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Standardization of pharmacognostic and preliminary phytochemical parameters of *Trianthema portulacastrum* Linn. Root: A halophytic plant

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Abstract
Medicinal plants are the major folk and traditional medicine for the prevention of diseases worldwide. *Trianthema portulacastrum* Linn. (Aizoaceae) is a small perennial weed, extensively used not only as medicine but also as vegetable for its various health benefits. The plant is reported to have analgesic, anti-pyretic and hepato-protective activity. Because of close morphological resemblance of the plant with crude drug "Punarnava", it is usually mistaken for the same. Pharmacognosy is an indispensable aid in standardization of herbal crude drug. In the present work, to ensure authenticity and maintain the therapeutic efficacy of the plant, evaluation of certain quality control parameters for the standardization of this plant was attempted. To achieve this, pharmacognostic studies of root of this plant was done. Pharmacognostical studies include systematics, macroscopy, microscopy, fluorescence study and physico-chemical analysis were determined. The extractive values in different polar and non-polar solvents were measured. For preliminary phytochemical analysis, as primary and secondary metabolites were evaluated along with HPTLC analysis. All the standard methods were followed for different estimations. The parameters evaluated in this study will safeguard the authenticity and efficacy of crude drug and also distinguish the drug from its adulterants. The observations of studied parameters will be useful and helpful in setting diagnostic indices for identification and preparation of monograph of the plant and in differentiating root of this species from closely related species of same genus and family.

Keywords: *Trianthema portulacastrum* Linn., biskhapra, aizoaceae, halophytic, adulteration, standardization, fluorescence study, HPTLC analysis

Introduction
There have been substantial contributions of plant resources to human welfare. Plants have been the source of remedies against different diseases from ancient time in many traditional medicine systems and continue to serve mankind with new remedies throughout the world (Shivanna & Koeteshwara 2009) [1]. The only shortcoming of traditional medicine is there are no stringent quality control parameters; in other words, there are no standardization parameters and hence they are prone to adulteration and substitution so puts doubt on their efficacy. Their chance of getting adulterated is directly proportional to their efficacy and availability. So, it is important to study pharmacognostic characters of each medicinal plant to differentiate the unadulterated plant sample (Singh & Prajapati, 2022 A) [2].

Trianthema portulacastrum Linn. belongs to the family-Aizoaceae. It is a prostrate, glabrous, branched annual herb with slightly fleshy leaves, found almost throughout India in cultivated and wastelands along road side and in fields. The whole plant is laxative, antipyretic, analgesic, antidote, astringent, appetizers, diuretic, expectorant, carminate, stomachic, antiasthematic and cure bronchitis, itche, piles, liver diseases, night blindness, chronic ulcer, skin diseases, blood disease and used in inflammation and rheumatism (Karim *et al*., 2015) [3]. The root is reported to be effective in eye corneal ulcers, itching, dimness of sight and night blindness, mild purgative or cathartic and abortion (Bereket & Tilahu, 2017) [4].

The only shortcoming of traditional medicine is there are no stringent quality control parameters; in other words, there are no standardization parameters and hence they are prone to adulteration and substitution and puts doubt on their efficacy. Their chance of getting adulterated is directly proportional to their efficacy and availability (Singh & Prajapati, 2022 B) [5]. So, it is important to study pharmacognostic characters of each medicinal plant to differentiate the unadulterated plant sample. Therefore, standardized procedures of crude drugs from different parts of medicinal plant are needed to ascertain its identity and to establish its quality, purity, different extractive values, content of moisture, fluorescence study and powder microscopy (Bhushan *et al*., 2015) [23].

There are many studies related to the therapeutic activity of this plant. However, phytochemical screening of different components in a wide range solvent system and development of quality control parameters for the standardization of different part specially root is still limited.

Thus, to ensure authenticity and maintain the therapeutic efficacy of this plant, evaluation of certain quality control parameters for the standardization of this plant was attempted. To achieve this, pharmacognostic, physico-chemical, phytochemical and TLC assay & HPTLC analytical studies of this plant were done. Systematics, macroscopic, microscopic and powder study of root was also done in detail. For physico-chemical analysis, loss on drying, total ash and acid insoluble was determined. The extractive values in different polar and non-polar solvents were measured. For phytochemical analysis, phenols, flavonoids, tannins, saponins, triterpenes, etc. were evaluated. All the standard methods were followed for different estimations.

Material and Methods

In the present investigation detailed pharmacognostical studies of plant genera *Trianthema portulacastrum* Linn., were undertaken and following methods were followed for investigation.

