

# Pharmacognostical and Phytochemical Analysis on Leaves of *Homalium ceylanicum* (Gardn.) Benth.

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## ABSTRACT

**Objective:** *Kakhara*, *Dhanimari* or *Kakhda* is one of the folklore plant of Odisha, which has been identified as *Homalium ceylanicum* (Gardn.) Benth. (Syn. *H. zeylanicum*) belonging to family Salicaceae (Flacourtiaceae). The leaves and bark of the plant is used in rheumatism, diabetes and wound healing. Review of literature revealed that the scientific evaluation on various parts of the plants has not been carried out, hence the present study has been designed to study leaves of *Homalium ceylanicum* Benth. For its morphology, anatomy, physiochemical and phytochemical aspects. **Methods:** The leaves samples were collected from Gandhamardana hills, Odisha, in month of September 2016, herbarium was provided with herbarium reference no. phm/6216/2016-17 and also certified by BSI Kolkata. Macroscopic observations were made with naked eyes and centimeter scale was used to measure the leaf size. Microscopy of leaf, Stomatal index and powder microscopy was done as per standard protocol. Physicochemical and qualitative analysis were done following standard API protocols. **Results:** Leaves are simple, alternate, with crenate margin and petiole is pubescent. T.S. of petiole shows boat shaped with two protruding arms supported by 2 meristele. The schematic diagram of T.S. of petiole shows somewhat orbicular to boat shaped with two arms protruding supporting two meristele in each arm. Stomatal index is 15.94-16.91, powder is bitter with leafy aroma, microscopic shows paracytic stomata key character of genus. LOD is  $8.66 \pm 0.72$  and carbohydrates are present in both extracts. **Conclusion:** The anatomical characters and values obtained from analytical study can help in standardization.

**Key words:** *Anukta Dravya*, Flacourtiaceae, *Homalium ceylanicum*, Leaf, Salicaceae.

## INTRODUCTION

India has one of the richest plant based traditional medicine in the world which are well documented in certain classical texts. Some of the plants are use traditionally, which are not a part of classical texts of *Ayurveda* or pharmacopoeia and are enumerated into the category of ethano medicinal plants or extra pharmacopoeial plants (*Anukta Dravya*).<sup>1</sup>

*Kakhara*, *Dhanimari* or *Kakhda*.<sup>2</sup> is one of the folklore plant of Odisha, stem bark is used in the management of fever and its leaves mixed with *jhuna* dust (*Shorea robusta* oleo gum resin) acts as mosquito repellent.<sup>3</sup> The leaves and bark of the plant is used in rheumatism, diabetes and wound healing.<sup>4</sup>

Botanically *Kakhara* has been identified as *Homalium ceylanicum* (Gardn.) Benth. (Syn. *H. zeylanicum*).<sup>5</sup> It belongs to family Salicaceae (Flacourtiaceae).<sup>6</sup>

*Homalium ceylanicum* is a large evergreen tree, with alternate crenate leaves bearing petiole and raceme inflorescence.<sup>7</sup> 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 *Homalium ceylanicum* Benth. leaves for its morphology, anatomy, physiochemical parameters and phytochemical screening including HPTLC.

## MATERIALS AND METHODS

### Collection and authentication

The leaves samples were collected by the SRF- from one of its natural habitat, Gandhamardana hills, Odisha, in month of September 2016 with help of local taxonomist. Herbarium was submitted to pharmacognosy laboratory authenticated by the Pharmacognosist of the institute and provided with herbarium reference no. phm/6216/2016-17 and also certified by BSI Kolkata with letter no. CNH/55b/2013/Tech. II/116. (Figure 1)

### Pharmacognostical study

Macroscopic observations were made with naked eyes and centimeter scale was used to measure the leaf size. The leaves were washed and transverse sections were taken cleared with choral hydrate to observe the anatomy of leaf with help of Quasmo binocular compound microscope. For histochemical test the thick Transverse sections of the leaves were exposed to Idoine, Phloroglucinol and HCl for observation of starch grain and lignified tissue.

