

Comparative Pharmacognostic Studies on Three Species of *Portulaca*

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ABSTRACT

To compare the structural features and physicochemical properties of three species of *Portulaca*. **Methods:** Different parts of *Portulaca* were examined for macroscopical, microscopical characters. Physicochemical, phytochemical and fluorescence analysis of the plant material was performed according to the methods of standardization recommended by World Health Organization. **Results:** The plants are succulent, prostrate herbs. Usually roots at the nodes of the stem. Leaves are opposite with paracytic stomata and characteristic Kranz tissue found in C-4 plants. Abundant calcium oxalate crystals are present in all vegetative parts of the plant. Quantitative determinations like stomatal number, stomatal index and vein islet number were performed on leaf tissue. Qualitative phytochemical screening revealed the presence of alkaloids, carbohydrates, saponins, steroids and triterpenoids. **Conclusions:** The results of the study could be useful in setting quality parameters for the identification and preparation of a monograph.

Key words: *Portulaca*, physicochemical, standardization, Kranz tissue, quantitative.

INTRODUCTION

Genus *Portulaca* (Purslane) is an extremely tough plant that thrives in adverse conditions and belongs to the flowering plant family of Portulacaceae. It comprises of about 40-100 species and commonly found in the tropics and warm temperate regions. The botanical name is derived from the Latin *Potare*, meaning to “carry,” and *Lac* or “milk”, referring to the milky sap of the plant.¹ *Portulaca* (*Portulaca* spp.) is a non-fussy plant and valued for its attractive foliage and brilliant flowers. Of the three selected *Portulaca* species, one is strictly ornamental (*Portulaca grandiflora*) and two are edible weeds (*Portulaca oleracea* and *Portulaca quadrifida*). They have been used as a folk medicine in many countries for skin rashes, inflammation, ulcers, abdominal complaints, detoxification, cough and urinary discharge.²⁻⁵ It has been suggested that from the therapeutic point of view they are quite similar and one can be used as a substitute for the other by the drug dealers. This can be solved by the standardization of the species as per the guidelines of World Health Organisation (WHO). The present study is focused to evaluate the pharmacognostical and physicochemical properties which will help in quality control of the plant species.

MATERIALS AND METHODS

Plant material: The plant material was obtained from Bhimavaram of East Godavari District and authenticated by P. Prasanna Kumari, Department of Botany, DNR College, Bhimavaram; a specimen is preserved in the college herbarium of Shri Vishnu College of Pharmacy (Voucher number: SVCP/Cognosy/2, 3 and 4).

Preparation of extract: The powdered plant material was extracted with methanol on a Soxhlet apparatus (Borosil Glass Works Ltd, Worli, Mumbai) for 48 h. The extract was filtered using a Buchner funnel and Whatman No. 1 filter paper and sterile cotton wool. The filtrate of the extract was concentrated on Rotary Flash Evaporator (Roteva Equitron, Medica Instrument Manufacturing Company, Mumbai) and dried in desiccators for 1 wk and later reconstituted in distilled water for conducting necessary tests in this study.

Pharmacognostic study: Fresh parts were taken for morphological and microscopical studies. Coarse powder (60#) was used to study microscopical characters, physicochemical parameters and for phytochemical investigation. Microscopical studies were performed as per standard procedure.⁶⁻⁸ The powder microscopy was performed according to the method of Khandelwal.⁷ For extraction of plant material the plant material was dried under shade for 45 days. The material was pulverized and powder was extracted with methanol. The extract was evaporated to dryness under reduced pressure at 45°C to give a solid residue. The residue was weighed and stored in refrigerator for further phytochemical study.⁹

Microscopical studies: Different parts of the plant were cut and fixed in a mixture of Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml (FAA). After 24 hrs the specimens were dehydrated with graded series of tertiary-butyl alcohol (TBA).¹⁰ Paraffin wax (melting point 58-60°C) was gradually added to the specimens. The specimens were cast into paraffin blocks. The paraffin embedded specimens were sectioned with the help of



Fig. 1: Macroscopic characters of *Portulaca* species

Table 1 : Summary of Phytochemical analysis

S. No	Phytoconstituent	<i>Portulaca grandiflora</i>	<i>Portulaca oleracea</i>	<i>Portulaca quadrifida</i>
1	Carbohydrates	+	+	+
2	Proteins	+	+	+
3	Lipids	-	-	-
4	Alkaloids	+	+	+
5	Glycosides	+	+	+
6	Flavonoids	+	+	+
7	Tannins	+	+	+
8	Saponins	-	-	-
9	Gums/mucilage	+	+	+
10	Steroids/triterpenoids	+	+	+

+ Present - Absent



Fig. 2 : Transverse section of *Portulaca grandiflora* root rotary microtome. Later sections were freed from the wax.¹¹ Finally the sections were mounted in glycerine and stained where ever necessary and observed under microscope. Powdered plant material was mounted in glycerine for examination. Different cell component were

studied and measured¹², their photographs were taken using photomicrography.

Quantitative Microscopy: Quantitative determinations like stomatal number, stomatal index, length of epidermal cell, vein islet number are measured as per the standard procedures.¹³

Table 2 : Summary of Fluorescence studies

	<i>Portulaca grandiflora</i>		<i>Portulaca oleracea</i>		<i>Portulaca quadrifida</i>	
	Visible	UV	Visible	UV	Visible	UV
Powder as such	Brown	Green	Brown	Green	Brown	Green
Powder+ Conc. HCl	Brown	Yellow	Brown	Green	Brown	Green
Powder+dil. HCl	Brown	Yellow	Brown	Green	Brown	Green
Powder+Conc. H ₂ SO ₄	Brown	Green	Black	Green	Black	Green
Powder+ dil. H ₂ SO ₄	Brown	Green	Brown	Green	Black	Green
Powder+ Conc. HNO ₃	Colourless	Yellow	Brown	Black	Brown	Black
Powder+ dil. HNO ₃	Yellow	Yellow	Brown	Black	Brown	Black
Powder+ Iodine	Yellow	Brown	Brown	Green	Brown	Green
Powder+ FeCl ₃	Brown	Green	Brown	Green	Green	Black
Powder+NaOH	Brown	Brown	Brown	Brown	Green	Green

UV- Ultra violet, Conc- Concentrated, dil- dilute, HCl- Hydrochloric acid, H₂SO₄- sulphuric acid, HNO₃- nitric acid, FeCl₃-ferric chloride, NaOH- sodium hydroxide

