Available online at www.ijrbp.net

DOI: http://dx.doi.org/10.18782/2322-0392.1260

ISSN: 2322 – 0392

Int. J. Rec. Biotech. (2020) *8*(4), 1-7

International Journal of Recent Biotechnology

Research Article

Peer-Reviewed, Refereed, Open Access Journal

Pharmacognostic Evaluation of Stem of Punarnawa (*Boerhavia diffusa* Linn.)

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Received: 10.10.2020 | Revised: 16.11.2020 | Accepted: 23.11.2020

ABSTRACT

The herbal medicine has ancient medicinal use in different societies from the times of the B.C. A number of plant products have been identified through phyto-chemistry and the extract of their different plant parts are useful in various diseases without side effects. Boerhaavia diffusa Linn. (family- Nyctaginaceae), commonly known as 'Punarnava' in the Indian system of medicine, is a perennial creeping herb found throughout the waste land of India. Root and aerial parts of Boerhaavia diffusa were used in Ayurveda for the treatment of diabetes. Punarnava corrects the digestive system, alleviates fluid retention and very useful in managing heart diseases. Punarnava also benefits in anemia, hernia and respiratory distress. Punarnava can also be taken in liver problems and managing lipids and cholesterol in healthy limits The present paper provides a detailed account of the pharmacognostical evaluation of Boerhaavia diffusa Linn. stem. The study includes macro and microscopic characters, powder microscopic characteristics, HPTLC fingerprinting, preliminary phytochemical screening, physicochemical parameters. The information generated by this particulars study provides relevant pharmacognostical and physicochemical data needed for proper identification and authentication of Boerhaavia diffusa Linn. Stem.

Keywords: *Boerhaavia diffusa Linn, Pharmacognostic evaluation, Preliminary phytochemical screening, Physico chemical analysis, HPTLC fingerprinting.*

INTRODUCTION

Punarnava *(Boerhavia diffusa* Linn.) is a flowering plant that is commonly known as punarnava which means rejuvenating or renewing the body. The other common names of the plant are tarvine, spreading hogweed and red spiderling. This herbal medicine is used to relieve pain and the leaves are used as a green vegetable in numerous parts of India. Punarnava (Hogweed) literally means 'bring back to life' or 'renewer'. Genus *Boerhavia,* consisting of 40 species is distributed in tropical and subtropical regions and warm climate.

Cite this article: Mishra, M. C., Shukla, S. P., Rajak, V., & Tripathi, M. (2020). Pharmacognostic Evaluation of Stem of Punarnawa (*Boerhavia diffusa* Linn.), *Int. J. Rec. Biotech. 8*(4), 1-7. doi: http://dx.doi.org/10.18782/2322-0392.1260

It is found in Australia, China, Egypt, Pakistan, Sudan, Sri Lanka, South Africa, USA and in several countries of the Middle East. Among 40 species of Boerhaavia, 6 species are found in India, namely *Boerhavia,* diffusa, *Boerhavia,* rependa, *Boerhavia,* chinensis, *Boerhavia,* hirsute *Boerhavia,* erecta, and *Boerhavia,* rubicunda. Boerhaavia diffusa in India is found in warmer parts of country and throughout up to 2,000 m altitude in the Himalayan region. It is a perennial, spreading hogweed, commonly occurring abundantly in waste places, ditches and marshy places during rains. The plant is also cultivated to some extent in West Bengal (Najam et al., 2008, & Chopra et al., 1923). It grows well on wastelands and in fields after the rainy season (Chopra et al., 1956). The whole plant and preferably the roots are effectively used to cure several diseases including Jaundice (Bajpay, 1993). Punarnava corrects the digestive system, alleviates fluid retention and very useful in managing heart diseases. It is also used to treat the anemia, hernia and respiratory distress, liver problems, managing lipids and cholesterol in healthy limits (Debjit et al., 2012). Despite the numerous medicinal uses attributed to this plant, there are no pharmacognostical studies on the stem of this plant have so far been carried out. Hence, the present work deals with the morphological, anatomical evaluation, physicochemical constants, preliminary pytochemical screening and HPTLC fingerprint profile of Punarnava stem which could serve as a valuable source of information and provide suitable standards for the further identification of this plant.

MATERIALS AND METHODS

Collection of specimens

The fresh plant stem of *Punarnawa was* collected from the Chitrakoot forest of Satna district (M.P.) in the month of March 2016. The voucher specimens were collected and placed in the herbarium of Department of Botany, Government P.G. (Autonomous) college, Satna. Fresh material was used for anatomical studies whereas shade dried material was powdered in electric grinder for physico-chemical, phytochemical and HPTLC studies.

Macroscopy

Macroscopic or organoleptic characters like appearance, colour, odour and taste were evaluated (Anonymous, 2011).

