



STUDIES ON FOLIAR EPIDERMAL MICROMORPHOLOGY, VEGETATIVE ANATOMY AND XYLEM ELEMENTS OF FOUR MEMBERS OF PORTULACACEAE

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ABSTRACT

This investigation deals with the comparative studies on leaf epidermal micromorphology, vegetative (stem and petiole) anatomy and xylem elements of four members of the family Portulacaceae. The species selected for the study are *Portulaca grandiflora* Hook., *Portulaca oleracea* L., *Portulaca quadrifida* L. and *Talinum fruticosum* (L.) Juss. which have medicinal importance. In each case, the epidermal cells are irregular in shape and cell wall outline is wavy. Size of epidermal cells ranges from 37.64 μm \times 71.25 μm to 48.91 μm \times 81.91 μm . Stomata are of paracytic type in all four taxa. Stomatal size varies from 23.52 μm \times 16.51 μm to 39.2 μm \times 23.36 μm . Stomatal index varies from 12.35 to 19.7. Palisade ratio ranges from 5.75 to 7.84. Stem anatomy of the investigated taxa does not show any variation in the basic pattern of tissue arrangement. Petiole anatomy of the investigated species shows variation in number and arrangement of the vascular bundles. Number of vascular bundle ranges from 4 to 14. Vascular bundles show the circular arrangement in all three species of *Portulaca*. But in *Talinum fruticosum*, it is horse - shoe shaped. Vessel elements have simple and transverse or obliquely placed perforation plate. Tracheids are with spiral side wall thickening and their width ranges from 15.68 μm to 26.41 μm . Fibres are long with thick wall and gradually tapering tips. Besides the morphological characters, this study may provide some diagnostic foliar micromorphological and anatomical features that can be used in proper identification of these taxa.

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INTRODUCTION

Research with medicinally important plants has now got a momentum throughout the World. The scientific evaluation of medicinally important plants is now being carried out by covering various aspects of their studies like efficacy of the crude drug, phyto-chemical evaluation of active principles, different pharmacognostic parameters including morpho-anatomical features. The morpho-anatomical features including foliar epidermal features, stomatal type and index, trichome types are now used as important parameters in identification and taxonomical characterization of various groups of vascular plants (Ahmed, 1979; Banerjee *et al.*, 2002; Choudhury *et al.*, 2009a; Choudhury *et al.*, 2009b; Choudhury *et al.*, 2012; Choudhury *et al.*, 2013; Cutler, 1984; De Bery, 1884; Hagerup, 1973; Lelavathi and Ramayya, 1983; Mukherjee *et al.*, 2000; Ogundipe and Olatungi, 1991; Parveen *et al.*, 2000; Rajagopal, 1979; Raja Shanmukha Rao and Ramayya, 1987; Saha and Rahaman, 2013; Tomlinson, 1979). Ontogeny and structure of stomata are now also considered as an important taxonomic character for many of the angiospermic taxa (Carpenter and Smith, 1975; Inamder, 1970; Kothari and Shah, 1975; Rajagopal, 1979). Through perusal of literature it has been found that no thorough work has been done earlier on

these four medicinally important taxa of the family Portulacaceae in respect of their epidermal micromorphology, vegetative anatomy and wood element features. Therefore, present investigation has been undertaken to study the foliar epidermal micromorphology, stem and petiole anatomy, and xylem elements of four members of the family Portulacaceae which will be used in proper identification of those taxa and also be a tool for pharmacognostic evaluation of the crude drug obtained from these plant taxa.

MATERIALS

Four medicinally important species of the family Portulacaceae namely *Portulaca grandiflora* Hook., *Portulaca oleracea* L., *Portulaca quadrifida* L. and *Talinum fruticosum* (L.) Juss. have been selected for this study. Plant specimens of those four selected taxa have been collected from Santiniketan and its adjoining areas, and voucher specimens have been kept in Visva-Bharati Herbarium, Department of Botany, Visva-Bharati, Santiniketan, India for future reference.

METHODS

Epidermal micromorphology :- Leaf samples were cleared following the Bokhari's method (1970). The cleared leaf samples then mounted on the slide with a drop of 10%

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glycerine & 1% aqueous safranin and observed under the compound light microscope.

Vegetative anatomy :- Free hand sections of different parts of stem and petiole of the selected taxa were cut, stained suitably following safranin-light green staining schedule (Johansen, 1940) and observed under compound light microscope.

Wood maceration :- The stem pieces of the respective plant species were macerated following the standard method (Johansen, 1940) and observed under compound light microscope.