Physicochemical and phytochemical analysis: Physicochemical parameters such as ash values and extractive values were determined according to the well-established official method and procedure.¹⁴⁻¹⁷ Preliminary Phytochemical screening was carried out using the standard procedure described by Khandelwal.^{8, 16}

Fluorescence analysis: When the sample is exposed to ultraviolet radiation many crude drugs exhibit the fluorescence. Evaluation of crude drugs based on fluorescence in daylight is not much used, as it is usually unreliable due to the weakness of the fluorescence effect. Fluorescence lamps eliminate visible radiation from the lamp as they are fitted with suitable filters and transmit ultraviolet radiation of definite wavelength. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation. The changes in appearance and colour were observed and recorded. Powdered plant material was treated with various chemical reagents and exposed to visible and ultraviolet (UV) light to study their fluorescence behavior.¹⁸

RESULTS

Taxonomic classification

Kingdom- Plantae; Subkingdom – Viridiaeplantae; Division – Tracheophyta; Subdivision – Spermatophyta; Class – Magnoliopsida; Superorder – Caryophyllanae; Order – Caryophyllales; Family – Portulacaceae; Genus – *Portulaca*.

Species 1 - *Portulaca oleracea* L; Synonyms of *P. oleracea* - *Portulaca olitoria* Pall, *Portulaca parviflora* Haw, *Portulaca suffruticosa* Thw, *Portulaca viridis* Hort. ex DC.

Species 2 - *Portulaca grandiflora* Hook; Synonyms of *P. grandiflora* - *Portulaca megalantha* Steud, *Portulaca mendocinensis* Gill. Ex Rohrb.

Species 3 - *Portulaca quadrifida* L; Synonyms of *P. quadrifida* - *Portulaca formosana*, *Portulaca meridiana* Linn, *Portulaca linifolia* Forssk.

Common names¹⁹

Portulaca grandiflora

English- Eleven o'clock, Moss-rose, Rose moss, Sunplant; Sanskrit- Paciri, Paviri; Telugu- gaddi roja, Goddu Pavelli;

Hindi – luaniya; Kannada- kaama sakkare; Marathi- chini-gulab; Tamil-Pasalai Keerai, Siru pasalai; Malayalam- Neelakeera; Manipuri - Pung mapan satpi; Urdu: Gul-shama.

Portulaca oleracea

English- common purslane; Sanskrit- gholika, ghotika, lonika; Telugu – boddupavilikoora, ganga paavilikoora, pedda pavilikoora; Hindi- chhotalunia, luniya, lonia; Kannada – budagora, dudagorai, golisoppu; Malayalam- koluppa, koricchira, kara-tsjira; Marathi – bhuigholi, ghole, gholbhaji; Tamil- karikkirai, karpakantam, kolikkirai, talvari; Urdu- boondaqi, khurfa.

Portulaca quadrifida

English- Chicken Weed; Sanskrit – laghughonika, laghulonika; Telugu - goddupaavili koora, payalaaku, sannapappu; Hindi - baralunia, chota luniya, lonika; Kannada - bachhale soppu, gonisoppu; Malayalam – nelatsjira; Marathi- bhumi gol, kathe chanval; Tamil- antarattacari, pasarai keerai, payviri.

Folklore uses of *Portulaca grandiflora*: The entire plant is depurative. It is used in the treatment of hepatitis, cirrhosis of the liver with ascites, swelling and pain in the pharynx. The fresh juice of the leaves and stems is applied externally as a lotion to snake and insect bites, burns, scalds and eczema.^{20, 21} Used for the relief of sore throat, skin rashes, and detoxification.²² Leaves used for scurvy. In Chinese medicine it is used to treat various tumors. It is one of the ingredients of the Chinese herbal medicine, Tumoclear (Kang zhong pian) formulated for tumor and cancer care. In Thai medicine aerial parts are used for the treatment of sore throat, skin rash and detoxification. Leaves and flowers are worn around the neck to relieve muscle spasms and neck stiffness. Flowers and leaves are placed on child's bed to drive away evil spirits.^{23, 24}

Folklore uses of *Portulaca oleracea*: It is used traditionally for alleviating pain and swelling. Leaves and seeds are eaten or applied topically to soothe skin. It is employed in Brazil against hemorrhoids and as vermifuge. It also exhibits a wide range of pharmacological effects, including antibacterial, analgesic, anti-inflammatory, skeletal muscle- relaxant and wound healing activities. It is also consumed as a vegetable.²⁵⁻²⁷ It is also useful in headache, stomachache, painful urination, dysentery, enteritis, lack of milk flow in nursing mothers, and in postpartum bleeding, inflammation, skin sores, and ulcers. Fresh herb

is used as poultice or juice⁴ and in Unani formulation “Qur Tabasheer” useful as antihyperglycemic and antihyperlipidemic drug.²⁸

Folklore uses of *Portulaca quadrifida*: Generally used as diuretic, to treat rheumatism, gynecological diseases, fever, urinary tract disorders, worm diseases, dysentery, as a tonic, sedative, analgesic, cardio tonic and to apply externally to ulcers, eczema and dermatitis.²⁹ Plant is used in the diseases of skin, kidneys, bladder and lungs. Used for asthma, cough, urinary discharges, inflammations and ulcers. Poultice of plant is applied to erysipelas, hemorrhoids and abdominal complaints. In Guam, the plant is used as antiscorbutic. In Egypt bruised leaves are used as an anticephalic. Zulus use a plant infusion as emetic. Used traditionally as leafy vegetable and famine food in many African countries.³⁰

Macroscopy (Figure 1)

Macroscopical characteristics of *Portulaca grandiflora*

Habit: annual or perennial, erect or prostrate herb with upright branches, about 20 cm long.

Root: tap root with fibrous secondary roots, 10 cm long, usually roots at the regions of node.

Stem: cylindrical, smooth, succulent, and glabrous. Stems and branches are purplish green in colour.

Leaves: fleshy and glabrous; alternate or irregularly scattered, sub sessile, semi-cylindrical or subterete, linear-oblong, acute tip, 2-3 mm broad, entire margin; few stipular white hairs in axils. Inflorescence: cymose; 1-3 or rarely 4 flowers in sessile clusters subtended by a dense growth of hairs and 5-8 leaved involucre. Flowers are large, showy, sessile, yellow, pink or scarlet, 2-5 cm across. Sepals slightly unequal and united at the base into a short tube, ovate, 6-7 mm long, 3.5-4.5 mm broad, somewhat hooded at the apex, acute apex. Petals 5 or multiples of 5, united at the base, obovate, pale brown or yellow spotted at the base, 16-18 mm long, 12-14 mm wide, emarginate. Stamens numerous, united at the base; filaments unequal, scarlet-purple in colour. Carpels 5, syncarpous ovary; style 5-fid; stigmas 5, linear, recurved. Fruit: capsule, 5 mm long, oblong, obtuse in shape, 3 mm in diameter.

