



Pharmacognostic and HPTLC Evaluation of Leaves and Roots of *Trianthema portulacastrum* L.

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Abstract

Trianthema portulacastrum L. (TP) belongs to the family Aizoaceae. It is rapidly growing, much branched, succulent, prostrate and annual terrestrial weed. The plant parts such as leaves, fruits and roots are used for various medicinal purposes. Some traditional uses reported are as alternative cure for bronchitis, heart disease, blood anemia, inflammation, piles, ascites and throat troubles. The root paste applied to the eye cures corneal ulcers, itching, dimness of sight and night blindness. Various pharmacological activities of the plant have been reported. The present work deals with the physico-chemical, botanical and HPTLC studies of leaf and root of TP. The physico-chemical parameters such as water soluble extractive, alcohol soluble extractive, loss on drying at 105°C, total ash, acid insoluble ash, pH (10% solution) and volatile oil were determined according to standard methods. The macroscopic, microscopic and powder microscopic characteristics of leaf and root were determined. HPTLC studies of chloroform extracts of leaf and root of TP were conducted at 254 nm, 366 nm and 575 nm after derivatisation using vanillin- sulphuric acid reagent and the results were documented. The total ash content and the solubility in alcohol and water for leaf are higher than that of root. The Physico-chemical parameters, Macroscopical, Microscopical and Powder Microscopical characters and the developed HPTLC fingerprint profiles obtained from this study can be used as reference standards in laying pharmacopoeial standards of the two plant materials.

Keywords

Aizoaceae, Botanical characters, HPTLC finger printing, Physico-chemical, Standardization, *Trianthema portulacastrum*.

INTRODUCTION

Trianthema portulacastrum L. (TP) belongs to the family Aizoaceae. It is an annual herb found in tropical and sub-tropical region and almost throughout India as a weed. Its infestation is very common in various agricultural and vegetable crops, such as mustard, maize, moong bean, potato, onion, cotton, pearl millet, and sugarcane, especially during the rainy seasons [1,2]. It is rapidly growing, much branched, succulent, prostrate and annual terrestrial weed. In India it is used as green leaf vegetable and is considered to be useful for the purpose of medicinal value [3]. The plant parts such as leaves, fruits and roots have various medicinal properties and are used as analgesic, antipyretic, anti-inflammatory and antibacterial [4,5]. Pharmacological activities like hypoglycemic, hypolipidemic, analgesic, hepatoprotective [6] anthelmintic, anticancer, diuretic, antioxidant activities [7] and mosquito larvicidal activities have been reported for different extracts of the plant and served as alternative cure for bronchitis, heart disease, blood anaemia, inflammation, piles and ascites. The plant is used against throat troubles and as an anti-fungal agent. The root paste applied to the eye cures corneal ulcers, itching, dimness of sight and night blindness [8]. In the Indian traditional medical systems, the plant is considered as a diuretic. The leaves possess diuretic properties [9]. The fleshy nature of leaves makes them suitable for use as a wound-dressing. It is also used as vegetable in various parts of the world due to its high nutritional value. Two forms of this plant is reported, a red coloured form in which the stem, leaf margin and flowers are red; and a green coloured form which has a green stem and white flowers and this variety was taken for the study. TP is usually misidentified with *Boerhaavia diffusa* [10]. In order to maintain the efficacy of the drug, standardization is essential and thereby preventing adulteration and substitution. Therefore, the need of the hour is to establish their correct botanical and chemical identity by subjecting them to botanical, physico-chemical and HPTLC studies. The different vernacular names of TP are Marathi - Pundharighentuli; Punjabi - Bishkatra; Sanskrit - Punarnavi; Hindi - Lalsabuni; Tamil - Vellai-sarvalai; Telugu - Galijeru; Kannada - Muchchugoni [11,12]

Geographical distribution

TP is found in tropical and sub-tropical countries of the world and indigenous to South Africa. It is widely distributed in India, Srilanka, Baluchistan, West Asia, Africa and Tropical America [8, <http://plants.usda.gov>].

MATERIALS AND METHODS

(A). Plant Material

The fresh leaves and roots of *T. portulacastrum* (Figure 1) were collected, authenticated and supplied by Siddha Medicinal Plants Garden, Mettur Dam.

(B). Macroscopy

The macroscopic studies were carried out using organoleptic evaluation method. The arrangement, size, shape, base, texture, margin, apex, venation, colour, odour, taste of leaves and roots were observed. Macroscopic characters were studied as described by [13] and photographs at different magnifications were taken.

(C). Microscopy

Microscopic study was carried out by preparing thin sections of root and leaf. The thin sections were further washed with water, stained with safranin and mounted in glycerin for observation.

(D). Powder microscopy

The powder microscopy of the leaf and root powders were studied using standard procedure by capturing the images of different fragments of tissues and diagnostic characteristic features were recorded [14].

