins and the prenylated phloroglucinol, hyperforin. The highest levels of the chemicals are found at full flowering stage when almost all the plants of the genus are widely used in folk medicine in different parts of the world. The growing interest in the usage of *Hypericum* in traditional medicine is due to several reasons the principal one being that modern medicine has invariably side effects and its costs are prohibitive. Among an estimated 27 species reported from India including the most popular and productized, *H. perforatum* and the recently investigated *H. hookerianum*, a high altitude shrub of the Himalayas and the Western Ghats. *Hypericum hookerianum* Wight and Arn. Also known as *Norysca hookeriana* Wight and Arn. commonly known in Nepal as Mehandiphul, is a round topped shrub with weakly spreading, non-erect branches with golden-yellow flowers also distributed in Sikkim, Khasiand Jaintia hills of Himalayas and in the high altitudes (>2300 m) of the Nilgiris and Palni hills of

the Western Ghats.²The terete shrubs are 2-2.5 m in height, with rather flaccid ovate leaves, set bifarious, mucronate to mucronate at apex.²The tribal people of the Shola forest (Tamil Nadu) in Palni hills use the aerial parts of the plant for treating burns and wounds demonstrated by Mukherjee and Suresh.³to have wound healing properties. The Toda tribe of the Palni hills uses it as a folklore medicine for its different therapeutic potentials, including anti-depressant, spasmolytic, stimulant, hypotensive and antifungalactivities.⁴Leaf anatomy of *Hypericum* species differ greatly and is the basis for not only identifying the species but also grouping them under different taxonomic criteria. Anatomical features in terms of types of glands present, secretions of the glands, interconnecting and extension of the ducts, quality of the products of the secretory glands and phytochemical profiles of the secretion play an important in differentiating the species. The anatomy and ultra-structure of the cavities in the internodes, leaves and petals varied extensively between species of *Hypericum*, especially in *H. hookerianum*. The secondary growth is more extensive in shrubs (*H. inodorum* and *H. forestii*). The petals also differ between species in the mesophyll structures and the occurrence and location of secondary structures.⁵ It is against this background, the pharmacognostic profile, anatomy of the secretory

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structures, phytochemical constituents, anti-bacterial activity of *H. hookerianum* were studied.

MATERIAL AND METHODS

Collection and authentication of the plant

Leaves of *H. hookerianum* were collected from Pambar Shola forest in the outskirts of Kodaikanal Town of Palni hills in the Western Ghats of Indian the month of June, 2015 (Figure. 1). The taxonomical identification of the plant was authenticated by Prof P. Jayaraman, Institute of Herbal Science, Plant Anatomy Research Centre, Chennai, Tamil Nadu. The voucher specimen (voucher no. CNBT/15/HH/01) was preserved in our laboratory for future reference.

Reagent and Chemicals

All the chemicals and reagents used were of analytical grade, purchased from Sigma chemical co. (St Louis, MO, USA) and Merck (Darmstadt, Germany).

Macroscopic Investigation

For morphological observations, fresh leaf approx. 3-5 cm in length was used. The macro-morphological characteristics of the leaf were observed under magnifying lens.⁶

Pharmacognostic studies

Pharmacognostic studies include organoleptic characters and microscopical features of the plant.

Morphological investigation

The morphology evaluation is used to find the closely related species and used to study the external texture and sensory characters such as color, odor, taste, size, shape etc. Fresh leaves of *H. hookerianum* were used for the morphological studies and reported.^{7,8}

Microscopical investigation of fresh leaf and dried powder

The transverse and longitudinal sections of the fresh leaves were prepared using standard procedures.⁹The normal leaf samples of *H. hookerianum* were cut and detached from the plant and fixed in F.A.A. (Formalin – 5ml+ Acetic acid – 5ml + 70% Ethyl alcohol – 90ml).After 24 hrs of fixing, the specimens were dehydrated with a graded series of tertiary-Butyl alcohol as per the schedule given by Sass.¹⁰Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks. The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was by customary procedure.¹¹ The sections were stained with Toluidine blue as per the method published by O'Brien *et al.*¹²Since Toluidine blue is a polychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink color 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 and IKI. For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections as well as clearing of leaf with 5% NaOH or epidermal peeling by partial maceration employing Jeffrey's maceration fluid.¹⁰were prepared. Glycerine mounted preparations were made and the different cell components were studied and measured. The dried powdered material of leaf was used to evaluate the powder analysis following the method of Brian and Turner.⁷

