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RESEARCH ARTICLE

Pharmacognostical and Physicochemical Evaluation of *Homalium zeylanicum Benth*

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ABSTRACT:

Homalium zeylanicum (Gardn.) *Benth*. (Syn. H. ceylanicum) belonging to family *Salicaceae* (*Flacourtiaceae*) and is distributed in evergreen and semi-evergreen forests, native to south India and srilanka. It is also found in Bangladesh, Laos. Myanmar, Nepal, Thailand and Vietnam. The various parts of plant having many traditional medicinal uses, include analgesic anti-inflammatory hepatoprotective, anthelimintic antioxidant, cytotoxic diabetes, wound healing and rheumatism. Review of literature revealed that the scientific evaluation on various parts of the plants has not been carried out. **Objective:** Objective of the present study is to evaluate the various parts of plant *Homalium zeylanicum Benth* with its morphology, anatomy, and physiochemical aspects. **Materials and Method:** The plant specimens for the proposed study were collected from Thenmala of Kollam District in Kerala, herbarium was provided and certified. Macroscopic and. Microscopic examinations were done as per standard protocol. Physicochemical parameters of both leaf and bark were done following standard procedure. The anatomical characters and values obtained from study can help in standardization. **Results and Conclusion:** Whole plant subjected for anatomical works, leaf and bark powder subjected for microscopic and physiochemical parameter measurement which provides relevant information about the plant. The current observation about this plant will also be helpful in differentiating this species from closely related species of same family.

KEYWORDS: Flacourtiaceae, *Homalium ceylanicum*, Leaf, stem bark, Salicaceae.

INTRODUCTION:

There are number of Indian medicinal plants have been used globally. There are many references to Indian medicinal plants and trade in spices in a number of historical documents. Herbal medicine has been used in India for thousands of years and is increasingly been used worldwide during the last few decades as evidenced by rapidly growing global and national markets of herbal drugs[1]. The world health organization (WHO) estimates that about 80% of the population is still depends up on these herbal medicines for their treatment of disease[2].

As the allopathic medication system is being widely followed but the reported side effects have posed an important cause of concern hence researches are being conducted on the use of herbal medications which have given us fruitful results and less complications are reported[3].

The genus *Homalium*, based on the single species *H. racemosum*, from Martinique, was established by Jacquin in 1760, and in 1763 a more extended description with a figure of the flower was given in his book entitled 'Selectarum Stirpium Americanarum Historia' There are more than 163 scientific plant names of species rank for the genus *Homalium* and among these 33 are accepted species, they are *H. densiflorum Spruce, H. guianense Warb, H. nicaraguense Blake, H. puberulum Klotzsch, H. mollicellum Blake, H. pleiandrum Blake, H. leiogynum Blake , H. hemisystylum Blake, H. racemosum Jacq, H. integrifolium Britton, H. pittieri Blake, H. trichocladum, H. pedicellatum Spruce,*

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H. eleutherostylum Blake, H. columbianum Blabe, H. stenosepalum Blake, H. eurypetalum Blake, H. trichostemon Blake[4].

Homalium ceylanicum (Gardn.) Benth. (Syn. H. zeylanicum)[5] belongs to family Salicaceae (Flacourtiaceae)[6]. Homalium ceylanicum is a large evergreen tree, and is distributed in evergreen and semi-evergreen forests, native to south India and srilanka.[7] Synonym: *Blackwellia ceylanica*, Common name: Liyan, mukki,

Taxonomy-Kingdom: Plantae, Phylum: Magnoliophyta, Class: Magnoliatae, Order: Violales, Genus: Homalium

Habit: Trees up to 25m tall, Habitate: Evergreen and Semi evergreen forests, native to south India and srilanka. Trunk and Bark: Bark smooth, grey, blaze white with orange speckles.

Branches and Branch lets: Branchlets slender, terete, glabrous., Leaves: Leaves simple, alternate, distichous; stipules caducous, apex abruptly acuminate, base acute or rounded to sub attenuate, margin crenate, chartaceous. Inflorescence/Flower: Inflorescence long, slender spikes with interrupted clusters of small flowers; flowers generally greenish white, sometimes few clusters crimson red in the same spike. Fruit and Seed : Capsule; seeds small, many, oblong or angular.[8,9]

The various parts of this plant having many traditional medicinal uses, include analgesic anti-inflammatory hepatoprotective, anthelimintic antioxidant, cytotoxic diabetes, wound healing and rheumatism. Review of literature revealed that the scientific evaluation on various parts of the plants, to establish its Pharmacognostical characters, has not been carried out. Hence the present study has been designed to study the various parts of *Homalium ceylanicum* Benth with its morphology, anatomy, physiochemical parameters. Many compounds namely, polyamines, isocoumarins and nickel complexes have been reported from genus Homalium. The scant phytochemical information on members of Homalium and the Flacourtiaceae in general has urged us to examine *Homalium zeylanicum*[10,11,12]

