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EXTRACTION, PHYTOCHEMICAL ANALYSIS AND ANTHELMINTIC ACTIVITY STUDY OF *PORTULACA QUADRIFIDA* LINN

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ABSTRACT

Portulaca quadrifida Linn. belongs to the family Portulacaceae. It is a small diffused, succulent, annual herb found throughout the tropical parts of India. It is used as a vegetable and also used for various curative purposes. It is said to be useful in asthma, cough, urinary discharges, inflammations and ulcers. In Rajasthan, the leaves are used in preparing bread by mixing with Bajra. In Tamilnadu, leaves and tender shoots cooked and eaten as greens. A poultice of the plant is applied in abdominal complaints, erysipelas and haemorrhoids. In Nigeria the leaves are used as a local application to swellings. The present study was aimed to undergo ethanolic extraction, phytochemical analysis of ethanolic extract and investigation of the anthelmintic potential of crude ethanolic extract of *Portulaca quadrifida* Linn. on Indian earth-worm (*Pheretima posthuma*). Three concentrations (25, 50, 100 mg/ml) of each extract were studied in activity which involved the determination of time of paralysis (vermifuge) and time of death (vermicidal) of the worms. Albendazole in 25mg/ml concentration was included as standard reference and normal saline water with 1% CMC as control. The ethanolic extracts exhibited significant anthelmintic activity at a concentration of 100 mg/ml. Findings of the present investigations confirms that, the ethno-medicinal claim of anthelmintic activity of this plant is genuine

INTRODUCTION

Parasitic infection including Helminthiasis is a critical serious problem in the tropical regions including the Asian countries which affects more than two billions of people worldwide. Helminthes produce serious problem in human and other animals around the world specifically to the third world countries. Different type of helminthes infects the human and animals out of which intestinal round worms (Ascardia sp.) are most common. Approximately 300 million people suffer severe morbidity associated with these parasites and half of which are school-going children affected by massive infections. Variety of several clinical symptoms arises due to this infection include dysentery, diarrhoea, nausea-vomiting, loss of appetite and weight, acidity and sometimes anaemia. Other manifestations of helminthic infections include respiratory symptoms, dermatological consequences and epilepsy as a result of neurocysticercosis. Helminthic infections may also subvert immune responses to pathogens of other diseases such as tuberculosis, HIV, and malaria.[1] The plant *Portulaca quadrifida* is commonly known as 'Chicken Weed', widely used as green vegetable, described in the Ayurvedic Literature. *Portulaca quadrifida*

Linn. belongs to the family Portulacaceae. It is a small diffused, succulent, annual herb found throughout the tropical parts of India. It is said to be useful in asthma, cough, urinary discharges, inflammations and ulcers. In Rajasthan, the leaves are used in preparing bread by mixing with Bajra. In Tamilnadu, leaves and tender shoots cooked and eaten as greens. A poultice of the plant is applied in abdominal complaints, erysipelas and haemorrhoids.[2] *Portulaca quadrifida* has been reported to possess antimicrobial activity against *Bacillus subtili*.[3] 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.[4] The present investigation was aimed to evaluate the presence of phytoconstituents and anthelmintic activity of ethanolic extract of aerial parts of *Portulaca quadrifida* Linn.

MATERIALS AND METHODS

Plant materials

The fresh *Portulaca quadrifida* Linn. plant was collected nearly 2.0 kg from the rural belt of the Jangalakandriga Village, Nellore Dist., Andhra Pradesh, India. The plant was authenticated by Dr. C.V.S. Bhaskar, Ph. D, Director and Principal, Department of Botany, Venkata Raja's College, Nellore Dist, Andhra Pradesh, India. A voucher specimen number 13 (*Portulaca quadrifida* Linn.) had kept in Department of Pharmacognosy for future references.

Fig 1: The Flower Part

Fig 2: The Whole Plant

Preparation of extract

Extraction is a process where the main focus is on the materials to be extracted and the type(s) of the compound that is being isolated. The best way to get the extract is boiling the plants parts in ethanol. The classical chemical procedure for obtaining organic constituents from dried plant tissue is to continuously extract powdered material in a soxhlet apparatus within a range of solvent, starting in turn with Hexane, Petroleum ether, Chloroform, Ethyl acetate, Ethanol, Methanol etc. This method is useful when working on the gram scale. But it is rarely possible to complete separation of constituents and the same compounds may be recovered in several fractions.[5]

Extraction of aerial parts of *Portulaca quadrifida* Linn.

