ORIGINAL ARTICLE

Pharmacognostical, physicochemical, and phytochemical evaluation of *Samarakhadyam* (*Byttneria herbacea* Roxb.) whole plant – An extra pharmacopoeial drug of Ayurveda

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Abstract

Introduction: *Byttneria herbacea* Roxb., family Sterculiaceae, is a small herb found in several parts of India. The plant as a whole is reported for traditional use in the management of dysentery, cuts, wounds, syphilis, cholera, diarrhea, leukorrhea, fracture of limbs, ulcers, and sprains, etc., in either powder or decoction form. Although used traditionally, whole plant powder has not been evaluated scientifically for its pharmacognostical and phytochemical characters till date. **Objective:** The objective of the study was to evaluate pharmacognostic, preliminary physicochemical, and phytochemical profiles including high-performance thin-layer chromatography of *B. herbacea* whole plant powder. **Materials and Methods:** *B. herbacea* was collected from Gandhamardan hill ranges of Odisha; shade dried and powdered through the mechanical grinder. Its powder microscopy, preliminary physicochemical, and phytochemical tests were investigated following standard recommended procedures of Ayurvedic Pharmacopoeia of India. Methanolic extract of the whole plant powder was determined for chromatographical evaluations. **Results:** Powder microscopy of the whole plant showed the presence of identifying characters such as ample amount of simple and compound starch grains, diacytic stomata, simple and stellate trichomes, annular vessels, fragments of spiral vessels, rhomboidal crystals, and brown content (tannin). Water-soluble extractive value (13.136%) was more than methanol soluble extractive value (5.652%), and pH was 6.5. The preliminary phytochemical study disclosed the presence of alkaloids, glycosides, tannins, flavonoids, and other phenolic compounds. Chromatography study showed eight spots at 254 nm and seven spots at 366 nm. **Conclusion:** The findings of the study are going to be helpful in the identification and standardization of *B. herbacea* whole plant.

Key words: *Anukta dravya, Byttneria,* HPTLC, microscopy, phytochemical, *Samarakhai*

INTRODUCTION

The plant *Samarakhadyam* is identified as *Byttneria herbacea* Roxb. belonging to Sterculiaceae family, is a branched, unarmed herb with a perennial woody rootstock, glabrous ovate-acuminate toothed leaves, small pale purple flowers in short cyme, and pale green capsule covered with soft subulate prickles. It is commonly found in peninsular India from Gujarat Southwards to Tamil Nadu and in Odisha and Bihar.[1,2] Review of literature

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Received: 23-02-2019
Revised: 17-07-2019
Accepted: 11-08-2018

reveals that few works have been reported on *B. herbacea* which include review on ethnomedicinal claims,[3] anti-edemogenic activity,[4] anti-inflammatory activity,[5] anti-asthmatic activity,[6] and antioxidant activity.[7]

Although various parts of plants are used by tribals, the whole plant is not evaluated in a scientific way for its pharmacognostical, physicochemical characters, and phytochemical constituents. Hence, the present study is

