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COMPARATIVE PHARMACOGNOSTICAL EVALUATION OF ROOT AND LEAF OF PUNARNAVA (*BOERHAVIA DIFFUSA* L.) AND VARSHABHU (*TRIANTHEMA PORTULACASTRUM* L.)

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INTRODUCTION

Punarnava (*Boerhavia diffusa* Linn.) is an important medicinal plant used in Ayurvedic system of Medicine since a long period. **Varshabhu (*Trianthema portulacastrum* Linn),** a common weed which grows very rapidly in rainy season, seems very similar to Punarnava in morphology. Generally there are found two varieties of Punarnava Rakta and Sveta according to many Acharya. But in Raja Nighantu another variety Nile Punarnava is also described. Some Acharya accept Rakta Punarnava as *Boerhavia diffusa* and Sveta Punarnava as *Trianthema portulacastrum*. Thakur Balvant Singh ji considered *Boerhavia repens* Linn as Sveta Punarnava and *Boerhavia diffusa* Linn. as Rakta Punarnava while mentioning *Trianthema* species as Sveta and Rakta Varshabhu.

Acharya Charaka has mentioned Punarnava in swedopaga, Anuvasnopaga, Kasahara and vayasthapana mahakashay whereas Acharya Sushruta has placed them in vidarigandhadi gana and Acharya Vagbhatta in Vidaryadi gana.

In **Bhava Prakash Nighantu**, Punarnava and Varshabhu are mentioned in Guduchyadi Varga; In **Kaiyedevanighantu**, it is mentioned in Aushadhivarga; In **Dhanwantari Nighantu,** they are mentioned in Guduchyadi Varga; In **Raja nighantu** Punarnava is mentioned in Parpatadi varga.

Punarnava is a traditional ayurvedic plant that is used to rejuvenate the whole body. The plant as a whole is used in the treatment of rheumatoid arthritis, fever, edema, eye problems, stomach issues, and liver disorders.

MORPHOLOGY

***Boerhavia diffusa* L.**

- **Stem:** Greenish purple, stiff, slender, cylindrical, swollen at nodes, minutely pubescent or nearly glabrous, prostrate divaricately branched, branches from common stalk, often more than a meter long.
- **Root:** Well developed, fairly long, somewhat tortuous, cylindrical, 0.2-1.5 cm in diameter, yellowish brown to brown coloured, surface soft to touch but rough due to minute longitudinal striations and root scars, fracture, short, no distinct odour, taste, slightly bitter, sweet, pungent.
- **Leaves:** Opposite in unequal pairs, larger ones 25-37 mm long and smaller ones 12-18 mm long ovate-oblong or suborbicular, apex rounded or slightly pointed, base subcordate or rounded, green and glabrous above, whitish below, margin entire or sub-undulate, dorsal side pinkish in certain cases, thick in texture, petioles nearly as long as the blade, slender.
- **Flowers:** Very small, pink coloured, nearly sessile or shortly stalked, 10-25 cm, in small umbells, arranged on slender long stalks, 4-10 corymb, axillary and in terminal panicles, bracteoles, small, acute, perianth tube constricted above the ovary, lower part greenish, ovoid, ribbed, upper part pink, funnel-shaped, 3 mm long, tube 5 lobed, stamen 2-3.
- **Fruit:** One seeded nut, 6 mm long clavate, rounded, broadly and bluntly 5 ribbed, viscidly glandular.

***Trianthema portulacastrum* L**

Root: Light yellow on the surface, creamish white inside, thin, slender, tapering with lateral branching fibrous root, 5 to 15 cm in length, 0.3 to 2.5 cm in diameter.

- **Stem:** Cylindrical, dichotomously branched, prostrate, glabrous with reddish tints at places and swollen nodes;
- **Leaves:** Entire, wavy with reddish and papillose border, sub-fleshy, obliquely opposite, unequally paired, exstipulate, larger leaves obovate to obcordate, 2 to 4 by 2 to 2.3 cm, the smaller one

narrow oblong and tapering to the base, rounded or apiculate at the apex, 10.2 to 6 mm, long petiolate, dilated into a membranous pouch at the base clasping the stem especially those of the smaller leaves, slightly hairy;

- **Flower:** Small, solitary, sessile, pinkish, nearly concealed by the pouch of the petiole, calyx tube scarious, thin, stamens 10 to 15, ovary superior, sessile, style single papillose, shorter than the stamens.