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon LabPhoto 2 microscopic Unit. For normal observations, bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent 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 anatomy book.¹³

Fluorescence Evaluation

The fluorescence analysis of the dried leaf powder was carried out by the method of Kokoski *et al.*¹⁴A small quantity of the sample treated using fluorescence reagents (such as 1 N NaOH, 1 N HCl, 50% H₂SO₄, FeCl₃, Iodine Solution, Acetic Acid Glacial, 0.1N NH₄OH, 1 % CH₃COOH) and analyzed under visible light and ultra violet light (Wavelength 254 nm).

Phytochemical determination

The dried leaf powder of *H. hookerianum* was extracted successively with different polarity solvents such as Hexane, Ethyl Acetate and Ethanol. After 24 hours, the dried extracts were obtained and were analyzed using chemical reagents for both qualitative phytochemical evaluation following standard procedure.¹⁵

Estimation of total phenol, anthocyanin and flavonoid

The Folin-Ciocalteu method was used to determine total phenolic content (TPC) of the various solvent extracts as described by Singleton and Rossi.¹⁶Gallic acid was used as a reference standard (20-100 µg/mL) for plotting calibration curve. A volume of 0.5 mL of the plant extract (100 µg/mL) was mixed with 1.5 mL of Folin-Ciocalteu reagent (diluted 1:10with de-ionized water) and were neutralized with 3 mL of sodium carbonate solution (7.5%, w/v). The reaction mixture was kept in dark at room temperature for 30 min with intermittent shaking for color development. The absorbance of the resulting blue color was measured by using double beam UV-Vis spectrophotometer at fixed wavelength of 765 nm. The TPCs were determined using linear regression equation obtained from the standard plot of gallic acid. The content of total phenolic compounds was calculated as mean±SD (n=3) and expressed as mg/g gallic acid equivalent (GAE) of dry extract.

Total anthocyanin content of the extract was determined by the pH differential method.¹⁷ A pH 1.0 buffer solution was prepared by mixing 125 ml of 0.2 N KCl with 385 ml of 0.2 N HCl and 490 ml of distilled water. The pH of the buffer was adjusted to pH 1.0 with 0.2 N HCl. A pH 4.5 buffer solution was also prepared by mixing 440 ml of 1.0 M sodium acetate with 200 ml of 1M HCl and 360 ml of distilled water. The pH of the solution was measured and adjusted to pH 4.5 with 1M HCl. The sample extract (0.5 ml) was diluted to 12.5 ml in the pH 1.0 and 4.5 buffers and allowed to equilibrate in the dark for 2 h. The absorbance of the samples at 512 nm (A₅₁₂ nm) and 700 nm (A₇₀₀ nm) was measured. The difference in absorbance (ΔA) between the anthocyanin extract diluted in pH 1.0 and pH 4.5 buffers was calculated and total anthocyanin content was also estimated.

The total flavonoid content (TFC) of each extract was investigated using the aluminum chloride colorimetry method.¹⁸ The calibration curve was prepared by diluting quercetin in 95% ethanol (0 - 100 µg/mL). The diluted extract or quercetin (2.0 mL) was mixed with 0.1 mL of 10% (w/v) aluminum chloride solution and 0.1 mL of 0.1 mM potassium acetate and 2.8 mL of distilled water in a test tube. The mixture was kept at room temperature for 30minutes to complete the reaction. Then the maximum absorbance of the mixture was measured at 415 nm with double beam

UV-Vis spectrophotometer against blank. A typical blank solution contained all reagents except aluminum chloride which is replaced by the same amount of distilled water. The amount of flavonoid was calculated from linear regression equation obtained from the quercetin calibration curve. TFC was expressed as mg/g quercetin equivalent (QE) of dry extract. All the values were expressed on dried weight basis and each value represents mean \pm SD (n=3).

