An Integrated Study On The Phytochemistry And Pharmacognosy Of Various Parts Of *Trianthema portulacastrum* L.

Elizabeth Robin*[1] and Mammen Daniel[2]

1. School of Science, Navrachna University, Vadodara, Gujarat, India.

2. Dr. Daniels Laboratories, Manjalpur, Vadodara, Gujarat, India.

ABSTRACT

Trianthema portulacastrum L. is a well-known medicinal plant of India. As a thorough study on the phytochemical and pharmacognostic characters of various parts of this plant is wanting , a complete and exhaustive study of phytochemical and pharmacognostic characters of all the three different organs like roots, stem and leaves were conducted. Ecdysone was located in all the three parts. Only stem contained the peculiar 5, 2'dihydroxy 7 methoxy 6, 8 dimethyl flavone while the roots and leaves possessed sesuvin and quercetin and similar phenolic acids. Mucilage was abundant in leaves and stem, and to a less extent in roots. Pharmacognostically all the three parts exhibited their distinct characters. Diarch protoxylem, included primary phloem, anomalous spiral thickening, short vessel segments are features of root, whereas the stem possessed reticulate secondary xylem having intraxylary phloem, long broad vessel segments and uniseriate and unicellular trichomes. The leaves are distinct with bundle sheath cells, spongy resembling palisade and unicellular trichomes.

Key Words: Sesuvin, Ecdysterone, Spiral anomaly, Intraxylary phloem.

INTRODUCTION

Trianthema portulacastrum L. (Aizoaceae) is a well-known medicinal plant of India, commonly known as "Punarnava" in South India. But it is *Boerhavia diffusa* L. (Nyctaginaceae), which is popularly known as Punarnava in the rest of India. Both these plants are treated as therapeutically equivalent[1,2] . The roots of *Trianthema* are highly medicinal as a rejuvenating drug. The plant is found to have anti-inflammatory, hypotensive, cardiovascular, antipyretic and analgesic activities. Ecdysterone[3], sesuvin, quercetin[4], trianthenol, 3-acetylaleuritolic acid, 5, 2'-dihydroxy,7- methoxy, 6,8- dimethyl flavones[5] and leptorumol are the compounds reported[6]. In addition a number of studies in which group tests for various secondary metabolites have been conducted resulted in certain wrong conclusions. Even the anatomical and pharmacognostic studies conducted earlier[7,8] are far from satisfactory. On a routine study conducted on roots of this plant we found a unique type of spiral secondary growth in roots[9]. Therefore in the present study individual parts such as roots, stem and leaves of *Trianthema portulacastrum* are thoroughly investigated for finding out all the secondary metabolites and pharmacognostic characters.

MATERIALS AND METHODS.

Fresh plant materials were collected from Botanical garden of M.S. University of Baroda and compared with the Herbarium (BARO) in Department of Botany. Voucher specimen (D/292) was deposited in Baroda University Herbarium. Plant material was collected, different parts separated and dried at 60^{o}C. They were then extracted with methanol and analysed for ecdysterone, flavonoids, tannins, phenolic acids and mucilage using standard procedures [10,11]. The plant residue after methanol extract was extracted with boiling water and the mucilage was precipitated adding methanol to the concentrated aqueous extract. The mucilage precipitated was filtered off and quantified gravimetrically.

Fresh plant materials were used for initial studies which involved hand sections. Later they were fixed in FAA[12]. Trimmed parts were embedded in paraffin and transverse sections

were taken in a rotary microtome. Sections were selected and stained with safranin and after dehydration mounted in DPX. The sections are then observed under microscope and photographs were taken. The size (dimensions) of various cells and crystals were measured using stage and ocular micrometers. The quantitative data are based on the average of 20 readings.

RESULTS:

Phytochemistry

Roots, leaves and stem.

The roots are found to possess ecdysterone, flavonols like quercetin and traces of sesuvin, phenolic acids like vanillic, syringic. p-methoxy benzoic and ferulic acids. Mucilage amounted to 8% (dry wt.) of roots.

The leaves contained sesuvin (6,7, dimethoxy - 3,5,4'-trihydroxy flavone), quercetin, vanillic, syringic, *p*-hydroxy benzoic acid and ferulic acid and ecdysterone. The mucilage content was much higher *i.e*. 40%.

The stem also contained the very same compounds as of leaves, but in addition possessed a C-methyl flavone, 5,2'dihydroxy7 methoxy 6,8 dimethyl flavone. The mucilage content was also the same, 40%.

PHARMACOGNOSY

Roots

The roots are found to have a unique anomalous spiral thickening which is recently reported by us[9]. The roots, when dry, appear twisted to right with many narrow ridges (Fig.1A). The root is diarch with 3-7 celled primary xylem consisting of two protoxylem points and 3-5 metaxylem trachieds. Alternate to them, at right angles, are the two primary phloem groups (Fig.1B). The secondary thickening is of three types. They are the following

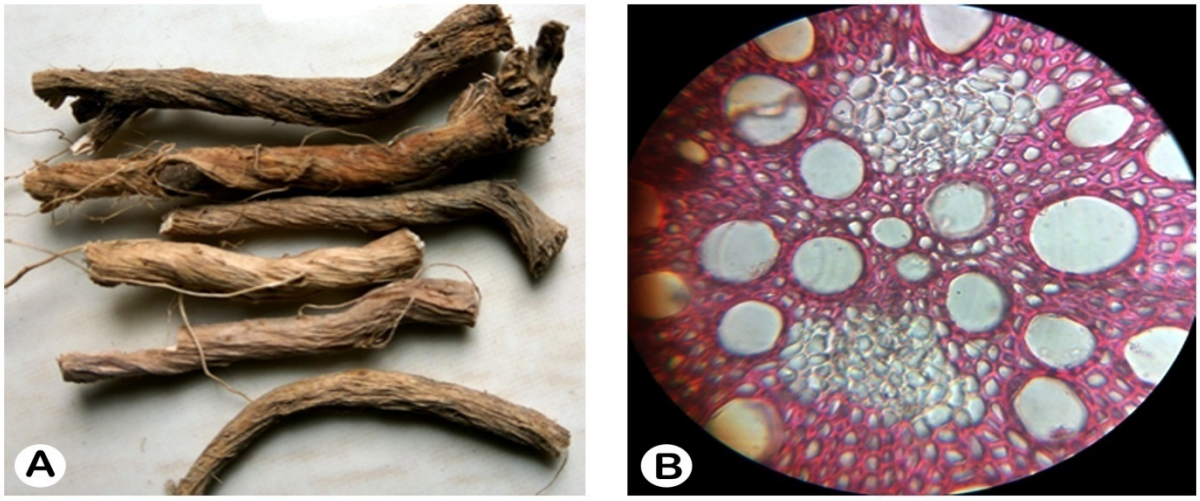

Fig.1: A. Dry roots of *T. portulacastrum*, ridges twisted to right, B. Primary vascular tissues of root with two protoxylem groups and two primary phloem groups.