RESULTS AND DISCUSSION

Foliar Micromorphology

General description and measurement of the epidermal cells, stomata, crystals, xylem elements of the investigated taxa are given below (Tables -1, 2, 3 and 4).

upper and lower surfaces are 337.14/ mm² and 282.07/ mm² respectively. Palisade ratio is 7.2 (Table-1).

Stomatal complex- Stomata are present on both the epidermal surfaces of the leaf i.e. leaves are amphistomatic. In both surfaces, stomata are strictly paracytic type. Stomatal size in the upper surface is 31.23µm × 15.68µm and in the lower surface it is 35.28µm × 19.6µm. Stomatal index is 17.45 in the upper surface and it is 19.6 in the lower surface. In the upper surface stomatal frequency is 36.18/ mm² and it is 80.76/ mm² in the lower surface (Table-2).

Trichome - Trichome is absent on both the epidermal surfaces.

Crystals- Star shaped crystals of calcium oxalate (sphaeraphide) are present on both the leaf surfaces. The diameter of the crystals in the upper epidermis is 31.36 µm and it is 32.76µm in the lower epidermis (Table-3).

Table 1. Epidermal cell characters of the investigated taxa

TAXA	LEAF SURFACE	EPIDERMAL CELL SHAPE	EPIDERMAL CELL LENGTH (µm)	EPIDERMAL CELL WIDTH (µm)	NUMBER OF EPIDERMAL CELL/ mm ²	EPIDERMAL CELL WALL OUT LINE	PALISADE RATIO
<i>Portulaca grandiflora</i>	Upper	Irregular	35.45	83.67	337.14	Wavy	7.2
<i>Portulaca grandiflora</i>	Lower	Irregular	47.81	74.15	282.07	Wavy	
<i>Portulaca oleracea</i>	Upper	Irregular	45.56	72.04	305.25	Wavy	5.75
<i>Portulaca oleracea</i>	Lower	Irregular	48.91	81.98	249.69	Wavy	
<i>Portulaca quadrifida</i>	Upper	Irregular	37.64	71.25	372.77	Wavy	7.84
<i>Portulaca quadrifida</i>	Lower	Irregular	39.12	75.76	337.41	Wavy	
<i>Talinum fruticosum</i>	Upper	Irregular	48.33	82.32	249.95	Wavy	6.92
<i>Talinum fruticosum</i>	Lower	Irregular	49.97	83.71	239.07	Wavy	

Table 2. Stomatal features of the investigated taxa

TAXA	LEAF SURFACE	STOMATAL TYPE	STOMATAL LENGTH (µm)	STOMATAL WIDTH (µm)	STOMATAL INDEX (%)	STOMATAL FREQUENCY/ mm ²
<i>Portulaca grandiflora</i>	Upper	Paracytic	31.23	15.68	17.45	36.18
<i>Portulaca grandiflora</i>	Lower	Paracytic	35.28	19.6	23.81	80.76
<i>Portulaca oleracea</i>	Upper	Paracytic	23.52	16.5	12.35	34.8
<i>Portulaca oleracea</i>	Lower	Paracytic	34.76	19.72	14.06	88.75
<i>Portulaca quadrifida</i>	Upper	Paracytic	29.4	15.85	15.13	42.67
<i>Portulaca quadrifida</i>	Lower	Paracytic	31.36	18.67	17.87	97.73
<i>Talinum fruticosum</i>	Upper	Paracytic	35.28	19.87	18.32	39.98
<i>Talinum fruticosum</i>	Lower	Paracytic	39.2	23.36	19.70	91.75

Table 3. Crystals of the investigated taxa

TAXA	LEAF SURFACE	TYPE	DIAMETER(µm)
<i>Portulaca grandiflora</i>	Upper	Sphaeraphide	31.36
	Lower	Sphaeraphide	32.76
<i>Portulaca oleracea</i>	Upper	Sphaeraphide	33.84
	Lower	Sphaeraphide	31.97
<i>Portulaca quadrifida</i>	Upper	Sphaeraphide	39.2
	Lower	Sphaeraphide	37.6
<i>Talinum fruticosum</i>	Upper	Sphaeraphide	42.12
	Lower	Sphaeraphide	34.56

1. *Portulaca grandiflora* Hook

a. Leaf micromorphology

Epidermis- Epidermal cells are irregular in shape and cell wall outlines are strictly wavy in both the surfaces. The size of the upper epidermal cell is 35.45µm × 83.67µm and it is 47.81µm × 74.15 µm on the lower surface. Cell frequencies in both

b. Stem Anatomy

The transverse section of the stem is more or less circular in outline. Following tissue organization from periphery towards the center of the stem is observed.

