Research Article

Pharmacognostic, Phytochemical and Pharmacological Investigation on Leaf and Root of *Mirabilis jalapa* Linn (Nyctaginaceae)

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ABSTRACT

Antioxidants play an important role in protecting against damage by reactive oxygen species. The antioxidant activity of different extracts of *Mirabilis jalapa* was evaluated by employing three in vitro experiments namely 1, 1-Diphenyl-2-picrylhydrazyl (DPPH), Nitric oxide method and Hydrogen peroxide scavenging**.** The leaf and root powder of the plant was extracted with different solvents by extraction method in order of decreasing polarity and then partitioned. The present study was designed to evaluate the plant potential as an antioxidant lead by using various *in vitro* models like DPPH, Hydrogen peroxide scavenging method, and, Nitric oxide method. The plant exhibited significant antioxidant properties and could serve as a free radical inhibitor or scavenger.

Keywords: *Mirabilis jalapa Linn*, Antioxidant, DPPH, Nitric oxide method, Hydrogen peroxide scavenging activity.

INTRODUCTION

Mirabilis is an interesting family containing 350 species in 34 classifications. The wild 'Four o' clock' (family: Nyctaginaceae) is a native of the Dakota prairies and has spread as a weed eastward to the Atlantic States. It was originally discovered by the French botanist Andre Michaux around 1792. It is known as Anthi-Mandhaari in Tamil, Naalumanipoovu in Malayalam, Gulabakshi in Marathi, GodhuliGopal in Assamese, Vieruurtjie in Afrikaans, Zi Mo Li in Chinese, Belle-De-Nuit in French, Belle Di Notte in Italian, Punkkot in Korean, Hoja De Xalapa in Spanish, Beauty of night, Four O' clock, Marvel of Peru in English[15].

Mirabilis jalapa Linn of family *Nyctaginaceae* has been called by various vernacular names around the world like 'Four o' clock' in English, Gulambasa' in Ayurveda, and 'Gul-abbas' in Hindi. 'Four o' clock' received the name because of habit of opening in the late afternoon. *Mirabilis jalapa* has been extensively used in almost all folklore remedies around the world for treating a variety of conditions. It has been reported that indigenous Mexican population uses various decoctions and preparations of *Mirabilis jalapa* for muscular pain, diarrhoea, dysentery, and abdominal colic[1]. *Mirabilis jalapa* is used in traditional medicine by the people from different countries for the treatment of diarrhea, dysentery, conjunctivitis, edema, inflammation, swellings and muscular pain. *Mirabilis jalapa* is widely used to treat dysentery, diarrhea, muscular pain, and abdominal colics in many countries and its extract has antibacterial, antiviral, and antifungal functions. The root is believed to be an aphrodisiac as well as diuretic and purgative. It is used in the treatment of dropsy[8]. An enzyme or other organic molecule that can counteract the damaging effects of oxygen in tissues. Although the term technically applies to molecules reacting oxygen, it is often applied to molecules that protect from any free radical (molecule with unpaired electron). Evidence indicates that harmful free radicals play an important role in most major health problem such as cancer, cardiovascular disease, and rheumatoid arthritis.

Antioxidants are beneficial components that neutralize free radicals before they can attack cells and hence prevent damage to cell proteins, lipids and carbohydrates[13].

MATERIALS AND METHODS

Collection and Identification of Plant Material

The fresh plants of *Mirabilis jalapa Linn* were collected in the months of July-August from K.T.H.M. campus, Nashik district, Maharashtra, India, and authenticated by Dr P.G. Diwakar, Botanical Survey of India, Pune.

Preparation of Extracts

The leaf and root of *Mirabilis jalapa* was collected, washed and dried at room temperature. Leaf and roots were grinded into the fine powder, extracted with different solvents in decreasing order of solvent polarity i,e Petroleum ether, Chloroform, Methanol each for 72 hrs. The extract was dried in a vacuum oven to obtained constant weight.

Phytochemical Evaluation

The methanolic extract was used to analyze qualitatively various phytoconstituents such as alkaloids, glycosides, steroids, phenolic compounds, tannins, flavonoids and carotenoids using standard procedures[5].

Estimation of Total Flavonoid Content

Material

Chemicals

Folin-ciocalteu reagent, Gallic acid, Sodium Carbonate, Distilled Water, Methanol.