(E). Determination of leaf constants

A number of leaf measurements are used to distinguish some closely related species not easily characterized by general microscopy [15]. Stomatal number, stomatal index, palisade ratio, trichome number, vein islet and vein let termination were observed under 4X objective of microscope.

(F). Physico-chemical analysis

The plant materials were cut, crushed, dried and kept in airtight containers and these were used for the experimental purposes. The physico-chemical parameters like ash content, acid insoluble ash, volatile oil, solubility in water and alcohol, loss on drying at 105°C, pH and foreign matter were determined as per WHO guidelines [16]

(G). High Performance Thin Layer Chromatographic analysis (HPTLC)

HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials and is the simplest separation technique today available to the analyst [17].

Preparation of extracts of the drug materials for HPTLC analysis

4 gm each of the dried and powdered leaf and root of TP was soaked in 40 ml chloroform at room temperature for overnight. The contents were filtered through different filter papers and the filtrates were concentrated on a water bath to 3 ml.

These extracts were used for chromatographic studies [18].

Development of HPTLC profile

Chloroform extracts of the plant materials were spotted in the form of bands with Camag microlitre syringe on two plates precoated with silica gel 60 F₂₅₄ (Merck) by using Automatic TLC Sampler 4 (ATS4). Mobile phase used was Toluene: Ethyl acetate: Formic acid (5: 1: 0.1) for root extract and Toluene: Ethyl acetate (5: 1) for leaf extract. Linear ascending developments were done in twin trough glass chambers saturated with the specified mobile phases. The plates were air dried and kept under UV 254 nm and 366 nm and white light after derivatisation using vanillin-sulphuric acid reagent and photo documentation were done. Then the plates were scanned in UV 254 nm, 366 nm and in 575 nm after derivatisation using TLC Scanner 4 with win CATS software for interpretation of data.

RESULTS AND DISCUSSION

(A). Plant materials

Fresh plant materials were used for anatomical studies and dried powder of plant materials were used for powder microscopic, physico-chemical and HPTLC studies.

(B). Macroscopic characters

TP is a prostrate somewhat succulent herb; stem more or less angular, glabrous or pubescent, much branched. Leaves sub fleshy, obliquely opposite, unequal, broadly obovate, rounded and often epiculate at the apex, cuneate at the base, glabrous; petioles 6-13 mm long, much dilated and membranous at the base. Flowers are solitary, sessile, almost concealed by the pouch of the petiole. Calyx-lobes are ovate, and acute. Stamens 10-20. Ovary is truncate; style 1. Capsule small, almost concealed in the petiolar pouch, lid truncate, slightly concave, with 2 spreading teeth carrying away at least one seed, the lower part 3-5 seeded. Seeds are reniform, muriculate and dull black in colour. Roots are thin, slender, tapering, and tortuous, with lateral branching fibrous root, 5-15 cm in length; 0.3–2.5 cm in diameter, light yellow externally, creamish white internally, fractures fibrous.

(C). Microscopic characters

1. Leaf

The T.S of TP leaf is shown in Figure 2. The leaf lamina was dorsiventral in nature. The upper and lower epidermis was single layered. It was covered with thin cuticle. Epidermis was followed by 2 layered palisade cells and 4-7 layered mesophyll tissues. Centrally located conjoint collateral vascular bundles were surrounded by spongy parenchymatous cells.

The paracytic stomata were present in upper and lower epidermis.

Petiole

The T.S of TP petiole is shown in Figure 3. The petiole was bean shaped. The single layered upper and lower epidermis was surrounded by thin cuticle layer. The epidermis was covered with unicellular and multicellular, 2-3 celled trichomes. Ground tissue was parenchymatous. Calcium oxalate crystals were scattered and vascular bundles were three in numbers. The size of the vascular bundles varied from centre to leaf margin i.e. large too small. They were centripetally arranged i.e. xylem surrounded by the phloem.

2. Root

The transverse section of TP root is shown in figure 4. Root shows anomalous secondary growth. Cork is 5 to 8 layered. Secondary cortex is a narrow zone consisting of round to polygonal, tangentially elongated, thin walled, parenchymatous cells. Below secondary cortex five concentric bands of vascular tissue; vessels of varying sizes occurring along with xylem fibres and phloem. A few rows of polygonal, thin walled, parenchymatous cells occur in rings. Medullary rays are found to be prominent in middle of the cortical region and in the second or third bundle ring. The central portion is mostly occupied by a single vascular bundle strand with two isolated groups of phloem. Alternate zone of xylem and phloem are found due to anomalous secondary growth and their numbers vary according to the age of the plant. Xylem consists of vessels of various sizes, tracheids and xylem parenchyma. Phloem consists of sieve tubes and companion cells.

(D). Powder microscopy

a. Leaf

Powder microscopy of leaves of TP is shown in Figure 5. The characters noted were calcium oxalate crystals, paracytic stomata, tannin filled cells, leaf veinlet termination and vessel with spiral thickening

b. Root

Powder microscopy of TP root is shown in Figure 6. The characters noted were starch grains, vessel with characteristic thickening, calcium oxalate crystals and warty trichomes.