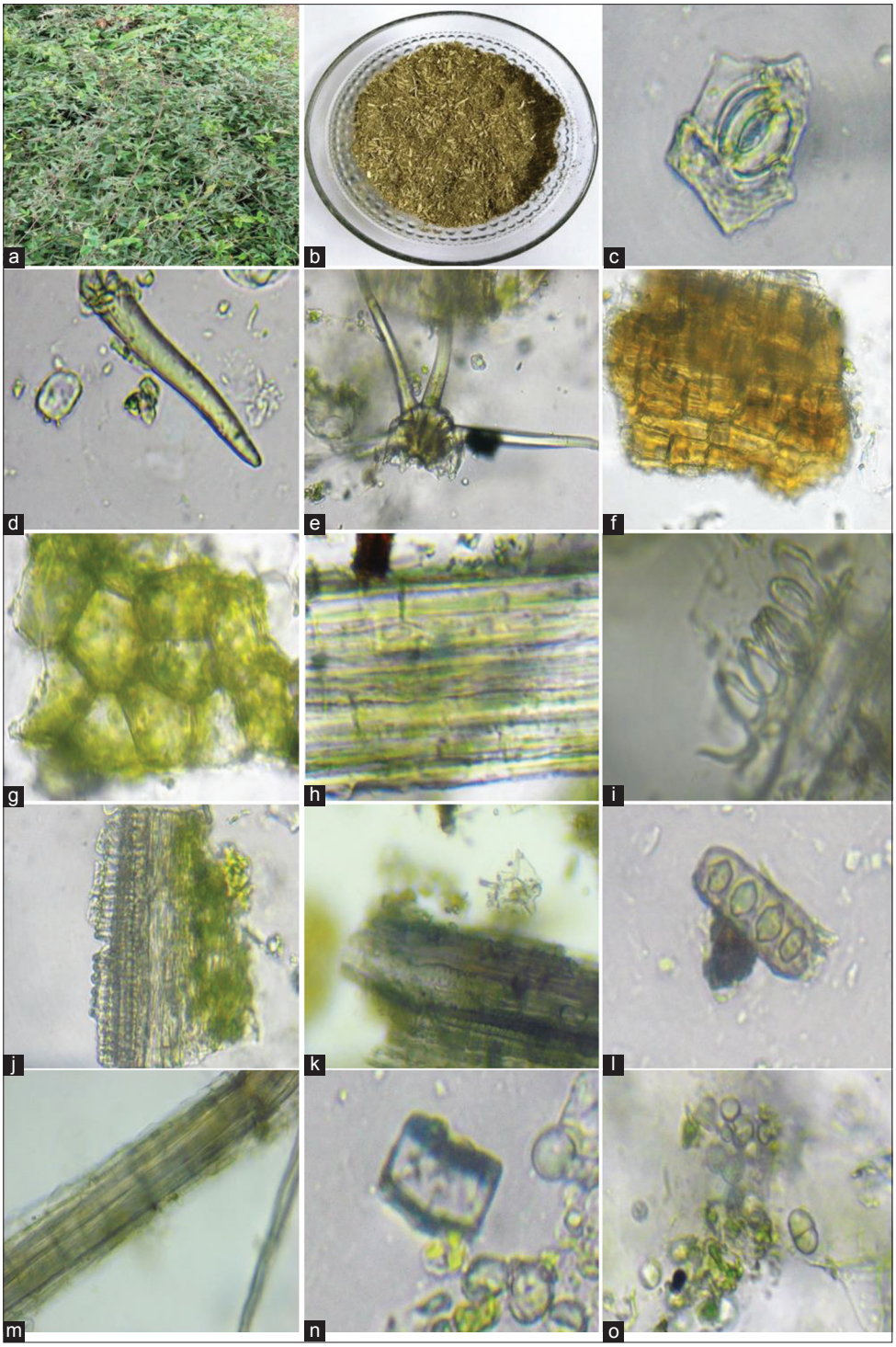

Figure 1: Powder microscopy of the whole plant of *Byttneria herbacea* Roxb. (a) *B. herbacea* in natural habitat, (b) whole plant powder, (c) anomocytic stomata, (d) simple trichome, (e) fragment of stellate trichome, (f) cork cells in tangential view with brown content, (g) epidermal cells in surface view, (h) group of fibers, (i) fragment of spiral vessel, (j) annular vessels, (k) pitted vessels, (l) fragment of crystal fiber, (m) fibers passing through medullary rays, (n) rhomboidal crystal, (o) simple and compound starch grains