For surface study, triplicate reading were taken for trichomes, stomata length width and stomatal index was calculated taking into consideration six consecutive readings along with Standard Deviation.

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**Figure 1A:** Plant in Natural Habitat, B: Flowering Twig of the tree, C: Herbarium phm/6216/2016-17, D: Certificate from BSI.

For powder microscopy, to obtain powder shaded dried leaves were grounded by mechanical grinder and sieved through 80#. For micrometry, triplicate reading were recorded and mean value was taken into consideration along with standard mean of deviation.<sup>8</sup>

### Physicochemical parameters and qualitative analysis

The leaf powder was exposed to Physico-chemical i.e. pH, loss on drying, total ash value, acid insoluble ash value, water soluble extractive value and alcohol soluble extractive value, protocols followed as recommended by API. For qualitative analysis, the presence of various secondary metabolites dissolved in water and alcohol extract was done as per reference.<sup>9,10</sup>

### HPTLC Study

Methanolic extract of leaves were exposed to HPTLC study. The solvent system used for the study is toluene: ethyl acetate (9:1)

### Chromatographic conditions

Application mode : Camag Linomat V  
 Development Chamber : Camag Twin trough Chamber.  
 Plates : Precoated Silica Gel<sub>GF254</sub> Plates.  
 Chamber Saturation : 30 min.  
 Development Time : 30 min.

Scanner : Camag Scanner III.  
 Detection : Deuterium lamp, Tungstan Lamp  
 Data System : Win cats software.

### Spray reagent<sup>11</sup>

Preparation: 0.5 g Vanillin is dissolved in 100 ml sulphuric acid-ethanol (40+10). Or 1 g of Vanillin was dissolved in 100 ml conc. Sulphuric acid. Treatment after spraying: heated at 120°C until maximum spot colour intensity was reached.

## RESULTS

### Macroscopic study

Leaves are simple, measures about 8.3-15 × 4.7-9 cm, 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 1.2-1.5 cm in length and is covered with minute hairs. (Figure 2, A)

### Microscopic study

#### T.S. of petiole

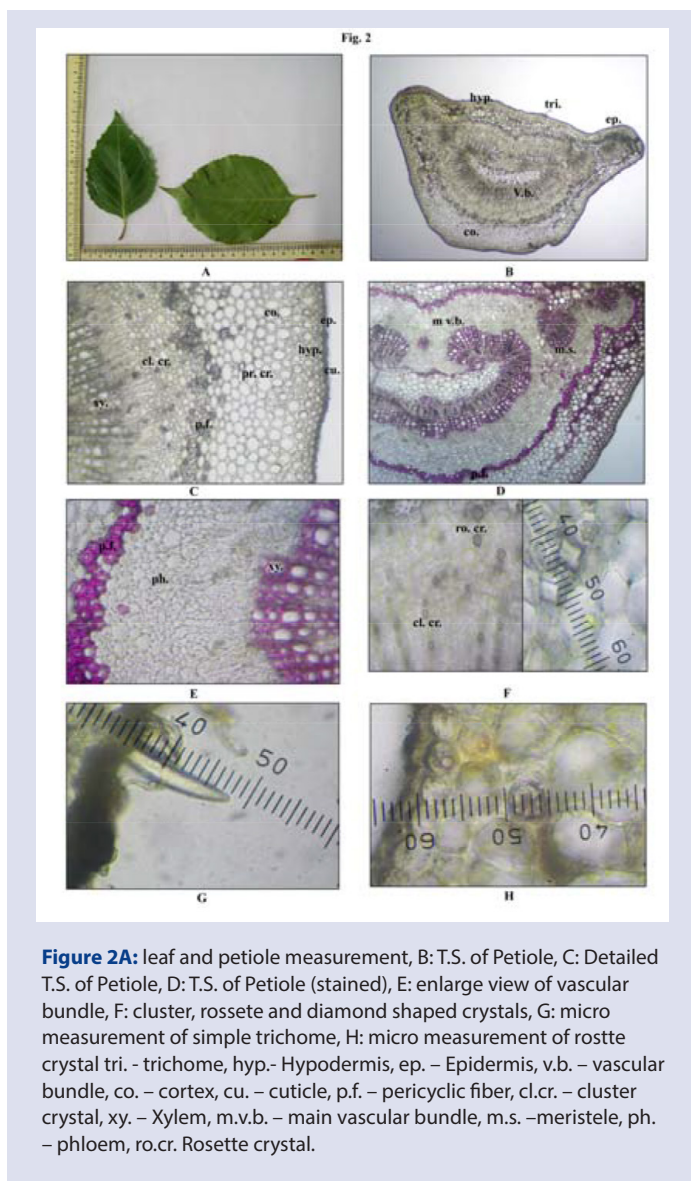
The schematic diagram of T.S. of petiole shows somewhat orbicular to boat shaped with two arms protruding supporting two meristele in each arm measuring about 0.36 μm × 0.50 μm near arm (4×). (Figure 2, B)

