RESEARCH ARTICLE

Detailed pharmacognostical and phytochemical screening of *Homalium ceylanicum* (Gardn.) Benth. mature stem bark and developing stem

Rabinarayan Acharya[1]*, Switu Jani[1], C. R. Harisha[2], Vinay J. Shukla[3]

[1]Department of Dravyaguna, IPGT & RA, Gujarat Ayurved University, Jamnagar, Gujarat, India, [2]Pharmacognosy Lab, IPGT & RA, Gujarat Ayurved University, Jamnagar, Gujarat, India, [3]Pharmaceutical Chemistry Lab, IPGT & RA, Gujarat Ayurved University, Jamnagar, Gujarat, India

Abstract

Introduction: *Kakhara*, *Dhanimari* or *Kakhda* is one of the folklore plants of Odisha, which has been identified as *Homalium ceylanicum* (Gardn.) Benth. (Syn. *H. zeylanicum* Benth.) belonging to family Salicaceae (Flacourtiaceae). The stem bark and stem of the plant are used in the treatment of rheumatism, diabetes, malaria, and wound healing. Although it is used by tribal, scientifically the plant is yet to be evaluated for its pharmacognostical characters. Hence, stem bark and stem of *H. ceylanicum* are explored from its microscopic including powder microscopy, physiochemical, and preliminary phytochemical aspects. **Materials and Methods:** The plant was collected from Gandhamardana hills Paikamal - Odisha. Herbarium (Phm/6216/16-17) was authenticated by the pharmacognosist of the Institute. Pharmacognostical study, physicochemical and phytoconstituent study was performed following standard procedures. **Results:** Stem bark is light greenish to brown and stem is brown in color. T.S. of stem bark shows outermost cork cell layers, followed by cortical region often interrupted by discontinuous, irregular shaped patches of sclereid, and stone cells while stem shows vascular bundle with central pith. Bitter taste was observed in both samples. Physicochemical study reveals loss on drying of stem bark is 8.67; stem is 8.92. High-performance thin-layer chromatography study reveals similar R_f in both samples, i.e., 0.69 and 0.94 before spraying. **Discussion and Conclusion:** *H. ceylanicum* stem bark and stem show the presence of solitary prismatic and cluster crystal which is key characters of Salicaceae family.

Key words: *Homalium ceylanicum, Kakhara,* pharmacognosy, physicochemical, Salicaceae

INTRODUCTION

The use of plants or animal products for healing is as old as human civilization. Our country has rich floristic and ethnic diversity. Due to inaccessibility to modern health care, the real knowledge of the usage of plants lies with the rural populations of country which consists of tribals, other forest dwellers, and many villagers.[1] Some plants are used traditionally, but are not a part of classical texts of *Ayurveda* or pharmacopoeia and are enumerated into the category of ethnomedicinal plants or extra pharmacopoeial plants (*Anukta Dravya)*.[2] *Kakhara, Dhanimari* or *Kakhda*[3] is one of the folklore plants of Odisha. The leaves and bark of the plant are used in rheumatism, diabetes, and wound healing.[4] Botanically *Kakhara* has been identified as *Homalium ceylanicum* (Gardn.) Benth. (Syn. *H. zeylanicum*).[5] It belongs to family Salicaceae (Flacourtiaceae).[6] *H. ceylanicum* is a large evergreen tree, with simple, alternate crenate leaves bearing petiole, and raceme inflorescence.[7] Moreover, *Homalium nepalense* Wall. Benth. stem bark, which belongs to the same genus is also used to cure puerperal fever by oral administration of stem bark paste 10 g each thrice for single day.[8,9] Review of literature revealed that isolation of chemical

Address for correspondence:
Prof. Rabinarayan Acharya, Department of Dravyaguna, IPGT & RA, Gujarat Ayurved University, Jamnagar, Gujarat, India. Phone: 9924585855.
E-mail: drrnacharya@gmail.com

Received: 08-12-2017
Revised: 04-04-2018
Accepted: 02-05-2018

moieties such as coumarin 1 and epigallocatechin 2 is obtained from leaves and bark, further recently explored for antioxidant and anticancer activity,[10] but the scientific evaluation on various parts of the plants to establish its pharmacognostical characters has not been carried out. The present study has been designed to study *H. ceylanicum* Benth. mature stem bark and developing stem for its morphology, anatomy, physiochemical, and phytochemical parameters include high-performance thin-layer chromatography (HPTLC).

