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Comparative study on morpho-anatomy of leaf, stem and root of *Boerhaavia diffusa* L. (Nyctaginaceae) and its adulterant plants

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Punarnava (*Boerhaavia diffusa* L.- Nyctaginaceae) is a promising drug to rejuvenate new cells in the body. It is well known in Ayurvedic medicine and locally called Tambadivasu. Superficially it is similar to other species of *Boerhaavia* and species of *Trianthema* and *Sesuvium.* Due to the minute morphological differences, the above plants are erroneously used in medicine as Punarnava, and at times on purpose as an adulterant. Therefore, it is necessary to highlight the anatomical features of Punarnava for proper identification of the medicinal plant species for local people and for scientific research. Due to the ambiguity in local names and similar apparent appearance, market samples of Punarnava are often adulterated with various species of *Trianthema* and *Sesuvium*. These adulterated samples contain neither the Punarnavine alkaloid, nor does it possess anisocytic stomata but possess paracytic stomata. Comparative study of stem anatomy showed two main characteristic differences. First, plenty of starch grains can be seen in both the ground parenchymatous tissues present in between successive cambia and xylem parenchyma of Punarnava which is not observed in species of *Trianthema*, and second, the phloem around the xylem of Punarnava root has semi-circular or eccentric patches, while that of *Trianthema* only has narrow strips. This study is focused on comparative SEM study of leaf morphologies and anatomy of leaf, stem, and root of *Boerhaavia diffusa* L.*, Trianthema portulacastrum* L. and *Sesuvium portulacastrum* L.

Uniterms: Medicinal plants/comparative study. Medicinal plants/Scanning Electron Microscopy. Ayurvedic medicine.

INTRODUCTION

Herbal medicine is an important branch in Ayurvedic Medical Sciences; however, there is lack of standardized identification methods of medicinal plants, active components, harvesting time, pharma-dynamics and pharmacokinetics. Medicinal plants is a subject plagued by a multitude of problems such as correct botanical identity since all the plants described in Ayurveda are in Sanskrit language, ambiguous local names due to several languages, active principles, pharmacognosy and so on (Firenzuoli, Gori 2007).

The efficiency of herbal medicines is attributed to synergistic action of wide components present in a particular plant. However, the scope of these plants in herbal cosmetics, and in herbal veterinary medicines is not properly assessed. Use of similar looking plants as an adulterant in place of the medicinally important plant is commonplace. It is generally agreed that the pink flowered variety is *B. diffusa,* while the white flowered plants have been identified as *B. rependa*. The roots of *T. portulacastrum* are often used as an adulterant for Punarnava. Both genera belong to different families, and *T. portulacastrum* neither contains Punarnavine (active constituent), nor has white to light pink flowers. Fresh as well as dried plants of *B. diffusa* are the genuine source of the Punarnava drug, and are officially considered as diuretic in Indian Pharmacopoeia (Chopra *et al.*, 1923). Similarly, dried sample of certain plants, e.g. *Sesuvium portulacastrum* also resembles Punarnava and is therefore used as an adulterant. Vernacular names also pose a problem, e.g. *B. diffusa* and *T. portulacastrum* are both called Satodi in Gujarat.

Boerhaavia diffusa L. or Spreading Hogweed,

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also known as Punarnava in Sanskrit, is a creeping and spreading perennial herb from family Nyctaginaceae. It is distributed throughout India and is extensively used in many parts of the country as a traditional medicine. Its utility as a drug to regenerate new cells in body is promising and is well known in Ayurvedic medicine too. It contains Punarnavine (Kirtikar, Basu, 1993), an alkaloid which is bitter in taste, cooling, astringent in nature, hot, light and dry in effect. It has been confirmed by experimental studies that Punarnava has diuretic properties, particularly the root (Gaintode, 1974). *B. diffusa* also has high antiviral activity; therefore it has very high medicinal value (Awasthi, Kumar, 2003; Verma, Awasthi, Saxena, 1979; Lohani, Jan, Verma, 2007).

A large number of studies have been carried out on the medicinal properties of *B. diffusa* but no much information is available on the adulterants that are added with genuine drug. Studies on the morphology of *B. diffusa* have been done by Bhargava (1932), Maheshwari (1930) and anatomical studies by researchers like Metcalf and Chalk (1983), Pant and Mehra (1961), and Rajput, Rao (1998).

The present work is therefore intended to analyze the leaf, stem and root morpho-anatomy of *B. diffusa* and its adulterant species like *Trianthema* and S*esuvium* in order to contribute to the knowledge base for medicinal plant identification. The principal aim of the study was to understand the structural differences in the leaf, stem and root structure, especially in the secondary xylem, cortex, secondary phloem, in the deposition of substances and any other structural differences to provide information for identification of genuine material.

