Development and standardization of quality control parameters of different parts of *Trianthema portulacastrum* L.

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Abstract

The present study about *Trianthema portulacastrum* (Linn.), family Aizoaceae, is an effort to evaluate physicochemical parameters, phytochemical screening, pharmacognostic properties of stem, root, leaf and seed and preparedness of different extracts by solvent system of wide-range polarity. Physicochemical analysis includes loss on dryness, total ash value, water-soluble ash value, acid-insoluble ash value, pH analysis, fluorescence study and extractive values in different solvent systems. All the parts of the plant showed loss on dryness in the range of 2 ± 0.69 to 7 ± 0.73 . Foreign organic matters recorded in different parts of plant were in the range of 0.8 ± 0.045 to 2.0 ± 0.081 . The total ash values were determined from 9.59 ± 1.87 to $15.54 \pm 1.21\%$ (w/w). The water-soluble ash values were from 7.5 ± 1.10 to $9.38 \pm 1.54\%$ (w/w), and acid-insoluble ash values were from 0.92 ± 0.84 to $4.19 \pm 0.75\%$ (w/w). Macroscopic, powder microscopy, fluorescence studies, extractive values, different properties of extracts and phytochemical screening studies were completed by a series of standard tests. The parameters evaluated under this study will safeguard the authenticity and efficacy of crude drug and provide referential information in distinguishing 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 this plant and in differentiating root, stem, leaf and seed of this species from closely related species of same genus and family.

Keywords Adulteration · Flavonoids · Macroscopic · Pharmacognosy · Phytochemical analysis · Standardization

1 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 [53]. There are many unwanted plants also called weeds which are very common, dominant and widespread in different crop fields and occupy open spaces [36]. These are produced in a large quantity and are competing to crops for nutrients, water and light. The estimated loss caused by weeds every year is more than 24%. Recently, many

phytochemicals from the weeds were investigated for their therapeutic potential against many chronic diseases [16].

About twenty species are reported in genus *Trianthema*; these are annual or perennial plants. They are usually characterized by fleshy, opposite, unequal, smooth-margined leaves, prostrate growth form, flower with five perianth segments, and subtended by a pair of bracts. Another important feature like superior fruit having a circumscissile capsule with a winged lid and net primary production represents the biomass which is incorporated into the plant parts during a specific time interval [51]. One of the novel plants of this genus is *Trianthema portulacastrum* Linn. which is a prostrate, diffuse, branched,

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glabrous, succulent annual herb and up to 30–65 cm length. This plant is commonly known as Biskhapra, galijeru, desert Horse Purslane, belonging to Aizoaceae family [6]. The plant and its different parts showed various applications in traditional system of medicine. The whole plant is used as laxative, antipyretic, analgesic, antidote, astringent, appetizer, diuretic, expectorant, carminative, stomachic, anti-asthmatic and cures bronchitis, itch, piles, liver diseases, night blindness, chronic ulcers, skin diseases, diseases of blood and heart and used in inflammation and rheumatism [26]. The leaves possess diuretic properties and applied for the treatment of strangury, jaundice and dropsy. The juice of the leaves dropped in the nostrils relieves migraine. The root is reported to be effective in eye corneal ulcers, itching, dimness of sight and night blindness, mild purgative or cathartic and abortion [18]. A study also reveals it as an antidote to alcoholic intoxication and is very effective in the reduction of diethylnitrosamine-induced hepatocarcinogenesis [4] and lowering effect of paracetamol- and thioacetamide-induced hepatotoxicity in rats as well as antioxidant activity [31]. Various parts of the *T. portulacastrum* extracts on experimental studies have been reported as antioxidant [62], antilithic, central analgesic, hepatoprotective, nephroprotective, antifertility, antihyperglycemic, antibacterial and antifungal, anthelmintic, and antihypolipidemic and anticancer activity [35].

For the past few years, scientists have changed their focus on drug development strategies to isolate and purify active biomolecules from plant sources, since these show lesser side effects in comparison with chemically synthesized drugs. Many bioactive molecules are qualitatively identified in different constituents of plant like alkaloids, flavonoids, phenols, saponins, terpenes and tannins that are the most significant [19]. Therefore, standardized procedures for the extraction 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 [9]. There are many studies related to the therapeutic activity of this plant. However, phytochemical screening of four different parts of *T. portulacastrum* in a wide-range solvent system and development of quality control parameters for the standardization of different part is still limited. This study investigates the microscopic study, pharmacognostical evaluation of plant and its fluorescence characterization, which aid in identification, adulteration and authentication of phytoconstituents that play a major role in the therapy of curing various disorders.

2 Materials and methods

2.1 Procurement and authentication

Trianthema portulacastrum L. plants were collected from local areas of Banasthali Vidyapith, Rajasthan, during December 2018. The geographical location of sampling site with latitude: 26°22'48" N, longitude: 75°51'19" E and 314 m elevation from sea level. These herbs were authenticated by ethnobotanist and deposited in herbarium at the department of Bioscience and Biotechnology, Banasthali Vidyapith, Rajasthan, and the authentication number is BURI-19086/2019.