Epidermis- It is uniseriate with compactly arranged broad barrel shaped cells; 3.98 µm wide, and cuticle is thick here.

Cortex- Cortex is broad, massive and differentiated into two distinct zones. The first zone is collenchymatous hypodermis of 1 to 2 cell layer broad, 11.76 μm in thickness, lying just beneath the epidermal layer. Below the hypodermis, 6 cells layered thick cortical zone is present. Cells are parenchymatous with intercellular spaces; some cells filled with mucilage and some cells contain chlorophylls. It is 425.5 μm in thickness. Starch sheath absent.

Vascular bundle – They are collateral, conjoint and open type with phloem and xylem. Vascular bundles are appearing as a continuous cylinder and arranged in a ring. Vascular cylinder is 256.8 μm thick. Peripheral zone of phloem tissue containing some sclerenchymatous cells. Cambium is indistinct.

Pith- At the center of the stem massive pith is present. Cells of it are very large, isodiametric, mucilaginous, thin walled parenchymatous with profuse intercellular spaces.

c. Petiole anatomy

Outline of the petiole in transverse section is more or less circular. Epidermis is uniseriate and cells are compactly arranged, cuticle is thin. 2-3 layers of collenchyma cells present just below the epidermal layer. Altogether 8 vascular bundles are arranged in more or less circular manner. At the center large parenchyma cells with intercellular spaces forming pith. Some of the parenchyma cells containing mucilage.

d. Xylem elements

General description along with measurements of the xylem elements of stem have been presented below-

Vessel elements are long with simple, transversely or obliquely placed perforation plate. Pits are simple, arranged in horizontal lines. Tail is absent. Size of the vessel is 121.52 μm \times 27.44 μm and frequency is 22.03/ mm^2 . Tracheids are very long with spiral side wall thickening. Average diameter of tracheids is 26.41 μm and frequency is 33/ mm^2 . Fibers are libriform type with blunt ends. Simple pits are sometimes found on the side wall, but septa are completely absent. Size of the fiber is 215.6 μm \times 14.6 μm and its frequency is 63.31/ mm^2 (Table-4).

2. *Portulaca oleracea* L.

a. Leaf micromorphology

Epidermis- Epidermal cells are irregular in shape and cell wall outlines are strictly wavy in both the surfaces. The size of the upper epidermal cells is 45.56 μm \times 72.04 μm and it is 48.91 μm \times 81.98 μm on the lower surface. Cell frequencies in both upper and lower surfaces are 305.25/ mm^2 and 249.69 / mm^2 respectively. Palisade ratio is 5.75 (Table-1).

Stomatal complex- Stomata are present on both the epidermal surfaces of the leaf i.e. leaves are amphistomatic. In the both surfaces, stomata are strictly paracytic type. Stomatal size in the upper surface is 23.52 μm \times 16.5 μm and in the lower surface, it is 34.76 μm \times 19.72 μm . Stomatal index is 12.35 in the upper surface and it is 14.06 in the lower surface. In the upper surface stomatal frequency is 34.8/ mm^2 and it is 88.75/ mm^2 in the lower surface (Table-2).

Trichome - Trichome is absent on both the epidermal surfaces.

Crystals- Star shaped crystals of calcium oxalate (sphaeraphides) are present on both the leaf surfaces. The diameter of the crystals in the upper epidermis is 33.84 μm and it is 31.97 μm in the lower epidermis (Table-3).

b. Stem anatomy

The transverse section of the stem is more or less circular in outline. Following tissue organization from periphery towards the center of the stem is observed-

Epidermis- It is uniseriate with compactly arranged barrel shaped epidermal cells, 3.75 μm thick, and cuticle is thick here.

Cortex- Cortex is broad, massive and parenchymatous. Below the epidermis 3 to 5 cell layer thick cork layer is present and it is 14.82 μm in thickness. Below the cork layer, 6 to 8 cells layer thick parenchymatous cortex zone is present and it is 416.59 μm in thickness. Some cells of this zone are filled with mucilage and few cells contain chlorophylls. Starch sheath absent.

Vascular bundle – They are collateral, conjoint and open type with phloem and xylem. Vascular bundle forms a continuous cylinder which is 286.29 μm thick. Phloem is very scanty in amount followed by xylem tissue. Cambium is indistinct.