Microscopy

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- 21*l* with Dig ieye camera using Caliper plus version 4.2 software (Anonymous, 1989, & Iyer & Kolammal, 1993).

Powder microscopy

The dried roots were subjected to powdered and completely passes through 355 µm IS Sieve (old sieve number 44) and not less than 50% pass on through 180 µm IS Sieve (old sieve number 85). About 2 g of powder washed thoroughly with potable water, pour out the water without loss of material. Mounted a small portion in glycerin, warmed a few mg with chloral hydrate solution, wash and mounted in glycerin, treat a few mg with iodine solution and mount in glycerin, about 1 g of powder warmed over water bath with 50% con. Nitric acid till brown fumes appear, cool and wash with water thoroughly and mount a small portion in glycerin and seen under microscope at 40 X 10X magnification of the trinocular research microscope (Anonymous, 2007, & Indian Herbal Pharmacopoeia, 1998).

Physico-chemical parameters

Physico-chemical parameters such as moisture content (loss on drying at 105^0C), water soluble extractive value, alcohol soluble extractive value, total ash value, acid insoluble ash value and water soluble ash were

calculated (Mukherjee, 2002, & Anonymous, 1999).

Fluorescence study

Fluorescence study was carried out with different chemicals and colour observed with the help of UV Spectrophotometer (Tripathi & Sikarwar, 2015).

Preliminary phytochemical studies

Preliminary tests were carried out on ethanolic and water extract for the presence\absence of phyto-constituents like alkaloids, flavanoids, tannins, resins, carbohydrates, proteins and saponins (Harborne, 1984, & Tripathi & Sikarwar, 2014).

High Performance Thin Layer Chromatography (HPTLC)

For HPTLC, the powdered roots 2 gm of sample was extracted with 50 ml of ethanol overnight, filtered and concentrated. It was applied by spotting extracted sample on pre-coated silica-gel aluminium plate 60 F_{254} (5x10 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe. The samples, 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 *Hexane: Ethyl acetate (6:4* 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 30 min. at room temperature. The length of chromatogram run was 8 cm. 20 ml of the mobile phase. Subsequent to the development, TLC plates 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 spots were made before and after derivatization (with 50% *Vanillin-sulphuric* reagent) at 254nm, 366nm and day light with Win cat software and R_f values noted (Ansari, 2013, Anonymous, 2007 & Tripathi, 2015).

RESULTS AND DISCUSSION

Macroscopy

Punarnawa stem colour is brownish green, taste slightly bitter and odour characteristics. Stem spreading on the ground longitudinally striated, branched, swollen nodes (Fig.1 & 2).

Microscopy

Punarnawa stem diagrammatic TS shows an epidermis, cortex, a continuous band of stellar tissue and a pith embedded with vascular bundles. (Fig.3).

Transverse section of Punarnawa stem shows a thin layer of epidermis covered with thick cuticle several stomata found in the epidermis. Several simple, multi cellular covering and glandular trichomes with multi cellular stalk and unicellular head found in the epidermis. Cortex is narrow, 2 to 3 rows of cells lying underneath the epidermis being collenchymatous unlike the remaining 3 to 4 rows which are chlorenchymatous, endodermis is distinct, phloem is very narrow, traversed with isolated lignified fibres, followed by a lignified continuous band of xylem consisting of radially running vessels, tracheids, uni-to biseriate medullary rays and fibres, forming the major portion of the rings; pith is well developed parenchymatous, embedded with circular to oval shaped fibro vascular bundles of various sizes. (Fig.4).

