Fingerprinting and Quality Control of *Boerhaavia erecta* L. Root

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Abstract: *Boerhaavia diffusa*, *B. erecta*, and *B. repanda* have been found in use as 'Punarnava.' *Boerhaavia erecta* L. (Nyctaginaceae). It is pantropical weed, common on plains, along roadside, moist areas and towards the coast. It is equated with 'Shwet Punarnava.' *B. erecta is a* rich source of various phytochemicals viz. alkaloids, steroids, tannins, flavonoids, saponins, and glycosides. It is used in the traditional system of medicine due to its significant pharmacological activities such as hepatoprotective, gastro-protective, analgesic, antioxidant, antipyretic, and anti-inflammatory. In view of the therapeutic importance of this plant and its potential to substitute *B. diffusa*, the quality control parameters viz. macroscopic, microscopic, ash values, extractive values, phytochemical screening, and chromatography has been evolved. TLC studies carried out employing solvent systems viz. chloroform: methanol (80:20) using visualizers such as UV 254 nm and 366 nm, iodine, Dragendorff's reagent, and 1%Vanilline-50%phosphoric acid. These findings might be useful to supplement the information about its identification, physicochemical evaluation, standardization, and quality control parameters of this potential medicinal herb. The physicochemical constants evolved in the present investigation will serve as the parameters for quality control and fingerprinting for plant-based pharmaceutical and cosmetic market products.

Keywords- *Boerhaavia erecta***,** hepatoprotective**,** quality control, pharmacognostic standardization, physicochemical evaluation, Shwet Punarnava

I. INTRODUCTION

Punarnava is an important therapeutic drug in Ayurveda, Siddha and Unani systems of medicine. In Ayurveda Punarnava is regarded as rejenuvating drug. It has been employed commonly since ancient period traditionally by native population for various ailments. This drug has been mentioned in Rigveda, Matsya Purana and Agni Punarana [1]. In Nighantus Punarnava drug is valued for cough, asthma, anemia, swellings, eye diseases, nervous disorders, etc. Punarnava species are used as laxatives, antipyretic, stomachic, cardiotonic, expectorant, hypotensive, diuretic, antibacterial, inflammatory and kidney stimulant. It is also used for asthma, jaundice, eye diseases, urine complaints and menstrual cramps [2-5]. In the pharmaceutical industries Punarnava is used in 52 preparations [6].

B.diffusa, *B.erecta*, *B.repanda*, and *T. portulacastrum* are considered as Punarnava. There is confusion regarding the real identity of 'Shwet Punarnava.' The Shwet Punarnava is equated with *Trianthema portulacastrum* L. While some of the research workers also consider *B. erecta* as Shwet Punarnava as it possesses 'white flowers. *B.punaranava* and *B. erecta* were considered as different species by some researchers; however, This is the same species, and the former becomes a superfluous name [7]. Some research workers mentioned Shwet Purnava more efficacious with *B. repens* [8]. *Boerhaavia erecta L.* (Nyctaginaceae) syn. *B.punarnava* Saha and Krish. is native to tropical America, introduced in India and naturalized in waste places and roadsides. It is pantropical weed that is common on plains, along roadside, moist areas and towards the coast. The bioactivity screening of *B. erecta* revealed anti-bacterial [9], anti-malarial [10], muscle contractility, anti-HIV [11] properties. This is mainly due to presence of some sterol compounds present in *B. erecta*. In view of therapeutic importance of this plant and its value in pharmaceutical industry, a detailed morphology, anatomy, pharmacognosy, phytochemistry and chromatographical studies have been carried out. The physicochemical constants serve as parameters for quality control and fingerprinting.

II. MATERIALS AND METHODS

Plant material: Fresh plant material of *B. erecta* was collected from Pune and other districts of Maharashtra viz. Dhulia, Jalgaon, Nasik, Sindhudurg, and Raigad. The bulk quantity of material was collected in the monsoon period. The authentication of the *B. erecta* L. was done from the Botanical Survey of India, Western Zone, Pune. Hand sections of plant material were used for histochemical studies.

Macroscopic characteristics: The morphological characteristics and organoleptic characteristics of root were recorded.

Microscopical characterization:

Sectioning: The standards methods were followed for organoleptic evaluation; the standard methodology was followed.

Photomicrograph: Microscopic descriptions of the selected tissues were supplemented with micrographs.