(E). Determination of leaf constants

Leaf constants of TP are shown in Figure 7. Epidermis with paracytic stomata and average number of stomata was 20-25. The average of vein islet and veinlet termination was 12 and 14 in number.

(F). Physico- chemical analysis

The results of the physico- chemical analysis of root and leaf of TP are pictorially represented in Table 2. Total ash value of the plant materials indicates the

amount of minerals and earthy materials attached to it. Acid insoluble ash usually represents the amount of silica present as sand and dust and indicates contamination. Total ash value of the leaf was 17.60 % while acid insoluble ash was 2.25 %. Total ash value of the root of the plant was 6.75% while acid insoluble ash was 0.95%. Loss on drying at 105°C shows the presence of moisture content and volatile oil in the drug. Loss on drying for leaf was 14.37% and for root was 14.07%. The water soluble extractive value indicates the presence of more polar constituents such as tannin, sugar, plant acid, mucilage and glycosides. The alcohol soluble extractive values indicated the presence of phenols, alkaloids, steroids, glycosides, flavonoids etc. The water soluble extractive value of leaf of the plant was 26.17 % and that of the root was 6.75%, which indicate the presence of high polar compounds. The alcohol soluble extractive values (5.23 % and 1.05%) of the plant materials are less when compared to water soluble extractive values. The pH values (7.5 & 7.9) of drug materials suggest its little alkaline nature. Presence of volatile oil was not detected in both the plant materials. Most of the physico-chemical parameters of the plant materials are different. The extractive values in alcohol and water for leaf are higher than that of root. These values are a measure of the quantity of the chemical constituents soluble in the solvents used. Ash values are also higher for leaf indicating the presence of more inorganics in the leaf than in the root.

(G). High Performance Thin Layer Chromatographic analysis (HPTLC)

The HPTLC fingerprinting patterns of chloroform extracts of the leaf and root of TP were developed in different plates at 254 nm, 366 nm and 575 nm after derivatisation with vanillin–sulphuric acid are given in Figure 8 and Figure 9 respectively.

R_f values and colour of bands of chloroform extracts of leaf and root of TP under UV 254 nm, UV 366 nm and white light after derivatisation are represented in the Table 3 and Table 4 respectively.

The HPTLC finger print profile of leaf of TP (Figure 10) at UV 254 showed eight peaks among which the peak at R_f 0.67 is the major peak with an area of 26.61% followed by the peak at R_f 0.55, 0.60 & 0.84 with areas of 17.25%, 11.69% and 9.97% respectively. Other peaks appeared at R_f 0.14, 0.19, 0.34 and 0.39 with area of 1.23%, 1.61%, 2.61% and 2.68%. The finger print profile at UV 366 showed twelve peaks among which the peak at R_f 0.51 is the major peak with an area of 20.59%. The other major peaks are at R_f 0.34 and 0.87 having areas of 16.03% & 14.03% respectively. All other peaks are minor peaks of area less than 10%. Ten peaks at R_f 0.16 (3.08%), 0.28 (8.87 %), 0.35 (6.78 %), 0.38 (5.42 %), 0.48 (6.35 %), 0.55 (7.01 %), 0.61 (3.55%), 0.69 (12.88%), 0.80 (5.39%) and 0.94 (22.08%) are seen in the finger print profile at 575 nm after derivatisation with vanillin-sulphuric acid. Among which the peak at R_f 0.69 (12.88%) and 0.94 (22.08%) are major peaks and all other peaks are minor peaks as the percentage area for the peaks were less.

HPTLC fingerprinting pattern, R_f values and their relative peak areas of the chloroform extract of root of TP at 254 nm, 366 nm and 575 nm after derivatisation are given in Figure 11. It is evident that in the HPTLC finger printing pattern at 254 nm there are 10 peaks at R_f values 0.08, 0.10, 0.17, 0.32, 0.37, 0.48, 0.60, 0.71, 0.83 and 0.98 indicating the occurrence of at least 10 different components in chloroform extract. Finger printing pattern at 366 nm also shows 10 peaks and R_f values at 0.58 and 0.70 are more predominant as the percentage area is 30.16% and 27.99% respectively. From the finger printing pattern at 575 nm after derivatisation, it is clear that there are minimum 11 different components in chloroform extract. It is also clear that out of 11 components, the components with R_f values 0.44 and 0.59 were found to be more predominant as the percentage area is 30.21% and 26.98% respectively. The other peaks were found to be minor as the percentage area for the peaks were less. First peak of every fingerprint has not been taken into account since it is at the loading position.

