ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Pharmacognistical and Phytochemical Analysis of *Portulaca quadrifida* Linn

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ABSTRACT

Herbs are staging a comeback and herbal renaissance is happening all over the globe. The herbal products today symbolize safety in contrast to synthetic drugs that are regarded unsafe to the human and environment. Portulaca quadrifida L., a traditional medicinal plant, valued for its benefits in the management of urinary and inflammatory disorders. The juice of leaves is applied to abscesses and decoction is given in dysentery. The decoction of the plant can act as antihelminthic and used in the treatment of stomach complaints and gonorrhea. The current study was therefore carried out to provide requisite pharmacognostic details about Portulaca quadrifida L. Pharmacognostic evaluation included examination of morphological and microscopical characters; physicochemical properties, phytochemical analysis and fluoresence study. In addition, HPTLC fingerprint was also carried out, which can be used for correct identification of the plant. The powder microscopy showed the presence of starch grains, spiral vessels and pitted vessels. Phytochemical screening reported the presence of alkaloids, tannins, mucilage, steroidal compounds and carbohydrates. The present study will provide the information with respect to identification and authentication of this medicinal plant.

Keywords: Portulaca quadrifida L., Pharmacognostic evaluation, phytochemical analysis, HPTLC fingerprint

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ISSN: 0975-8585

INTRODUCTION

After decades of serious obsession with the modern medicinal system, people have started looking at the ancient healing systems like Ayurveda, Siddha and Unani. This is because of the adverse effects associated with synthetic drugs. Herbal drugs play an important role in health care programs especially in developing countries. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers 'all' plant parts to be potential sources of medicinal substances [1]. In the last few decades there is an exponential growth in the field of herbal medicine. It is getting popularize in developing countries owing to its natural origin and lesser side effects. Nowadays, herbal medicines are being manufactured on a large scale in mechanical units. The manufacturers are facing many problems such as availability of good quality of raw material, authentication of raw material, availability of standards, proper standardization methodology of drugs and formulations, quality control parameters etc. [2].

Portulaca quadrifida L. is an important medicinal herb belonging to the family Portulacaceae. The plant is a small, diffuse, annual and erect herb. The plant is sour, bitter, hot, alterative, laxative, causes biliousness and "Kapha"; cures fevers, asthma, cough, urinary disorders, inflammations, good for eye diseases, skin diseases and ulcers. In Indo-China the juice of leaves is applied to abscesses and used as a collyrium; a decoction is given in dysentery. In Nigeria the leaves are used as a local application to swellings [3].

Portulaca quadrifida L. has been reported to possess antifungal activity against Aspergillus fumigates and Candida albicans [4]. The effect of ethanolic extract of Portulaca quadrifida L. on central and peripheral nervous system were studied by using spontaneous motor activity, in vivo muscle relaxant activity (Grip strength) and anticonvulsant activity and it is also found to have good effect on central nervous system [5].

Inspite of the numerous medicinal uses attributed to this plant, there is no detailed pharmacognostical report on the macroscopy, anatomical markers, microscopy etc. and HPTLC fingerprint, required for the quality control of the crude drug. Therefore, the present investigation of Portulaca quadrifida L. plant is taken up to establish pharmacognostic profile of the plant which will help in identification as well as in standardization of the quality and purity of the plant drug.

MATERIALS AND METHOD

Herbarium of Portulaca quadrifida L. was prepared and authenticated from Blatter Herbarium, St. Xavier's College, Mumbai. Fresh plants of Portulaca quadrifida L. were collected from local vegetable market, Dombivli, M.S., India, washed under running tap water and blotted dry for further studies. The plant was dried in preset oven at 40 ± 2˚C for about one week, ground into powder and used for further analysis. Physicochemical constants such as the percentage of total ash, acid insoluble ash, water soluble ash and extractive values using various solvents viz. water, alcohol, methanol, chloroform, acetone and petroleum ether were

calculated according to the methods described by Mukherjee [6]. Preliminary phytochemical analysis of powdered leaf was performed as described by Khandelwal [7] and Kokate [8]. Fluorescence analysis was carried out using methods of Kokashi [9] and Chase and Pratt [10] and histochemical analysis was carried out using methods described by Madhavan et al. [11]. Phytochemical analysis was carried out using Thin Layer Chromatography as per methods described by Wagner and Bladt [12].

