

COMPARATIVE PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDY OF VARIOUS SPECIES OF THE CONTROVERSIAL DRUG 'PUNARNAVA'

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ABSTRACT

Objective: Punarnava is a controversial drug i.e. its true botanical identity has not been ascertained, and several different species are used as Punarnava. It is a part of many medicinally important traditional formulations. Ayurvedic Pharmacopoeia of India describes three different species—Roots of *Boerhaavia diffusa*, *Trianthema portulacastrum* and *Boerhaavia verticillata*. The present work deals with the microscopic, macroscopic and phytochemical comparison of the roots of these species of Punarnava, so as to differentiate them and establish their quality parameters.

Methods: Roots of these three species were collected, and their comparative morphological, microscopical (transverse section, powder study) and the phytochemical (screening, tannin estimation) study was performed.

Results: Roots of *Boerhaavia diffusa* showed morphological characteristics which clearly distinguished it from the other species. They showed the presence of well developed and stratified cork, abnormal vascular bundles which were discontinuous, and a central cavity, presumably formed due to the disintegration of tissues. *Boerhaavia verticillata* showed the presence of xylem bundles arranged in the form of a 'X'-shaped cross in the central region. Thick-walled cork cells and pitted xylem vessels were present only in *Boerhaavia diffusa* root powder, whereas bundles of fibrovascular tissue were present only in the powder of *Boerhaavia verticillata*. Tannin content was found to be highest in *Boerhaavia diffusa* roots.

Conclusion: The present investigation will help herbal industries and traditional medicinal practitioners to detect adulteration of the medicinally important immunomodulator *Boerhaavia diffusa* by *Trianthema portulacastrum* and *Boerhaavia verticillata*, which are considered to have no immunomodulatory activity.

Keywords: *Boerhaavia diffusa*, *Boerhaavia verticillata*, Controversial drug, Immunomodulator, Microscopy, Nyctaginaceae, Punarnava, Quality Control, Standardization, *Trianthema portulacastrum*

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INTRODUCTION

Punarnava is a 'controversial drug' because its true botanical identity has not been ascertained yet. Various species are known by the name of 'Punarnava'. Ayurvedic Pharmacopoeia of India describes three species—Roots of *Boerhaavia diffusa*, *Trianthema portulacastrum* and *Boerhaavia verticillata*.

Roots of *Boerhaavia diffusa* (*B. diffusa*) syn. *Boerhaavia repens*, *Boerhaavia procumbens* (Family—Nyctaginaceae, also known as Rakta Punarnava) are traditionally used as an immunomodulator, adaptogenic, diuretic, anti-arthritis, analgesic, anti-fibrinolytic, blood purifier, hypotensive, hepatoprotective, anti-anemic, antioxidant, anti lymphoproliferative and in edema and dropsy [1, 2]. Punarnava roots available in the market are normally the roots of *B. diffusa*.

Roots of *Trianthema portulacastrum* (*T. portulacastrum*) syn. *Trianthema monogyna* (Family—Ficoidaceae, also known as Varshabhu, Shwet Punarnava) are used traditionally as antipyretic, analgesic, spasmolytic, deobstruent, cathartic, anti-inflammatory, in diseases of liver and spleen, anaemia and edema [3, 4].

Roots of *B. verticillata* (Family—Nyctaginaceae, also known as Vrischiva, Shwet Punarnava) are used traditionally as digestive, appetizer, antipyretic, the antidote for poisonous bites of insects, anti-leprotic, anti-cough and in disorders of the heart, spleen and gastrointestinal tract [5, 6].

Roots of *T. portulacastrum* and *B. verticillata* are rarely available in the market, and none of them being immunomodulators, are considered to be adulterants of *B. diffusa*.

No work has been done so far to distinguish the three species from each other, which is crucial for ascertaining the actual species of 'Punarnava' and for its quality control. The present investigation deals

with pharmacognostic and phytochemicals evaluation of roots of the three species, thereby setting parameters which will help herbal industries to detect adulteration of *B. diffusa* by *T. portulacastrum* and *B. verticillata*, thereby ultimately benefiting the patients.