Table 1: Leaf constants of TP

Sl. No.	Parameters	Results
1	Stomatal number (20x)	22
2	Stomatal index (20x)	14
3	Palisade ratio (20x)	3
4	Vein islet number (4x)	12
5	Vein let number (4x)	14
6	Epidermal number (20x)	14.5

Table 2: Physico-chemical parameters of Leaf and Root of TP

Sl. No.	Parameters	Leaf	Root
1	Total ash (%)	17.60	6.75
2	Acid insoluble ash (%)	2.25	0.95
3	Loss on drying (%)	14.37	14.07
4	Water soluble extractive (%)	26.17	6.75
5	Alcohol soluble extractive (%)	5.23	1.05
6	pH value (10 % solution)	7.5	7.9
7	Volatile oil	Nil	Nil
8	Foreign Matter	< 2	< 2

Table 3: R_f values and colour of bands of chloroform extracts of TP leaf

Sl. No.	TP Leaf					
	Under UV 254nm		Under UV 366 nm		Under White light	
	R _f	colour	R _f	colour	R _f	colour
1	0.10	Light green	0.06	Brown	0.16	Light purple
2	0.23	Light green	0.13	Red	0.32	Light purple
3	0.38	Light green	0.34	Red	0.48	Light purple
4	0.55	Dark green	0.42	Brown	0.53	Bluish green
5	0.60	Dark green	0.54	Light pink	0.60	Yellow
6	0.66	Dark green	0.58	Fluorescent red	0.68	Bluish green
7	0.75	Dark green	0.64	Fluorescent red	0.79	Brown
8	0.88	Light green	0.81	Brownish red	0.92	Purple
9			0.93	Dark red		

Table 4: R_f values and colour of bands of chloroform extract of TP root

Sl. No.	TP Root					
	UV 254nm		UV 366 nm		575 nm	
	R _f	colour	R _f	colour	R _f	colour
1	0.10	Light green	0.05	Red	0.09	Green
2	0.17	Light green	0.10	Red	0.16	Light purple
3	0.30	Light green	0.16	Light green	0.44	Purple
4	0.68	Dark green	0.26	Violet	0.58	Purple
5	0.91	Light green	0.36	Blue	0.75	Light purple
6			0.39	Brown	0.84	Light purple
7			0.55	Fluorescent blue	0.97	purple
8			0.60	Pink		
9			0.67	Dark Pink		
10			0.78	Blue		
11			0.90	Blue		


Figure 1: Habit and root of *Trianthema portulacastrum* L.

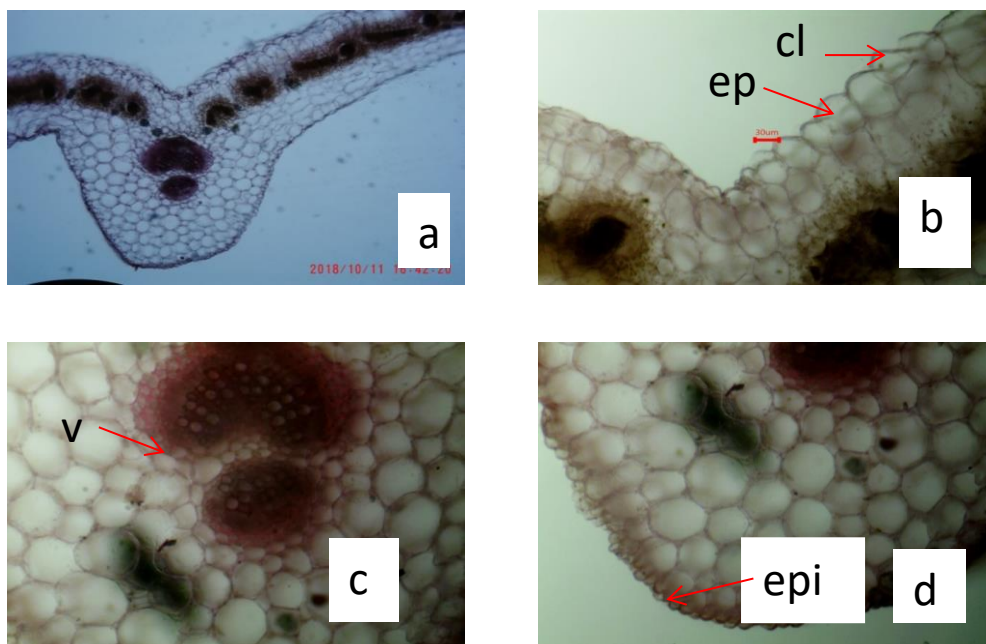


Figure 2: a: T.S of leaf, b: upper epidermis (epi) & cuticle (cl), c: vascular bundle (vb), d: lower epidermis (epi).