Detailed T.S. of petiole shows single layered epidermis interrupted by unicellular covering trichome, along with thick layer of cuticle, 1-2 layer of hypodermis followed by parenchyma cells filled with chlorophyll pigments forming 5-9 layers of cortical cells, some cells are embedded with rosette crystals, rarely prismatic crystals of calcium oxalate and red colored pigments. Some of the cortical cells are pitted and lignified. Single layered endodermis continuous all over the ground tissue. 2-3 layered thick lignified pericyclic fibres running all around the ground tissue just beneath the endodermis. Rectangular and diamond shaped prismatic crystal present adjacent to the pericyclic fiber. Vascular bundle consist of phloem towards cortex made up of fibers and sieve elements, xylem radially arranged. Protoxylem towards pith and metaxylem towards phloem made up of xylem fibers and parenchyma. Reduced pith is occupying the center of the section, cells often filled with rosette and cluster crystals. Two identical vascular bundle representing meristele at the corner of the two arms. (Figure 2, C-H)

#### T.S. of leaf passing through Midrib

The schematic diagram of T.S. of leaf passing through midrib shows bulged center with lamina extension in the same line. The midrib region measures about 0.25 μm in diameter and lamina region measures about 0.09 μm in diameter (4×). (Figure 3, A)

Detail T.S. passing through mid-rib shows two layered upper and lower epidermis with thick layer of cuticle, which slowly merges into single layer epidermis in extended arms of lamina. Some of the lower epidermal cells are thick and lignified often interrupted by stomatal openings and simple unicellular trichome. Beneath the epidermis 1-2 layers of compactly arranged collenchyma cell, some of the collenchymatous cells shows lignification, parenchymatous cells forming 4-8 layered ground tissue often filled with cluster and rosette crystals. Single layered bundle sheath encircled by 2- 3 layers of pericyclic fibers followed by several layers of parenchyma cells embedded with rosette, cluster and few prismatic crystals of calcium oxalate. Centrally located main vascular bundle radially arranged with continuous ring of xylem. Xylem made up of xylem parenchyma and it is fibers. Phloem is situated at the base of xylem and towards lower epidermis. Two meristele represents the vascular



**Figure 2A:** leaf and petiole measurement, B: T.S. of Petiole, C: Detailed T.S. of Petiole, D: T.S. of Petiole (stained), E: enlarge view of vascular bundle, F: cluster, rossete and diamond shaped crystals, G: micro measurement of simple trichome, H: micro measurement of rosette crystal tri. - trichome, hyp.- Hypodermis, ep. - Epidermis, v.b. - vascular bundle, co. - cortex, cu. - cuticle, p.f. - pericyclic fiber, cl.cr. - cluster crystal, xy. - Xylem, m.v.b. - main vascular bundle, m.s. -meristele, ph. - phloem, ro.cr. Rosette crystal.

