ORIGINAL ARTICLE

Pharmacognostic study and establishment of quality parameter for medicinal plant of Dipterocarpaceae in Northeastern state Tripura

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Abstract

Background: *Dipterocarpus turbinatus* and *Shorea robosta* are widely distributed in the Northeastern state of Tripura. Tribals of Tripura used both the plant and plant derived product for various purposes in their daily life. **Aim:** The present investigation deals with the macroscopic, microscopic, and preliminary phytochemical screening to establish quality parameters for evaluation of the bark and bark powder of *D. turbinatus* and *S. robosta*. **Materials and Methods:** The microscopic study was done by Magnus lab photomicroscope and physicochemical properties were studied by standard procedure. Barks for these studies were procured from Tripura. **Results:** Chief characters of the transverse section and powder of the drugs include cork, cortex, parenchymatous cells, stone cell, lignified fiber, and calcium oxalate crystals along with the abundant amount of starch grains. In quantitative, microscopy length and width of lignified fiber and diameter of starch grains had been measured, which shows a significant difference between the two plants of the same family. Physiochemical and preliminary phytochemical evaluation of both the plants show the difference in alkaloid and sponin content. The chief chemical entity present in both the plants includes steroids, flavonoids, terpenoids, and tannins. **Conclusion:** The established parameter can be used as a biological standard to identify both the plants.

Key words: *Dipterocarpus turbinatus*, microscopy, morphology, phytochemical analysis, *Shorea robosta*

INTRODUCTION

Tribal people are the ecosystem people who lived in harmony with nature and maintain a close link between man and environment.[1] Primitive tribal people have used various plants to cure a variety of ailments, but they did not keep any record and the information about their practices is mainly passed verbally from generation to generation.[2] The Northeast India is in two biodiversity hotspots (Indo Burma and Himalayan) in the world map. The Northeast India is the richest reservoir of plant diversity hotspot of the world and contributing about 50% in India's biodiversity. Botanical survey of India Eastern circle, Shillong, has documented 194 plants species for 50 diseases and ailments, among which most of them were traditionally practiced by indigenous people of Eastern states of India.[3] *Shorea robosta* is a large deciduous tree found extensively in part of Northeast and central India.[4-6] Traditionally, it is used in anti-diarrheal, astringent, wound, ulcer, and in obesity.[7-10] *Dipterocarpus turbinatus* is a large deciduous tree attaining a height of 100-120 ft. and a girth of 8-15 ft.[4-6,11] It is found in the semi evergreen or evergreen mist tropical forest of Tripura, Assam and Andaman.[9] Traditionally, oleoresin of this plant is recommended for tuberculosis, leprosy, psoriasis, indolent ulcer, and gonorrhea.[10] The biological evaluation of the plant products, which were and are, being used in the traditional herbal system of medicine, develops a basic

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platform for the newer drug discovery. Therefore, the present study is aiming to develop morphological and microscopical standards for both the plants and also aiming to screen the phytoconstituents. This will further helpful to establish cell content and constituents parameters for identification of the plants and also helpful to study the various therapeutic important chemical entities of both the Dipterocarpaceae plants.

MATERIALS AND METHODS

Plants were collected from the forest of the Gomuti District of Tripura. Plant materials were dried under shade and made as a coarse powder by a pulverizer. The plant materials were identified and authenticated by Professor P. Jayaraman, M.Sc, Ph.D., Director; Plant Anatomy Research Centre, Tambaram, Chennai. (Voucher specimens No. PARC/2012/1276 & No. PARC/2012/ 1277). The chemicals used in this study were of laboratory grade and obtained from SD Fine chemicals. Magnus lab photomicroscope was used for the study of microscopic characters.