MATERIALS AND METHODS:

Collection and Authentication:

The plant specimens for the proposed study were collected from Thenmala hills of Kollam district in Kerala in the month of December 2017 with the help of local taxonomist. Care was taken to select healthy plants and normal organs. Herbarium was submitted to Pharmacognosy laboratory authentified by the Pharmacognostic of the institute and also certified with register No. PARC/2018/3750 (Figure 1.)

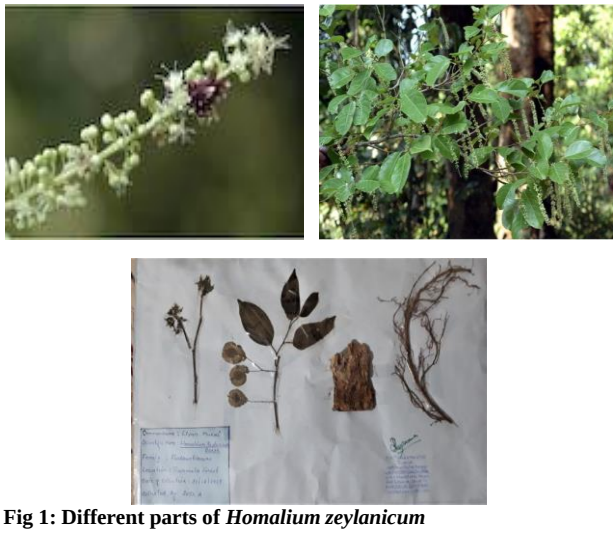

Fig 1: Different parts of *Homalium zeylanicum*

Pharmacognostical Study:

The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin 5 ml+ Acetic acid 5ml+70% Ethyl alcohol90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol[13]. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60^{o}C) until TBA solution attained super saturation. The specimens were cast in to 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 procedures[14]. The sections were stained with Toluidine blue[15]. Since Toluidine blue is a polychromatic stain. The staining results were remarkably good; and some Cytochemical reactions were also obtained. 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 and KI (for starch).

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 were prepared[13]. Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerine medium after staining. Different cell component were studied and measured.

Quantitative Microscopy:
Quantitative Microscopy includes surface constants like stomatal number, stomatal index, Vein-islets number, Vein termination number, Palisade ratio were studied. Palisade ratio was determined based on Wallis. These values help in the evaluation of purity of drugs. The cleared materials were washed thoroughly and stained with safranin for quantitative microscopic studies[16,17].

Powder microscopy is one of the cheapest and simplest method to start with establishing the correct identification of the source materials[18]. For powder microscopy, to obtain powder shaded dried leaf and bark specimens were grounded by mechanical grinder and sieved through 80#. 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 were studied using polarized light Since these structures have birefringent property, under polarized light they appear bright against dark background.[19] Magnifications of the figures are indicated by the scale –bars.

Physicochemical Parameters:
The leaf and bark powder were exposed to Physico-chemical parameter analysis i.e. loss on drying, total ash value, acid insoluble ash value, water soluble ash, sulphated ash, water soluble extractive value and alcohol soluble extractive value, and ether soluble extractive value. The procedures recommended in IP, 1996 and WHO guidelines, 1992 were followed to calculate the physicochemical constants.[20,21]

RESULTS:

Macroscopic study:
Leaves are simple, measures about 8.3-15 × 4.7-9cm, alternate, stipulate, petiolate, ovate to oblong, crenate, acuminate apex, glabrous, dark green above, parrot green beneath and having reticulate venation. 6-10 pairs of main nerves arising from mid rib, appearing yellowish green in colour on upper surface of leaf. Petiole measure about 1.2-1.5cm in length and is covered with minute hairs.

