Fingerprinting and Quality Control of *Trianthema portulacastrum* L. Root

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Abstract: *Boerhaavia diffusa*, *Boerhaavia erecta*, *Boerhaavia. repanda* and *Trianthema portulacastrum* L. are equated with 'Punarnava.' *T. portulacastrum* L. (Ficoidaceae). It is a pantropical weed, common on plains, along the roadside, moist areas, and towards the coast. Many researchers consider it as 'Shwet Punarnava.' *T. portulacastrum* is a rich source of various phytochemicals viz. alkaloids, steroids, flavonoids, saponins, and glycosides, etc. It is used in the traditional system of medicine due to its significant pharmacological activities such as hepatoprotective, anti-fertility, anti-inflammatory, diuretic, etc. TLC studies were carried out by employing a solvent system such as chloroform: methanol (80:20) using UV 254 nm and 366 nm, iodine, Dragendorff's reagent, and 1%Vanilline-50%phosphoric acid visualizers. In view of the therapeutic importance of this plant and its potential to substitute *B. diffusa*, the quality control parameters viz. macroscopic, microscopic, ash values, extractive values, phytochemical screening, and chromatography have been evolved. The physicochemical constants evolved in the present investigation will serve as the parameters for quality control and fingerprinting for plant-based pharmaceutical and cosmetic market products.

Keywords: Hepatoprotective, quality control, pharmacognostic standardization, physicochemical evaluation, Shwet Punarnava, *Trianthema portulacastrum.*

I. INTRODUCTION

Trianthema portulacastrum. *L. Syn. T. monogyna L., T. obcordata Roxb.* belongs to Ficoidaceae**.** In Greek tri = three and anthemis = a flower; flowers are usually clustered in threes and hence the name *Trianthema*. It is known as **'**Horse purslane' or Shweta Punarnava. It is a prostrate, succulent, branched herb with white flowers. It is pantropical in distribution and found throughout Srilanka, Malaya, Western Asia and tropical Africa. It is common in South India, Maharashtra, Gujarat, Rajasthan, Uttar Pradesh, Bengal, Haryana, etc. It is a weed in the cultivated fields, wastelands, and in dried beds of nala. It is abundant during the rainy season. *B. diffusa*, *B. erecta*, *B. repanda*, and *T. portulacastrum* are considered as Punarnava. There is a controversy regarding the real identity of 'Shwet Punarnava'[1-3]. In Ayurveda, Punarnava is used in Ayurveda, Siddha and Unani systems of medicine. In Shwet, Purnarnava is considered more potent than Rakta Punarnava.

The Shwet Punarnava is equated with *Trianthema portulacastrum* L. While some of the research workers also consider *B. erecta* as Shwet Punarnava as it possesses 'white flowers. *T. portulacastrum* L. is used as an adulterant or substituted in market samples of Punarnava. *T. portulacastrum* L. has important therapeutic properties viz. hepato-protective diuretic, anti-fertility, anti-inflammatory [4-7]. *T. portulacastrum* L. possesses alkaloids, carbohydrates, steroids, phenolic compounds, tannins, etc. [8].

Thin Layer Chromatography (TLC) is mainly used for the detection, standardization, and determination of the ingredients, adulterants or substitutes for material under consideration. TLC can be used for the standardization of Ayurvedic drugs [9]. So in the present investigation using extracts in ethanol and petroleum ether TLC fingerprints have been developed. The spots have visualized using ultraviolet (254 nm and 366 nm wavelengths), Iodine, Dragendorff's reagent, and 1%Vanilline-50% phosphoric acid to detect the complete array of chemicals. Despite the paramount importance of this technique, very few workers [10-12] have carried out TLC studies in Punarnava species. In view of the therapeutic importance of T. *portulacastrum* and its value in the pharmaceutical industry, a detailed morphology, anatomy, pharmacognosy, phytochemistry, and chromatographical studies have been carried out. The physicochemical constants serve as parameters for quality control and fingerprinting.

II. MATERIALS AND METHODS

Plant material: Fresh plant material of *Trianthema portulacastrum* was collected from Pune and other districts of Maharashtra viz. Dhulia, Jalgaon, Nasik, Sindhudurg and Raigad. The bulk quantity of material was collected in the monsoon period. The authentication of the *Trianthema portulacastrum*. was done from the Botanical Survey of India, Western Zone, Pune. Hand sections of plant material were used for histochemical studies.

Macroscopic characteristics: The morphological characteristics and organoleptic characteristics of root were recorded.

Microscopical characterization:

Sectioning: The standards methods were followed for organoleptic evaluation; the standard methodology was followed.