MATERIALS AND METHODS

Collection and Authentication

The mature stem bark and developing stem samples were collected by the first and second author from one of its natural habitat, Gandhamardan hills, Odisha, in the month of September 2016 with the 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].

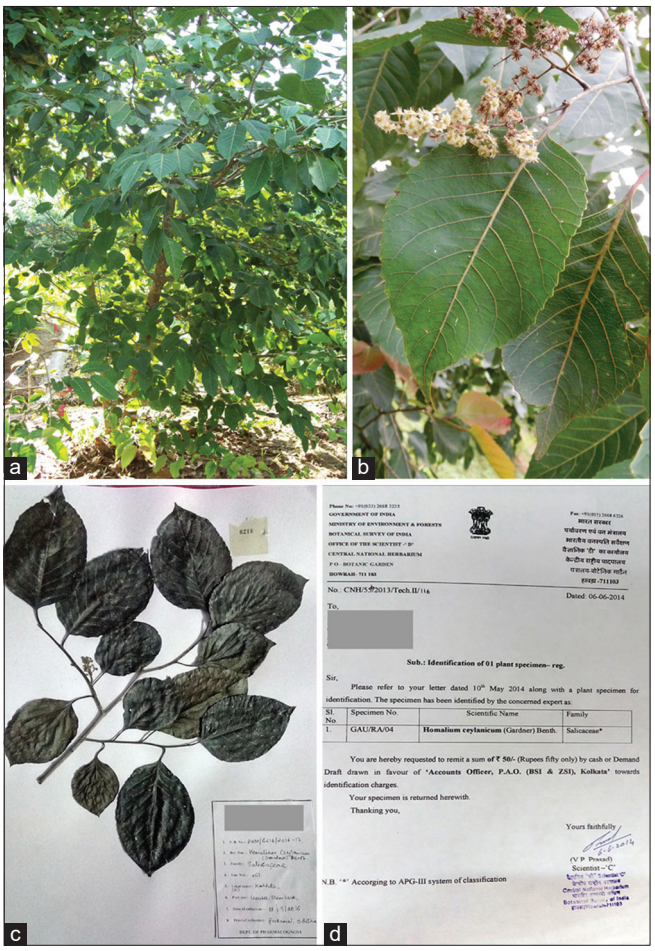

Figure 1: (a) Plant in natural habitat, (b) flowering twig of the tree, (c) herbarium phm/6216/2016-17, and (d) certificate from BSI

Pharmacognostical Study

Macroscopic observations were made with naked eyes and centimeter scale was used for measurement. The stem bark and stem were washed and transverse sections were taken cleared with chloral hydrate to observe the anatomy of stem bark and stem with help of Quasmo binocular compound microscope.

For powder microscopy, to obtain a powder shaded dried pieces of stem bark and stem were grounded by the mechanical grinder and sieved through 60#. For micrometry, triplicate reading was recorded and mean value was taken into consideration along with standard deviation.[11]

Physicochemical Parameters and Qualitative Analysis

Powders of stem bark and stem were exposed to physicochemical, 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.[12,13]

HPTLC Study

Methanolic extract of stem bark and stem was 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_{GF254} Plates.
Chamber saturation: 30 min.
Development time: 30 min.
Scanner: Camag Scanner III.
Detection: Deuterium lamp, Tungsten Lamp
Data system: Win cats software.

Spray Reagent

Preparation: 0.5 g vanillin is dissolved in 100 ml sulphuric acid-ethanol (40+10). Treatment after spraying: Heated at 120°C until maximum spot color intensity was reached.[14]

RESULTS AND DISCUSSION

Macroscopic Study

Mature stem bark

Outer surface of mature stem bark is light greenish to brown in color whereas inner surface is yellowish white in color,

slightly aromatic odor with short fracture. The cut pieces measures about 6.5–2.8 × 3.2–2.5 in cm. Stem bark rolls into single quill only from one side [Figure 2].

Developing stem

Developing stem cut pieces measures about 21.5–30 cm in length and 0.3–0.7 cm in diameter. Outer surface light to dark brown in color while the inner surface is creamish white, stem possess proper nodes and internodes, creamish white lenticels, longitudinal striations, and slightly aromatic odor with a short fibrous fracture [Figure 3].