2.2 Chemicals, reagents and solvents

All the chemicals, reagents and solvents used in this study were of analytical grade. The solvent included petroleum ether (PE), benzene (B), chloroform (C), ethyl acetate (EA), ethanol (E) and distilled water (AQ).

2.3 Processing, preservation and preparation of extracts

Fresh whole plant of *T. portulacastrum* L. was washed under running tap water and blot-dried. The plant material was separated into leaf, root, seed and stem, further shade-dried for nearly 3 weeks and pulverized into powder. Powder was passed through the #40 mesh sieve and stored in airtight closed container for further analysis. All four parts of plant powder were used for the successive solvent extraction process by Soxhlation method. About 50 g of the dried pulverized material was uniformly packed into a thimble and extracted with 250 ml of different solvent systems of increasing polarity, using by hot continuous extraction method in Soxhlet apparatus at a temperature of 30–85 °C. The Soxhlet extraction continues till the solvent in the siphon tube of an extractor become colorless [61]. The solvents used sequentially are petroleum ether, benzene, chloroform, ethyl acetate, and ethanol and were finally macerated with distilled water. Shade-dried powder was extracted with water by macerating 50 g in 250 ml for 1 week with occasional stirring. Each time the pulverized drug material was dried in open air and subjected to further extraction. At the end of effective extraction, macerated and Soxhleted extracts were filtered using Whatman filter paper No. 1. Finally, the excess solvent was evaporated to solventlessness at 37 °C under reduced pressure (– 760 mmHg) on a rotary evaporator (HEIDOLPH, Germany) to produce a sticky material and stored at 4 °C into sterile vials for their pharmacognostical,

phytochemical and physiochemical screening [33]. The percentage of extractive yield is calculated by formula [30] as mentioned below.

$$\% \text{ Extraction yield (w/w)} = \frac{\text{Weight of dried extract}}{\text{Weight of the Sample used for extraction}} \times 100$$

2.4 Organoleptic evaluation

Organoleptic evaluation is an important step for the identification of any adulterant in the medicinal plant. It refers to sensory evaluation, and the characteristics are evaluated such as color, odor, size, taste, shape and texture [29].

2.5 Powder microscopy

The dried powders of root, stem, leaf and seeds were observed under high-power (10X, 40X) microscope (Metzer) with stain (safranin and fast green) and without stain, mounted in glycerin, and various diagnostic characters such as stoma, trichomes, lignified fibers, xylem, sclereids are studied [21, 29, 40].

2.6 Physicochemical analysis

The physicochemical parameters such as percentage of total ash value, acid-insoluble ash, water-soluble ash value, extractive values, foreign organic matter, loss on dryness (LOD), pH analysis and fluorescence study are the deciding factors to evaluate purity, quality and adulteration of

crude drugs. All the analysis was performed according to WHO guidelines [3, 12].

2.7 Phytochemical screening

Preliminary qualitative phytochemical screening of different solvent extracts of crude powder of four different parts of *T. portulacastrum* was carried out to identify different phytoconstituents by using standard conventional procedures [47]. The presence and absence of phytochemicals are indicated with signs “+” and “–”, respectively.

3 Results and discussion

3.1 Organoleptic characters

Organoleptic analysis helps to assess the physical and chemical quantity of plant sample. *T. portulacastrum* L. grows in a variety of habitat as weed. It is an annual herb-forming prostrate mat or club with a meter-long stem. It has glabrous surface and smooth slightly succulent texture, and features are represented in Table 1 and Fig. 1.

3.2 Powder microscopy

The fine powder of root, stem, seed and leaf was mounted in glycerin and treated with lactophenol before staining with iodine, phloroglucinol + HCl and Sudan III. Various micro-morphological features observed in different parts of plant powder and revealed numerous square-, prismatic-, polygonal-, rosette-shaped calcium oxalate crystals in root, stem, leaf and seed powders. Different arrangements of trichomes are observed with xylem vessels and

Table 1 Organoleptic and morphological features of *T. portulacastrum* L.

Parameters	Observations
Color	Dark green areal surface with pink to red margins leaf
Taste	Leaf—Bitter, root—faint, stem—bitter
Odor	Aromatic
Shape	Succulent, glabrous, pubescent herb with spread branches
Leaf	Subfleshy, obliquely opposite unequal or subopposite, 1.2–3.5 × 0.8–2.2 cm, stipulate with truncate base, entire wavy margin and apiculate apex
Size	Middle leaflet and 6–12 cm long
Stem	Green to brown, a meter long
Stomata	Anomocytic
Flowers	7 mm across, axillary, solitary, sessile, calyx tube 1.5 mm long
Seeds	Black with muricate concentric lines, one seed may be carried on the detached cap of the fruit, other seeds may fall out of the remaining part of the fruit or remain on the plant after it dies

Fig. 1 a–c, i Plant, e, h leaf, f stem, d, g root—organoleptic and morphological features of *T. portulacastrum* L.

lignified fibers. Powders also manifest the presence of various lactiferous granules, polyhedral or sharp angles starch grains in stem, root and leaf and oil bodies and aleuronic grains in seeds. Epidermal cells, stomatal openings, spongy parenchyma, vittae-volatile contain schizogenous cells; somewhat irregular shaped parenchymatous cells were also observed in leaf powder microscopy, as represented in Fig. 3.

