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RESEARCH ARTICLE

Key distinguishing characters (KDCs) of official (*Boerhaavia diffusa* L.) and commonly mistaken (*Trianthema portulacastrum* L.) sources of *Mukkirattai* of Siddha

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ABSTRACT
Boerhaavia diffusa L. has been used extensively in *Siddha* system of medicine and is often confused with *Trianthema portulacastrum* L. due to morphological similarities. This particular study compares, analyses and identifies the key distinguishing features of the two whole plant drugs based on pharmacognostical and phytochemical aspects. The samples were studied for macroscopy, microscopy, physicochemical analysis, preliminary phytochemical analysis and HPTLC following standard procedures. Macroscopic studies showed few notable differences in macro-microscopy of root, stem, leaves and flowers of both the plants. Microscopically root, stem, leaf and petiole of both the plants showed differences in layers of cork, presence of crystals and medullary rays (in root), the thickness of cuticle, cortex and arrangement of vascular bundles (stem), presence of characteristic type of trichomes (leaf) and shape of the petiole. Powder microscopy showed differences in epidermis, crystal types and shape of pollen. Preliminary phytochemical analysis showed the presence of phenol, saponins and coumarins in *B. diffusa* and was not detected in *T. portulacastrum*. There were significant differences in the values of quantitative microscopy, physicochemical parameters and HPTLC of both the whole plant samples. The finding of this study will be helpful for the correct identification of the plant.

Introduction

Plants have been used as a source of medicine since the prehistoric era due to the availability of potent phytochemicals. *Siddha* is one among the ancient systems of medicine which indicates that each plant has a specific medicinal activity. In many instances, the *Siddhars* had specified the use of the whole plant as medicine. The plants which look alike morphologically seem to be challenging for common people who use this medicinal plant. In such cases, the pharmacognostic and phytochemical studies which involve comparing the macroscopic, microscopic, powder microscopic and phytochemical aspects of different species, comes to their rescue. These studies help us to spot the key distinguishing characters of plants which show similarities only in morphology.

Boerhaavia diffusa L. and *Trianthema portulacastrum* L. are two weeds that are found commonly throughout India. *B. diffusa* is a perennial diffuse herb (1) whereas *T. portulacastrum* is a prostrate, glabrous, annual, succulent herb (2). The former finds its extensive use in the *Siddha* system of medicine, however, is often confused with the latter due to the similar looks and hence often adulterated with the same.

In *Siddha*, the whole plant of *B. diffusa* is used in the preparation of *Talakaccenturam* which is used in the treatment of indigestion associated with children, eye diseases and painful diseases; root is used in the preparation of *Manturatiataikkutinir* for treating anaemia, jaundice, mars in abdomen, ascites, inflammation etc (3). *Mukkirattaichuranam* made from *B. diffusa* is used for the treatment of stomach

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ache, constipation, numbness, leprosy, splenomegaly and skin diseases (4).

The major chemical constituents of *B. diffusa* include punarnavoside (5), boeravinone C (6), liriodendrin (7), hypoxanthine-9-L-arabinofuranoside (8) and eupalitin-3-O-beta-D-galactopyranoside (9). *B. diffusa* had been found effective in patients suffering from oedema, dermatopathies, heart disorders, anaemia, hepatic disorders, inflammatory disorders and wound (10). The plant has been found to induce diuresis, decrease in albumin urea and rise in serum protein. It also reduced the serum cholesterol level in patients with nephrotic syndrome (11). Ecdysterone is a major chemical constituent of *T. portulacastrum* (12). Ethanolic extract of *T. portulacastrum* showed antipyretic, anti-inflammatory, antibacterial, central nervous system depressant (13) and analgesic (13, 14) properties. The plant was also found to possess hepatoprotective (15, 16) and diuretic activities (17, 18).

Both the plants are medicinally important, but, have different pharmacological actions and chemical composition. The current paper attempts to differentiate the two plants pharmacognostically to obtain key distinguishing characters (KDC) to have a clear picture on differentiating the two crude drugs when sold in the market as *Mukkirattai*.

Materials and methods

Collection of samples

Whole plant samples of *B. diffusa* and *T. portulacastrum* were collected from Anna Hospital Campus, Arumbakkam, Chennai during the month of June 2019. The samples were authenticated by a qualified botanist referring to the flora of the region (19). The herbarium specimens were deposited in the SCRI herbarium (Voucher No.: *B. diffusa*-SCRIP022 and *T. portulacastrum*- SCRIP023) for the future reference.

Processing of samples

Parts of both the samples were preserved in FAA for anatomical and quantitative studies. Fifty grams of air-dried samples were dried and powdered for powder microscopic and phytochemical studies.

Macroscopy

Macroscopical characters were observed and recorded using Nikon COOLPIX4500 camera and ZEISS stereo microscope (20).

Microscopy

FAA preserved plant parts were transversely cut using 7 O'Clock platinum blade and stained with safranine and photographed using Nikon ECLIPSE E200 trinocular microscope attached with ZEISS AxioCam Erc5s digital camera under bright field light. Observations were recorded and magnification was indicated by scale bars.

The powdered sample was passed through sieve no. 60 and a pinch of it were taken and mounted in glycerine after treating with 2% KOH on a clean microscopic slide. The characters were identified and labelled along with magnification indicated by scale bars.

The leaf fragments of about 5×5 mm were heated with 5 ml of 4% KOH solution in a test tube for about 15 min or until the fragments became transparent and epidermal layers got separated. The layers of both the samples are separately transferred to a microscopic slide after staining with safranin, mounted in glycerol solution and examined with a 40x objective and a 10x eyepiece, to which a microscopic apparatus (Camera lucida– Prism type) is attached. Epidermal number, stomatal number, stomatal index, vein islets and vein termination were calculated as per standard procedures (20).

Physico-chemical analysis

Both the samples were tested for loss on drying at 105ºC, total ash, water-soluble ash, acid insoluble ash, water-soluble extractives, acid insoluble extractives and pH value as per standard protocol (21).

Preliminary phytochemical analysis

Tests were done to detect the presence of phenol, tannin, flavonoids, triterpenoids, proteins, glycosides, reducing sugar, anthraquinones, quinones, alkaloids, saponins, cardiac glycosides, steroids, coumarin and acids in both the samples (20).

HPTLC

One gram of the whole plant samples was taken separately in a conical flask and 10 ml of ethanol was added, boiled for a few minutes, cooled, filtered and then concentrated to 2 ml. The mobile phase used was toluene: ethyl acetate: acetic acid (4:3:0.5 v/v/v). Six and 10 µl of the samples were applied on silica gel (60 F254) pre-coated TLC plate using CAMAG (Muttenz, Switzerland) ATS4 applicator. The plate was developed in a previously saturated twin trough chamber (CAMAG) of 10 cm × 10 cm size with the prepared mobile phase. The plate was developed up to 90 mm from the bottom. After development, the plate was photo-documented using CAMAG TLC Visualizer under λ254 nm and λ366 nm. Then the plate was scanned using CAMAG Scanner 4 at λ254 nm (D2 lamp, absorption mode) and λ366 nm (Hg lamp, fluorescence mode) respectively and fingerprint profiles of the sample were documented. Subsequently, the plate was dipped in vanillin sulphuric acid reagent followed by heating at 130ºC until the development of the coloured spots. The plate was then documented in white light using CAMAG TLC Visualizer and scanned at λ520 nm (W light, absorption mode) (20).

Results

Boerhaavia diffusa

Macroscopy

Root yellowish to dark brown measuring about 20 cm in length and 2 cm in diameter with occasional branches, rootstock woody with a rough, longitudinally wrinkled and fissured surface; stem greenish-purple, spreading with swollen nodes; leaf simple, opposite, petiolate, thick, ovate to orbicular

with size varying 2 to 4 cm in length, 1.5 to 3 cm in width, with round apex and subcordate base; upper surface green and lower silvery white; flower pink very small, in long pedunculated umbels which are both axillary and terminal, bracteates, epigynous with fused cup-shaped perianth lobe, stamens 5, ovary inferior (Fig. 1).