A qualitative densitometric HPTLC analysis was performed with methanolic extract for the development of characteristic fingerprint profile which may be used for quality evaluation and standardization of the drug. 10 µl of extract was spotted on precoated silica gel G60 F_{254} HPTLC plates (Merck) with the help of CAMAG Linomat V applicator. The plate was developed in glass twin trough chamber (20cm x 10 cm) pre-saturated with mobile phase (Toluene: Ethyl acetate: Glacial Acetic acid in the ratio 5: 3: 1). The plate was derevatised using methanolic H_2SO_4 and scanned using TLC Scanner 3 (CAMAG).

RESULTS

MACROSCOPIC CHARACTERS

Stem is cylindrical, filiform, glaborous, succulent, purple in colour less than a millimeter in diameter. Stem is rooting at the nodes. The diameter of stem was found to be 0.1 cm.

Leaves are opposite, fleshy, ovate, acute; stipulate with a ring of silvery hairs. Petiole is very short. Stem and leaves are mucilaginous on crushing; mucilage is slimy. Internodes are 1.5 to 3.0cm long. The length of leaf was found to be 0.9 cm. The breadth of leaf was found to be 0.3 cm (Plate 1A and Plate 1B).

MICROSCOPIC CHARACTERS

The cross section of the stem was circular in outline. The epidermal cells were polygonal in shape and were surrounded externally by thick cuticle. The outer wall of the epidermal cells slightly bulged out. Epidermis was followed by parenchyma. The parenchyma consists of thin walled, more or less isodiametric cells without any intercellular spaces. The parenchyma was followed by endodermis. Collateral vascular bundles were arranged in ring. Pith consists of thin walled isodiametric cells some of which contain calcium oxalate crystals (Plate 1C).

The transverse section of leaf showed outermost layer of epidermis. Epidermal cells are rectangular to polygonal in shape. Epidermis was followed by spongy and palisade mesophyll. The palisade cells showed the presence of crystals of calcium oxalate arranged in groups which were referred to as spheraphids (Plate 2D). Stomata occur on both adaxial and abaxial surfaces of the leaf. Surface preparation of leaf showed presence of paracytic stomata (Plate 1E).

The transverse section of root showed epiblema as the outermost layer. Epiblema was

followed by cortex which consists of 4 to 6 layers of thin walled, polygonal parenchymatous cells. Transverse of section of root showed presence of triarch primary xylem (Plate 1F).

Powder Microscopy

The powder microscopy of the plant powder revealed the presence of starch grains, spiral vessels, pitted vessels and fibres (Plate 1G, Plate 1H, Plate 1I and Plate 1J).

Quantitative determination

The number of stomata, vein islet number and measurement of stomatal index and size of stomata of leaves were obtained with the help of calibrated ocular micrometer and results are tabulated in Table - 1.

Table 1: Quantitative leaf microscopy of *Portulaca quadrifida* L.

S No.	Parameters	Vein Islet Number
1.	Stomatal Index	
	a. Upper surface	21.41%
	b. Lower surface	23.59%
2.	Stomatal Number	
	a. Upper surface	8-9
	b. Lower surface	10-12
3.	Vein islet number	10.4
4.	Stomatal size	
	Length(µm)	0.3-0.4
	Breadth(µm)	0.3

PHYSICOCHEMICAL PARAMETERS

Ash of any organic material is composed of their non-volatile inorganic components. Controlled incineration of crude drugs results in an ash residue consisting of an inorganic material (metallic salts and silica). This value varies within fairly wide limits and is therefore an important parameter for the purpose of evaluation of crude drugs [6]. Therefore percentage of the total ash, acid insoluble ash and water soluble ash were calculated (Table 2).

Table 2: Physicochemical Analysis of powdered plant of *Portulaca quadrifida* L.

S No.	Physicochemical Constant	Observation (%)
1.	**Ash Values**	
a)	Total ash	10.40
b)	Acid insoluble ash	1.14
c)	Water soluble ash	6.73
2.	**Extractive Values**	
a)	Petroleum ether	2.48
b)	Acetone	3.92
c)	Chloroform	5.12

ISSN: 0975-8585

d)	Ethanol	10.64
e)	Methanol	13.04
f)	Aqueous	19.68

The extraction of any crude drug with a particular solvent yields a solution containing different phyto-constituents. Extractive value is also useful for evaluation of crude drug, which gives an idea about the nature of the chemical constituents present in a crude drug and is useful for the estimation of specific constituents, soluble in that particular solvent used for extraction [7]. Loss on drying is the loss of mass expressed as percent w/w [6]. The results are tabulated in Table -2