Gallic Acid Stock Solution

In 100 ml of volumetric flask, 0.5 gm of dried Gallic acid dissolved in 10 ml of methanol and diluted up to the volume with water.

Preparation of Stock Solution

Stock solution of 1000 ppm of methanolic extract was prepared in methanol.

Sodium Carbonate Solution

200 gm Sodium carbonate of dissolved at 70-80^{0}C volume was made with distilled water up to 1 lit. Solution was filtered through glass wool and allows standing overnight.

Method

Preparation of Calibration Curve

To prepare a calibration curve 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 ml of Gallic acid stoke solution transferred in 100ml of volumetric flask, dilute up to volume with water to produce 0, 25, 50, 100, 125, 150, and 200 mg/lit of concentrations of Gallic acid.

One ml of standard Gallic acid solution in 25 ml of volumetric flask, added with 10 ml water, 1.5 ml of Folin-ciocalteu reagent and allow to stand for 10 minute.

Four ml of Sodium carbonate solution was added in each volumetric flask and volume adjusted with water. Absorbance was taken after 1hr at 760 nm by UV spectrophotometer against reagent blank. Absorbance Vs Concentration calibration curve was plotted.

Estimation of Phenolic Content

One ml of stock solution of extract was taken in 25 ml of volumetric flask, added with 10 ml water, 1.5 ml of Folin-ciocalteu reagent and allow to stand for 10 minute. Four ml of Sodium carbonate solution was added in each volumetric flask and volume adjusted with water. Absorbance was taken after 1hr at 760 nm by UV spectrophotometer against reagent blank.

Total phenolic content was expressed as mg Gallic acid equivalents (GAE) /g of sample, calculated from the formula:

$$T = C \times V/M$$

Where, T = Total phenolic content in mg/g plant extract, in GAE

C = Concentration (mg/ml) of Gallic acid obtained from calibration curve

V = Volume of extract (ml)

M = Weight (g) of plant extract

Determination of Total Tannin Content

Total phenolics content was determined according to the Folin-Ciocalteu method, using gallic acid as standards. Extract (1 mg) was dissolved in 1 ml 50% methanol solution. Extract solution (0.5 ml) was mixed with 0.5 ml of 50% Folin-Ciocalteu reagent. After 2-5 min, 1.0 ml of 20% Na_2CO_3 was added to the mixture and incubated for 10 min at room temperature. The mixture was centrifuged at 150 g for 8 min and the absorbance of the supernatant was measured at 730 nm. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram sample[6].

In Vitro Antioxidant Activity

DPPH Free Scavenging Assay

Chemical

DPPH (1.3 mg/ml), Fraction of extract of leaf and root (i.e. KPML and KPMR), Methanolic extract of leaf and root (i.e MEL and MER), Methanol, Ascorbic acid.

Equipment

UV-Spectrophotometer, Graduated pipetts.

Preparation of DPPH Stock Solution

1.3mg of DPPH dissolved in 10ml methanol.

Preparation of Ascorbic Acid Solution

1000µg/ml stock solution was prepared by dissolving 100mg of ascorbic acid dissolved in 100 ml of distilled water.

Sample Preparation

1000µg/ml stock solution was prepared by dissolving 100mg of extract in 100 ml of distilled water. And various concentration of extracts 25, 50, 75, 100 and 125 µg/ml solutions were prepared from stock solution.

Procedure

Three ml of different concentration of extract solution and standard were taken in vials. Add 5ml of methanolic solution of DPPH, shaken well and mixture was incubated at 37°C for 25 min. Measure the Absorbance against Methanol as blank at 517nm. Absorbance of DPPH as control was recorded. Percent antiradical activity was calculated by using formula[7].

$$\%\ Anti-radical\ Activity = \frac{(Control\ Abs - Sample\ Abs)}{Control\ Abs} \times 100$$

Nitric Oxide Method

Preparation of Stock Solution

1000µg/ml Stock solution was prepared by dissolving 100mg of Ascorbic acid in 100 ml of distilled water.

Preparation of Sample

Different concentration (25, 50, 75, 100, 125 µg/ml) of pet ether extract and methanolic extract, Ascorbic acid dissolved in phosphate buffer (pH 7.0).