Collection of genuine plants material

Specimens of plant genera *Trianthema portulacastrum* Linn. were collected from their natural habitat. The plant were identified with the help of floras and by matching them with the type specimens deposited in the institute's herbarium. The herbarium specimens were made and deposited to national herbarium of National Botanical Research Institute (NBRI), Lucknow, India.

Botanical name: *Trianthema portulacastrum* Linn.
Family: Aizoaceae (Ficoidaceae)
Vernacular name: Bishkhapra
Place of collection: Biznour farm of NBRI, Banthra, Lucknow
Voucher No: 262528
Part used: Whole root

Processing of plant material for study

The plant materials were properly dried in shade at 40°c and powdered. The fresh material was preserved in FAA solution (formaldehyde: acetic acid: alcohol: water in a ratio of 10:5:50:35) for microscopic studies.

Studies of organoleptic characters

This study include surface markings, texture, fracture, internal appearance, cut surface, odour and taste of the crude drug.

Microscopic methods for herbal raw material

Microscopic evaluation deals with identification of the various characters of tissues, cells and cell contents by microscopic methods by preparing specimens of crude material. Microscopic studies vary, depending on the part used like, leaf, stem, root, bark, flower, and fruit and also on the nature of the material *i.e.* entire, cut or powdered.

A. Disintegration of hard and woody tissues

Cut the material into small pieces and transfers few pieces to test tube containing 4ml of dil. HNO_3 and heat to boiling. Add powdered potassium chlorate warm it gently and allow to react. Tissue starts to disintegration, when completely bleached. Apply pressure with glass rod for complete disintegration of the tissues. Allow the material to settle down, decant the liquid and wash the bottled material repeatedly with waters until the acidity is removed.

B. Preparation of sections

For microscopically studies, the sections were cut by the razor/ blade or through microtome and double staining were performed in safranin and hematoxylin. The sections of 13-18 µm thickness were taken from the plant genera. The permanent slides (T.S. /T.L.S.) were prepared by using dehydration method.

Maceration

To observe the shape, size and structure of isolated thick walled elements, small pieces of material are placed in a test tube and boil with 40% HNO_3 for 15-45 minutes. Wash thoroughly with water, place the material on the microscopic slide and then macerate with the help of a needle then add 1 drop of glycerol and 1 drop of safranin, cover with a cover slip.

Powder Studies

Different characters of powdered drugs like organoleptic characters *viz.* color, odour, fineness, degree of uniformity of the particles and sensation of smoothness were recorded. For examining characters of the powder, take sufficient amount of powder in chloral hydrate solution on a slide and cover it with a cover slip, warm over a low flame for a short time. Fluorescence test of powder (under UV light and visible light) were performed according to the method described by Chase and Pratt (1949) [7] and Kokoski *et al*. (1958) [8].

Physico-chemical parameters for the standardization of crude drugs

The physico-chemical analysis often plays an important role in herbal drug standardization. These tests are simple and quick to perform and give valuable information about the nature and purity of a crude drug. The values given in the results are replicate of six samples. The tests which are normally performed include:

A. Determination of foreign matter

Drug should be entirely free from visible sign of contamination by moulds or insects and other animal contamination. No abnormal odour, discoloration, slime or sign of deterioration should be detected. It is seldom possible to obtain marketed plant materials that are entirely free from harmful foreign matter or residue. Morphological examination can conveniently be employed for determining the presence of foreign matter in whole or cut plant materials. However, microscopy is indispensable.

Procedure

100-500g of the drug sample to be examined weighed it and spread out in a thin layer. Detect the foreign matter by inspection with the unaided eye or by the use of a lens (6 xs). Separate the other material weight it and calculated the percentage present. The amount of foreign matter shall not be more than the percentage prescribed in the pharmacopoeia (2%).

B. Determination of moisture content (loss on drying)

Determination of the amount of volatile matter in the drug is measure of loss on drying for substances.

Procedure
10 gram of drug were kept in oven at 100°c for 3h and made it moisture free, weighted till constant weight was attained and calculated the percentage of moisture by the following formula-

$$\text{Moisture percentage} = \frac{Pw - Fw}{W} x100$$

Where's,
Fw = Final constant weight of the sample
Pw = Pre weight of sample
W = Total weight of sample

C. Ash Value
Ash value is determined to estimate the total amount of the inorganic salts present in the drug. This includes total ash and acid insoluble ash.
(a) The total ash: Method is designed to measure the total amount of material remaining after ignition. This includes both "Physiological ash" which is derived from the plant tissue itself, and "Non physiological ash" which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface.