3.3 Physicochemical analysis

Evaluation of different physicochemical parameters is an important tool in detecting adulteration or improper handling of drugs. Physicochemical characterization of powder of studied parts of *T. portulacastrum* L. is represented in Figs. 2, 4.

3.4 pH analysis

The pH values of different parts of the plant powder of 1.0% (w/v) were slightly acidic in the range of 6.0 to 6.8,

for root 6.8, for stem 6.1, for leaves 6.0 and for seeds 6.4. The pH-acceptable range of fruits, vegetable, grasses, flowers, trees, shrubs and annual is 4.0–7.5, while that of food is given as pH 2.0–9.0 [59]. According to various guidelines, biopharmaceutical characteristics of drugs are depend on acid/base properties, and moreover, absorption, distribution, metabolism, extraction and toxicity (ADMET) are profoundly affected by the charge state of compounds under varying pH conditions. Indeed, acid/base character affects drug potency and selectivity and has a great impact on both pharmacokinetic and biopharmaceutical properties [34] (Fig. 3).

3.5 Loss on drying (LOD)

The percentage of weight loss on drying or moisture content at 105 °C of root, stem, leaf and seed is, respectively, 6.2 ± 0.58 , 7 ± 0.73 , 5.7 ± 0.43 and $2 \pm 0.69\%$ of *T. portulacastrum* L. Moisture content of drug should be at minimum level to discourage the growth of bacteria, yeast or fungi during storage. Low moisture content

Fig. 2 Shade-dried parts of *T. portulacastrum* L. a Root, b stem, c leaf, d seed and powdered form, e root, f stem, g leaf, h seed

Fig. 3 Powder study *T. portulacastrum* L. Root—(a) calcium oxalate crystal, (b) lignified cells, (c) reticulate xylem vessels, (d) spiral xylem vessels, (e) lignified cells with spiral vessels, (f) starch granules); stem—(g) starch granules, (h) calcium oxalate crystals, (i) phloem fiber, (j) trichome, (k) cork cells, (l) lignified cells, (m) spongy cells, (n) embedded

Ca-oxalate crystals); seed—(o) Ca-oxalate crystals, (p) epidermal fiber, (q) trichome, (r) polygonal cellulosic oil globules and aleurone grains); leaf—(s) Ca-oxalate crystals, (t) spongy parenchyma, (u) stone cells, (v) cork cells, (w) lignified cells, (x) spiral xylem vessels, (y) starch granule, (z) trichome)

Fig. 4 Evaluation of different physicochemical properties of *T. portulacastrum* L.

indicates the appropriate standard, quality and stability of plant material and can be considered in future study or application. If the drying process is not efficient, it may lead to the degradation of phytoconstituents of the drug during storage [17]. Deterioration period of the crude drug powder is dependent on the presence of water quantity in plant material. High water content may easily support the growth of fungal colonies and lead to interfere with the quality of drug easily [33]. The

present study supported the previous investigations of same plant where loss on drying was reported as 9.5% [40]. The present investigations made on each part of *T. portulacastrum* L., where minimum LOD was found in seed with respect to root, stem and leaf. This indicates that drying process was efficient, loss on drying for leaves of 10.7% for *Gynura segetum* [49] and 8.8% for *Garcinia indica* [44].

3.6 Foreign matter

Foreign organic matters recorded in the powder of four different parts of plant under study were 0.8 ± 0.045 , 1.2 ± 0.75 , 1.9 ± 0.089 and 2.0 ± 0.081 for root, stem, leaf and seed, respectively. In the present study, the amount of foreign organic matter detected was low as per WHO [60].

3.7 Ash values

The ash value of all the four parts of plant under study was determined by three different forms, viz. total ash, water-soluble ash and acid-insoluble ash, and the results are presented in Fig. 4. The total ash values for root,

stem, leaf and seed were determined as 15.54 ± 1.21 , 15.54 ± 1.21 , 9.59 ± 1.87 and $11.41 \pm 1.91\%$, respectively. The water-soluble ash values were 7.5 ± 1.10 , 8.83 ± 1.32 , 9.38 ± 1.54 and $7.64 \pm 1.29\%$ for root, stem, leaf and seed, respectively, while, acid-insoluble ash values for root, stem, leaf and seed were 3.74 ± 0.85 , 4.19 ± 0.75 , 1.68 ± 0.92 and $0.92 \pm 0.84\%$, respectively. The ash values were in accordance with earlier study about this plant and comparable to such studies for other plants. Total ash value was reported as 11.83%, water-soluble ash as 1.5% and acid-insoluble ash as 0.83% [40] and total ash value 22%, water-soluble ash value 13% and acid-insoluble ash value as 2% [2] for leaf *T. portulacastrum*. The present investigation also supports the earlier reports on *Boerhavia diffusa* L. [55]. 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 content of inorganic compounds in the drug is represented by the water-soluble ash values. The acid-insoluble ash is used to estimate the amount of silica present, especially sand which is the indication of contamination with earthy material [46]. Relatively less amount of these three parameters indicate low inorganic matter and silica were detected in root, stem, leaf and seed powders of *T. portulacastrum* L.