Sectioning and Staining

The required sample was cut and removed from the plant and fixed in FAA (Formalin - 5 ml + acetic acid 5 ml + 70% ethyl alcohol 90 ml). After 24 h of fixing, the specimens were dehydrated with tertiary butyl alcohol (TBA). Infiltration of the specimen was carried out by gradual addition of paraffin wax (melting point 58-60°C) until TBA attained supersaturation. The specimens were cast into paraffin blocks.[12] The paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the produced sections was between 10 and 12 µm.[13-15] Dewaxing of section was carried by the customary procedure. The sections were boiled with a bleaching solution for decolourization and to remove unwanted pigments.[16] The sections were stained with phloroglucinol for microscopic study (with 2 min of exposure time) and for lignified tissue few drops of concentrated hydrochloric acid were added over the stained section. Bleached section was stained with Iodine and potassium iodide for starch grains. Acetic acid was used to identify calcium oxalate crystals, Sudan red III was used for oil globules, and Picric acid was used for the identification of aleurone grains.[16-19]

Photomicrograph

Microscopic descriptions of tissues are supplemented here with photomicrographs wherever necessary. Photograph of different magnification was taken in Magnus lab photomicroscope. The bright field was used for normal observations. For the study of crystal, starch grains and lignified cells polarized light were employed. Descriptive terms of the anatomical feature are as given in the standard anatomy books.[13,14,20] For powder microscopic study, powder drug was treated with bleaching solution and then treated with different stains (phloroglucinol, con HCl, iodine solution, Sudan red III, picric acid solution and acetic acid) and observed under ×10 and ×45 magnifications.[21] For quantitative microscopy, magnification was done by using stage micrometer and eyepiece micrometer for powder drugs. Stage micrometer was replaced by stained powdered sample and ocular micrometer was used for measuring the diameter of starch grains, length and width of lignified fiber.[19]

Physicochemical Study

Various physicochemical parameters such as loss on drying (LOD), ash values (total ash, acid insoluble ash, water soluble ash) extractive values (by cold extraction) of *D. turbinatus* and *S. robosta* were established by using the powdered crude drugs as per the method mentioned in WHO guidelines.[22]

Phytochemical Study

Shade dried powders of *D. turbinatus* and *S. robosta* were extracted separately with different solvents by hot percolation method.[22,23] Choice of the solvent was selected according to their polarity index (petroleum ether, chloroform, ethanol and water),[24] Solvents were recovered by a rotary evaporator, and concentrated extracts were dried in desiccators. Various phytoconstituents present in the various extracts were detected by respected chemical tests[21,23,25].The solvent system for thin layer chromatography (TLC), for various extracts of *D. turbinatus* and *S. robosta*, was selected, on the basis of Solvent system for unknown polar and non-polar compounds, as per the method given in Wagner *et al*.[26] The chromatograms were examined in the ultraviolet (UV) chamber at 254 nm and 365 nm. Retention factor (RF) values were calculated for identifying spot using formula[27] RF = X/Y (where X is distance travel by solute in TLC plate and Y is the distance traveled by the solvent in same system).

RESULTS

Morphology

The bark of *D. turbinatus* is re-curved with bordered pits and farrows on the outer surface. Inner bark is fibrous. The thickness of the bark is in between 0.5 and 1.5 cm and length varies. The color of the outer bark is grayish; green because of the deposition of lichen and moss, and the inner bark is dark brown color. Fresh bark is strongly aromatic odor and astringent in taste. Fracture is splintery and fibrous. Whereas bark of *S. robosta* is channeled to flat with longitudinal lenticels on the outer surface, inner surface is smooth and shiny. Thickness of the bark is in between 1.5 and 2.5 cm and length varies. The outer bark shows radish brown in color and

inner bark is dark red in color. Fresh bark is slight aromatic odor and taste is astringent. Fracture is short [Figure 1a and b].

Transverse Section

A microscopic study in *D. turbinatus* shows cork and slightly larger parenchymatous cell. Phellogen is not clearly visible. Parenchyma region is abundant of spherical shaped starch grains and rhombic calcium oxalate crystals. Lignified fiber and stone cell layer is found in the bark. Whereas in *S. robosta* large tabular shape, with intercellular space parenchymatous cells were found in cortex region, in cortex region abundant of rectangular shaped calcium oxalate crystals were found, Cork cells were rectangular shaped and brown in color. Phelleogen was easily distinguishable [Figure 2a for *D. turbinatus* and Figure 2b *S. robosta*].