1. In a majority of cases, the secondary cambium originated from the conjunctive tissue outside of only one of the two primary phloem groups (Figs. 2A), and it proceeded in a spiral manner encircling the primary vascular tissues, producing xylem towards centre and secondary phloem towards periphery while other primary phloem group remained embedded in secondary xylem (Figs. 2B.). Secondary xylem spiral consisted of long curved patches separated by medullary rays or traversed by root traces. In a few cases there is an abruptness in spiral in that one xylem patch is seen continuous with another patch placed slightly above. This spiral ends abruptly in cortical region.

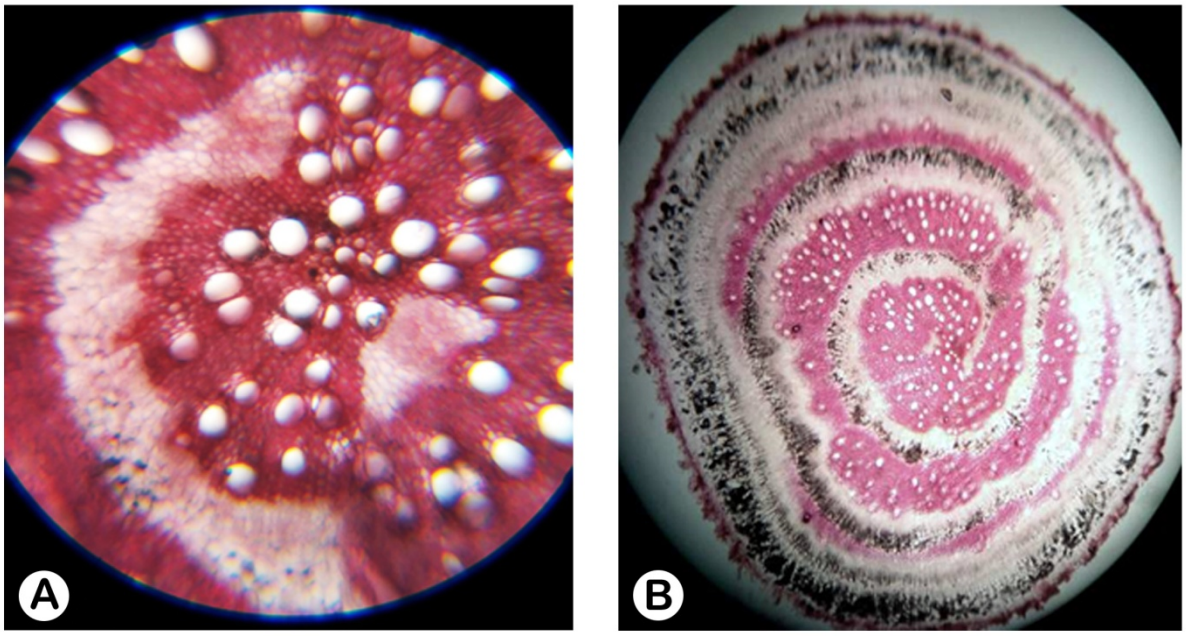

Fig.2: A. Initiation of secondary growth from one of the primary phloem groups; B. Secondary cambium proceeding as a spiral.

2. In some other (a few) cases, as reported earlierby Vidya[7], the secondary cambium developed the conjunctive tissues outside primary vascular tissues especially outside the xylem, which produced only xylem towards centre and phloem towards outside. This results in both primary phloem groups getting included within the secondary xylem (Fig.3A). This ring of cambium produced a complete ring of xylem towards inside and a ring of phloem outside. It stopped functioning after some time. This is followed by development of another secondary cambium from conjunctive tissue outside the secondary phloem. This secondary ring also produced a ring of sec xylem towards inside and sec. phloem outside and ceased it's activity after producing a second ring of xylem and phloem. This process was continued and successive rings of sec. xylem and sec. phloem were produced.

3. In a few cases the initial secondary cambia originate from conjunctive tissue connecting both the primary phloem groups on one side and they join one another producing an incomplete ring. Further secondary growth is by a new ring of cambium developing from outside this phloem. But

here also the spiral nature of the secondary tissues is visible (Fig.3B). In many cases all the three types of anomalies are observed at different lengths of the same root.

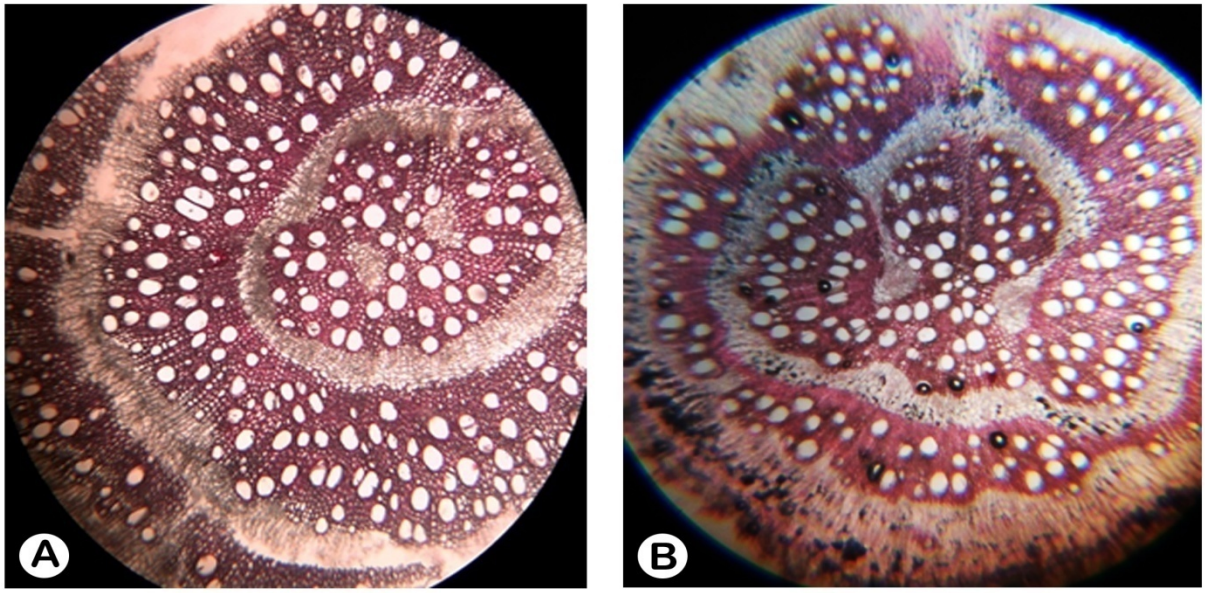

Fig. 3: A. Both primary phloem groups included and concentric rings of secondary xylem and secondary phloem, B. Secondary cambium develops from outside both primary phloem groups