Microscopy

Root

TS 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

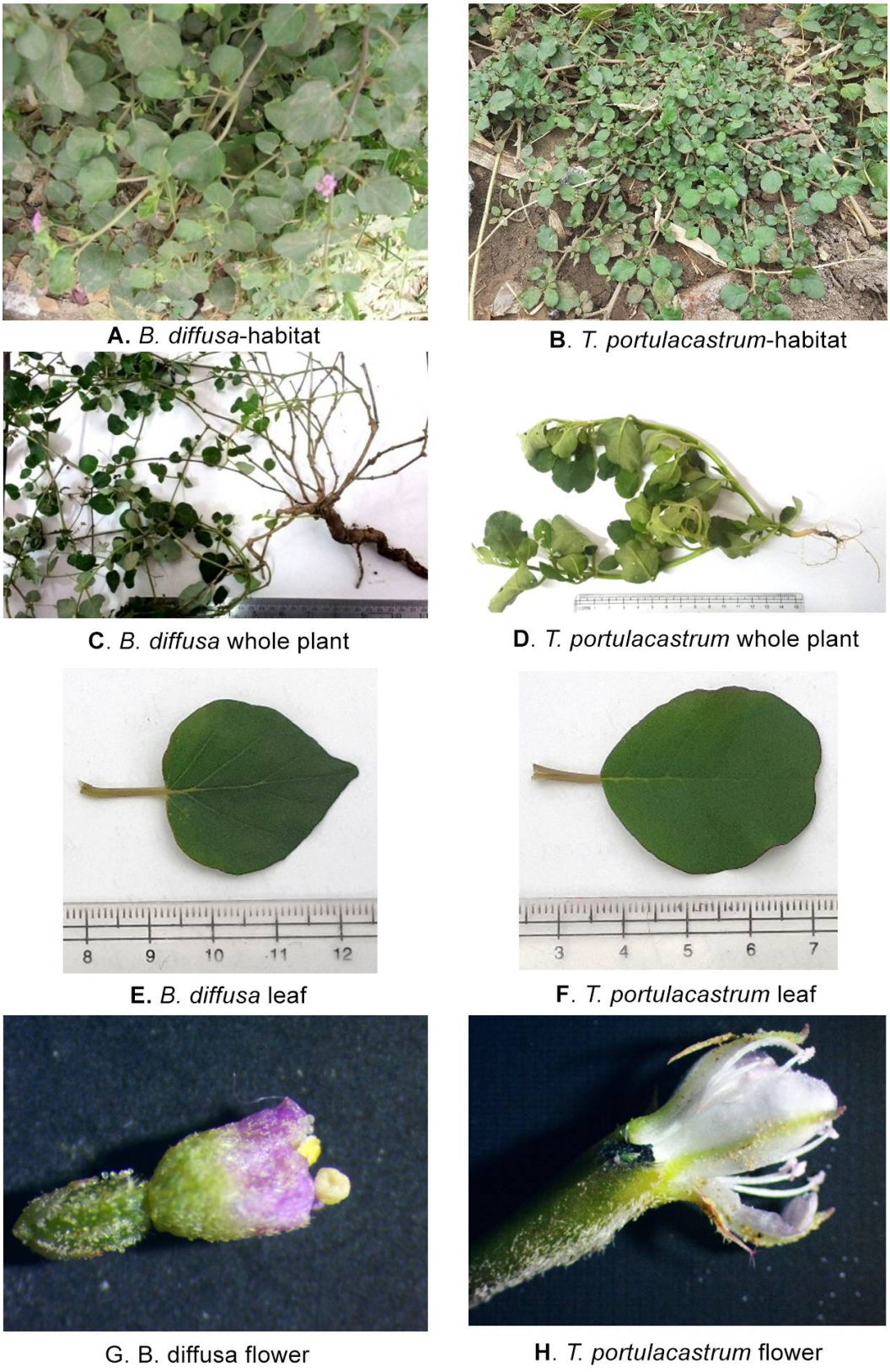
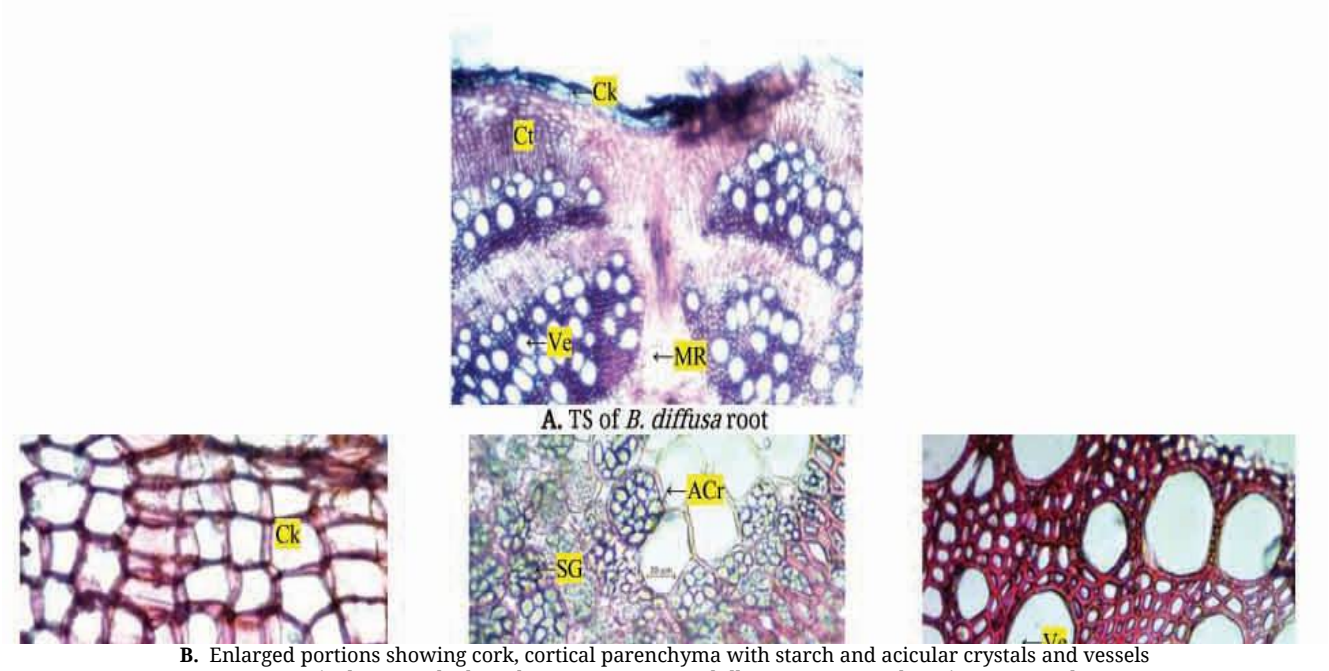


Fig. 1. Macroscopic features of *B. diffusa* and *T. portulacastrum.*

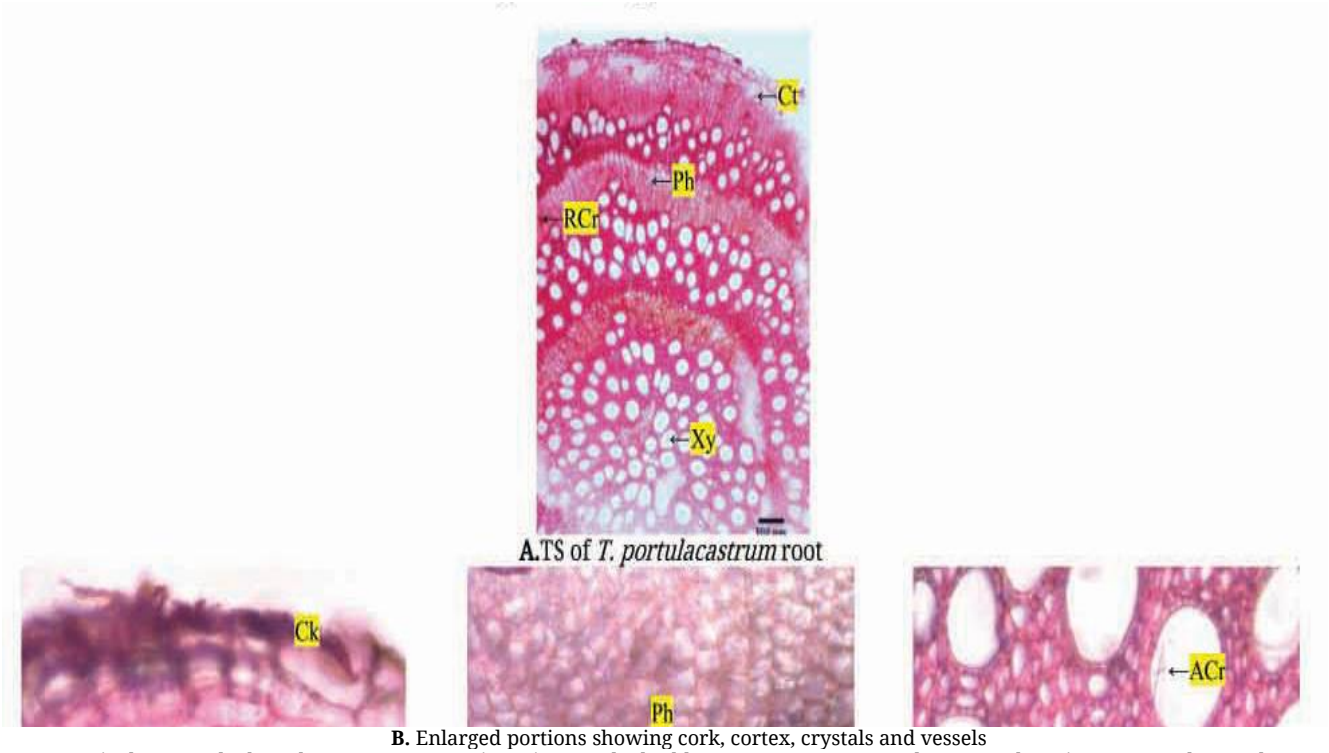
grains. The stellar region shows centrally located primary xylem surrounded by phloem which is composed of phloem fibres and parenchyma. Surrounding this, a discontinuous band of secondary rings are observed which are separated by medullary rays (Fig. 2).

collenchymatous cells which are followed by five to six layers of chlorenchyma with acicular crystals. A layer of endodermis is followed by cortical vascular bundle which consists of a narrow band of phloem tissue with few isolated thick-walled fibres. Below the phloem layer, xylem tissue with thick-walled fibres



A. TS of *B. diffusa* root

B. Enlarged portions showing cork, cortical parenchyma with starch and acicular crystals and vessels
ACr-Acicular crystal; Ck-Cork; Ct-Cortex; MR-Medullary ray; SG-starch grains; Ve-Vessel
Fig. 2. Microscopy of Root of *B. diffusa*