Procedure
Place 2 gm of ground air-dried material was accurately in a previously ignited and tarred crucible. Spread the material as an even layer and ignite it by gradually increasing the temperature not exceeding 450°c, until it become white, indicating the absence of carbon. Cool in desiccators and weight. If carbon free ash cannot be obtained in this manner, cool the crucible and moisten the residue with about 2 ml of water. Dry on a plate and ignite to constant weight. Allow the residue to cool in suitable desiccators for 30 minutes, then weight without delay. Calculate the content of total ash of air-dried material.

$$\text{Total ash percentage} = \frac{Pw - Fw}{W} \times 100$$

Where's,
Pw = Pre weight of crucible
Fw = Final weight of crucible
W = Total weight of powdered plant material

(b) Acid insoluble ash
Procedure
Boil the ash obtained as total ash with 25 ml of dilute hydrochloric acid in the crucible, cover with a watch glass and boil gently for 5 minutes. Rinsed the watch glass with 5ml of hot water and add this liquid in the crucible. Collect the insoluble matter on ash-less filter paper and wash with hot water until the filtrate neutral. Transfer the ash-less filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccators for 30 minutes, and then weight with delay. Calculate the content of acid-insoluble ash of air-dried material.

$$\text{Acid insoluble ash percentage} = \frac{FWb - FWa}{W} \times 100$$

Where's,
FWa = Final weight of crucible with acid insoluble ash
FWb = Final weight of crucible with total ash
W = Total weight of powdered plant material

D. Extractive values
It is the amount of soluble constituents (active or otherwise) extracted with solvents like alcohol, water, methanol, hexane and other solvents from a given amount of medicinal plant material. These are used to determine the amount of the matter, which is soluble in the solvents used; it includes alcohol soluble extractive, water soluble extractive, and hexane soluble extractive etc.

(a) Determination of alcohol soluble extractive
Procedure: Macerate 5 g of the coarsely powdered air- dried drug with 100 ml of alcohol in a closed flask for twenty-four hours, shaking frequently during six hour and loss of solvent. Take 25 ml of the filtrate in a tarred flat-bottomed shallow dish, evaporate and dry at 105 °C to constant weight. Calculate the percentage of alcohol soluble extractive with reference to the air-dried drug (Anonymous, 1966) [7].

(b) Determination of water soluble extractive
Procedure: Macerate 5 g of the coarsely powdered air-dried drug with 100ml of chloroform water (0.1%) in a closed flask for twenty-four hour, shaking frequently during six hours and allowing standing for eighteen hours. Filter rapidly, taking precaution against loss of solvent. Take 25 ml of the filtrate in a tare-bottomed shallow dish, evaporate and dry at 105°c to constant weight. Calculated the percentage of water–soluble extractive with reference to the air-dried drug (Anonymous, 1966) [9].

(c) Determination of successive soxhlet extractive values
Procedure: Extract 5g of the air dried coarsely powder drug exhaustively with hexane, chloroform, acetone, alcohol and water in a successive order. Collect the hexane, chloroform, acetone, alcohol and water soluble extractives obtained separately, concentrate and dry. Calculated the percentage of each extractive with reference to the air dried drug.

(d) Sugar estimation (Montgomery 1957) [10]: Total amount of sugar present in the drug can be calculated.

Procedure
Prepare 10 percent homogenate of the plant tissue in 80 percent ethanol. Centrifuge at 2000 rpm for 50 minutes. The supernatant obtained is made up to known volume (generally up to 10 ml or depending on the expected concentration of sugar). Take 0.1 ml aliquot and add 0.1 ml of 80 percent phenol and 5 ml conc. H_2SO_4. Cool and then read the absorbance at 490 nm. Calculate the percentage according to the absorbance.

$$\text{Total amount of sugar percentage} = \frac{3.1 \times \text{Absorbance}}{\text{Sample amount}}$$

Phytochemical screening / tests (Qualitative analysis)
Determination of various class of primary (carbohydrates, lipids, proteins, etc.) as well as secondary (alkaloids, glycosides, saponins, flavanoids, terpenoids, tannins etc.) metabolites was estimated. General screening of the alcoholic, aqueous and other extracts of the plant material is used for quantitative determination of the group of organic compound present in them. The preliminary phytochemical studies are used for testing the different chemical groups present in plant

extracts. 10% (w/v) solution of extract is taken unless otherwise mentioned in the respective individual test. General screening of the extracts of the plant material is used for qualitative determination of the groups of organic compound present in them.