Secondary xylem consisted of vessels and tracheids. Vessels appeared mostly oval in T.S, elongated radially with about 60 mμ in height and 40 mμ in breadth. Vessel segments (members) were short about 130 to 150 mμ in length, broad (50-60 mμ) with 20-25 pits in a transverse line. Tracheids were long with thin walls and ovate or broadly pointed ends, 130 to 200 mμ in length, 3-4 mμ in breadth and single or 2 rows of transversely elongated pits (Fig.4A &B). Medullary rays were rare. When present they were multiseriate, spindle shaped, 12- 15 cells in breadth and 60 to 70 cells in height containing druses in cells of the margin (Fig 4C). Each druse (crystal aggregate) consisted of square or rectangular crystals (Fig.4D). But large rectangular crystals filling entire cell also were noted (see powder study of stem). Two to three layers of secondary cortex adjoining the phloem commonly contained druses (clusters of 28-30 square or rectangular crystals each having 6-7 mμ in length) or large rectangular (70 x 20mμ) or square (20 x 20mμ) crystals. Outer cortex consisted of 3-4 layers of rectangular suberised cells.

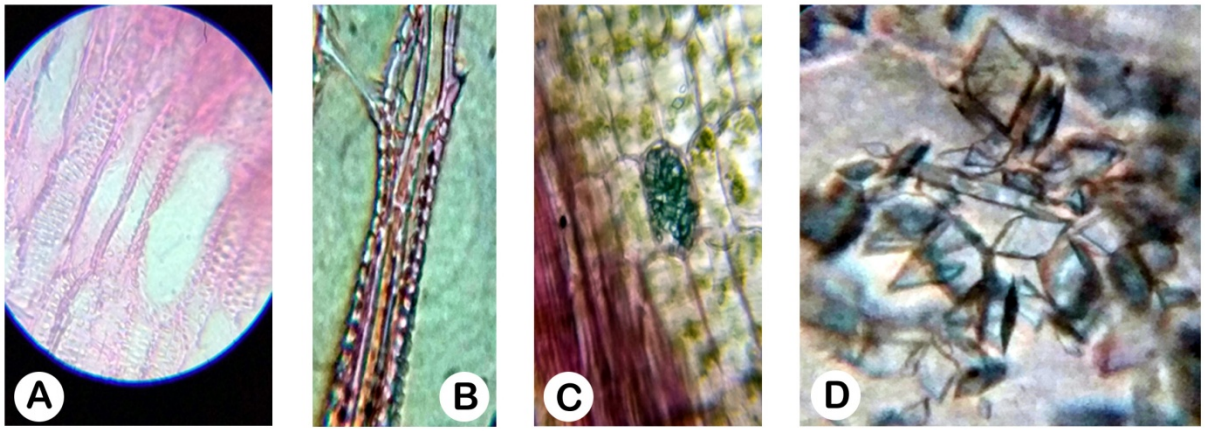

Fig. 4; A & B: Vessel members and adjoining tracheids; C. Druces in Cortex; D. Square or rectangular components of Druses.

Leaves

Leaf was dorsiventral with a single prominent midrib and 3-4 pairs of lateral veins. The midrib was slightly sunken and was obconical or semicircular on the lower side (Fig 5A). There were three vascular bundles arranged radially in a group resembling a stele with all protoxylem pointing towards centre. Each bundle consisted of 5 to 9 mostly uniseriate rows of tracheids separated by single rows of medullary ray cells. Large tracheids had a diameter of about 20 mμ. Phloem formed a small patch outside. Outer to phloem was 2-3 layers of sclerenchymatous pericycle with each sclenchyma cell having a height of 25 mμ and breadth of 20 mμ. In the centre of 3 vascular bundle was a large cluster crystal (30-35 mμ) consisting of rectangular crystals radiating from centre. Ground tissue consisted of large isodiametric parenchymatous cells, the size varied with relative position. The isodiametric small parenchyma next to epidermis had a diameter of 50 to 60 mμ while the larger had a diameter of 70 to 80 mμ with small intercellular spaces. The upper epidermis of midrib was continuous with that of leaf lamina with barrel shaped cells with 65 to 70 mμ in length and 50 to 55 mμ in height. There was a parenchymatous hypodermis consisting of smaller oval or round cells having an approximate size of 50 to 75 mμ. There were a few cluster crystals scattered in ground tissue. The lower epidermis consisted of very small barrel shaped cells 25 to 35 mμ breadth and 25 to 30 mμ in height (Fig 5B). Somewhere when the midrib joined lamina, these small epidermal cells joined with the large lower epidermal cells of lamina. The cells next to lower epidermis was at least 4

times bigger. This epidermal layer contained large (80 to 90 mμ diameter) single-celled glands protruding out.

Lamina was dorsiventral in nature with the entire mesophyll grouped around the numerous vascular bundles to form spherical clusters which ran parallel to each other (Fig 5C). Each cluster consisted of a vascular bundle surrounded by a bundle sheath which, in turn, was surrounded by one layer of palisade on the upper half and one layer of spongy tissue in the lower half. Sometimes the whole vascular bundle got surrounded by palisade issue. In such cases the palisade tissues of upper side were slightly longer and those on the lower side appear shorter and broader looking like an intermediate stage of palisade and spongy tissue. Each vascular bundle consisted of a few pitted tracheids occupying the upper side and phloem of a few sieve tubes and companion cells on lower side. The bundle sheath consisted of a single layer of large broadly triangular (conical at the inner side and obconical at outer side) with a breadth (at the broadest part) of 60 to 100 mμ and a height of 60 to 70 mμ and contained light green round chloroplasts of average diameter of 6-7 mμ (Fig 5D).. The palisade consisted of tubular cells having a height of 60 to 65 mμ and a breadth of 15 to 20 mμ. These cells contained dark green chloroplasts (Fig 5E). The spongy cells were bigger, mostly square with one side of 65 to 70 mμ in length. They contained light green chloroplasts scattered within. The bundle clusters were often separated by sphaeraphides. The upper epidermis was similar to that explained in midrib but contained a few paracytic stomata. But the lower epidermis consisted of big rectangular cells having a breadth of about 130-140 mμ and a height of 50- 60 mμ. This was interrupted by many paracytic stomata. There is a layer of hypodermis above the lower epidermis (and below the spongy) having the same cellular dimensions as of lower epidermal cells.