Pith- At the center massive pith is present. Cells of it are very large, isodiametric, mucilaginous and thin walled parenchymatous with profuse intercellular spaces.

c. Petiole anatomy

The outline of petiole in transverse section is more or less circular. Epidermis is uniseriate, cells are compactly arranged, cuticle thin. 2-3 layers of collenchyma cells present just below the epidermal layer. Altogether 10 vascular bundles are arranged in more or less circular manner. At the center large, isodiametric parenchyma cells with intercellular spaces forming pith. Some of the parenchyma cells containing mucilage.

d. Xylem element

General description along with measurements of the xylem elements of stem have been presented below-

Vessel elements are long with simple, transverse or obliquely placed perforation plate. Pits are simple, arranged in horizontal lines. Tail is absent. Size of the vessel element is 98 μm \times 39.2 μm and frequency is 22.76/ mm^2 . Tracheids are very long with spiral side wall thickening, 15.68 μm diameter and frequency is 21.98/ mm^2 . Fibers are libriform type, ends blunt, pits simple. Size of the fiber is 258.72 μm \times 15.60 μm and frequency is 65.54/ mm^2 (Table-4).

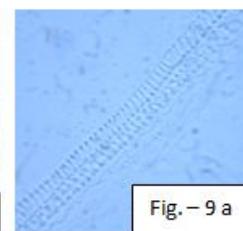
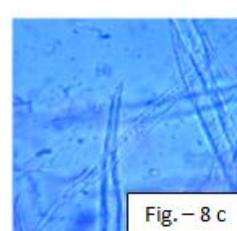
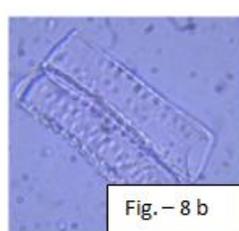
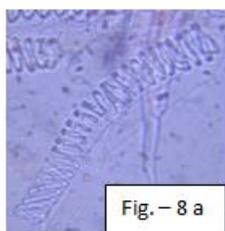
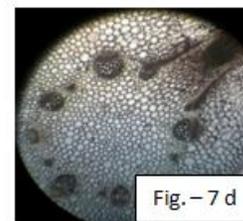
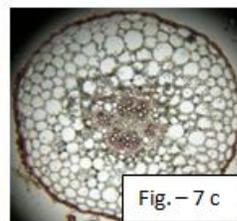
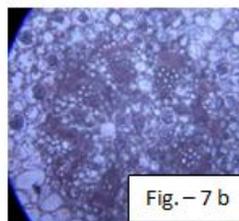
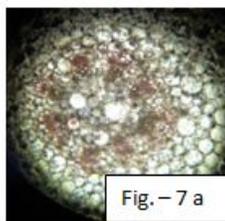
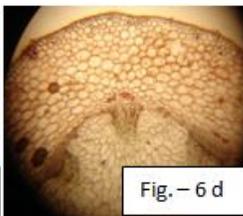
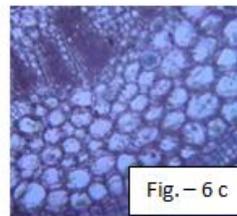
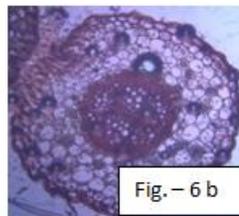
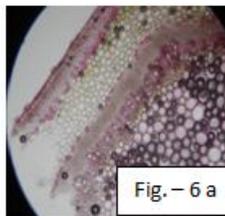
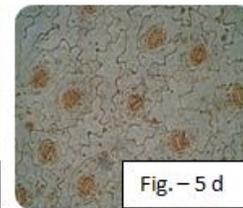
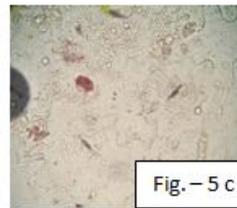
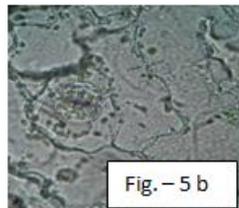
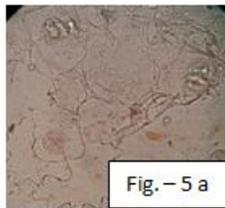
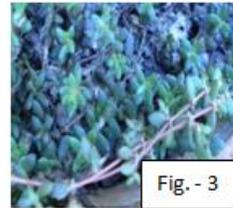
3. *Portulaca quadrifida* L.

a. Leaf micromorphology

Epidermis - Epidermal cells are irregular in shape and cell wall outlines are strictly wavy in both the surfaces. The size of the upper epidermal cell is 37.64 μm \times 71.25 μm and it is