3.8 Extractive values

The extractive values of different parts of *T. portulacastrum* are manifested in Fig. 4. The highest extractive value was found in water followed by alcohol and minimum extractive value in petroleum ether. So far, as the different parts of plant are concerned, stem shows the highest water extractive value followed by root, leaf and seed. The maximum alcohol extractive value was in leaf powder followed by stem, seed and root. All the four parts had shown minimum petroleum ether extractive values. The extractive values of organic solvent of *T. portulacastrum* were found in the range of 1.66 to 22.5%. The above results indicate the presence of higher amount of polar compounds than nonpolar. The present investigation also supports the earlier reports on *T. portulacastrum* and other such plants. The range of extractive value from 0.54% in petroleum ether to 10.94% in methanol and water extractive value was reported as 7.93% in halophyte *Cyperus conglomerates* [40]. In other such study of *T. portulacastrum*, water-soluble extractive value, alcohol-soluble extractive value and chloroform-soluble extractive value were reported as 37.6%, 8.8% and 3.2%, respectively [2]. The extractive value ranged from 0.52 to 8.64%, and water-soluble extractive value 17.7% reported in *T. portulacastrum* [41]. The alcohol-soluble extractive value and water-soluble extractive value

of *Z. armatum* were reported to be 14.66% and 10.33%, respectively [58]. The alcohol-soluble extractive value and water-soluble extractive value of *Moringa Oleifera* leaves were reported as 13.20% and 08.63%, respectively [20].

When the chemical composition of drug cannot be easily estimated by any other means [24], the extractive values can be important tool to evaluate and identify the chemical constituents present in the crude drug and helpful in estimating the chemical proportions soluble in a particular solvent system [25]. 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, phenolics, flavonoids and glycosides [52].

3.9 Fluorescence study

The powder of root, stem, leaf and seed of *T. portulacastrum* showed different behaviors recorded upon treatment with different chemical reagents. The root, stem, leaf and seed powders as such showed dark brown to light brown, whereas when they are treated with 1 N NaOH, root turned into lemon green, stem turned into light yellow, leaf turned into green and seed turned into light green. When they were treated with 1 M HCl and concentrated HCl, the root powder turns into colorless and light brown, respectively, and it was observed as fluorescent blue and light brown under UV (low 254 nm and high 365 nm), stem turned into light brown and dark brown, respectively, and was light green and dark brown under UV, leaf turned into light brown and black, respectively, and was light brown and black under UV, seed turned into light brown and brown, respectively, and observed as fluorescent blue and dark brown under UV. These drugs were treated with 40%NaOH + 10% CH₃COOPb: the root powder turns into yellow and under UV it turned into light yellow; stem turned into brown and was light brown under UV; leaf turned into yellow and under UV it was light brown; and seed turned into light brown and was fluorescent blue and dark brown under UV. When these drugs were treated with concentrated acetic acid, the root powder turns into colorless and it was fluorescent blue under UV; stem turned into lemon yellow and under UV it was light brown; leaf turned into green and was light brown under UV; and seed turned into yellowish green and under UV it was brown. Likewise, the fluorescence characteristics of powdered root, stem, leaf and seed after treatment with different reagents emitted various color radiations under ultraviolet and ordinary light which are tabulated in Table 2.

The fluorescence study is a simple, useful and one of the rapid methods to evaluate pharmacognostic properties of crude drugs. This is a valuable analysis tool that can authenticate the identification and recognition of adulterants of crude drugs. Different plant parts in powder form are examined with various nonpolar to polar reagents [64]. The colors produced by the powdered drug in the presence of different reagents represent the presence of active constituents in visible range in daylight. The fluorescence characteristics are very distinctive and distinguishing features for the determination of a drug. Some of the bioactive compounds like alkaloids produce fluorescence under UV, which does not fluoresce during normal daylight. Some other components of drug may be converted into fluorescent derivatives in the presence of different chemical reagents [42]. Therefore, fluorescence study is the most important parameter to assess the pharmacognostic property of crude drug qualitatively. The present investigation also supports the earlier fluorescence analysis for other plants such as *Trichosanthes dioica* [28], *Terminalia arjuna*, [15] and *Abutilon indicum* L. [11].