MATERIALS AND METHODS

Dried roots of *B. diffusa* were obtained from Yucca Enterprise, Mumbai, whereas roots of *T. portulacastrum* and *B. verticillata* were collected from the herbal garden of School of Pharmacy, RK University (22 ° 14' 27.1284" N, 70 ° 54' 3.4308" E) in flowering season in July, 2014. The samples were authenticated were authenticated by Dr. Kunjal Soni, Botanist, School of Science, RK University, and deposited in the institute repository. The roots were compared morphologically and used for transverse section study. The roots were dried, powdered, stored in airtight containers and used for phytochemical studies. For microscopical studies, safranin was used for staining [7]. Photomicrography of the transverse sections and the powdered drug was performed using the camera. Phytochemical screening of both the species was performed using the appropriate extracts (aqueous and alcoholic) (table 2) [8-12]. Total tannins were estimated in all species [13].

RESULTS

Macroscopy

Roots of *B. diffusa* were cylindrical, deep brown, thick, 6-8 cm X 0.5-1 cm, with a rough surface and no lateral roots. Roots of *T. portulacastrum* were also cylindrical, creamish-white, very thin, 4-5 cm X 0.1-0.2 cm, with a smooth surface and many hairy secondary rootlets. Roots of *B. verticillata* were cylindrical, creamish-white, very thin, 6-10 cm X 0.1-0.4 cm, with a smooth surface and many hairy secondary rootlets (fig. 1).



Fig. 1: Morphology of various species of Punarnava A: Entire plant of *T. portulaca strum* (Right) and *B. verticillata* (Left); B: Roots of *T. portulaca strum* (Right), *B. verticillata* (Centre) and *B. diffusa* (Left)

Microscopy: transverse section

In *B. diffusa*, the cork was well developed and stratified, consisting of 8-10 layers of large, thin-walled cells followed by 4-5 layers of small, compact, suberized cells. Underlying the cork were 4-5 layers of parenchymatous cortical cells containing acicular crystals of calcium oxalate, brownish matter and starch grains. Underlying the cortex were the discontinuous anomalous vascular bundles consisting of successive and alternate layers of phloem and xylem. The phloem in the centre normally degrades to form a cavity (fig. 2 a, b).

In *T. portulaca strum*, the cork was not well-developed and not stratified, consisting of 2-3 layers of large, thin-walled cells. Underlying the cork were 4-5 layers of large, parenchymatous cortical cells containing acicular crystals of calcium oxalate, brownish matter and

starch grains. Underlying the cortex were the continuous anomalous vascular bundles consisting of successive and alternate layers of phloem and xylem. No cavity was present in the centre due to the presence of xylem elements and absence of phloem (fig. 3 a, b).

In *B. verticillata*, the cork was not well-developed and not stratified, consisting of 2-3 layers of large, thin-walled cells. Underlying the cork was a cortex similar to *T. portulaca strum*. Underlying the cortex were the continuous anomalous vascular bundles consisting of successive and alternate layers of phloem and xylem. No cavity was present in the centre due to the presence of xylem elements. The most striking feature in the central region of the root was the presence of four xylem bundles arranged perpendicular to each other in the form of a 'X'-shaped cross (fig. 4 a, b).



Fig. 2a: Detailed T. S. of *B. diffusa* root in 10x cork and cortex (Left); vascular bundles (right)