Table 4. Wood element characters of the investigated taxa

Wood elements	<i>Portulaca grandiflora</i>	<i>Portulaca oleracea</i>	<i>Portulaca quadrifida</i>	<i>Talinum fruticosum</i>
Vessel elements:				
Type of perforation plate	Simple	Simple	Simple	Simple
Arrangement of perforation plate	Transverse or oblique	Transverse or oblique	Transverse or oblique	Transverse or oblique
Pits	Simple	Simple	Simple	Simple
Tail	Absent	Absent	Sometimes present	Sometimes present
Length (μm)	121.52	98	101.92	133.2
Breadth (μm)	27.44	39.2	33.48	31.36
Frequency ($/\text{mm}^2$)	22.03	22.76	66.78	66.08
Tracheids:				
Wall thickening	Spiral	Spiral	Spiral	Spiral
Diameter (μm)	26.41	15.68	15.88	18.98
Frequency ($/\text{mm}^2$)	33	21.76	24.72	32
Fibers:				
Ends	Blunt	Blunt	Blunt	Blunt
Pits	Present	Sometimes present	Present	Not found
Length (μm)	215.6	258.72	196	307.52
Diameter (μm)	14.6	15.60	28.89	23.12
Frequency ($/\text{mm}^2$)	63.31	65.45	62.46	61.18



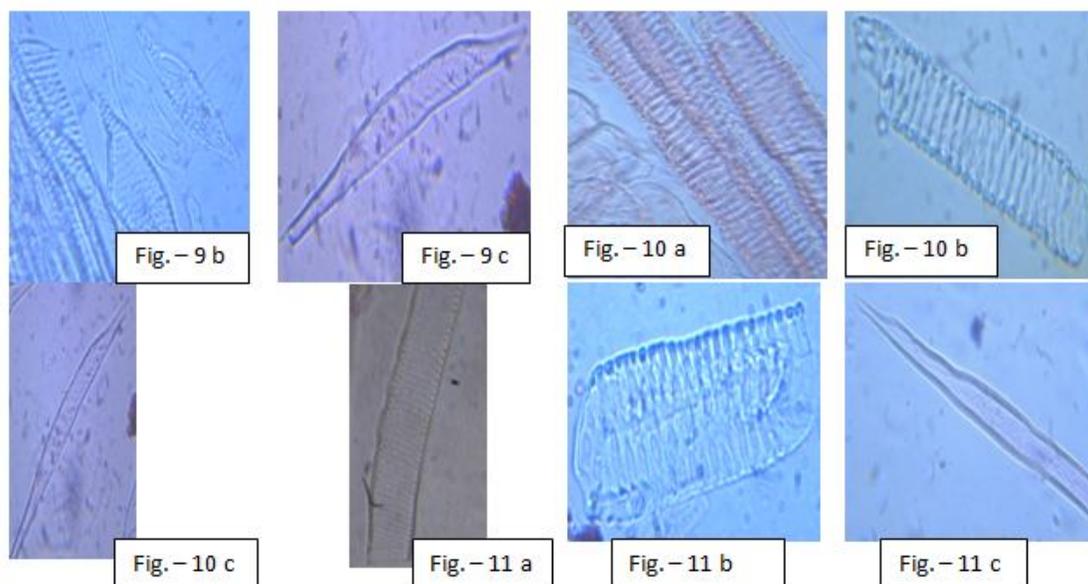


Fig. 1- *Portulaca grandiflora* Hook.; Fig. 2 - *Portulaca oleracea* L.; Fig. 3 - *Portulaca quadrifida* L. ; Fig. 4 - *Talinum fruticosum* (L.) Juss. ; Fig.5 - Paracytic stomata and epidermal cells - a. *P. grandiflora*, b. *P. oleracea*, c. *P. quadrifida*, d. *T. fruticosum* ; Fig. 6 - T.S. of stem- a. *P. grandiflora*, b. *P. oleracea* c., *P. quadrifida*, d. *T. fruticosum*; Fig. 7- T.S. of petiole- a. *P. grandiflora*, b. *P. oleracea*, c. *P. quadrifida*, d. *T. fruticosum*; Fig. 8 – Xylem elements of *P. grandiflora* : a. Tracheid, b. Vessel element, c. Fiber ; Fig. 9- Xylem elements of *P. oleracea* : a. Tracheids, b. Vessel element, c. Fiber ; Fig. 10- Xylem elements of *P. quadrifida* : a. Tracheid, b. Vessel element, c. Fiber ; Fig. 11- Xylem elements of *T. fruticosum* : a. Tracheid, b. Vessel element, c. Fiber.