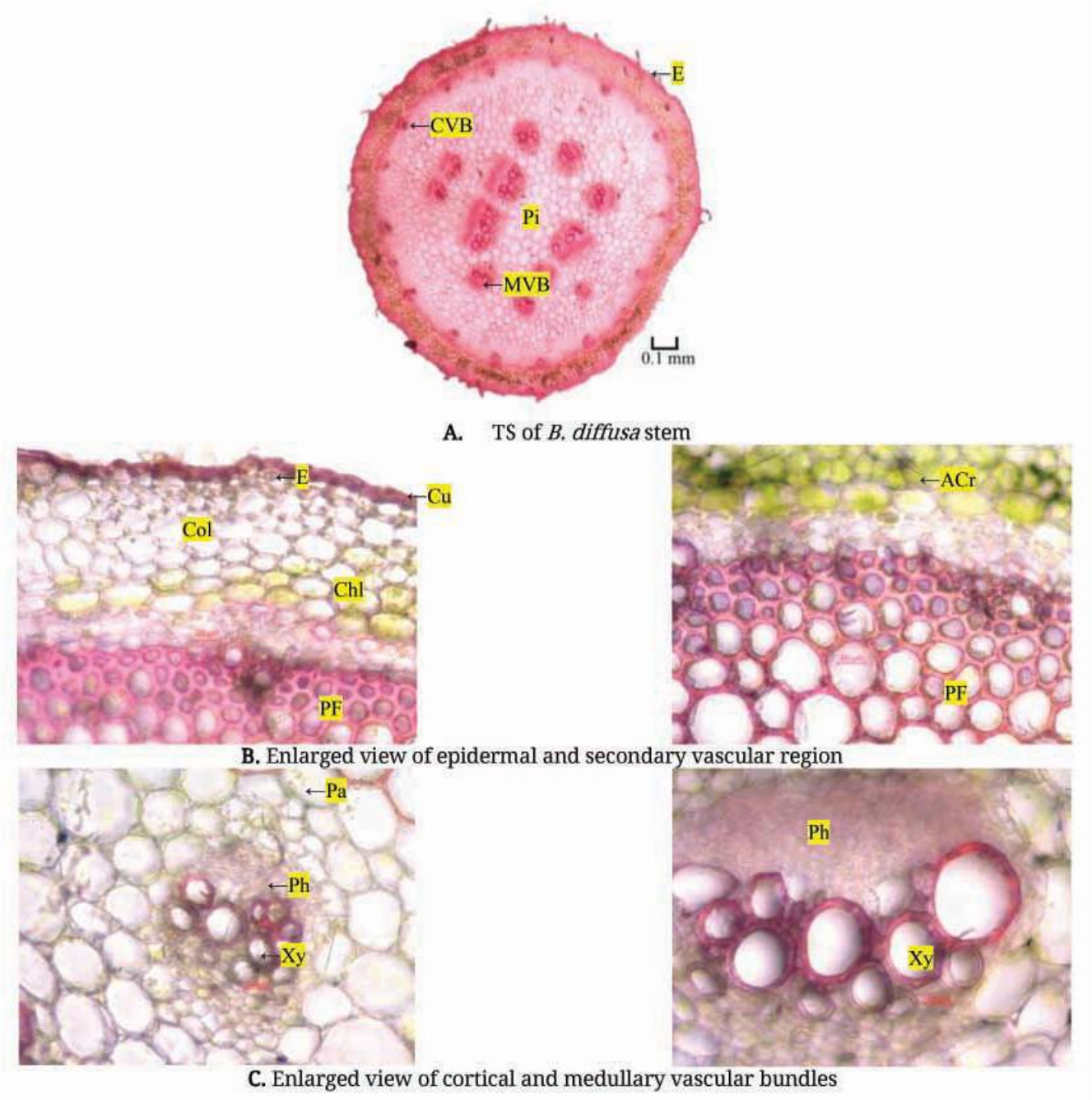
A.TS of *T. portulacastrum* root

B. Enlarged portions showing cork, cortex, crystals and vessels
ACr-Acicular crystal; Ck-Cork; Ct-Cortex; PCr-Prismatic crystal; Ph-Phloem; RCr-Rosette crystal; SG-Starch grains; Ve-Vessel; Xy-Xylem
Fig . 3. Microscopy of Root of *T. portulacastrum.*

Stem

TS of the stem is circular, shows outermost epidermal layer covered with thick cuticle with many unicellular to multicellular trichomes. The epidermal layer is followed by two to three layers of

and vessels are observed. The pith is composed of loosely arranged parenchymatous cells with acicular crystals embedded with heteromorphic vascular bundles that are scattered. The endodermis is found above these bundles (Fig. 4).



ACr-Acicular crystal; Chl-Chlorenchyma; Col-Collenchyma; Ct-Cortex; Cu-Cuticle; CVB-Cortical Vascular bundle; E-Epidermis; MVB-Medullary Vascular bundle; Pa-Parenchyma; PF-Phloem fibres; Ph-Phloem; Pi-Pith; T-Trichome; Ve-Vessel; Xy-Xylem

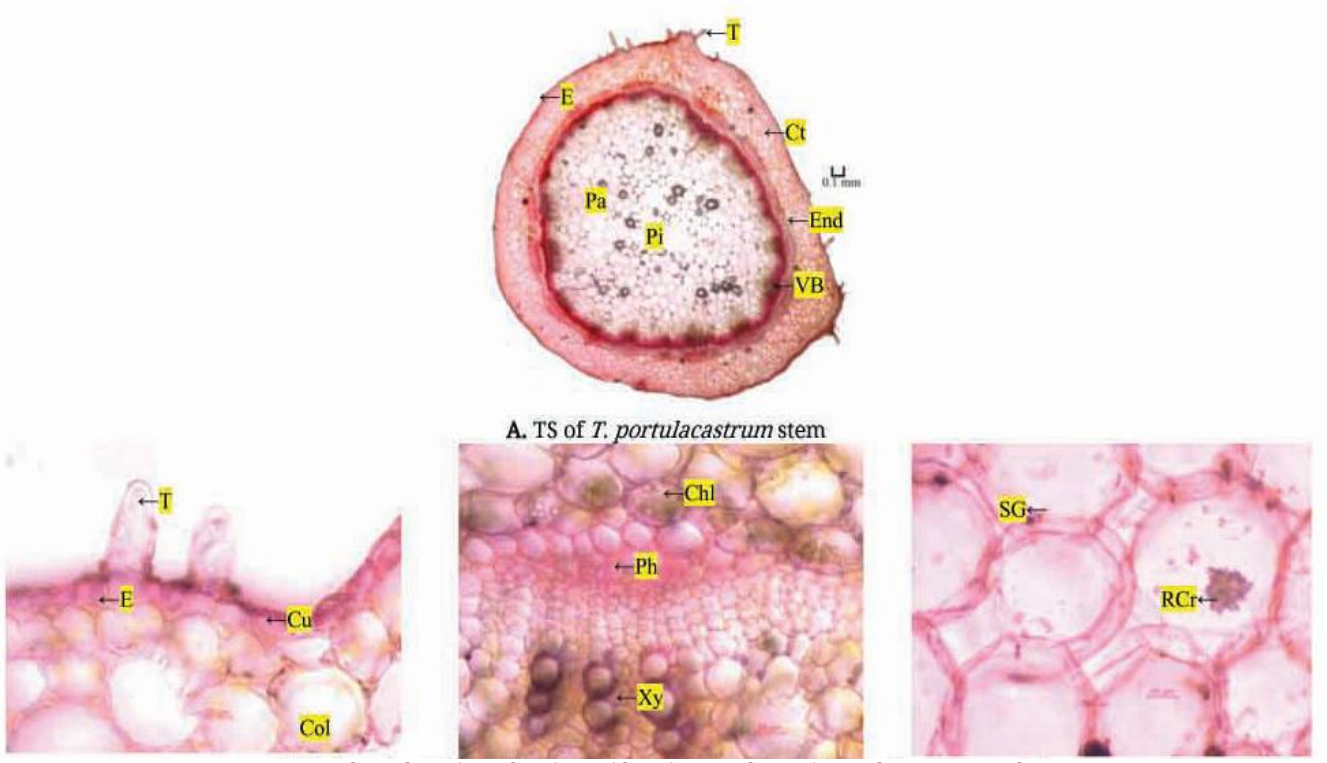
Fig. 4. Microscopy of Stem of *B. diffusa.*

Petiole

TS of the petiole is oval-shaped with a 'V' shaped depression on the upper side. The outermost layer is composed of epidermis with many uni and multicellular trichomes. The ground tissue is composed of seven to eight layers of collenchymatous cells followed by two to three layers of parenchyma in which vascular bundles are embedded in a pattern of 'U'. Acicular crystals are randomly scattered in the collenchyma. A total of 5 vascular bundles are observed. The three bundles that are located in the lower portion of 'U' is found to be predominant, whereas the bundles on either side of the upper portion are smaller; xylem surrounded by phloem (Fig. 6).

