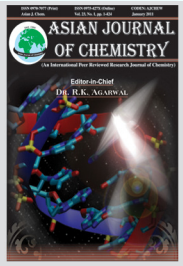
Asian Journal of Chemistry; Vol. 23, No. 2 (2011), 799-801

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Free Radical Scavenging Potential of *Jatropha gossypifolia*

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(*Received*: 5 May 2010; *Accepted*: 5 October 2010) AJC-9166

The leaves of *Jatropha gossypifolia* Linn. known as Bellayche bush is an important Indian medicinal plant. Preliminary phytochemical screening of the plant showed the presence of high amount of phenolics, tannins and flavonoids. Subsequent quantification showed the presence of 8.6 % w/w total phenolics and 7.4 % w/w of flavonoids. This high amount of phenolics and flavonoids prompted us to evaluate its antioxidant activity. We studied the antioxidant activity of *J. gossypifolia* in three *in vitro* models: DPPH free radical, ferric thiocyanate and nitric oxide scavenging method. The methanol, ethyl acetate and aqueous extracts of *Jatropha gossypifolia* leaves showed significant antioxidant activity. Free radical scavenging activity might be due to the presence of flavonoids.

Key Words: *Jatropha gossypifolia*, DPPH, Nitric oxide, Ferric thiocyanate.

INTRODUCTION

The plants from the genus Jatropha were reported for the various activities such as antibacterial, analgesic, anticonvulsant, antiinflammatory and useful as emmanogogue, emetic and purgative. The leaves are applied to boils and carbuncles, eczema and itches. In the pith of the old thick stems a yellowish brown substance is found which is sold in gold coast medicine markets. The leaves are boiled and used in the bath to cure fever. Their juice is used to cure sores on the tongues of babies[1,2]. However literature reveals that *Jatropha gossypifolia* Linn., is not studied thoroughly for both pharmacognostically and pharmacologically. It was also evident from literature that various ethnomedicinal claims as well as modern pharmacological investigations and rationalization chemotaxonomical tracing is another parameter that stimulates the detailed investigation of plant.

EXPERIMENTAL

The fresh leaves of *Jatropha gossypifolia* Linn. were collected from Amravati district, Maharashtra, India and authenticated by Dr. (Mrs.) Bhogaonkar HOD Dept. of Botany, VMV College Amravati.

Preparation of the plant extract: The collected plant leaves were dried under shade and pulverized into coarse powder. The powder was used for preparation of extracts and was successively extracted in soxhelt extractor with solvent of increasing polarity *i.e.*, petroleum ether (60-80 ºC), chloroform, ethyl acetate, methanol and water. The extract was concentrated under vacuum and evaporated to dryness.

1,1-Diphenyl-2-picryl hydrazyl (DPPH), Sigma Aldrich; ethylene diamine tetra acetate (EDTA), sodium nitroprusside, naphthalene diamine dihydrochloride and folin ciocaltu reagent SD fine chemicals and all the solvents used were of A.R. grade.

UV/Vis spectrophotometer (Shimadzu) and CAMAG Linomat V automatic sample spotter (Switzerland) were used in this study.

Phytochemical evaluation: Five hundred milligrams of the dried extracts were reconstituted in 10 mL of respective solvents and used for preliminary phytochemical testing for the presence of different chemical groups of compounds. Preliminary phytochemical investigation showed that presence of alkaloids, flavonoids and tannins[3].

Estimation of total phenolics: The total phenolic content of the plant was estimated according to the method described by Singleton and Rossi and expressed as percentage gallic acid[4].

Estimation of flavonoids: The flavonoids content was estimated by the aluminum chloride method[5].

Free radical scavenging activity: Free radical scavenging activity was carried out by three *in vitro* model.

Assay for antiradical activity: Antiradical activity was measured by a decrease in the absorbance at 516 nm of the methanolic solution of coloured DPPH brought about by the sample. A stock solution of DPPH (1.3 mg/mL methanol) was prepared such that 75 mL of it in 3 mL methanol gave an initial absorbance of 0.9. This stock solution was used to measure the antiradical activity. Decrease in the absorbance in the presence

of different extracts of *Jatropha gossypifolia* at different concentrations was noted after 15 min. EC_{50} was calculated from percentage inhibition. Ascorbic acid was used as positive control. Experiment was repeated in triplicate[6-8].

Nitric oxide method: Sodium nitroprusside (5 Mm) in standard phosphate buffer solution was incubated with different concentration of test solution dissolved in standard phosphate buffer and tubes were incubated at 25 ºC for 150 min. Control experiment without test sample but sample with equivalent amount of buffer was conducted in identical manner. After 150 min, 1.5 mL of incubated solution was removed and diluted with 1.5 mL of Griess reagent (10 % sulphanilamide 2 % H_3PO_4 and 1.0 % naphthalene diamine dihydrochloride). The absorbance of chromophore formed during diazotization of nitrite with subsequent coupling with naphthalene diamine was read at 546 nm. Experiment was repeated in triplicate[9,10].