3.10 Solvent extraction and yield

The extracts of *T. portulacastrum* were prepared by hot continuous Soxhlet extraction method using solvents—petroleum ether, benzene, chloroform, ethyl acetate, ethanol and aqueous with the ascending order of polarity 0.0, 2.7, 4.1, 4.4, 5.2 and 9.0, respectively. The percentage w/w yields of all the six extracts for four different parts of the plant under study were calculated and tabulated. The color, nature, consistency and physical appearance of each of six extracts for four different parts of *T. portulacastrum* are represented in Figs. 5, 6 and Table 3. For six different extracts of root, their colors varied from light brown, green to off white, consistency from sticky to dry, nature of extracts was solid to semisolid and %w/w yields minimum in ethyl acetate (0.67%) and maximum in aqueous (18.39%). The extracts of stem color, consistency, physical state varied from dark green to brown, from sticky, oily to dry and solid to semisolid, respectively, and minimum %w/w yield of chloroform was 0.42% and maximum %w/w yield of aqueous 13.83%. The extracts of leaf color, consistency, physical state varied from green to dark brown, from dry, sticky to oily and solid to semisolid, respectively, and minimum %w/w yield of chloroform was 0.23% and maximum %w/w yield of aqueous 25.15%. The extracts of seed color, consistency, physical state varied from light green to light brown, from sticky to dry and solid to semisolid, respectively, and minimum %w/w yield of benzene was 0.72% and maximum %w/w yield of aqueous 21.43%.

Successful isolation and determination of bioactive compound from the experimental plant largely depend

on the selection of solvent system used in the extraction procedure. Ideal solvent for the extraction process includes low boiling point, low toxicity and preservative action and does not degrade the complex extract. There are other factors which affect the choice of solvent such as quantity of phytochemicals extracted, diversity of different compounds in extracts, rate of extraction, diversity of inhibitory compounds in extract, ease of handling of extract, potential health hazard and toxicity of solvent in the bioassay [33]. High % yield of extract was detected with ethanol and water due to their higher polarity than others, and ethanol was found easier to penetrate the cellular membrane to extract intracellular ingredients from the test plant material [13]. During each cycle, a portion of the nonvolatile compound dissolves in the solvent. After many cycles, the desired compound is concentrated in the distillation flask. Usually, for longer time solvent and solute remain in contact to each other and therefore favor the system to have maximum mass transfer and finally reach to an equilibrium following the Fick's second law of diffusion [10]. The advantage of Soxhlet system is that single batch of solvent is recycled multiple times through the sample leading to maximum dissolution of plant bioactive compounds. Prolong heating can degrade such compounds in this system; therefore, the method is not suitable for thermolabile drug extraction. At the same time, maceration is suitable for thermolabile compound extraction [57]. The present study also supports the earlier solvent extraction of crude drug and %yield analysis on other medicinal plants such as *Azadirachta indica* [8], *Spondias purpurea* [10] and *Mangifera indica* [39].

3.11 Phytochemical screening

The qualitative phytochemical screening of different solvent extracts from root, stem, leaf and seed powder of *T. portulacastrum* plant is arranged in Table 4. Primary metabolites present in different solvent extracts of root, viz. fixed oil in PE and B extract, amino acids and fixed oil in C extract, amino acids in EA extract, protein, amino acids and carbohydrates in E extract and protein, amino acids, carbohydrates in AQ extract. So far, secondary metabolites in different solvent extracts of root are concern maximum polyphenolics present in E extract followed by EA and AQ extracts, trace amount of flavonoids in EA and AQ extracts, saponin in EA, E and AQ extracts, tannin in B, E and AQ extracts, terpenoids in E and AQ extracts and terpenoids in EA and AQ extracts. Primary metabolites are present in different solvent extracts of stem, viz. fixed oil in E and AQ extracts, appreciable amino acids and protein in EA extract, moderate in E, amino acids in EA extract, protein, amino acids and carbohydrates in E and EA extracts and trace in PE, B and C extracts. So far, secondary metabolites

Table 2 Florescence characteristics of powders of *T. portulacastrum* L. root, stem, leaf and seed in different means