MATERIAL AND METHODS

The botanical materials were collected from the campus and Botanical garden of S.P. Pune University. Dried materials were submitted to Herbarium (BSI), Pune, for authentication and the corresponding voucher numbers were VP01, VP02 and VMK01. Mature leaves and stem materials were also collected and fixed in FAA (Johansen, 1940). Suitably trimmed samples were dehydrated in tertiary butyl alcohol series and embedded in paraffin after infiltration with paraffin (Berlyn, Miksche, 1976). Serial transverse, tangential and radial longitudinal sections of 12–15 µm thickness were obtained with the help of a rotary microtome while sections of mature stem and branches were cut with the help of a sliding microtome. Sections were stained with Safranin–fast green combination (Johansen, 1940), after dehydration through ethanol-xylene series, they were mounted in DPX.

For Scanning Electron Microscopic analysis (SEM), samples were fixed and dehydrated in an ascending ethanol series (Souza, 1998) and finally in graded series of ethanol: isoamyl alcohol (IAA). Samples were kept in pure IAA and then sputter coated with gold. The leaf surface was then observed using a scanning electron microscope (JEOL JSM-6360A, Japan) at a different magnification ×100 to ×10,000.

Small pieces of stem were macerated with Jeffery's solution (Berlyn, Miksche, 1976) at 55 ºC to 60 ºC for 24-38 hours and stained with 0.5% aqueous Safranin to obtain the length and width of vessels elements and fibers as well as to study the morphology. One hundred readings were taken randomly to obtain mean and standard deviation. Important results were micro-photographed with a Leica DM 3000 LED research microscope.

RESULTS

General morphology of *Boerhaavia diffusa* L. and other adulterant plants species like *Trianthema portulacastrum* and *Sesuvium portulacastrum* are given in Table I, Figure 1 and Micro-morphology is given in Table II.

Leaf anatomy

In all studied species, the transverse section of leaf showed uniseriate epidermis covered with a thin cuticle. It consisted of stomata and trichomes on both, upper and lower surface. SEM study of leaves showed anisocytic stomata with uniseriate or multicellular trichome with enlarged globose or elongated terminal cell in *Boerhaavia diffusa*, while paracytic stomata was observed in *Trianthema* and *Sesuvium* (Figures 2A-D). Epidermis was followed by mesophyll tissue. It revealed dorsiventral organization, comprising one-two stratum of palisade and about 3-4 layers of spongy tissue (Figures 2E, F; 3A-D). Length and width of the palisade varied in different plant species. They were measured from 50-72 µm length and 24-38 µm width in *B. diffusa*, and 33-48 µm length and 24-26 µm width in *T. portulacastrum*.

In *B. diffusa*, transverse section of leaf passing through midrib showed a single convex shape on the abaxial face (Figure 2E). Collateral vascular bundle along with thick walled parenchyma cells adjoining the phloem were embedded in the ground parenchyma tissue. The vascular bundles varied in number and arrangement from species to species. It had crescent shaped three large central vascular bundles, while two lateral smaller bundles formed the central midrib (Figures 2E; 3A, B).

In the lamina portion, minor collateral vascular bundles were surrounded by 4-5 parenchyma sheath containing raphide crystals at places and fused sheath cells, joined together forming horizontal rows of cells, while druses

TABLE I - *Boerhaavia diffusa* are differentiated on the basis of following morphological characters

	***Boerhaavia diffusa* L.**	***Trianthema portulacastrum* L.**	***Sesuvium portulacastrum* L.**
Habit	Erect/diffuse branched herbs	Glabrous, pubescent, diffuse, prostrate branched herbs.	Herbs, undershrubs, erect/prostrate, branched, succulent.
Leaves	Opposite often in unequal pairs, petiolate, ovate, sub orbicular/ cordate	Petiolate, opposite, unequal, ovate-obovate, petiole dilated	Opposite, subfleshy, petiole connate.
Flowers	Small, paniculate, umbellate/ subcapitate, articulated with pedicel, bracteolate, pink, purple/ white	Axillary, sessile/peduncled, solitary, cymose/panicled	Axillary, sessile/peduncled bracteolate, solitary not in cluster. Purple/flesh coloured.
Calyx	Calyx and corolla not differentiated forming perianth.	Calyx tube short/long, 5-lobes, coloured within mucronate at back.	Calyx tube turbinate, 5-lobed, oblong/triangular, coloured within.
Stamens	1/ 2-5, connate below exserted, filaments capillary, unequal.	5-10/many inserted near the top of the calyx tube.	Many/5 inserted round the top of the calyx tube, filaments filiform, didynamous.
Ovary	Oblique, stipitate, ovule erect, stigma peltate.	Free, sessile, 1-2 celled often truncate at the apex, ovules 1-∞, basal, styles ½.	3-5 celled, free ovules numerous, styles 3-5, longitudinal, papillose inside.
Fruit	Fruit enclosed in perianth tube. Round 5-ribbed/5-angled viscidly glandular	Capsule membranous below with hard thick cap.	Capsule ellipsoid/ovoid, membranous, dehiscent.