Portulaca quadrifida Linn. plant was collected, then they were separated out from unwanted ingredients and washed with plenty of water. Then the plants were dried in shade and then milled in to coarse powder by a mechanical grinder. The powder was kept in air tight container for further use. The powdered material was then packed in Soxhlet Apparatus and continuous hot extraction was carried out using Ethanol as solvent. The extract was filtered through Whatman filter paper to remove any impurities if present. The extract was then concentrated by using vacuum distillation unit. The concentrated extract was placed in vacuum desiccators to remove the excess moisture. The dried extract was weighed and the yield of the extract was calculated as 11.6% w/w. The extracts were then kept in airtight container in desiccators for further use.

Preliminary Phytochemical Screening

To identify the phytoconstituents present in ethanolic extract of *Portulaca quadrifida* Linn. was subjected to undergo following preliminary Phytochemical screening.[6-9]

a. Test for Alkaloids

Mayer's reagent: It is used for the detection of alkaloids. (i) Dissolve 1.36gm of mercuric chloride in 60ml distilled water. (ii) Dissolved 5gm. Potassium iodide in 60ml distilled water. Mix (i) and (ii) and adjust the volume to 100ml with distilled water. With alkaloids it shows white to buff precipitate.

Wagner's reagent: Dissolve 1.27gm of iodine and 2gm of potassium iodide in 5ml of water and volume make up to 200ml with distilled water. With alkaloids it shows reddish brown precipitate.

Dragendroff's reagent: Boil 14gm of sodium iodide with 5.2gm bismuth carbonate in 50ml glacial acetic acid for a few minutes. Allow it to stand overnight and filter off the precipitate of sodium acetate crystals. To 40ml of the red-brown filtrate add 160ml of acetate and 1ml of water. Preserve the stock solution in amber colored bottle. When needed, add 20ml of acetic acid to 10ml of this stock solution and make up to 100ml with water. With alkaloids it gives orange brown precipitate.

Hager's reagent: A saturated aqueous solution of picric acid used for detection of alkaloids. It gives characteristics crystalline precipitate with many alkaloids.

b. Test for carbohydrates

Molisch test: Aqueous or alcoholic solution of substance + 10% alcoholic solution of α-napthol → shake → add conc. H_2SO_4 along the side of the tube. A violet ring at the junction of to orange colour will be seen.

d. Test for anthraquinone glycosides

Borntrager's test: Boil 0.1gm of the powdered drug with 5ml of 10% sulphuric acid for 2 minutes. Filter while hot, cool the filtrate and shake gently with equal volume of benzene. Allow the benzene layer to separate completely from the lower layer. Pipette out and transfer the benzene layer to clean test tube. Add about half its volume of aqueous solution of ammonia (10%). Shake gently and allow the layer to separate. The lower ammonical layer will acquire red pink colour due to the presence of free anthraquinones.

Modified Borntrager's test: The C-glycosides of anthraquinones requires more drastic conditions for hydrolysis and thus a modification of the above test is to use ferric chloride and hydrochloric acid to affect oxidative hydrolysis. 0.1gm of the drug + 5ml of dil. HCL and 5ml of 5% solution of ferric chloride and boil for 5 minutes, cool the solution and filter. This filtrate is shaken with benzene. Separate the benzene layer and add an equal volume of dilute ammonia. The ammonical layer shows pink colour.

two liquids confirms the presence of carbohydrates.

Fehling's test: 2ml of Fehling's solution A + 2ml of Fehling's solution B + 2ml of extracts. Boil, if yellow or brick precipitate appears then reducing sugars are present.

Benedicts test: 5ml of Benedict's reagent + 3ml of test solution → boil in water bath. If appearances of brick red ppt at the bottom of the test tube then monosaccharide is present.

c. Test for cardiac glycoside

Keller-Killani test: To an extract of the drug in glacial acetic acid, few drops of ferric chloride and conc. H_2SO_4 acid are added. A reddish brown colour is formed at the junction of two layers and the upper layer turns bluish green.

Legal test: To a solution of Glycoside in pyridine, Sodium nitroprusside solution and Sodium hydroxide solution are added. A pink to red colour is formed.

Baljet test: Take a piece of thick section of the aerial part and add Sodium picrate reagent, if glycoside is present, yellow

e. Test for gums and mucilage

Precipitate with 95% alcohol: Gums and mucilage precipitate with addition of 95% alcohol, being insoluble in alcohol.