39.12 $\mu\text{m} \times 75.76\mu\text{m}$ on the lower surface. Cell frequencies in both upper and lower surfaces are 372.77/ mm^2 and 337.41/ mm^2 respectively. Palisade ratio is 7.84 (Table-1).

Stomatal complex- Stomata are present on both epidermal surfaces of the leaf i.e. leaves are amphistomatic. Stomata are strictly paracytic type. Stomatal size in the upper surface is 29.4 $\mu\text{m} \times 15.85\mu\text{m}$ and in the lower surface, it is 31.36 $\mu\text{m} \times 18.67\mu\text{m}$. Stomatal index is 15.13 in the upper surface and it is 18.32 in the lower surface. In the upper surface stomatal frequency is 42.67/ mm^2 and it is 97.73 / mm^2 in the lower surface (Table-2).

Trichome - Trichome is absent on both the epidermal surfaces.

Crystals- Star shaped crystals of calcium oxalate (sphaeraphides) are present on both the leaf surfaces. The diameter of the crystals in the upper epidermis is 39.2 μm and it is 37.6 μm in the lower epidermis (Table-3).

b. Stem anatomy

The transverse section of the stem is more or less circular in outline. Following tissue organization from periphery towards the center of the stem is observed.

Epidermis- It is uniseriate with compactly arranged barrel shaped cells, 3.92 μm thick, cuticle thick.

Cortex- Cortex is broad, massive and differentiated into two distinct zones. The first zone is collenchymatous hypodermis of 1 to 2 cell layer thick lying just beneath the epidermis,

4.72 μm in thickness . Below the hypodermis, 5 to 7 cells layer thick parenchymatous middle cortex present which is 87.56 μm thick. Some cells of it contain mucilage and some cells are chlorophyllous. Starch sheath absent.

Vascular bundle – They are collateral, conjoint and open type with phloem and xylem. Vascular cylinder is composed of scanty amount of phloem and massive xylem which is 26.75 μm thick.

Pith- At the center massive parenchymatous pith is present. Cells of it are very large, isodiametric, mucilaginous, thin walled with profuse intercellular spaces. Some of the pith cell contains starch granules.

c. Petiole anatomy

Outline of the petiole in transverse section is more or less circular. Epidermis is uniseriate, cells compactly arranged, cuticle thin. 2-3 layers of collenchyma cells present just below the epidermis. Altogether 4 vascular bundles are arranged in a cross like manner. At the center, large parenchyma cells with intercellular spaces forming pith. Some of the parenchyma cells containing mucilage.

d. Xylem element

General description along with measurements of the xylem elements of stem have been presented below-

Vessel elements are long with simple, transverse or obliquely placed perforation plate. Pits are simple, arranged oppositely i.e. in horizontal rows. Tail is present. Size of the vessel element is 101.92 $\mu\text{m} \times 33.48\mu\text{m}$ and frequency is 66.78/ mm^2 .

Tracheids are very long with spiral side wall thickening, diameter $15.88\mu\text{m}$ and frequency is $24.72 / \text{mm}^2$. Fibers are libriform type with blunt ends, pits simple. Size of the fiber is $196\mu\text{m} \times 28.89\mu\text{m}$ and frequency is $62.46 / \text{mm}^2$ (Table-4).

4. *Talinum fruticosum* (L.) Juss.

a. Leaf micromorphology

Epidermis- Epidermal cells are irregular in shape and cell wall outlines are strictly wavy in both the surfaces. The size of the upper epidermal cell is $48.33\mu\text{m} \times 82.32\mu\text{m}$ and it is $41.90\mu\text{m} \times 73.71\mu\text{m}$ on the lower surface. Cell frequencies in both upper and lower surfaces are $612.87 / \text{mm}^2$ and $604.76 / \text{mm}^2$ respectively. Palisade ratio is 5.92 (Table-1).

Stomatal complex- Stomata are present on both the epidermal surfaces of the leaf i.e. leaves are amphistomatic. Stomata are strictly paracytic type. Stomatal size in the upper surface is $35.28\mu\text{m} \times 19.87\mu\text{m}$ and in the lower surface, it is $39.2\mu\text{m} \times 23.36\mu\text{m}$ Stomatal index is 18.32 in the upper surface and it is 19.70 in the lower surface. In the upper surface stomatal frequency is $39.98 / \text{mm}^2$ and it is $91.75 / \text{mm}^2$ in the lower surface (Table-2).

Trichome - Trichome is absent on both the epidermal surfaces.

