



CHARACTERIZATION AND EVALUATION OF ANTIOXIDANT ACTIVITY OF *PORTULACA OLERACEA*

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ABSTRACT

The present study was designed to investigate the anti-oxidant activity of the methanolic extract of *Portulaca oleracea*. The methanolic extract was evaluated by TLC and HPTLC fingerprint method. Anti-oxidant activity of methanolic extract was determined by DPPH free radical scavenging activity, reducing power by FeCl₃, nitric oxide free radical scavenging activity, super oxide scavenging activity by alkaline DMSO method.

Key words: *Portulaca oleracea*, Anti-oxidant, TLC, HPTLC, DPPH, DMSO.

INTRODUCTION

Portulaca oleracea (Linn.) family *Portulacaceae* is commonly called as Brihalloni, Gholika, Lona, Lonamla, Loni, Lonika and Lunia. *Portulaca oleracea* (Common Purslane, also known as Verdolaga, Pigweed, Little Hogweed or Pusley), is an annual succulent in the family *Portulacaceae*, which can reach 40 cm in height. It is a native of India and the Middle East, but is naturalised elsewhere and in some regions is considered an invasive weed.

The whole plant is considered antiphlogistic (takes the heat out), bactericide, antidiabetic, anaphrodisiac (opposite to aphrodisiac), emollient, calmative, diuretic, a refreshing agent¹.

The herb is used as a gastric sedative, to

allay excessive heat and pain, and applied to the eyes, to remove inflammation². Except for the roots, the entire plant is used as an antibacterial, anti-inflammatory and anthelmintic. It is used in treating bacillary dysentery and dysuria, Poultices of fresh leaves are used to treat mastitis, boils and impetigo³. Herb is chiefly valued as a refrigerant and alterative pot herb, particularly useful as an article of diet in scurvy and liver diseases⁴. It acts as a refrigerant and alterative in scurvy and liver diseases⁵. The use of this plant as a vegetable, spice and medicinal plant has been known since the times of the ancient Egyptians and was popular in England during the middle Ages⁶. Sour leaves are used as a vegetable⁴. Plant

and seeds are used in diseases of the kidney and bladder, as strangury, dysuria, haematuria, gonorrhoea etc. and also for diseases of the lungs. They are beneficial to the intestinal mucous membrane and therefore relieve tormina, tenesmus and other distressing symptoms in dysentery and mucous diarrhoea, particularly when combined with other drugs of similar nature⁴. The seeds are said to be used as a vermifuge, and to be useful in dyspnoea⁵.

MATERIAL AND METHODS

Extraction

Air dried and coarsely powdered (350 gm.) *Portulaca oleracea* herb was taken. Extraction was carried out in a soxhlet extractor using methanol. The extract was then concentrated to dryness under reduced pressure and it was preserved in a refrigerator^{7,8}. The soxhlation process was carried out until the solvent was found to be colourless. Then the solvent was filtered and distilled off. Final traces of methanol were removed under pressure by using rotary vacuum flask evaporator.

Thin layer chromatography

Thin layer chromatography is an important analytical tool in the separation, identification and estimation of different components. Here the principles of separation are adsorption and the stationary phase acts as an

adsorbent. Depending on the particular type of stationary phase, its preparation and use with different solvent can be achieved on the basis of partition or a combination of partition and adsorption. The plant methanolic extract showed good resolution in solvent system by trial and error method^{8,9}.

TLC procedure

The TLC plate prepared with silica gel-G (activated) was the stationary phase having a thickness of about 0.5mm. 20µl each of test solution was applied on silica gel-G plate (5x15 cm.). The TLC plate was developed in the saturated chromatographic chamber containing Toulene: Ethylacetate: Diethylamine (7:2:1) solvent systems.

HPTLC finger printing

Application of sample

Commercially available precoated aluminium sheets silica gel G60 F 254 [E. Merck], 10 × 10 cm plate were used for this study. The methanolic plant extract was used for analysis.

Chromatogram development

The plates were developed in Camag Twin Trough Chamber using the solvent system as used in TLC. After developing the plate was air dried and observed under UV chamber (Camag TLC Scanner).