S No.	Reagent treatment (Powder + Reagent)	Root			Stem			Leaf			Seed		
		Visible	Low UV (254 nm)	High UV (365 nm)	Visible	Low UV (254 nm)	High UV (365 nm)	Visible	Low UV (254 nm)	High UV (365 nm)	Visible	Low UV (254 nm)	High UV (365 nm)
1.	Only Powder (P)	Dark brown	Dark brown	Dark brown	Light brown.	Light brown.	Light brown.	Brown	Brown	Brown	Dark brown	Dark brown	Dark brown
2.	P + 1 N NaOH(aq.)	Light yellow	Light brown	Light brown	Dark brown.	Brown	Brown	Brown	Brown.	Dark brown	Brown	Brown	Brown
3.	P + 1 N NaOH(aic.)	Lemon green	Fluorescent blue	Fluorescent green	Light yellow	Light green	Light green	Green	Light yellow	Yellowish green	Light green	Fluorescent blue	Fluorescent blue
4.	P + 1 M HCl	Colorless	Fluorescent blue	Fluorescent blue	Light yellow	Light brown	Light green	Light brown	Light brown	Light brown	Light brown	Fluorescent blue	Fluorescent blue
5.	P + Conc. H ₂ SO ₄	Dark brown	Black	Black	Black	Black	Black	Black	Black	Black	Dark brown	Black	Black
6.	P + 1 M H ₂ SO ₄	Colorless	Fluorescent blue	Fluorescent blue	Very light brown	Fluorescent blue	Fluorescent blue	Light brown	Fluorescent green	Fluorescent green	Colorless	Fluorescent blue	Fluorescent blue
7.	P + Conc. HNO ₃	Light yellow	Yellow	Yellow	Pale yellow	Pale yellow	Pale yellow	Light orange	Light orange	Light orange	Light yellow	Pale yellow	Light brown
8.	P + Conc. HCl	Light brown	Light brown	Light brown	Dark brown	Brown	Brown	Dark brown	Black	Black	Brown	Dark brown	Dark brown
9.	P + 1 M HNO ₃	Light yellow	Fluorescent blue	Fluorescent blue	Light yellow	Light brown	Light brown	Light brown	Light brown	Light brown	Light brown	Fluorescent green	Fluorescent green
10.	P + 40% NaOH + 10% CH ₃ COOPb	Yellow	Light yellow	Light yellow	Brown	Brown	Brown	Yellow	Brown	Brown	Light brown	Brown	Brown
11.	P + Conc. acetic acid	Colorless	Fluorescent blue	Fluorescent blue	Lemon yellow	Light brown	Light brown	Green	Light brown	Light brown	Light green	Fluorescent blue	Fluorescent blue
12.	P + 5% FeCl ₃	Pale yellow	Pale yellow	Pale yellow	Brown	Brown	Brown	Yellowish brown	Brown	Brown	Yellowish green	Brown	Brown
13.	P + 1 M HNO ₃ + 5% NH ₄ OH	Colorless	Light brown	Light brown	Light brown	Fluorescent green	Fluorescent green	Light brown	Fluorescent blue	Fluorescent blue	Transparent	Blue	Fluorescent blue
14.	P + 5% NH ₄ OH	Light brown	Light brown	Light brown	Light brown	Light brown	Light brown	Light brown	Light brown	Light brown	Translucent	Blue	Fluorescent blue
15.	P + Benzene	Colorless	Fluorescent blue	Fluorescent blue	Very light brown	Fluorescent blue	Fluorescent blue	Lemon green	Fluorescent blue	Fluorescent blue	Colorless	Fluorescent blue	Fluorescent blue
16.	P + Pet ether	Colorless	Fluorescent blue	Fluorescent blue	Colorless liquid	Fluorescent blue	Fluorescent blue	Colorless liquid	Fluorescent blue	Fluorescent blue	Colorless	Fluorescent blue	Fluorescent blue
17.	P + Chloroform	Colorless	Fluorescent blue	Fluorescent blue	Colorless liquid	Fluorescent blue	Fluorescent green	Light yellow	Light green	Light green	Colorless	Blue	Blue
18.	P + Hexane	Colorless	Fluorescent blue	Fluorescent blue	Colorless liquid	Fluorescent blue	Fluorescent blue	Colorless	Fluorescent blue	Fluorescent blue	Colorless	Fluorescent blue	Fluorescent blue
19.	P + Ethyl acetate	Colorless	Fluorescent blue	Fluorescent blue	Light green	Fluorescent blue	Fluorescent green	Lemon green	Light brown	Light brown	Colorless	Fluorescent blue	Fluorescent blue
20.	P + Ethanol	Colorless	Fluorescent blue	Fluorescent blue	Light yellow	Fluorescent blue	Fluorescent blue	Light green	green	Green	Light green	Fluorescent blue	Fluorescent blue

The colors mentioned in the table are based on the color identification chart, Royal Botanical Garden, Edinburgh (1996)

Fig. 5 Sequential extracts of *T. portulacastrum* root—(a pet. ether extract, b benzene extract, c chloroform extract, d ethyl acetate extract, e ethanol extract, f aqueous extract); seed—(s pet. ether extract, t benzene extract, u chloroform extract, v ethyl acetate extract, w ethanol extract, x aqueous extract); stem—(g pet. ether extract, h benzene extract, i chloroform extract, j ethyl acetate extract, k ethanol extract, l aqueous extract); leaf—(m pet. ether

extract, n benzene extract, o chloroform extract, p ethyl acetate extract, q ethanol extract, r aqueous extract); seed—(s pet. ether extract, t benzene extract, u chloroform extract, v ethyl acetate extract, w ethanol extract, x aqueous extract)

Fig. 6 Crude extracts of different parts of *T. portulacastrum* L. root, stem, leaf and seed

in different solvent extracts of stem are concern appreciable polyphenolics present in E and AQ extracts, moderate flavonoids in EA, E and AQ extracts, appreciable saponin in E and trace in EA and C extracts, moderate tannin in B and trace in C, EA and E extracts, moderate terpenoid in PE, B,

C, EA and E extracts, trace alkaloid, anthraquinone in AQ extract and moderate glycosides in AQ. Primary metabolites are present in different solvent extracts of leaf, viz. fixed oil in PE, C, EA, E and AQ extracts, trace amino acids and protein in EA and E extracts and trace carbohydrates