A. Alkaloids

Dragendorff's test: Dissolve few mg of alcoholic or aq. extract of the drug in 5 ml of distilled water, add 2 M hydrochloric acid until an acidic reaction occur, then add 1 ml of Dragendorff's reagent, an orange or orange - red ppt. produced immediately indicate the presence of alkaloid.

B. Carbohydrates

Anthrone test: To 2 ml of anthrone solution, add 0.5 ml of aq. extract of the drug. A green or blue color indicates the presence of carbohydrates.

C. Flavonoids

Schinoda test: In a test tube containing 0.5 ml of alcoholic extract of the drug, add 5-10 drops of dil. hydrochloric acid followed by a small piece of magnesium. In the presence of flavonoids a pink, reddish pink or brown color is produced.

D. Triterpenoids

Liebermann -Burchard's test: Add 2 ml of acetic anhydride solution to 1 ml of petroleum ether extract of the drug in chloroform followed by 1 ml of conc. sulphuric acid through the side. A violet color colored ring formed indicating the presence of triterpenoids.

E. Proteins

Biuret's test: To 1ml of hot aq. extract of the drug add 5-8 drops of 10% w/v sodium hydroxide solution followed by 1 or 2 drops of 3% w/v copper sulphate solution. A red or violet color is obtained.

F. Resins

Dissolve the extract in acetone and pour the solution into distilled water. Turbidity indicates the presence of resins.

G. Saponins

In a test tube containing about 5 ml of an aqueous of the drug add a drop of sodium bicarbonate solution, shake the mixture vigorously and leave for 3 mnts. Honeycomb like forth formed indicates saponins.

H. Steroids

Liebermann-Burchard's test: Add 2 ml of acetic anhydride solution to 1 ml petroleum ether extract of the drug in chloroform followed by 1 ml of conc. sulphuric acid. A greenish color is developed which turns to blue.

I. Tannins

To 1-2 ml of extract of the drug add a few drops of 5% $FeCl_3$ solution. A green color indicates the presence of Gallo tannins while brown color indicates tannins.

J. Starch

Dissolve 0.015 g of iodine and 0.075 g of potassium iodide in 5 ml of distilled water and add 2-3 ml of an extract of drug. A blue color is produce.

Chromatographic Analysis

A. Thin layer chromatography (TLC)

Thin layer chromatography (TLC) is frequently used for the rapid and positive analysis of herbal medicines. The time required for the demonstration of most of the characteristic constituents by TLC is very short and in addition to qualitative detection, the TLC also provides semi- quantitative information on the chief constituents of the plant drug and thus enables an assessment of drug quality. It is a open bed technique in two phases a stationary phase acting through adsorption and a mobile phase in the form of a liquid. Identification can be effected by adsorption of spots of identical R_f value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

TLC is used for the separation of simple mixtures where speed, low cost, simplicity are required. It provides a chromatographic drug fingerprints. It is therefore suitable for monitoring the identity and purify of drug. In TLC the various steps involved are.

- Application of sample
- Chromatographic development
- Detection of spots
- Quantification
- Documentation

1. Application of sample- A known quantity of sample is dissolved in a known volume of solvent and the sample applied on percolated TLC plate in the form of a spot or a band.
2. Chromatographic development (separation) - Development of the chromatographic is affected after the solvent of the applied sample is completely evaporated. Rectangular glass chamber or twin through chamber is commonly used for TLC development.
3. Detection of spots- For detection of spot UV light is generally preferred.
4. Quantification and documentation- Densitometry is *in situ* instrumental measurement of visible UV absorbance, fluorescence quenching directly. The scanner convents the spot/band on the layer into a chromatogram consisting of peaks similar in appearance of HPLC.

The portion of the scanned peaks on the recorder chart is related to R_f value of the spots on the layer and the peaks light or area is related to the concentration of the substance on the spot.

B. High performance thin layer chromatography (HPTLC)

HPTLC is an advanced versatile chromatographic technique for quantitative analyses with high sample throughout and is complementary to HPLC/GLC. It provides a chromatographic drug fingerprint. It is therefore suitable for monitoring the identity and purity of drugs. In HPTLC the various steps involved are

- Application of sample.
- Chromatographic development
- Detection of spots
- Quantification
- Documentation

Applications of sample– An automatic applicator (Linomat) is used for sample application. A known quantify of sample is dissolved in a known volume of solvent and the sample on

percolated TLC plate either in the form of a spot or a band. However a band form is preferred because:

- Larger quantities of sample can be handled for application.
- Better separation because of rectangular area in which compounds are present on the plate.
- Response of densitometry in better due to variable concentration of substances in a spot.