Seeds: minute, 0.5-1 mm in diameter, compressed, metallic grey or greyish-black in colour.

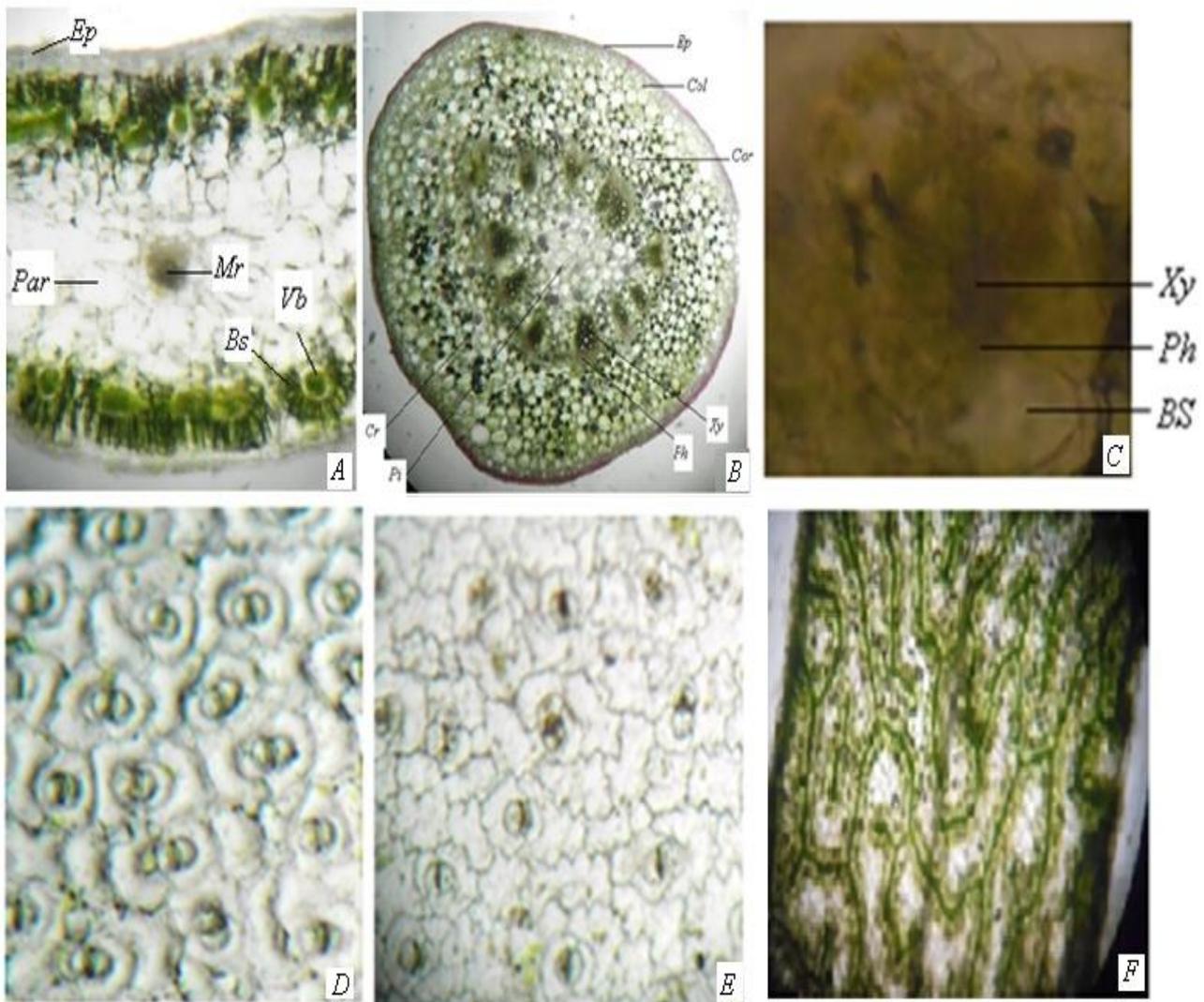


Fig. 3 : Microscopy of *Portulaca grandiflora* leaf and stem

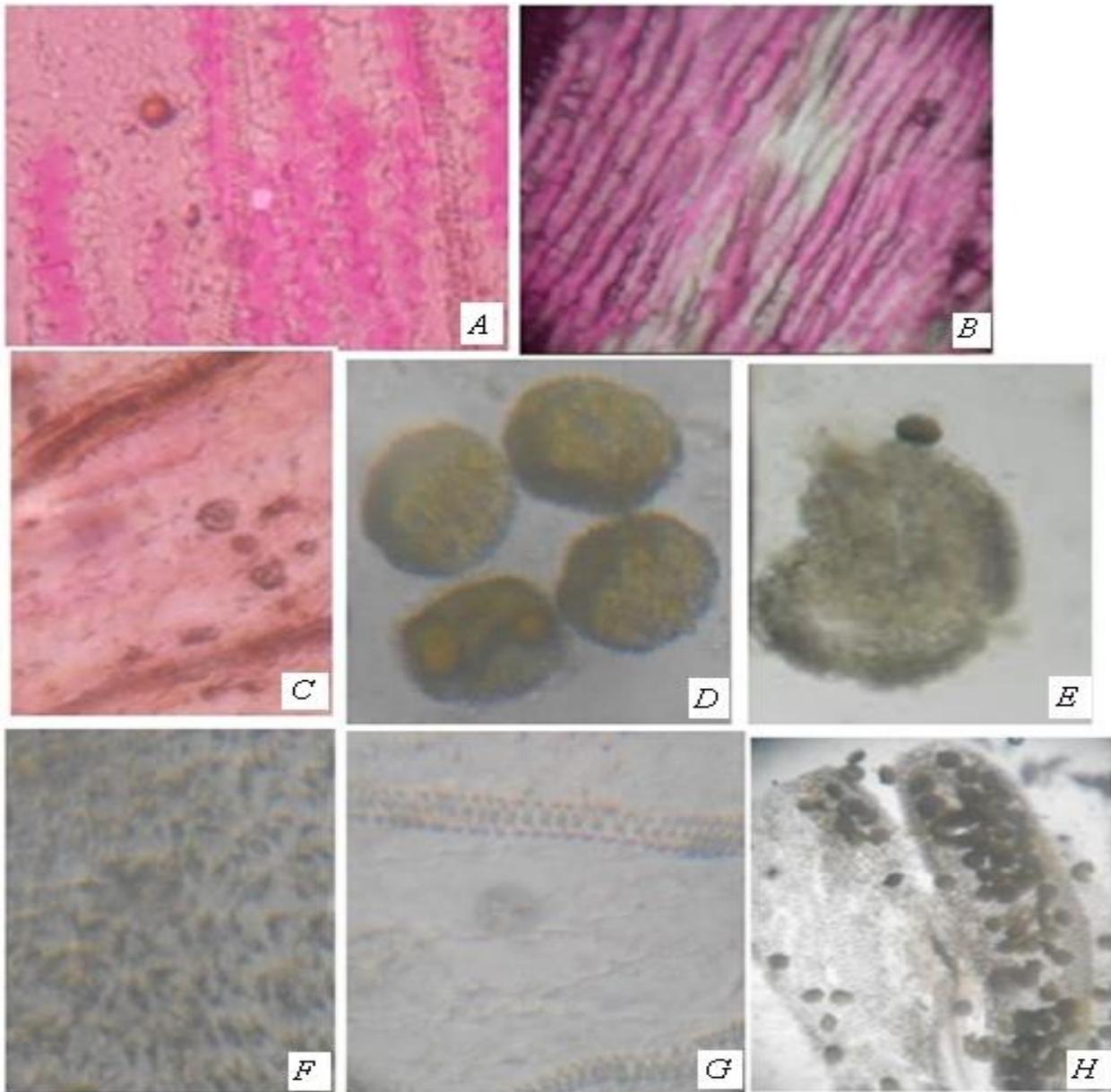


Fig. 4 : Microscopy of *Portulaca grandiflora* flower

Table 3: Summary of Physicochemical analysis

S. No	Physicochemical constants	<i>Portulaca grandiflora</i>	<i>Portulaca oleracea</i>	<i>Portulaca quadrifida</i>
1	Total ash(% W/W)	18.6	8.12	9.54
2	Acid insoluble ash	1.01	0.92	0.95
3	Water insoluble ash	10.11	11.23	4.94
4	Foreign organic matter	1.98	2.28	8.4
5	Moisture content not more than	8.5	3.52	8.65
6	Extractive Value (Methanol)*	6.3	4.6	2.4

* g/Kg

Macroscopical characteristics of *Portulaca oleracea*

Habit: succulent, copiously branched, erect or prostrate herb, about 50 cm long

Root: tap root, usually roots at node regions.

Stem: green to reddish or brownish in colour, glabrous but with hairs at the nodes when young.

Leaves: alternate to more or less opposite or in whorls on branchlets, simple, obovate to spatulate; petiole 1-3 mm long, cuneate at base, rounded at apex, entire margin.

Inflorescence: a sessile cluster at the tip of branches, up to 8-flowered, often overtopped by branches growing from leaf axils. Flowers bisexual, regular; sepals 2, connate at base, ovate-triangular, 3-5 mm long, keeled; petals 5, adnate at base to sepals, broadly obovate, 3-8 mm long,

yellow, emarginated; stamens 7-12, connate at base; half inferior ovary; one celled, style with 3-6 arms.

Fruit: capsule, 4 mm long, circum sessile just below the middle, many seeded.

Seeds: orbicular - reniform, 0.5-1 mm in diameter, black in colour, smooth to tuberculate surface.

Macroscopical characteristics of *Portulaca quadrifida*