Table 3 Properties of crude extracts in different solvent systems *T. portulacastrum* L. root, stem, leaf and seed

Plant parts	Solvents	Extraction method	Extract color	Consistency	Nature of extracts	Extract weight (g)	% Yield
Root	PE	S	Light brown	Sticky	Solid	0.05 ± 0.01	1.0 ± 0.15
	B	S	Green	Dry	Solid	0.06 ± 0.01	1.31 ± 0.24
	C	S	Off white	Dry	Solid	0.04 ± 0.01	0.89 ± 0.18
	EA	S	Green	Dry	Solid	0.03 ± 0.01	0.67 ± 0.06
	E	S	Brown	Dry	Semi solid	0.60 ± 0.03	13.51 ± 1.21
	Aq	M	Brown	Dry	Solid	3.06 ± 0.27	18.39 ± 1.14
Stem	PE	S	Dark green	Sticky	Solid	0.74 ± 0.16	1.48 ± 0.37
	B	S	Light green	Sticky	Solid	0.37 ± 0.07	0.75 ± 0.53
	C	S	Green	Dry	Solid	0.20 ± 0.06	0.42 ± 0.26
	EA	S	Green	Dry	Solid	0.22 ± 0.08	0.48 ± 0.09
	E	S	Brown	Dry	Semi solid	3.76 ± 0.19	9.23 ± 0.23
	Aq	M	Brown	Dry	Solid	5.44 ± 0.31	13.83 ± 1.22
Leaf	PE	S	Dark green	Oily	Solid	0.43 ± 0.03	0.86 ± 0.61
	B	S	Dark green	Sticky	Solid	0.34 ± 0.04	0.69 ± 0.23
	C	S	Green	Dry	Solid	0.11 ± 0.03	0.23 ± 0.02
	EA	S	Light green	Dry	Solid	0.13 ± 0.02	0.29 ± 0.19
	E	S	Brown	Sticky	Solid	4.25 ± 0.85	9.74 ± 1.72
	Aq	M	Dark green	Dry	Semi Solid	9.03 ± 1.35	25.15 ± 1.52
Seed	PE	S	Light brown	Oily	Solid	1.15 ± 0.03	11.5 ± 1.25
	B	S	Green	Sticky	Solid	0.06 ± 0.02	0.72 ± 0.31
	C	S	Green	Sticky	Solid	0.09 ± 0.02	1.12 ± 0.28
	EA	S	Green	Dry	Solid	0.14 ± 0.03	1.85 ± 0.36
	E	S	Brown	Sticky	Semi solid	0.75 ± 0.06	9.93 ± 1.73
	Aq	M	Brown	Dry	Solid	1.46 ± 0.38	21.43 ± 1.78

PE petroleum ether, B benzene, C chloroform, EA ethyl acetate, E ethanol, Aq aqueous, M maceration, S Soxhlation, Ex extraction

in E and EA extracts. So far, secondary metabolites in different solvent extracts of stem are concern appreciable glycosides in B extract, appreciable polyphenolics in E extract, moderate polyphenolics in AQ and C extracts, trace polyphenolics in EA extract, appreciable saponin in E and AQ extracts, moderate saponin in EA extract, moderate-free sugars in E, trace flavonoids in B extract, moderate tannin in B extract, trace tannin in PE, C and E extracts, moderate terpenoids in PE, EA and E extracts and trace terpenoid in C extract. Primary metabolites are present in different solvent extracts of seed, viz. trace carbohydrate in C and AQ extracts and trace fats in B, C, EA, E, AQ extracts. So far, secondary metabolite in different solvent extracts of seed are concern trace alkaloid in C and AQ extracts, cardiac glycosides in E and AQ extracts, trace flavonoids in E and AQ extracts, moderate polyphenolics in E and AQ extracts, trace saponin in EA, E and AQ extracts, moderate tannins in E extracts, trace terpenoid in B and C extracts and moderate terpenoid in E extract.

The qualitative phytochemical screening of medicinal plants is an important parameter of quality control.

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 [27]. Phytochemical studies performed on different plant extracts reveal the presence of bioactive molecules which were known to possess medicinal and physiological activities. Different solvent extracts of different parts of plant analysis revealed the presence of various types of phytoconstituents in different amounts that help in the selection of specific extract for isolating the active principle [48]. The pharmacological activity studies are not part specific of medicinal plant rather all organs are important and evaluated for various therapeutic efficacies of plant such as leaf of *Ageratum conyzoids* and *Homalium ceylanium* [1, 7, 22], root of *Argyreia pilosa* [45], stem of *Psychotria rubra* and *Acacia auriculiformis* [38, 50, 56], bark of *Chloroxylon swietenia* [14], fruit of *Benincasa Hispida* [37], flower of *Woodfordia fruticosa* [5], seed of *Aegle marmelos* [42], aerial parts of *Heracleum persicum* and *Achyranthes aspera* [54, 63], rhizome [23] and pseudobulbs of *Coelogyne cristata* [43].