Physicochemical parameters

The proximate analysis to assess the physicochemical parameters like total ash value, loss on drying and water soluble extractive values, pH value, solubility, etc. were determined as per WHO guidelines.¹⁹

Antibacterial activity

The plant extracts were analyzed for their antibacterial activity against selected human pathogens including *P. vulgaris* (MTCC - 426), *E. coli* (MTCC - 443), *B. subtilis* (MTCC - 441) and *M. luteus* (MTCC - 1538). In this method, discs of 4 mm were loaded with different solvent extracts of *H. hookerianum*. The discs were placed on the solidified Mueller - Hinton Agar (MHA) plates spread with test bacterial strains to which the sensitivity was measured (Figure. 3). The plates were incubated at 37°C for 24 h and after incubation the zones of inhibition were measured.²⁰

Statistical analysis

All experiments were repeated at least three times. Results are reported as Mean \pm S.E.M. (Standard Error of Mean).

RESULTS

Macroscopical investigation

The classification of this plant through the process of identification was done at the Institute of Herbal Science, Plant Anatomy Research Centre, Chennai, and Tamil Nadu as follows:

Description of plant:

Family : Hypericaceae

Genus : *Hypericum*

Species : *hookerianum* (Wight & Arn.)

Synonym : *Norysca hookeriana* (Wight & Arn.)

Leaves are simple alternate stipulate and petiolate. Leaves with petiole 1-4 mm; blade narrowly lanceolate to oblong-lanceolate or broadly ovate, main lateral veins 2,3 or 4 paired, apex acute to rounded with entire margin. Inflorescence 1-5 flowered, nearly round-topped, Pedicels 3-16 mm. The leaves are dark to light greenish in colour with slight odour and characteristic in taste, size width : 2.5 – 3.5 cm and Length : 3.5 – 5.5cm (Table 1)

Microscopical investigation of the fresh and dried powder

Leaf: The transverse section of the leaf found that the presence of anacytic stomata, epidermal cells often contain oil cells, guard cell with many chloroplast in lower epidermis and absence of stomata in upper epidermis. Palisade cells are arranged to sub adjacent to upper epidermis with very long cells, in midrib upper surface consists of one layered epidermal cells, cuticles are colored, collenchymatous are less greenish with less contents and innermost colorless. Xylem vessels are radially arranged, phloem cells are arranged with more dark contents. The leaf consists of a planoconvex midrib and thick smooth lamina. The midrib is flat on the adaxial side and conically thick on the abaxial side. The midrib is 750 μ m thick and 700 μ m wide (Figure. 2.1). The epidermal layer of the



Figure 1: Habitat of *Hypericum hookerianum*

midrib is thin with even outer periclinal walls. The epidermal cells are squarish with thick walls. The ground tissue of the abaxial conical part consists of large, polygonal thick walled and compact. The palisade cells are extended up to the lateral parts of the adaxial region of the midrib (Figure 2.2)

The vascular bundle of the midrib is single and collateral. It consists of horizontally extended thick segment of several vertical rows of xylem elements. The xylem elements are narrow, thick walled and elliptical in outline. The abaxial part of the xylem segment bears thick walled arc of phloem elements. The phloem cells are small, thick walled and darkly stained. A thick cup shaped sclerenchymatous sheath is located on the lower end of the phloem (Figure 2.2)

Lamina (Figure 2.3): The lamina has distinct adaxial and abaxial sides. It is 320 μ m thick. The adaxial epidermis is thin and smooth; the cells are squarish and have thick walls. The abaxial epidermis consists of hemispherical outer tangential walls and the cells appear prominently papillate. The mesophyll tissue is differentiated into adaxial layer of single row of palisade cells and abaxial zone of about seven layers lobed spongy parenchyma cells. The spongy mesophyll forms reticulate partitions and wide air-chambers. There are wide circular secretory cavities. (Figure 2.4).

Epidermal cells and stomatal morphology

The epidermal structure was studied from the paradermal sections of the lamina. The adaxial epidermis is apostolates; the cells are rectangular to polyhedral and have thick straight anticlinal walls. The abaxial epidermis is stomatic ferous (Figure 2.5). The epidermal cells small, polygonal and have slightly wavy ad thick walls. The stomata are thick and elliptical with narrow stomatal aperture. The stomata are actinocytic type. The guard cells are surrounded by six or more radiating rectangular subsidiary cells. In surface view the stomatal complex appear star shaped (Figure 2.6).