Microscopic study:
T S of Leaf:
The leaf consists of fairly thick biconvex midrib and thick lamina (fig. 1. a). The midrib consists of adaxial broadly conical and shallow part and wide and thin abaxial part. The midrib measures 650µm in vertical plane and 600µm in horizontal plane. The adaxial epidermal layer of the midrib consists of thick walled squarish cells the adaxial epidermal cells are conical and thick walled (fig. 1. b). The vascular system of the midrib consists of sclerenchyma fibris cells

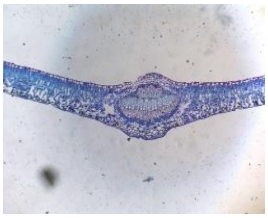
Figure 1 a

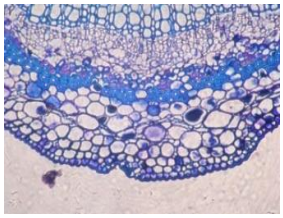
Figure 1 b

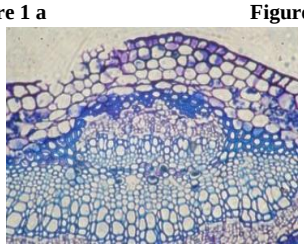
Figure 1 c

Figure1: Fig 1.a Leaf T S, Fig 1. b and c Leaf midrib

Crystal distribution in the midrib:
Calcium oxalate crystals are abundant in the phloem parenchyma of the midrib. The crystals are druses, which are spherical bodies with spiny surface. Mostly the crystals are seen in uniseriate vertical lines (fig. 2). Rarely some prismatic crystals are also seen along with the druses.

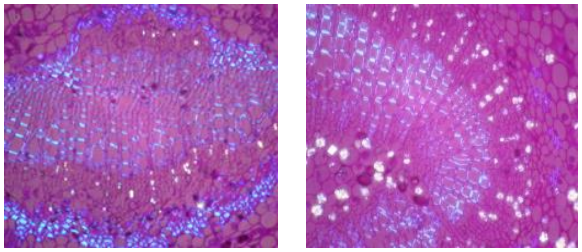
Figure 2: Crystal distribution in the midrib

Epidermal tissues of the lamina:
The adaxial epidermis of the lamina is apostomatic (without stomata). The epidermis cells are angular or lobed with sinuous anticlinal walls (fig. 3. a). The abaxial epidermal layer is densely stomatiferous (fig. 3. b,). The stomata are 10x20 µm in size, and it has two subsidiary cells, one on either side or parallel to the guard cells. So the stomata are paracytic type.

The lamina is heteromorphic and dorsiventral. It is differentiated in to adaxial palisade zone and abaxial spongy mesophyll zones (fig. 3. d).

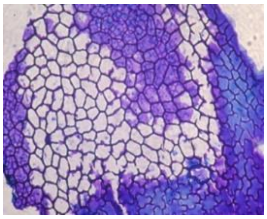
Figure 3. a

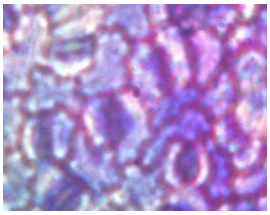
Figure 3. b

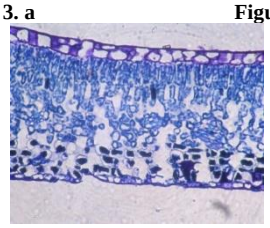
Figure 3. d

Figure 3: (Fig 3.a-apostomatic adaxial epidermis of lamina. fig 3. b, c- Stomatiferous epidermal layer. fig 3.d-lamina)

Petiole:

The petiole is semicircular at the lower part and flat with thick and wide lateral wings on the adaxial side. The petiole has a uniseriate epidermal layer, homogenous parenchymatous ground parenchyma, circular small vascular bundles and planoconvex major vascular cylinder (fig. 4).

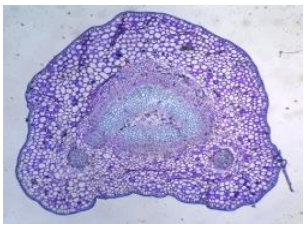
Figure 4:

T S Of Stem:

The stem is circular in cross sectional view with even outline (fig. 5. a). The stem consists of the thick cuticle of the epidermal cells. The epidermis is uniseriate at certain region and becomes three layered perioderm in other regions (fig. 5. b).

Figure. 5.

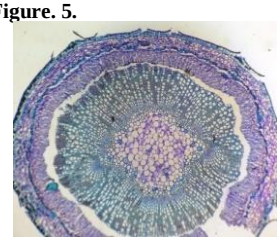
Figure 5. a

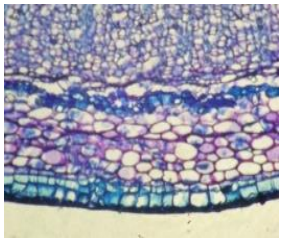
Figure 5. b

T S Of Root:

Root measuring 1.75 mm was studied. It includes thick periderm which irregularly fissured at several places. The periderm is heterocellular comprising outer and inner thin walled tabular suberised phellem cells and middle layers of curved, thick walled lignified phelloid cells. (fig. 6. a, b).

