

A Comparative Diagnostic account of Punarnava (*Boerhaavia diffusa* L, *B. erecta*, *B. repanda* and *Trianthema portulacastrum* L.)

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Abstract: Punarnava is an important therapeutic drug in Ayurveda, Siddha, and Unani systems of medicine. In Ayurveda, Punarnava is regarded as a rejuvenating drug. It has been employed commonly since the ancient period traditionally by the native population for various ailments. This plant has a variety of applications such as laxative, antipyretic, stomachic, cardiogenic, expectorant, hypotensive, diuretic, antibacterial, anti-inflammatory, and kidney stimulant. Punarnava is potent medicine on asthma, jaundice, eye diseases, urine complaints and menstrual cramps. However, there is confusion regarding the real identity of 'Punarnava.' Rakta Punarnava is equated with *B. diffusa* L., Neel Punarnava with *B. repanda*, and Shwet Punarnava with *B. erecta* and *Trianthema portulacastrum* L. In the present work, detailed comparative morphology, anatomy, pharmacognosy, phytochemistry, and chromatographical studies, along with the biological activity of all four Punarnava species, have been carried out. The detailed studies of these species revealed that all these species are very distinct. The anatomical parameters, physico-chemical constants and chromatography studies carried out in the present investigation serves as measures of a quality control and fingerprinting for the four plants i.e. . *B. diffusa*, *B. erecta*, *B. repanda*, and *Trianthema portulacastrum* L.

Keywords- Anti-inflammatory, *Boerhaavia erecta*, *Boerhaavia erecta*, *Boerhaavia repanda*, Chromatographic studies, quality control, pharmacognostic standardization, physicochemical parameters, Shwet Punarnava, *T. portulacastrum*.

I. INTRODUCTION

Punarnava is an important therapeutic drug in Ayurveda, Siddha, and Unani systems of medicine. In Ayurveda, Punarnava is regarded as rejuvenating drug. It had been employed since the ancient period traditionally by the native population for curing various ailments. Punarnava has been mentioned in Rigveda, Matsya Purana, and AgniPunarana, Vagbhatta, Caraka Samhita, Sushruta, Bhava Prakash, Dhanvatariya Nighantu and other related texts [1-5]. In Nighantus, these drugs are valued for cough, asthma, anemia, swellings, eye diseases, nervous disorders, etc. Punarnava has various therapeutic properties and used as laxatives, antipyretic, stomachic, cardiogenic, expectorant, hypotensive, diuretic, antibacterial, anti-inflammatory, and kidney stimulant. It is also used for asthma, jaundice, eye diseases, urine complaints and menstrual cramps [6-12]. The roots of all these plants are used for the medicinal purpose. In the pharmaceutical industries, Punarnava is used in 52 preparations viz. Livemap drop, Restone syrup, Livotrit liquid, Livo-ton syrup, etc. for liver disorders and to promote urination in dropsy [13]. The literate survey revealed that in the Punarnava species viz. *B.diffusa*, *B.erecta*, *B.repanda*, and *T. portulacastrum*, the detailed comparative studies have not been worked out so far. In view of the medicinal importance, the controversy of related to identity of Punarnava, to identify adulteration, and to evolved parameters for quality control a detailed study of all the four species *B.diffusa*, *B.erecta*, *B.repanda*, and *T. portulacastrum* has been carried out.

II. MATERIALS AND METHODS

Plant material: Fresh plant material of all the four species *B.diffusa*, *B.erecta*, *B.repanda*, and *T. portulacastrum* 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 all the four species was done from the Botanical Survey of India, Western Zone, Pune. Hand sections of plant material were used for anatomical and histochemical studies. The histochemical Studies were carried out using standard methods [14]. Root powder analysis was carried out as per the method described by following Trease and Evans method [15]. Fluorescence analysis was carried out by Chase and Pratt method [16]. The ash analysis and percentage of extractive was determined by using standard methods Ayurvedic Pharmacopoeia of India [17]. Preliminary phytochemical screening was by using coarse root powders that were 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 studies performed as per the methods described by Stahl [18]. 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:

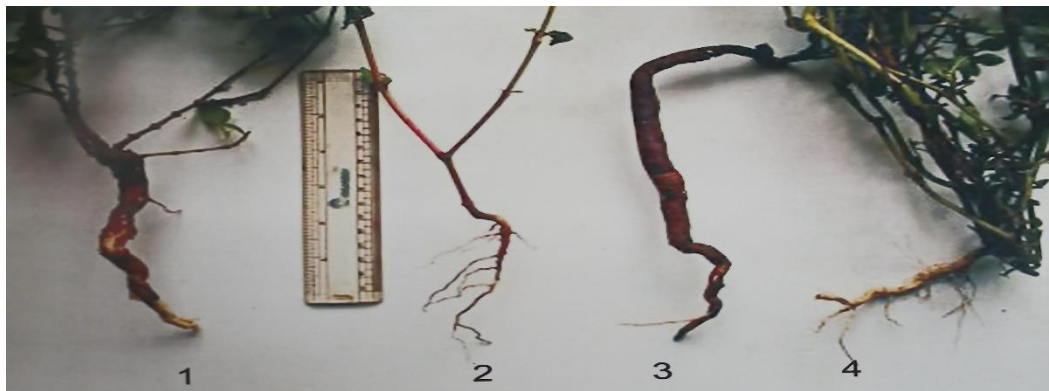


Figure 1. 1. *B.diffusa* 2. *B. erecta* 3. *B.repanda* 4. *T. portulacastrum* Root.

Table no. 1. Comparative characteristics of root

Characters	<i>B.diffusa</i> L.	<i>B.erecta</i> L.	<i>I. B.repanda</i> Willd.	<i>T.portulacastrum</i> . L
Root type	Large, fusiform, thick, woody, no lateral roots	Small, conical, slender, woody, not rough, few lateral roots	Large, tapering at both ends, thick brittle, no lateral roots.	Small, conical, cylindrical, extensive lateral roots.
Root colour	Dark brown	Pale yellow	Brownish	Whitish
Root diameter in cm	9-15.5-22 X 0.6-1.6-2.0	5.5-10.85-17.0 X 0.1-0.17-0.3	11.0-19.39-28 X 0.5-0.9-1.5	4.5-16.97-30.0 X 0.12-0.3-0.6

Table no. 2. Organoleptic characteristics of root

Organoleptic character	<i>B.diffusa</i>	<i>B.erecta</i>	<i>B.repanda</i>	<i>T.portulacastrum</i>
Colour	Whitish gray	Gray	Dull white	Gray
Smell	Indistinct	Indistinct	Indistinct	Indistinct
Taste	Slightly sweetish	No specific taste	Sweetish	No specific taste
Feel	Course	Course	Very fine	Slightly course
Fracture	Incomplete, fibrous	Complete, smooth	Complete, smooth	Incomplete, fibrous

Table no. 3. Starch grains of root

Features	<i>B. diffusa</i>	<i>B.erecta</i>	<i>B.repanda</i>	<i>T.portulacastrum</i>
Type of starch grains	Simple and compound	Simple and compound	Simple and compound	Simple and compound
Shape (single)	Rounded, polyhedral, ovoid.	Rounded, subspherical, ovoid.	Ovoid to ellipsoidal, polyhedral	Ovoid elliptical, polyhedral
Dimensions (single)	6.0-8.35-11.0 X 7.0-8.46-10.0 μ	4.0-5.12-7.0 X 4.0-5.25-7.0 μ	7.0-11.80-19.0 X 7.0-11.28-18.0 μ	7.0-10.12-12.0 X 8.0-9.87-13.0 μ
Hilum	line	'Y' shaped	2-5 rayed fissure	line
Striations	Faintly marked	Faintly marked	Faintly marked	Faintly marked
Aggregation	Mostly compound	Very few compounds	Very few compounds	Very few compounds

Table no. 4. Anatomical peculiarities of root

Anatomical features	<i>B.diffusa</i>	<i>B.erecta</i>	<i>B.repanda</i>	<i>T.portulacastrum</i>
Cork	10-12 layered, cells large, suberized and uneven	2-3 layered, cells small, suberized and uneven	10-12 layered, cells large and uneven	1-2 layered, cells large and uneven
Cortex	6-7 layered, with polygonal cells	5-6 layered, with polygonal cells	20-22 layered, with polygonal cells	4-5 layered, with rectangular cells
Endodermis and pericycle	Not distinct	Not distinct	Not distinct	Not distinct
Phloem	Occurs as crescent-shaped patch outside xylem vessels	Forms patch outside xylem vessels	Forms hemispherical patch outside xylem vessels	In the form of narrow strips on the outside of xylem
Xylem	Vessels are in a group of 2-8, polygonal to oblong-shaped	Vessels are in a group of 1-4, polygonal to oblong.	Vessels are in the group of 2-6, two-vessel elements are quite big, polygonal to oblong shaped.	Vessels arranged in a row, polygonal to oblong shaped.