Lamina

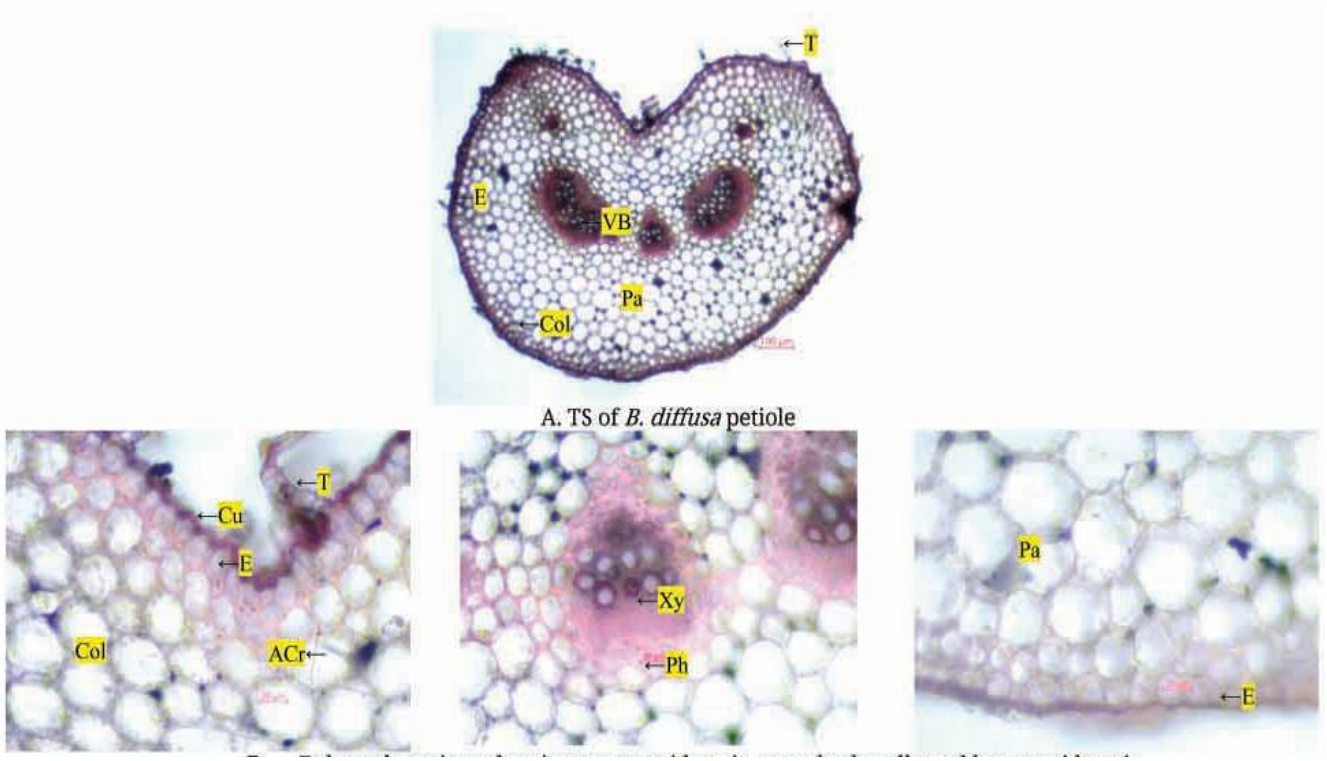
TS of lamina through midrib shows a layer of upper and lower epidermis covered with a thick cuticle. Both the epidermis shows many unicellular, multicellular and glandular trichomes. Three to four rows of collenchymatous cells are found below the upper epidermal layer of midrib and a row of palisade 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



B. Enlarged portions showing epidermis, vascular region and rosette crystal

ACr-Acicular crystal; Chl-Chlorenchyma; Col-Collenchyma; Ct-Cortex; Cu-Cuticle; CVB-Cortical Vascular bundle; E-Epidermis; End-Endodermis; Pa-Parenchyma; PF-Phloem fibres; Ph-Phloem; Pi-Pith; RCr-Rosette crystal; SG-Starch grain; T-Trichome; VB-Vascular bundle; Xy-Xylem

Fig. 5. Microscopy of Stem of *T. portulacastrum.*



B. Enlarged portions showing upper epidermis, vascular bundle and lower epidermis

ACr-Acicular crystal; Col-Collenchyma; Cu-Cuticle; E-Epidermis; Pa-Parenchyma; Ph-Phloem; T-Trichome, VB-Vascular bundle; Xy-Xylem

Fig. 6. Microscopy of Petiole of *B. diffusa*.

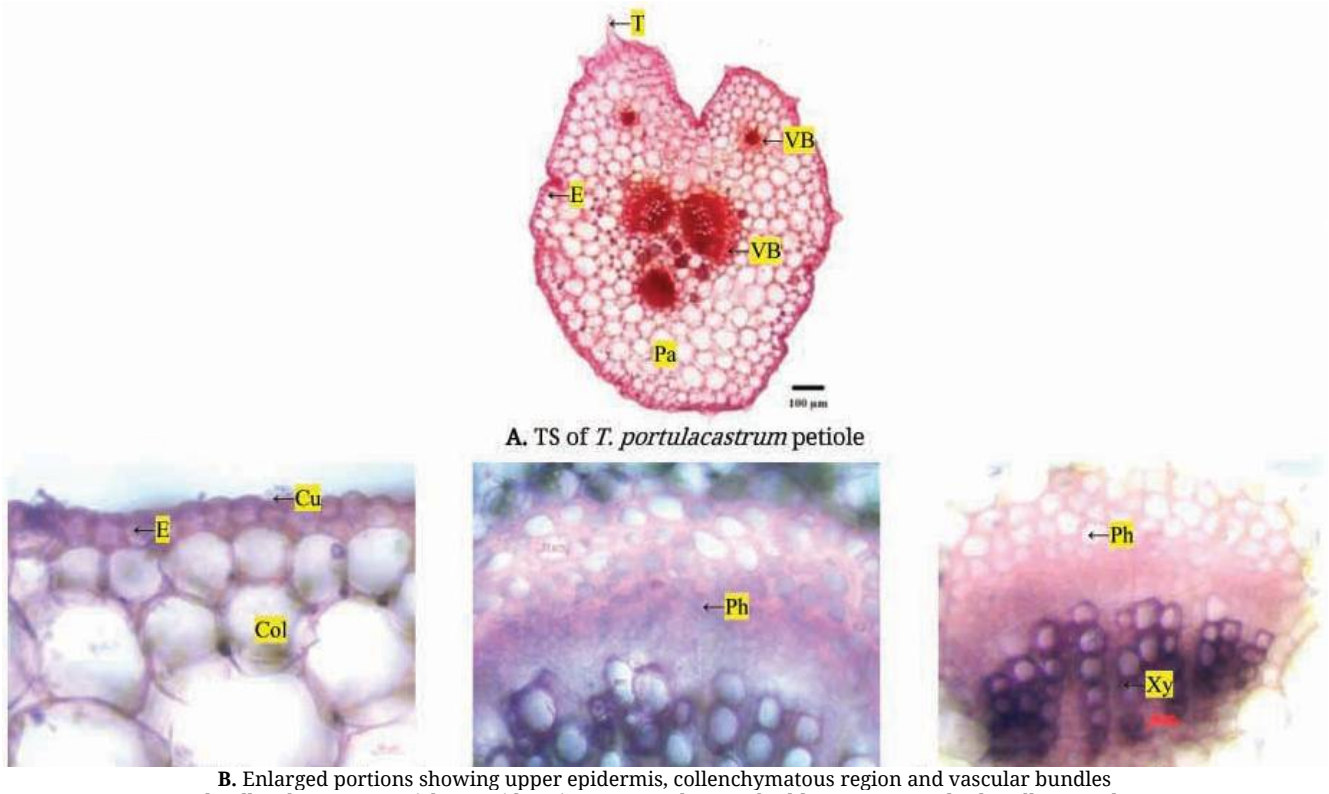


Fig. 7. Microscopy of Petiole of *T. portulacastrum.*

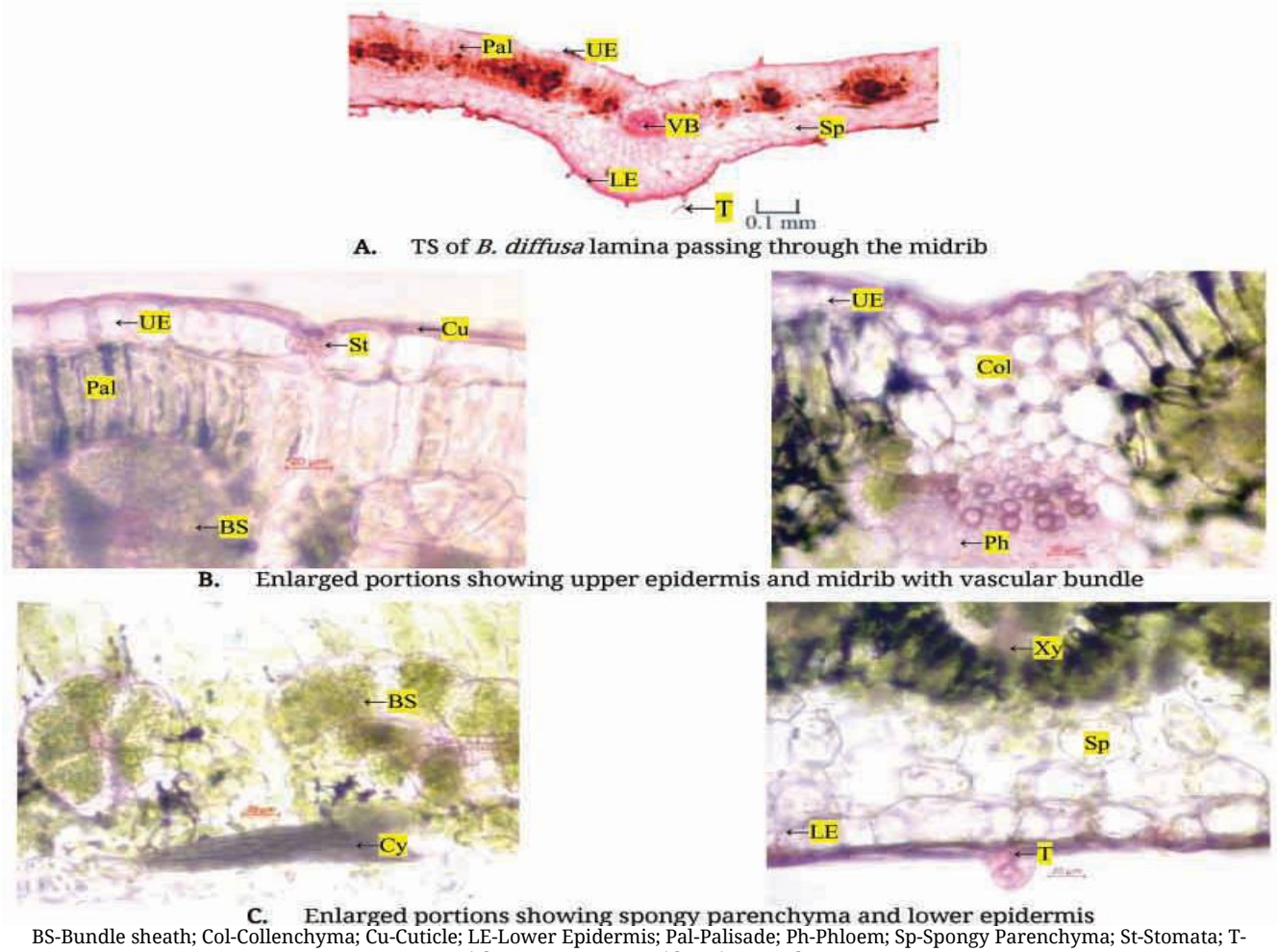


Fig. 8. Microscopy of Lamina through midrib of *B. diffusa*.

tissue of midrib is composed of parenchymatous cells and a centrally located vascular bundle (Fig. 8).