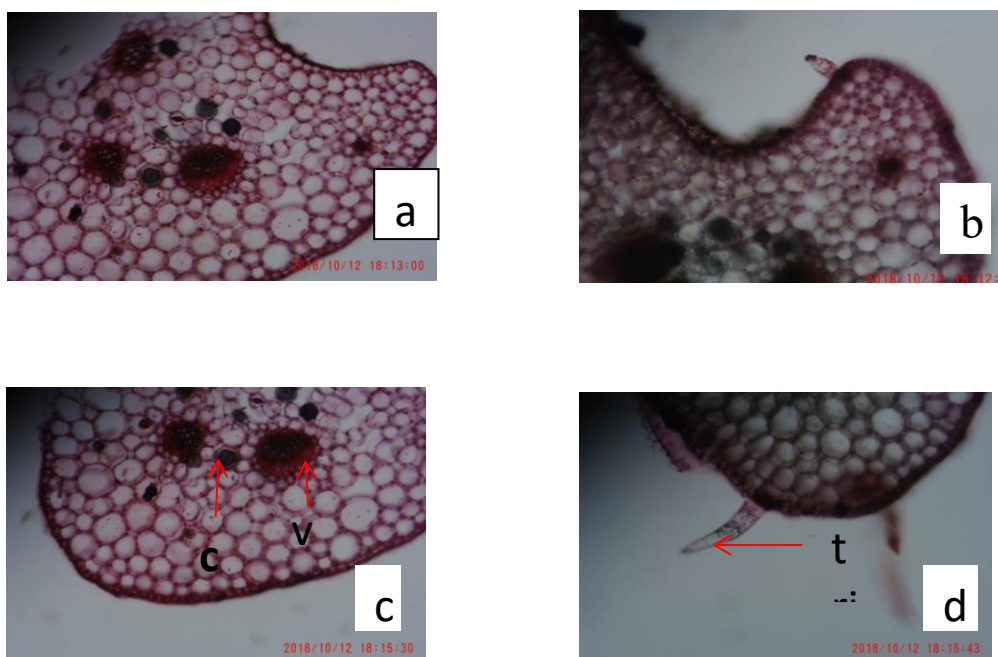


Figure 3: a: T.S of petiole & a portion enlarged, b: epidermis, c: vascular bundle (vb) & calcium oxalate crystal (cr), d: lower epidermis with trichome

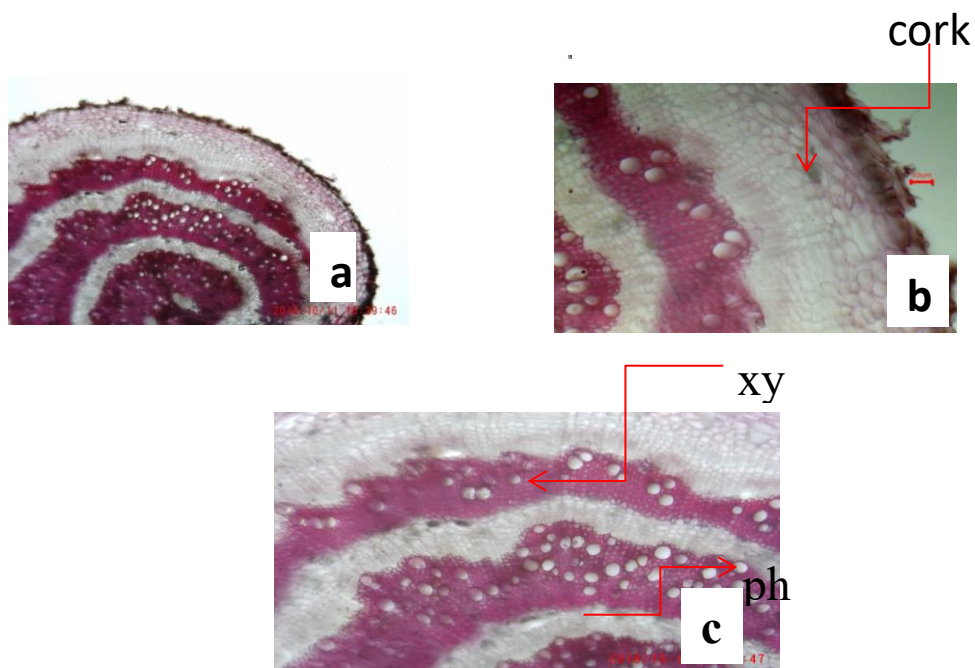


Figure 4: a: T.S of root, b: cork, c: xylem (xy), phloem (ph)