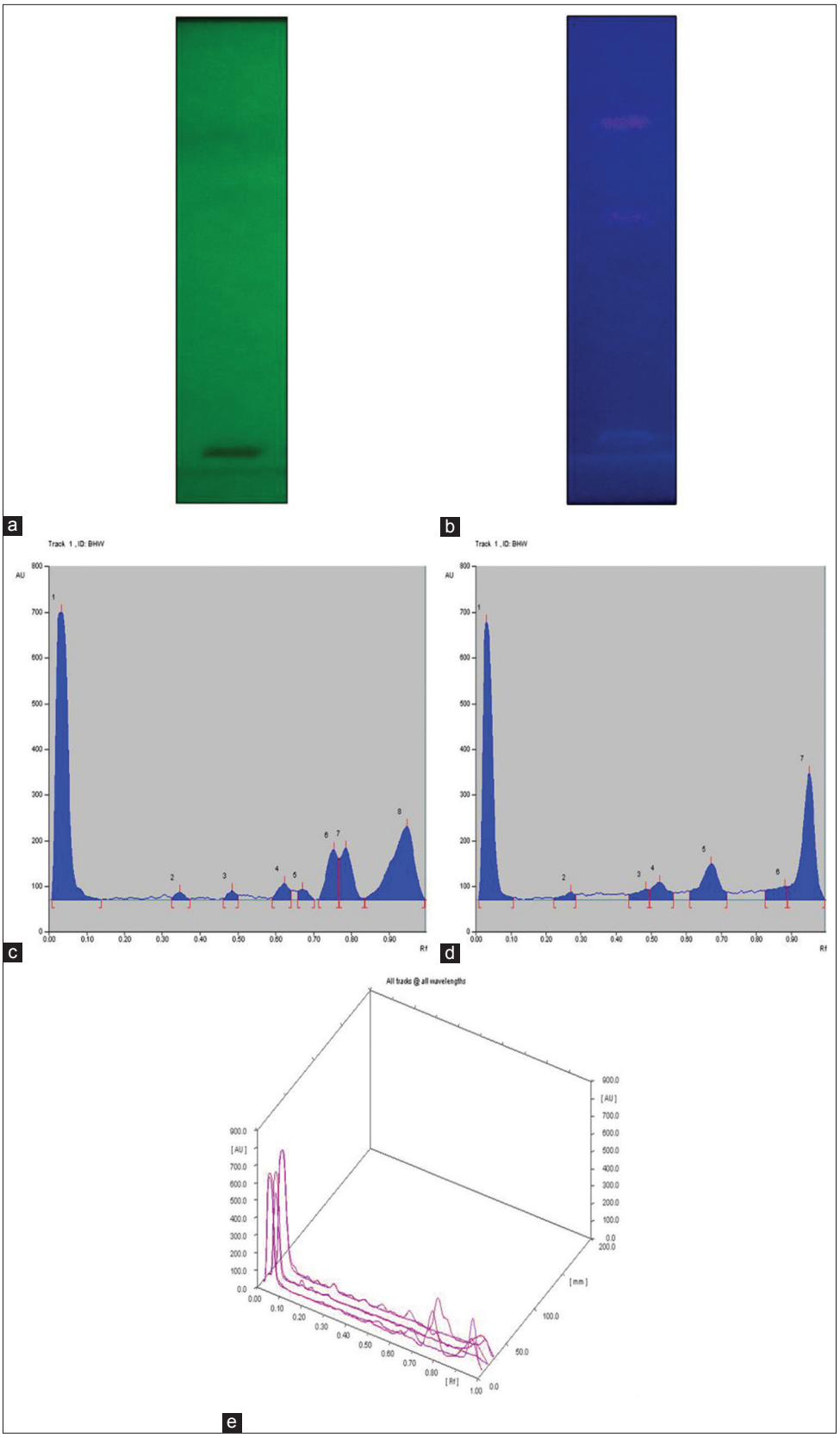

Figure 2: High-performance thin-layer chromatography (HPTLC) of whole plant of *Byttneria herbacea* Roxb. (a) HPTLC plate at 254 nm, (b) HPTLC plate at 366 nm, (c) peak display at 254 nm, (d) peak display at 366 nm, (e) 3 d graph at 254 nm and 366 nm

planned to evaluate microscopical and physicochemical characters together with different qualitative tests including high-performance thin-layer chromatography (HPTLC) of *B. herbacea* whole plant powder.

MATERIALS AND METHODS

Collection, Authentication, and Preservation of the Sample

Samarakhadyam, growing in Gandhamardan hill ranges of Paikmal, Bargarh district of Odisha, India, was identified as *B. herbacea* Roxb., belonging to Sterculiaceae family, on the basis of its morphological characters. Plant specimen was authenticated at BSI Kolkata with letter no. CNH/2016/Tech.II/68. The herbarium was prepared and submitted to Pharmacognosy lab., Institute for Postgraduate Teaching and Research in Ayurveda, Jamnagar, vide herbarium no. Phm. 6200/16-17, for future reference. The collected plant samples were shaken to remove adherent soil and dirt. The roots and leaves were separated from the stem, washed under running fresh tap and then with reverse osmosis water. Then, they were shade dried and powdered by mechanical grinder and sieved through 60# for powder microscopy, physicochemical parameters, and qualitative analysis. The whole plant powder was stored in airtight glass container.