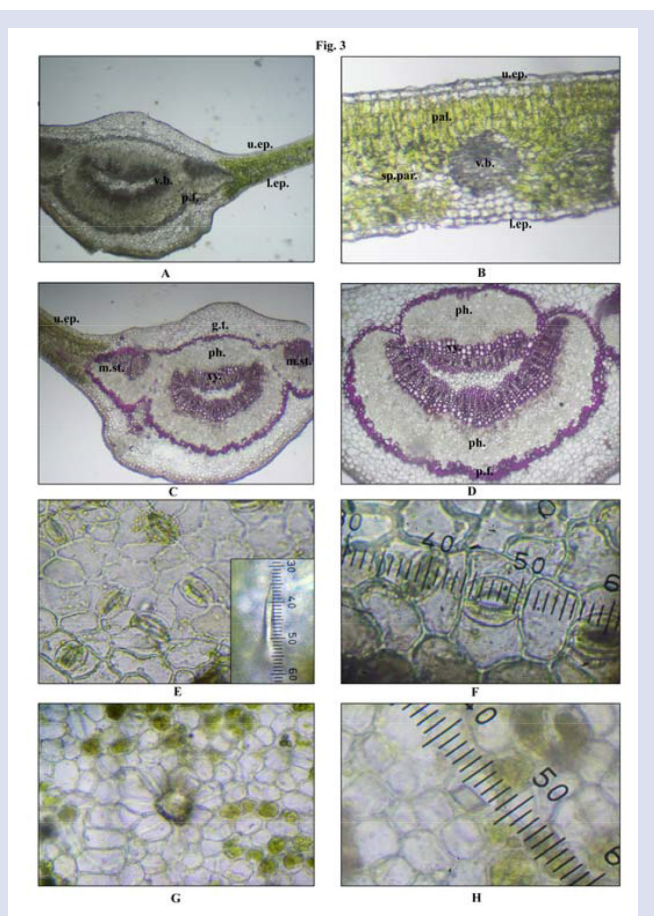
bundle just attached to the upper side of the main vascular bundle with more layers of pericyclic fibers and devoid of pith.

T.S. passing through lamina consists of single layer of upper and lower epidermis, both covered with cuticle. Followed by the mesophyll region, divided further into 2-4 layers below the upper epidermis elongated cells filled with chlorophyll pigments known as palisade cell layer and compactly arranged spongy parenchyma cells towards the lower epidermis, filled with green pigments and isolated oil globules. Small vascular bundles, made of xylem and phloem are found embedded in the lamina at regular intervals. (Figure 3, B-D)

### Surface study

The lower epidermis shows paracytic or rubiaceous type of stomata,<sup>11</sup> few cells are embedded with prismatic crystals of calcium oxalate. Cicatrix cells and unicellular simple trichomes. The stomata measures about  $0.775 (\pm 0.15) \times 0.625 (\pm 0.09) \mu\text{m}$  and the palisade ratio is 1/6.

Stomatal index of lower epidermis ranges from 15.94-16.91, and mean of all the six readings of stomatal index is  $16.44 \pm 0.36$  deviation. Stomata were not found in the upper epidermis. Presence of rectangular crystals



**Figure 3 A:** T.S. of leaf passing through mid rib, B: T.S. of Lamina, C: T.S. of leaf passing through mid-rib (Stained), D: Enlarged view of main vascular bundle, E: Paracytic stomata micro measurement of trichome, F: Micro measurement of stomata (lower surface), G: Cicatrix present in upper epidermis surface, H: Micro measurement of rectangular crystal u.ep. -upper epidermis, lep. - Lower epidermis, sp. par. - Spongy parenchyma, m.st. - Meristele, g.t. - ground tissue.

of calcium oxalate and cicatrix are found in surface of upper epidermis of leaf. (Figure 3, E-H)