Table 3: Fluroscence Analysis of *Portulaca quadrifida* L. Powder

S no.	Tests	Visible light	254nm	366nm
1.	Powder as such	Greenish buff	Black	Dark green
2.	Powder + nitrocellulose	Greenish buff	Black	Brownish green
3.	Powder + 1N NaOH in methanol	Dark green	Black	Dark green
4.	Powder + 1N NaOH in methanol+ nitrocellulose in amyl acetate	Brownish black	Black	Dark green
5.	Powder + 1N HCl	Brown	Black	Green
6.	Powder + 1N HCl + nitrocellulose in amyl acetate	Greenish brown	Black	Dark brown
7.	Powder + 1N NaOH	Brownish green	Black	Black
8.	Powder + 1N NaOH + Nitrocellulose in amyl acetate	Brownish black	Black	Blackish green
9.	Powder + HNO_3 (1:1)	Brown	Black	Black
10.	Powder + H_2SO_4 (1:1)	Greenish black	Black	Dark green
11.	Powder + 1% Picric acid	Yellowish green	Black	Blackish green
12.	Powder + acetic acid	Blackish green	Black	Blackish green
13.	Powder + 5% Iodine	Dark brown	Black	Blackish green
14.	Powder + 5% $FeCl_3$	Dark green	Black	Blackish green
15.	Powder + 25% Ammonia + HNO_3	Brown	Black	Blackish green
16.	Powder + methanol	Brown	Black	Blackish green
17.	Powder + conc. HNO_3	Brown	Black	Blackish green
18.	Powder + 10% $K_2Cr_2O_7$ solution	Brown	Black	Black
19.	Powder + 50% KOH	Green	Black	Blackish green

The leaf powder of Portulaca quadrifida L. was treated with various chemical reagents and examined under long UV (254 nm), short UV (366 nm) and visible light. The changes in the fluorescence observed are presented in Table – 3. The histochemical colour reactions on the leaf and stem were performed for the identification of major cell inclusions. The results are tabulated in Table – 4.

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Table 4: Histochemical Tests of stem and leaf of *Portulaca quadrifida* L.

S No.	Test for	Stem Section	Leaf Section
1.	Starch	+	+
2.	Tannins	+	+
3.	Lignin	+	+
4.	Ca oxalate crystals	+	+
5.	Mucilage	+	+
6.	Oil globules	-	-
7.	Aleurone grains	-	-
8.	Stone cells	-	-
9.	Alkaloids	+	+
10.	Steroids	+	+

Key Words: + = Present; - = Absent

PRELIMINARY PHYTOCHEMICAL STUDIES

The leaf powder was extracted with various solvents viz. water, alcohol and chloroform. These extracts were tested for presence of different phytoconstituents. The results of phytochemical analysis are tabulated in Table – 5.

Table 5: Phytochemical Analysis of *Portulaca quadrifida* L.

S No.	Phytoconstituents	Aq.E	AE	CE
1	Acid compounds	-	-	-
2	Aleurone grains	-	-	-
3	Alkaloids	+	+	-
4	Amino acid	+	+	-
5	Carbohydrates	+	+	+
6	Fats & fix oils	-	-	+
7	Glycosides	+	-	-
8	Mucilage	+	-	-
9	Tannins	+	+	-
10	Proteins	+	+	-
11	Starch	+	-	-
12	Steroids /Triterpenoids	+	+	+
13	Flavonoids	-	+	-
14	Essential oils	-	-	-
15	Resins	-	-	-
16	Saponins	-	-	-
17	Anthraquinone	-	-	-

Key Words: + = Present; - = Absent; AqE = Aqueous Extract; AE = Alcoholic Extract; CE = Chloroform Extract

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HPTLC FINGERPRINT

A densitometric HPTLC was performed for the development of characteristic fingerprint profile, which may be used for quality evaluation and standardization of the drug. Rf values of the separated compounds are recorded in Table -6; Plate 1.

Table 6: R*f* values of the separated phytoconstituents by HPTLC fingerprint of *Portulaca quadrifida* L.