Powder Microscopic Evaluation

The sample was kept on a slide and studied under a microscope using distilled water. The samples were additionally examined after staining with different appropriate reagents, i.e., phloroglucinol (20 mg/ml of alcohol) together with hydrochloric acid (6 N), ferric chloride (5% w/v in 90% alcohol), and iodine solution (2 g iodine and 3 g potassium iodide in 100 ml water)[8] under compound microscope and photographs were taken using Kodak EasyShare C140 HD camera.

Physicochemical Analysis

Assessment of the parameters such as moisture content, foreign matter, ash value, acid insoluble ash, water-soluble extractive, alcohol soluble extractive, and pH was carried out by following standard procedures recommended by Ayurvedic Pharmacopoeia of India and other standard texts.[9,10]

Phytochemical Analysis

Exactly weighed 5 g of *B. herbacea* whole plant powder macerated with methanol (100 ml), keeping it for overnight with initial occasional shaking up to 6 h, and then set aside. After 24 h, it was filtered, and the methanolic extract was collected. Various qualitative tests were performed for methanolic extract of whole plant powder, following standard procedure.[11]

HPTLC Study

Extraction of plant material

Sample of the whole plant of *B. herbacea* (2 g) was soaked in 20 ml of methanol and kept overnight. Next day, it was boiled for 10 min. and filtered. The filtrate was concentrated to 10 ml in a standard flask.

Method

HPTLC study of the extract was carried out by pre-coated silica gel 60 F_{254} plates which possess standardized adsorption layers, at room temperature. All the solvents systems were selected by trial and error method. The chromatogram was developed in CAMAG Twin through glass chambers on 10 cm × 10 cm plates till the mobile phase traveled up to distance of 8 cm from starting point. After development, the plate was dried at room temperature for 5–10 min and observed under ultraviolet (UV)-254 nm and UV-366 nm wavelength and R_f value were recorded.[12]

HPTLC fingerprinting profile

One track as 10 µL of the sample was applied on an E. Merck aluminum plate pre-coated with silica gel 60 F_{254} of 0.2 mm thickness using Linomat IV applicator.

Development of solvent system

A number of the solvent system was tried to find out the best mobile phase. The solvent system toluene:ethyl acetate:diethylamine (7:2:1) gave best resolution and maximum no. of spots.

Development of chromatogram

The chromatogram was developed in CAMAG Twin through glass pre-saturated with mobile phase, i.e., toluene:ethyl acetate:diethylamine (7:2:1) up to distance at 80 mm.

Scanning and detection of spots

The developed and air-dried chromatoplate was at 254 nm and 366 nm to obtain planer chromatogram. Scanning was performed by CAMAG HPTLC densitometer in absorbance mode at 254 nm and 366 nm and color of the resolve bands, R_f values were noted.

RESULTS AND DISCUSSION

Powder Microscopy

Organoleptic characters of whole plant powder show light greyish green color, slightly aromatic odor, astringent-bitter taste, and coarse fibrous touch.

The diagnostic characters of the *B. herbacea* whole plant powder show presence of diacytic stomata, fragment of simple trichome and stellate trichome, cork cells in tangential view and surface

view filled with brown (tannin) content, lignified cork cells, epidermal cells in surface view and along with starch cells, group of fibers, fragment of spiral vessels and crystal fibers, annular vessels and pitted vessels, rhomboidal crystals with oil globule, simple starch grains, and compound starch grains [Figure 1a-o].

Preliminary Physicochemical Analysis

Whole plant powder was found to be devoid of any foreign matter. Detailed results of physicochemical analysis are given in Table 1.

Preliminary Phytochemical Analysis

The preliminary phytochemical test revealed presence of alkaloids, steroids, tannins, flavonoids, and phenolic compounds in methanolic extract of whole plant. The results of tests performed are portrayed in Table 2.