Microscopic Study

T.S. of stem bark (mature)

T.S. of stem bark measures about 5.5–6.7 µm × 1.7–2.3 µm. Outermost layer consists of 7–9 layers cork cells which are tabular in shape arranged one after the other followed by several layers of cortical cells often interrupted by discontinuous and irregular shaped patches of scleride and stone cells.

Cortical cells often embedded with prismatic crystals, cluster crystals and rhomboidal crystals along with oil globules. Initial layers of cortical cells contain green contents.

T.S. of stem bark after staining reveals 2–3 initial layers of cork cells are lignified. Irregular shaped patches of stones cells and scleride are also lignified. Some scleride are with the wide lumen and pitted [Figure 3].

T.S. of stem (developing)

Schematic diagram of young stem T.S. is circular in outline often interrupted by simple unicellular trichomes, followed by cortex, pericyclic fiber, endodermis, and centrally located stellar region with wide pith.

Detailed T.S. of developing stem shows single layer epidermis covered with thick layer of cuticle often interrupted by simple unicellular trichome. Trichomes mostly filled with yellow content, followed by 10–12 layer of cortical cells embedded with prismatic, rhomboidal crystals of calcium oxalate, starch grains, and oil globules. Cortex is followed by a discontinuous ring of lignified pericyclic fibers. Single layer

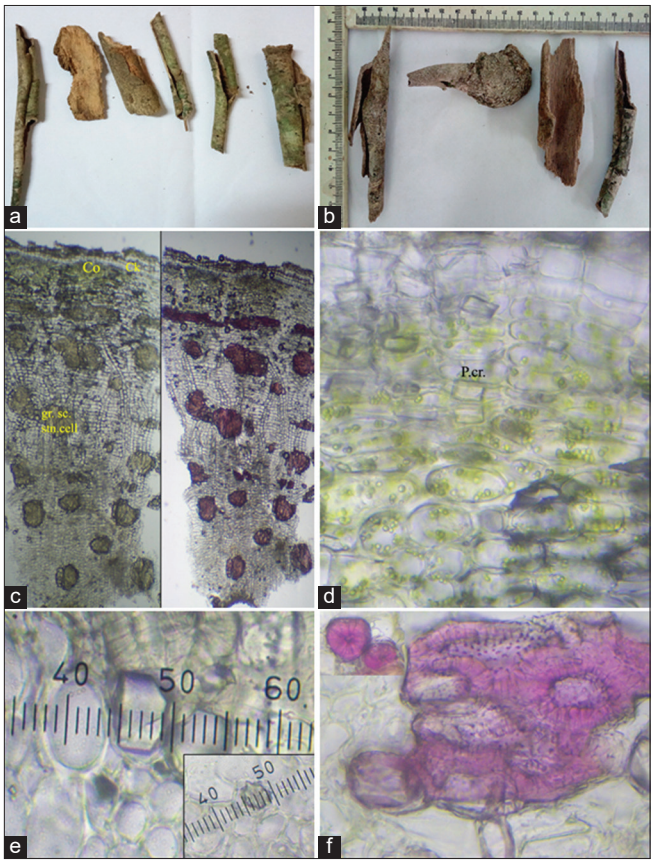

Figure 2: (a) Fresh mature stem bark cut pieces, (b) dry mature stem bark cut pieces, (c) T.S. of mature stem bark unstained and stained, (d) prismatic crystal in cortex, (e) micro-measurement of rhomboidal and cluster crystals, and (f) group of lignified stone cells and sclereids. Co: Cortex, Ck: Cork, gr.sc.stn.cell: Group of sclereids and stone cells, P.cr.:Prismatic crystal, s.ph.: Secondary phloem