Taxonomical Classification

	Punarnava	**Varshabhu**
Kingdom	Plantae	Plantae
Phylum	Tracheophyta	Spermatophyta
Class	Magnoliopsida	Dicotyledonae
Order	Caryophyllales	Caryophyllales
Family	Nyctaginaceae	Ficoidaceae (Aizoaceae)
Genus	*Boerhavia*	*Trianthema*
Species	*diffusa*	*portulacastrum*

Rasapanchak

Properties of Punarnava and Varshabhu mentioned in various text

Rasapanchaka	**D.N.**	**S.N.**	**M.P.N.**	**K.N.**	**Bh.N.**	**R.N.**
Tikta Rasa	+		+	+	+	+
Kashaya Rasa				+		
Madhura Rasa				+		
Sara Guna			+	+		
Laghu Guna	+		+		+	
Ruksha Guna	+			+		
Ushna Guna	+					
Ushna Virya	+	+		+	+	
Sheeta Virya			+			
Madhura Vipaka						
Katu Vipaka			+		+	

(D.n. – Dhanvantari Nighantu, S.n.- Shodhal Nighantu, M.p.n.- Madanpal Nighantu, K.n.- Kaiyadeva Nighantu, Bh.n.- Bhavprakash Nighantu, R.n.- Raj Nighantu)

Raspanchak of Punarnava in API
Rasa- Madhura, Tikta, Kashaya
Guna- Ruksha
Virya- Ushna
Vipaka- Madhura

Raspanchak of Varshabhu in API
Rasa- Madhura, Katu, Tikta, Kashaya
Guna- Laghu, Ruksha
Virya- Ushna
Vipaka- Katu

Effect of Punarnava and Varshabhu on Dosha

Effect of the drug on dosha, as mentioned in different texts is given below in the table

S.N.	**Ayurvedic text**	**Effect on Tri-Dosha**	
		Punarnava	**Varshabhu**
1.	Dhanvantari Nighantu	Pittanashak	
2.	Sodhala Nighantu	Kapha-vata nashak	
3.	Madanpal Nighantu	Vatakarak, Rakta-pitta nashak	
4.	Kaiyadev Nighantu	Vata-kapha nashak	Vatakarak, Rakta-pitta-kapha shamak
5.	Bhavprakash Nighantu	Vatakarak, Kapha-pitta-rakta vikara nashak	
6.	Gunaratnamala	Kaphanashak	
7.	Raj Nighantu	Pitta nashak	
8.	Shankar Nighantu	Pitta nashak	
9.	Nighantu Adarsa	Kapha-vata nashak	

Prayogyaanga (Usable part)

The official usable part of Punarnava and Varshsbhu is the **root and leaf**.

Matra (Posology)

- As per the **API** the dose of Punarnava is 20-30 gm of the drug for decoction.
- As per the **API** the dose of Varshabhu is 2-5 gm of the drug in powder form.

- As per **Dravyaguna Vigyan (Ach. P. V. Sharma)** doses of Punarnava are as follow
- Powder-3to 6 g,
- Expressed juice- 5 to 10 ml,

Comparative pharmacognostical study

For pharmacognostical studies laboratory work was conducted in laboratory of State Ayurvedic College, Lucknow and CSIR-NBRI, Lucknow.

Material – Root and leaf of Punarnava (Boerhavia diffusa L.) and Varshabhu (Trianthema portulacastrum L.) were used as sample material.

Collection of Sample- Root and Leaf of Punarnava and Varshabhu was collected in Varsha Ritu i.e., best time for collection of Varshabhu as mentioned in Ayurvedic text.

Organoleptic characters of crude samples of Punarnava and Varshabhu.