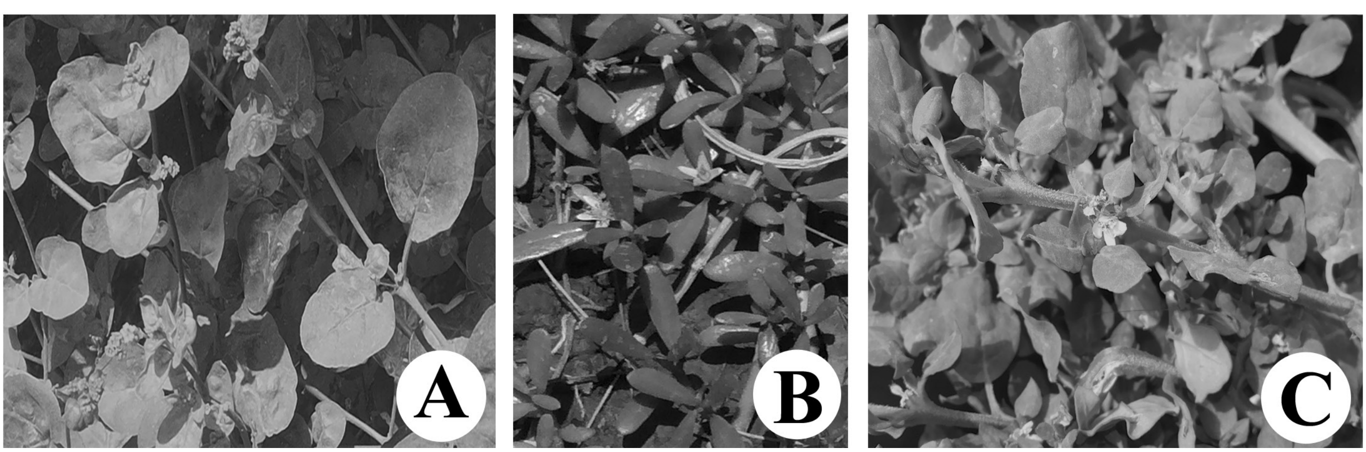

FIGURE 1 - General morphology of aerial organs in *Boerhaavia diffusa* L. (A), *Trianthema portulacastrum* L. (B) and *Sesuvium portulacastrum* L. (C)

TABLE II - Micro-morphology of plants studied

Micro-morphology	***Boerhaavia diffusa* L.**	***Trianthema portulacastrum* L.**	***Sesuvium portulacastrum* L.**
Stomata	Anisocytic	Paracytic	Paracytic
Trichome	Uniseriate-multicellular with elongated head	Unicellular-uniseriate	Unicellular-uniseriate
Crystals	Starch-grains, raphides	Druses	Druses