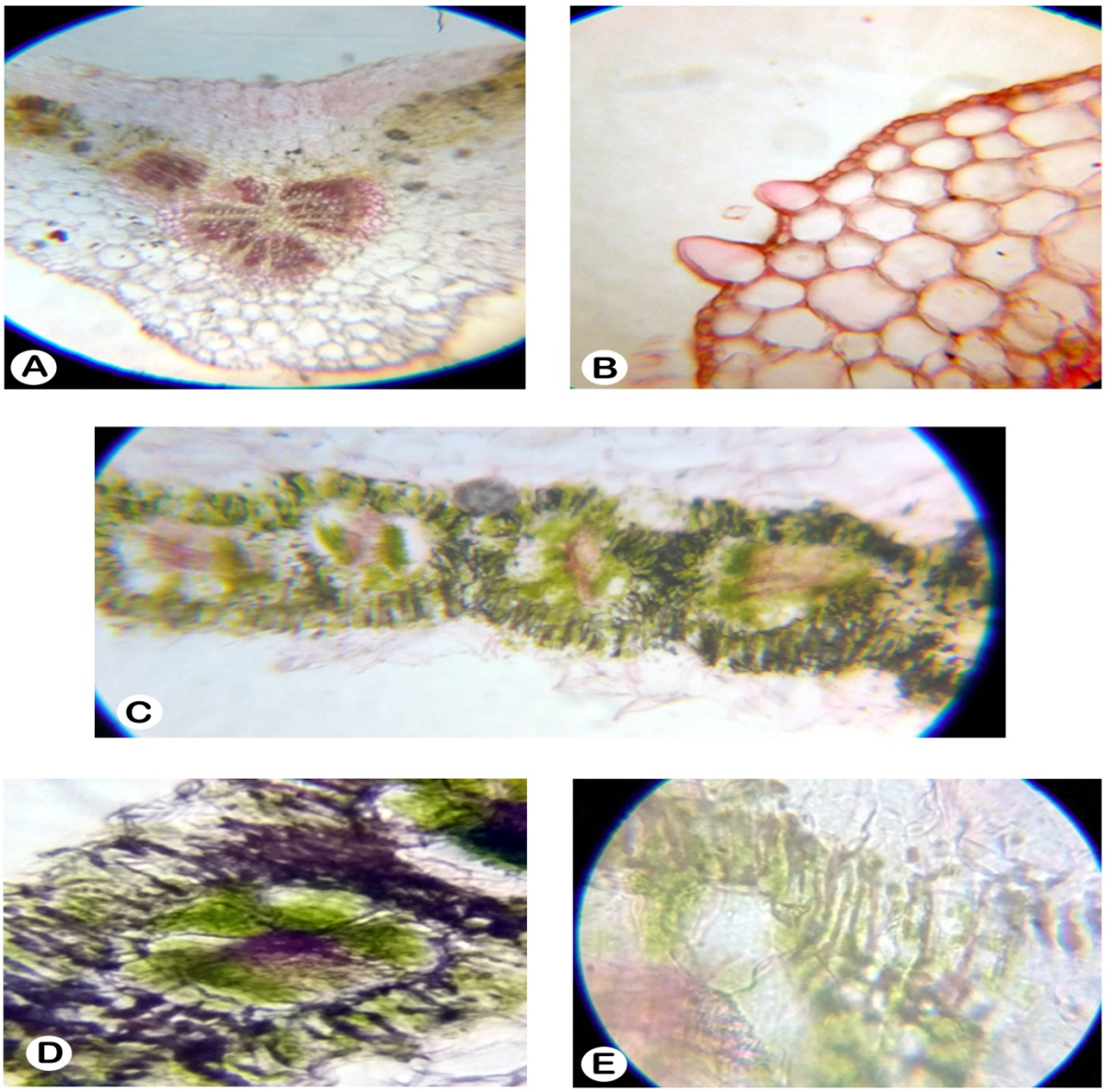

Fig. 5; A: Midrib showing 3 vascular bundles. B: Lower epidermis of petiole. C: Lamina T.S. showing vascular bundles. D: Bundle sheath of vascular bundle. E: Xylem - Bundle sheath cell and palisade cells.

Stem

The young stem was circular in outline having a large stele composed of 18 to 22 conjoint collateral and open vascular bundles in a ring enclosing a very large pith (Fig. 6A). Xylem was composed of 8 to 10 rows of tracheids (almost circular in outline) separated by uniseriate medullary rays. Phloem was a small patch outside the xylem. Pericycle was of 2-

layered sclerenchyma almost throughout. But at times regions consisting of prosenchymatous pericycle also observed. Endodermis was single layered. Pith which amounted to about 70-80 percent of stele, is of parenchyma which were getting bigger when one proceeds towards the centre. These parenchyma contained a few chloroplasts also (Fig 6B). Scattered in pith were sphaeraphides consisting of trapezoid or rectangular crystals of calcium oxalate (Fig 9E). Cortex was composed of parenchyma which contained chloroplasts and a few cluster crystals.. Epidermis was of a single layer of parenchyma cells, which are heavily cutinized. The cuticle is serrated on the outside. Hypodermis was of parenchyma as against collenchyma seen in most of dicot stems (Fig 6C). A few hairs were seen emerging from epidermis. They were mostly single celled and elongate. But very few multicellular uniseriate trichomes with very long cells structurally resembling unicellular trichomes were also seen (Fig 6D.)