Ferric thiocynate method: Each extract [500 µg/mL] and BHT (50 µg/mL) was mixed with 4.0 mL of 95 % (v/v) ethanol, 4.0 mL of linoleic acid (2.51 % v/v) in 99.5 % ethanol, 8.0 mL 0.05 M phosphate buffer pH 7.0 and 4.0 mL distilled water. The vials were incubated at 40 ºC for 5 day's in dark with the sampling interval of 24 h. To 0.1 mL of sample withdrawn, 9.7 mL of 75 % (v/v) ethanol and 0.1 mL of 30 % (w/v) ammonium thiocynate was added. Precisely after 3 min 0.1 mL of 20 mM ferrous chloride was added. The absorbance at 500 nm was measured[1].

RESULTS AND DISCUSSION

Preliminary phytochemical screening revealed the presence of flavonoid glycosides, alkaloids, carbohydrates and proteins. Subsequent quantification showed the presence of 8.6 % w/w total phenolics and 7.4 % w/w of flavonoids. The methanolic extract showed promising antioxidant activity in all three types of method. While ethyl acetate extract showed moderate activity and aqueous extract showed poor activity. Table-1 illustrates a significant decrease in the conc. of DPPH radical due to the scavenging ability of soluble solids in the various extracts and the standard ascorbic acid as a reference compound presented the highest activity at all concentrations.

The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine was read at 546 nm and referred to the absorbance of standard solution of curcumin treated in the same way with Griess reagent. Table-2 illustrates a significant decrease conc. of nitric oxide radical as increase in concentration of extracts.

Delay or prevention of LDL peroxidation is an important function of antioxidants. Table-3 shows the time course plots for antioxidant activity of the different extract fractions from *J. gossypifolia* and BHT using the FTC method. Methanol extract had the highest antioxidant activity, followed by ethyl acetate. Water extract had lower antioxidant activity. Hence, water extract of leaf may contain some compounds that can enhance peroxidation. There were significant differences among the different extract fractions in antioxidant activity.

As phytochemical screening revealed the presence of flavonoids, which could be responsible for its antioxidant activity. This result substantiates the ethanobotanical use of this plant. Further study needed to evaluate *in vivo* antioxidant activity. More detailed phytochemical studies are, however, necessary to identify the active principle(s).

Statistical analysis: The experimental results were expressed as the mean ± SD.

TABLE-1
FREE RADICAL SCAVENGING POTENTIAL OF *J. gossypifolia* BY DPPH RADICAL SCAVENGING METHOD

Extract	Concentration (µg/mL) and Inhibition (%)						IC_{50} (µg/mL)
	12.5	25	37.5	50	75	100	
Water	12.47 ± 0.4445	28.14 ± 0.6115	35.40 ± 0.2768	45.18 ± 0.0635	59.88 ± 0.335	73.96 ± 0.2821	63.36
Ethyl acetate	14.10 ± 0.8844	32.03 ± 042	46.73 ± 0.4619	57.51 ± 0.70	62.47 ± 0.2768	73.62 ± 0.3536	43.47
Methanol	15.66 ± 0.2910	24.18 ± 0.2289	57.32 ± 0.555	70.99 ± 0.2250	87.25 ± 0.4221	89.25 ± 0.1680	32.71
Vitamin C	30.95 ± 0.2345	59.73 ± 0.065	91.66 ± 0.11	93.47 ± 0.0635	93.84 ± 0.0635	95.18 ± 0.1789	20.92

TABLE-2
FREE RADICAL SCAVENGING POTENTIAL OF *J. gossypifolia* BY NITRIC OXIDE SCAVENGING METHOD

Extract	Concentration (µg/mL)					IC_{50} (µg/mL)
	100	200	300	400	500	
Water	14.37 ± 0.010	23.27 ± 0.005	44.41 ± 0.010	69.17 ± 0.010	76.70 ± 0.010	289.14
Ethyl acetate	13.68 ± 0.010	29.44 ± 0.010	45.88 ± 0.015	64.30 ± 0.011	73.40 ± 0.020	311.04
Methanol	19.16 ± 0.005	25.33 ± 0.010	49.88 ± 0.015	66.40 ± 0.026	78.54 ± 0.005	301.20
CR	30.81 ± 0.015	48.62 ± 0.005	62.32 ± 0.020	75.33 ± 0.020	91.09 ± 0.015	265.49

TABLE-3
FREE RADICAL SCAVENGING POTENTIAL OF *J. gossypifolia* BY INHIBITION OF LINOLIC ACID PEROXIDATION

Extract	Inhibition (%)/h				
	24	48	72	96	120
Water	50.16037 ± 0.027	53.70479 ± 0.005	64.71719 ± 0.015	65.07301 ± 0.004	64.71771 ± 0.017
Ethyl acetate	52.40400 ± 0.013	57.46800 ± 0.004	67.21000 ± 0.008	70.97400 ± 0.004	78.93300 ± 0.018
Methanol	59.38700 ± 0.007	63.30700 ± 0.003	73.27600 ± 0.015	74.19400 ± 0.021	83.16100 ± 0.008
BHT	57.99700 ± 0.004	63.84100 ± 0.004	73.91600 ± 0.005	74.73400 ± 0.004	83.96400 ± 0.007

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