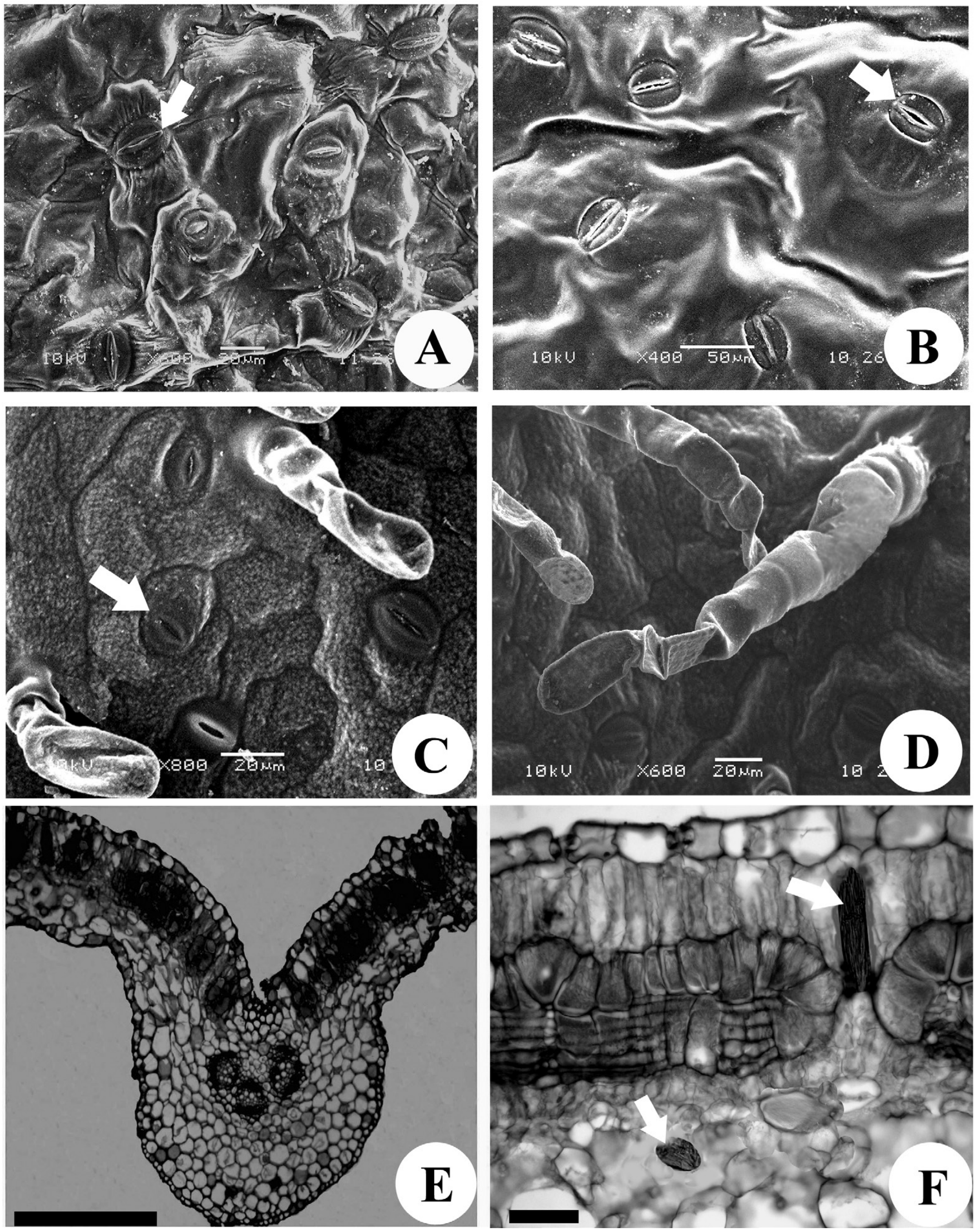

FIGURE 2 - Scanning electron micrograph leaf peel (A-D) and transverse view of *Trianthema portulacastrum*, Sesuvium portulacastrum and *Boerhaavia diffusa* L. A, B: *Trianthema portulacastrum* and *Sesuvium portulacastrum* leaf peel showing paracytic stomata. C: *Boerhaavia diffusa* leaf peel showing anisocytic stomata. D: uniseriate, multicellular trichome with elongated head cell on the leaf peel of *Boerhaavia diffusa*. E: Transverse view of *Boerhaavia diffusa* leaf showing dorsiventral orientation of leaf, crescent shape vascular bundle in midrib region, bundle sheaths in lamina. F: Transverse view of *Boerhaavia diffusa* leaf showing bundle sheaths cells, Arrowhead showing raphide.

were present in *Trianthema* and *Sesuvium* (Figures 2E, F; 3D). Comparative leaf anatomical characters of other plants species are given Table II.

Stem and root anatomy

Mature stems of all the species (*S. portulacastrum*, *T. portulacastrum* and *B. diffusa*) were composed of three to five rings of successive cambia (Figures 3A; 4B, E). The cambium was comprised entirely of fusiform cambial initials (at least in the early part of secondary growth) while ray cells were either absent or showed paedomorphic nature. A single layered epidermis was made up of isodiametric cells and covered with thin cuticle that