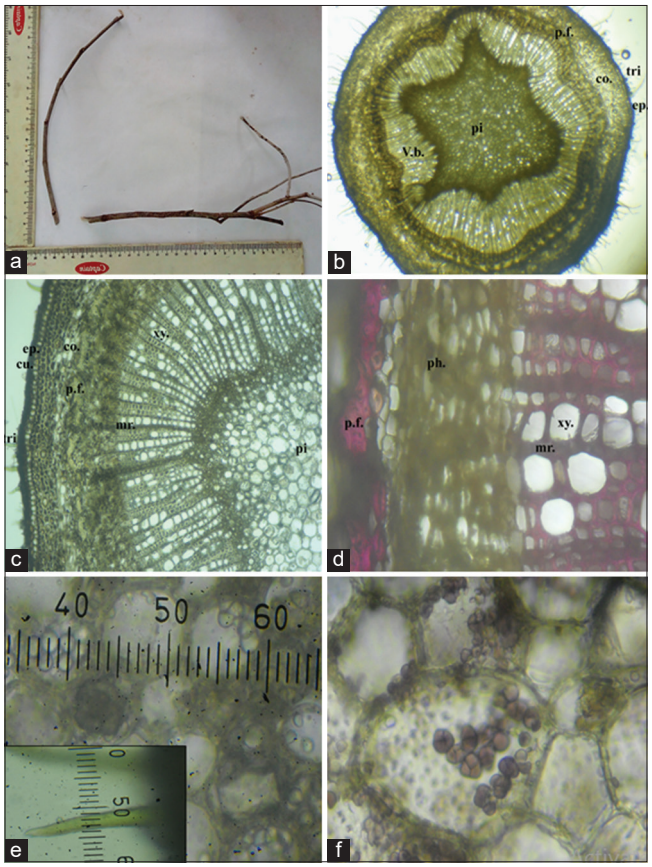

Figure 3: (a) Tender stem cut pieces, (b) Schematic T.S. of developing stem, (c) detailed T.S. of developing stem, (d) lignified pericyclic fibers, xylem and its parenchyma, (e) micro-measurement of rosette crystal and trichome, and (f) compound starch grains stained (iodine). tri – trichome, cu.- cuticle, co. – cortex, p.f.- pericyclic fiber, ep. – epidermis, mr. – medullary rays, pi- pith, ph. – phloem, xy.- xylem

endodermis is followed by main stellar region consisting of xylem, phloem medullary rays, and centrally located pith. Phloem is located just beneath the endodermis; metaxylem is situated toward periphery and protoxylem toward the pith along with uni-biserrate medullary rays often occupied with ergastic substances. Centrally located pith consists of parenchyma cells, some of which are embedded with starch grains and prismatic crystals of calcium oxalate. Some of the parenchymatous pith cells are lignified and pitted [Figure 3].

Powder Microscopy

Stem bark (mature)

Organoleptic characters

Color brownish cream, odor slightly aromatic, and taste initially sweet followed by bitter and touch are rough and fibrous.

Powder microscopy

Powder microscopy reveals the presence of stone cells, scleride, prismatic crystals, starch grains, cork cells in tangential view, pitted cortical cells in surface view, pitted scleride, and brown content [Figure 4]. The micrometric values of cellular substances are enumerated in Table 1.

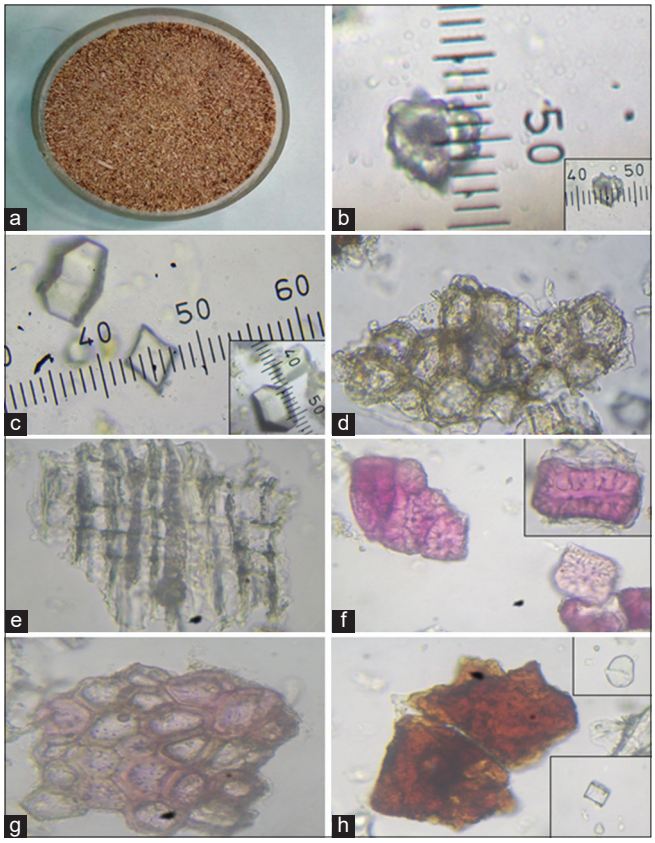

Figure 4: (a) Powder of mature stem bark, (b) micro-measurement of rosette crystal, (c) micro-measurement of prismatic and rhomboidal crystals, (d) cork cells in surface view, (e) fiber passing through medullary rays, (f) lignified stone cells and sclereids, (g) lignified cork cells in surface view, (h) brown content, starch grain, and rectangular prismatic crystal

Stem (developing)

Organoleptic characters

Color creamish brown, odor slightly aromatic, taste bitter and touch are rough and fibrous.