Crystals- Star shaped crystals of calcium oxalate (sphaeraphides) are present on both the leaf surfaces. The diameter of the crystals in the upper epidermis is $42.12\mu\text{m}$ and it is $34.56\mu\text{m}$ in the lower epidermis (Table-3).

b. Stem anatomy

The transverse section of the stem is more or less circular in outline. Following tissue organization from periphery towards center is observed.

Epidermis- It is uniseriate with compactly arranged large barrel shaped cells, $3.92\mu\text{m}$ thick and cuticle is thick here.

Cortex- Cortex is broad, massive and differentiated into two distinct zones. The first zone is collenchymatous hypodermis of 2 cell layers, $14.82\mu\text{m}$ in thickness lying just beneath the epidermis. Below the collenchymatous zone, 10 to 12 cells layer thick, parenchymatous zone of cortex is present. Some cells of it contain mucilage and some cells are chlorophyllous. Few parenchymatous cells contain sphaeraphide. It is $510.78\mu\text{m}$ in thickness. Starch sheath absent.

Vascular bundle – They are collateral, conjoint and open type with phloem and xylem. Seven vascular bundles are interconnected with interfascicular cambium which indicates on setting of its secondary growth. Some sclerenchyma cells are present in the phloem. Vascular cylinder is $492.76\mu\text{m}$ thick.

Pith- At the center of the stem pith is present. Cells of it are very large, isodiametric, mucilaginous, thin walled parenchymatous with profuse intercellular spaces. It is $843.76\mu\text{m}$ thick.

c. Petiole anatomy

The outline of petiole in transverse section is more or less circular. Epidermis is uniseriate, cells are compactly arranged, cuticle thin. 2-3 layers of collenchyma cells present just below the epidermis. Altogether 14 vascular bundles are arranged in horseshoe shaped manner. 7 of them are smaller in size and other 7 are larger. At the center pith is present which is composed of large, isodiametric parenchyma cells. Some of the parenchyma cells containing mucilage.

d. Xylem element

General description along with measurements of xylem elements of the stem have been presented below-

Vessel elements are long with simple, transverse or obliquely placed perforation plate. Pits are simple, horizontally arranged. Tail is present at the end of the element. Size of the vessel element is $133.2\mu\text{m} \times 31.36\mu\text{m}$ and frequency is $66.08 / \text{mm}^2$. Tracheids are very long, side wall with spiral thickening, diameter $18.98\mu\text{m}$ and frequency is $32.92 / \text{mm}^2$. Fibers are libriform type with blunt ends, pits simple. Size of the fiber is $307.52\mu\text{m} \times 23.12\mu\text{m}$ and its frequency is $61.18 / \text{mm}^2$ (Table-4).

DISCUSSION

The present study reveals the foliar epidermal features, vegetative anatomy (stem and petiole) and stem xylem elements of four members of the family Portulacaceae which are of some taxonomic importance in identification of the investigated taxa in their fresh as well as dried form. Some of the characters of the investigated taxa confirmed the features identified earlier by different workers (Banerjee and Mukherjee, 2001; Metcalfe and Chalk, 1950, 1979).

Studies of stomata can have great taxonomic as well as pharmacognostic value in proper identification of different plant taxa including medicinal plants (Choudhury *et al.*, 2009; Choudhury, *et al.*, 2013; Inamder, 1970; Kothari & Shah, 1975; Pant & Mehera, 1963; Rahaman *et al.*, 2008; Saha and Rahaman, 2013). Here in all the four investigated species the stomata are of paracytic type which may help along with other stomatal features in identification of those taxa (Fig. 5a- 5d). Stomatal index which is used very commonly as marker character for taxonomical identification of plant species. In this investigation, marked difference in stomatal index among the investigated taxa has been found. It is 12.35 in *Portulaca oleracea* and 23.81 in *P. grandiflora*. In *Portulaca oleracea* stomatal index is 12.35 that confirm the observation of previous workers (Banerjee and Mukherjee, 2001). The differences in stomatal size and frequency here among the investigated taxa have also been found which can have also usefulness in proper identification of the species. Stomatal size ranges from $23.52\mu\text{m} \times 16.5\mu\text{m}$ in *Portulaca oleracea* to $35.28\mu\text{m} \times 19.87\mu\text{m}$ in *Talinum fruticosum* in the upper epidermal surface and $31.36\mu\text{m} \times 18.67\mu\text{m}$ in *P. quadrifida* to $39.2\mu\text{m} \times 33.36\mu\text{m}$ in *Talinum fruticosum* in lower epidermal surface. Stomatal frequency varies from 34.8 in *Portulaca oleracea* to 39.98 in *Talinum fruticosum* on upper epidermis and in case of lower epidermis it ranges from 80.76 in