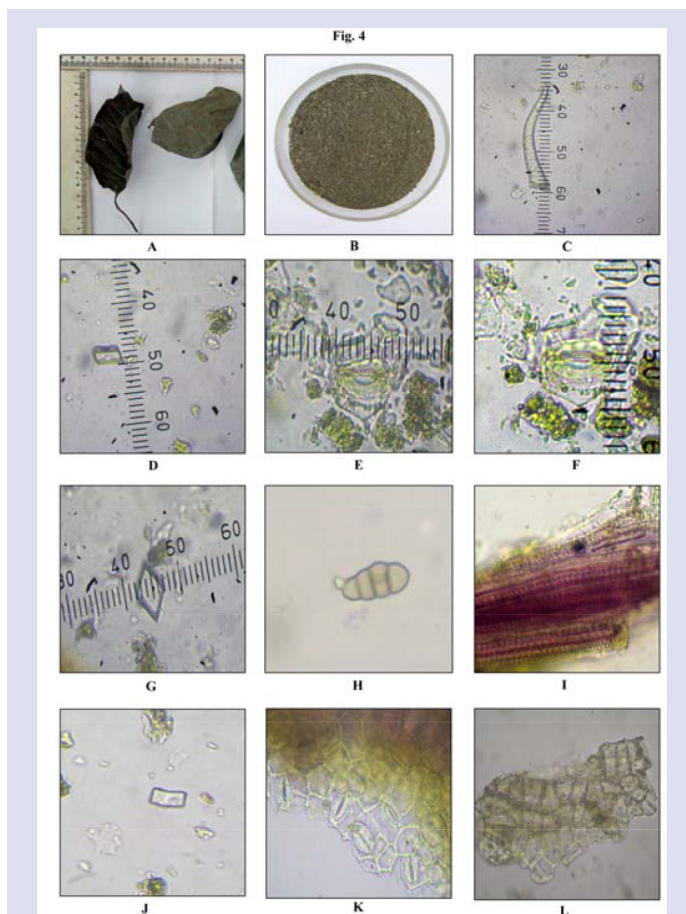
### Powder microscopy

Powder was green in colour with leafy smell, fine texture and bitter astringent taste. Diagnostic powder characters observe under microscope are simple unicellular trichome, prismatic crystals, rhomboidal crystals, fragment of paracytic stomata, fragment of upper epidermal cell in surface view, diamond shaped prismatic crystals and fragment of spiral and pitted vessels. (Figure 4, A-B)

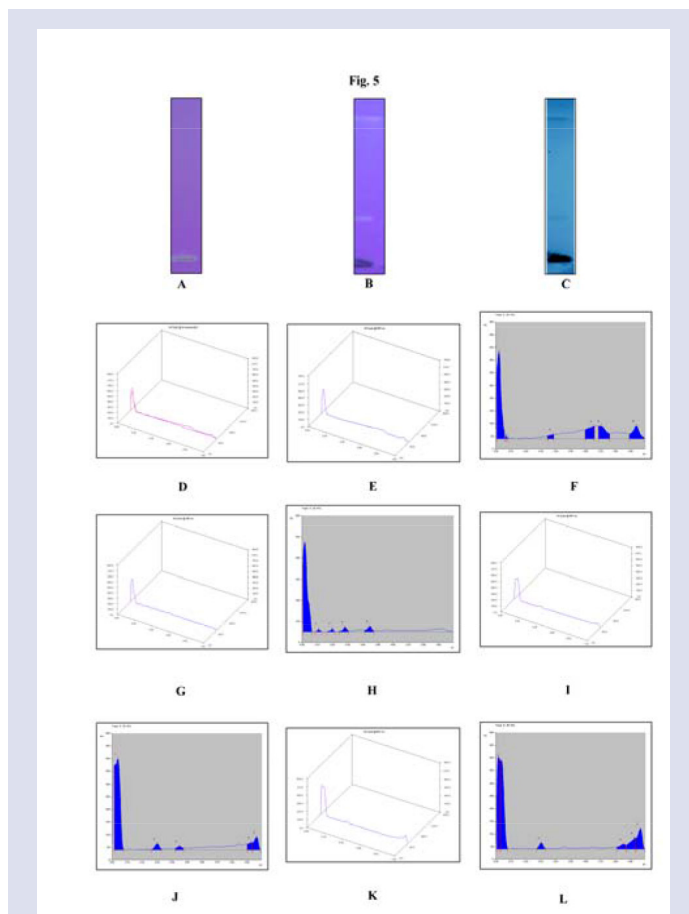
Trichome  $2.16 (\pm 0.55) \times 0.4 \mu\text{m}$ , prismatic crystals  $0.4 \times 0.5 \mu\text{m}$ , rhomboidal crystal  $0.4 \times 0.3 \mu\text{m}$ , fragment of stomata  $0.5 \times 0.3 \mu\text{m}$ , diamond shaped crystal  $0.8 \times 0.6 \mu\text{m}$ . (Figure 4 C-L).