Table 1: Depicts the micrometric values of ergastic substances observed in stembark and stem powder microscopy

Cellular content	Measurement (μm) at 40×	
	Stem bark (mature)	Stem (developing)
Rectangular prismatic crystal	0.63×0.4	0.7×0.46
Rhomboidal prismatic crystal	0.63×0.43	0.75×0.55
Cluster crystal	0.65×0.45	0.5×0.5
Diamond shaped prismatic crystal	0.65×0.47	Absent
Rosette crystal	0.5×0.5	Absent
Trichomes	Absent	3.2×0.37
Starch grains	Absent	0.3×0.35

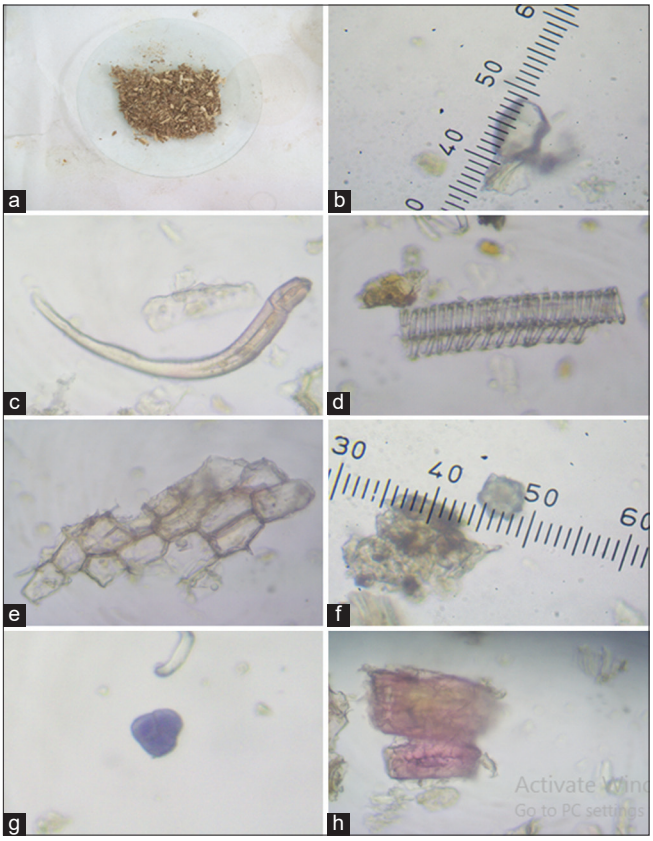

Figure 5: (a) Powder of developing stem, (b) micro-measurement of rhomboidal crystal, (c) trichome, (d) lignified spiral vessel, (e) cork cells in tangential view, (f) micro-measurement of rosette crystal, (g) compound starch grains, (h) lignified stone cells

Powder microscopy

Powder microscopy shows presence of crystal fiber, pitted vessel, rhomboidal crystals, prismatic crystals, fragment of trichomes, compound starch grains, group of stone cells, fragment of stomata with brown content, simple starch grains, oil globules, border pitted vessels, fragment of cork cells in surface view, fragment of parenchyma cells, scleride, brown content, and fragment of fibers [Figure 5].

Physicochemical Parameters

The pH of mature stem bark and developing stem water extract was found to be 5.7 and 5.4 at 31.7°C. The other values are described in Table 2 along with calculated deviation.

Table 2: Physicochemical parameters of *Homalium ceylanicum* (Gardn.) Benth. stem bark and stem

Parameters	Results (%w/w)	
	Stem bark (mature)	Stem (developing)
Loss on drying	8.67±0.25	8.92±1.25
Total ash value	3.46±0.28	7.12±0.25
Acid insoluble ash value	1.89±0.18	0.17±0.05
Water extractive value	7.00±0.49	10.87±1.02
Alcohol extractive value	3.22±0.46	17.15±1.18

Qualitative Tests

Qualitative analysis on water and alcohol soluble extracts of stem shows the presence of carbohydrate, alkaloid, and tannin. Results of other tests conducted are mentioned in Table 3.