Densitometric Scanning

The developed plate was scanned using densitometric at 254 nm, 366 nm. [range 200 – 400 nm.]. The developed chromatogram of this extract is shown in the HPTLC Chromatogram.

***In-vitro* antioxidant activity**

DPPH free radical scavenging activity

Preparation of standard solution

Required quantity of Ascorbic acid was dissolved in methanol to give the concentration of 5, 10, 20, 30, 40 and 50 µg/ml.

Preparation of test sample

Stock solutions of samples were prepared by dissolving 10 mg of dried methanolic extract in 10 ml of methanol to give concentration of 1mg/ml.

Preparation of DPPH solution

4.3mg of DPPH was dissolved in 3.3 ml methanol: it was protected from light by covering the test tubes with aluminum foil.

Protocol for estimation of DPPH scavenging activity

- 150µl DPPH solution was added to 3 ml methanol and absorbance was taken immediately at 516 nm for control reading.
- Different volume levels of test sample (100, 120, 140, 160, 180 and 200 µl) were screened and made 200 µl of each dose level by dilution with methanol.

- Diluted with methanol with up to 3 ml.
- 150µl DPPH solution was added to each test tube.
- Absorbance was taken at 516 nm in UV-visible spectrophotometer (Shimadzu, UV-1700, Japan) after 15 min using methanol as a blank.
- The % reduction and IC₅₀ were calculated as follows
- The free radical scavenging activity (FRSA) (% antiradical activity) was calculated using the following equation:

% antiradical activity =

$$\frac{\text{Controlabsorbance} - \text{Sampleabsorbance}}{\text{Controlabsorbance}} \times 100$$

- Each experiment was carried out in triplicate and results are expressed as mean % antiradical activity ± SD¹⁰.

Reducing power by FeCl₃

Preparation of standard solution

10mg of ascorbic acid dissolved in 10 ml of distilled water. Dilutions of this solution with distilled water were prepared to give the concentration of 5, 10, 20, 30, 40 and 50 µg/ml.

Preparation of test sample

Required quantities of the test samples were dissolved in minimum quantity of methanol and volume were made up to 10 ml with phosphate buffer. Separately all the samples were diluted in 10 ml volumetric flask with phosphate buffer

to give (100, 500, 1000, 2000 and 3000 µg/ml concentration.

Preparation of reagents

- Phosphate buffer: 0.2M phosphate buffer of pH 6.6 was prepared according to I.P.
- 1% Potassium ferricyanide solution: 2 gms of potassium ferricyanide was dissolved in 200ml of distilled water.
- 10% Trichloro acetic acid: 40 gms of Trichloro acetic acid was dissolved in 400ml of distilled water.
- 0.1% ferric chloride solution: 0.1 gm of ferric chloride was dissolved in 100ml of distilled water.

Protocol for reducing power

- 2ml of each sample and standard solutions were spiked with 2.5ml of 1% Potassium ferricyanide solution.
- This mixture was kept at 50° C in water bath for 20 min.
- After cooling, 2.5 ml of 10% Trichloro acetic acid was added and centrifuged at 3000 rpm for 10 min.
- 2.5 ml of supernatant was mixed with 2.5 ml of distilled water and 1 ml of 0.1% ferric chloride and kept for 10 min.
- Control was prepared in similar manner excluding samples.
- The absorbance of resulting solution was measured at 700 nm².

Nitric oxide free radical scavenging activity

Preparation of standard solution

10 mg of curcumin was dissolved in methanol to give the concentration of 1 to 13 µg/ml.

Preparation of test sample

Stock solutions of samples were prepared by dissolving 10 mg of dried methanolic extract in 10 ml of methanol to give concentration of 1mg/ml.