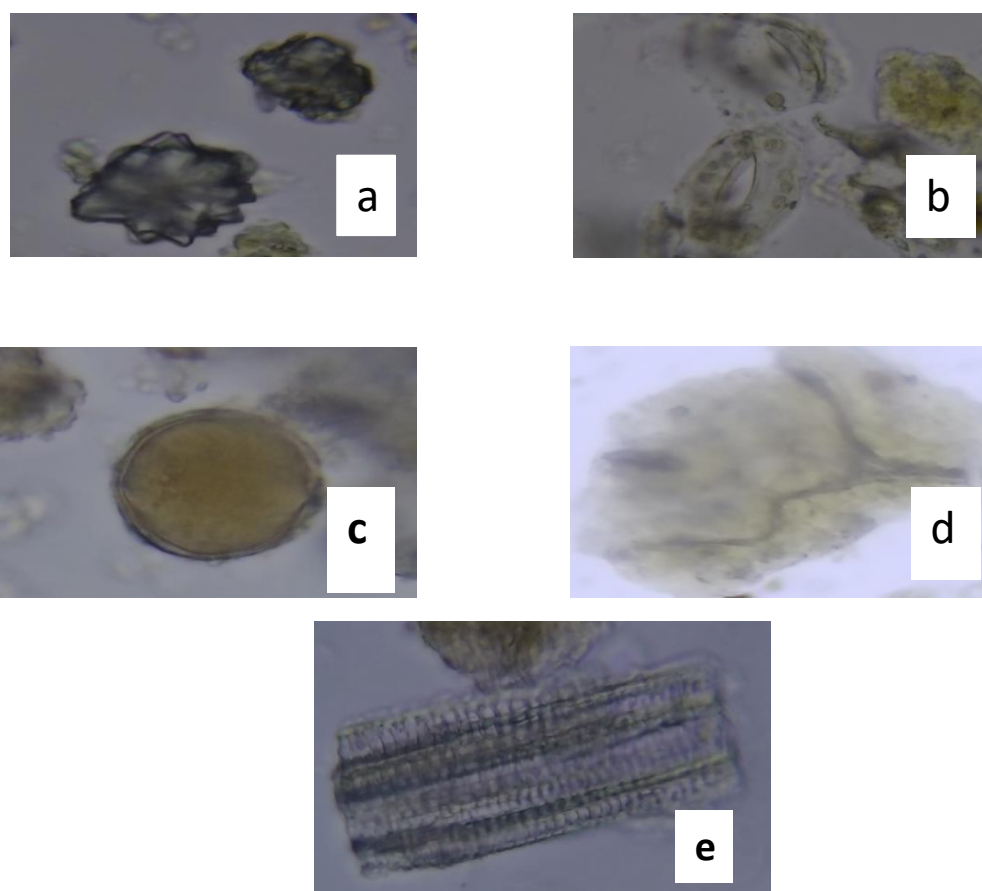


Figure 5: a: calcium oxalate crystal, b: paracytic stomata, c: tannin filled cell, d: vein let termination, e: xylem tracheid with spiral thickening.

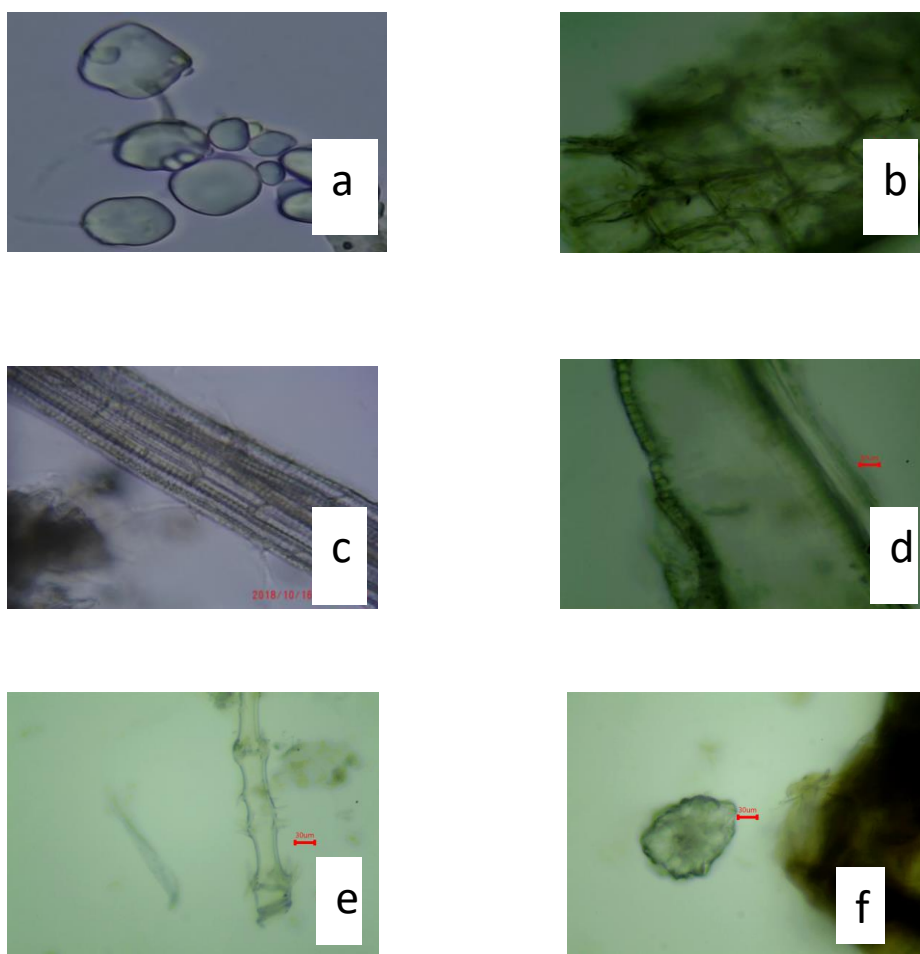


Figure 6: a: Starch grain, b: cork cell, c & d: vessel with characteristic thickening, e: wartytrichome, f: calcium oxalate crystal