Trianthema portulacastrum

Macroscopy

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 (Fig. 1).

Microscopy

Root

TS 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 (Fig. 3).

Stem

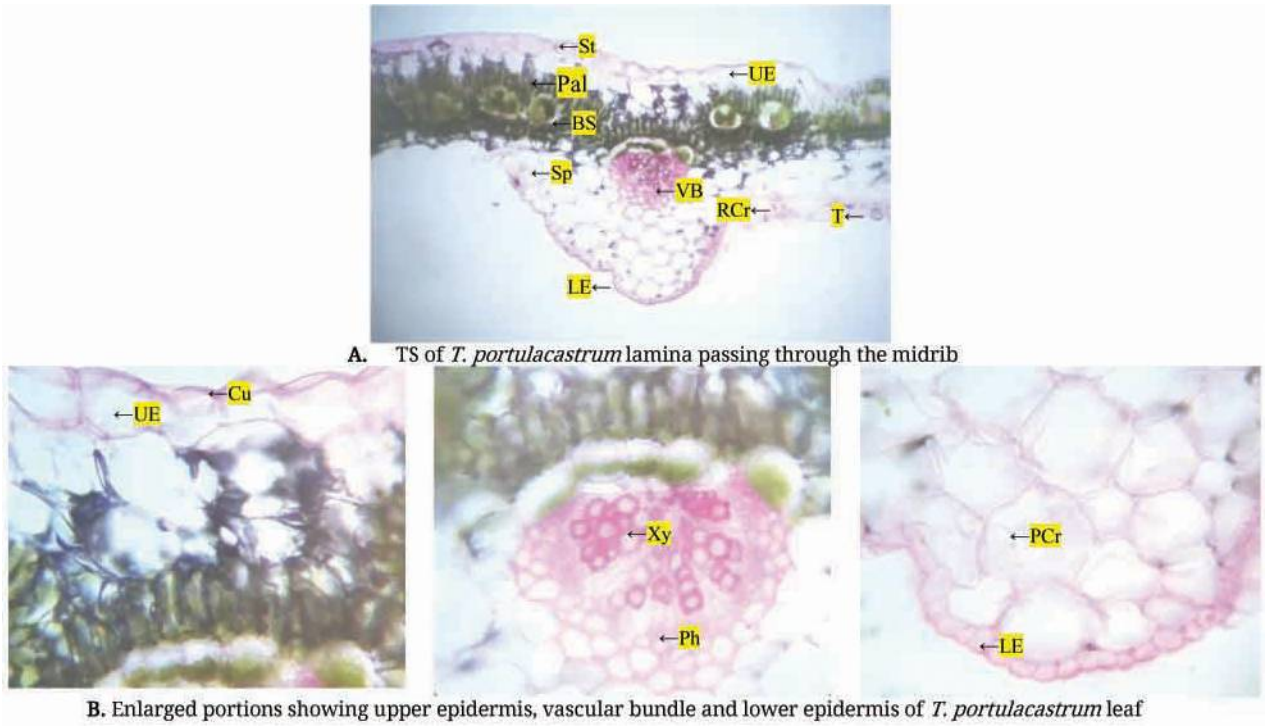
TS of the stem is nearly circular. The outermost layer is composed of epidermis with thin cuticle. Many unicellular trichomes are observed on the epidermis. Below the epidermis lies cortex region which is composed of six to eight layers of collenchymatous cells followed by two to three layers of chlorenchyma. Below the chlorenchyma lies endodermis followed by vascular bundles that are arranged in a ring. Thick-walled phloem fibres are found followed by phloem parenchyma underneath which lies the xylem composed of vessels and parenchyma. Vessels are arranged radially. Pith is distinct and consists of parenchymatous cells with simple, compound starch grains and rosette crystals (Fig. 5).

Petiole

TS of the petiole is almost circular in shape with a 'V' shaped depression on the upper side. The outermost layer constitutes the epidermis with a thick cuticle. The epidermis is interrupted by many unicellular to multicellular trichomes, followed by five to seven layers of collenchyma. There are three large vascular bundles in the centre and two smaller bundles on either side of the V-shaped depression. Phloem consists of phloem fibres and parenchyma; xylem consists of vessels (Fig. 7).

Lamina

TS of leaf passing through midrib shows a single layer of upper and lower epidermis covered with cuticle. Epidermal layers are interrupted by stomata,



A. TS of *T. portulacastrum* lamina passing through the midrib

B. Enlarged portions showing upper epidermis, vascular bundle and lower epidermis of *T. portulacastrum* leaf

BS-Bundle sheath; Cu-Cuticle; LE-Lower Epidermis; Pal-Palisade; Ph-Phloem; PCr-Prismatic crystals; RCr-Rosette crystals; Sp-Spongy Parenchyma; St-Stomata; T-Trichome; UE-Upper Epidermis; Xy-Xylem

Fig. 9. Microscopy of Lamina through midrib of *T. portulacastrum.*

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 (Fig. 9).

Quantitative microscopy

The quantitative parameters such as stomatal number and index, palisade ratio, vein islet and vein termination number were quantified and tabulated (Table 1). The peelings of leaf epidermis showed amphistomatic and anomocytic stomata in *B. diffusa* and paracytic stomata in *T. portulacastrum* (Fig. 10).

Table 1. Leaf constants of *B. diffusa* and *T. portulacastrum*

Parameters	*B. diffusa*		*T. portulacastrum*	
	Upper epidermis	Lower epidermis	Upper epidermis	Lower epidermis
Epidermal Number	430-445	440-450	150-160	180-195
Stomatal Number	90-105	110-125	60-70	70-80
Stomatal Index	15-20	18-23	28-35	25-30
Vein islets	3-5		6-8	
Vein termination	10-16		15-20	

Powder microscopy

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 (Fig. 11).

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 (Fig. 12).

Table 2. Physicochemical analysis of the whole plant drugs

Physicochemical parameters	Mean±SEM	
	B. diffusa	*T. portulacastrum*
Loss on Drying (%w/w)	7.12±0.01	10.16±0.165
Total Ash (%w/w)	10.70±0.045	20.88±0.175
Water soluble ash (%w/w)	3.96±0.09	12.20±0.65
Acid insoluble ash (%w/w)	0.81±0.08	2.60±0.25
Water Soluble Extractives (%w/w)	17.14±0.0725	25.45±1.05
Acid Insoluble Ash extractives (%w/w)	11.49±0.085	7.55±0.45
pH Value (10 % solution)	6.33±0.025	5.71±0.01

Physicochemical analysis

During the physicochemical analysis of both the plants, loss on drying, total ash, water-soluble ash, acid insoluble ash, water-soluble extractives, acid insoluble ash extractives, pH Value (10 % solution) was recorded and is summarized in Table 2.

Preliminary phytochemical analysis

The dried powder of the samples was taken for the preliminary phytochemical analysis (phenol, tannin, flavonoids, triterpenoids, proteins, glycosides, reducing sugar, anthraquinones, quinones, alkaloids, saponins, cardiac glycosides, steroids, coumarin, acids) and the observations were recorded in Table 3.

Table 3. Preliminary phytochemical analysis of the whole plant drugs

Phytochemical	*B. diffusa*-whole plant	*T. portulacastrum*-whole plant
Phenol	+	-
Tannin	+	+
Flavonoids	+	+
Triterpenoids	+	+
Proteins	-	-
Glycosides	+	+
Reducing sugar	-	-
Anthraquinones	-	-
Quinones	-	-
Alkaloids	-	-
Saponins	+	-
Cardiac glycosides	-	-
Steroids	+	-
Coumarin	+	-
Acids	-	-

+ present, - absent

HPTLC

TLC chromatograms of ethanol extract of *B. diffusa* whole plant (Fig. 13) revealed 1 band with Rf 0.08 (green) at 254 nm whereas that of *T. portulacastrum* whole plant showed 4 bands with Rf 0.07, 0.57, 0.63 and 0.70 (green); *B. diffusa* whole plant showed a total of 6 bands with Rf 0.16, 0.19 (blue), 0.33, 0.47, 0.56 and 0.68 (red) at 366 nm whereas that of *T. portulacastrum* showed 9 spots with Rf values 0.07 (bluish-red), 0.10, 0.20, 0.35, 0.33, 0.47, 0.57, 0.63 and 0.68 (red). *B. diffusa* whole plant showed a total of 3 spots with Rf 0.32 (grey), 0.45 (violet) and 0.59 (grey) after dipping vanillin sulphuric acid whereas that of *T. portulacastrum* showed 7 spots with Rf 0.08, 0.11, 0.33 (grey), 0.41, 0.45 (violet), 0.59 and 0.69 (grey) (Table 4). In the fingerprint profile of *B. diffusa* showed 8 peaks (Conc. 5 µl), 9 peaks (Conc. 10 µl) and 6 peaks (Conc. 15 µl) with the maximum area covered under peaks 7,8,5 respectively (Fig. 14). In the fingerprint profile of *T. portulacatrum* showed 12 peaks (Conc. 5 µl), 13 peaks (Conc. 10 µl) and 14 peaks (Conc. 15 µl) with the maximum area covered under peaks 11,12,13 respectively (Fig. 15).