Physicochemical evaluations: Physicochemical parameters of *B. erecta* root powder were determined as per standard methods. Total ash, water-soluble ash, acid-insoluble ash, and sulfated ash values were worked out. Alcohol and water-soluble extractive values were determined to find out the amount of water and alcohol-soluble components

Root powder analysis and percentage of extractives were determined as per the method given in Indian Pharmacopoeia [12].

Histochemical Studies: Carried out as per the standard protocol [13]

Thin Layer Chromatography studies: The methods described by standard methods [14].

Total ash, water-soluble ash, acid-insoluble ash, and sulfated ash values were worked out. Alcohol and water-soluble extractive values were determined to find out the amount of water and alcohol-soluble components.

Preliminary phytochemical screening: About 25g **c**oarse root powder of *B. erecta* was subjected to Soxhlet for successive solvent extraction. The extract was concentrated and subjected to various chemical tests to detect the presence of different phytoconstituents.

Thin Layer Chromatography (TLC): TLC studies have been carried out employing solvent systems viz. chloroform: methanol (80:20). TLC plates were developed in the Camag Twin Developing chamber, and plates were observed under a Camag UV cabinet at 254 nm and 366 nm. TLC plates were developed in iodine, Dragendorff's reagent, and 1%Vanilline-50%phosphoric acid.

III. RESULTS AND DISCUSSION

Root:

Figure 1.A. *B.erecta* habit Fig. 1.B Root of *B. erecta*

Table 1. Characteristic features of *B. erecta* root.

Parameters	*B. erecta L.*
Type	Small, conical, slender, woody, with few lateral roots
Root colour	Pale yellow
Dimensions (cm)	5.5-10.85-17.0 X 0.1-0.17-0.3

Microscopical studies:

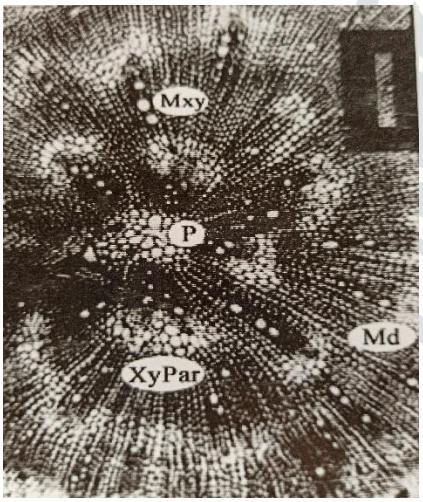


Figure 2. T.S. of *B. erecta*. Root(400X)

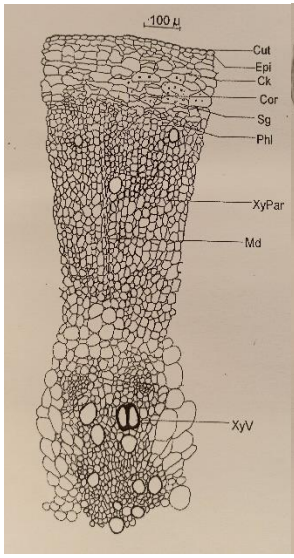
P-Pith, Md-Medullary rays, Mxy-Metaxylem, XyPar-Xylem parenchyma

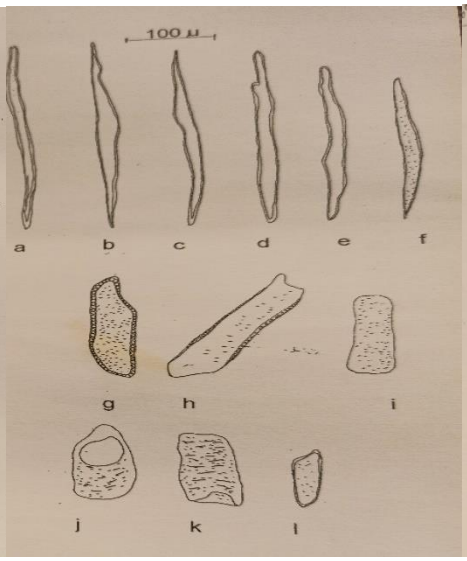
Table 2. Anatomical peculiarities of *B.erecta* root.