S.N.	Parameter	DESCRIPTION OF ROOT	
		***B. diffusa* L.**	***T. portulacastrum* L.**
1.	**Color**	Outer surface yellowish brown, Inner surface whitish	Outer surface light yellowish grey, inner surface whitish
2.	**Odour**	Characteristic	Characteristic
3.	**Taste**	Bitter some sweet	Not characteristic
4.	**Texture**	Hard, outer surface with small transverse and longitudinal cracks	Short, fibrous, uneven with wrinkles
5.	**Touch**	Rough	Rough

Organoleptic characters of powder of both samples

S.N.	Parameter	DESCRIPTION OF POWDER OF BOTH SAMPLE			
		Sample- PR	**Sample-PL**	**Sample- VR**	**Sample- VL**
1.	**Texture**	Coarse, fibrous	Fine to coarse	Fine to coarse	Fine to coarse
2.	**Color**	Light brown	Blackish green	Brown	Yellowish green
3.	**Odour**	No particular smell	Slightly bitter	Slightly sweet	Sweet
4.	**Taste**	Astringent	Bitter	No taste	Slightly sweet
5.	**Touch**	Rough	Smooth	Rough	Smooth

Microscopic study of root of *B.diffua* L.

Tranverse section of the root shows well-differentiated cork and cortex. There are about 10 to 12 layers of cork cells followed by several layers of cortical parenchyma which consists of many acicular crystals and starch grains. The stellar region shows centrally located primary xylem surrounded by phloem which is composed of phloem fibres and parenchyma. Surrounding this, a discontinuous band of secondary rings are observed which are separated by medullary rays.

❖ **Microcopic study of leaf**

TS of lamina through midrib shows a layer of upper and lower epidermis covered with a thick cuticle. Both the epidermis shows many unicellular, multicellular and glandular trichomes. Three to four rows of collenchymatous cells are found below the upper epidermal layer of midrib and a row of palisade cells is found below the upper epidermal layer of the lamina. About two to three layers of spongy parenchymatous cells are found below the palisade layer which is followed by the lower epidermis. Mesophyll consists of numerous vascular bundles surrounded by prominent bundle sheath and randomly distributed acicular crystals. The ground tissue of midrib is composed of parenchymatous cells and a centrally located vascular bundle.

❖ **Powder microscopy of *B.diffusa***

The powder was brown in colour, bitter in taste and has a characteristic smell. The powder showed the presence of cork, epidermal cells with anomocytic stomata, epidermis of petiole with stomata, trichome, cystolith, bordered pitted vessels, acicular crystals, starch grains and pollen grains.

Microscopic study of root of *T. portulacastrum* L.

Transverse section of the root is circular in shape. Outermost cork region constitutes of about two to three layers of tangentially elongated cells. Below the cork is the cortex region which is composed of several layers of polygonal parenchymatous cells with prismatic and acicular crystals of calcium oxalate. The cortex region is followed by secondary growth rings which are found predominantly in the section. The phloem and xylem cells occur alternately in the growth rings. Phloem is composed of phloem parenchyma with few acicular and rosette crystals, xylem is composed of vessels of different sizes and thick-walled fibres.

❖ **Microscopic study of leaf**

TS of leaf passing through midrib shows a single layer of upper and lower epidermis covered with cuticle. Epidermal layers are interrupted by stomata, uni to multicellular trichomes. Some trichomes are balloon shaped. Lamina shows upper epidermis followed by two layers of palisade cells interspersed by vascular bundles

that are surrounded by a parenchymatous sheath. There are about five layers of loosely arranged spongy parenchyma. Rosette and prismatic crystals are observed in the mesophyll. Midrib portion shows three vascular bundles arranged in an arc, with xylem vessels in rays and phloem in between. About two to three layers of parenchyma are seen surrounding the vascular region.

❖ **Powder microscopy of *T. Portulacastrum***

The powder was yellowish green coloured, sour taste and had a characteristic odour. The powder showed the presence of cork, epidermal fragment with paracytic stomata, surface view of epidermis with striations, sclerenchyma, rosette crystals, starch, vessels, tracheids with bordered pits and pollen grains.