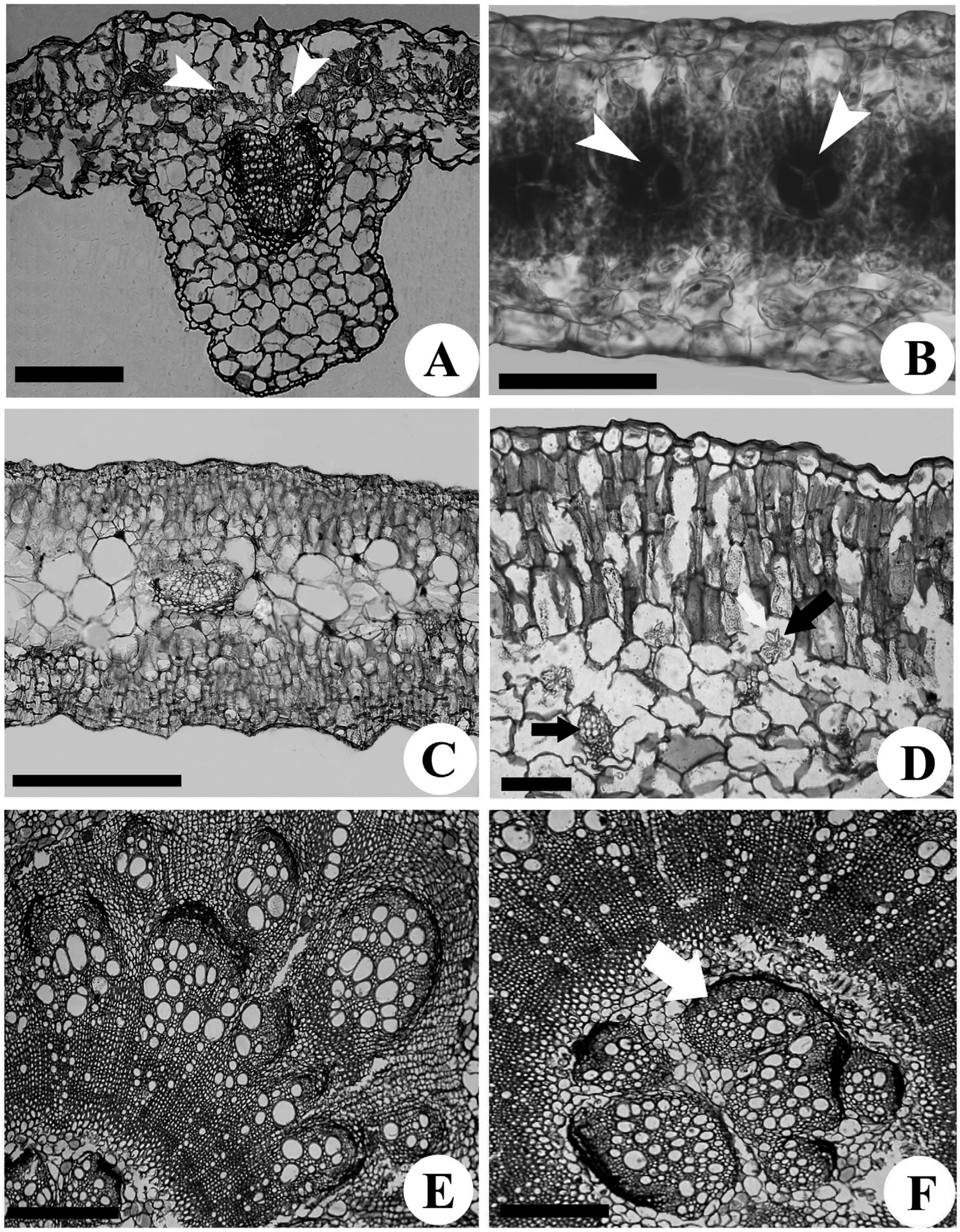

FIGURE 3 - Transverse view of *Trianthema portulacastrum* and *Sesuvium portulacastrum* leaf (A-D) and *Boerhaavia diffusa* stem (E-F) showing structural variations. A: *Trianthema portulacastrum* leaf showing midrib portion with single vascular bundle, irregularly arranged parenchyma cells. Arrowheads indicate druces. B: Leaf lamina region of *Trianthema portulacastrum* L. showing bundle sheaths (arrowhead). C: *Sesuvium portulacastrum* leaf showing isobilateral orientation of leaf, four layered palisade tissue, single vascular bundle in midrib region. D: Enlarged view of *Sesuvium portulacastrum* showing lateral vascular bundle (small arrow) in lamina and druces (arrow). *E: Boerhaavia diffusa* stem showing successive rings secondary xylem. F: Pith region of *Boerhaavia diffusa* stem showing medullary bundles (arrow).

enclosed multilayered parenchymatous cortex. Raphides were present in the cortical region of mature stem and root of *B. diffusa*, while druces were present in *S. portulacastrum* and *T. portulacastrum*. Crystals were more abundant in roots rather than stems of all studied plants. In stem and root of *B. diffusa* raphides were observed while druses were common in other two species. In thick stems, upright cells appearing like rays were noticed in the xylem, but in radial sections of the cambial zone such cells were found absent. Fusiform cambial cells were arranged in semi-storied fashion measuring from 210-220 μm in length and 17-23 μm in width. In transverse view, non-dividing cambium appeared

