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Comparative root anatomy and thin layer chromatographic identification of *Boerhavia diffusa* L. and *Boerhavia erecta* L.

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ABSTRACT

“*Punarnava*” is an important rejuvenative drug, widely used in the indigenous system of medicine. The root is the officinal part and used in various formulations in Ayurveda and found two varieties in the commercial market namely *Boerhavia diffusa* L. and *B. erecta* L. In the present study, a comparative root anatomy and thin layer chromatographic profile of both species have been examined and provided detailed diagnostic characters for the identification of commercial drug.

Keywords: *Boerhavia diffusa*, *B. erecta*, *Punarnava*, Pharmacognosy, Thin layer chromatography.

INTRODUCTION

“*Punarnava*” botanically known as *Boerhavia diffusa* L. belongs to the family Nyctaginaceae, is an important rejuvenative drug, widely used in the indigenous system of medicine, since the time immemorial. It is bitter, astringent, cooling, stomachic, laxative, diuretic, expectorant, antipyretic and cardiogenic. The medicinal part is the root and it forms major ingredient of several Ayurvedic formulations like *Punarnavasavam*, *Kumaryasavam*, *Dhanvantaram kulambu*, *Cyavanaprasam*, *Vastyamayantaka ghritam* etc. [1-2] and exhibits various phyto-constituents and biological activities [3-7].

In the Ayurvedic texts, two varieties of *Punarnava* are mentioned viz. *Raktapunarnava* (red variety) and *Svetapunarnava* (white variety). Both attribute different properties. With regard to the identification of the different varieties of *punarnava*, there is difference of opinion among the Ayurvedic physicians. *Raktapunarnava* is invariably equated with *Boerhavia diffusa* L. and *svetapunarnava* has since been equated with white-flowered species *Boerhavia erecta* L. (syn: *B. punarnava* Saha & Krishnamurthi), *Trianthema portulacastrum* L. and *Commicarpus verticillatus* (Poir.) Standl. Some author states that *B. erecta* is an American weed, seemingly introduced in India rather recently and hence cannot be considered as the original source of the drug [8]. However, *B. erecta* intentionally or unintentionally adulterated/substituted with *B. diffusa*. Hence, the present investigation was undertaken to diagnose the both species roots using anatomical characters and thin layer chromatographic profiles.

MATERIALS AND METHODS

Anatomical study

The roots of *B. diffusa* and *B. erecta* were collected freshly from Redhills, Chennai and authenticated using regional flora. Samples were cut in to small pieces and fixed immediately in Formalin-Acetic-Alcohol for 24h. After fixation they were washed thoroughly in distilled water, dehydrated, embedded in paraffin wax after infiltration and sectioned using rotary microtome to the thickness of 12 to 14 µm [9]. Sections were stained with toluidine blue, observed and photographed under Nikon Eclipse 400 microscope.

Thin Layer Chromatographic (TLC) analysis

Freshly collected root samples were dried in shade and pulverized. 5 g of both the root samples were extracted with methanol (25 ml) 3 times and combines extracts were dried in water-bath. The extract residue re-dissolved in 5 ml methanol and used for TLC analysis.

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The following conditions are used for the TLC analysis.

- Stationary phase** : Precoated Silica Gel F254 (E. Merck) plate
Mobile phase : Toluene: Ethylacetate (3:1)
Dipping reagent : 1% Vanillin Sulphuric acid
Observation : Before dipping reagent - UV-254 nm
 After dipping reagent - Visible light

RESULTS

***Boerhavia diffusa* L.**

Macroscopic characters of the root (Figure – 1: 1-2)

Root up to 8-12 cm long, 1-2 cm thick, strong, hard, pale brownish colour when fresh, dark brown when dry with characteristic odour.

Microscopic characters of the root (Figure – 2: 1-6)

A cross section of about 5 mm thickness root shows well developed spongy tissue of periderm with small fissures (Figure – 2: 3). Presence of large bundles raphides in the cells of cortex region (Figure – 2: 3-4). Vascular elements show a clear anomalous secondary growth structure. Xylem and phloem elements shows clear circular ring one above (Figure – 2: 1-2). Starch grains present in the parenchyma cells just above phloem region (Figure – 2: 5). Vessel elements are few, mostly distributed in the external region of the xylem, arranged in group or short radial multiples (Figure – 2: 5-6).