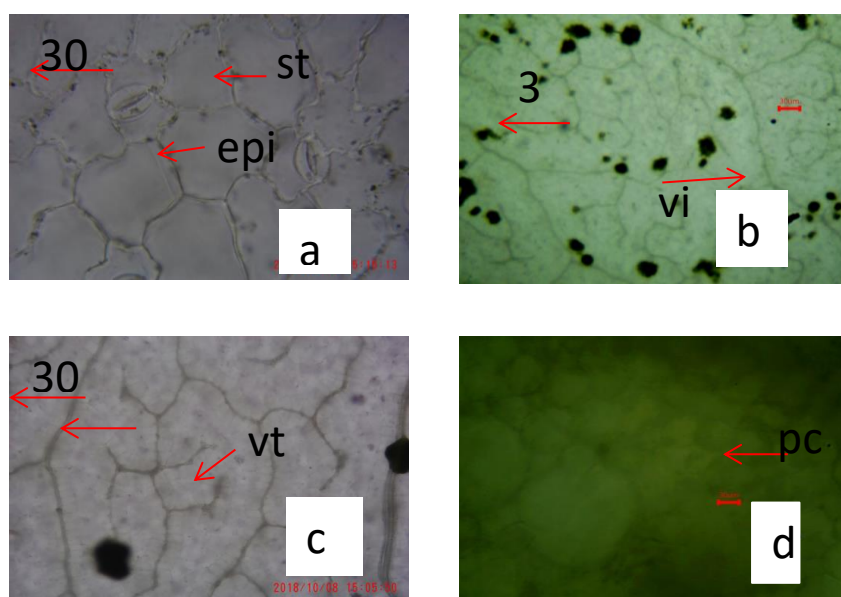


Figure 7: a: epidermis (epi) with paracytic stomata (st), b: Vein islet (vi), c: Veinlet termination (vt) d: Palisade cell (pc)

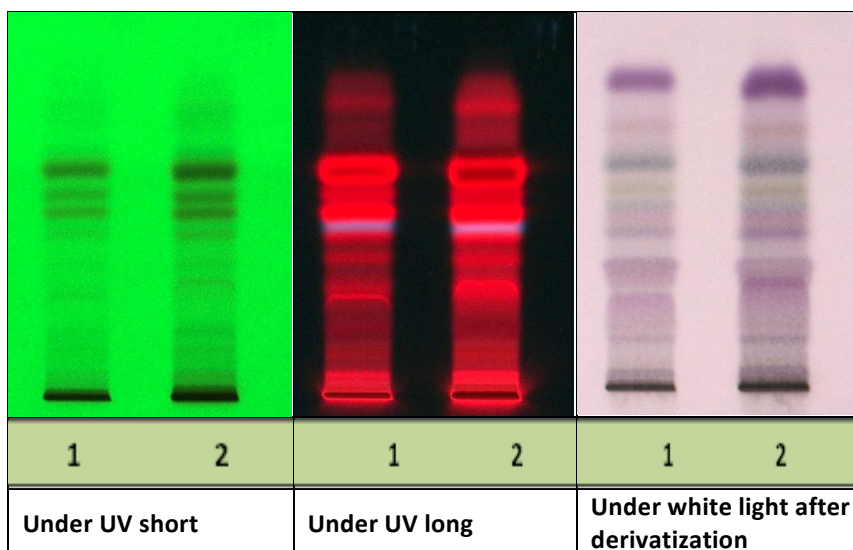


Figure 8: HPTLC photo documentation profile of the chloroform extract of leaf of TP. Track 1: 3 µl; Track 2: 6 µl

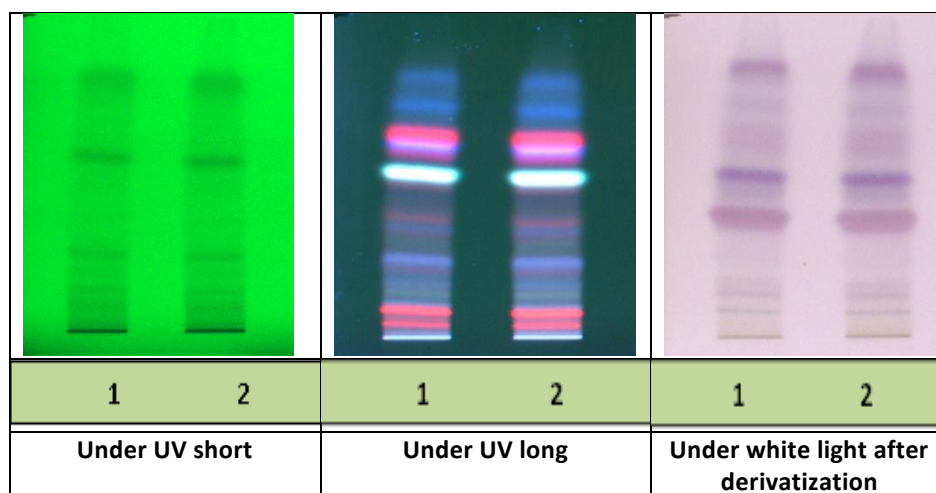


Figure 9: HPTLC photo documentation profiles of the chloroform extract of root of TP. Track 1: 15 µl; Track 2: 20 µl