TABLE III - Summery of distinctive anatomical characters of studied plants

Plants studied	*Boerhaavia diffusa* L.	*Trianthema portulacastrum* L.	*Sesuvium portulacastrum* L.
		Stem anatomy	
Cambium-successive cambia	Successive cambium rings are found in patches, not continuous due to interfascicular parenchyma formation in between.	Successive cambium rings are continuous due to activity of fusiform cambial cells.	Successive cambial rings are formed due to activity of axial parenchyma cells which first form cortex and after by repeated divisions forms the ring.
Medullary bundles	Medullary bundles are present.	Medullary bundles are absent.	Medullary bundles are absent.
		Root anatomy	
Cambium successive cambia	Successive cambium rings are found in patches, not continuous due to interfascicular parenchyma formation in between.	Successive cambia are continuous due to activity of fusiform cambium and are more in number compared to stem.	Successive cambial rings are formed due to activity of axial parenchyma cells which first form cortex and after by repeated divisions forms the ring.
Medullary bundles	Medullary bundles are present.	Medullary bundles are absent.	Medullary bundles are absent.
		Leaf anatomy	
Leaf	Leaf- dorsiventral	Leaf-dorsiventral	Leaf- isobilateral and flat.
Palisade tissue	Single layered.	Two layered.	Three-four layered.
Mid-rib	In the mid-rib the vascular bundle vary in number and arrangement from species to species have a crescent shape three larger central vascular bundles while two lateral smaller, which forms the central midrib.	In midrib 4-5 vascular bundles unite to form horseshoe shaped resembling solitary vascular bundle forming single midrib.	Mid rib consist of single vascular bundle surrounded by irregular parenchyma cells.

two to three layered and four to six layered in dividing cambial zone. Cambial variant (anomalous structure of cambium) was observed namely successive cambia. In this type first ring of cambium ceased to divide after a limited period of activity and the second ring of cambium was developed from the parenchyma cells at a distance of about three to six cell layers outside the phloem produced by the previous cambium referred as ground or conjunctive parenchyma. Prior to the initiation of the second cambial ring, these parenchyma cells underwent repeated periclinal divisions to form a wide band of cells that provided a site for the origin of the new ring of cambium. The second new cambium divided further and gave rise to additional layers of secondary cortex. In each successive ring, the cambium is functionally two distinct types: i) small segment of it formed conducting elements of xylem towards the center and phloem towards the periphery and ii) rest of the alternate segments gave rise to thick walled conjunctive tissue centripetally and thin walled parenchymatous conjunctive tissue centrifugally in first two successive cambial rings (Figs. 3A-C, E). In every successive ring of cambium, relatively small cambial segments produced conducting elements of secondary xylem and phloem in centripetal and centrifugal directions respectively (Figures 3E, 4A-C, E). These small segments were interconnected by wider segments of cambium that produce thin walled parenchyma in both centripetal and centrifugal directions (Figure 4E). Successive cambium rings were found in patches, non-continuous due to interfascicular parenchyma formation in *Boerhaavia* and *Sesuvium* (Figures 3E, 4A, E) and continuous in *Trianthema.* Structurally, cambium and secondary xylem in the stem and roots remained almost similar, except that conjunctive tissues were relatively more in roots (Figures 4B, C).

In the mature stem, xylem was composed of vessel elements, tracheids and fibers, while xylem rays were absent in the early stages of xylem development in all species. Each xylem ring consisted vessels with associated parenchyma cells and fibers that alternate with thick walled conjunctive tissue. In *Boerhaavia diffusa*, the successive rings of secondary xylem alternate with the phloem rings. In *Trianthema* and *Sesuvium* xylem was composed of tracheids, vessels and fibres while xylem rays were found to be absent in all studied samples of these plants. The