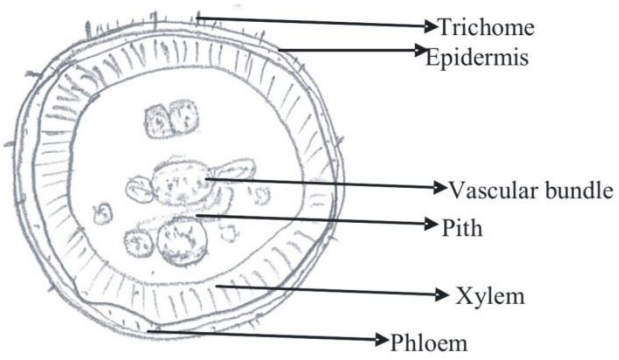


Fig. 3 Diagramatic TS of stem

Fig. 1 Plant

Fig. 2 Stem

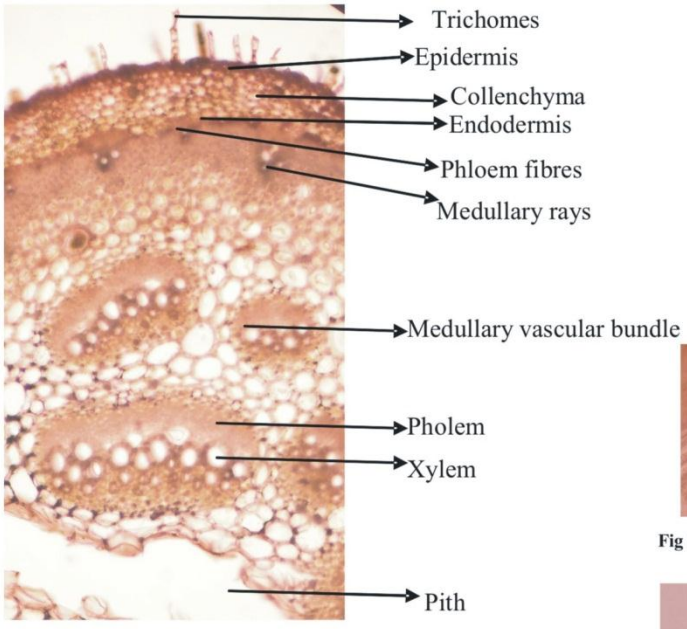


Fig. 4: Detailed TS of stem

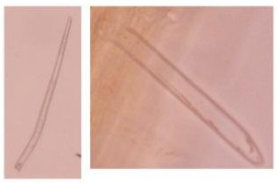


Fig.5 Lignified fibres

Fig 6 Epidermal cells stomata

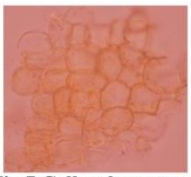


Fig.7 Collenchymatous cells

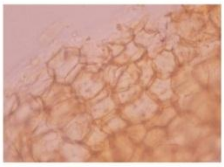


Fig. 8 Thick walled parenchymatous cells

Fig. 9 Reticulate and spiral thickening

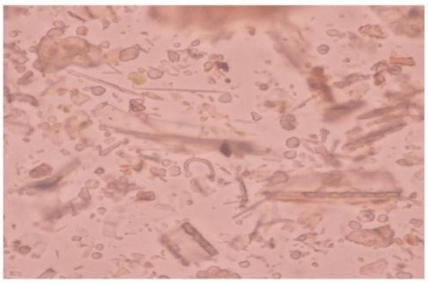


Fig. 10 Starch grains, Prismatic & acicular crystals of calcium oxalate

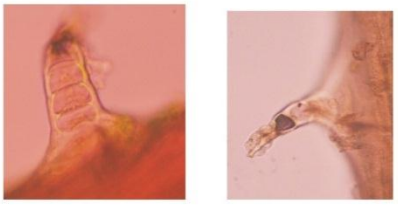


Fig. 11Trichomes

Table 1: Physico-chemical analysis of Punarnawa stem

S. N0.	Tests	Result
1	Foreign matter	1.12%
2	Loss on drying(at 105^{o}C)	3.13%
3	Alcohol soluble extractive value	2.2%
4	Water soluble extractive value	14.75%
5	Total ash value	12.32%
6	Acid in soluble ash value	1.38%

Table 2: Fluorescence study of Punarnawa stem

S. No.	Powder + reagent	Observation at day light	Observation at 366nm
1	Powder a sit +P	Cream colour	Dark cream
2	1 N HCl + P	Brown	Dark green
3	1 NaoH (methanol) + P	Green	Light green
4	1 NaoH +P	Dark brown	Dark green
5	50% KOH + P	Dark brown	Green
6	50% H2SO4 + P	Dark brown	Dark green
7	Con. H2SO4 + P	Black	Black
8	50% HNO3 +P	Brown	Black
9	Con. HNO3 + P	Brownish red	Black
10	Glacial acetic acid	Brownish green	Green
11	Iodine water + P	Brown	Black
12	50% HCl + P	Brown	Green
13	Con. H Cl+ P	Brownish black	Green
14	Picric acid +P	Brown	Light green
15	Acetone + P	Brown	Whitish green
16	50% FeCl3 + P	Dark green	Black
17	50% Ammonia +P	Brown	Green