Habit: succulent, prostrate, mat forming annual or short lived perennial herb.

Root: tap root, usually roots at the regions of node.

Stems: fleshy, often red in colour, grows up to 30 cm long, a dense whorl of whitish hairs is present at the nodes.

Leaves: opposite, simple, sessile, narrowly elliptical to ovate, 0.5-1.5 cm x 1-1mm, obtuse to sub acute apex, smooth surface with distinct veins.

Flowers: solitary, present at the tips of short lateral branches, surrounded by an involucre of four leaves and copious hairs, bisexual; sepals 2, ovate, 3-6mm long; petals 4, obovate, 3.5-10 mm x 4 mm, usually yellow; stamens 7-16, arranged in one whorl; ovary half inferior, style usually with 4 arms.

Fruit: capsule, 2-3.5 mm long, dehiscent near the base leaving only a very thin persistent rim and many seeded.

Seeds: semi orbicular in outline, 1 mm in diameter, dull grey in colour.

Distribution of the plant

Cosmopolitan in distribution. Found in the agricultural fields, waste lands and banks of streams.

Microscopical characteristics

Microscopical characteristics of *Portulaca grandiflora* root (Figure 2)

The transverse section of root showed periderm as the outermost layer. Periderm was followed by cortex. The mature root shows secondary growth. It has a central core of primary xylem and wide secondary xylem. The cortex consists of 4-6 layers of thin walled, tangentially elongated parenchyma cells. Cells of cortex consist of abundant deposits of starch grains. The vascular tissue is wide occupies remaining cortex region. The xylem bands are thick walled; phloem occurs only along the xylem bands. Calcium oxalate crystals are fairly abundant in the xylem cells of old root. Pith is almost reduced in young roots and is visible only in roots with secondary growth.