Physico-chemical Study

S.N.	Experiments	Punarnava			Varshabhu		
		Root	Leaf	API (Panchang)	Root	Leaf	API (Root)Total
1.	**Foreign matter**	0%	0%	Not more than 2%	0%	0%	Not more than 2%
2.	**Moisture Content**	5%	6.9%	Not mentioned	11.9%	17.8%	Not mentioned
3.	**Total Ash**	11.8%	18%	Not more than 15%	7.25%	18.3%	Not more than 11%
4.	**Acid insoluble Ash**	2.8%	1.7%	Not more than 6%	0.8%	1.45%	Not more than 2%
5.	**Alcohol soluble extractive**	6.1%	8.7%	Not less than 1%	12.4%	14.1%	Not less than 2%
6.	**Water soluble extractive**	7.4%	8.3%	Not less than 4%	13.7%	19.5%	Not less than 11%

Phytochemical screening

Phytochemical analysis of both samples

Name of tests	Sample- PR	Sample- PL	Sample- VR	Sample- VL
Flavonoids	+	+++	+	+++
Tannins	+	+	+	+
Phenol	+	+	_	_
Starch	+	++	+	++
Alkaloid (Hegarand meyar)	++	++	_	_
Reducing sugar	++	+	++	+

(PR- Punarnava root, PL- Punarnava Leaf, VR- Varshabhu root, VL- Varshabhu leaf)

Chromatographic Study

TLC (Thin Layer Chromatography)

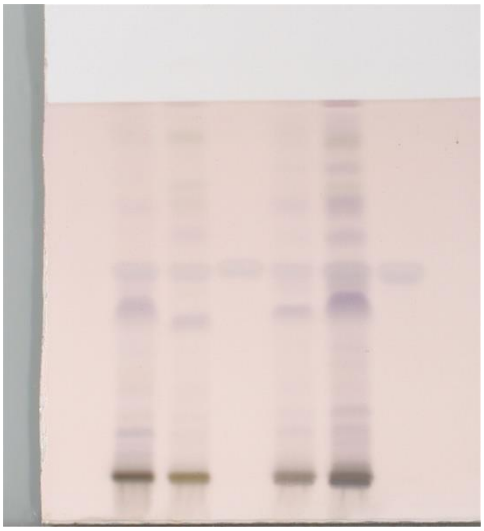
- **Samples stoksolution:** 100 mg methanolic Extract of each sample was dissolved with 5 ml chloroform and filtered solution with filter paper.
- **Samples working solution:** 20mg/ml Concentration of chloroform solution of each sample was used for TLC profile.
- **Reference stok solution:** 0.5 mg Betasitosterol marker components was dissolved with 1 ml of methanol.
- **Reference working solution:** 0.5mg/ml & 0.8mg/ml concentration of Betasitosterol
- **Mobile phase/Solvent system-** Toluene: Ethyl acetate: formic acid (8:2:0.1).
- **Procedure:** Prepared sample of Boerhavia diffusa L. (10µl&10µl applied volume) and Trianthema portulacastrum L. (10 µl & 10µl applied volume) and reference marker betasitosterol (5µl & 8µl applied volume) was applied on a pre-coated silica gel GF254 plate of uniform thickness (0.2 mm). Then develop the plate in the solvent system to a distance of 8 cm.
- **Visualization:** Observed of the plate under UV 254 and 366 nm and after derivatization with Annisaldehyde reagent under visible light. Band was shown after spraying with Annisaldehyde reagent dried in air and heated for 5 min at 1050 C temperature. After heating plate was observed in visible light and 366 nm UV light.

HPTLC (High Performance Thin Layer Chromatography)

- **Samples Stock Solution:** 100mg Extract of each sample was dissolved with 5 ml methanol.
- **Samples working solution:** 20mg/ml stock solution of each sample was used for HPTLC profile.
- **Reference working solution:** 0.5 mg /ml concentration Betasitosterol marker was used for HPTLC profile.

Working solutions of samples (20 mg/ml) and reference solution of marker (0.5 mg/ml) were prepared freshly from stock on same day for analytical work.