HPTLC Study

The methanol extract of mature stem bark shows 7 peaks, 4 peaks, and 7 peaks at UV Vis range of 254 nm, 366 nm, and 600 nm, respectively. After spraying with spray reagent, 5 peaks are obtained at 366 nm. The R_f values are presented in Table 4, and the photographs and peak display are shown in Figure 6. The methanolic extract of developing stem shows 6 peaks, 5 peaks, and 7 peaks at UV Vis range of 254 nm, 366 nm, and 600 nm, respectively. After spraying with spray reagent, 6 peaks are obtained at 366 nm. The R_f values are presented in Table 4, and the photographs and peak display are shown in Figure 7. Comparative spectra at similar R_f, i.e., 0.69, 0.94 at 254 nm and 366 nm of stem bark and stem obtained before spraying are displayed in Figure 8.

DISCUSSION AND CONCLUSION

The outer surface of mature stem bark in fresh condition is greenish in color due to the presence of molds, which are thoroughly removed after proper washing hence green color is absent in dry stem bark. Transverse section of mature stem

Table 3: Qualitative tests of an aqueous and methanolic extract of *Homalium ceylanicum* (Gardn.) Benth. stem bark and Stem

Tests	Stem Bark (mature)		Stem (developing)	
	Water extract	Alcohol extract	Water extract	Alcohol extract
Molisch's test	--	--	++	++
Fehling test	--	--	--	--
Dragendorff test	++	--	--	--
Haggers test	--	--	--	--
Wagner's test	++	++	--	--
Biuret	--	--	--	--
Ninhydrin test	--	--	--	--
Borntrager's test	--	--	--	--
Neu. Ferric chloride	--	--	++	++
Lead acetate	--	++	--	--
Copper sulfate	--	++	--	--
Seliwanoff's test	--	--	--	--
Salkowski test	--	--	++	
Shinoda test	--	--	--	--
Vanillin+sulfuric acid	--	--	++	++

--: Negative, ++: Positive

bark and developing stem shows the presence of solitary rhomboidal crystals and few cluster crystals in the cortical region which are key identification characters of genus *Homalium*.[15]

The above-mentioned values from physicochemical parameters and preliminary qualitative analysis can be helpful in identification and further standardization of stem bark and stem of *H. ceylanicum* (Gardn.) Benth. The