### Physicochemical parameters

The pH of leaf water extract was found to be 5.02 at  $31.1^\circ\text{C}$ , other values are described in Table 1 along with calculated deviation.



**Figure 4 A:** Dry leaves, B: Dried leaf powder, C: Simple warty trichome, D: Micro measurement prismatic crystals, E: Micro measurement stomata (length), F: Micro measurement stomata (width), G: Micro measurement of prismatic crystal, H: Pleuricellular trichome, I: lignified group of vessels, J: Rhomboidal crystal, K: Fragment of lower epidermis, stomata (surface view), L: Fragment of upper epidermis (surface view).



**Figure 5 A:** HPTLC plate before spray at 366 nm, B: HPTLC plate after spray at 366 nm, C: HPTLC plate after spray in visible light, D: 3 d graph at uv vis range, E: all tracks 3 d graph at 254 nm, F: Peak display at 254 nm, G: 3 d graph at 366 nm, H: peak display at 366 nm, I: 3 d graph at 366 nm after spray, J: peak display after spray at 366nm, K: 3 d graph at 600 nm, L: peak display at 600 nm.

**Table 1: Physicochemical parameters of *Homalium ceylanicum* (Gardn.) Benth. Leaves.**

Parameters	Results (%W/W)
Loss on drying	8.66 ± 0.72
Total Ash value	7.83±0.19
Acid insoluble ash value	1.15 ±0.07
Water extractive value	19.80 ±1.98
Alcohol extractive value	11.17 ±0.88

### Qualitative study

Qualitative analysis on water and alcohol soluble extracts shows presence of carbohydrate, alkaloid and tannin. Results of other tests conducted are mentioned in Table 2. Carbohydrate and phenolic compounds are present in water and alcoholic extract of leaf, while alkaloid is found to be present only in alcoholic extract of leaf.

### HPTLC Study

The methanol extract of leaf shows 6 peaks, 5 peaks and 6 peaks at UV Vis range of 254nm, 366nm and 600 nm respectively. After spraying with

spray reagent 5 peaks are obtained at 366nm. The  $R_f$  values are presented in the Table 3 and the photographs along with peak display are shown in Figure 5.

### DISCUSSION

Leaves are simple, alternate with crenate, acuminate tip and glabrous which are key characters for identification of Family Flacourtiaceae.<sup>7</sup>

T.S. of petiole shows presence of unicellular trichomes and one main vascular bundle with three meristele which are becoming united to form an arc on the adaxial side are typical characters of Flacourtiaceae, leaf petiole's anatomy. T.S. of leaf shows simple unicellular trichome, cuticle showing split markings and presence of cluster crystals are special character of *Homalium* genus. Surface study shows paracytic type of stomata. Absence of stomata on upper surface are identifying characters of *Homalium* genus.<sup>12</sup> Moreover, the stomatal index marks as one of the main identification parameter for standardization. Micro-measurement of paracytic stomata in powder microscopic study can help in identification of the plant even in powder form. Values obtained from analytical study can be useful in identification and further in preparation of monograph. Carbohydrate and phenolic compounds are present in both extracts supporting the reference.<sup>13</sup> The chromatographic profile

**Table 2: Results of Qualitative analysis *Homalium ceylanicum* (Gardn.) Benth. Leaves.**

Tests	Water extract	Alcohol extract
Molisch's test	++	++
Fehling test	++	++
Dragendroff test	--	++
Haggers test	--	--
Wagner's test	--	--
Biuret	--	--
Ninhydrin test	--	--
Bountrager's test	--	--
Neu. Ferric chloride	++	++
Lead acetate	++	++
Copper sulphate	--	++
Seliwenoff's test	--	--
Salkowski test	--	--
Shinoda test	--	--
Vanillin + sulphuric acid	--	--