Table 1: Preliminary physicochemical analysis of *Byttneria herbacea* whole plant powder

Physicochemical parameters	Results (%)
Loss on drying	10.8568 w/w
Ash value	5.6855 w/w
Acid insoluble ash value	0.3368 w/w
Water-soluble extractive value	13.1369 w/w
Methanol soluble extractive value	5.6528 w/w
pH value (in aqueous extract)	6.5

Table 2: Qualitative analysis of whole plant of *Byttneria herbacea* in methanol extract

Phytochemical tests	Results
Test for carbohydrates	
Molisch's test	-
Test for glycosides	
Keller–Killiani test	+
Test for saponins	
Foam test	-
Test for alkaloids	
Dragendorff's test	+
Test for flavonoids	
Alkaline reagent test	+
Test for phenolic compounds and tannins	
Lead acetate test	+
Test for steroids	
Salkowski test	+
Test for proteins	
Biuret test	-
Test for amino acids	
Ninhydrin test	-

"+": Present, "-": Absent

Table 3: R_f values obtained at short ultraviolet (UV) light (254 nm) and long UV light (366 nm) of whole plant powder of *Byttneria herbacea*

R_f at 254 nm	R_f at 366 nm
0.03	0.03
0.35	0.27
0.48	0.48
0.62	0.52
0.67	0.67
0.75	0.88
0.79	0.95
0.95	-

HPTLC

The methanol extract of the whole plant powder showed 8 peaks and 7 peaks at UV range of 254 nm and 366 nm, respectively. Common Rf values 0.03, 0.48, and 0.67 were found under UV range of 254 nm and 366 nm. The Rf values are mentioned in Table 3. The photos of HPTLC plates, 3d graphs, and peak display at UV ranges are depicted in Figure 2 (a-e).

DISCUSSION

B. herbacea Roxb. is branched, unarmed herb with perennial woody rootstock and glabrous ovate-acuminate toothed leaves. Whole plant powder shows that the presence of diacytic stomata, simple and stellate trichomes, brown (tannin) content, fragment of spiral vessels and crystal fibers, annular vessels and pitted vessels, rhomboidal crystal with oil globule and ample amount of simple and compound starch grains, are the key characters.

Whole plant powder was found to be devoid of any foreign matter, which may be due to the good harvesting practice followed during the collection of the drug. Loss on drying of whole plant was 10.8568% w/w. The loss on drying of any sample is directly related to its moisture content. An excess of water in medicinal plant materials can encourage microbial growth, the presence of fungi or insects should have an effect on its preservation.[13] Water-soluble extractive value (13.1369% w/w) of whole plant has been found more in compare to methanol soluble extractive value (5.6528% w/w) which indicates the probability of the presence of high water-soluble constituents than the alcohol-soluble constituents. Water-soluble and alcohol soluble extractive values are indicative of the bioavailability of the plant. pH value of water extract of whole plant was 6.5 which indicates its weak acidic nature.

The ash value indicates the presence of inorganic and salt materials within the sample. This includes both "physiological ash" which comes from the plant tissue itself, and "non-physiological" ash, which is the residue of the extraneous matter (sand and soil) adhering to the plant surface.[13] Ash value of whole plant was found to be 5.6855% w/w. Acid-insoluble ash designates the presence of more siliceous matter in the drug. Acid-insoluble ash value was 0.3368% w/w.

The results acquired from physicochemical parameter, preliminary phytochemical, and HPTLC study will serve as standardization values providing information regarding the authentification of the plant *B. herbacea*.

CONCLUSION

B. herbacea Roxb., family Sterculiaceae, is a small perennial branched herb. The powder form of the whole plant depicts that the presence of diacytic stomata, stellate trichome, brown (tannin) content, fragment of spiral vessel, crystal fibers, annular vessels, pitted vessels, and rhomboidal crystal, are the key identification characters of the plant. Whole plant powder portrays the presence of different types of functional groups such as cardiac glycosides, alkaloids, flavonoids, tannins, and steroids. Physicochemical, phytochemical and HPTLC results will help in further standardization and acts as standards for quality assurance.

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