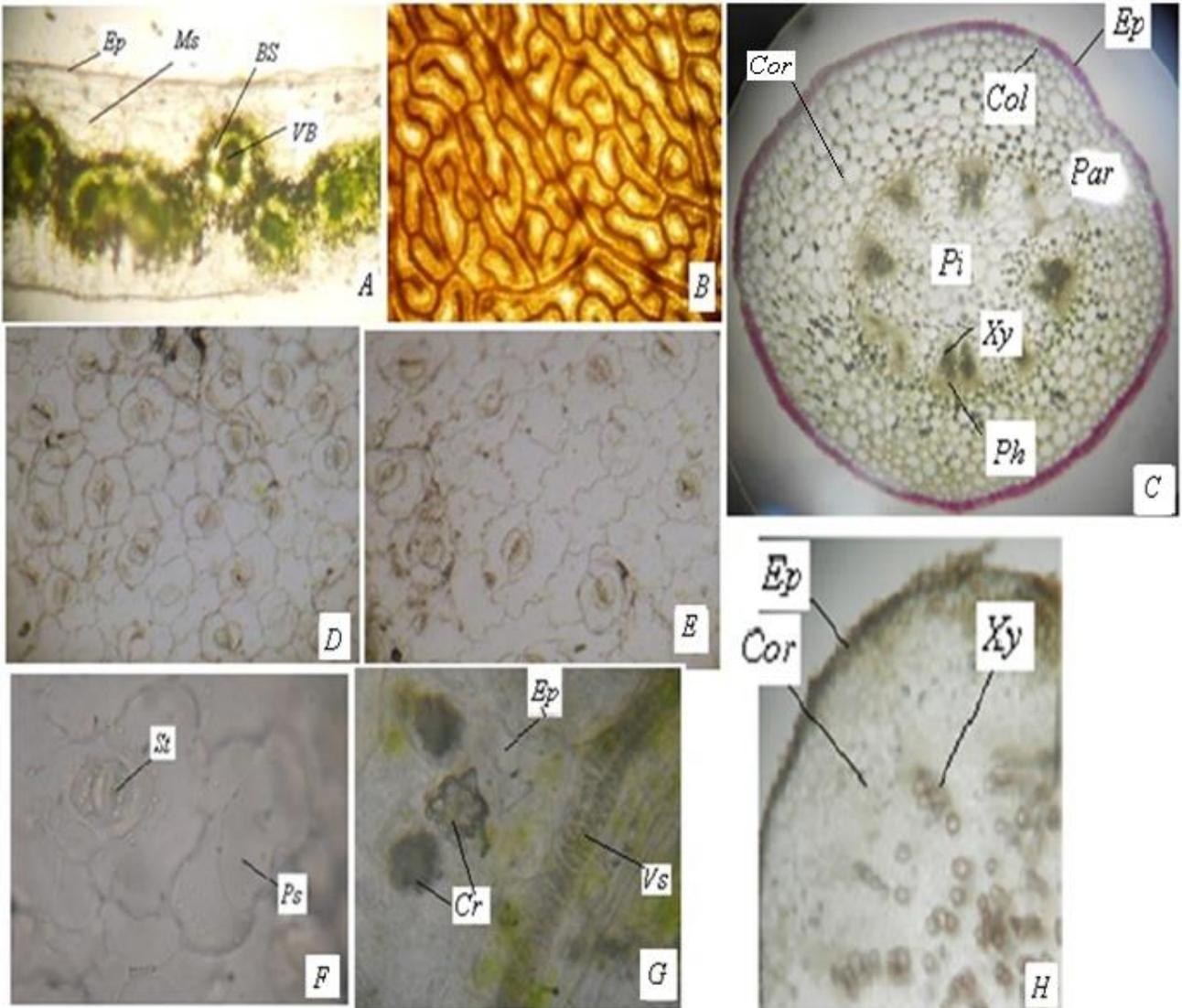


Fig. 5 : Microscopy of *Portulaca oleracea*

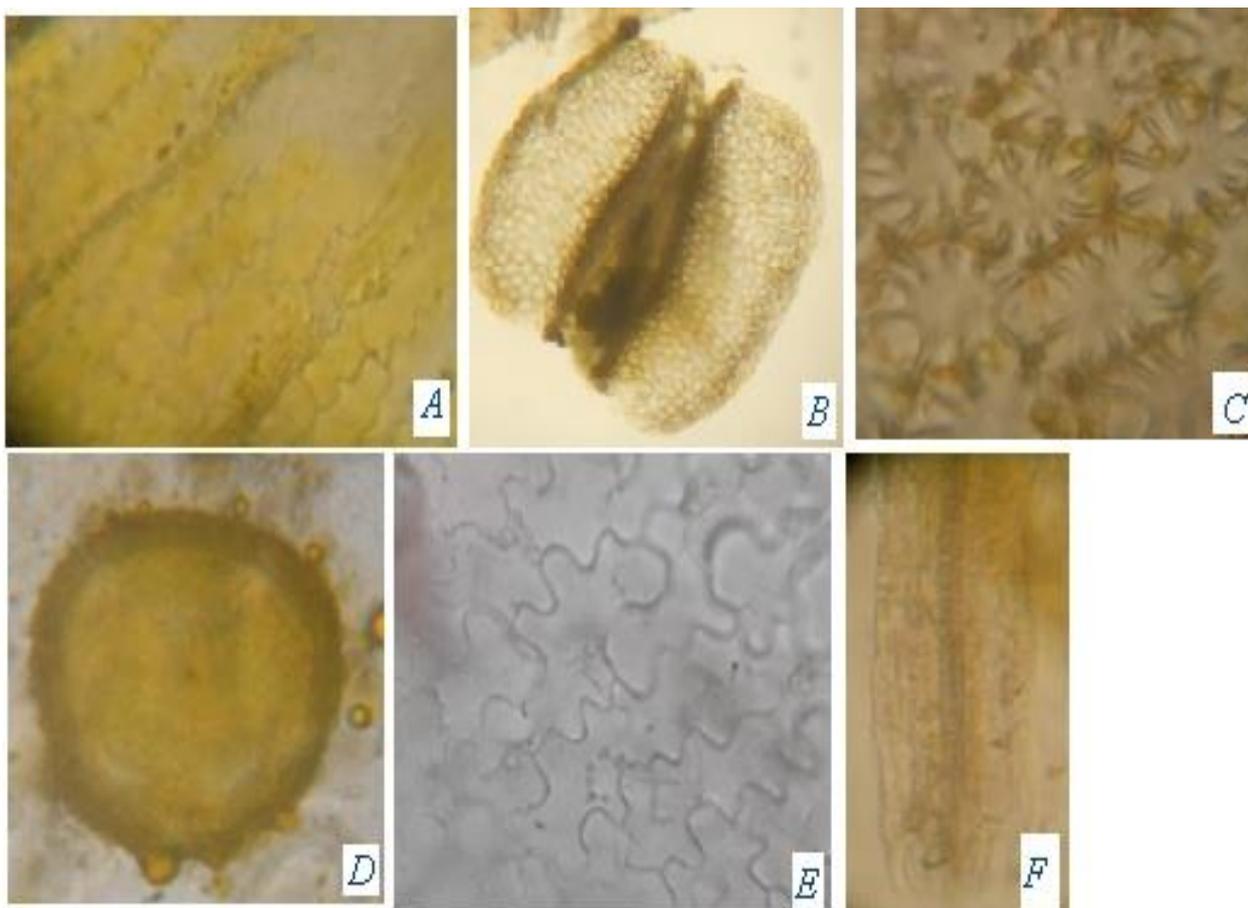


Fig. 6 : Microscopy of *Portulaca oleracea* flower

A: Root with primary growth, B: Root with secondary growth; Pe-Periderm, Ep- epidermis, Pi- pith, Co- cortex, Xy- xylem, Col-collenchyma, St- starch grains, Sec Xy- Secondary Xylem