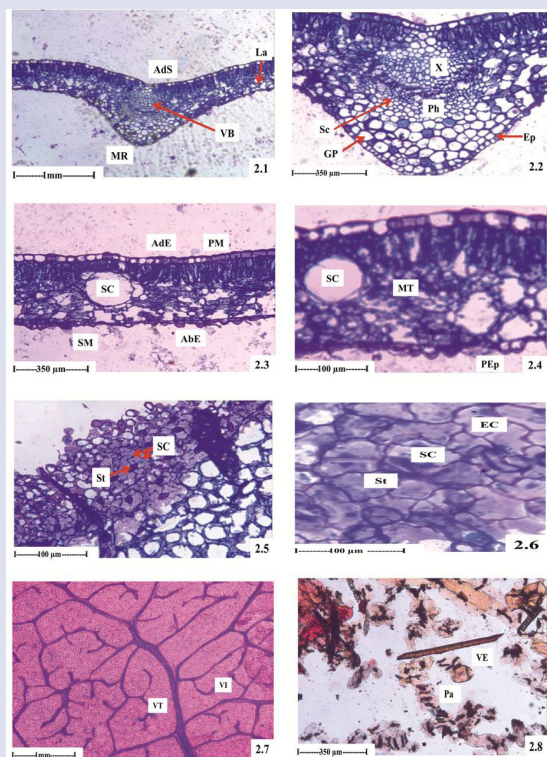


Figure 2: Pharmacognostic investigation of *H. hookerianum* leaves 2.1. T.S. of leaf through midrib; 2.2. T.S. of Midrib enlarged; 2.3 & 2.4. T.S. of Lamina; 2.5 Abaxial Epidermis; 2.6: Stomata enlarged; 2.7: Venation pattern; 2.8: Vessel Element in the powder

Note: AdS:- Adaxial Side; La : Lamina; MR : Midrib; VB : Vascular Bundles, EP: Epidermis; GP : Ground Parenchyma; Sc : Sclerenchyma; X : XylemPh : Phloem, AbE : Abaxial Epidermis; AdE : Adaxial Epidermis; Mesophyll Tissue; PEp : Papillate Epidermal Cells; PM : Palisade Mesophyll; SM : Spongy Mesophyll; SC : Secretory Cavity, SC : Subsidiary Cells; St : Stomata, EC : Epidermal Cells; VI : Vein Islets; VT : Vein Terminations; Pa : Parenchyma Cells; VE : Vessel Elements

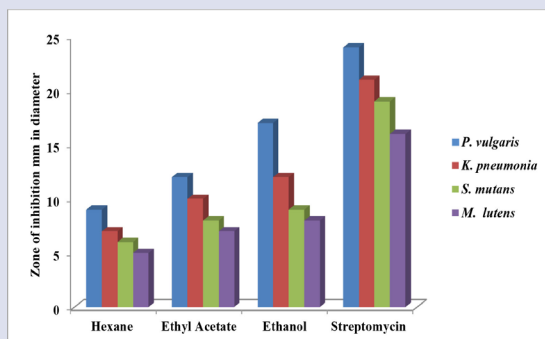


Figure 3: Antibacterial activity of different solvent extracts of leaves of *H. hookerianum* against human pathogens

Venation pattern:

The venation is densely reticulate; the thickness of the vein is gradually reduced from primary veins to secondary and tertiary veins. The vein-islets are wide and polygonal in outline. The vein boundaries are thick and straight. The vein terminations are short and simple (unbranched) or repeatedly branched to form dendroid outline (Figure. 2.7).

Powder characteristics

The powder preparation of the plant exhibits the following inclusions, i) Fragments of small epidermal peelings are common in the powder. The epidermal peelings are usually adaxial epidermis. The epidermal cells are polygonal in outline and their anticlinal walls are straight. The cells possess prominent nuclei and Vessel elements (Figure. 2.8) Thin, long, cylindrical vessel elements are occasionally dense bordered pits and oblique narrow end wall perforations. The vessel elements are up to 470µm long and 40µm thick.

Fluorescence Evaluation

The fluorescence analysis is adequately susceptible and enables the precise and accurate determination of the analyte over a pleasing concentration range without several time-consuming dilution steps prior to analysis of pharmaceutical samples.²¹ The fluorescence analysis of the powdered leaves of *H. hookerianum* is presented in the Table 1.

Phytochemical Determination

The phytochemical evaluation of the different solvent extracts of the leaves of *H. hookerianum* showed the presence of various phytochemical constituents which contribute to the medicinal activity of the plant listed in Table 2. The presence of various bioactive compounds justifies it might be utilized for the development of traditional medicines and further investigation is needed to elute novel active compounds from the medicinal plants which may create a new way to treat many incurable diseases.