Procedure

Incubate stock solution with different concentration of sample at 25°C for 150 minute. Control experiment

without the test sample but equivalent amount of buffer was conducted in identical manner.

After incubation take 0.5 ml of solution, add 0.5 ml of Griess reagent (1% Sulphanilamide, 0.1% N-1-naphylethylenediamine dichloride, 2% phosphoric acid) was added.

The absorbance was taken at 546 nm. Ascorbic acid used as standard and % inhibition was calculated[7].

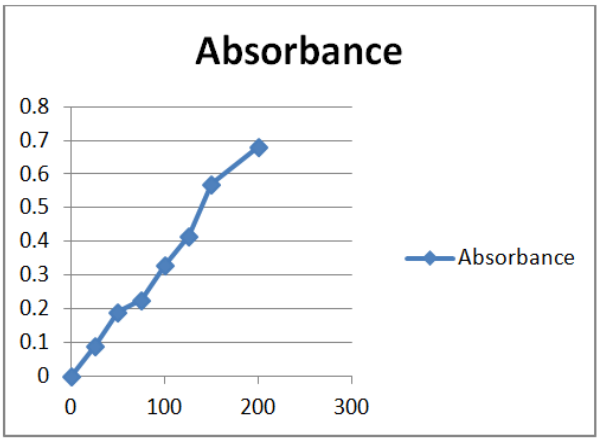

Figure 1: Plot of Calibration Curve of Standard Gallic Acid

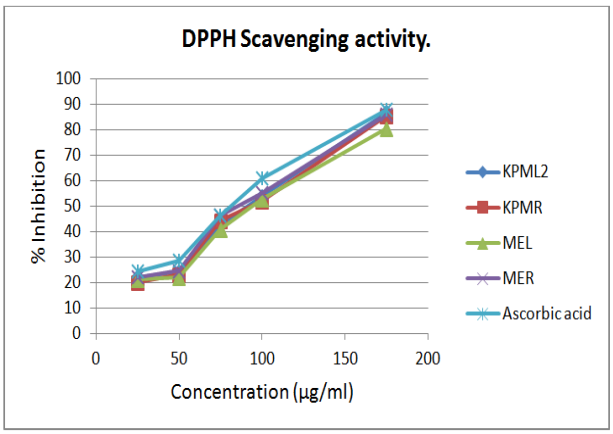

Figure 2: Effect of ME and Ascorbic Acid on Free Radical Scavenging Activity by DPPH Activity

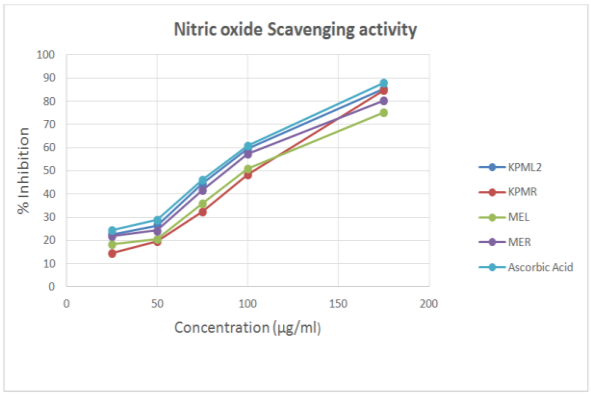

Figure 3: Effect of ME and Ascorbic Acid on Free Radical Scavenging Activity by Nitric Oxide Activity

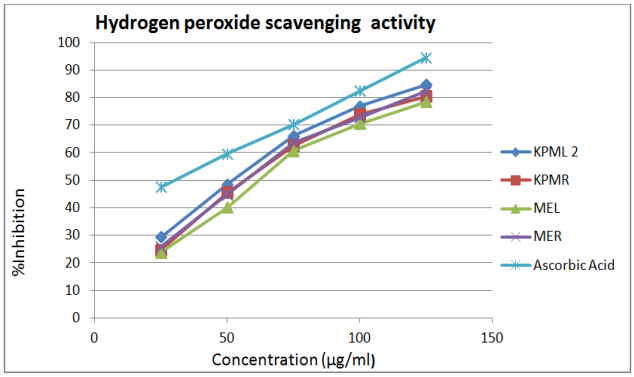

Figure 4: Effect of ME and ascorbic acid on free radical scavenging activity by Hydrogen peroxide scavenging activity.