Microscopical characteristics of *Portulaca grandiflora*
 Leaf : The transverse section of leaf (Figure 3, Plate A) is somewhat ovoid in outline and exhibits isobilateral nature with characteristic Kranz tissue (Figure 3: Plate C). The outermost layer is epidermis with rectangular to polygonal cells and contains paracytic stomata (Figure 3, Plate D). Number of stomata is more on the abaxial surface than in the adaxial surface (Figure 3: Plate E). Epidermis was followed by palisade parenchyma and spongy parenchyma. The mesophyll showed the presence of crystals of calcium oxalate. Stomata occur on both adaxial and abaxial surfaces of the leaf. The ground tissue consists of large, thin walled compact parenchyma cells. The midrib consists of collateral vascular bundle. The vascular strand has arranged in shallow arc, the phloem cells occur on the abaxial convex part of xylem strand. A ring of dilated bundle sheath cells surrounds the vascular bundles of the lamina region; these bundle sheath cells are called Kranz tissue, which are characteristic C4 type of photosynthesis of some selected plants. Crystals of calcium oxalate are in the form of druses and are fairly abundant in the mesophyll cells. The lateral veins are prominent and form distinct vein islets. The islets are wide, rectangular and mostly one vein termination in each islet (Figure 3: Plate F).

Microscopical characteristics of *Portulaca grandiflora*
 Stem: The cross section of the stem was semi circular in outline (Figure 3: Plate B). It consists of epidermis, cortex and pith. The epidermal cells were polygonal in shape and the outer wall of the epidermal cells slightly bulged out. The epidermis was followed by cortex. Outer cortex is composed of 2-4 layers of collenchyma. Inner cortex is composed of thin walled parenchyma, more or less isodiametric cells without any intercellular spaces. Collateral vascular bundles were arranged in a ring with endarch xylem and phloem towards outer surface. Pith is composed of thin walled parenchymatous cells. Calcium oxalate crystals are abundant in the cortex and pith regions.
 A: Transverse section of leaf, B: Transverse section of Stem, C: Kranz tissue in leaf, D: Upper epidermis of leaf, E: Lower epidermis of leaf, F: Vein islets; Ep- epidermis, Mr- Midrib, Par- parenchyma, Bs- bundle sheath cells, Vb- Vascular bundle, Col-collenchyma, Cr- Calcium oxalate crystal, Pi- pith, Cor - cortex, Ph- phloem, Xy- xylem
 Microscopical characteristics of *Portulaca grandiflora*
 Flower (Figure 4)

Epidermal cells are showing two types of cells. Cells of upper region are tubular with wavy margins (Figure 4: Plate A). Cells in the lower part are tubular in outline with oblique walls (Figure 4: Plate B). Much branched vascular tissue is seen (Figure 4: Plate C, G). Abundant calcium oxalate crystals are present (Figure 4: Plate C). Anthers are bilobed with many circular pollen grains.

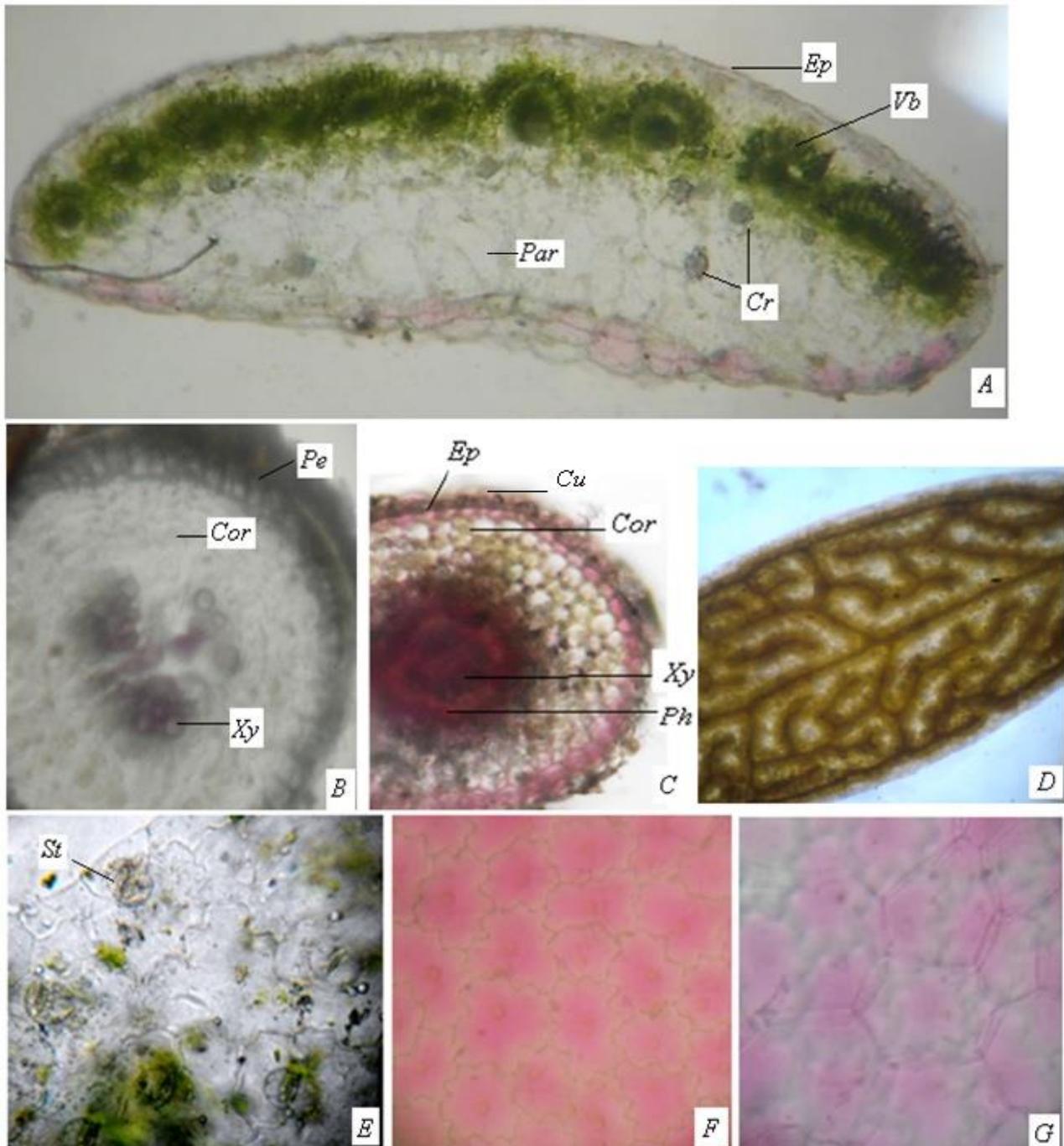


Fig.7 : Microscopy of *Portulaca quadrifida*

A: Cells of corolla in the upper part, B: Cells of corolla in the lower part, C: Calcium oxalate crystals, D: Pollen grains, E: seed, F: anther wall, G: Vascular tissue, H: Anther lobes with pollen grains