Powder Microscopy

Shade dried powder of *D. turbinatus* and *S. robosta* were reddish brown in color, with astringent taste and with the characteristic order. In *D. turbinatus*, fibers were lignified in a group, with uniform thickness, long, slender and cylindrical. Some fibers were associated with cork cells and a measure of 1464-775-366 µm and width 43.92-26.61-14.64 µm [Figure 1c]. Whereas in *S. robosta* fibers were thin, long, single or in group, linear, straight, strongly lignified and measured about 1171.2-580.18-219.6 µm and 87.84-39.63-29.28 µm [Figure 2c]. In *D. turbinatus*, occasional fragment of cork cells were found with thin wall, rectangular to a polygonal cell on surface view and containing brownish matter [Figure 1e]. Corks were spherical to polygonal in *S. robosta* on surface view [Figure 2e]. An abundant amount of rectangular calcium oxalate crystal in parenchyma were observed in both the plants [Figure 1i and 2i]. Starch grains were minute, simple, present abundantly and measuring about 35.44-2082-8.36 µm in *D. turbinatus* [Figure 1h]. Similar shape of starch grains were found in *S. robosta,* but their diameter was small in size and measuring of 26.58-12.84-4.43 µm [Figure 2h].

Physicochemical Evaluation

Bark powder of *D. turbinatus* and *S. robosta* were subjected to various physicochemical studies. The LOD for the bark was found to be 8.125 ± 0.125% and 8.625 ± 0.314% for *D. turbinatus* and *S. robosta* respectively. Ash values were determined in percentage for both the powdered drugs. For *D. turbinatus*, values were found to be, 6.238 ± 0.36, 5.3 ± 0.897 and 2.195 ± 0.104, respectively, for the total ash, acid insoluble ash and water soluble ash and for *S. robosta,* values were found to be 5.158 ± 0.376, 1.063 ± 0.7398, and 1.970 ± 0.027, respectively for the total ash, acid insoluble ash and water soluble ash. Extractive values for Petroleum ether, chloroform, ethanol, and water were established for *D. turbinatus* and *S. robosta.* Petroleum ether soluble extractive value for *D. turbinatus and S. robosta* were found to be 4.548 ± 0.0295% and 3.133 ± 0.1965%, respectively. The chloroform soluble extractive value of 9.295 ± 0.1916% and 8.005 ± 0.092%, ethanol soluble extractive value of 16.56 ± 0.3825 and 13.56 ± 0.44% and water soluble extractive value of 11.96 ± 0.216% and 19.46 ± 0.459% were obtained for *D. turbinatus and S. robosta,* respectively.

Phytochemical Analysis

Phytochemical screening revealed the presence of various therapeutic important classes such as alkaloids, flavonoids, steroids, terpenoids and tannin in both the drugs. The petroleum ether and chloroform extracts from the bark of both the plants shows the presence of terpenoids, steroids and