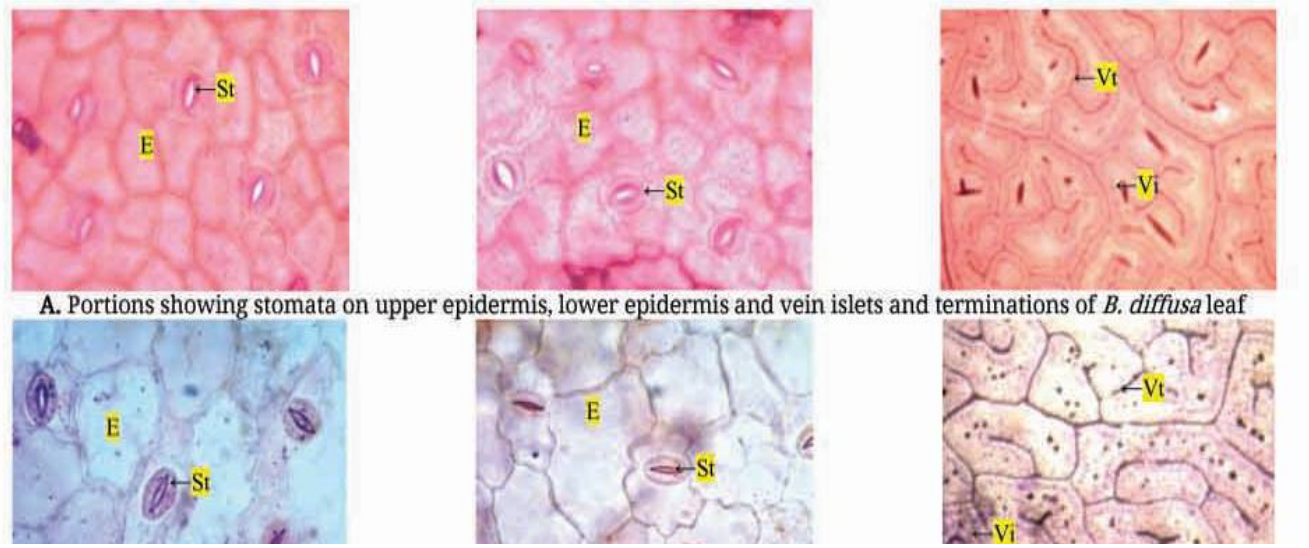
Table 4. Rf values and colour spots of ethanolic extracts of *B. diffusa* and *T. portulacastrum*

254 nm				366 nm				After dipping vanillin sulphuric acid			
B. diffusa		***T. portulacastrum***		***B. diffusa***		***T. portulacastrum***		***B. diffusa***		***T. portulacastrum***	
Rf	**Colour**	**Rf**	**Colour**	**Rf**	**Colour**	**Rf**	**Colour**	**Rf**	**Colour**	**Rf**	**Colour**
0.08	Green	0.07	Green	0.16	Blue	0.07	Bluish Red	0.32	Grey	0.08	Grey
		0.57	Green	0.19	Blue	0.10	Red	0.45	Violet	0.11	Grey
		0.63	Green	0.33	Red	0.20	Red	0.59	Grey	0.33	Grey
		0.70	Green	0.47	Red	0.35	Red			0.41	Violet
				0.56	Red	0.33	Red			0.45	Violet
				0.68	Red	0.47	Red			0.59	Grey
						0.57	Red			0.69	Grey
						0.63	Red				
						0.68	Red				

Mobile phase: Toluene: ethyl acetate (5:1)

Table 5. Key distinguishing features

Sl.No.	Character/parameter	*B. diffusa*	*T. portulacastrum*
1.	Macroscopy: Root	Dark brown, Stout, very long, occasionally branched	Light yellow externally and creamish internally, Thin slender, branching fibrous root
	Stem	Greenish purple coloured	Reddish tint at places
	Leaf	Cuspidate and rounded at the base	Retuse and tapering at the base
	Flower	Pink-coloured with 5 stamens, inferior ovary	Pinkish white coloured with 10-15 stamens, superior ovary
2.	Microscopy: Root	Cork: 10-12 layers Only acicular crystals are present Presence of parenchyma loaded with starch and medullary rays	Cork: 2-3 layers Presence of prismatic, acicular and rosette crystals -
	Stem	Cuticle: thick Cortex: Narrow Presence of Acicular crystal VB: Heteromorphic, scattered found both in cortex and medulla	Cuticle: thin Cortex: Broad Presence of Rosette crystal VB: Arranged as a radial band
	Leaf	Cuticle: thick - -	Cuticle: thin Presence of balloon-shaped trichomes Presence of rosette and prismatic crystals
	Petiole	Oval shaped	Circular shaped
3.	Powder microscopy	- - Cystolith Pollen-round	Presence of striated epidermis Rosette crystal - Pollen-triangular



B.Portions showing stomata on upper epidermis, lower epidermis and vein islets and terminations of *T. portulacastrum* leaf
E-Epidermal cell; St-Stomatal cell; Vi-Vein islet; Vt-Vein termination

Fig. 10. Quantitative microscopy of *B. diffusa* and *T. portulacastrum.*

Discussion

B. diffusa is often adulterated with *T. portulacastrum* because of the common availability of the latter in South India and hence a complete study which providing a comparison of the whole plants in all aspects of pharmacognosy and phytochemistry is required to identify the adulterant. Several studies

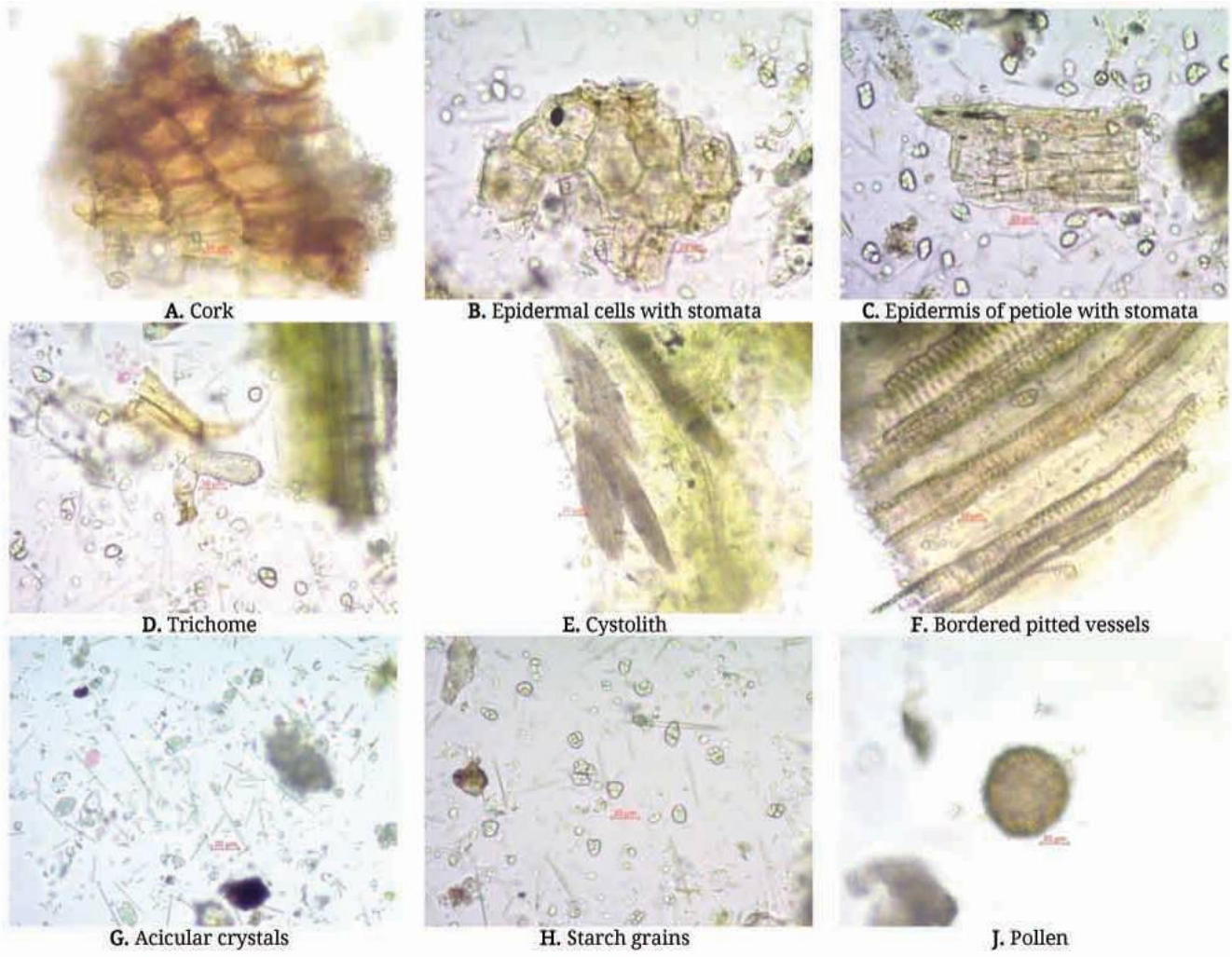


Fig. 11. Powder microscopic characters of *B. diffusa* whole plant.

are available comparing different species of *Boerhaavia*. These include the study (22), dealing with the absence of rays and the formation of cambium rings in different species (23), In a study African species of *Boerhaavia* were compared and discussed on various aspects based on SEM images of trichomes, the anatomy of stem, leaf and anthocarp of the samples. Two species of *Boerhaavia* and reported the differences on grounds of macroscopy, microscopy and TLC (24). *T. portulacastrum* was individually studied and results were discussed concerning macroscopy, microscopy, powder microscopy and phytochemicals of root, stem and leaf (25) and the phytochemical constituents based on preliminary phytochemical analysis and physicochemical parameters (26).