Medullary rays	Uniseriate with narrow cells	Uniseriate, with rectangular cells	Not distinct	Uniseriate to biseriate, with narrow to squarish cells
Pith	Primary bundles occupy the central region	Pith becomes sclerified in later stages	The abundant pith of parenchymatous tissues	Pith is crushed by secondary growth at maturity
Crystals	Few starch grains and raphides	Few starch grains and raphides	Abundant starch grains and raphides	Few starch grains and rhomboidal prisms

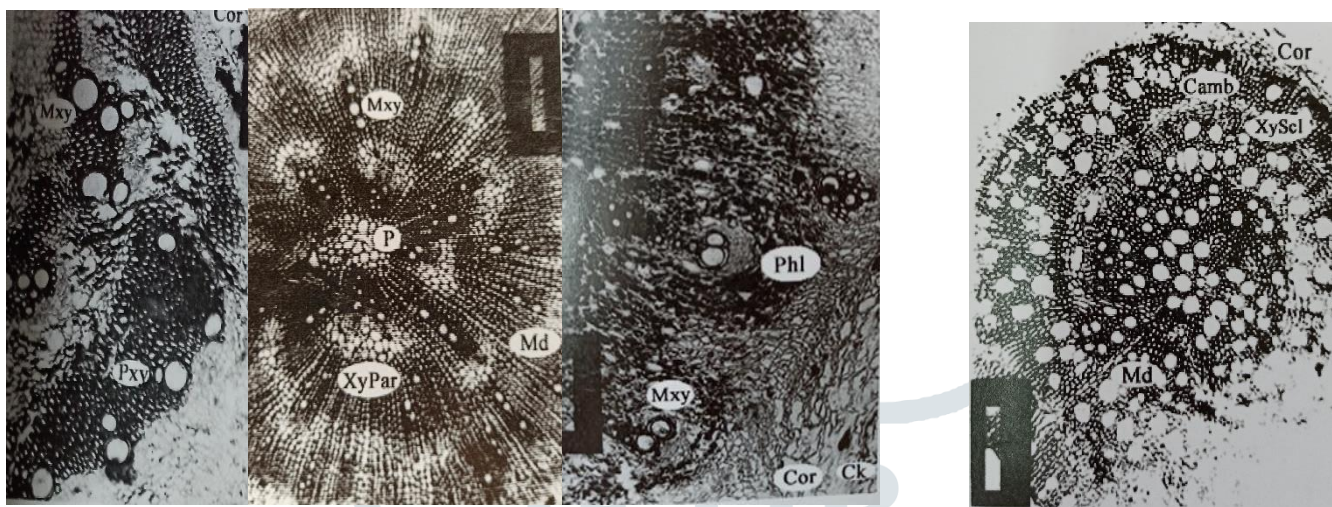


Figure 2. 2.1. *B. diffusa* 2.2 T.S. of *B. erecta* Root 2.3 T.S. of *B. repanda* Root 2.4 T.S. of *T. portulacastrum* Root

P-Pith, Md-Medullary rays, Mxy-Metaxylem, XyPar-Xylem parenchyma Ck-cork; Cor-cortex; Md-medullary rays; M.xy-Metalxylem; P-pith

Earlier workers reported zigzag medullary rays while in the present investigation did not show zigzag medullary rays in *B. diffusa* [19]. The cork and cortex are recorded many-layered in *B.diffusa* and *B.repanda* while *B.erecta* and *T. portulacatrum* are few-layered. The crystal analysis showed that in *T. portulacastrum*, the crystals are prismatic with 11.0-21.76-30.0 X 8.0-14.11-25.0 μ while in other Punarnava species, these are needle-shaped (raphides). Structural differences and variations in the dimensions in tissue elements serve as indemnification mark for the closely related species.

Table no. 5. Maceration studies of Root

Dimensions of cells	<i>B.diffusa</i>	<i>B.erecta</i>	<i>B.repanda</i>	<i>T.portulacastrum</i>
Vessel elements (μ)	Reticulate thickenings 85.0-140.0-160.2X52.0-74.5-97.0	Reticulate or sclariform 102.4-137.0-190.0X50.0-54.4-70.0	Spiral 60.0-75.0-80.5X20.0-55.16-80.0	Reticulate 70.0-140.7-160.0X25.0-32.1-65.0
Tracheids (μ)	110.0-195.7-260.0X16.5-22.0-43.0	100.0-187.2-270.0X15.0-20.66-40.0	52.0-130.2-170.0X10.0-18.12-19.5	80.0-146.0-173.0X12.0-15.6-25.0
Xylem fibres (μ)	220.0-310.0-417.0X16.0-20.0-27.0	210.0-295.5-400.0X15.0-19.93-25.0	120.0-310.7-410.0X13.0-18.0-20.0	120.0-240.0-293.0X10.0-15.8-25.0
Xylem parenchyma (μ)	70.5-95.0-100.0X13.4-18.1-23.0	70.2-80.2-92.0X15.3-20.2-35.0	80.0-100.0-110.0X15.2-20.2-25.0	100.0-120.2-140.0X35.0-40.0-45.8
P+ D.W.	Flax blue	Citrine	Flax blue	Flax blue