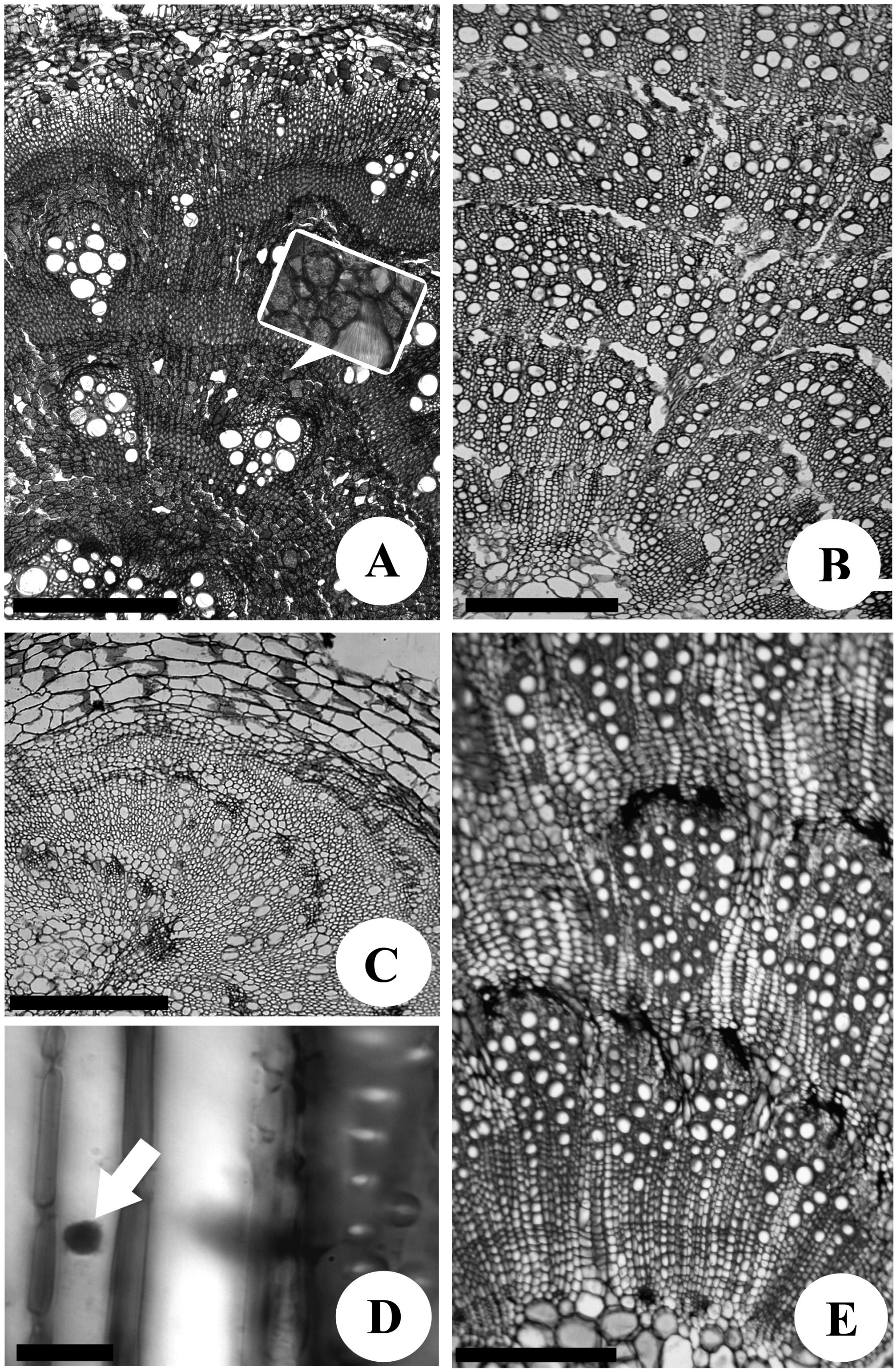

FIGURE 4 - Transverse view (A-C, E) and longitudinal (D) view of root of *Boerhaavia diffusa, Trianthema portulacastrum* and *Sesuvium portulacastrum.* **A.** Root portion of *Boerhaavia diffusa* L. Enlarge area showing raphides and starch grains accumulation in parenchymatous cells. **B.** Matured stem of *Trianthema Portulacastrum* L. showing incomplete successive rings of cambia, arrow showing druses present in ground parenchymatous region. **C.** *Trianthema portulacastrum* L. root showing more number of continuous successive cambial rings. **D.** Xylem fibre with a nucleus (arrow). E. Mature stem *Sessuvium portulacastrum* showing successive rings of xylem embedded in parenchymatous groundmass of conjunctive tissue.

average length and width measurements of vessels were 140-145 µm and 45-50 µm in *B. diffusa*, 159-195 µm and 48-78 µm in *Trianthema* and 120-180 µm and 35-50 µm in *Sesuvium*. In *Trianthema,* vessels were solitary, oval to oblong or angular in transverse view. Vessel elements had a simple perforation plate on transverse, slightly oblique end walls. The pits on walls were bordered and arranged alternately in all studied plants.

In *B. diffusa*, the length of fibers ranged from 260-465 µm and they showed heavy accumulation of starch whereas in *Trianthema* they were 380-460 µm and 13-23 µm in length and width respectively. In contrast, fibers in *Sesuvium* retained their living protoplast and showed distinct nuclei even after deposition of secondary wall material (Figure 4D), they were 442 µm to 489 µm in length and 17-22 µm in width.

DISCUSSION

In spite of the importance of Punarnava in medicine, perusal of literature indicates that there is no published data available on the adulterant used and their identification from the genuine drug plant. From an anatomical point of view, *Boerhaavia* and its different species have been worked out by various researchers (Joshi 1937; Rajput, Rao, 1998; Varier, 1944). India is known for several regional languages or sometimes even within the same state resulted in several vernacular names to it. *Boerhaavia* is a very good example and is known by various names such as Punarnava, Raktakanda, Shothaghni, Varshabhu in Sanskrit, Tambadivasu in Marathi, Snathikari in Hindi, Mukaratee-Kirei in Tamil and Dholia-saturdo, Moto-satodo in Gujarati etc, while in certain regions, Satodo is the local name for *Trianthema* or *Sesuvium*. Such variation in names has ultimately resulted in adulteration due to improper knowledge of plants to plant collectors. Seemingly identical morphology of *Boerhaavia* with *Trianthema* is also a contributing factor. This adulteration may be due to lack of proper knowledge of the particular species, and sometimes deliberate with a profit motive. Therefore, it is necessary to put forward the anatomical features shown by Punarnava for accurate identification of the medicinal plant species.