Ruthenium red test: Dissolve 0.008gm of ruthenium red in 10 ml of 10% solution of lead acetate. It stains mucilage to red colour.

f. Test for proteins and amino acids

Biuret test: 2ml of extract + 2ml of 10% NaOH solution + 2-3 drops of 1% $CuSO_4$ solution → mixing are done. If violet or purple colour appears then proteins are present.

Ninhydrin test: 2ml of extract + 0.5ml of Ninhydrin solution → boil for 2minutes then cool. If blue colour appears then proteins are present.

Millon's test: 2ml of extract + 2ml of Millon's reagent → boil → cool. Few drops of $NaNO_2$ solution were added. Appearance of red precipitate indicates presence of proteins.

g. Test for tannins and phenolic compounds

With ferric chloride: A 5 % w/v solution of ferric chloride in 90% alcohol is used for detection of phenols.

With lead acetate: Tannins are precipitated with lead acetate.

With gelatin solution: To a solution of tannins (0.5-1%) aqueous solution of gelatin (1%) and sodium chloride (10%) are added. A white buff coloured precipitate is formed.

h. Test for steroids and sterols

Libermann burchard reagent: To about 2ml of a solution extract in chloroform in a dry test tube, add 2ml of acetic anhydride and 2-3 drops of conc. H_2SO_4. Mix and stand for a few minutes. An emerald green colour develops if steroids or sterols are present.

Salkowski's test: To 5ml of a solution of extract in chloroform in a dry test tube add gently along the sides; on equal volume of conc.H_2SO_4 observe the upper chloroform layer and the lower acid layer. The acid layer develops a yellow color with a green fluorescence. The chloroform layer will give a play of colors first from bluish red to gradually violet red.

i. Test for Triterpenoids

Tin + Thionyl chloride: Extract dissolved in chloroform; add a piece of metallic tin. Add 1 drop of thionyl chloride. If pink color develops then triterpenoids are presents.

j. Test for Saponins:

Foam test (1ml. of extract + 9ml. of water): About 1ml of alcoholic and aqueous extracts is diluted separately with distilled water to 10ml. and shaken in a graduated cylinder for 15min and kept aside. One cm layer of foam after standing for 30min indicates the presence of saponins.

Haemolysis test: 3 drops of blood, to it 1 drop of extract is added.

k. Test for flavonoids

With NaOH: The extract dissolved in water, filter, and the filtrate is treated with sodium hydroxide, a yellow color is observed if flavonoids are present.

With H_2SO_4: A drop of conc. H_2SO_4 acid when added to the above, the yellow color disappears.

Shinoda Test: The extract is dissolved in water, filter and the filtrate is treated with Magnesium turnings, add drop of conc. HCL. A pink color develops which indicates presence of flavonoids.

Anthelmintic activity study

All the experiments were carried out in Indian adult earthworms (*Pheretima posthuma*) due to its anatomical resemblance with the intestinal roundworm parasites of human beings. They were collected from moist soil and washed with water to remove all fecal matters.

Administration of extract

The suspension of Ethanolic extract of *Portulaca quadrifida* Linn. of different concentration (25, 50 & 100 mg/ml) were prepared by using 1.0 % w/v of CMC as a suspending agent and final volume was made up to 20 ml for respective concentration. Albendazole was used as standard. Groups of approximately equal size worms consisting of two earthworms individually in each group were released into in each 20 ml of desired concentration of drug and extracts in the Petridis.

Administration of Albendazole

Albendazole (25 mg/ml) was prepared by using 1.0 % w/v of CMC as a suspending agent as administered as per method of extract.

Experimental design

The anthelmintic activity was performed according to the method.[10] *Pheretima posthuma* was placed in petridish containing three different concentrations (25, 50 & 100 mg/ml) of ethanolic extract of *Portulaca quadrifida* Linn. Each petridish was placed with 2 worms and observed for paralysis or death. Mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; the time death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. The test results were compared with Reference compound Albendazole (25 mg/ml) treated samples.

RESULTS

Percentage yield of ethanolic extract of *Portulaca quadrifida* Linn: The powdered material of *Portulaca quadrifida* Linn. was extracted in Soxhlet apparatus by using ethanol and the solvent was evaporated by vacuum distillation process.

The percentage yields of extract was obtained had been tabulated in the following Table No. 1.

Phytochemical investigation of ethanolic extracts of *Portulaca quadrifida* Linn.