Figure 6:

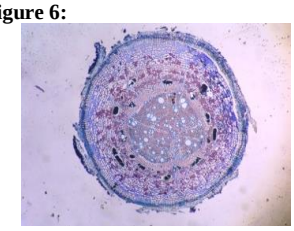
Figure 6. a

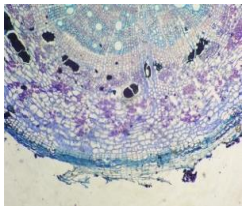
Figure 6. b

T S of Stem Bark:

The stem bark consists of outer thick heterocellular periderm and secondary phloem cylinder which occupies major part of the bark. The bark includes outer periderm which is differentiated in to thick radial segments tabular, thin walled, suberised phellem cells, and inner region of tangentially elongated, thin walled tabular phellam cells. Alternating the vertical bands of phellam occur broad region of tabular thick walled, nonsuberised, but lignified phelloid cells (fig. 7. a).

Secondary phloem is the major constituent of the bark,it is differentiated in to outer collapsed phloem and inner noncollapsed phloem. The collapsed phloem consists of collapsed and compressed sieve elements and companion cells. Phloem rays are distorted and discontious. Noncollapsed phloem (fig. 7. b) is narrow region as compared to the collapsed phloem. The phloem rays are very high and non-storied. The rays range from multiseriate, biseriate and uniseriate (fig. 7. c). The rays are heterocellular having two types of cells. The cells in the middle part of the rays are square shaped and are called procumbent cells. The cells at ends of the rays are vertically elongated and rectangular; these cells are called upright-cells

Calcium oxalate druses type of crystals is abundant in the phloem rays. They occur in uniseriate vertical rows in the rays (fig. 7. d).

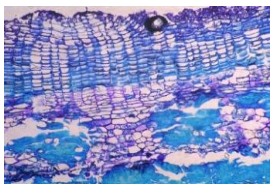
Figure .7. a

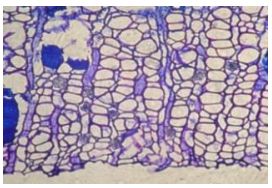
Figure 7. b

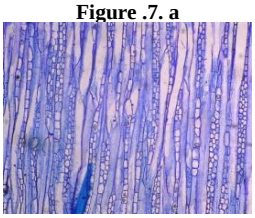
Figure 7. c

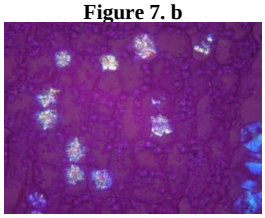
Figure 7. d

Figure. 7: (7. a: T S of stem bark with thick walled nonsuberised lignified phelloid cells, 7. b: Collapsed phloem, 7. c: Non collapsed phloem, 7. d: crystals in phloem rays)

Quantitative Microscopy:

The observed values for stomatal number, stomatal index, vein islets, vein termination number and palisade ratio of *H. zeylanicum* are given in table.

Table 1. Quantitative microscopic data of *H. zeylanicum*

S. No	Parameters	Values in 1sq.mm (average of 10 fields)
1.	Stomatal number Adaxial Abaxial	 18.20 16.82
2.	Stomatal index Adaxial Abaxial	 16.74 14.60
3.	Vein-islets number	5.16
4.	Vein termination number	4.21
5.	Palisade ratio	6.18

Powder Microscopy:

The powder preparations of the plant exhibits the following elements:

1. Small fragments of periderm of the bark are seen in surface view. The periderm cells are seen in surface view are angular, thick walled compact and some of them possess dark contents. (fig. 8. a).
2. Long, narrow, rectangular, thick walled darkly coloured cells are frequently seen in the powder. These cells are components of the views of the leaf. They occur in compact bundle and in the powder are seen separate and scattered. The cells are up to 80 µm long and 20µm thick (fig. 8. b).
3. Long, thin libriform fibres are common in the powders. They are uniformly thin with pointed ends (fig. 8. c). The fibres have thick lignified walls and narrow lumen. Minute simple pits are seen in vertical row in the fibre. The fibres are 750µm long and 15µm thick.
4. Septate fibres - fairly thick and short wide fibres are also equally common. These fibres have wide lumen and have thin septa along the length of the fibres. Granular cell contents are seen in fibre. The fibre is 600µm long and 20µm thick.
5. Tangentially cut small fragments of the bark are common in the powder. These fragments exhibits spindle shaped rays, bark fibres and parenchyma cells. The rays are mostly multiseriate; some are bi or uniseriate rays (fig. 8. d).
6. Adaxial epidermal peeling of the leaf is frequently seen. The epidermal layer is apostomatic, the cells being rectangular to polygonal and thick walled.
7. On the lamina are seen densely distributed circular structures. These circular structures have an outer ring of radially elongated rosette cells and central group angular cells. The circular cells vary in diameter. The larger structure is 100µm in diameter, the smaller one are 40µm in diameter. (fig. 8. e)
8. Adaxial epidermal tissue – The epidermal peeling of the adaxial surface of the lamina shows, polyhedral, thick walled compact parenchyma cells. Distributed among the parenchyma tissue are seen numerous circular structures. These structures have a central core of compact cells and several radiating rosette cells. Stomata are absent on the epidermis.
9. Abaxial epidermis - The epidermal peeling of the abaxial epidermal layer is seen in powder. The epidermis is densely stomatiferous. The stomatal pore wide and elliptical or slit –like. The epidermal cells are polyhedral, highly thick walled. The anticlinal walls are straight. The stomata are cyclocytic type. Each stoma is encircled by six subsidiary cells of equal size and shape.