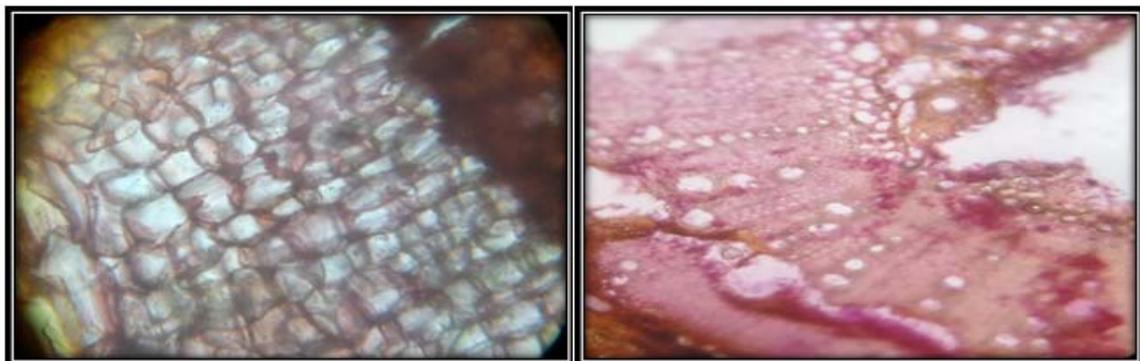


Fig. 2b: Detailed T. S. of *B. diffusa* root in 45x cork and cortex (Left); vascular bundles (right)

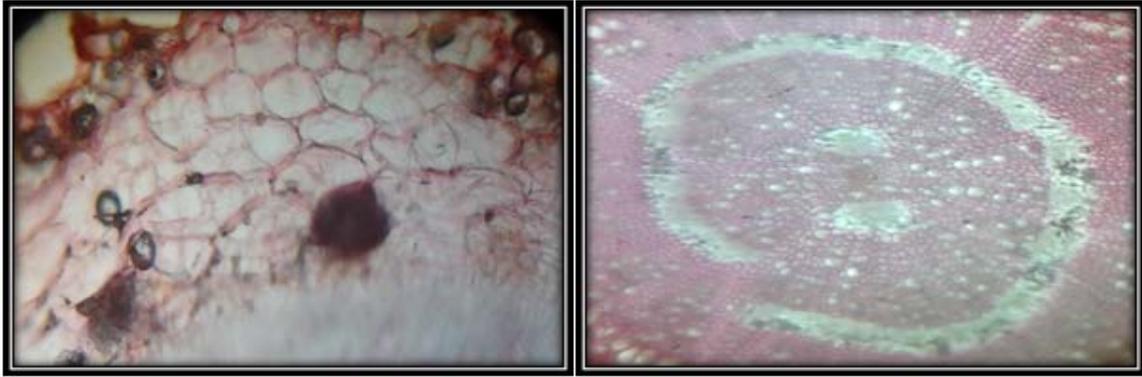


Fig. 3a: Detailed T. S. of *T. portulaca strum* root in 10x Cork and cortex (Left); vascular bundles (right)



Fig. 3b: Detailed T. S. of *T. portulaca strum* root in 45x Cortex (Left); vascular bundles (right)

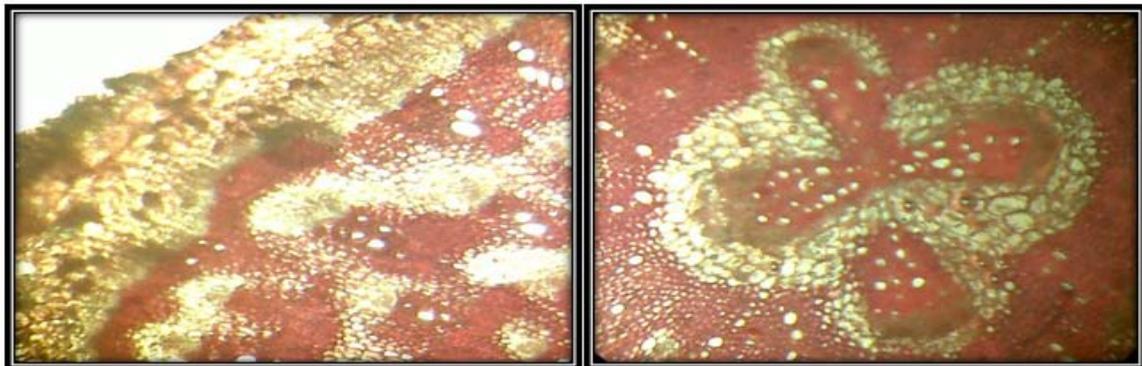


Fig. 4a: Detailed T. S. of *B. verticillata* root in 10x cork and cortex (Left); vascular bundles (right)