Parameters	*B. erecta*
Cork	2-3 layered, small, suberized and uneven cells
Cortex	5-6 layered, with polygonal cells
Endodermis and pericycle	Not distinct
Phloem	Forms patch outside xylem vessels
Xylem	Vessels are in the group of 1-4, polygonal to oblong.
Medullary rays	Uniseriate with rectangular cells
Pith	Pith becomes slcerified in later stages
Crystals	Few starch grains and raphides

Table no. 3. Characteristics of Starch grains of *B. erecta*

Features	*B.erecta*
Type of starch grains	Simple and compound
Shape (single)	Rounded, subspherical, ovoid.
Dimensions (single)	4.0-5.12-7.0 X 4.0-5.25-7.0 µ
Hilum	'Y' shaped
Striations	Faintly marked
Aggregation	Very few compounds





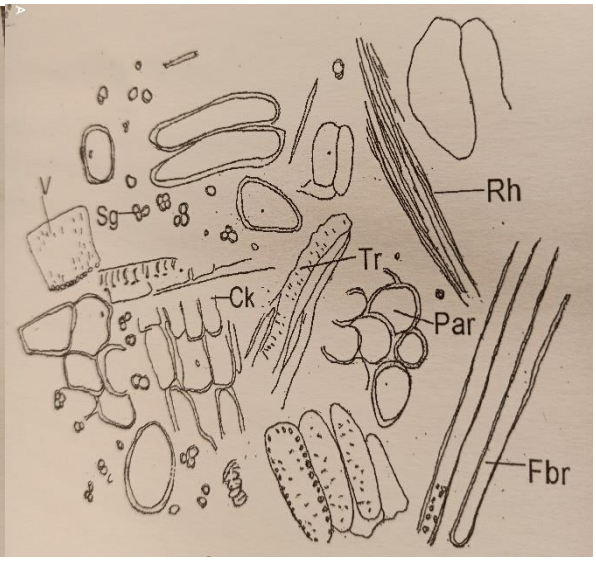


Fig.3 T.S. root (diagrammatic) **Fig. 4 Maceration studies** a-f-fibres;g-i-tracheids,j-k-vessels, i-rayparenchyma **Fig.5 Root powder** (Ck-cork, Rh-raphides, Fbr- Fibres, Tr- tracheids Par-Parenchyma

Table no. 4. Maceration studies of *B. erecta*

Dimensions of different cells	*B.erecta*
Vessel elements (µ)	102.4-137.0-190.0X50.0-54.4-70.0
Tracheids (µ)	100.0-187.2-270.0X15.0-20.66-40.0
Xylem fibres (µ)	210.0-295.5-400.0X15.0-19.93-25.0
Xylem parenchyma (µ)	70.2-80.2-92.0X15.3-20.2-35.0

The peculiarities of starch grains and calcium oxalate crystals serve as the diagnostic characters for identification and differentia of the closely related Punarnava species. Starch grains are distinct in their type, dimensions, hilum, and striations.

Organoleptic characteristics: These characteristics are important in the identification of Ayurvedic drugs. However, these characteristics of the crude drug are very subjective.

Table 5. Organoleptic characteristics of *B. erecta* root powder

Organoleptic characters	Observations
Colour	Brown
Smell	Indistinct
Taste	No specific taste
Feel	Slightly coarse

Table no. 6. Fluorescence Characteristics of root powders of *B. erecta*

Mountant medium	Fluorescence colour
P+ nitrocellulose in amylacetate (A)	Pale luteous
P+ 1N NaOH in MeOH + nitrocellulose in amylacetate (B)	Umber
P+ 1N NaOH in MeOH	Umber
P+ D.W.	Citrine

Fluorescence analysis was used for quick identification of powers. However, it has limited applications in drug evaluation, and it can be used as an additional parameter for the differentiation of closely related species.

Microscopic analysis of root powder: Cork cells are large-sized and large in number. Broken fragments of vessels; pieces of lignified, long, slender, cylindrical fibres with tapering ends; abundant simple (7.0-12.0 µ) and compound starch grains; the presence of characteristic prismatic crystals. Fluorescence was observed with 1 N NaOH in methanol and aq. NaOH. The microscopical examination of root powder shown characteristic abundant prismatic or rhomboid crystals of calcium oxalate in the cortex region.