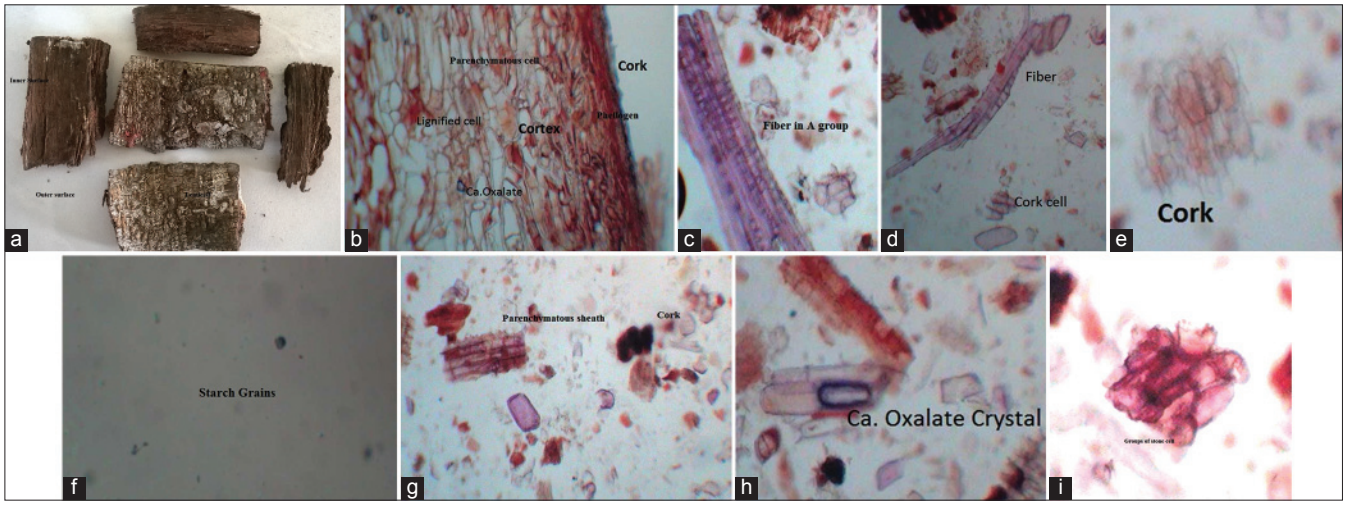

Figure 1: (a) Morphology *Dipterocarpus turbinatus*, (b) TS of *Dipterocarpus turbinatus*, (c) lignified fiber of *Dipterocarpus turbinatus*, (d) cork and fiber of *Dipterocarpus turbinatus*, (e) cork of *Dipterocarpus turbinatus*, (f) starch grains of *Dipterocarpus turbinatus*, (g) parenchymatous cell of *Dipterocarpus turbinatus*, (h) calcium oxalate of *Dipterocarpus turbinatus*, (i) stone cell of *Dipterocarpus turbinatus*

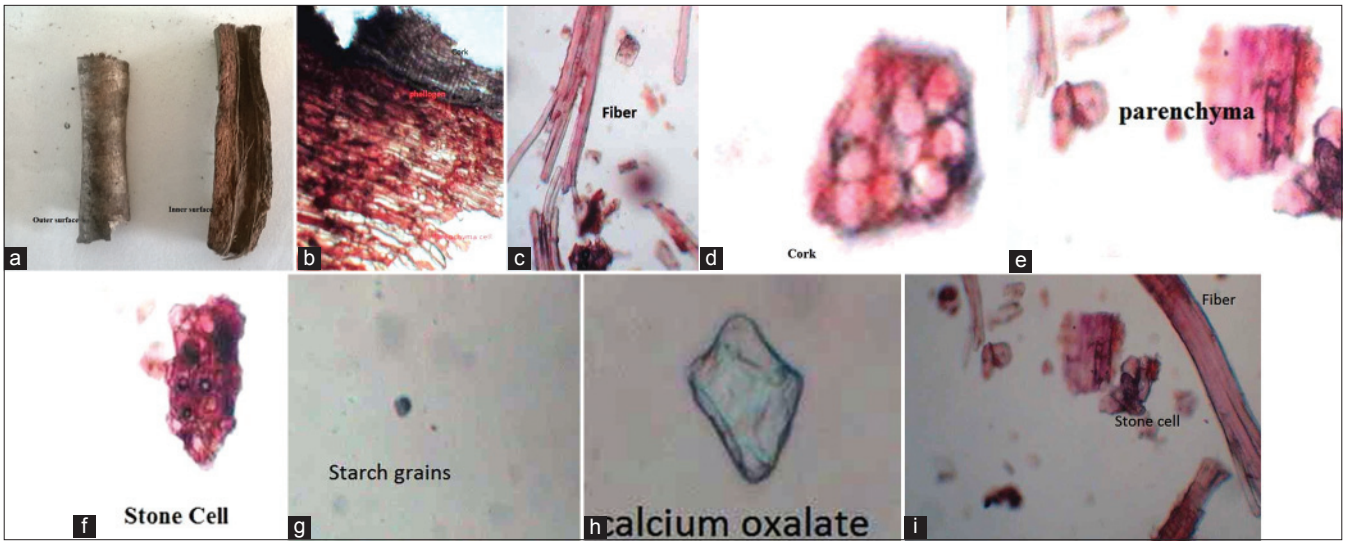

Figure 2: (a) Morphology *Shorea robosta*, (b) TS of *Shorea robosta*, (d) lignified fiber of *Shorea robosta*, (c) cork of *Shorea robosta*, (e) parenchymatous cell of *Shorea robosta*, (f) stone cell of *Shorea robosta*, (g) starch grains of *Shorea robosta*, (h) calcium oxalate *Shorea robosta,* (i) cork and fiber of *Shorea robosta*