'++' - presence, '--' - absence

**Table 3: R<sub>f</sub> values obtained at UV and visible range of *H. ceylanicum* (Gardn.) Benth. Leaves.**

R <sub>f</sub> at 254 nm	R <sub>f</sub> at 366 nm	After spray (366 nm)	Visible (600 nm)
0.02	0.02	0.04	0.02
0.07	0.11	0.30	0.04
0.38	0.20	0.45	0.30
0.65	0.28	0.93	0.85
0.70	0.45	0.97	0.93
0.94	-	-	0.97

obtained for Methanolic extract of leaf can be helpful for further standardization of plant.

## CONCLUSION

The macroscopic characters like simple leaf, crenate margin, pubescent petiole and microscopic characters like presence of unicellular trichome, main vascular bundle uniting with three meristele forming an arc is observed in T.S. of petiole, cuticle showing split marking and presence

of cluster crystals in T.S. of leaf are identifying character of *Homalium ceylanicum* Benth. Presence of paracytic stomata in lower surface and absence of it in upper surface, stomatal index 15.94-16.91 are the key characters of *Homalium ceylanicum* Benth. The observed physicochemical, qualitative test and HPTLC results will help in further standardization of the plant.

## ACKNOWLEDGMENT

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## ABBREVIATIONS USED

**T.S:** Transverse section; **cm:** centimeter; **g:** gram/s; **w/w:** weight by weight; **nm:** nanometer; **HPTLC:** high performance thin layer chromatography; **R<sub>f</sub>:** retardation factor.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

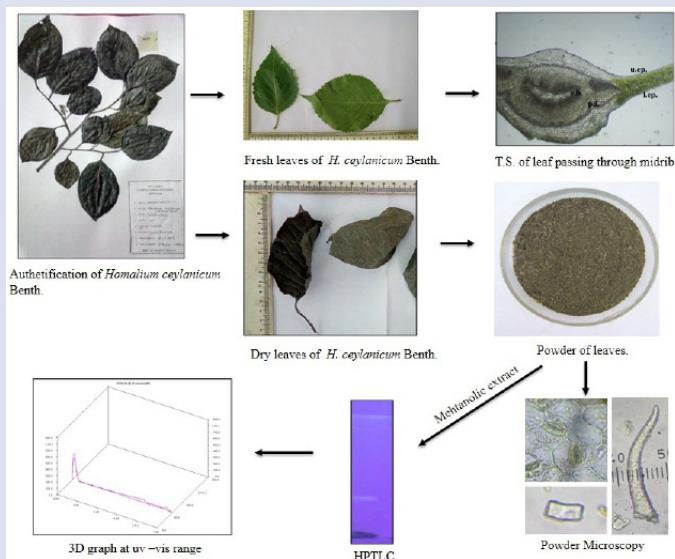
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## SUMMARY

- Kakhara*, *Dhanimari* or *Kakhda* is one of the folklore plant of Odisha, which has been identified as *Homalium ceylanicum* (Gardn.) Benth. (Syn. *H. zeylanicum*) belonging to family Salicaceae (Flacourtiaceae). The leaves and bark of the plant is used in rheumatism, diabetes and wound healing. The present study has been designed to study leaves of *Homalium ceylanicum* Benth. for its morphology, anatomy, physiochemical and phytochemical aspects. The leaves samples were collected from Gandhamardana hills, Odisha, in month of September 2016. The Leaves are simple, alternate, with crenate margin and petiole is pubescent. T.S. of petiole shows boat shaped with two protruding arms supported by 2 meristele. The schematic diagram of T.S. of petiole shows somewhat orbicular to boat shaped with two arms protruding supporting two meristele in each arm. Stomatal index is 15.94-16.91, powder is bitter with leafy aroma, microscopic shows paracytic stomata key character of genus. LOD is 8.66 ± 0.72 and carbohydrates are present in both extracts. the anatomical characters and values obtained from analytical study can help in standardization.

**GRAPHICAL ABSTRACT**



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