S No.	Wavelength		
	254 nm	366 nm	540 nm
	R*f*	R*f*	R*f*
1.	0.09	0.02	0.09
2.	0.21	0.04	0.11
3.	0.37	0.09	0.20
4.	0.42	0.20	0.28
5.	0.52	0.39	0.38
6.	0.74	0.48	0.47
7.	0.93	0.51	0.53
8.	-	0.56	0.55
9.	-	0.59	0.65
10.	-	0.71	0.70
11.	-	0.76	0.80
12.	-	-	0.93

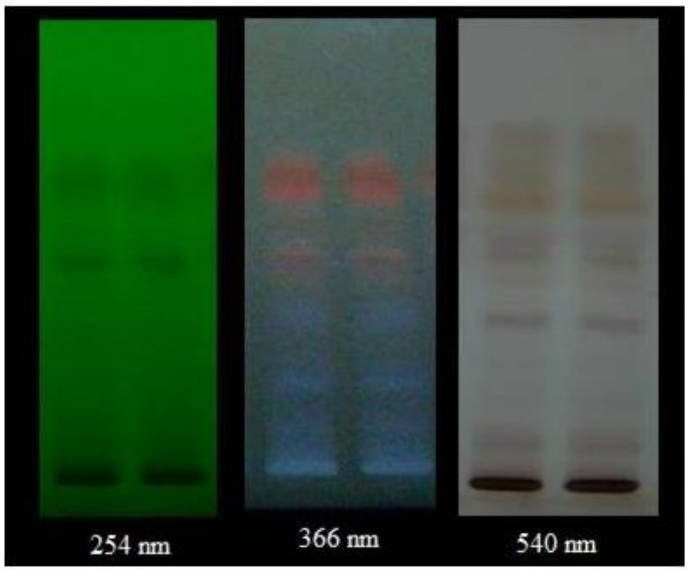

Plate 1: HPTLC fingerprint of *Portulaca quadrifida* L.

ISSN: 0975-8585

DISSCUSSION

The standardization of a crude drug is an integral part of establishing its correct identity. For inclusion of a crude drug in Pharmacopoeia, pharmacognostic parameters and standards must be established. The results of these investigations could, therefore, serve as a basis for proper identification, collection and investigation of the plant.

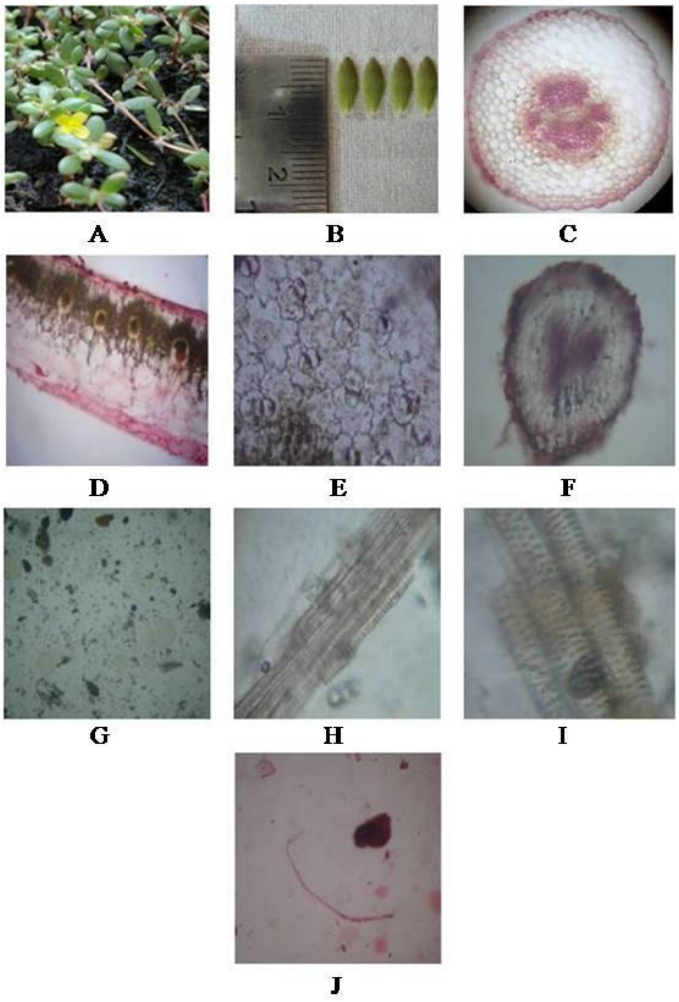

Plate 2:- A: Plant of *Portulaca quadrifida* L.; B: Leaf size of *Portulaca quadrifida* L.; C: Transverse section of Stem; D: Transverse section of Leaf; E: Surface preparation of Stomata; F: Transverse section of root; G: Starch grain; H: Spiral vessels; I: Pitted vessels ; J: Fibre

The transverse section of leaf is distinguishable into upper spongy and lower palisade cells. The stomata are present on both the epidermi. The anatomical markers of leaf are presence of stomata and presence of calcium oxalate crystals either single or in groups. The powder microscopy of leaf shows presence of starch grains, spiral vessels and pitted vessels.