Despite many studies being published, there are no comparative studies of the key distinguishing features of the plants as these studies play a major role in identifying and ruling out the adultery in the herbal system of medicines (Table 5). The stem anatomy of *B. diffusa* and *T. portulacastrum* were compared. The differences in the availability of starch grains and phloem patches in both the species has been reported (27). Another comparative study on stem leaf and root anatomy of *Boerhaavia, Trianthema* and *Sesuvium* has been recorded (28). However, all these studies provided information on anatomical and morphological aspects but did not give information regarding the quantitative, powder microscopic and phytochemical comparisons of *B. diffusa* and *T. portulacastrum* nor did they provide the anatomy of petiole as the shape of the anatomical section has been identified as a key distinguishing feature among both the plants.

This particular study deals with a complete analysis and identification of key distinguishing characters of both the plants with the help of various pharmacognostic and phytochemical analytical methods.

Conclusion

Macroscopic, Microscopic and phytochemical parameters of the two whole plant drugs were assessed and recorded with its description. These key diagnostic features can be used for the identification of the correct plant of interest and thus prevent adulteration during the preparation of herbal medicines.

Authors' contributions

SB contributed towards the conceptual and intellectual content and design of topic, AR carried out the microscopical studies of both plants, KGD

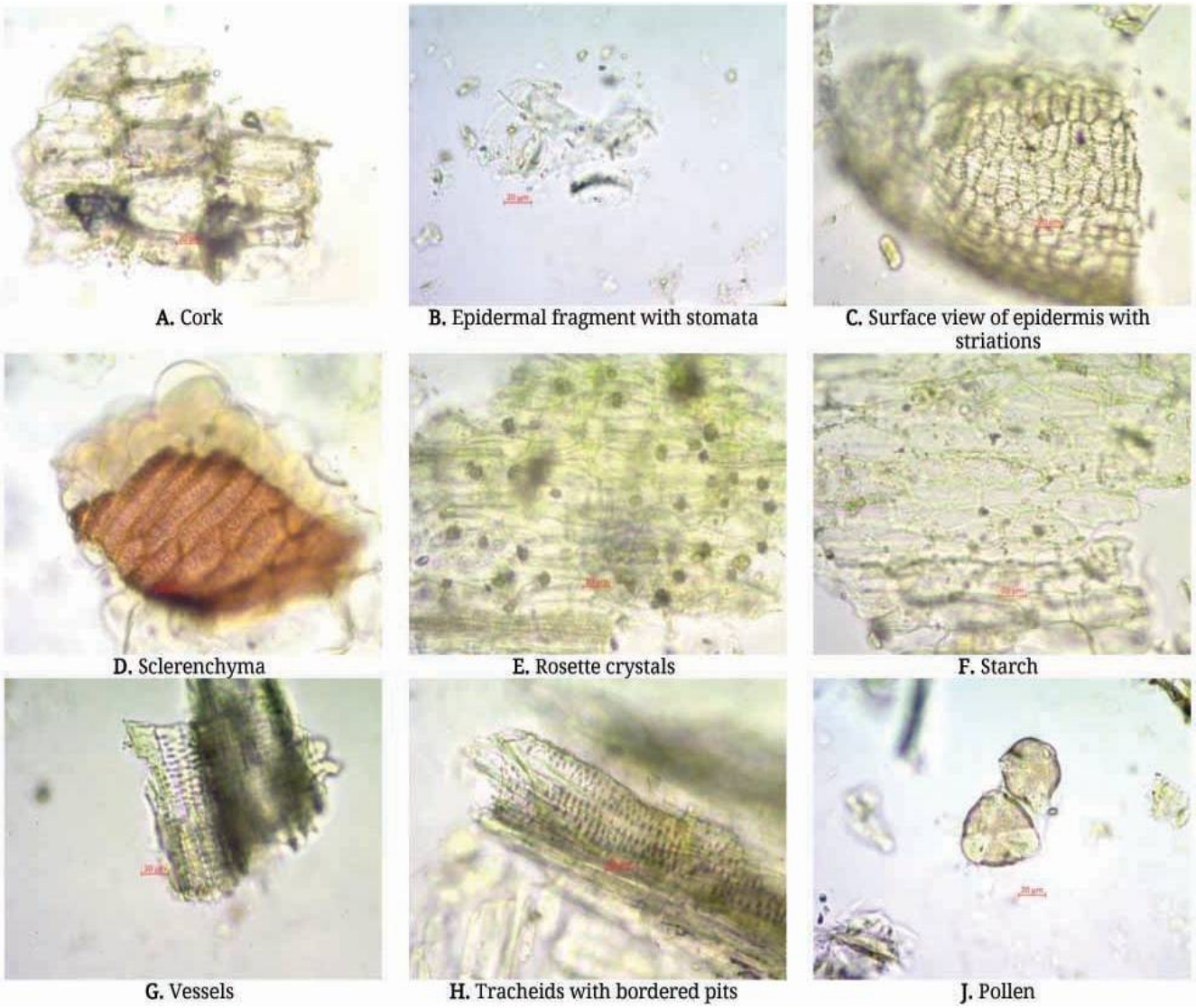
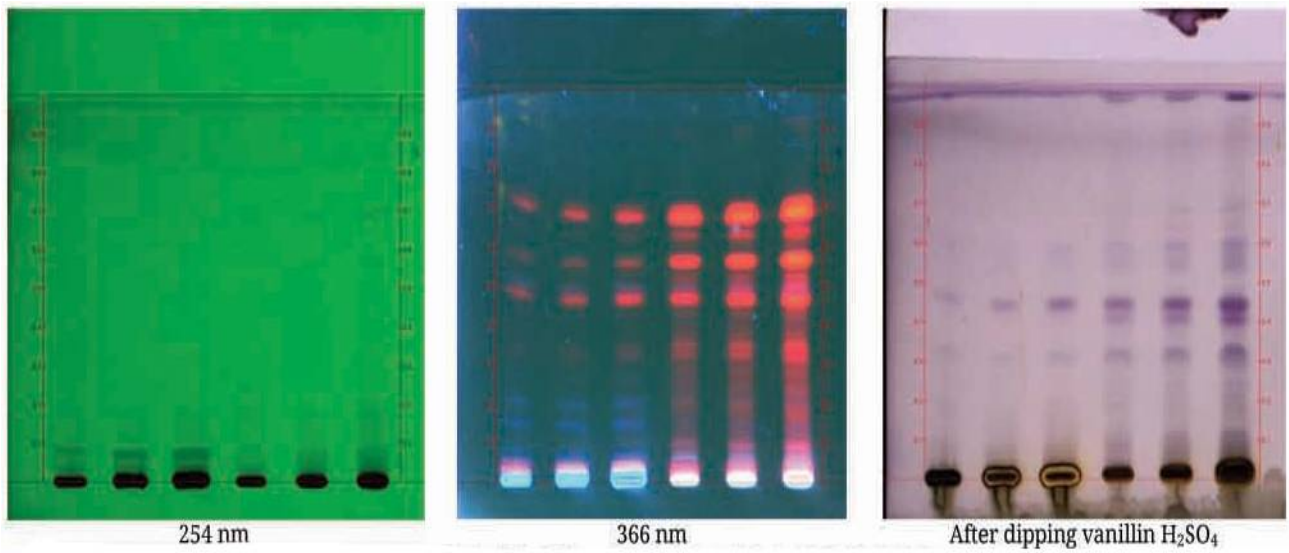
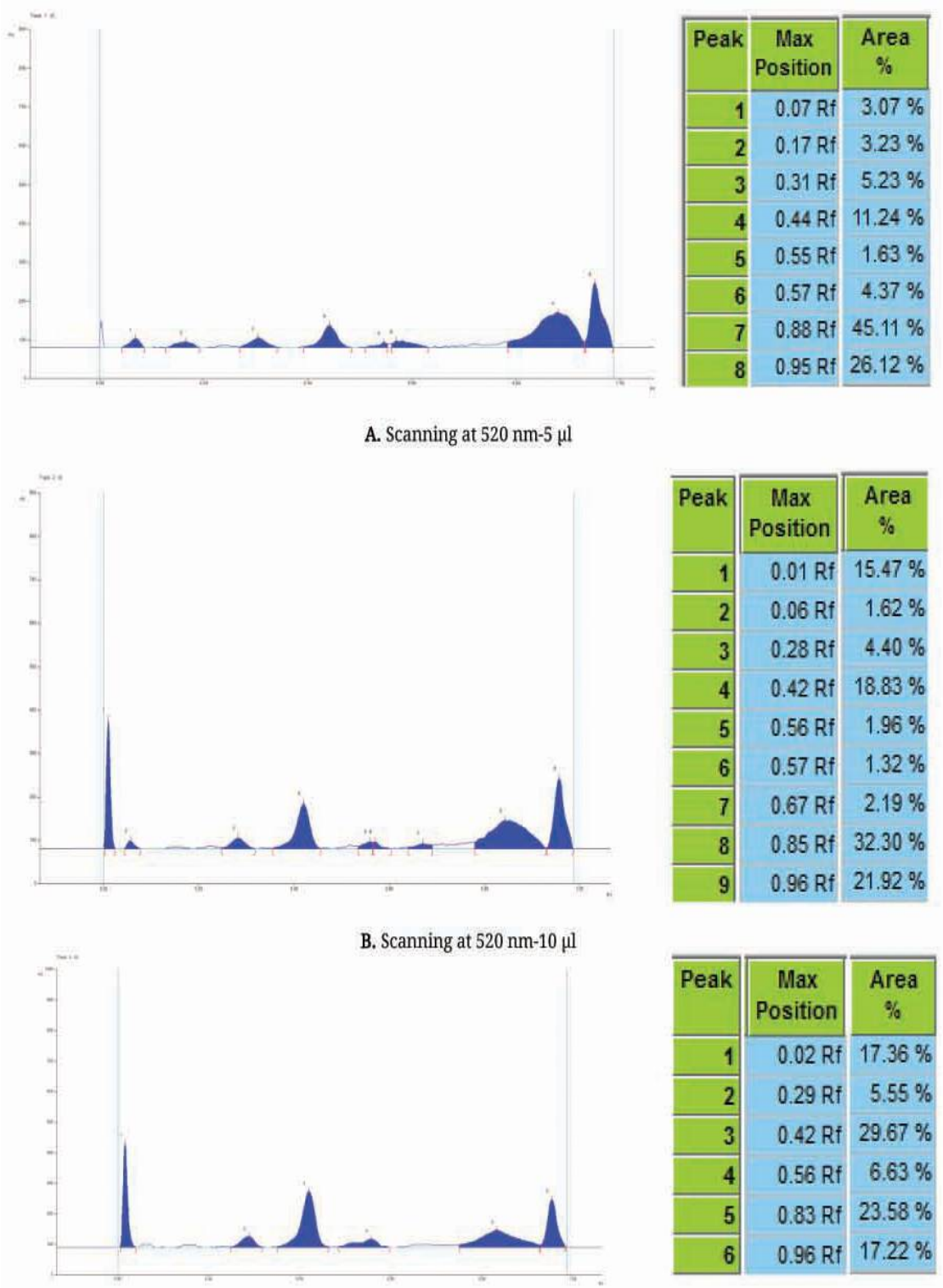


Fig. 12. Powder microscopic characters of *T. portulacastrum* whole plant.