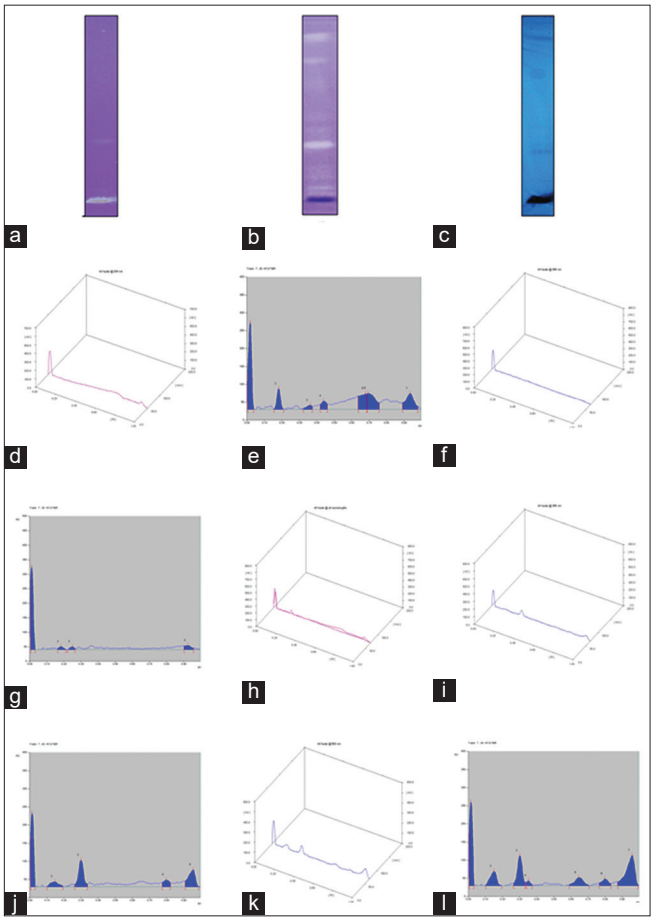

Figure 6: (a) High-performance thin-layer chromatography (HPTLC) plate of stem bark at 366 nm (before spray), (b) HPTLC plate of stem bark at 366 nm (after spray), (c) HPTLC plate of stem bark at 600 nm, (d) 3d display at 254 nm, (e) peak display at 254 nm, (f) 3D display at 366 nm, (g) peak display at 366 nm, (h) all tracks at all wave lengths, (i) 3D display at 366 nm after spray, (j) peak display at 366 nm after spray, (k) 3D display of all tracks at 600 nm, (l) 2D peak display at 600 nm

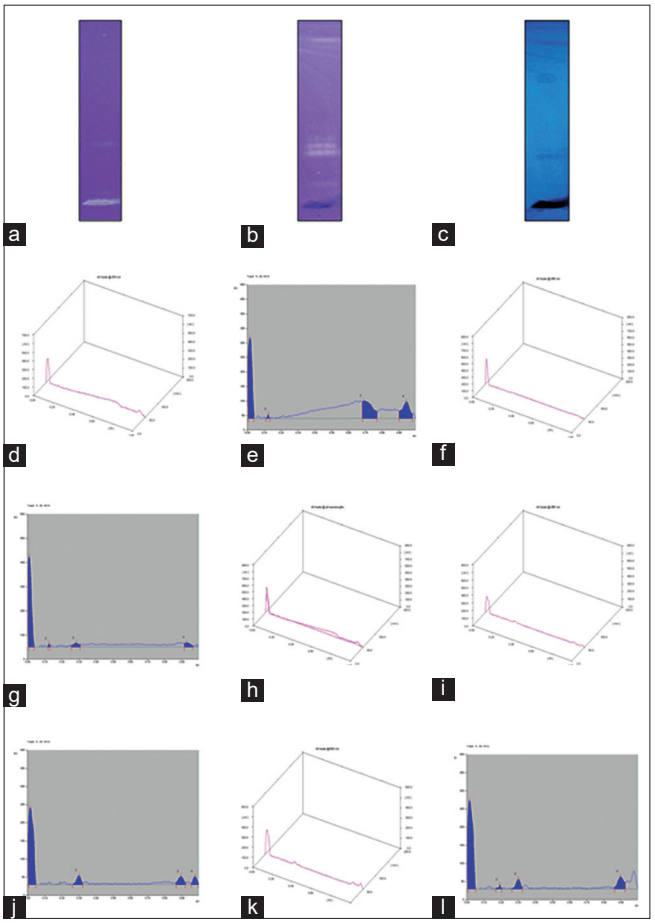

Figure 7: (a) High-performance thin-layer chromatography (HPTLC) plate of stem at 366 nm (before spray), (b) HPTLC plate of stem at 366 nm (after spray), (c) HPTLC plate of stem at 600 nm, (d) 3D display at 254 nm, (e) peak display at 254 nm, (f) 3D display at 366 nm, (g) peak display at 366 nm, (h) all tracks at all wave lengths, (i) 3D display at 366 nm after spray, (j) peak display at 366 nm after spray, (k) 3D display of all tracks at 600 nm, (l) 2D peak display at 600 nm

Table 4: Various R_f values at Uv-vis range methanolic extract of *Homalium ceylanicum* (Gardn.) Benth. Mature stem bark and developing stem

R_f at 254 nm		R_f at 366 nm		After spray (366 nm)		Visible (600 nm)	
Stem bark	Stem	Stem bark	Stem	Stem bark	Stem	Stem bark	Stem
0.02	0.02	0.01	0.02	0.01	0.01	0.01	0.02
0.18	0.07	0.18	0.11	0.15	0.26	0.15	0.04
0.36	0.38	0.25	0.20	0.30	0.42	0.30	0.30
0.44	0.65	0.93	0.28	0.80	0.90	0.35	0.85
0.68	0.70	No spot	0.45	0.96	0.94	0.65	0.93
0.69	0.94	No spot	No spot	No spot	No spot	0.80	0.97
0.94	No spot	No spot	No spot	No spot	No spot	0.96	No spot