***Boerhavia erecta* L.**

Macroscopic characters of the root (Figure – 1: 3-4)

Root up to 5-7 cm long, 0.5-1.0 cm thick, strong, hard, pale yellowish brown colour when fresh, brown when dry with characteristic odour.

Microscopic characters of the root (Figure – 3: 1-6)

A cross section of about 4-5 mm thickness root exhibit well developed clear tangential shaped cells of periderm with distinct fissures (Figure – 3: 3). Small bundles raphides present in the cells of cortex region (Figure – 3: 3-4). Vascular elements show a clear anomalous secondary growth structure as in *B. diffusa*. But xylem and phloem regions are not in clear circular ring, instead wavy in outline (Figure – 3: 1-2). Phloem regions are not in continuous ring, observed as long or small patches in between the xylem (Figure – 3: 6). Starch grains absent in the parenchyma cells of phloem region. Vessel elements are numerous, distributed entire xylem region, arranged in mostly short radial multiples or few group (Figure – 3: 5-6).

Thin Layer Chromatographic (TLC) analysis (Figure – 4)

The TLC profile of both *B. diffusa* and *B. erecta* as follows

UV – 254 nm		Visible light	
<i>Boerhavia diffusa</i>	<i>Boerhavia erecta</i>	<i>Boerhavia diffusa</i>	<i>Boerhavia erecta</i>
--	0.08	--	0.15 (Blue)
0.10	--	0.18(Blue)	--
0.19	--	0.27(Pale yellow)	--
0.28	0.28	0.31(Blue)	0.29(Blue)
--	0.32	0.38(Orange)	--
0.39	--	0.50(Purple)	0.50(Purple)
--	0.43	0.56(Blue)	0.56(Blue)
0.54	0.54	0.61(Blue)	0.61(Blue)
0.60	0.60	0.76(Light green)	--
0.65	--	0.84(Violet)	0.84(Violet)
0.67	0.67		



Figure 1: Habit & Root. 1) *Boerhavia diffusa* aerial part, 2) *Boerhavia diffusa* root, 3) *Boerhavia erecta* aerial part, 4) *Boerhavia erecta* root

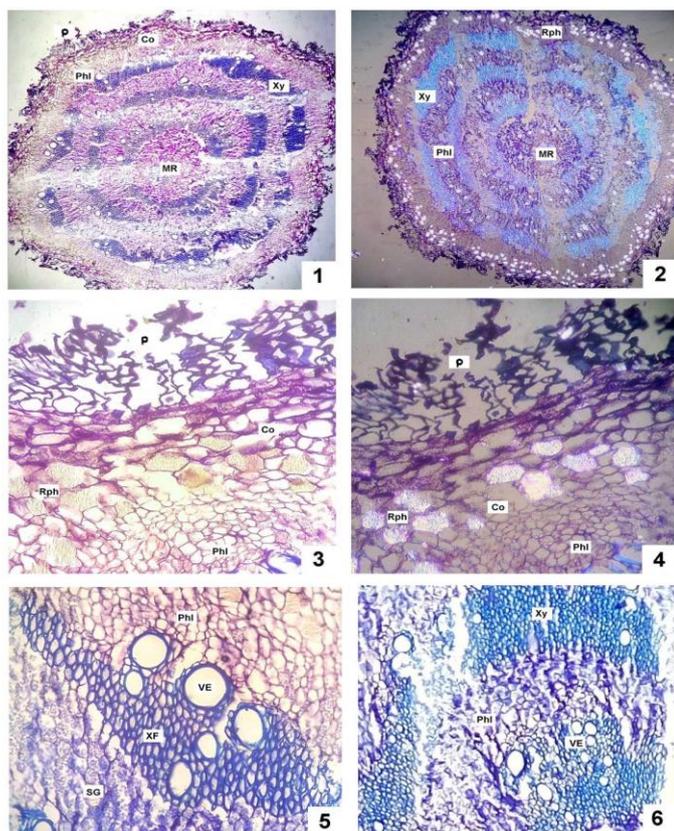


Figure 2: Root anatomy of *Boerhavia diffusa*. 1) Cross section of root, 2) Cross section of root under polarized light, 3) Periderm and cortex region, 4) Periderm and cortex region under polarized light, 5- 6) Xylem and phloem region enlarged