Photomicrograph: Microscopic descriptions of the selected tissues were supplemented with micrographs.

Physicochemical evaluations: Physicochemical parameters of *Trianthema portulacastrum* root powder were determined as per standard methods. Total ash, water-soluble ash, acid-insoluble ash, and sulfated ash values were worked out. Alcohol and water-soluble extractive values were determined to find out the amount of water and alcohol-soluble components

Root powder: It was sieved (# 60 mesh sieve) separately, cleared in chloral hydrate and mounted in glycerin for further observations. The qualitative analysis of powders was carried out as per the method described by Trease and Evans [13]. The colours recorded during organoleptic evaluation and fluorescence analysis are confirmed from 'A Mycological colour chart' of Rayner [14].

Ash values and extractive values: Total ash, water-soluble ash, acid-insoluble ash, and sulfated ash values were worked out. Alcohol and water-soluble extractive values were determined to find out the amount of water and alcohol-soluble components [15].

Histochemical Studies: Carried out as per the standard protocol [16].

Preliminary phytochemical screening: The coarse root powder of *T. portulacastrum* (25 g) was subjected to Soxhlet for successive solvent extraction. The extract was subjected to various chemical tests to detect the presence of different phytoconstituents.

Thin Layer Chromatography studies: The standard methods followed [17]. TLC studies have been carried out employing solvent systems viz. chloroform: methanol (80:20). TLC plates were developed in Camag Twin Developing chamber, and plates were observed under Camag UV cabinet at 254 nm and 366 nm. TLC plates were developed in iodine, Dragendorff's reagent, and 1%Vanilline-50%phosphoric acid.

III. RESULTS AND DISCUSSION

Root:

Figure 1.A. *T. portulacastrum* habit Fig. 1.B Root of *T. portulacastrum*

Table 1. Characteristic features of *T.portulacastrum* root

Parameters	*Peculiarities*
Type	Small, conical, cylindrical, woody, extensive lateral roots.
Root colour	Whitish
Dimensions (cm)	4.5-16.97-30.0 X 0.12-0.3-0.6

Microscopical studies:

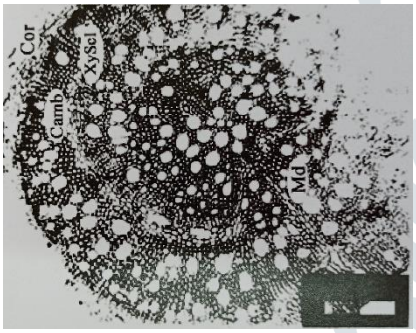

Figure 2. *T. portulacastrum* root (400X)
Md-Medullary rays, XyScl-Xylem sclerenchyma, Cam-cambium, Cor-Cortex

Table 2. Anatomical peculiarities of *T. portulacastrum* root.

Parameters	*Peculiarities*
Cork	1-3 layered
Cortex	4-5 with layered polygonal cells
Phloem	Forms the narrow strips on the outer side of xylem
Xylem	Vessels in radial rows, Polygonal to oblong in shape.
Medullary rays	Uniseriate with squarish cells and prominently seen in T.S.
Pith	Scanty, Pith is crushed by secondary growth at maturity
Cell contents	Abundant starch grains and rhomboidal or prism-shaped crystals.

Table no. 3. Characteristics of Starch grains of *T.portulacastrum*

Features	*Peculiarities*
Type of starch grains	Simple and compound
Shape (single)	Ovoid or polyhedral
Dimensions (single)	7.0-10.12-12.0 µ X 8.0-9.87-13.0 µ
Hilum	Line
Striations	Faintly marked
Aggregation	Few compound

Root powder of *T. portulacastrum:* The microscopical examination of root powder shown characteristic abundant prismatic or rhomboid crystals of calcium oxalate in the cortex region

Organoleptic characteristics: These characteristics are essential in the identification of Ayurvedic drugs. However, these characteristics of the crude drug are very subjective.

Table 4. Organoleptic characteristics of *T.portulacastrum* root powder

Organoleptic characters	observation
Colour	Gray
Smell	Indistinct
Taste	No specific taste
Feel	Slightly coarse

The peculiarities of starch grains and calcium oxalate crystals serve as the diagnostic characters for the identification and differentiation of the closely related Punarnava species. Starch grains are distinct in their type, dimensions, hilum, and striations. Calcium oxalate crystals are present in prismatic or rhomboidal forms. The prismatic crystals are with 11.0-21.76-40.0 µ X 8.0-14.11-35.0µ dimensions have been recorded. However, raphides were not recorded in the root power.