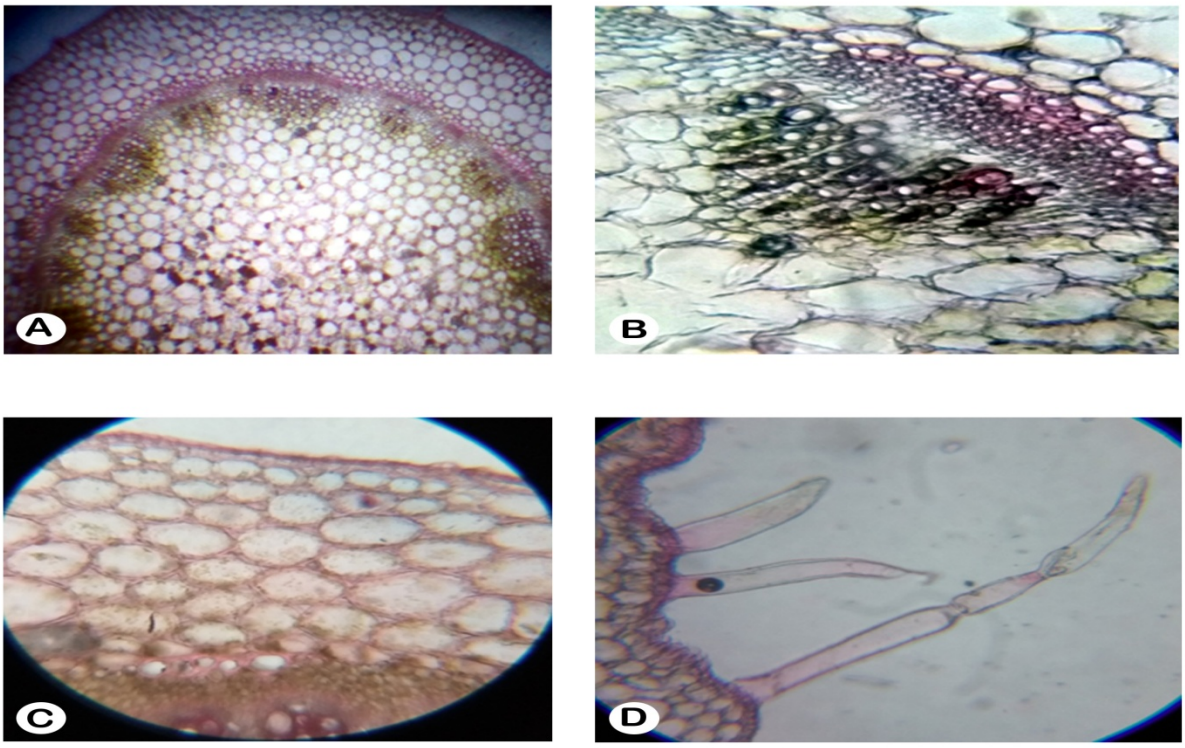

Fig. 6; A: Vascular bundles in a ring and a large pith. B: Vascular bundle in detail with pericyclic sclerenchyma. C: Hypodermal parenchyma. D: Unicellular and uniseriate trichomes.

As this plant is a herbaceous spreading annual, secondary thickening is seen in older stems closer to the stout main stem attached to tap root. The secondary thickening starts with initiation

of cambia from secondary cortical cells (as evidenced by the presence of chloroplasts) closer to the outermost phloem tissue. In many cases as in *Boerhavia,* these cambial cells produce xylem towards inside and phloem towards outside producing alternate rings of secondary xylem and phloem. But such complete rings are wanting here. It is seen that the second ring of cambium (arise in patches and independent of other patches) is discontinuous arising from the adjacent cortical parenchyma with the result the cambium of first ring grow outward through the spaces left by second ring of cambium. This results in xylem of first ring (where second ring of cambium is not produced) is seen continuous with the xylem of second ring (Fig. 7A). This results in the formation of apparent xylem bridges between the rings and patches of phloem and 2-3 layers of secondary cortex get included with in xylem (Fig 7B). It is apparent that cambial initials did not arise from outside those areas of xylem bridges. The third ring of cambium also behaves like this, but are formed outside the xylem bridges. This pattern continues and a reticulate type of secondary thickening occurs with a large number of included phloem patches with a little bit of secondary cortex of chlorenchyma, trapped among sec. xylem (7 C&D).

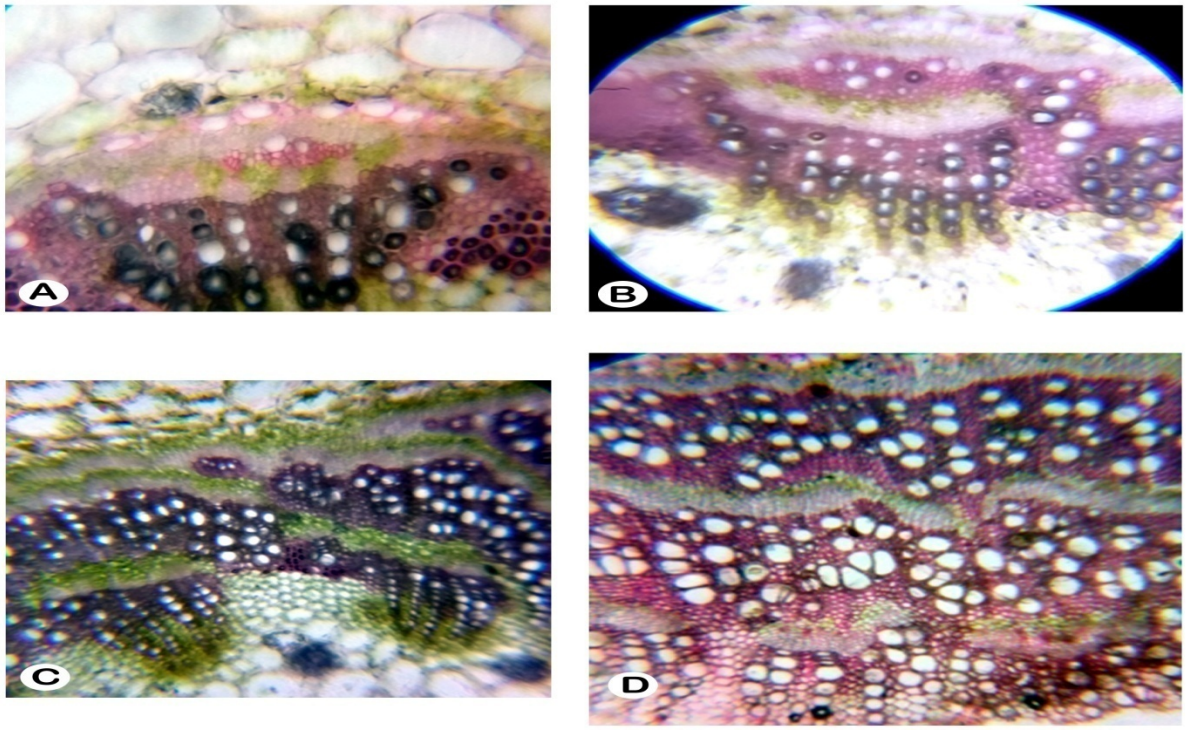

Fig. 7; A: Initiation of 2nd ring of cambium from innermost layers of secondary cortex. B: Establishing xylem bridges. C: Formation of third ring like wise. D: islands of Phloem and part of secondary cortex in a reticulated secondary xylem.

The primary xylem does not contain vessels. But the secondary xylem possesses many large slightly radially elongated vessels having a diameter of 40-50mμ. In L.S., the vessel segment is broad (diameter of 33 to 40 mμ) and long (150 to 180mμ) having 5-8 vertical rows of transversely elongated pits (Fig 8A) and slightly oblique end walls. Tracheids also are almost equal in length with a breadth of 10 to 15mμ with single or double vertical rows of bordered pits. Fibres also have similar length and breadth but no pits. The pericyclic sclerenchyma are 35 to 40 mμ.mu diameter. The rays contain rectangular (60 x 20mμ) and spherical (100mμ diam.) or elliptical crystals (Fig.8B).The prosenchyma cells were slightly larger having a diameter of 45 to 50 m. The cortical parenchyma appeared square (90- 100mμ) or rectangular (130 to 80mμ) (Fig 8C). The cork consists of smaller square (20- 30mμ) thick walled cells.