Ash analysis and extractive values: Physical evaluation of drugs is an important parameter in detecting adulteration or improper handling of drugs. The total ash is particularly important in the evaluation, for the purity of the drugs, that is, to identify the presence or absence of foreign inorganic matter such as metallic salts and silica. Ash analysis and extractive values have been recorded in Table no. 7 & 8

Table 7 Ash values of *B. erecta* root

Ash peculiarities	Values w/w
Total ash	10.10(10.00-10.32)
Water insoluble	7.29(7.10-7.32)
Acid insoluble	0.95(0.94-0.96)

Table 8 Extractive values for *B. erecta* root

Extractives	Values w/w
Petroleum ether (60-80^{0}C)	0.18 (0.175-0.190)
Ethanol	2.49 (1.198-2.801)
Water	11.875 (9.83-12.0)

Table no.9. Histo-chemical tests for *B. erecta* roots

Chemical	Tissues
Lignin	Cu,P.xy,S.xy, Xy.f.
Cellulose	Ck, Cor, P.,Md.
Suberin and cutin	Cu., Ck.,
Chitin	-
Mucilage	-
Starch	Hyp.Cor.,P.
Proteins	Pd., Cor., Phl.par., Xy.
Amino acids	-
Fats and oils	Cor.,
Tannins	-
Alkaloids	Epi.,
Saponins	-
Glycosides	Epi.,X.par.
Flavonoids	-
Steroids and terpenes	-

Ck-cork; **Cor**-cortex; **Cu**-cuticle; **Endo**-endodermis; **Epi**-epidermis; **Hyp**-hypodermis; **Md**-medullary rays; **M.xy**-Metalxylem; **P**-pith; **Pal**.- palisade; **Per**-pericycle; **Pd**-phelloderm; **Phl**-phloem; **Phl.par**- Phloem parenchyma; **P.xy**- Primary xylem; **S.xyl**-Secondary xylem; **Scl**-sclerenchyma; **St**-spongy tissue; **Xyl.V.**-Xylem vessel; **Xy.par**-xylem parenchyma; **Xy**-xylem.

Chitins, mucilage, tannins, flavonoids and steroids and terpenes were not detected in Punarnava species.

Phytochemical studies of *B. erecta* root: Phytochemical analysis showed the presence of alkaloids, glycosides, and sugars.

Table 10 Preliminary phytochemical studies of root

Sr.no	Chemical	Detection
1	Carbohydrates	+
2	Starch	+
3	Reducing sugars	+
4	Pentoses	+
5	Proteins	-
6	Amino acids	-
7	Fats/ oils	+
8	Tannins	-
9	Alkaloids	+
10	Glucosides	+
11	Flavonoids	+
12	Resins	-
13	Steroids and terpenes	+

Table no. 11. Proximate analysis of *B. erecta* root.

Constituents	*B.erecta*
Total carbohydrates	76.16
Total proteins	6.30
Total fats	0.20
Total crude fibres	9.70
Total ash	7.62
Total percentage	100.0

Very few researchers have studied fluorescence with 1 N NaOH in methanol and aq. NaOH differs from the fluorescence studies, Phytochemical analysis, and acid ash values and extractive values recorded by earlier research workerd [15]. The values recorded by earlier research worker are especially acid ash value and extractive values 19.15, 4.0, 18.3, 0.95 for water, alcohol, solvent ether,

and petroleum ether (60-80°C), respectively, in *B.erecta* Root. The results depicted in present research work are serves as paramers for identification, authentication, detection of adulteration and quality control for crude drugs.

TLC studies of *B. erecta*: TLC studies have been recorded of the root of *B. erecta* ethanol, and petroleum ether extract with chloroform: methanol (80:20) system for U.V. developers, iodine and 1% vanillin-50% phosphoric acid has been recorded in Table no. 12

Table 12. TLC profile of *B. erecta* root extract (Petroleum ether)

Spot no	*Rf value	254nm	366nm	Iodine	1%Vanillin- 50% phosphoric acid	Dragendorffs reagent
1	0.30	-	-	yellow	purple	-
2	0.37	intense blue	faint blue	-	-	-
3	0.44	-	-	faint yellow	faint purple	-
4	0.56	intense blue	intense blue	-	-	-
5	0.70	faint blue	-	-	faint purple	-
6	0.74	-	-	yellow	-	-
7	0.83	orange	faint blue	-	-	orange
8	0.96	intense blue	intense blue	-	-	-

TLC conditions:
Solvent system: **Chloroform: methanol (80:20)**
Silica layer G of 0.3 mm thickness.
Activation Time: 60 min.
Saturation time: 40 min.
Temperature: 28-30^{0}C
Plates were observed under the CAMAG UV cabinet.
*Mean of 10 observations.