Microscopical characteristics of *Portulaca oleracea* leaf (Figure 5)

Transverse section of leaf (Figure 5, Plate A) is broadly concave on the adaxial side and convex on the abaxial side. The lateral veins are thick and prominent (Plate B). They form distinct vein islets. The islets are wide, rectangular and mostly one vein termination in each islet. The terminations are long and thick. The leaf is isobilateral. It consists of following regions epidermis, palisade parenchyma, spongy parenchyma and ground tissue.

Epidermal cells are rectangular to polygonal with slightly lobed cells; the cell walls are thin and slightly wavy. Stomata are paracytic in nature and present on both the surfaces (Plate D, E). The ground tissue consists of large, thin walled compact parenchymatous cells. The vascular strand has arranged in shallow arc, the phloem cells occur on the abaxial convex part of xylem strand. The midrib region has collateral vascular bundle. Each bundle has a vertical file of xylem elements and small nest of phloem elements. A ring of dilated bundle sheath cells surrounds the bundle; these bundle sheath cells are called Kranz-tissue, which are characteristic C4 type of photosynthesis of some selected plants. Crystals of calcium oxalate (Plate G) are in the form of druses and are fairly abundant in the

mesophyll cells. The crystals are found in the ground tissue as well as along the veins, mostly along the major veins.

Microscopical characteristics of *Portulaca oleracea* Stem: The stem is circular in outline with smooth and even surface (Figure 5, Plate C). It consists of epidermis, cortex and pith. Epidermis is thin with tangentially elongated rectangular cells and distinct cuticle; epidermal cells are pink in colour. The cortex is wide and consists of 2-3 layers of outer collenchyma and remaining portion composed of thin walled less compact parenchyma cells. Vascular bundles are collateral with endarch xylem elements and phloem elements are present on the outer part. The xylem elements are thick walled and angular. The pith is wide and cells are similar to cortical parenchyma and possess abundant calcium oxalate crystals.

Microscopical characteristics of *Portulaca oleracea* Root: The transverse section of the root is circular in outline with thick periderm (Figure 5, Plate G). It consists of periderm and cortex and a central core of primary xylem. It has diarch or triarch primary xylem at the center and narrow cortex. The periderm consists of 2-3 layers of phellem, a single layer of phelloderm and phellogen layer. The cortex consists of 4-6 layers of thin walled, tangentially stretched parenchyma cells. The xylem bands have narrow, thick walled elements; phloem occurs only along the xylem

bands. Calcium oxalate crystals are abundant in the ground tissue.

A: Transverse section of leaf, **B:** Vein islets and vein terminations, **C:** Transverse section of Stem, **D:** Upper epidermis of leaf, **E:** Lower epidermis of leaf, **F:** Stomata; **G:** Calcium oxalate crystals, **H:** Transverse section of Root; **Ep-** epidermis, **Ms-** Mesophyll, **BS-** Bundle sheath cells, **VB-** Vascular bundle, **Col-** collenchyma, **Par-** Parenchyma, **Cor-** cortex, **Xy-** Xylem, **Ph-** Phloem, **Pi-** Pith, **St-** Stomata, **Ps-** Palisade cell, **Vs-** Vascular strand, **Cr-** Calcium oxalate crystal

Microscopical characteristics of *Portulaca oleracea* Flower (Figure 6)

Epidermal cells of corolla are having wavy margins (Figure 6, Plate A), cells of calyx has more wavy margins than corolla (Figure 6, Plate E). Anthers (Plate B) are bilobed with angular projections on the surface (Plate C). Pollen grains are rounded (Plate D).

A- Cells of corolla, **B-** Transverse section of anther, **C-** Anther wall, **D-** Pollen grain, **E-** Cells of calyx, **F-** Transverse section of filament

Microscopical characteristics of *Portulaca quadrifida* Leaf: The transverse section of leaf shows dorsiventral arrangement with characteristic Kranz tissue (Figure 7,