Market samples of Punarnava are often adulterated with various species of *Trianthema* and *Sesuvium*. These adulterated samples lack the Punarvine. Varier (1994), compared stem anatomy and reported two main characteristic differences: 1) Plenty of starch grains could be seen in both the ground tissues and xylem parenchyma of Punarnava which was not observed in *Trianthema*, and 2) The phloem around the xylem of Punarnava root had semi-circular or eccentric patches, while *Trianthema* possess narrow strips only. However, no detailed study has been done on comparative stem and root anatomy of *Boerhaavia*, *Trianthema* and *Sesuvium*.

Occurrence and development of successive cambia has been studied in *Boerhaavia, Sesuvium* and *Trianthema* by earlier workers (Metcalf, Chalk, 1930; Rao, Rajput, 1998; Rajput, Patil, Shah, 2008). Development of cambium in *Boerhaavia* diffusa, the fascicular and inter fascicular segments of the first cambial ring maintain their identity giving rise to conducting elements and inter fascicular parenchyma respectively on both sides. In *Sesuvium* and *Trianthema,* the first ring of cambium ceases its activity after producing xylem centripetally and phloem centrifugally. Second ring of cambium is formed by the differentiation of phloem parenchyma cells produced by the previous cambium mainly in *Trianthema*. In *Sesuvium,* second ring of cambium is formed from the axial parenchyma cells, which underwent periclinal divisions to form a wide band of cells referred as secondary cortex, which served as site for formation of next cambial ring by their repeated divisions, reported by Rajput and Rao (1998, 2008). Here with, we reconfirmed the feature as mentioned by previous worker in our study. However, these studies are mainly focused on the developmental and anatomical aspects of these plants while information regarding position of crystals has been neglected. *Boerhaavia* possess raphides i.e. needle like crystals in the leaf, however druses were observed in *Sesuvium* and *Trianthema*. Various functions have been attributed to plant crystals and idioblasts (Franceschi, Horner, 1980). Some evidences have pointed towards the ionic balance which prevents the oxalate toxic accumulation, to the storage of calcium in cells, and to mechanical support of the plant body.

Metcalf, Chalk (1983), reported Rananculaceous (anomocytic) stomata in Nyctaginaceae, Rubiaceous (paracytic) and Rananculaceous (anomocytic) in Aizoaceae. Similarly present study revealed that *Boerhaavia diffusa* shows anomocytic stomata while *Trianthema sp*. and *Sesuvium sp*. show paracytic stomata. Compared to traditional methods, SEM showed many structures very distinctly, which facilitated accurate identification. Finally, our observations on trichomes are in agreement with those described by Metcalf, Chalk (1983).

Absence of rays occurs in plants having limited cambial activity and deposits little secondary xylem (Carlquist, 1970). When a single cambium no longer produces more xylem, then successive cambia increases stem or root diameter. Such xylem always shows absence of rays, as has been reported by Barghoorn (1941) and

Carlquist (1970). However, in the present investigation all plants showed scanty accumulation of secondary xylem by forming successive cambia. Raylessness is not a common feature and it remains confined to a few species of dicotyledons belonging to different families (Carlquist, 1988; Lev-Yadun, Aloni, 1995; Rao, Rajput, 1998; Rajput, Rao, 1998, 1999a). All the species investigated in the present study showed absence of rays and is reported by earlier workers (Rajput, Rao, 1998; Rajput, Patil, 2008; Rao, Rajput 1998).

Occurrence of nucleated xylem fibers is associated with the rayless nature of xylem. According to Fahn and Leshem (1963) nucleated xylem fibers is an adaptive feature, which is associated with diminishing supportive function. They display a transition towards parenchyma cells that occur in herbaceous plants. Accumulations of starch in nucleated fibers further suggest that beside mechanical support, they also perform the storage function (Rajput, Rao, 1999b, c). Parameshwaran and Liese (1969) correlated the ability of xylem fibers to store starch during paucity of storage parenchyma. In *Sesuvium portulacastrum*, presence of nucleated xylem fibers suggests that these fibers not only act as a reservoir of photosynthate but also play an important role in radial conduction. They are performing the dual function as storage cells and mechanical tissue in the absence of rays.

CONCLUSION

The morpho-anatomical characters are described for *Boerhaavia* and its adulterants, in which Punarnava differs from other species in presences of anisocytic stomata, uni-multicellular with elongated head trichome. Central midrib is crescent shape, raphide crystals and starch-grains were distributed in leaves, stem and root. Stem anatomy of Punarnava showed successive cambia along with medullary bundles, while medullary bundles are absent in other two species. All these characters may be used for the correct identification of *Boerhaavia*.

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