Marker Betasitosterol was quantified using Camag scanner equipped with Camag Visioncat software in absorption mode. HPTLC fingerprinting of samples of Boerhavia diffusa L. (10µl&10µl applied volume) and Trianthema portulacastrum L. (10 µl & 10µl applied) and reference marker (5µl, & 8µl, applied volume) was done. The mobile phase consisting Toluene: Ethyl acetate: formic acid (8:2:0.1). of was optimized for quantitative study. Under UV 254 nm and UV 366 nm wavelength plate was observed but presence of marker Betasitosterol was observed after derivatization of plate by Annisaldehyde.



PR PL BS VR VL BS
(BS: Bitasitosterol)

S. n.	Sample	% of Betasitosterol marker
1	PR	.0198%
2	PL	.0204%
3	VR	.0178%
4	VL	.0185

RESULT AND DISCUSSION

❖ Physico-chemical Study

Determination of foreign matter was done to check the presence of any adhering matter whether it is soil derived or by other source. According to API foreign matter in Boerhavia diffusa L. and Trianthema portulacastrum L. both should not be >2%. Both samples were collected by myself and washed with tap water, dried in shed. No foreign mater was observed. Moisture content of crude drug is related to its stability and consequently with the shelf life of crude drug. PR have 5.0% moisture content and PL have 6.9% VR have 11.9 % moisture content and VL have 17.8%.

The total Ash value method is design to measure the total amount of material remaining after ignition. This include both physiological ash which derived from the plant itself and non-physiological ash which is the residue of the extraneous matter (eg. sand and soil) adhering to plant surface. According to API (Punarnava API part I vol. 1) Total Ash value of Punarnava and Varshabhu should not be more than 15% and 11%. Total Ash value found in sample of PR and PL was 11.8% and 18% respectively and in VR and VL was 7.25% and 18.31%. Punarnava has higher value of ash content in comparison to Varshabhu.

The acid insoluble ash content indicates the presence of siliceous matter. Acid insoluble ash content found in PR and PL was 2.8% and 1.7% and in VR and VL was 0.89% and 1.45%. It signifies that Punarnava has more siliceous matter in comparision to Varshabhu. In API limit value of acid insoluble ash of Punarnava was mentioned as not more than 6 % and for Varshabhu it is not more than 2%.

Estimation of extractive values determines the amounts of active constituents in a given amount of plant material when extracted with a particular solvent. The extraction of any crude drug with a particular solvent yields a solution containing different phytoconstituents.

The Extractive values was done in 2 solvents; Alcohol and water. Limit of Alcohol soluble extractive value of Punarnava in API should be not less than 1%. The Alcohol soluble extractive value of PR and PL was found 6.1 % and 8.7% and of VR and VL was found 12.4% and 14.1%. Varshabhu have more alcohol soluble extractive value than Punarnava means that Varshabhu have more alcohol soluble active compounds.

Limit of Water-soluble extractive value of Punarnava in API should not be less than 4%. Water soluble extractive value in sample of PR and PL was found 7.4% and 8.3% and in VR and VL it was found 13.7% and 19.5%. Varshabhu have more water-soluble extractive value content in comparison to Punarnava, this indicates that Varshabhu have more water-soluble active constituents than Punarnava.

❖ Phytochemical screening

Identification of phytochemicals indicates pharmacological active metabolites present in the plant. Phytochemical screening of Aqueous extract and Methanolic extract samples of Punarnava revealed the presence of Alkaloid, carbohydrate, phenolic compound, flavonoids, tannin, saponin. Phytochemical screening of

Methanolic extract of Varshabhu revealed the presence of Carbohydrate, Alkaloid, phenolic compound, flavonoid, tannin (weakly positive), saponin, Steroid, terpenoid.

❖ **Chromatographic study**

TLC-Prepared sample of concentration 10mg/ml of Boerhavia diffusa (10µl&10µl applied volume) and Trianthema portulacastrum (10µl & 10µl applied volume) and reference marker betasitosterol (5µl and 8µl applied volume) was applied on a precoated silica gel GF254 plate of uniform thickness (0.2 mm). Then develop the plate in the solvent system of Toluene: Ethyl acetate: formic acid (8:2:0.1) to a distance of 8 cm. Observed of the plate under UV 254 and 366 nm and after derivatization with Annisaldehyde reagent under visible light. Band was shown after spraying with Annisaldehyde reagent dried in air and heated for 5 min at 1050 C temperature. After heating plate was observed in visible light and 366 nm UV light. Reference Marker was found in both samples.