Portulaca grandiflora to 97.73 in *Portulaca quadrifida*. In the lower surface of the leaf, the stomatal frequency is greater than the upper surface in case of all the investigated taxa of the present study which confirms the observation made in *Portulaca oleracea* by the earlier workers (Banerjee and Mukherjee, 2001). Earlier investigators like Metcalfe and Chalk (1950, 1979) reported that number of subsidiary cells for each stoma in the family Portulacaceae varies from 2 to 4. But, in the present investigation number of subsidiary cells is strictly 2 in all four investigated taxa. Palisade ratio ranges from 5.75 in *Portulaca oleracea* to 7.84 in *Portulaca quadrifida* which is distinct in these two taxa.

Trichome features have successfully been employed by many workers in the field of plant taxonomy for identification and delimitation of plant taxa (Cutler, 1984; Lelavathi and Ramayya, 1983; Raja Shanmukha Rao and Ramayya, 1987). But in present investigation there is no scope to use this parameter in species identification because no trichome has been observed in all four investigated taxa. Although Metcalfe and Chalk (1950) reported papillate type of trichome in the genus *Talinum* without mentioning any species. Here in *Talinum fruticosum* any trichome has not been recorded which differs from the earlier workers' observation.

Sphaeroidal crystals of calcium oxalate (Sphaeraphide) are present on both the leaf surfaces of all the investigated taxa which confirm the observation made by previous workers (Metcalfe and Chalk, 1950, 1979). The diameter of the crystals found distinct among the investigated species here in this study which ranges from 31.36 μm in *Portulaca grandiflora* to 42.12 μm *Talinum fruticosum*. Stem anatomy does not show any distinction among the investigated taxa here. In each species, the outline of the stem in transverse section is more or less circular and basic tissue arrangement in the stem is quite similar with each other. In some cases, very minor variations have been observed here in the stem anatomy. The amount of phloem is comparatively greater in *Portulaca grandiflora* (Figure – 6 a) and it is very scanty in the species *Portulaca quadrifida* (Figure – 6 b). There is a variation in number of collenchyma layers in the hypodermal zone of cortex of the species studied here. Number of cell layer of collenchymas in hypodermis ranges from 1 to 2 cell layer in *Portulaca grandiflora* and it is 3 to 5 in *Portulaca oleracea* (Figure – 6 c). Petiole in transverse section is almost circular in outline in each species here. Number of vascular bundle in petiole is very distinct to the species investigated. The minimum number of vascular bundle found in *Portulaca oleracea* (Figure – 7 c) is 4 and its highest number is 14 found in the petiole of *Talinum fruticosum* (Figure – 7 d).

In each species, perforation plate of vessel elements is simple and it is transverse or obliquely placed. But the size of the vessel elements shows variation among the investigated species. There is a distinct trend of evolutionary advancement in vessel elements among the investigated taxa which supports the view of other workers (Metcalfe and Chalk, 1950, 1979). It is short and very wide in *Portulaca oleracea* (98 $\mu\text{m} \times 39.2 \mu\text{m}$) which is considered most advanced type among the investigated taxa (Figure – 9 b). Fibres are long with thick wall and tapering tips which do not show any distinctiveness. Although size of fibers has been found distinct among the

investigated taxa. Size of fibers varies from 307.52 $\mu\text{m} \times 23.12 \mu\text{m}$ of *Talinum fruticosum* (Figure – 11 c) to 215 $\mu\text{m} \times 14.6 \mu\text{m}$ of *Portulaca grandiflora* (Figure – 8 c).

Key to the investigated taxa

Number of vascular bundle in petiole ranges from 4-6; tail absent in vessel elements:

Number of vascular bundle is 4 in petiole; stomatal index of lower epidermis is 14.06

.....*Portulaca oleracea*

Number of vascular bundle is 6 in petiole; stomatal index of lower epidermis is 19.6

.....*Portulaca grandiflora*

Number of vascular bundle in petiole ranges from 12-14; tail present in vessel elements :

Number of vascular bundle is 12 in petiole; Palisade ratio is 7.84

..... *Portulaca quadrifida*

Number of vascular bundle 14 in petiole; Palisade ratio is 6.92

.....*Talinum fruticosum*

Conclusion

These micromorphological features, vegetative anatomy and wood element features can be used as a tool in proper identification of these four members of Portulacaceae. This study does also have equal importance in authentication of respective crude drugs obtained from those respective investigated taxa and in detection of drug adulterants. Thus it will help in maintaining the quality of the drug obtained from these four plant species.

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