ABSTRACT

Boerhavia diffusa Linn. Family Nyctaginaceae is the most important herbaceous plant. It is commonly known as Punarnava in Sanskrit. People and other tribal communities have long employed the entire Boerhaavia diffusa plant as well as its specialized parts—the root, stem, and leaf for their medical purposes. This practice is widespread throughout India. Warm climates and tropical and subtropical regions are common places to find it. It is found in warmer regions of these countries and can be found in, Ceylon, Sudan, Pakistan, Australia and Malay Peninsula. It can also be found in Africa, China, America and Pacific Islands. It is used in traditional medicinal and ayurvedic system of medicines. The present communication provides a detailed account of the physicochemical study carried out of Punarnava stem. The study includes macroscopy, microscopy and powder microscopic studies, preliminary phytochemical investigation, physicochemical tests and development of HPTLC (High Performance Thin Layer Chromatography) fingerprints profile. Established parameters can be used as standards for quality control and identification of the plant in herbal compound formulations and also preparation of a monograph of the plant.

Keywords: Boerhavia diffusa, Physicochemical, HPTLC analysis, Phytochemical

INTRODUCTION

Boerhavia diffusa Linn. family Nyctaginaceae is a herbaceous perennial plant growing ascending upward and prostrate in various habitats such as agricultural fields, grasslands, roadsides, wastelands, residential area, ditches and marshy places during rains. Boerhaavia diffusa plant is consisting of 40 species and found in warmer regions of these countries and can be found in, Ceylon, Sudan, Pakistan, Australia and Malay Peninsula. It can also be found in Africa, China, America and Pacific Islands.

Boerhaavia diffusa is a very popular medicinal plant in India, whole plants are used in Indian system of medicine. Plant root is registered in the Ayurvedic Pharmacopoeia of India.
Various parts of Punarnava are used to treat different types of human disorder such as cardiac problem, stomach disorder, hepatoprotective, reduces cough, laxative, diuretic, and anthelmintic. It is useful in treatment of enlarged spleen, diabetes, jaundice, poisoning, glaucoma, gonorrhoea, asthma, congestive heart failure and other internal inflammations. A decoction of the roots of Punarnava is also useful to treat night blindness, corneal ulcers and boiled root are useful to ulcers. Aerial parts decoction is used to maintain gastrointestinal pains and intestinal worms. Whole parts of this plant are used to preparation of ayurved formulations. It is also used as an ethno-medicine. Despite the numerous medicinal uses of Punarnawa, so far task has been undertaken. Hence the present work deals with the morphological, anatomical evaluation, physicochemical tests, preliminary phytochemical screening and High-Performance Thin Layer Chromatography.

MATERIALS AND METHODS
Collection of samples
The fresh Punarnawa plant was collected from Arogyadham campus, Deendayal Research Institute, Chitrakoot, Satna, (M. P.). The plant was identified and authenticated by Dr. Manoj Tripathi Senior Scientist, Arogyadham, Deendayal Research Institute Chitrakoot. The voucher specimen (AD/AS/266/2023) prepared as per standard procedure. Fresh material was used for anatomical studies whereas shade dried material was powdered in electric grinder for physico-chemical study, phytochemical investigation and development of High Performance Thin Layer Chromatography fingerprint profile.

Macroscopic study
Macroscopic or organoleptic characters Gloriosa stem like appearance, colour, odour and taste were evaluated of the Punarnawa stem.

Microscopic study
Fresh stem section was cut by free hand sectioning and numerous sections examined Microscopically. Photographs of the microscopical sections were captured with the help of Olympus Trinocular Research Microscope CX- 211 with Digi-eye camera using Caliper plus version 4.2 Software.
Powder microscopic study
The dried stem was powdered and completely passed through 355 μm IS Sieve (old sieve number 44). About 2 g of powder washed thoroughly with potable water, poured out the water without loss of material. Mounted a small portion in glycerin were used to all characters of the Punarnawa stem, small quantity of sample cleared by heating with chloral hydrate solution, wash and mounted in glycerin, treat a few mg with iodine solution and mount in glycerin, another small quantity of sample stained with sudan red solution and mounted with glycerin, all mounted slide were seen under microscope at 40 x 10x magnification of the Trinocular Research Microscope

Physico-chemical parameters
Physico-chemical parameters such as moisture content (loss on drying at 105°C), water soluble extractive value, alcohol soluble extractive value, total ash value, acid insoluble ash value was calculated

Preliminary phyto-chemical investigation
Preliminary phyto-chemical tests were carried out on ethanolic and water extract for the presence\absence of phyto-constituents like alkaloids, flavonoids, tannins, resins, carbohydrates, proteins and saponins