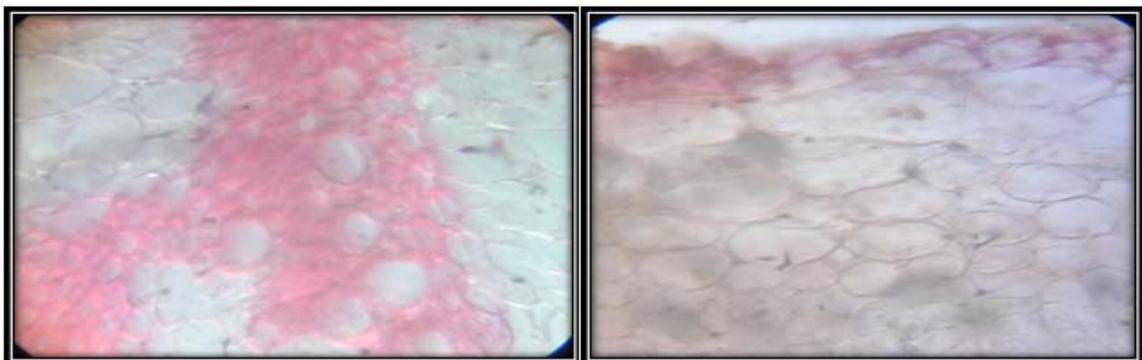


Fig. 4b: Detailed T. S. of *B. verticillata* in 45x cork and cortex (Right); vascular bundles (left)

Microscopy: powder characteristics

In *B. diffusa*, the important diagnostic features of the powder included parts of cork cells in surface view having thick-walled cells, xylem fibers with a pointed end, pitted xylem vessels, single and compound starch grains and acicular calcium oxalate crystals (fig. 5a).

Powder of *T. portulaca strum* consisted of cork cells in surface view having thin-walled cells, xylem fibers with a pointed end, reticulately-thickened xylem vessels, single and compound starch grains and acicular calcium oxalate crystals (fig. 5b).

In powder of *B. verticillata*, the chief features included cork cells in surface view having thin-walled cells, a fibro-vascular tissue consisting of bundles of xylem vessels and xylem fibers and acicular crystals of calcium oxalate (fig. 5c).



Fig. 5a: Powder study of *B. diffusa* root (45x) (a; crk cel: cork cell, b; fbr: xylem fiber, d; xyl vesl: pitted xylem vessel, e; srch: starch grain, cal oxlt: calcium oxalate acicular crystals)

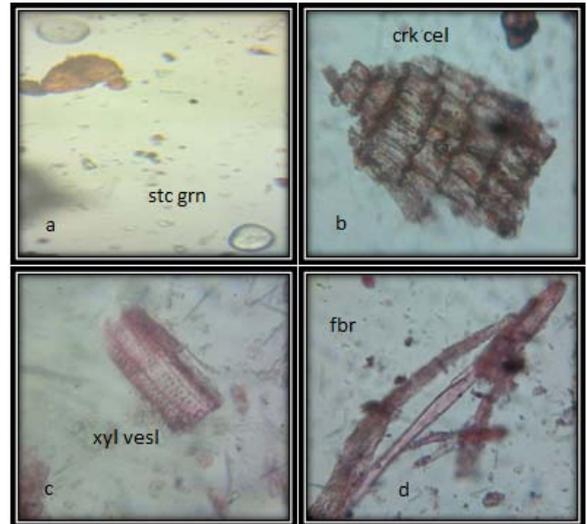


Fig. 5b: Powder study of *T. portulaca strum* root (45x) (a; stc grn: starch grain, b: crk cel: cork cell in surface view, c; xyl vesl: xylem vessel, d; fbr: xylem fiber)