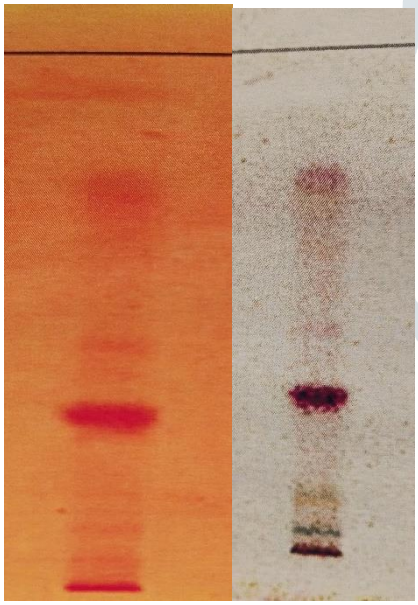


Fig.6 Visualizer-Iodine

Fig. 7 Vanillin-phosphoric acid

Solvent system- Chloroform: Methanol (80:20)

TLC profiling of *B. erecta* studies in chloroform: methanol (80:20) system recorded for Petroleum ether.

The present study on the pharmacognostic standardization and the physico-and phytochemical evaluation of *B. erecta* will be useful to supplement the information about its identification parameters, which are assumed significant for the acceptability of herbal drugs in the present scenario.

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REFERENCES

[1] Guha Bakshi, D.N., Sensarma, P. and Pal, D.C. (1999), A Lexicon of Medicinal Plants in India. Vol. I. NayaProkash 206, Bidhan Sarni, Calcutta.

[2] Kirtikar, K.R. and Basu, B.D. (1933, 1958 Repr.), Indian Medicinal Plants. Vol. III. Lalit Mohan Basu, Allahabad.

[3] Datta, S.C. and Mukerji, B. (1952), Pharmacognosy of Indian leaf Drugs. Pharmacognosy Laboratory Bulletin no.2. Delhi. Ministry of Health, Govt. of India. Govt. of India Press, Calcutta.

[4] Gupta, R.B.L., Singh, S., and Dayal, Y. (1962) Effect of Punarnava on visual acuity and refractive errors. **Indian J. Med. Res. 50 (3):** 428-434.

[5] Chakraborti KK, Handa SS.1989. Antihepatotoxic investigations on *Boerhaavia repanda* will. Indian Drugs 27(1):19-24.

[6] Tiwari, K.P., Shrivastav, J.L. and Sharma, M.C. (1998), Medicinal plants of Madhya Pradesh- Distribution, Cultivation, and Trade. **Bulletin no. 31:** 92 State Forest Research Institute (SFRI), Jabalpur, India.

[7] Nair, N.C. (1967) On the identity of *Boerhaavia punarnava* Saha et Krishnamurthy. **Bull. Bot. Surv. India 9:** 283.

[8] Chakravarty, H.L. (1942), The identity of Punarnaba. **J. Ind. Bot. Soc. 21 (122):** 87-92.

[9] Ordonez, M. G.; Montalvo, R. V.; Martinez, R. R.; Castilo, R. M.; Pulido, A. G.; Sardinas, I. G., Activadad antimicrobianay toxicidad de un extracto acuoso de Boerhavia erecta L. Abstract-Rev. Cubana Planta Med. 2004, 9.

[10] Hilou, A.; Nacoulma, O. G.; Guiguemde, T. R., In vivo antimalarial activities of extracts from Amaranthus spinosus L. and Boerhaavia erecta L. in mice. J. Ethnopharmacol. 2006, 103, (2), 236-40.

[11] Nugraha, A. S. Pharmacochemical screening on an African medicinal plant (*Boerhavia erecta* L.): Pursuing anti-HIV agent; University of Wollongong, Wollongong, 2008.

[12] Anonymous. 1999. The Ayurvedic Pharmacopoeia of India.1:191–2. Ministry of Health and Family Welfare, Department of Health, Govt. of India.

[13] JOHANSEN, D.A., 1940. PLANT MICRO-TECHNIQUES. FIRST ED. MCGRAW HILL BOOK COMPANY. INC., NEW YORK**.**

[14] Stahl, E. (1969), Thin Layer Chromatography. Springer-Verlag, Berlin.

[15] S R Surange and Pendse G.S. 1972. Pharmacognositc studies of the root of Boerhaavia repanda Wild. (Purnanva) J. Res. Indian Med.7.(1) 1-7.