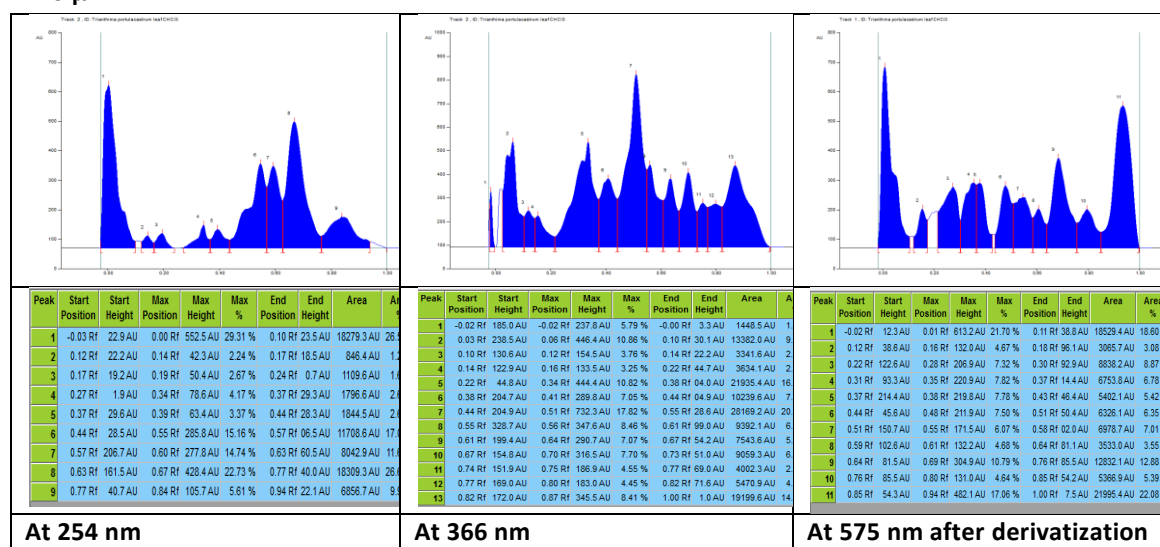


Figure 10: Finger print profile and Rf tables of chloroform extract of leaf of TP

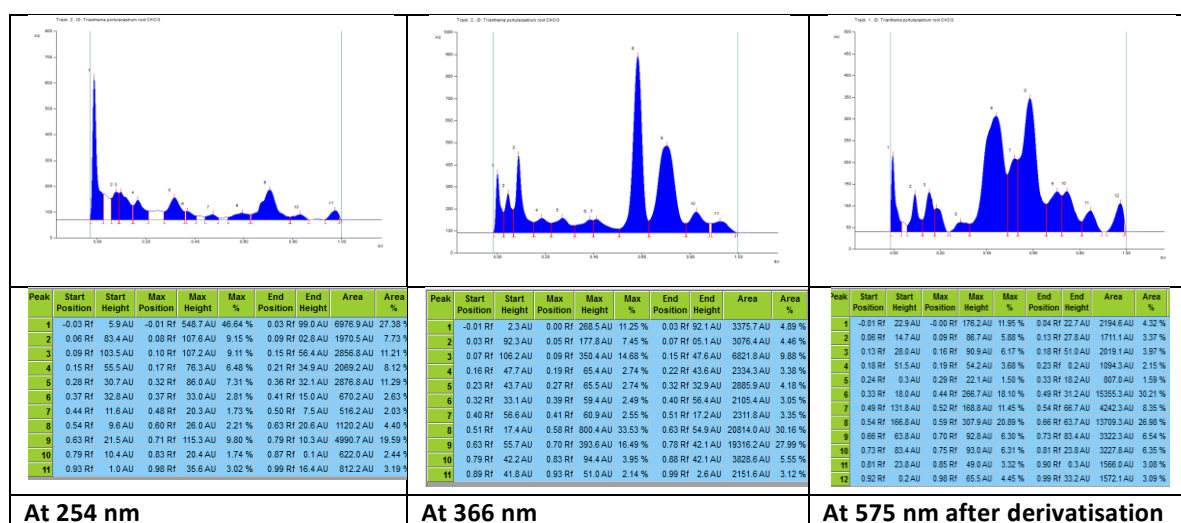


Figure 11: Finger print profiles and R_f tables of chloroform extract of root of TP

CONCLUSION

The pharmacognostic results and the developed HPTLC finger print profiles obtained from this study help to ensure sample identification, quality and purity of leaf and root of *Trianthema portulacastrum*.

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