Table no. 6. Histochemical tests of Purnanava root

Phytochemicals	<i>B. diffusa</i> L.	<i>B. erecta</i> L.	<i>B. repanda</i> Willd.	<i>T.portulacastrum</i> L.
Alkaloids	*Epi.	Epi.	Epi.	Epi.
Amino acids	..**	-	-	-
Cellulose	Ck, Cor, P, Md	Ck, Cor, P., Md.	Ck, Cor, P., Md.	Ck, Cor, P., Md.
Chitin	-	-	-	-
Fats/lipids	Cor.	Cor.	Cor.	Cor.
Glycosides	Epi., Xyl.Par.	-	-	-
Lignin	Cut, P.Xyl, S.Xyl.	Cut, P.Xyl, S.Xyl	Cut, P.Xyl, S.Xyl.	Cut, P.Xyl, S.Xyl
Mucilage	-	-	-	-
Proteins	Pd., Cor., Phl.Par., Xyl.	Pd., Cor., Phl.Par., Xyl.	Pd., Cor., Phl.Par., Xyl.	Pd., Cor., Phl.Par., Xyl.
Reducing sugars	-	-	-	-
Saponins	-	-	-	-
Starch	Hyp, Cor., P.	Hyp. Cor., P.	Hyp., Cor., P.	Hyp., Cor., P.
Steroids and terpenes	-	-	-	-
Suberin and cutin	Cut., Ck.	Cut., Ck.	Cut., Ck.	Cut., Ck.
Tannins	-	-	-	-

Ck-cork; **Cor**-cortex; **Cu**-cuticle; **Endo**-endodermis; **Epi**-epidermis; **Hyp**-hypodermis; **Md**-medullary rays; **M.xy**-Metalxylem; **P**-pith; **Pal**- pallsade; **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.

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; Simple and compound starch grains; the presence of characteristic prismatic crystals were observed.

Table no. 7. Fluorescence analysis of root

P+ nitrocellulose in amyloacetate (A)	Pale luteous	Pale luteous	Flax blue	Luteous
P+ 1N NaOH in MeOH + nitrocellulose in amyloacetate (B)	Umber	Umber	Umber	Hazel
P+ 1N NaOH in MeOH	Isabellite	Umber	Citrine	Isabellite
P+ D.W.	Flax blue	Citrine	Flax blue	Flax blue
P+ nitrocellulose in amyloacetate (A)	Pale luteous	Pale luteous	Flax blue	Luteous
P+ 1N NaOH in MeOH + nitrocellulose in amyloacetate (B)	Umber	Umber	Umber	Hazel
P+ 1N NaOH in MeOH	Isabellite	Umber	Citrine	Isabellite
P+ D.W.	Flax blue	Citrine	Flax blue	Flax blue

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

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. 8 & 9

Table no. 8 Ash analysis of root

Ash values	<i>B.diffusa</i> L.	<i>B.erecta</i>	<i>B.repanda</i>	<i>T.portulacastrum</i> L.
Total ash	14.909 (14.815-15.101)	10.102(10.000-10.320)	10.402(10.388-11.395)	14.760(14.650-14.810)
Water insoluble	11.405(11.100-11.541)	7.290(7.102-7.325)	9.150(9.000-9.302)	5.500(5.390-5.650)
Acid insoluble	0.620(0.592-0.645)	0.955(0.945-0.965)	2.200(2.182-2.319)	1.000(0.950-1.125)

Table no. 9. Extractive values of root

<i>B. diffusa</i>	<i>B. erecta</i>	<i>B. repanda</i>	<i>T. portulacastrum</i>
Pet-ether (60-80°C) 254nm	0.248 (0.225-0.280) Vinacious buff	0.184 (0.175-0.190) Olivacious buff	0.213 (0.131-0.286) Buff
Ethanol 254nm	1.670 (1.102-2.159) Vinaceous buff	2.488 (1.198-2.801) Vinaceous buff	1.698 (1.550-1.882) Buff
Water 254nm	9.840 (8.545-10.534) Vinaceous buff	11.875 (9.83-12.0) Mauve	8.175 (7.512-9.542) Vinaceous buff