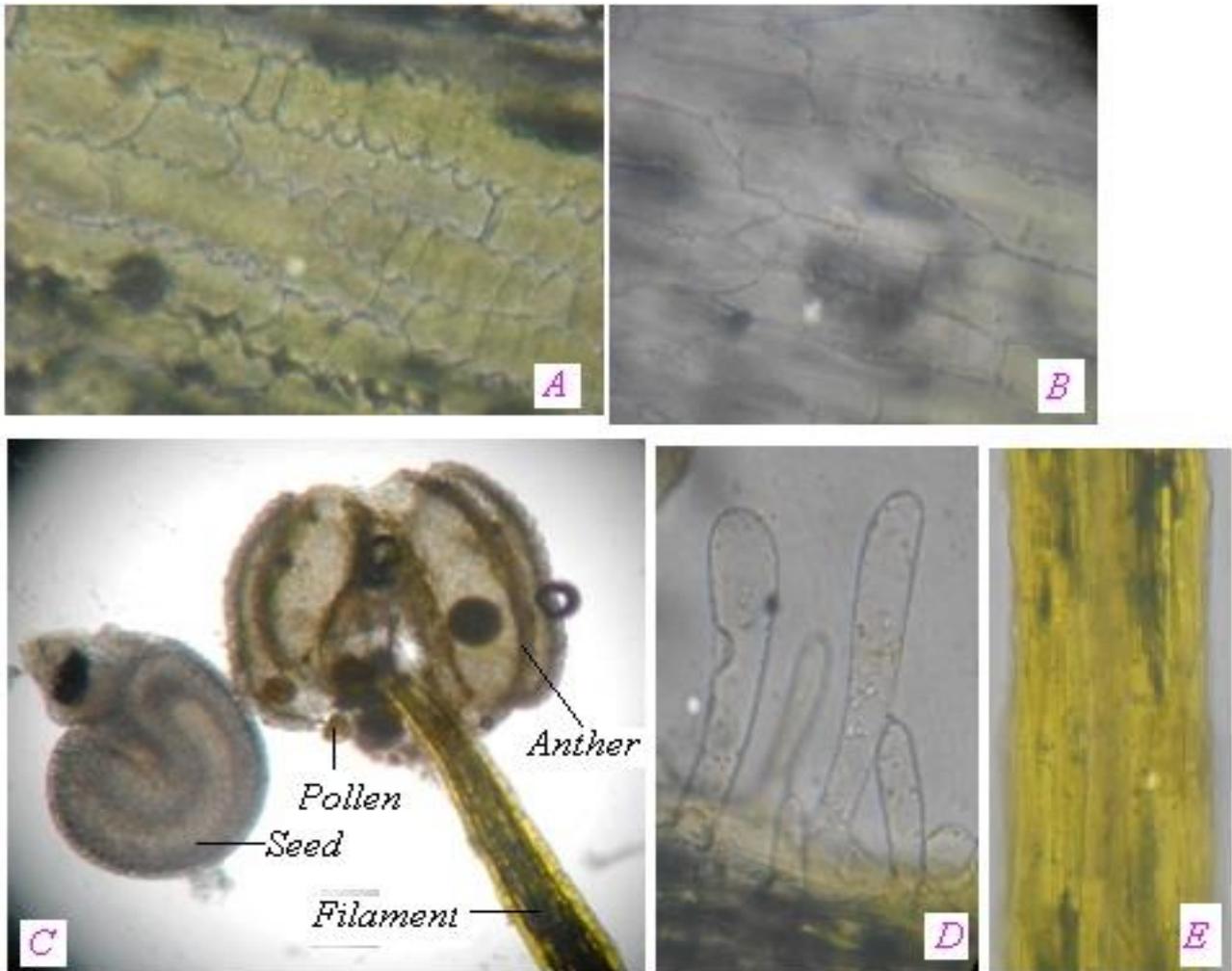


Fig. 8 : Microscopy of *Portulaca quadrifida* Flower

Table 4 : Summary of Quantitative microscopy

Parameter	<i>Portulaca grandiflora</i>	<i>Portulaca oleracea</i>	<i>Portulaca quadrifida</i>
Type of Stomata	Paracytic	Paracytic	Paracytic
Length of Stomata (UE)*	3.96 ± 0.14	3.64 ± 0.18	2.64 ± 0.15
Length of Stomata (LE)*	4.67 ± 0.23	4.42 ± 0.12	-----
Width of Stomata (UE)*	3.58 ± 0.12	2.88 ± 0.16	2.16 ± 0.24
Width of Stomata (LE)*	4.11 ± 0.29	3.68 ± 0.24	-----
Stomatal Number (UE)**	4.12 ± 0.16	3.84 ± 0.18	2.64 ± 0.12
Stomatal Number (LE)**	4.87 ± 0.21	4.62 ± 0.14	-----
Stomatal Index (UE)***	21.04 ± 0.42	17.33 ± 0.57	34.75 ± 0.78
Stomatal Index (LE)***	22.84 ± 0.37	17.22 ± 0.41	-----
Vein islet number**	10.6	13.2	10.8
Length of epidermal cell upper	21.14 ± 1.26	12.75 ± 0.34	11.83 ± 0.54
Length of epidermal cell lower	22.94 ± 0.78	14.84 ± 0.78	13.46 ± 0.43

* μm , ** per mm^2 , *** %, LE- Lower epidermis, UE- Upper epidermis

Plate A). The outermost layer is epidermis composed of rectangular to polygonal cells with paracytic stomata. The lateral veins are thick and prominent (Figure 7, Plate D). They form distinct vein islets. The islets are longer than wide, rectangular and mostly with more than one vein termination in each islet. The terminations are thick and short. Epidermis was followed by mesophyll which is differentiated into palisade and spongy parenchyma. The ground tissue is made up of thin walled parenchymatous cells with calcium oxalate crystals arranged in groups. Stomata occur only on adaxial surface of the leaf (Plate E) and absent on the abaxial surface (Plate F).

Microscopical characteristics of *Portulaca quadrifida* root: The transverse section of root is circular in outline with exfoliative periderm as the outermost layer (Figure 7, Plate B). Periderm is of 2-5 layers and followed by wide cortex consisting of 6 to 8 layers of thin walled, polygonal parenchymatous cells. Transverse of section of root showed the presence of triarch primary xylem at the center. Microscopical characteristics of *Portulaca quadrifida* Stem: The cross section of the stem was circular in outline (Figure 7, Plate C). It consists of epidermis, cortex and pith. The epidermal cells were polygonal in shape and were surrounded externally by thick cuticle. The outer wall of the epidermal cells slightly bulged out. Cortex is composed of thin walled, more or less isodiametric parenchymatous cells without any intercellular spaces. Collateral vascular bundles were arranged in a ring at the center. Pith is nearly absent.

A: Transverse section of Root, B: Transverse section of stem, C: Transverse section of Stem, D: Vein islets, E: Upper epidermis of leaf, F: Lower epidermis of leaf, G: palisade cells of leaf; Ep-epidermis, Vb-Vascular bundle, Cr-Calcium oxalate crystals, Par- Parenchyma, Pe-periderm, Cor- cortex, Xy- Xylem, Ph- Phloem, Cu-cuticle

Microscopical characteristics of *Portulaca quadrifida* flower (Figure 8)

Cells of corolla are tubular with wavy walls and transverse septa (Figure 8, Plate A). Cells of calyx are more or less rectangular with transverse to oblique septa (Figure 8, Plate B). Unicellular covering trichomes are seen at the base of corolla (Figure 8, Plate D). Anthers are bilobed with rounded pollen grains (Figure 8, Plate C).

A: Cells of corolla, B: Cells of calyx, C: Seed and Transverse section of Anther, D: Trichomes, E: Transverse section of filament

Physicochemical and phytochemical analysis: Preliminary phytochemical screening revealed the presence of carbohydrates, proteins, alkaloids, glycosides, flavonoids, tannins, mucilage, steroids and triterpenoids (Table 1). The results of fluorescence analysis are summarized in Table 2. The results of physicochemical investigations are summarized in Table 3.

Quantitative Microscopy: Length of stomata and epidermal cells, number of stomata were found to be more on the abaxial surface. Stomata was absent on the abaxial surface of *Portulaca quadrifida*. Value of the vein islet number is higher for *Portulaca oleracea* among the three species. Quantitative determinations of leaf constants are summarized in Table 4.

CONCLUSIONS

It is concluded that the above pharmacognostic and phytochemical parameters are very useful for the identification of the species. The results of the present study will also be helpful in the preparation of monograph. The reported phytochemical studies on the species support its traditional uses. Further research will help in the isolation of active compounds for therapeutic importance.

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