HPTLC- HPTLC fingerprinting of samples of Boerhavia diffusa L. (10µl&10µl applied volume) and Trianthema portulacastrum L. (10 µl & 10µl applied) and reference marker (5µl, & 8 µl, applied volume) was done. Marker Betasitosterol was quantified using Camag scanner equipped with Camag Visioncat software in absorption mode. The mobile phase consisting Toluene: Ethyl acetate: formic acid (8:2:0.1). of was optimized for quantitative study. Under UV 254 nm and UV 366 nm wavelength plate was observed but presence of marker Betasitosterol was observed after derivatization of plate by Annisaldehyde reagent. On scan under 500nm, 550nm, 600nm wavelength, different colored band was observed. maximum band was observed in 550nm wavelength.

Presence of Marker Betasitosterol in sample of Boerhavia diffusa L. root and leaf were 0.0198% and0.0204% respectively. Presence of marker Betasitosterol in sample of Trianthema portulacastrum L. root and leaf were 0.0178% and 0.0185% respectively.

CONCLUSION

On the basis of present research work entitled ***"Comparative Pharmacognostical evaluation of root and leaf of Punarnava (Boerhavia diffusa Linn.) and Varshabhu (Trianthema portulacastrum Linn)"*** following conclusions can be drawn.

For Identification of root and leaf of Punarnava and Varshabhu all standard parameters were used to establish Identity, Purity and Strength of both species mentioned in API. On Pharmacognostical evaluation it was observed that sample of root and leaf of Punarnava (Boerhavia diffusa Linn.) full fills the parameters of Punarnava mentioned in API Part- I, Volume – I at serial number 60, page no.126. But authentic parameter of Varshabhu is mentioned in API Part-I, Volume-IV at serial number 64, page no.154. Ash values are used to determine quality and purity of crude drug. It indicates presence of various impurities like carbonate, oxalate and silicate Punarnava has higher value of ash content in comparison to Varshabhu means that Punarnava have more carbonate, oxalate and silicate like impurities than Varshabhu. The acid insoluble ash content indicates the presence of siliceous matter. Punarnava has more siliceous matter in comparison to Varshabhu.

Estimation of extractive values determines the amounts of active constituents in a given amount of plant material when extracted with a particular solvent. Varshabhu have more alcohol soluble extractive value than Punarnava, means that Varshabhu have more alcohol soluble active constituents as compare to Punarnava. Varshabhu have more water-soluble extractive value in comparison to Punarnava, this indicates that Varshabhu have more water-soluble active constituents than Punarnava.

Phytochemical screening of Methanolic extract samples of Punarnava revealed the presence of Alkaloid, carbohydrate, phenolic compound, flavonoids, tannin, saponin. Phytochemical screening of Methanolic extract of Varshabhu revealed the presence of Carbohydrate, Alkaloid, phenolic compound, flavonoid, tannin, saponin, Steroid. Punarnava and Varshabhu have approximately similar phytochemicals. Similar phytochemical would have similar therapeutic effects.

HPTLC study is the key of Pharmacognostical evaluations. HPTLC was donalong with Identification marker Betasitosterol. On scanning of HPTLC plate under UV 360 nm and after derivatization of plate different bands of different color was observed.

On the basis of Physiochemical parameters, I can conclude that Varshabhu is more potent medicinal plant part as compare to Punarnava, because Punarnava have more ash value and less extractive values as compare to Varshabhu.

In Phytochemical screening I observed that Varshabhu have approximately similar phytochemicals as compared to Punarnava. Because of similar phytochemicals, Varshabhu can be used as substitute of Punarnava, if it is not available. Availability of Punarnava is enough in surrounding area, but Varshabhu is found in huge quantity in rainy season. Therefore if Punarnava is not available due to any reason then Varshabhu can be used as a authentic substitute. But comparative clinical study of both species is necessary to establish clinical efficacy and safety of both species in particular disease.

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