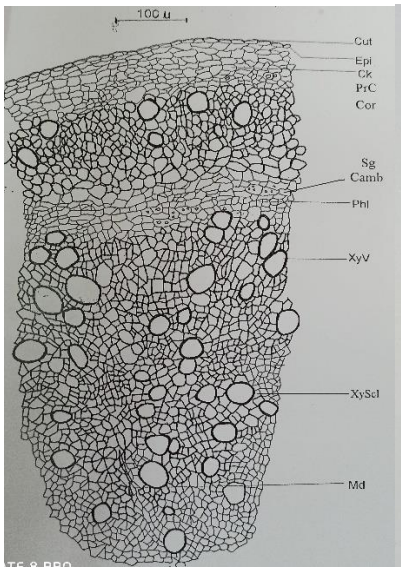

Fig.3 T.S. root (diagrammatic)

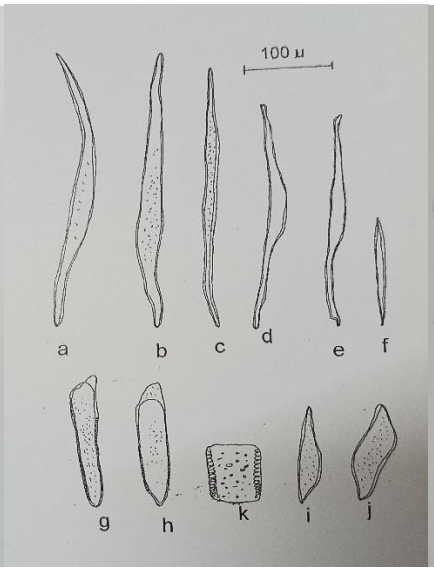

Fig. 4 Maceration studies
a-f-fibres;g-i-tracheids,j-k-vessels
i-rayparenchyma

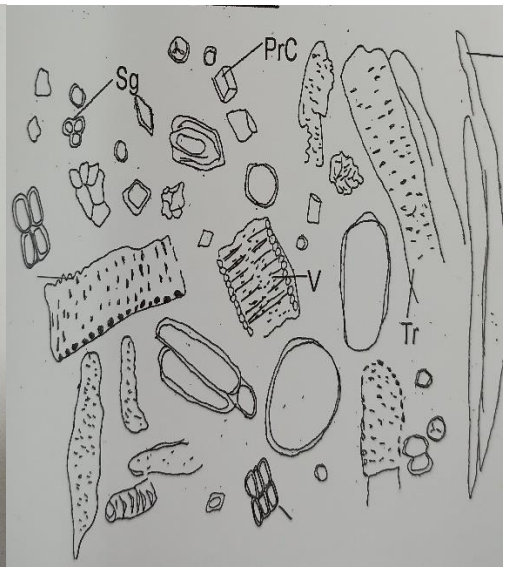

Fig.5 Root powder(Ck-cork,Rh-raphides,Fbr-Fibres,Tr-tracheids

Table no. 5. Maceration studies of *T. portulacastrum*

Elements	Dimensions
Vessel elements (µ) with reticulate thickenings	70.0-140.7-160.0 X 25.0-32.1-65.0
Tracheids (µ)	80.0-146.0-173.0 X 12.0-15.6-25.0
Xylem fibres (µ)	120.0-240.0-293.0 X 10.0-15.8-25.0 µ
Xylem parenchyma (µ)	100.0-120.2-140.0 X 35.0-40.0-45.8

Table no. 6. Fluorescence Characteristics of *T. portulacastrum*(Root powder)

Mountant medium	**Fluorescence colour**
P+ nitrocellulose in amylacetate (A)	Luteous
P+ 1N NaOH in MeOH + nitrocellulose in amylacetate (B)	Hazel
P+ 1N NaOH in MeOH	Isabellite
P+ D.W.	Flax blue

Fluorescence analysis was used for quick identification of powers. However, it has limited applications in drug evaluation, and it can be used as an additional parameter for the differentiation of closely related species.