Table 3: Rf values of HPTLC fingerprint profile of Punarnawa stem

Rf values	At 366nm Before derivatization		At 366nm After derivatization		At visible light	
	Test solution S1	Test solution S2	Test solution S1	Test solution S2	Test solution S1	Test solution S2
R_f1	0.12(sky blue)	0.12(sky blue)	0.12(sky blue)	0.12(sky blue)	0.08(light brown)	0.08(light brown)
R_f2	0.14(sky blue)	0.14(sky blue)	0.14(sky blue)	0.14(sky blue)	0.24 (light brown)	0.24 (light brown)
R_f3	0.22(sku blue)	0.22(sku blue)	0.24(sky blue)	0.24(sky blue)	0.30 (light yellow)	0.30 (light yellow)
R_f4	0.24(yellowish green)	0.24(yellowish green)	0.30 (sky blue)	0.30 (sky blue)	0.40(pinkish brown)	0.40(pinkish brown)
R_f5	0.30 (sky blue)	0.30 (sky blue)	0.36(sky blue)	0.36(sky blue)	0.52(brown)	0.52(brown)
R_f6	0.36(sky blue)	0.36(sky blue)	0.40 (yellow)	0.40 (yellow)	0.60 (yellow)	0.60 (yellow)
R_f7	0.40 (sky blue)	0.40 (sky blue)	0.58(light pink)	0.58(light pink)	0.70(brown)	0.70(brown)
R_f8	0.58(sky blue)	0.58(sky blue)	0.60 (yellow)	0.60 (yellow)	0.80(black)	0.80(black)
R_f9	0.60 (whitish yellow)	0.60 (whitish yellow)	0.80(brown)	0.80(brown)	-	-
R_f10	0.80(sky blue)	0.80(sky blue)	-	-	-	-

Powder microscopy

Fragments of thin walled spindle shaped fibres, fragments of pitted and spiral vessels, surface view with anomocytic stomata, multicellular, uniseriate, glandular and covering trichomes with unicellular head and multicellular stalk, simple to compound starch grains scattered throughout the powder, Prismatic crystals of calcium oxalate, crystal fibres and acicular crystals of calcium oxalate. (Fig.5 -11).

Physico-chemical analysis

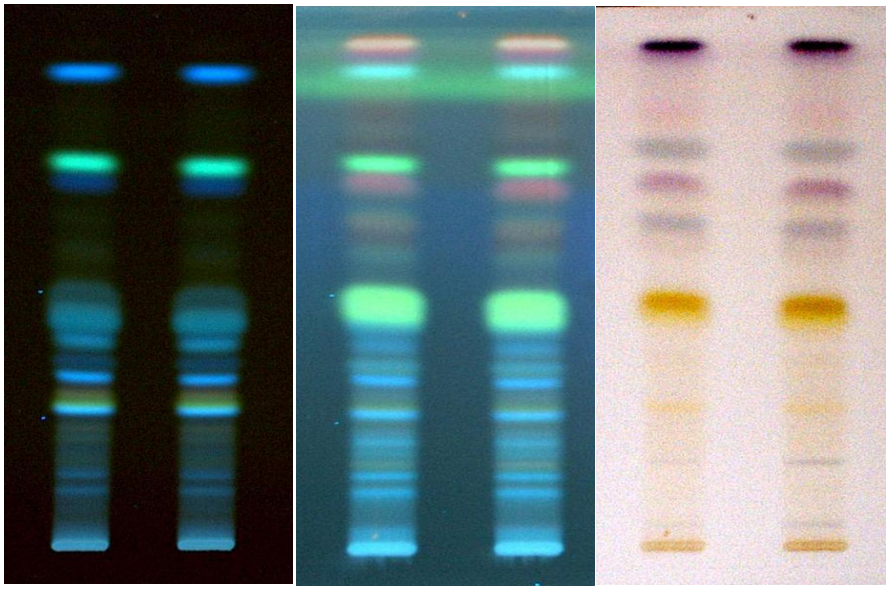
The physico-chemical parameters such as extractive values are useful for the determination of exhausted or adulterated drug; ash values of the drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Physico-chemical results of the drug are given in (Table1).

Preliminary phytochemical studied

Qualitative phyto-constituents were screened in the extracts taken in water and ethyl alcohol. The screening exhibited presence of saponin, alkaloids, tannin and resin.

HPTLC finger print profile

High performance thin layer chromatography (HPTLC) study of the ethanolic extract two spots of the sample extracts applied in the TLC plate. Major spots R_f values with colour were recorded under 366nm, after derivatization 366nm and UV light. Chromatogram profile and R_f values are given (Fig.12 - 14 & Table 3).



S_1 S_2 **S_1 S_2** **S_1 S_2**

Fig. 12 HPTLC at 254nm **Fig. 13 HPTLC at 366nm** **Fig. 14 HPTLC at day light**

CONCLUSION

The pharmacognostic characters and phytochemical values reported in this work may play a major role in setting some diagnostic indices for identification and preparation of a monograph of the plant, which might broaden its pharmacological, botanical and economical importance. With the help of this referential information, a researcher can easily reject the fake and adulterated plant products which are deviated from the above mentioned characters and select the correct herbal specimen for further investigations.

Acknowledgement

The authors are grateful to the Organizing Secretary Deendayal Research Institute, Chitrakoot, Satna (M.P.) for providing necessary facilities.

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