Presence of triarch vascular tissue is characteristic of root. T.S. of stem showed pith consisting of thin walled isodiametric cells, some of which contain calcium oxalate crystals.

Equally important in the evaluation of the crude drugs, is the ash value, water soluble ash value and acid insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign organic matter such as metallic salts and/or silica [13]. The total ash, water soluble ash and acid insoluble ash of Portulaca quadrifida L. are found to be is 10.40 %, 1.14% and 6.73 % respectively. Since the ash value is constant for the given drug, this value is one of the diagnostic parameter of the drug. Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The aqueous extractive value was found to be higher (19.68%) than the other solvents used viz. acetone, petroleum ether, chloroform, ethanol and methanol, revealing presence of large amount of water soluble constituents in the plant. The loss on drying was reported to be 85.74 %.

The fluorescent method is adequately sensitive and enable the precise and accurate determination of the analyze over a satisfactory concentration range without several time consuming dilution steps prior to analysis of pharmaceutical samples [14]. Kalidas et al. [15] suggested that a non-fluorescent compound may fluoresce if mixed with impurities that are fluorescent. Therefore, the results obtained from the present fluorescent studies will also help to check any impurities present in plant powder of Portulaca quadrifida L.

The histochemical tests revealed the presence of lignins, tannin, starch, mucilage, alkaloids and steroidal compounds in stem and leaf.

Presence or absence of certain important compounds in an extract is determined by colour reaction of the compound with specific chemicals. This procedure is a simple preliminary prerequisite before going for detailed phytochemical investigation. Various tests have been conducted qualitatively to find out the presence or absence of bioactive compounds [15]. Different phytoconstituents such as tannins, mucilage, steroids and alkaloids were detected in Portulaca quadrifida L. plant extracts which could make the plant useful for treating different ailments as having a potential of providing useful drugs for human use. HPTLC fingerprint profile along with their Rf values were recorded, which would serve as a reference standard for the scientists engaged in research on the medicinal properties of this plant.

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Figure 1a: Densitogram of *Portulaca quadrifida* L. at 254 nm

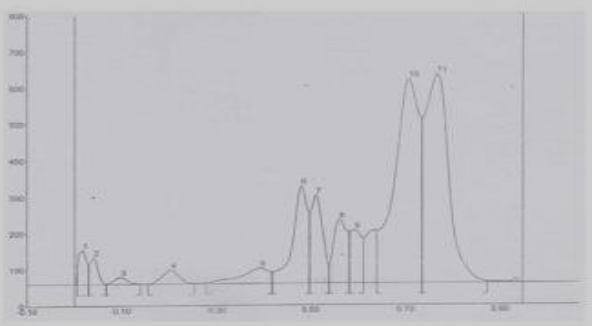

Figure 1b: Densitogram of *Portulaca quadrifida* L. at 366 nm

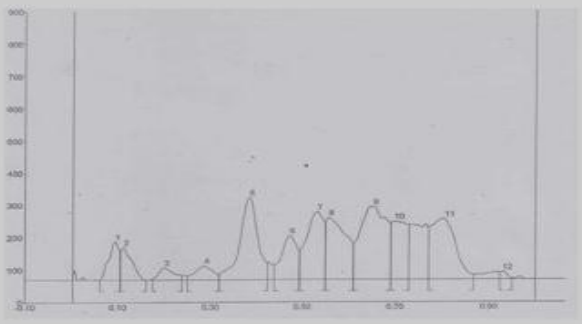

Figure 1c: Densitogram of *Portulaca quadrifida* L. at 540 nm

CONCLUSION

Thus the organoleptic, microscopic characters, physico-chemical, fluorescence study, preliminary phytochemical screening and HPTLC fingerprint of Portulaca quadrifida L. can be used as a diagnostic tool for the correct identification of the plant, which could be incorporated in the preparation of Indian Herbal Pharmacopoeia.

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