High Performance Thin Layer Chromatography (HPTLC) fingerprint profile
For High performance thin layer chromatography, the powdered 5 gm of sample was extracted with 100 ml of ethanol overnight, filtered and concentrated. It was applied by spotting extracted sample on pre-coated silica-gel aluminium plate 60 F254 (5x10 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 μl Hamilton syringe. The sample, in the form of bands of length 6 mm, were spotted 15 mm from the bottom, 15 mm from left margin the plate and 10 mm part. Plates were developed using mobile phase consisting of toluene: ethyl acetate (7: 3 v/v). Linear ascending development was carried out in 10x10cm twin through glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 20 minute at room temperature. The length of chromatogram run was 8 cm. 20 ml of the mobile phase. Subsequent to the development, Thin Layer Chromatography plate was dried with the help of Hot Air Oven. The peak area for samples and standard were recorded with Camera photo documentation system Camag Reprostar 3. Visualization of spot was made before and after derivatization (with 5%
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Methanolic - sulphuric acid reagent) at UV light with Win cat software and Rf values
noted\(^{17,18,19}\).

RESULTS AND DISCUSSION

Macroscopic characters

*Boerhaavia diffusa* stem is cylindrical shape, swollen at the nodes, prostrate, branched, branch about 1-1.5 meter long. Stem colour is pale greenish ventral side and light reddish brown in dorsal side, odourless and taste slightly bitter (Fig.1a).

Microscopic characters

Transverse Section of Punarnava stem showed outer layer of epidermal cell containing uniseriate, multicellular glandular trichomes with 8-10 stalked cells and a head. Cortex consists of 1–2 layers of parenchyma and endodermis indistinct, cortex have wide area which is about 140–230 \(\mu\)m long, pericycle 1–2 layered and thick walled, which is often containing isolated fibers, stele is consisting of different types of small vascular bundles and various large vascular bundles scattered in the ground tissue, intrafascicular cambium present (Fig.1b).

Powder microscopic characters

Punarnava stem powder colour is pale greenish, taste not characteristics and odour odourless. Under microscopic various types of anatomical structure showed *viz.* lignified fibres, epidermal cells stomata, acicular crystals of calcium oxalate, epidermal cells, thick walled parenchymatous cells associated with starch grains, reticulate and spiral thickening and simple covering and glandular trichomes with unicellular head and multicellular stalk (Fig.1c).

Physico-chemical analysis

Physicochemical analysis were performed and obtained the results such as Loss on drying (LOD) at 105\(^\circ\)C was found 5.45% w/w, total ash value 5.30% w/w, acid insoluble ash value 0.98% w/w, alcohol soluble extractive value 20.70% w/w and water soluble extractive value 29.80% w/w. The physico-chemical parameters such as extractive values are useful for the determination of exhausted or adulterated drug; ash values of the drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug.
Fig. 1b - TS of Punarnawa Stem

Fig. 1a - Punarnawa plant

Fig. 1c - Lignified fibres

Fig. 1d - Epidermal cells

Fig. 1e - Acicular crystals of calcium

Fig. 1f - Epidermal cells

Fig. 1g - Thick walled parenchymatous cells associated with starch grains

Fig. 1h - Reticulate and spiral

Fig. 1i - Multicellular, simple covering and glandular trichomes with unicellular head and multicellular stalk
Preliminary phyto-chemical investigation

Qualitative phyto-chemicals were screened in the extracts taken in water and ethyl alcohol. The screening exhibited presence of protein, carbohydrates, alkaloids, tannin, flavonoids, and saponin.

**HPTLC finger print profile**

High performance thin layer chromatography (HPTLC) study of the ethanolic extract three spots of the Gloriosa stem sample extract applied in precoated TLC plate. Applied 6 μl of the test solution as 8 mm bands and develop the plate in a solvent system toluene: ethyl acetate
(7: 3 v/v) to a distance of 8 cm. Dry the developed plate in room temperature and examined. Derivatized the plate using 5% Methanolic-sulphuric acid reagent and heating at 105°C till the bands are clearly visible. Major spots $R_f$ values with colour were recorded before derivatization at 366nm, after derivatization at 366nm and at UV light. Chromatogram profile and $R_f$ values are given (Fig. 2a, 2b & Table 1).

The macroscopic, microscopic and powder microscopic distinguished characters have been established to identify Punarnawa stem. The physicochemical parameters can be used for checking the adulteration and purity of this drug. HPTLC finger print profile helps in identification of various phytochemical constituents present in the crude drug thereby substantiating and authenticating of crude drug. The TLC profile also helps to identify and isolate's important phyto-constituents. Heavy metal elements are found under limits as per guideline WHO. These finding could be helpful in identification and authentication of Punarnawa stem.

CONCLUSION

Punarnawa is a well-known medicinal plant and used to indigenous systems of medicine such as Ayurveda, Siddha and Unani. This plant is using several herbal preparations in the world. The plant has a number of traditional uses for multiple diseasesPunarnawa is a plant that is used extensively nowadays and has a great deal of potential for future research, even though there are gaps in the studies that have been done thus far that need to be addressed in order to fully realise the plant's medicinal potential. These studies conducted on various aspects on Punarnawa stem by different parameters like that physicochemical parameters, phytochemical tests, pharmacognostical study and HPTLC fingerprints profile.

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REFERENCES