Phenolic, anthocyanin and flavonoid contents

The total phenolic content, total anthocyanin content and total flavonoid content of the ethanolic leaf extract, calculated from the calibration curves were 14.15 ± 0.43 gallic acid equivalents/g, 13.59 ± 0.45 cyanidin equivalent/g and 28 ± 1.37 quercetin equivalent/g respectively (Table 3). Phenolic compounds have redox properties, which allow them to act as antioxidants. As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity. Flavonoids, including flavones, flavanols and condensed tannins, are plant secondary metabolites, the antioxidant activity of which depends on the presence of free OH groups, especially 3-OH.

Among three organic extracts, maximum amount of phenol, anthocyanin and flavonoid content were found in ethanol followed by ethyl acetate and hexane extracts.

Physicochemical Parameters

The proximate analysis to assess the physicochemical parameters like total ash value, loss on drying and water soluble extractive values, pH value, solubility, etc. The percentage yield (% w/w) with color and consistency are mentioned in Table 4.

Antibacterial activity

Among the different solvent extracts of *H. hookerianum* leaves tested for antibacterial activity, ethanol extract showed better activity when compared to other solvent extracts. *Proteus vulgaris* showed highly sensitive to plant extract followed by *M. luteus*, *B. subtilis* and *E. coli*.

Table 1: Fluorescence analysis of powder of *H. hookerianum* Wight & Arn. (Leaf) with various chemical reagents

Reagents	Color observed under Visible Light	Color observed under UV Light (254 nm)
Powder as such	Green	No Changes
Powder + Concentrated HCL	No Changes	Pale Brown
Powder + Concentrated HNO ₃	No Changes	No Changes
Powder + Concentrated H ₂ SO ₄	Reddish	Colorless
Powder + Glacial acetic acid	Dark Green	Pale Green
Powder + 5% NaOH solution	No Changes	Yellowish Green
Powder + 5% KOH solution	No Changes	Colorless
Powder + 5% Ferric chloride solution	Black	Brown
Powder + Picric acid	Greenish Yellow	Colorless
Powder + Ammonia	No Changes	Pale green

Table 2: Qualitative analysis of phytochemicals present in different polarity solvents of leaf extracts *H. hookerianum* Wight & Arn.

Phytochemical tests	Hexane	Ethyl Acetate	Ethanol
Alkaloids	+	+	++
Anthroquinones	-	-	+
Flavonoids	+	++	++
Glycosides	-	+	+
Phenols	-	++	++
Saponins	-	+	+
Steroids	+	+	+
Tannins	-	+	+
Terpenoids	-	+	+

(++) : Present in high concentration, (+) : Present in moderate concentration and (-) : Absent

Table 3 : Quantitative analysis of phytochemicals present in different polarity solvents of leaf extracts of *H. hookerianum* Wight & Arn.

Bioactive compounds	Hexane	Ethyl Acetate	Ethanol
Phenol	9.14 ± 0.33	12.33 ± 0.25	14.15 ± 0.43
Anthocyanin	8.65 ± 0.05	11.18 ± .038	13.59 ± 0.45
Flavonoids	19.47 ± 1.21	23.28 ± 0.14	28.47 ± 1.37

Total phenolics: mg of GAE/g of dry extract; Total anthocyanins : mg of Cyanidin 3-glucoside /g of dry extract, Total flavonoids: mg of QE/g of dry extract; All the values are given means of triplicates determinations. Data presented as the Mean±SD.

Table 4: Physico-chemical parameters of the leaves of *H. hookerianum* Wight & Arn.