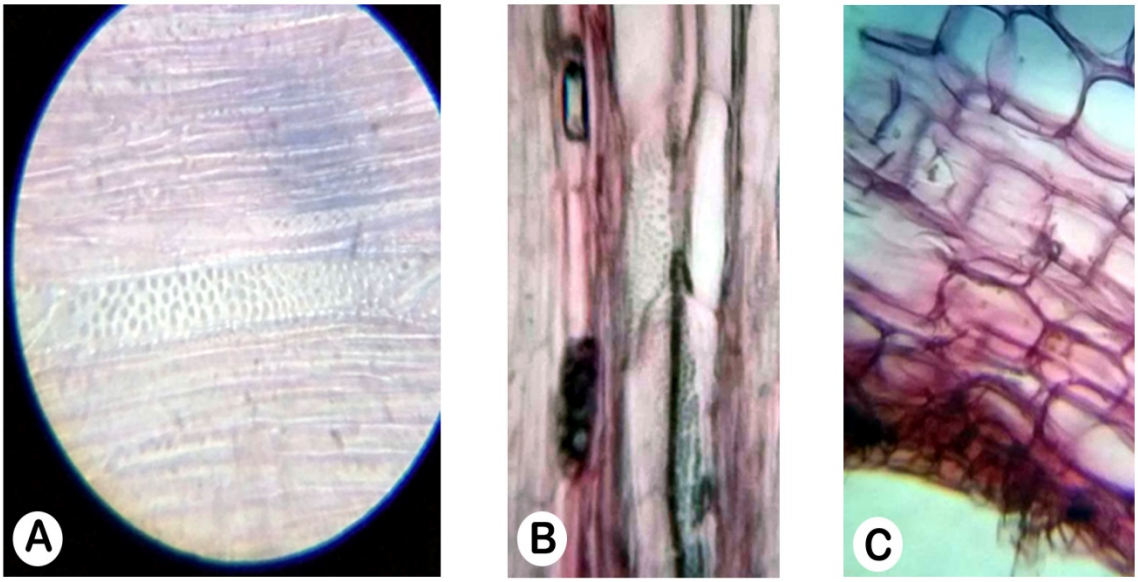

Fig. 8; A: Vessel members and tracheids in stem. B: Rectangular and cluster crystals in ray cells. C: Cortical parenchyma and cork.

POWDER STUDY

Root

Root powder showed short vessel segments with oblique walls (Fig 9A), tracheids with spiral thickenings (fig 9B), phloem tissue (9C), druses (9D) square cortical parenchyma cells and cork cells (Fig 9 E).

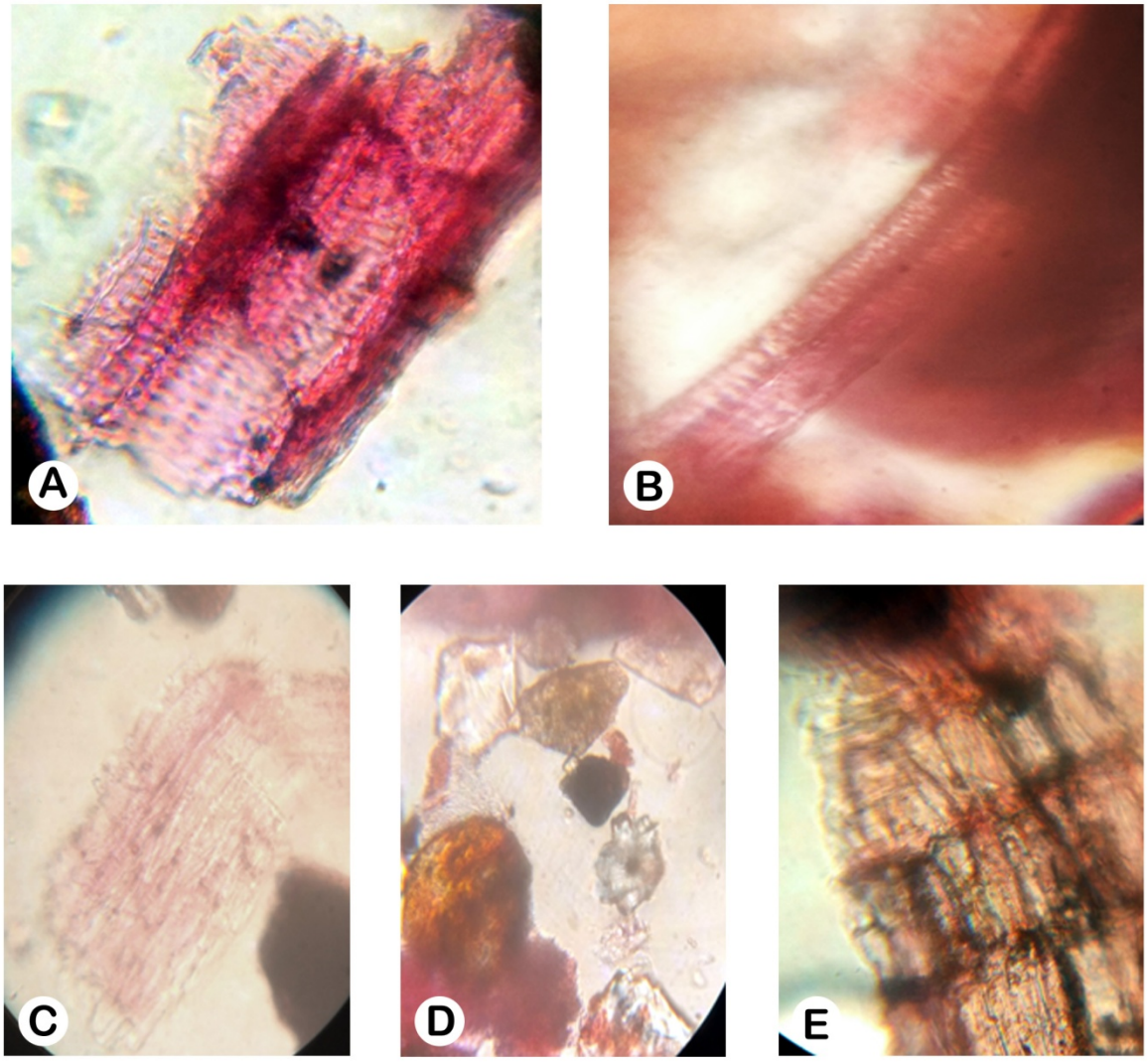

Fig. 9, Root powder with; A: Short vessel segments with oblique walls, B: tracheids with spiral thickenings, C: phloem tissue, D: druses, E: rectangular cork cells

Leaf

Leaf powder showed vascular bundle with sheath (Fig 10A), xylem tracheids with adjoining sheath cells (10B), individual sheath cells (10C), palisade cells (10D) and unicellular globose trichomes (10E).