Table no. 10 Phytochemical studies of root

Name of chemical	<i>B. diffusa</i>	<i>B. erecta</i>	<i>B. repanda</i>	<i>T. portulacastrum</i>
Reducing sugars	+	+	+	+
Pentoses	+	+	+	+
Proteins	-	-	-	-
Amino acids	-	-	-	-
Fats/ oils	+	+	+	+
Tannins	-	-	-	-
Alkaloids	+	+	+	+
Glucosides	+	+	+	+
Flavonoids	+	+	+	+
Resins	-	-	-	-
Steroids and terpenes	+	+	+	+

Table no. 11 Proximate analysis of Root

Constituents	<i>B.diffusa</i>	<i>B.erecta</i>	<i>B.repanda</i>	<i>T.portulacastrum</i> L.
Total carbohydrates	71.007	76.165	78.1	75.02
Total proteins	7.820	6.30	5.0	6.74
Total fats	0.202	0.205	0.225	0.348
Total crude fibres	8.19	9.705	6.313	10.217
Total ash	12.781	7.625	10.362	7.675
Total percentage	100.0	100.0	100.0	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. The ash values and extractive values recorded by earlier research workers are quite high than the presently recorded ash vales [20]. The results are depicted in present research work is for the identification, authentication, and detection of adulteration, as also for the compilation of quality control standards for crude drugs.

TLC studies has been carried out for the root of of all the four species. 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 no. 12. TLC studies of ethanol extracts of Punarnava species in chloroform: methanol (80:20) system

Sp of no	*Rf value	254nm				366nm				Iodine				1%Vanilline-50% phosphoric acid			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
4	0.30	faint blue	-	-	-	faint blue	-	-	-	yellow	yellow	-	-	faint violet	purple	-	purple
6	0.37	intense blue	intense blue	intense blue	intense blue	faint blue	faint blue	faint blue	faint blue	-	-	-	-	-	-	-	-
7	0.44	Faint blue	-	-	-	-	-	-	-	-	faint yellow	yellow	-	faint purple	faint purple	purple	-
8	0.47	-	-	-	-	-	-	-	-	-	-	-	yellow	-	-	-	faint purple

9	0.56	intense blue	intense blue	intense blue	-	intense blue	intense blue	faint blue	faint blue	-	-	-	-	yellow	-	faint yellow	-
10	0.58	-	-	-	-	-	-	-	-	-	-	-	-	purple	-	-	-
12	0.65	faint blue	-	-	faint blue	-	-	-	-	-	-	-	-	-	-	-	-
13	0.70	intense blue	faint blue	intense blue	-	-	-	-	-	yellow	-	yellow	-	blue	faint purple	purple	purple
14	0.74	-	-	-	-	-	-	-	-	-	yellow	-	yellow	dark purple	-	-	-
15	0.83	brown	orange	intense blue	intense blue	faint blue	faint blue	faint blue	faint blue	-	-	-	-	-	-	-	-
17	0.96	intense blue	intense blue	intense blue	intense blue	intense blue	intense blue	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 mean time: 40 min

Temperature: 28-30 degree Celsius

Plates were observed under CAMAG UV unit.

*Mean of 10 observations.

1. *B. diffusa* L. root (ethanol extract)
2. *B. erecta* L. root (ethanol extract)
3. *B. repanda* Willd. root (ethanol extract)
4. *T. portulacastrum* root (ethanol extract)

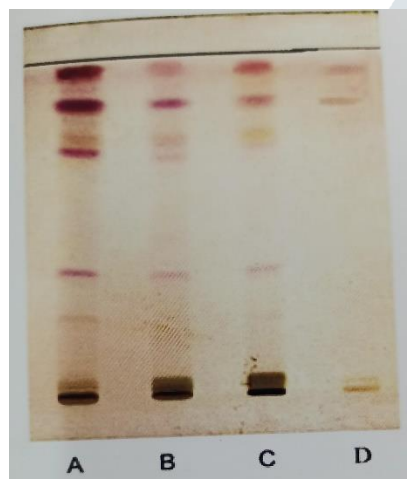


Fig.6

Fig. 7

Vanillin-phosphoric acid

Solvent system- Chloroform: Methanol (80:20)

- A. *B. diffusa* L. root (ethanol extract)
- B. *B. erecta* L. root (ethanol extract)
- C. *B. repanda* Willd. root (ethanol extract)
- D. *T. portulacastrum* root (ethanol extract)

Conclusion: The present study on the pharmacognostic standardization and the physico-and phytochemical evaluation of all the four species 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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