1. **Chromatographic development (separation):** Development of the chromatogram is affected after the solvent of the applied sample is completely evaporated. Rectangular glass chambers or twin trough chambers are commonly used for TLC development.
2. **Detection of spots:** For densitometry scanning, detection under UV light is generally preferred. But post chromatographic derivatisation reactions are essentially required for detection when individual compounds does not respond to UV light or do not have intense fluorescence.
3. **Quantification and Documentation:** Densitometry is *in situ* instrumental measurement of visible, UV absorbance, fluorescence quenching directly. The scanner converts the spot/band on the layer into a chromatogram consisting of speaks similar in appearance to HPLC. The portion of the scanned peaks on the recorder chart is related to R_f values of the spots on the layer and the peak height or area is related to the concentration of the substance on the spot.

Result

Systematics

Botanical name: *Trianthema portulacastrum* Linn.
Family: Aizoaceae (Ficoidaceae)

Vernacular names

Hindi – Pathar Bishkharpra, Saphed Punarnava, Sweet punarnava, Lalsabuni
Sanskrit – Punarnavi
Bengali – Sabuni
English – Hoase Purslane
Kannade – Sihi Punarnava, Muchchugane
Malayalam – Jamizhama, Thazuthama
Marathi – Sweta Punarnava
Oriya – Sweta Purni, Gothapurni
Punjabi – Sanaya
Tamil – Saranai
Telgu – Galijeru
Urdu – Bishkhapra

Identification

Plants with seeds, ovules enclosed within the ovary Angiosperms
Venation reticulate, flowers pentamerous Dicotyledonae
Petals free, thalamus cup-shaped, ovary inferior Calyciflorae
Calyx tube elongated, stamens inserted on the calyx tube Aizoaceae
Ovary and capsule 1-2 celled *Trianthema*
Style-1, calyx tube scarious thin closely sheathed by the base of the petiole *portulacastrum*

Taxonomic description of the plant

A brief taxonomic description of the plant is as follows.
Habit: A decumbent much branched herbs.
Habitat: Found throughout India, in plains as a weed in waste lands.

Root

Root mostly thin, twisted, slender, tap root, 5–15 cm in length, 0.3–2.5 cm in diameter with several lateral rootlets, light yellow externally.

Stem

Stem cylindrical, dichotomously branched, prostrate, somewhat glabrous, at places reddish tinted, nodes swollen, fresh stem succulent.

Leaf

Leaves entire, wavy with reddish and papillose border, sub-fleshy, obliquely opposite, unequally paired, exstipulate, large leaves obovate to obcordate, 2–4 × 2–2.3 cm, the smaller one narrow oblong and tapering to the base, rounded at the apex.

Flower

Small, solitary, sessile, pinkish, nearly concealled by the pouch of the petiole, calyx tube scarious, thin, stamens 10–15, ovary superior, sessile, style single papillose, shorter than the stamens.

Fig 1: Plant of *Tarianthema portulacastrum* Linn.

Fruit: Capsule 3–5mm long beak like upper part.
Seed: 2–3 reniform, blackish rough seeds.
Flowering & Fruiting: August (rainy season).
Macroscopy of the crude drug: Drug consists of the dried root of *Tarianthema portulacastrum* (Aizoaceae). Following are the morphological standards of the dried root crude drug.
Occurrence: Roots
Size: 5–15 cm length 3–7 cm in diameter
Shape: Tap root
Color: Light yellowish gray
Surface markings: Wrinkles
Texture: Short, fiberous, uneven
Internal appearance: Creamish-white
Cut surface: Cream colored, smooth, ringed
Odour: Not characteristic
Taste: Not characteristic

Mircroscopic study of root

Mature root shows anomalous secondary growth. The outer most layer of the cork is somewhat obliterated fallowed by three or four rows of closely arranged, thin walled cubical to

tangentially elongated rectangular cells. Distinct cork is not clear. Underneath this lies a narrow zone of cortex, composed of fairly large, oblong or polygonal elongated thin walled parenchymatous cells. A few cells containing groups of prismatic crystals of calcium oxalate, a few rows of polygonal, thin walled, parenchymatous cells occur in rings, medullary rays prominent in middle of the corticle region and in the second or third vascular bundle ring, centre mostly occupied by a single vascular bundle stand with two isolated group of phloem. Traces of lateral branches are also observed at some places.