Protocol for estimation of nitric oxide scavenging activity

- Sodium nitro prusside (10 mg) in phosphate buffer saline was mixed with different volume levels of test sample (100, 120, 140, 160, 180 and 200 µl) made 200 µl of each dose level by dilution with methanol.
- Incubate the solution at room temperature for 150 minutes.
- The same reaction mixture without the extract but equivalent amount of methanol served as control.
- After the incubation period 5ml of Griess reagent was added
- The absorbance was taken in UV-visible spectrophotometer at 546 nm.
- Curcumin was used as positive control¹¹.
- The % reduction and IC₅₀ were calculated as follows.

- The free radical scavenging activity (FRSA) (% antiradical activity) was calculated using the following equation :

% antiradical activity =

$$\frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

- Each experiment was carried out in triplicate and results are expressed as mean % antiradical activity \pm SD.

Super oxide scavenging activity by alkaline DMSO method

Chemicals and Reagents

- Dimethyl sulfoxide was purchased from Merck Co. (Germany), Mumbai.
- Nitro-blue tetrazolium was purchased from SD Fine Chemicals.

Preparation of standard solution

10 mg of curcumin dissolved in 10 ml of distilled water. Dilutions of this solution with distilled water were prepared to give the concentration of 5, 10, 20, 30, 40 and 50 μ g / ml.

Preparation of test sample

Dissolve 25 mg of methanolic extract in 25ml of dimethyl sulfoxide to give stock solution of 1 mg/ml. Dilution were done with same dimethyl sulfoxide to give concentrations of 100, 200, 300, 400, 500 and 600 μ g/ml.

Preparation of reagents

Alkaline DMSO: 1 ml alkaline DMSO containing, 5 mM NaOH in 0.1 mL water and 0.9 ml Dimethyl sulfoxide.

NBT: 25 mg of nitro-blue tetrazolium was dissolved in 25 ml of Dimehtyl sulfoxide to give concentration of 1 mg/mL .

Protocol for estimation of superoxide scavenging activity

- To the reaction mixture containing 0.1 mL of NBT (1 mg/mL solution in DMSO) and 0.3 mL of the extract and standard in DMSO, 1 mL of alkaline DMSO (1 mL DMSO containing, 5 mM NaOH in 0.1 mL water) was added to give a final volume of 1.4 mL and the absorbance was measured at 560 nm.
- Extracts (100-600 μ g/ml) were added to a hydrogen peroxide solution (0.6ml, 40mM).
- 300 μ l of plain DMSO, 0.1 ml NBT solution and 1 ml alkaline DMSO was mixed and absorbance was taken at 560 nm and this was taken as control reading.
- The percentage of super oxide radical scavenging by the *Portulaca oleracea* extracts and standard compounds was calculated as follows :

% super oxide scavenging activity =

$$\frac{\text{test absorbance} - \text{control absorbance}}{\text{test absorbance}} \times 100$$

RESULT AND DISCUSSION

Thin Layer Chromatography

In the present experiment, different solvent systems were tried to resolve the components of methanolic extract of *Portulaca oleracea*. TLC plate of methanolic extract was developed by using Toulene:Ethylacetate: Diethylamine (7:2:1) solvent system and visualized by using dragendroff's reagent as a spraying reagent. TLC of methanolic extracts of *Portulaca oleracea* is shown in Table 1 and photographs of TLC plate is shown in Fig. 1.

HPTLC finger printing

HPTLC is now a days applied to obtain "Finger Print" patterns of herbal formulations, quantification of active ingredients and also detection of adulteration. The HPTLC chromatograms were developed for both the plant extracts using the same solvent system utilized for TLC.

Plate : Aluminium plate precoated with

Silica Gel GF254

Thickness : 0.2 mm

Plate size : 10 X 10 cm

Sample Application : 10 μ l, 20 μ l, 30 μ l

Solvent System : Toulene: Ethylacetate: Diethylamine (7:2:1)

Detection : 366 nm

Instrument : CAMAG TLC Scanner 3 and LINOMAT-V

Photograph of HPLTC finger print and Chromatogram of HPTLC profile of methanolic extract is shown in Fig. 2 and Fig. 3 respectively.