Table 4 Qualitative tests for various metabolites in extracts of *T. portulacastrum* L. root, stem, leaf and seeds

Primary Metabolites	Plant constituents	Test performed	<i>T. portulacastrum</i> Root						<i>T. portulacastrum</i> Stem						<i>T. portulacastrum</i> Leaf						<i>T. portulacastrum</i> Seed					
			PE	B	C	EA	E	AQ	PE	B	C	EA	E	AQ	PE	B	C	EA	E	AQ	PE	B	C	EA	E	AQ
Primary Metabolites	Protein and Amino acids	Millon's test	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+
		Biuret test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Carbohydrates	Xanthoproteic test	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-
		Ninhydrin test	-	-	+	+	+	++	+	+	+	+++	+++	++	-	-	-	+	+	+	-	-	-	-	-	-
	Fixed oils and fats	Fehling's test	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+
Secondary metabolites	Alkaloids	Benedict's test	-	-	-	-	+	+	-	-	-	-	+	-	-	-	+	-	+	+	-	-	+	-	+	+
		Stain test	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-
	Anthraquinone	Soap test	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+
		Mayer's test	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	+
	Cardiac glycosides	Wagner's test	-	-	-	-	-	-	+	-	-	-	-	+	-	+	++	-	-	-	+	-	+	-	-	-
		Borntrager's test	-	-	-	-	-	-	-	-	-	-	-	+++	-	-	-	-	-	+	-	-	-	-	-	-
	Glycosides (free sugar)	Keller–Kiliani test	-	-	-	-	-	-	-	-	-	-	+	-	-	+++	+	-	-	-	+	-	-	-	-	+
		Legal's test	-	+	-	-	-	-	++	++	++	++	+	-	-	-	-	-	-	++	-	-	-	-	-	-
	Flavonoids	Ammonia test	-	-	-	-	+	+	+	-	-	-	++	++	-	+	-	-	-	-	-	++	-	-	-	+
		Lead acetate test	-	-	+	-	+	+	-	-	+	++	++	++	-	-	-	+	+	+++	++	-	-	-	-	+
Secondary metabolites	Phenolic	Alkaline reagent	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	++	++	-	-	-	-	+	+
		Ferric chloride test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Phlobatanin	Hydro chloride test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Folin–Ciocalteu test	+	+	+	++	+++	++	-	-	-	-	+++	++	++	+	++	+	+	+++	++	+	+	+	+	++
	Saponin	Frothing test	-	-	-	+	+	+	-	-	+	+	+++	-	-	-	-	++	++	+++	+++	-	-	+	+	+
		Olive oil test	-	-	-	+	+	+	-	-	+	+	+	-	-	-	-	+	+	+	+	-	-	+	+	+
	Tannin	Ferric chloride test	-	+	-	-	+	+	+	+	++	+	+	+	+	+	+	-	-	-	-	+	-	-	++	-
		Salkowski test	-	-	-	-	-	+	+++	++	++	++	++	++	-	+	+	++	++	++	+	-	+	+	++	+
	Terpenoids	Ruthenium red test	+++	++	++	++	++	-	-	+	+	++	++	+++	-	+	+	++	++	++	+++	-	+	+	+++	++
		Gums and mucilage	+++	++	++	++	++	-	-	+	+	++	++	+++	-	+	+	++	++	++	+++	-	+	+	+++	++

The significance of bold denotes its high quantity available in that part of the plant

(+++ appreciable amount, (++) moderate amount, (+) trace amount, (-) completely absent, PE petroleum ether, B benzene, C chloroform, EA ethyl acetate, E ethanol, Aq aqueous

T. portulacastrum thus comes across their therapeutic values due to the presence of respective phytochemical constituents. Various phytoconstituents present in different parts of this plant revealed that it can be a rich source of new drugs for various ailments.

4 Conclusion

There is no detailed and comparative physicochemical and phytochemical screening work of four different parts of *T. portulacastrum* L. which has been reported till date. Different parts of this plant have been used in traditional system of medicine. Although herbal drugs are well known for their safe action, they may lead to adverse reactions and may be due to incorrect identification and adulteration of crude drug. Therefore, the aim behind this study was to establish quality control, standardization and genuineness of drugs. Macroscopic and microscopic study of plant part will help in correct identification of drug. The comparative extractive values and ash values will be helpful in determining quality of drug material. The fluorescence study and phytochemical screening will support in analyzing the purity of the drug. The present investigations were taken up with a view to facilitate herbal industries and traditional medicinal people to establish the standards, authenticity of drug, quality, purity, pharmacological benefits, diverse versatility of the plant and their potential against various ailments and diseases. There is a need to conduct further studies to explore exact bioactive molecule isolation and their pharmacokinetics and also to know the effects on structure–function relationship. This study finally concludes that *T. Portulacastrum* L. is an extraordinary and incredible source with so much to offer the world of medicine.

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Author's contribution Purnima Pathak and Sneha Bhatt did literature search and compilation of results. Anand Prakash and Pracheta carried out all experiments as well as compilation of results. Anand Prakash and Vinay Sharma drafted the manuscript.

Compliance with ethical standards

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