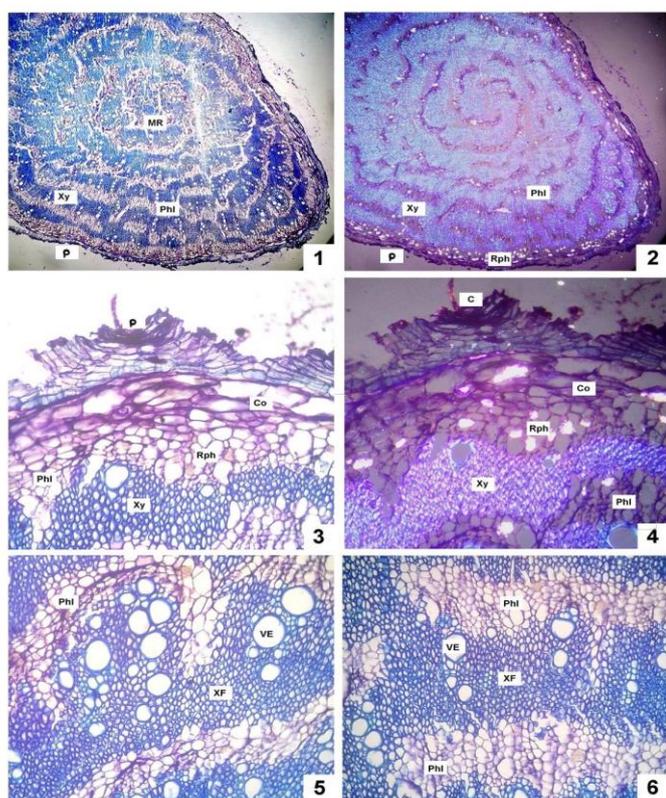


Figure 3: Root anatomy of *Boerhavia erecta*. 1) Cross section of root, 2) Cross section of root under polarized light, 3) Periderm and cortex region, 4) Periderm and cortex region under polarized light, 5-6) Xylem and phloem region enlarged

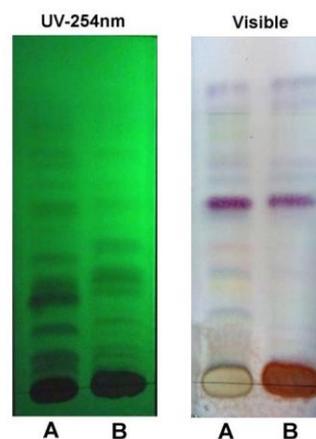


Figure 4: TLC Profile. A) *Boerhavia diffusa*, B) *Boerhavia erecta*

(Co – Cortex; MR – Medullary region; P – Periderm; Phl – Phloem; Rph – Raphides; SG – Starch grains; VE – Vessel element; XF – Xylem fibre; Xy – Xylem)

DISCUSSION

The root of *B. diffusa* (Punarnava) is the most renowned medicinal drug used to treat large number of human ailments. The whole plant or its root have a numerous medicinal property viz. antibacterial, antinociceptive, hepatoprotective, hypoglycemic, antiproliferative, antiestrogenic, antiinflammatory, anticonvulsant, antistress and antimetastatic [10]. The crude root drug, because of its restricted availability, is often adulterated or substituted with easily available root drugs. Surange and Pendse [11] studied the pharmacognostic characters of roots of *B. diffusa*. However, there is no comparative study with adulterant roots such as *B. erecta*. Hence, the present study was undertaken. The following comparative anatomical features of root and TLC profile can be employed for the identification of original drug.

Characters	<i>Boerhavia diffusa</i>	<i>Boerhavia erecta</i>
Periderm	Spongy tissue with small fissures	Tangentially elongated cells with distinct fissures
Raphides	Comparatively large bundles in size	Comparatively small bundles in size
Vascular elements	Xylem and phloem regions forms clear circular ring one above	xylem and phloem regions not in circular ring
Starch grains	Present in phloem parenchyma	Absent in phloem parenchyma
Vessel elements	Few, mostly distributed in the external xylem region, arrangements in group or short radial multiples	Numerous, distributed entire xylem region, arrangements in mostly short radial multiples or few group

In TLC profile, the following spots such as 0.08, 0.32 & 0.43 (under UV254nm) and 0.15 (under visible light) are absent in *B. diffusa*. In *B. erecta*, spots such as 0.10, 0.19, 0.39 & 0.65 (under UV254nm) and 0.18, 0.27, 0.38 & 0.76 are absent.

CONCLUSION

We reported in the current comparative study on the basis of anatomical characters and TLC profile of *B. diffusa* and *B. erecta*, can

be easily differentiated or distinguished from adulterant in fragmentary form.

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