In-vitro antioxidant activity

DPPH free radical scavenging activity

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 516 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in ethanol was at 516 nm. The decrease in

Table 1: TLC solvent system

Mobile phase for methanolic extract (<i>Portulaca oleracea</i>)	Spraying reagent	Rf value	Spot colour
Toulene:Ethylacetate: Diethylamine (7:2:1)	Dragendroff's Reagent	0.65	Pink
		0.73	Purple

Table 2: IC₅₀ value of different antioxidant activity

Model	<i>Portulaca oleracea</i> methanolic extract (µg/ml) ± S.D	Ascorbic acid (µg/ml) ± S.D	Curcumin (µg/ml) ± S.D
DPPH scavenging	12.67 ±1.2	15.621±1.33	-----
Nitric Oxide scavenging	667.12±16.02	-----	41.37±5.05
Super oxide scavenging	182.02±9.64	15.78±1.87	-----

Value are mean ± S.D.; n=3

absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical progresses, which results in the scavenging of the radical by hydrogen donation. Fig. 4 illustrates increase scavenging of DPPH radicals in dose dependent manner due to the scavenging ability of the *Portulaca oleracea* methanolic extract. IC₅₀ value of ascorbic acid is 15.621±1.33 µg/ml.

Reducing power by FeCl₃

For the measurement of the reductive ability, we investigated the Fe⁺³→Fe⁺² transformations in the presence of *Portulaca oleracea* methanolic extract using the method of Oyaizu (1986)¹². The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Standard curve of ascorbic acid was shown in Fig. 5. Like the antioxidant

activity, the reducing power of *Portulaca oleracea* methanolic extract increase with increasing concentration, as shown in Fig. 6.

Nitric oxide free radical scavenging activity

Active oxygen species and free radicals are involved in a variety of pathological events. In addition to ROS, nitric oxide is also implicated in inflammation, cancer and other pathological conditions. A potential determination of oxidative damage is the oxidation of tyrosine residue of protein, peroxidation of lipids, and degradation of DNA and oligonucleosomal fragments. Nitric oxide or reactive nitrogen species formed during its reaction with oxygen or with superoxide such as NO₂, N₂O₄, N₃O₄, nitrate and nitrite are very reactive. These compounds alter the structure and function of many cellular

components. Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of this damage. Methanolic extract of *Portulaca oleracea* shows increase in nitric oxide, as shown in Fig. 7. IC_{50} value of Standard Curcumin is $41.37 \pm 5.05 \mu\text{g/ml}$.

Super oxide scavenging activity by alkaline DMSO method

Superoxide radicals are known to be very harmful to the cellular component. Super oxide free radical was formed by alkaline DMSO which reacts with NBT to produce coloured diformazan. The methanolic extract of *Portulaca oleracea* scavenges super

oxide radical and thus inhibits formazan formation. Fig. 8 illustrates increase scavenging of superoxide radicals in dose dependent manner due to the scavenging ability of the *Portulaca oleracea* methanolic extract. IC_{50} value of ascorbic acid is $15.78 \pm 1.87 \mu\text{g/ml}$.

IC_{50} value of different antioxidant activity

IC_{50} value of different antioxidant activity of *Portulaca oleracea* methanolic extract was shown in Table 2.

Active oxygen species and free radicals involved in variety of pathological events. Herbal drug containing free radical scavengers are gaining importance in treating disease.

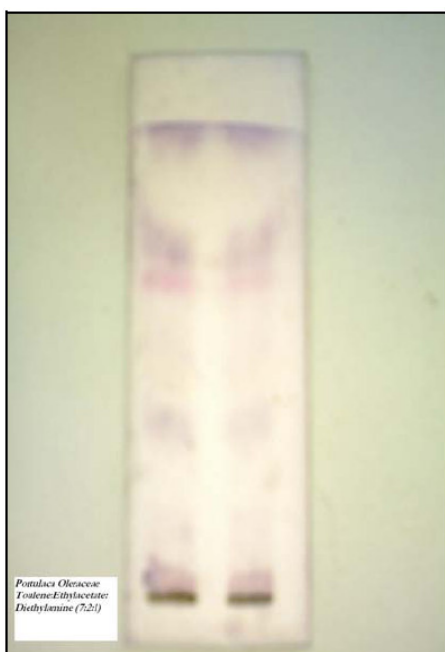


Fig. 1 : TLC of methanolic extract of *Portulaca oleracea* by using solvent system of Toulene: Ethylacetate: Diethylamine (7:2:1)