The extract was tested for the presence of alkaloids, carbohydrates, cardiac glycosides,

anthraquinones glycosides, gums and mucilage, proteins and amino acids, tannins and phenolic compounds, steroids and sterols, triterpenoids and flavonoids. Ethanolic extract had shown the presence of alkaloids, triterpenoids, flavonoids, tannins and saponins. The phytochemical results were tabulated in the Table No. 2.

Anthelmintic Activity study:

The anthelmintic activity was performed on adult Indian earth worm *Pheretima posthuma* as it has anatomical and physiological resemblance with the intestinal round worm parasites of human beings. Ethanolic extract of *Portulaca quadrifida* Linn. of different concentration and Albendazole as standard were used for the study. The results had tabulated in Table No. 3.

DISCUSSION

The study was carried out to extract the aerial parts of *Portulaca quadrifida* Linn. By using simple solvent extraction process using ethanol as solvent and the percentage yield was calculated. By using the ethanolic extract the various phytochemical screening was carried out to confirm the presence of phytoconstituents. This phytochemical analysis indicated the presence of alkaloid, tannins, phenolic compounds, saponins, triterpenoids and flavonoids. From the observations made, a dose dependent paralytic effect much earlier and the time of death was observed (Table no. 3). Although all the different concentration of ethanolic extract showed significant anthelmintic activity in a dose dependent manner but the higher concentration appeared to be more effective. Evaluation of anthelmintic activity was compared with reference standard albendazole. The Phytochemical analysis of extract revealed the presence of tannins & flavonoids. Tannins have been reported to produce anthelmintic activities [11-12] as they can bind to free proteins in the gastrointestinal tract of host animal [13] or glycoprotein on the cuticle of the parasite and thereby cause deaths [14]. The potent wormicidal activity of the extract fraction against earthworms suggests that it is effective against parasitic infections of humans.

Table: 1 Percentage Yields of Ethanolic Extract of *Portulaca quadrifida* Linn.

Sl. No.	Name of the Extract	Percentage Yield (%w/w)
1.	Ethanol	11.6%

Table 2: Phytochemical screening of ethanolic extract of *Portulaca quadrifida* Linn.

Sl. No.	Name of the test	Ethanolic Extract
Test for Alkaloids		
1.	Mayer's Test	+
2.	Wagner's Test	+
3.	Dragendroff's Test	+
4.	Hager's Test	+
Test for Carbohydrates		
5.	Molisch's Test	-
6.	Fehling's Test	-
7.	Benedicts Test	-
Test for Cardiac glycosides		
8.	Keller-Killiani Test	-
9.	Legal's Test	-
10.	Baljet's Test	-
Test for Anthraquinones glycosides		
11.	Borntrager's Test	-
12.	Modified Borntrager's Test	-
Test for Gums and Mucilage		

13.	Ruthenium red Test	-
14.	Precipitation with alcohol (95%)	-
Test for Proteins and Amino acids		
15.	Biuret Test	-
16.	Ninhydrin Test	-
17.	Millon's Test	-
Test for Tannins and Phenolic compounds		
18.	With ferric chloride	+
19.	With lead acetate	+
20.	With gelatin solution	+
Test for Steroids and Sterols		
21.	Libermann Burchard Test	-
22.	Salkowski's Test	-
Test for Triterpenoids		
23.	Tin + Thionyl chloride	+
Test for Saponins		
24.	Foam Test	+
25.	Haemolysis Test	+
Test for Flavonoids		
26.	With NaOH	+
27.	With H_2SO_4	+
28.	Shinoda Test	+

(+) = Presence and (-) = Absence

Table 3: Anthelmintic activity study of *Portulaca quadrifida* Linn.

Sl. No.	Compounds	Concentration	*Pheretima posthuma*	
			Paralysis	Death
1.	Control	--	--	--
2.	Ethanolic extract of *Portulaca quadrifida* Linn.	25 mg/ml	74.35 ± 2.805	125.83 ± 5.23
3.		50 mg/ml	51.34 ± 1.24	112.33 ± 5.87
4.		100 mg/ml	37.86 ± 3.03	76.21 ± 1.75
5.	Standard (Albendazole)	25 mg/kg	55.66 ± 4.59	124.83 ± 6.99

CONCLUSION

The Phytochemical tests were confirmed the presence of alkaloid, tannins, phenolic compounds, saponins, triterpenoids and flavonoids in the ethanolic extract, The ethanolic extract of *Portulaca quadrifida* Linn. exhibited significant anthelmintic activity against earthworms in a dose dependent manner. The observed activity may be due to the presence of tannins and phenolic content (flavonoids) which is worth for further investigations on isolation of the specific constituents.

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