Powder studies

A. Organoleptic characters: Following are the organolaptic characters of plant root drug.

Color: Creamy yellow
Taste: Tasteless
Odour: Slight

C. Microscopic study

On powder microscopy of *Trianthema portulacastrum* root shows fragment of fibers, tracheids, vessels with spiral scalariform and reticulates secondary wall thick wings, idioblast with single rosette crystal of calcium oxalate, fragments of cork and starch grains.

D. Fluorescence analysis

The behavior of the powdered drug with different chemicals reagents has been shown in the table.

Table 1: The behavior of the powdered drug with different chemicals reagents

S. No.	Treatment	Day light	UV – 254 nm	UV – 336 nm
1	Powder(P) as such	Creamy white	Brown yellow	Whitish yellow
2	P + NaOH in water	Yellow	Pale brown	Whitish yellow
3	P + 1N NaOH in methanol	Light yellow	Brown	Whitish yellow
4	P+50% KOH	Greenish yellow	Green	Whitish yellow
5	P+1N HCL	Pale yellow	Pale yellow	Whitish yellow
6	P+50% H2SO4	Pale yellow	Pale yellow	Greenish yellow
7	P+50% HNO3	Orange	Light green	Greenish yellow
8	P+ Conc. HNO3	Red	Yellow	Black
9	P+Acetic acid	Brown	Yellow	Black
10	P+ Conc. H2SO4	Black	Black	Yellow
11	P+ Iodine water	Pale yellow	Pale yellow	Black yellow

Physico–chemical studies

The different physico–chemical values obtained are recorded for identity, purity and strength.

Table 2: The different physico–chemical values obtained are recorded for identity, purity and strength

S. No.	Parameter	Range (in percentage)	Mean (in percentage)
1	Foreign matter	1.20-1.40	1.30
2	Moisture content	8.63-11.85	10.24
3	Total ash	7.96-8.78	8.37
4	Acid soluble ash	0.68-1.21	0.99
5	Hexane soluble extractive	2.60-3.80	3.20
6	Alcohol soluble extractive	2.88-3.32	3.10
7	Water soluble extractive	13.07-15.17	14.12
8	Sugar	0.330-0.643	0.450

Phytochemical studies

The preliminary phytochemical screening of whole root drug are recorded for different chemical groups present in different extractives are as follows.

Table 3: The preliminary phytochemical screening of whole root drug are recorded for different chemical groups

S.	Phytochemicals	Water	Alcohol	Chloroform	Acetone	Hexane
1	Alkaloids	+		+	+	
2	Carbohydrate			+		
3	Flavanoides					
4	Triterpenoids					
5	Protein	+	+	+		+
6	Resin	+		+		+
7	Saponins	+		+		+
8	Steroids		+			
9	Tannins		+			
10	Starch					

TLC Assay and HPTLC Analytical studies

Test solution– Extract 5 gm of powdered drug in soxlet apparatus with methanol. Filtrate and concentrate the methanolic extract. Take 10 mg of the residue and dissolve in 1 ml of methanol and use the same for TLC and HPTLC analysis of the drug.

Solvent system– Toluene: Ethyl acetate: Formic acid (8:2:05).

Procedure– Apply 10ml of the test solution on precoated silica gel 60 F254 TLC plate (E. Merck) of uniform thickness of 0.2 mm. Develop the plate in solvent system at distance of 8cm.

Visualization and Evaluation– Visualize the plate under UV light at 366nm (Fig.- 3&4) shows two fluorescence zones at Rf 0.1 and 0.45 which are not identical and corresponding to substituent's like *Boerhavia diffusa*, conforms in the variation of chemical contents.

Discussion

The use of herbs to treat diseases is almost universal. Herbal drugs are popular for their safe action. However, it may lead to adverse reactions at times. The major reason for this may be incorrect identification of the drug plant and adulteration due to it. So, it is of paramount importance to establish quality control pharmacopoeia standards for every herbal drug (Mukherjee, 2002) [24].

In the present study, pharmacognostic standards of root of crude drug with these parameters are very important because once the plant is dried and powdered it loses its morphological identity and is easily prone to adulteration. Pharmacognostic studies ensures plant identity, lays down standardization parameters which prevent the drug from adulterations. Such study helps in authentification of the plants and ensures reproducible quality of herbal products, which lead to safety and efficacy of natural products (Chandra, 2014 and Singh *et al*., 2017) [12-13]. Standardization