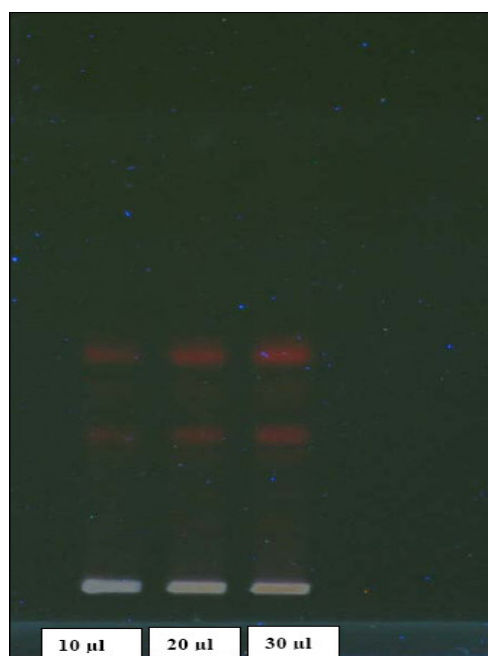


Fig. 2 : HPTLC Fingerprint

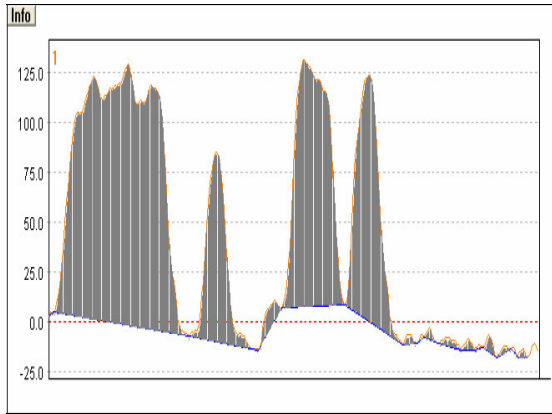


Fig. 3 : Chromatogram of HPTLC fingerprint profile for methanolic extract of *Portulaca oleracea*

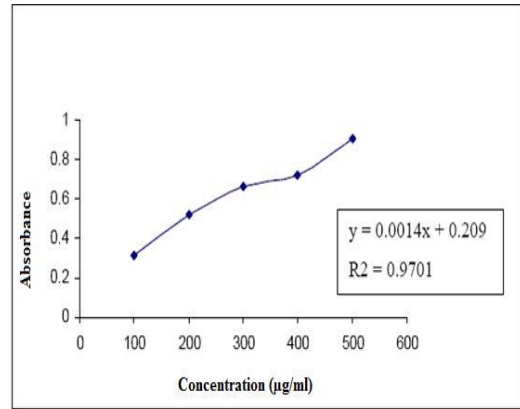


Fig. 6 : Reducing power by FeCl_3 method for *Portulaca oleracea* methanolic extracts

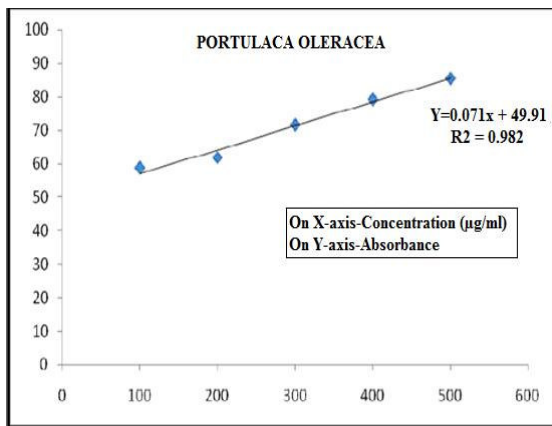


Fig 4: DPPH radical scavenging activity of *Portulaca oleracea* methanolic extract. IC_{50} value is $12.67 \pm 1.2 \mu\text{g/ml}$.