Ash analysis and extractive values: Physical evaluation of drugs is an important parameter in detecting impurity, improper handling of drugs, or adulteration. The total ash is particularly important in the evaluation, for the purity of the drugs and % of the absence of foreign inorganic matter such as metallic salts and silica. Ash analysis and extractive values have been recorded in Table no. 7 & 8

Table 7 Ash values of *T.portulacastrum* **roots**

Ash peculiarities	Values w/w
Total ash	14.76(14.65-14.81)
Water-insoluble	5.50(5.39-5.65)
Acid insoluble	1.00(0.95-1.13)

Table 8 Extractive values for *T.portulacastrum* **root**

Extractives	Values w/w	Colour
Petroleum ether (60-80^{0}C)	0.10 (0.08-0.12)	Buff
Ethanol	1.17(0.77-1.73)	Buff
Water	6.59 (5.59-7.06)	Pale luteous

Table no.9. Histochemical tests for *T. portulacastrum* root

Chemical	*T. portulacastrum*
Lignin	Cu, P.Xy, S.Xy
Cellulose	Ck, Cor, P., Md.
Suberin and cutin	Cu., Ck.
Chitin	-
Mucilage	-
Starch	Hyp., Cor., P.
Proteins	Pd., Cor., Phl.Par., Xy.
Fats and oils	Cor.
Tannins	-
Alkaloids	Epi.
Saponins	-
Flavonoids	-
Steroids and terpenes	-

Ck-cork; **Cor**-cortex; **Cu**-cuticle; **Epi**-epidermis; **Hyp**-hypodermis; **Md**-medullary rays; **P**-pith; **Pd**-phelloderm; **Phl.par**-Phloem parenchyma; **P.xy**- Primary xylem; **S.xy**-Secondary xylem; **Xy.par**-xylem parenchyma; **Xy**-xylem.

Chitins, mucilage, tannins, saponins, flavonoids and steroids and terpenes were not detected in *T. portulacastrum*

Phytochemical studies: Refer Table no. 10 & Table no.11.

Table no. 10 Microchemical tests for root *portulacastrum* root

Phytochemical	**Detection**
Carbohydrates	+
Starch	+
Reducing sugars	+
Pentoses	+
Proteins	-
Amino acids	-
Fats/ oils	+
Tannins	-
Alkaloids	+
Glucosides	+
Flavonoids	+
Resins	-
Steroids and terpenes	+

Table no. 11. Proximate analysis of *T.*

Constituent	%
Total carbohydrates	70.700
Total proteins	6.740
Total fats	0.348
Total crude fibres	6.557
Total ash	14.760
Total percentage	0.895

'+' indicates the detection of phytochemicals. '-'indicates the phytochemical is not detected.

Phytochemical analysis of *T. portulacastrum* root showed the presence of alkaloids, glycosides, steroids, and terpenes and sugars.

TLC studies of *T.portulacastrum*: TLC studies have been recorded for the *T. portulacstrum* root using ethanol and petroleum ether extracts with chloroform: methanol (80:20) system for UV developers, iodine, and 1% vanillin-50% phosphoric acid has been recorded in Table no. 11

Table 11. TLC profile of *T.portulacastrum* extract (ethanol)

Spot no	***Rf value**	254nm	**366nm**	Iodine	1%Vanillin- 50% phosphoric acid	Dragendorffs reagent
1	0.30	-	-	-	purple	-
2	0.37	intense blue	faint blue	-	-	-
3	0.47	-	-	yellow	faint purple	-
4	0.56	-	faint blue	-	-	-
5	0.65	faint blue	-	-		-
6	0.70	-	-	-	purple	-
7	0.74	-	-	yellow	-	-
8	0.83	intense blue	faint blue	-	-	orange
9	0.96	intense blue	intense blue	-	-	-
10		-	-	-	yellow	-

TLC conditions:
Solvent system: **Chloroform: methanol (80:20)**
Silica layer G of 0.3 mm thickness.
Activation Time: 60 min.

Saturation time: 40 min.
Temperature: 28-30^{0}C
Plates were observed under the CAMAG UV cabinet.
*Mean of 10 observations.

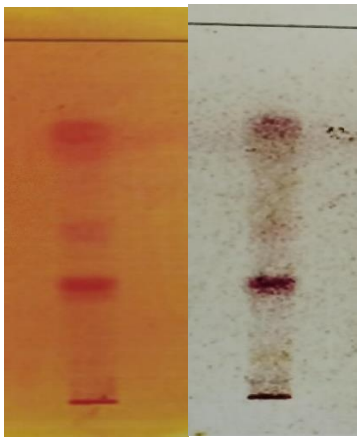

Fig.6 Fig. 7
Visualizer-Iodine Vanillin-phosphoric acid
Solvent system- Chloroform: Methanol (80:20)

TLC profiling of *T. portulacastrum* studies in chloroform: methanol (80:20) system recorded for Ethanol.

The present study on the pharmacognostic standardization and the physico-and phytochemical evaluation of *T. portulacastrum* will be useful to supplement the information about its identification parameters, which are assumed significant for the acceptability of herbal drugs in the present scenario. The results depicted in present research work is for the identification, authentication, and detection of adulteration, as also for the compilation of quality control standards for crude drugs.

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