is a system to ensure that every packet of medicine that is sold has the correct amount and will induce its therapeutic effect. Taxonomy of medicinal plants gives a systematics idea about the plant. Organoleptic and macroscopic evaluation is a qualitative evaluation based on the study of morphological profile of the plant. The powder of root of *Trianthema portulacastrum* Linn. showed different colour behaviours recorded upon treatment with different chemical reagents under fluorescence analysis. It is simple, rapid pharmacognostic procedure, which is useful in the identification of authenticity of crude drugs and recognizes adulterants. In the fluorescence analysis, the plant parts or crude drugs are examined as such or in their powdered form with a number of various polar and non-polar reagents. It is a valuable analytical tool in the identification of plant samples and crude drugs (Denston, 1946) [14]. The fluorescence analysis of *Trianthema portulacastrum* Linn. displayed an array of colours that could be employed for identification of probable classes of compounds in the plant. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material in the visible range in day light. The ultraviolet light produces fluorescence in many natural products which do not visibly fluorescence in day light. Some of the substances may be often converted into fluorescent derivatives by using different chemical reagents though they are not fluorescent, hence we can often assess qualitatively crude drugs using fluorescence.

Evaluation of different physico-chemical parameters is an important tool in detecting adulteration or improper handling of drugs. Foreign matter should not be 2% as per pharmacopeia standards. Moisture content of drug should be at minimum level to discourage the growth of bacteria, yeast or fungi during storage. Low moisture content indicates the appropriate standard, quality and stability of plant material and can be considered in future study or application. The degradation of phyto constituents of the drug during storage dependent on the presence of water quantity in plant material (Evans, 2005) [15]. High water content may easily support the growth of fungal colonies and lead to interfere with the quality of drug easily (Longanatghan *et al*., 2017) [16]. The present study supported the previous investigations, as on drying of roots was reported as 10.24% (Pande *et al*., 2018) [17].

The ash value of roots of plant was determined by two different forms, *viz*. total ash and acid-insoluble ash. The ash values of the drug are also a significant parameter for the detection of nature of material, adulteration, impurities, authenticity of drug, quality and purity of the test sample. The total ash value indicates the impurities like carbonate, oxalate and silicate. The acid-insoluble ash is used to estimate the amount of silica present, especially sand which is the indication of contamination with earthy material (Rakholiya *et al*., 2016) [18]. Relatively less amount of these two parameters indicate low inorganic matter and silica were detected in roots of *Trianthema portulacastrum* Linn.

The extractive values of root of *Trianthema portulacastrum* Linn. are manifested and the highest extractive value was found in water followed by hexane and minimum extractive value in alcohol. The above results indicate the presence of higher amount of polar compounds than nonpolar. The amount of extract yield in a solvent system is often an approximate measure of the amount of certain constituents that drug contains. High water-soluble extractive value indicates the presence of acids, sugars and inorganic compounds and high alcohol soluble extractive value indicates the presence of polar constituents such as steroids, flavonoids and glycosides (Sharma & Pracheta 2013) [22].

The qualitative phytochemical analysis was done in crude powder is an important parameter of quality control. It reveal the presence of bioactive molecules which were known to possess presence of various types of phytoconstituents in different amounts that help in the selection of specific extract for isolating the active principle (Sampath Kumar *et al*., 2011) [20]. So, it is a necessary step in the study of pharmacognostic attribute of the plant that may be used in the protection against chronic diseases, pharmaceutical formulation and further research (Kasthuri & Ramesh, 2018) [21]. Phytochemical studies performed on different plant extracts reveal the presence of bioactive molecules which were known to possess medicinal and physiological activities. TLC and HPTLC analytical methods commonly applied for the identification, the assay and the testing for purity, stability, dissolution or content uniformity of raw materials extract and formulation products. HPTLC finger print profile helps in identification of various phytochemical constituents present in the crude drug thereby substantiating and authenticating of crude drug. The HPTLC profile also helps to identify and isolates important phyto constituents. These finding could be helpful in identification and authentication.

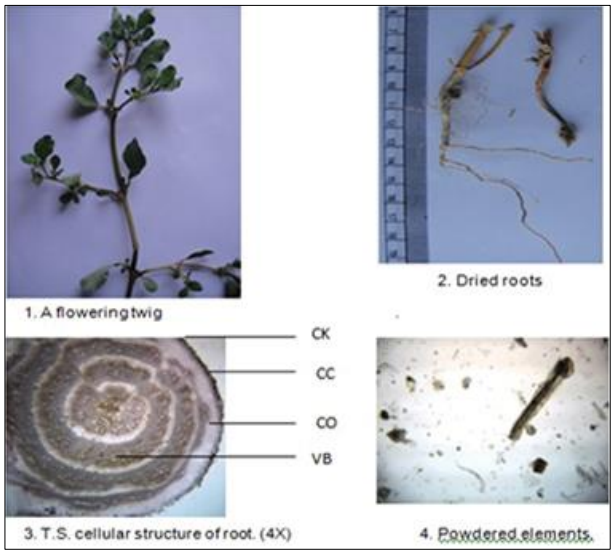

Fig 2: Macroscopic and microscopic characters of *Trianthema portulacastrum*. Linn.