Parameter	Values (% w/w)
Loss on Drying	7.86±0.34
Ash Values	
Total Ash	8.70±0.705
Acid insoluble ash	2.80±0.037
Water soluble ash	2.30±0.242
Extractive Values	
Water soluble extractive	18.0±0.526
Methanol soluble extractive	22.8±0.921
Dichloromethane soluble extractive	1.50±0.049

Standard deviation (SD) = ±SD; Number of readings (N) =3

DISCUSSION

Plants possess contain numerous phytochemical constituents, many of which are known to be biologically active compounds and are responsible for exhibiting diverse pharmacological activities.²² According to the previous records of pharmacognosy sciences plants have been used in native medicinal systems globally. Medicinal plant based research are rapidly increasing worldwide and further study with emphasis on efficiency, safety and quality of plant secondary metabolites is the need of the hour.⁹ Macroscopic characters involve size, arrangement, venation, texture, surface characters, markings and hardness of the plant materials. The microscopical studies (anatomical and histochemical) are often necessary to establish the botanical identity of commercial samples of medicinal plants and may play an important part in checking adulteration and substitution. It involves longitudinal and transverse sectional views of the leaves of the plant.²³

The leaves of *H. hookerianum* is characterized by the presence of different types of internal secretory oil cells present in the epidermal region. The lamina of the leaves has prominent wide circular secretory cavities responsible for secretion of secondary metabolites. The bioactive secondary metabolites have been shown to decrease the risk and development of many human diseases through various biological mechanisms. The results of preliminary chemical testing confirmed the presence of various classes of bioactive chemical constituents in ethanolic extract of *H. hookerianum* leaves including phenols, flavonoids, anthocyanin's, steroids, alkaloids, anthroquinones, glycosides and terpenoids. Therefore, based on the quantitative phytochemical analysis results, the total phenolic and flavonoid contents of different extracts of *H. hookerianum* leaves were estimated. The ethanol extract, was found to contain the highest content of total phenol (14.15 mg/g), anthocyanin (13.59mg/g) and flavonoid (28.47 mg/g) as compared to hexane/ethyl acetate extracts (9.14/8.65/19.47 mg/g and 12.33/11.18/23.28 mg/g, respectively). The physicochemical evaluation using percentage yield (w/w) color and consistency parameters is useful in the identification and authentication of crude extract. The antibacterial investigation revealed the potency of active principles in each extract which may be attributed to the high content of flavonoids, which have been reported to be involved in inhibition of nucleic acid biosynthesis and other metabolic processes.²⁴ The present study is a preliminary data for further studies. Moreover, this data was first time reported from this high altitude plant of Palni Hills, Western Ghats of India.

CONCLUSION

Pharmacognostic, phytochemical, physicochemical investigation were carried out for the first time in the high altitudes (>2300 M) plant of the Palni Hills, *H. hookerianum* and the results were computed to predict the utility of the species to treat common human values. The macroscopical, anatomical and powder microscopical studies provides the basis for further identification and to lay down standards for leaves. The preliminary phytochemical screening of the solvent extracts reveals the presence of flavonoids, anthocyanins, phenols, etc. The results suggest that, *H. hookerianum* is found a prospective versatile high value species having a wide range of phytochemicals with immense medicinal properties. Further exploration may reveal many novel features and its extraction may bring out novel products of specialized kind which needs to be upgraded / popularized before the remaining species disappear from the face of the Earth.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

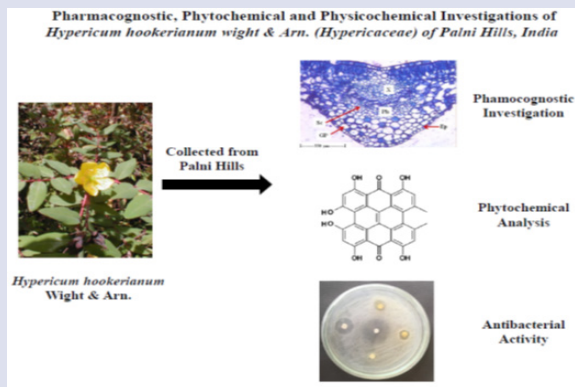
ABBREVIATION USED

µg/ml: Micrograms per milliliter; **w/v**: Weight per volume; **nm**: Nano-meter.

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GRAPHICAL ABSTRACT



HIGHLIGHTS OF PAPER

- This is the first study to report the pharmacognostic investigation of *Hypericum hookerianum* from Palni hills
- The study highlighted the major phytochemicals present in *Hypericum hookerianum*
- Further exploration of *Hypericum hookerianum* may reveal many novel products of specialized kind with immense medicinal properties.

AUTHOR PROFILE



Mahendrakumar M, a doctoral student at the PG and Research Department of Plant Biology and Plant Biology, Government Arts College for Men, Nandanam, Chennai, Tamil Nadu, India. The doctoral research focused on the scrutiny of traditional plants for their active phytochemical compounds and bioactivity.



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