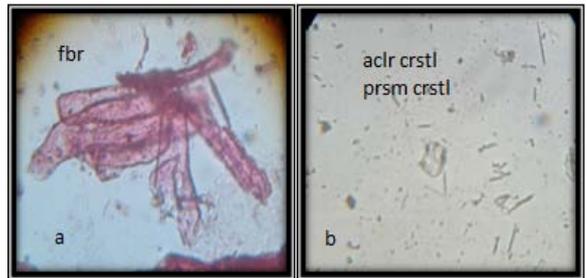


Fig. 5c: Powder study of *B. verticillata* root (45x) (a; fbr: fibro-vascular tissue, b; aclr crstl: acicular crystal, prsm crstl: prism crystal of calcium oxalate)

Table 1: Phytochemical screening

Phytoconstituent	Test	Result		
		<i>Boerhaavia diffusa</i>	<i>Trianthema portulaca strum</i>	<i>Boerhaavia verticillata</i>
Alkaloids	Dragendorff's test	+ve	+ve	+ve
	Hager's test	+ve	+ve	+ve
	Wagner's test	+ve	+ve	+ve
	Mayer's test	+ve	+ve	+ve
Flavonoids	Shinoda test	-ve	-ve	-ve
	Ferric chloride test	-ve	-ve	-ve
Phenolics	Folin Ciocalteu test	-ve	-ve	-ve
	Salkowski test	-ve	-ve	-ve
Sterols and triterpenoids	Libermann-Buchardt test	-ve	-ve	-ve
	Legal test	-ve	-ve	-ve
Cardiac glycosides	Baljet test	-ve	-ve	-ve
	Keller Killiani test	-ve	-ve	-ve
	Foam test	-ve	-ve	-ve
Saponin glycosides	Lead acetate test	-ve	-ve	-ve
	Modified Borntrager test	-ve	-ve	-ve
Anthraquinone glycosides	Fehling's test	+ve	+ve	+ve
	Molisch test	+ve	+ve	+ve

Table 2: Estimation of tannins

<i>B. diffusa</i>	<i>T. portulacastrum</i>	<i>B. verticillata</i>
12.99±0.5%w/v	14.14±0.5%w/v	12.48±0.5%w/v

DISCUSSION

Punarnava is a 'controversial drug'. Its true botanical identity has not yet been ascertained. Various species are known by the name of 'Punarnava'. Ayurvedic Pharmacopoeia of India describes three species—Roots of *Boerhaavia diffusa*, *Trianthema portulaca strum* and *Boerhaavia verticillata* [1, 3, 5]. The present work deals with the microscopic, macroscopic and phytochemical comparison of the roots of these three species of 'Punarnava'. Roots of *B. diffusa* showed morphological characteristics which clearly distinguished it from the other species. Microscopically, roots of *B. diffusa* showed the presence of well developed and stratified cork, abnormal discontinuous vascular bundles, and a central cavity, which distinguishes it from the other species. The unique microscopic feature of *B. verticillata* was the presence of xylem bundles arranged in the form of a 'X'-shaped cross in the central region of the root. The presence of thick-walled cork cells and pitted xylem vessels were the diagnostic features of *B. diffusa* root powder, whereas bundles of fibrovascular tissue were present only in the powder of *B. verticillata*. Tannin content was highest in *B. diffusa* roots. Since no work has been reported so far distinguishing the three species, these diagnostic pharmacognostic characters & phytochemicals data are vital. As the Indian traditional system of medicine is getting worldwide acceptance nowadays, it is highly necessary to bring an end to controversies related to ascertaining the true botanical identity of species by establishing quality testing parameters for the medicinally important genuine species and distinguishing it from its adulterants and substitutes. Such a comparative study is particularly important in the case of Punarnava since only *B. diffusa* is considered to possess vital immunomodulatory properties, and so it needs to be distinguished from the other species.

CONCLUSION

'Punarnava' is present in various Ayurvedic formulations and thus has considerable medicinal importance. However, confusion still prevails over the true botanical identity of 'Punarnava'. The present investigation will help herbal industries and traditional medicinal practitioners to detect adulteration of the medicinally important *B.*

diffusa by *T. portulacastrum* and *B. verticillata* by simple microscopic and phytochemical methods, thereby helping in quality control and standardization of Punarnava formulations, ultimately benefiting the end-users.

CONFLICT OF INTERESTS

Declared None

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