(Track 1, 2 and 3: 5, 10 &15µl of *B. diffusa*; Track 4, 5 and 6 : 5, 10 & 15µl of *T. portulacastrum*)

Fig. 13. TLC photodocumentation of *B. diffusa* and *T. portulacastrum.*



Peak	Max Position	Area %
1	0.07 Rf	3.07 %
2	0.17 Rf	3.23 %
3	0.31 Rf	5.23 %
4	0.44 Rf	11.24 %
5	0.55 Rf	1.63 %
6	0.57 Rf	4.37 %
7	0.88 Rf	45.11 %
8	0.95 Rf	26.12 %

A. Scanning at 520 nm-5 µl

Peak	Max Position	Area %
1	0.01 Rf	15.47 %
2	0.06 Rf	1.62 %
3	0.28 Rf	4.40 %
4	0.42 Rf	18.83 %
5	0.56 Rf	1.96 %
6	0.57 Rf	1.32 %
7	0.67 Rf	2.19 %
8	0.85 Rf	32.30 %
9	0.96 Rf	21.92 %

B. Scanning at 520 nm-10 µl

Peak	Max Position	Area %
1	0.02 Rf	17.36 %
2	0.29 Rf	5.55 %
3	0.42 Rf	29.67 %
4	0.56 Rf	6.63 %
5	0.83 Rf	23.58 %
6	0.96 Rf	17.22 %

Fig. 14. HPTLC fingerprint profile of *B. diffusa* (Ethanol extract).

helped in data acquisition, MR did the powder microscopical studies of the plants and literature study, BE assisted macroscopic studies and helped in tabulating the data, KNS edited and revised the manuscript with valuable suggestions, SM and RS performed the phytochemical fingerprinting studies.

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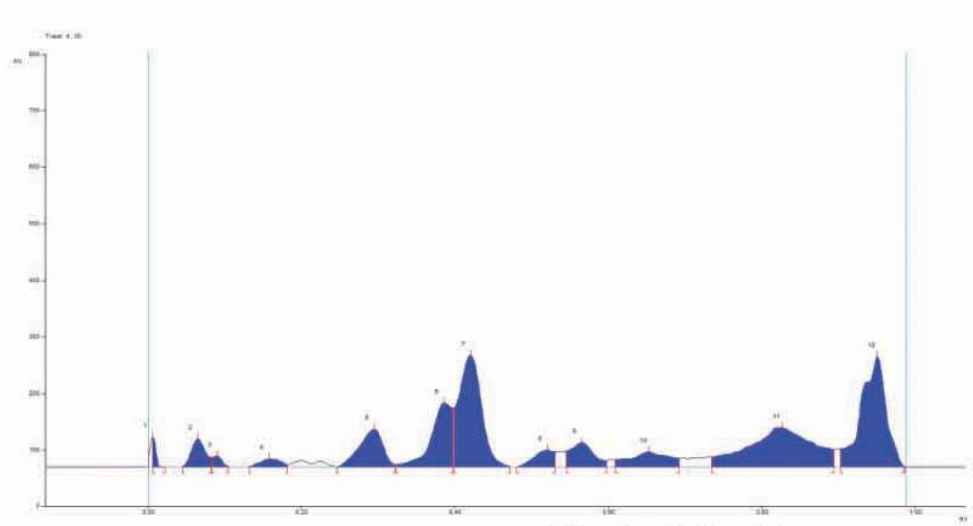
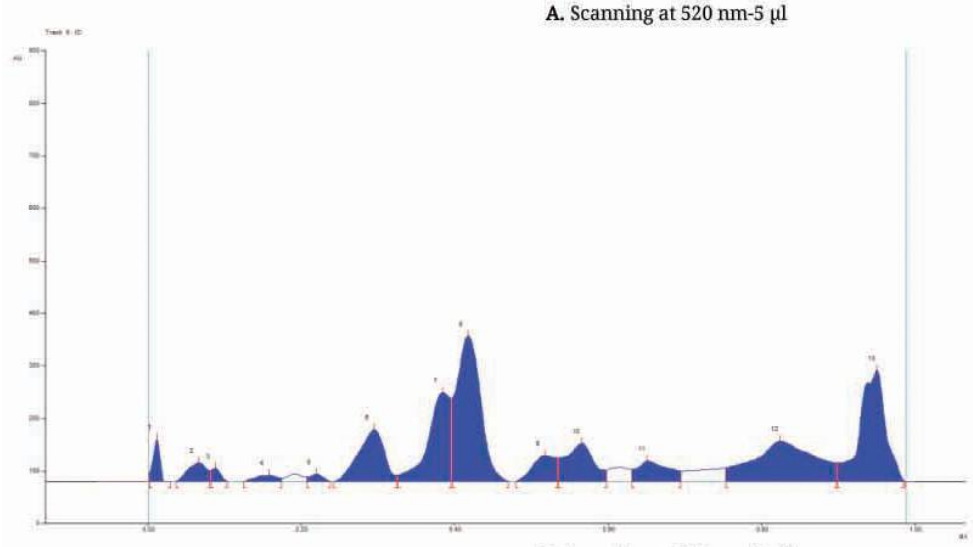
Conflict of interest

The authors do not have any conflict of interest to declare.



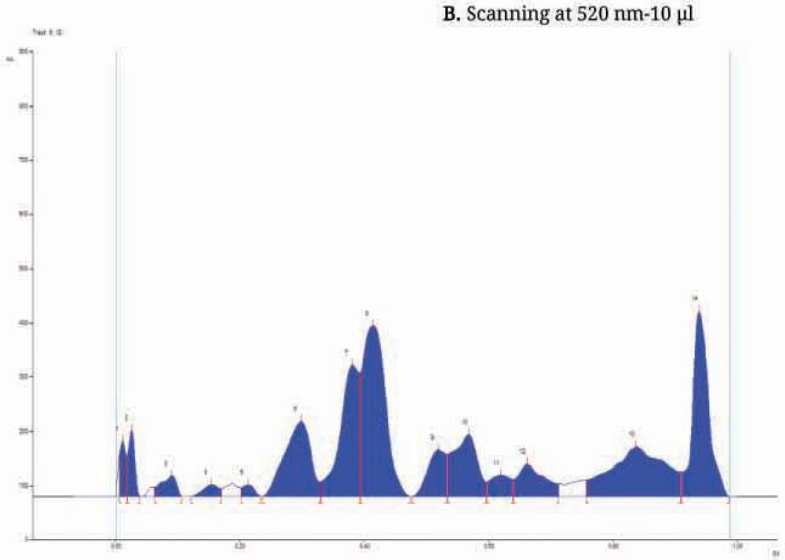
A. Scanning at 520 nm-5 µl

Peak	Max Position	Area %
1	0.01 Rf	0.82 %
2	0.07 Rf	3.15 %
3	0.09 Rf	0.83 %
4	0.16 Rf	1.33 %
5	0.30 Rf	7.29 %
6	0.39 Rf	10.87 %
7	0.42 Rf	20.47 %
8	0.52 Rf	2.73 %
9	0.57 Rf	4.60 %
10	0.65 Rf	4.77 %
11	0.83 Rf	20.64 %
12	0.95 Rf	22.51 %



B. Scanning at 520 nm-10 µl

Peak	Max Position	Area %
1	0.01 Rf	1.84 %
2	0.07 Rf	2.28 %
3	0.09 Rf	0.77 %
4	0.16 Rf	0.87 %
5	0.22 Rf	0.61 %
6	0.30 Rf	8.70 %
7	0.39 Rf	12.65 %
8	0.42 Rf	22.46 %
9	0.52 Rf	3.88 %
10	0.57 Rf	6.93 %
11	0.65 Rf	4.21 %
12	0.83 Rf	16.23 %
13	0.95 Rf	18.57 %



C. Scanning at 520 nm-15 µl

Peak	Max Position	Area %
1	0.01 Rf	1.81 %
2	0.03 Rf	2.15 %
3	0.09 Rf	1.71 %
4	0.15 Rf	0.99 %
5	0.21 Rf	0.70 %
6	0.30 Rf	10.35 %
7	0.38 Rf	13.00 %
8	0.41 Rf	20.43 %
9	0.52 Rf	4.37 %
10	0.57 Rf	7.95 %
11	0.62 Rf	2.28 %
12	0.66 Rf	4.49 %
13	0.84 Rf	14.18 %
14	0.94 Rf	15.60 %

Fig. 15. HPTLC fingerprint profile of *T. portulacastrum* (Ethanol extract).

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