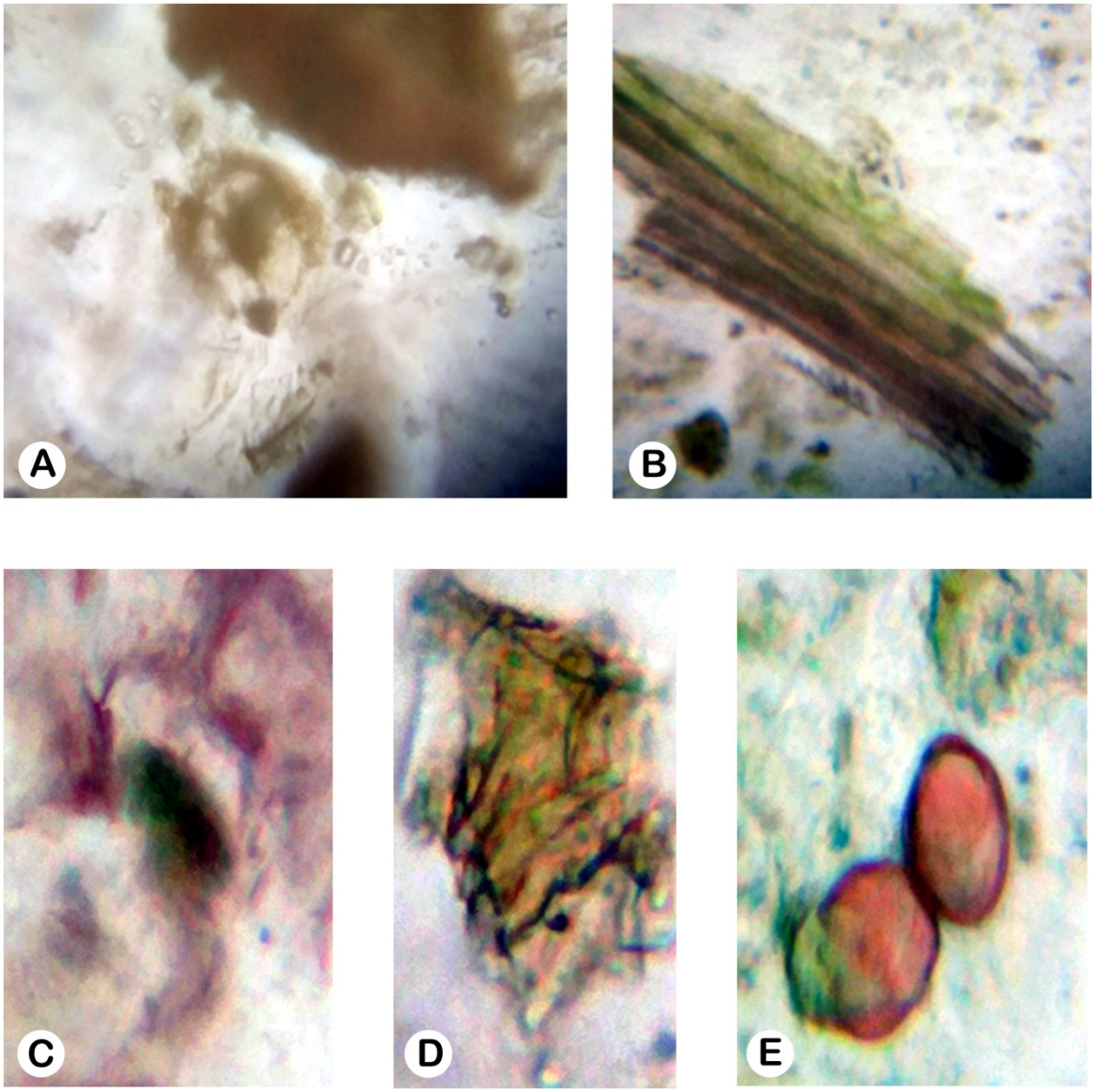

Fig. 10, Leaf powder showing; A: Vascular bundle with sheath, B: Xylem Tracheids with adjoining sheath cells, C: Individual sheath cells, D: Palisade cells, and E: Unicellular globose trichomes.

Stem

Stem powder is characterised by vessel segments with obique end walls (Fig 11A),fibers and adjoining chlorenchyma(11B), phloem cells with tracheids on both sides (11C), pith parenchyma

(11D), rectangular cells of outer cortex (11E)both rectangular and cluster crystals (11 F) and unicellular elongated trichomes (11G).