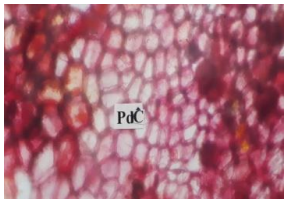

Figure 8. a

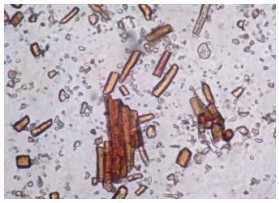

Figure 8. b

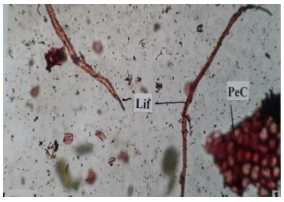

Figure 8. c

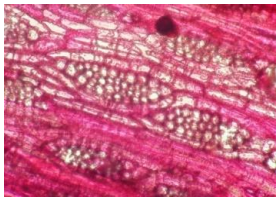

Figure 8. d

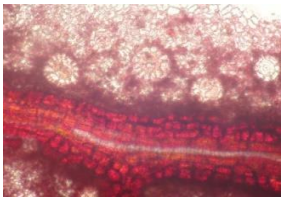

Figure 8. e

Figure .8: (Fig: 8. a-Thick walled periderm cells, Fig: 8. b-Leaf cells, fig: 8. c-libriform fibres,, fig: 8. d spindle rays,fig:8.e-circular structures on the lamina with an outer layer of rosette cells.)

Physicochemical Parameters:

The leaf and bark powder were exposed to Physico-chemical parameter analysis and the values are described in Table along with calculated deviation.

Table 2. Physico-chemical constants of leaf and Bark powders of *H. zeylanicum*

S. No.	Parameters	Leaf powder Percentage (%W/W)*	Bark powder Percentage (%W/W)*
1.	Total ash	4.64 ± 0.62	13.97 ± 1.85
2.	Acid insoluble ash	1.20 ± 0.17	02.35 ± 0.71
3.	Water soluble ash	2.35 ± 0.63	03.65 ± 1.83
4.	Sulphated ash	0.85 ± 0.32	04.50 ± 0.52
5. 6. 7.	Solubility Ethanol soluble extractive Water soluble extractive Ether soluble extractive	 10.75 ± 0.13 17.35 ± 1.25 08.23 ± 0.10	 04.58 ± 1.13 28.78 ± 2.33 04.18 ± 1.10
8.	Loss on drying	6.15	6.65

DISCUSSION:

Simple, alternate with crenate, acuminate tip and glabrous leaves, thick cuticle stem, stem bark with outer thick heterocellular periderm and secondary phloem cylinder, and root with irregularly fissured thick periderm which are key characters for identification of Family Flacourtiaceae. Presence of squarish cells in the adaxial epidermal layer and Calcium oxalate crystals are abundant in the phloem parenchyma of the midrib. Suberised phellem cells in the thick periderm of root and lignified phelloid cells and collapsed and non collapsed phloem of the bark are the main features of *Homalium zeylanicum*. Measurement of apostomatic and cyclocytic stomata in the powder microscopy of leaf and spindle shaped rays in the bark can help in identification of plant in powder form. Quantitative microscopy and values of physiochemical parameter measurements helps in the further standardisation of plant.

CONCLUSION:

No detailed anatomical study has been reported in literature for this plant. Whole plant subjected for anatomical works, leaf and bark powder subjected for microscopic and physiochemical parameter measurement which provides relevant information about the plant. The current observation about this plant will also be helpful in differentiating this species from closely related species of same family.

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