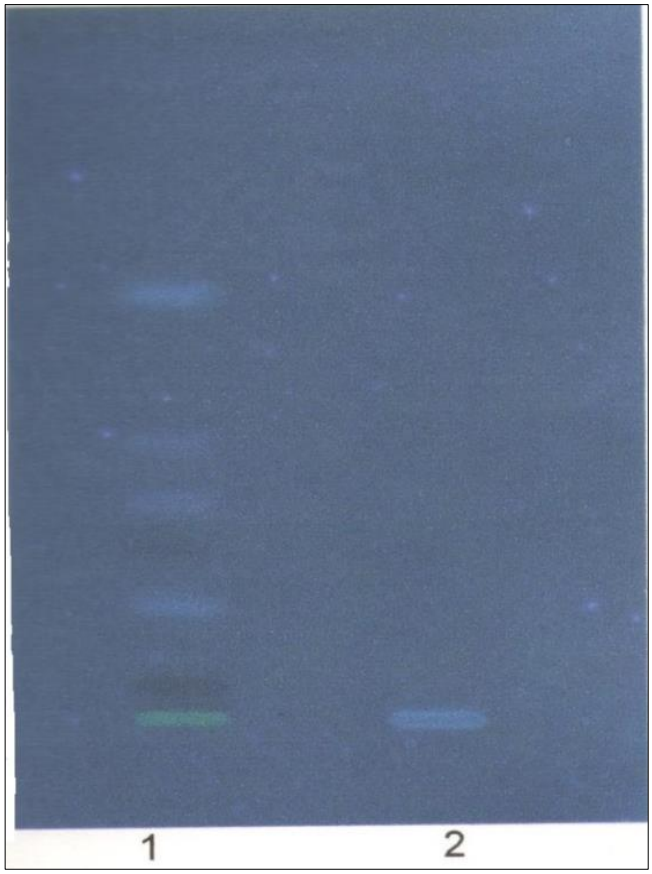

Fig 3: Comparative TLC profile of root test solution of *Boerhavia diffusa* (1) and *Trianthema portulacastrum* (2)

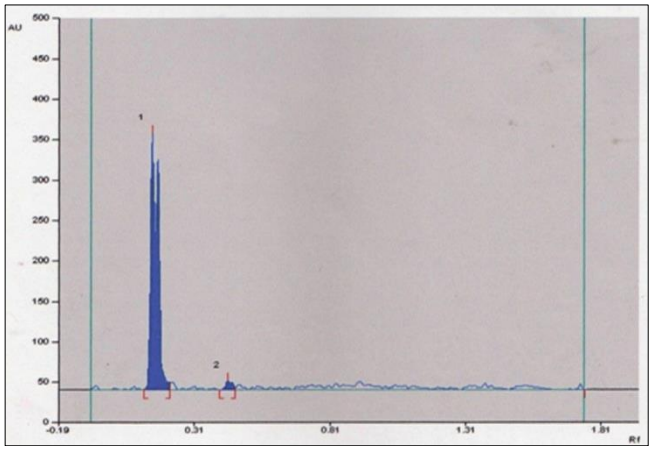

Fig 4: HPTLC chromatogram (densitometry scan at 366 nm) of *Trianthema portulacastrum* plant root extract

Abbreviations

% °C	Percentage - Degree centigrade	Dr. wt. g	Dry weight Gram
aq.	Aqueous	h	Hours
cm	Centimeter	l	Liter
conc.	Concentrated	ml	Milliliter
mm	Millimeter	sps.	Species
ppt	Precipitate	v/v	Volume per volume
s	Second	w	Weight
sq. mm	Square millimeter	w/v	Weight per volume
TLC	Thin Layer Chromatography	dil.	Dilute
µm	Micrometer	VB	Vascular Bundel
UV	Ultraviolet	CC	Cork cambium
v	Volume	CK	Cork
		CO	Cortex

Conclusion

The systematics, organoleptic, macro-microscopic characters, fluorescence studies, physico-chemical, phytochemical analysis results of this study could be used for the quality control of the crude drug. They will also help to maintain the efficacy and identity of the drug and will prevent mishandling of the drug. Therefore, the aim behind this study was to establish quality control, standardization and genuineness of drugs.

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