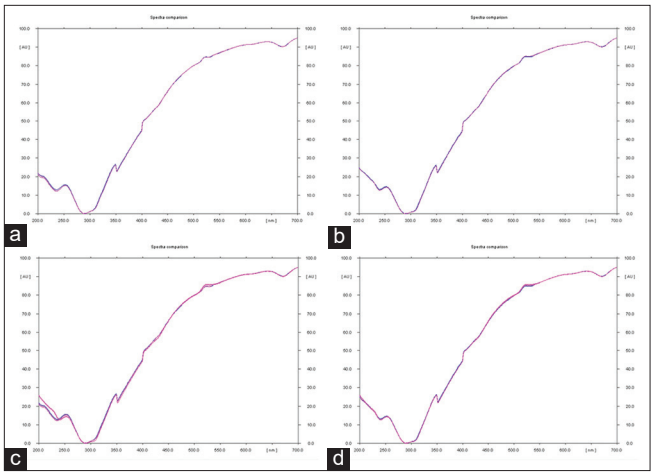

Figure 8: (a) Comparative spectra R_f 0.69 at 254 nm, (b) comparative spectra R_f 0.94 at 254 nm, (c) comparative spectra R_f 0.69 at 366 nm, (d) comparative spectra R_f 0.94 at 366 nm

comparative spectra at similar R_f obtained from HPTLC study may be indicative of the presence of similar chemical moiety in stem bark and stem which could be useful for further standardization of biomarker for this plant.

ACKNOWLEDGMENT

We would like to thank Ministry of AYUSH, Government of India, and Director of IPGT and RA, Gujarat Ayurved University, for funding the project and all the supporting laboratory heads and staff members of IPGT and RA.

REFERENCES

1 Anonyumous. An Appraisal of Tribal Folk Medicines. 1st ed. New Delhi: CCRAS; 1999. p. 1-2.
2 Retnam KR, Martin P. Ethnomeidicinal Plants. Jodhpur: Agrobios; 2008. p. 1.
3 Brahmam M, Saxena HO, Flora of Orissa. Vol 1. Orissa: Regional Research laboratory; 1994. p. 522.
4 Prasad KS, Shirivastava B, Yanadaiah JP. Assessment of analgesic and anti-inflammatory effect of *Homalium zeylanicum.* J Glob Trends Pharm Sci 2014;5:1886-90.
5 International Plant Name Index; 2004. Available from: http://www.ipni.org/ipni/idplantnamesearcg.do. [Last accessed on 2016 Aug 16].
6 Krikup SZ, Kew H. Interactive Key to Genera of *Salicaceae* sensu lato and *Achariaceae* sensu lato. Available from: http://www.kew.org/herbarium/keys/flacs/_baks/index.html.0006.d323.bak. [Last accessed on 2016 Aug 16].
7 Gamble JS. Flora of the Presidency of Madras. Vol. 1. Dehradun: Bishen Singh, Manhendra Pal Singh; 2011. p. 522.
8 Rao JK, Seetharami Reddi TV, Kumar OA. Ethnobotany of stem bark of certain plants of Visakhapatnam district, Andhra Pradesh. Curr Bot 2011;2:1-6.
9 Rao JK, Suneetha J, Seetharami Reddi TV, Kumar A. Ethnomedicine of the *Gadabas*, a primitive tribe of Visakhapatnam district, Andhra Pradesh. Int Multidiscip Res J 2011;1-2:10-4.
10 Rachala VK, Katakam B, Lakuma D, Niranjan KA, Kvns S, Kotesh K, *et al*. Phytochemical constituents and biologicalactivities of *Homalium zeylanicum* benth. Int J Pharm Biosci 2014;5:176-82.
11 Saunders Elsevier. Trease and Evans. Pharmacognosy. 16th ed. London: Saunders Elsevier; 2009. p. 567-68.
12 Anonymous. The Ayurvedic Pharmacopoeia of India. Part- II. 1st ed., Vol. 2. New Delhi: Government of India, Ministry of Health and Family Welfare, Department of AYUSH; 2008.
13 Khandelwal KR. Practical Pharmacognosy. 19th ed. Pune: Nirali Prakashan; 2008.
14 Egon S. Thin Layer Chromatography. Berlin Heidelberg: Springer Publication; 1969, Reprint 2005.
15 Metcalfe CR, Chalk L. Anatomy of the Dicotyledons. Vol. 1. Great Britain: Oxford University Press; 1950. p. 121.

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