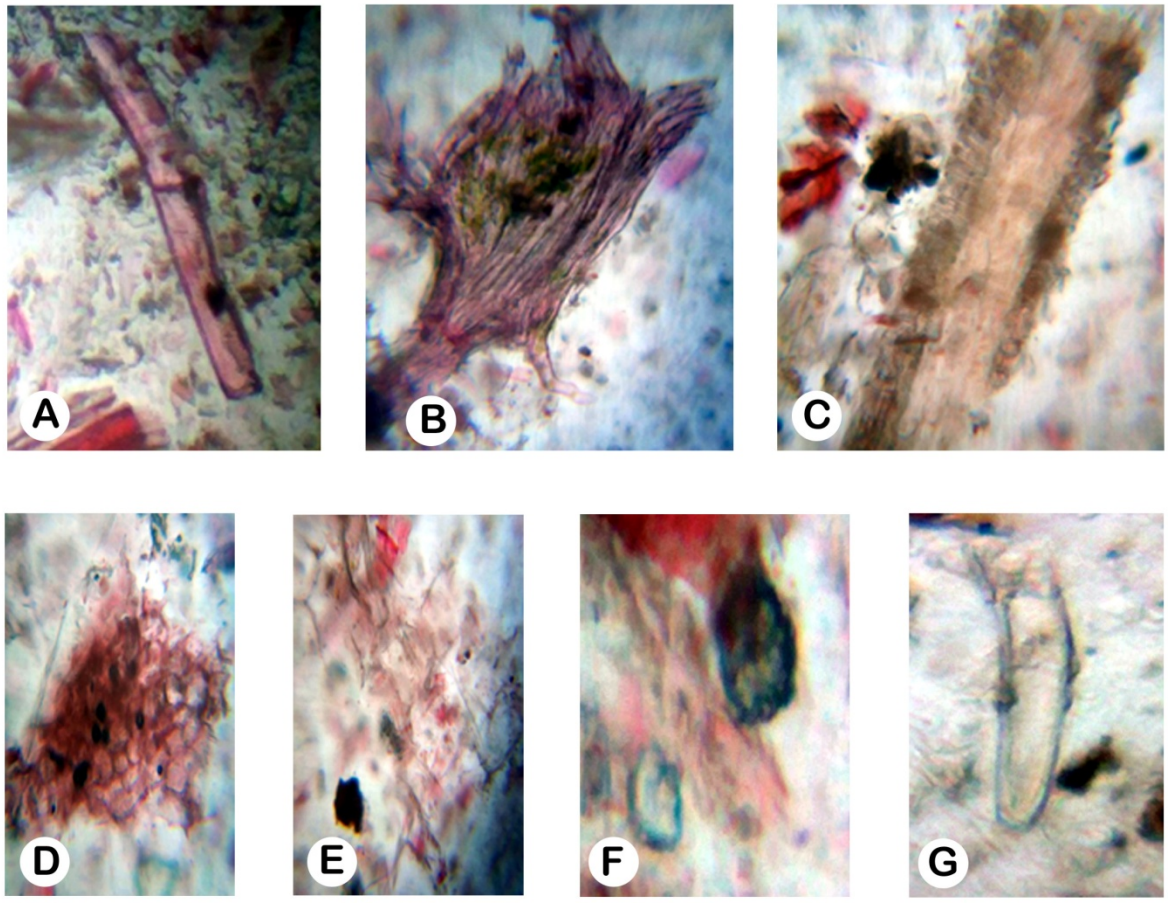

Fig. 11, Powder of stem showing; A: Long vessel segments with oblique end walls, B: Fibers and adjoining chlorenchyma, C: Phloem cells with tracheids on both sides, D: Rectangular cells of outer cortex, E: Pith parenchyma, F: Both rectangular and cluster crystals, and G: Unicellular elongated trichomes.

DISCUSSION

The present study unearths a large number of new features unnoticed by all previous workers. All the previous chemical studies were done on aerial parts as a whole and never the individual organs like roots, leaves and stem were studied separately. Roots were never studied earlier. Therefore, all reports of flavonols like, sesuvin, 5,2'dihydroxy 7 methoxy 6,8 dimethyl

flavone, quercetin; phenolic acids, ecdysone in leaves, stem and roots are new additions to the chemistry of *Trianthema portulacastrum.* Though the plant is said to be mucilaginous in all descriptions, the presence or amount of mucilage is never recorded. This may be due to the poor awareness of beneficial pharmacological aspects of mucilage. Instead, almost all workers used crude tests and the presence of foam (due to mucilage) was erroneously attributed to saponins. The plant is a very rich source of mucilage, especially in stem and leaves where it amounts to 40% and above, which acts as a dietary fibre and has innumerable health benefits. We could not detect saponins in this plant.

With regards to the pharmacognosy of the plant, a number of new data are added by the present study. The discovery of anomalous spiral thickening in root is a new knowledge added to Plant Sciences. Earlier, the roots are reported to have rings of secondary xylem alternating with rings of secondary phloem. Similarly the continuous outward growth of secondary vascular tissues with formation of patches of internal phloem in a reticulated secondary xylem as reported here is a new knowledge. Single large crystals as well as rhomboidal crystals forming a part of cluster crystals of calcium oxalate are never reported. Another very glaring omission by a few previous workers (except Vidya *et al*., 2016[7]) is the presence of clear bundle sheaths in leaf vascular bundles, though this was earlier reported by Riyadh and co-workers[13] in a paper on anatomy (Botany) in a number of members of Aizoaceae including *Trianthema portulacastrum.* In addition the present paper provides the quantitative measurements of all tissues which are key characters for a pharmacognostic work.

CONCLUSION

The present paper provides a complete and exhaustive study of phytochemical and pharmacognostic characters of all the three different parts like roots, stem and leaves separately of the important medicinal plant *Trianthema portulacastrum.* Ecdysterone is located in all the three parts. Only stem contained the peculiar 5,2'dihydroxy7 methoxy 6,8 dimethyl flavone while the roots and leaves possessed sesuvin and quercetin. Mucilage was abundant in leaves and stem, and to a less extent in roots. Pharmacognostically all the three parts exhibited their distinct

characters. Diarch protoxylem, included primary phloem, anomalous spiral thickening, short vessel segments are features of root, whereas the stem possessed reticulate secondary xylem having intraxylary phloem, long broad vessel segments, uniseriate and unicellular trichomes. The leaves are distinct with bundle sheath cells, spongy resembling palisade and unicellular trichomes.

REFERENCES

1. Sivarajan, V.V. and I. Balachandran. *Ayurvedic Drugs and Their Plant Sources*, Oxford & IBH, New Delhi, 1994
2. Sukhdev. *A Selection of Prime Ayurvedic Plant Drugs*, Anamaya Publishers, New Delhi, 2006
3. Banerji , A. *et. al*. Isolation of ecdysterone from Indian plants. *Phytochem.* 1971; **10**:2225–6.
4. Daniel. M and S.D.Sabnis. Chemotaxonomy of Aizoaceae-Molluginaceae. *Biome.* 1986; **1**, 65-69
5. Udom K. *et al*. A C-methylflavone from *Trianthema portulacastrum* **.** *Phytochemistry.* 1997; **44:** 719-722.
6. Gaddeyya, G. and P.K. Ratna Kumar. A Comprehensive review on Ethnobotany and Phytochemistry of a Herbal Weed, *Trianthema portulacastrum*. *J.Phacgn. and Phytochem*. 2016; **5** (4): 25-31
7. VidyaS.P., Rajput, K.S and N.P. Malpathak. Comparative study on morpho-anatomy of leaf, stem and root of *Boerhaavia diffusa* L. (Nyctaginaceae) and its adulterant plants, *Braz. J. Pharmaceu. Sci*. 2016; **52** (3) 433-442.
8. Sakkeena A. *et al*. Pharmacognostic and HPTLC Evaluation of Leaves and Roots of *Trianthema portulacastrum* L. *Int.J. Pharm. Biol. Sci*. 2019, 9 (2): 117-126
9. Daniel M and Elizabeth Robin. A novel anomalous spiral secondary growth in roots of *Trianthema portulacastrum L.,* Indian Journal of Applied Research. 2020; 10(5): 1-2
10. Harborne J.B. *Phytochemical Methods* (Revised Edn) Chapman and Hall, London, 1984

11. Daniel, M and M. Denni. *Analytical Methods for Medicinal plants and Economic Botany.* Scientific Publishers, Jodhpur, 2016

12. Johansen, D.A. *Plant microtechnique.*: McGraw Hill, New York, 1940

13. Riyadh, M, Rowan F.S and G.D. Nancy. Diversity of Kranz anatomy and biochemistry in C4 Eudicots. *Amer. J. Bot*. 2007; 94(3): 362–381.