was devoid from alkaloid. Ethanolic extracts of *D. turbinatus* show the presence of alkaloids, terpenoid, steroids, flavonoids and tannin. Whereas ethanolic extracts of *S. robosta* shows the presence of terpenoid, steroids, flavonoids and tannin but not of alkaloids. Whereas the aqueous extract did not show the presence of steroid and alkaloid in both the plants but shows the presence of tannin and flavonoids. Saponins are only present in the aqueous extract of *S. robosta.*

TLC Profile

The chromatograph for petroleum ether, chloroform, ethanol, and aqueous extract of *D. turbinatus* and *S. robosta* had shown various spots in TLC.

In the case of *D. turbinatus,* a prominent brown color spot was found with the solvent system of methanol, ethyl acetate, and water (100:13.5:7). The RF value of the brown color spot was found to be 0.84 for ethanolic extract and 0.88 for aqueous extracts. With toluene and ethyl acetate (93:7) solvent system, petroleum ether extract of *D. turbinauts* shows a florescent spot in UV 254 nm with the RF value of 0.45 and chloroform extract of the same plant shows a slight yellowish shiny spot (RF values 0.50).

Whereas ethanolic extract of *S. robosta* was found to produce two prominent brown color spots, with the solvent system of methanol, ethyl acetate and water (100:13.5:10), with different RF values of 0.51 and 0.84. The aqueous extract also found to produce two prominent brown color spots in the same solvent system with the RF values of 0.57 and 0.88. With toluene, ethyl acetate (93:7) solvent system, Pet. ether extract shows a florescent spot in UV 254 nm with the RF values of 0.50 and a shiny spot was observed for chloroform extract with the RF value of 0.81 with the same solvent system.

DISCUSSION

As per WHO norms an examination to determine the sensory, macroscopic characteristics is the first step toward the establishing the identity and the purity of the medicinal plant materials and should be carried out before any further test are undertaken.[28] The present study deals with macroscopic, microscopic, physicochemical, and preliminary phytochemical evaluation of the bark and bark powder of *D. turbinatus* and *S. robosta,* widely used by people of Tripura in various purposes. Macroscopic studies reveal various characters like shape, size, taste, color and odor of both the barks of plants. Both are found to have aromatic odor and astringent taste though differs is color, size, and shape. Microscopic study of both bark and powder shows cork, cortex, with stone cell, starch grains, and calcium oxalate crystals. *D. turbinatus* shows number of starch grains with large in shape as compared to *S. robosta.* The size of the cork cell is slight larger in *D. turbinatus* than in *S. robosta*. Cortex region of *S. robosta* shows intercellular space in the parenchymatous layer, whereas compact parenchymatous layer is found in *D. turbinatus*. In powder drugs, starch grains are small in *S. robosta* compared to *D. turbinatus*. The length of lignified fibers are less and width are more in the case of *D. turbinatus* compare to *S. robosta,* but in both case, they are associated with calcium oxalate crystals. Though both barks are found to contain strongly lignified Stone cells, *D. turbinatus* contains significantly larger stone cells than *S. robosta*.

D. turbinatus shows more extractive values with Petroleum ether and chloroform. *S. robosta* gives more extractive values with ethanol and water.

Phytochemical analysis for both plant barks shows, various biological important constituents such as flavonoids,

terpenoid, tannin, and steroids. Alkaloids are found to present in ethonolic extract of *D. turbinatus* but not in *S. robuta* extracts. Aqueous extract of *S. robusta* shows the presence of saponins, but absence in *D. turbinatus* extracts. TLC shows various spot with different RF values with the selected (methanol, ethyl acetate and water and toluene and ethyl actetate) solvent systems.

This monograph will help in standardization and differentiation of *D. turbinatus* and *S. robusta* and also helpful for performing further studies and investigation of individual compounds in the plants.

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