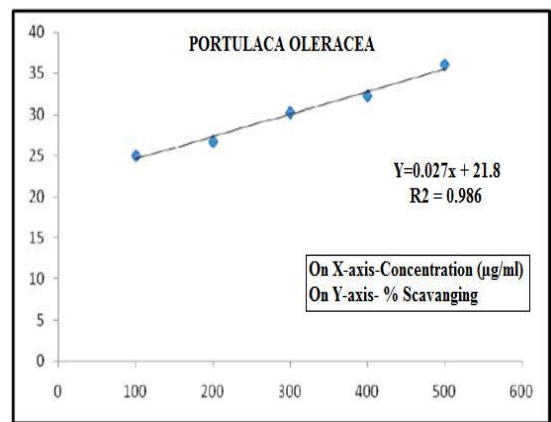


Fig. 7 : Nitric oxide radical scavenging activity of methanolic extract of *Portulaca oleracea*. IC_{50} value is $667.12 \pm 16.02 \mu\text{g/ml}$.

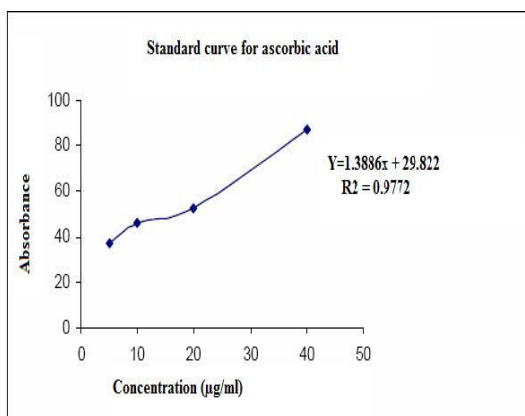


Fig. 5 : Standard curve for ascorbic acid (FeCl_3 method)

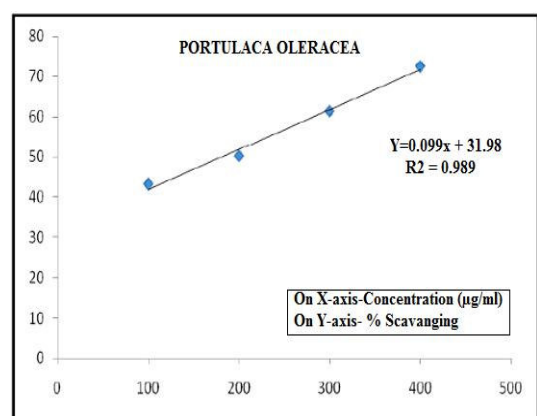


Fig. 8 : Super oxide radical scavenging activity of methanolic extract of *Portulaca oleracea*. IC_{50} value is $182.02 \pm 9.64 \mu\text{g/ml}$.

CONCLUSION

The present study of *Portulaca oleracea* herb might be useful to supplement information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario of lack of regulatory laws to control quality of herbal drugs.

TLC was performed for the methanolic extract by using solvent system Toulene: Ethylacetate: Diethylamine (7:2:1 v/v/v) with using Dragendroff's reagent which gives R_f value of 0.65 & 0.73 (pink and purple colour respectively).

The HPTLC chromatogram was developed for methanolic extract using the same solvent system utilized for TLC. HPTLC fingerprinting of *Portulaca oleracea* methanolic extract was also performed according to WHO guideline for quality control and standardization. Quantitative estimation of major compound, compound responsible for significant *in vitro* antioxidant activity and will be evaluated in future studies.

The DPPH free radical scavenging activity, reducing power by FeCl₃, Nitric oxide free radical scavenging activity and Super oxide scavenging activity by alkaline DMSO method, of methanolic

extract were determined. The methanolic extract shows significant *in vitro* antioxidant activity in a higher dose than standard antioxidant. The phytochemical analysis of the crude extracts indicated the presence of major phytochemicals, including phenolics, flavanoids which may have been responsible for the observed antioxidant activity.

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