Table 1: Characterization of extracts by Chemical Tests

S No.	Chemical Test	Leaf Extract			Root Extract		
		Petroleum Ether	Chloroform	Methanol	Petroleum Ether	Chloroform	Methanol
1	Test for sterol	Present	-	-	Present	-	-
2	Test for Glycosides	-	-	Present	-	-	Present
3	Test for Alkaloids	-	Present	-	-	Present	-
4	Test for Flavonoids	-	-	Present	-	-	Present
5	Test for Tannins	-	-	Present	-	-	Present
6	Test for Carotenoids	Present	-	-	Present	-	-

Observation Table

Table 2: Curve data of Gallic Acid

S. No.	Concentration (µg/ml)	Abs at 760 nm
1	0	0.0
2	25	0.089
3	50	0.19
4	75	0.226
5	100	0.332
6	125	0.418
7	150	0.572
8	200	0.682

Table 3: Observation Table for Percent Antiradical Activity by DPPH Scavenging Activity

S. No.	Concentration (µg/ml)	% Inhibition				
		KPML	KPMR	MEL	MER	Ascorbic Acid
1	25	22.10	20.12	21.51	22.31	24.5
2	50	24.32	23.15	22.0	24.69	28.84
3	75	42.63	44.25	40.98	46.23	46.26
4	100	53.98	51.98	52.69	55.25	60.78
5	175	86.85	85.36	80.53	86.11	87.98
6	IC50	16.27	16.27	17.17	15.98	14.59

Table 4: Antioxidant Activity by Nitric Oxide Scavenging Activity.

S. No.	Concentration (µg/ml)	% Inhibition				
		KPML	KPMR	MEL	MER	Ascorbic Acid
1	25	22.56	14.63	18.42	21.89	24.5
2	50	26.39	19.58	20.69	24.36	28.84
3	75	44.58	32.44	35.85	41.69	46.26
4	100	59.42	48.32	50.86	57.36	60.78
5	175	85.32	84.82	75.25	80.45	87.98
6	IC_{50}	15.48	17.96	18.40	16.61	14.59

Table 5: Hydrogen Peroxide Scavenging Activity

S. No.	Concentration (µg/ml)	% Inhibition				
		KPML	KPMR	MEL	MER	Ascorbic Acid
1	25	29.40	24.65	23.80	25.98	47.51
2	50	48.53	45.48	40.21	44.86	59.58
3	75	66.14	62.57	60.78	63.77	70.18
4	100	76.93	73.98	70.56	72.89	82.47
5	125	84.78	80.48	78.54	82.44	94.52
6	IC_{50}	16.89	18.45	19.58	17.15	15.10

Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide scavenging activity was measured with titrimetric method of estimation.

Procedure

1ml of 0.01Mm of H2O2, 2 drops of 3% Ammonium molybdate indicator, 10ml sulphuric acid and 7 ml of 2M KI. The mixed solution was titrated with 5 mM Sodium thiosulphate until yellow colour was disappeared. Ascorbic acid was used as positive control and percentage hydrogen scavenging was determined[16].

$$\%\ Inhibition = \frac{(Blank - Test)}{Blank} \times 100$$

RESULTS AND DISCUSSION

The fraction isolated from Leaf (KPML) is ß-Sitosterol and the fraction isolated from root (KPMR) is oleanolic acid.

Total phenolics content in leaf and root was found to be0.310, 0.365 respectively.

Total tannin content in leaf and root was found to be 9.28% w/w, 10.5% w/w respectively.

In the biological evaluation in the antioxidant activity in the DPPH method IC_{50} of KPML, KPMR, MEL, MER was found to be 16.27, 17.57, 17.17, and 15.98 respectively. By nitric oxide scavenging method IC_{50} of KPML, KPMR, MEL, MER was found to be 15.48, 17.96, 18.40, and 14.17 respectively. By Hydrogen peroxide scavenging activity IC_{50} of KPML, KPMR, MEL, MER was found to be 16.52, 18.28, 20.15, and 17.18 respectively.

CONCLUSION

Mirabilis jalapa plant showed presence of sterols, carotenoids, glycosides, alkaloids, flavonoids, tannins.

Leaf shows maximum activity as compare to oleanolic acid isolated from root more than standard Ascorbic acid.

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