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In vitro free radical scavenging activity of *Jatropha gossgypifolia* Linn. containing phenolic compounds

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To evaluate the *in vitro* free radical (FR) scavenging activity of leaves *Jatropha gossypifolia* Linn. petroleum ether, ethanol, aqueous extracts of *J. gossypifolia* were prepared, with successive extraction in Soxhlet apparatus. Each extract was selected to study the FR scavenging activity by superoxide scavenging assay and 2, 2-diphenyl-1 picrylhydrazyl hydrate (DPPH) radical scavenging assay method. It was found that aqueous extract contained carbohydrates, glycosides amino acids flavonoids, tannins, alkaloids and steroids; ethanolic extract contained glycosides amino acids flavonoids, tannins, alkaloids and steroids. Radical scavenging activity of plant extracts against stable DPPH was determined spectrophotometrically. Extract solutions were prepared by dissolving 0.025 g of dry extract in 10 ml of methanol. The solution of DPPH in methanol was prepared daily. The samples were kept in the dark for 15 min at room temperature and then the decrease in absorption was measured. Ethanolic extract of *J. gossypifolia* showed 58.7 ± 0.62% inhibition in superoxide scavenging model. Aqueous extract also showed almost similar activity (54.9 ± 0.53% compared to ethanolic extract), while petroleum ether extract showed poor inhibition of superoxide scavenging activity. All extracts showed dose and time dependent inhibition of superoxide scavenging activity. *J. gossypifolia* had the highest total phenolic content (42.60 mg tannic acid equivalent (TAE) /100 g fresh weight). Total phenolic content had positive correlation with antioxidant capacity. This shows that the plants, especially *J. gossypifolia*, may be potent source of natural antioxidants.

Key words: *Jatropha gossypifolia,* 2, 2-diphenyl-1 picrylhydrazyl hydrate (DPPH), phenolic content, superoxide scavenging, antioxidant activity.

INTRODUCTION

Jatropha gossypifolia, a common garden plant in tropical countries has been used as a traditional medicine. Plants are well known as a major source of modern medicines. From ancient times, humans have utilized plants for the treatment or prevention of diseases, leading to the dawn of traditional medicine. *J. gossypifolia* is one of the genera that are used in Chinese, Ayurvedic and Thai traditional medicine for the treatment of fever, pain and dysentery (Geronikaki et al., 2003; Wasana et al., 2008). Literature reveals that, the carbonyl groups are responsible for free radical (FR) scavenging activity (Nicholls and Budd, 2000). FR are atoms or groups of atoms with an odd number of electrons and can be formed when oxygen interacts with certain molecules. To prevent FR damage, the body has a defense system of antioxidants (Patil et al., 2003; Sharma et al., 2002). Antioxidants are able to give FR, which becomes a companion to their unpaired electron, thus, eliminating the threat of gene alteration leading to cancer (Patil et al., 2003; Sharma et al., 2002). Medicinal plants have attracted attention of not

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attracted attention of not only professionals from various systems of medicines, but also the scientific community belonging to different disciplines. (Chhajed el al., 2007; Khandelwal, 2000). In continuation of search in potential FR scavenging agents (Kokate, 1999), the present investigation was aimed to determine FR scavenging activity of *J. gossypifolia* leave (Linn.). FR scavenging properties help in strengthening the immune system of the body, which helps to overcome cancer.

Plant phenolics are commonly found in both edible and non-edible plants, and have been reported to have multiple biological effects, including antioxidant activity. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. In addition, they have a metal chelation potential (Rice-Evans et al., 1995). The phenolic compounds are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food (Kähkönen et al., 1999). The importance of natural phenolic compounds from plants materials is also raising interest among scientists, food manufacturers, and consumers due to functional food with specific health effects (Löliger, 1991). Several studies had been conducted to evaluate the correlation between phenolic compounds and antioxidant activity. The antioxidant activity of Du-Zhong (*Eucomnia ulmoides*) (Yen and Hsieh, 1998), ear mushrooms (Chao, 2001) and anise (*Pimpenella anisum* L.) seed (Gülçin et al., 2003) were found to correlate with the phenolic compounds. Studies on local plants such as turmeric (*Curcuma domestica*), betel leaf (*Piper betel*), pandan leaf (*Panadanus odorus*), asam gelugur (*Garnicia atroviridis*), mengkudu (*Morinda citrifolia*), pegaga (*Centella asiatica*), ginger (*Zingiber officinale*), cassava shoot (*Manihot asculenta*), kesum (*Polygonum minus*) and selom (*Oenathe javanica*) (Huda-Faujan et al., 2007; Jayamalar and Suhaila, 1998; Mohd. Zin et al., 2002; Noriham et al., 2004; Zainol et al., 2003) also exhibit good antioxidant activity. The antioxidative properties of some vegetables and fruits are partly due to the low molecular weight phenolic compounds, which are known to be potent as antioxidants (Wang et al., 1999).

MATERIALS AND METHODS

Collection and preparation of extract

Leaves of *J. gossypifolia* (Linn.) were collected from Indore (Madhya Pradesh). The authentication was done by Prof. S. R. Upadhyaya (Ex. Professor, Govt. Girls Post graduate College, Indore (M.P.) India.

Preparation of extracts

The leaves of *J. gossypifolia* were collected and shade dried. The dried leaves were coarse powdered and the powder was packed into a Soxhlet column and extracted successively with petroleum ether (60 to 80°C), ethanol (64.5 to 65.5°C) and distilled water. The extracts were concentrated under reduced pressure (bath temperature 50°C). The dried extracts were stored in airtight container in refrigerator.

Preliminary phytochemical screening

The preliminary phytochemical screening was carried out on petroleum ether, ethanol and aqueous extracts of *J. gossypifolia* leaves for the detection of various phytochemicals. Tests for common phytochemicals were carried out by standard methods (Bagul et.al., 2005; Richards and Sharma, 1991).

Determination of total phenolic contents

The amount of total phenolics in the extract was determined with Folin–Ciocalteu reagent according to the method of Singleton and Rossi (1965) with slight modification using tannic acid as a standard. Briefly, 1.0 ml of extract solution (5 mg/ml) was added in a 100 ml volumetric flask that contained about 60 ml distilled water. Then, 5.0 ml of Folin–Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 1 to 8 min, 15.0 ml Na_2CO_3 (20%) was added and the volume was made up to 100 ml using distilled water. The mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm using an ultraviolet-visible (UV-Vis) spectrophotometer (Jenway 6100, Dunmow, Essex, U.K). The total phenolic content was determined as mg of tannic acid equivalent (TAE) using an equation obtained from the standard tannic acid calibration graph.

Superoxide scavenging activity

Petroleum ether, aqueous and ethanolic extracts were screened for antioxidant activity using superoxide FR scavenging activity in dose and time dependent manner (Matill, 1947). The assay was based on the capacity of the samples to inhibit blue formazan formation by scavenging the superoxide radicals generated in riboflavin-light-nitroblue tetra-zolium (NBT) system. The reaction mixture contains 50 mM phosphate buffer, pH 7.6, 20 µg riboflavin, 12 mM ethylene diamine tetraacetic acid (EDTA), 0.1 mg/3 ml NBT, added in that sequence. The reaction was started by the reaction mixture with different concentrations (5 to 100 µg/ml) of samples for 15, 30 and 45 min, and immediately after illumination, the absorbance was measured at 590 nm (Bagul et al, 2005). Ascorbic acid was used as standard drug. Percentage inhibition and half maximal inhibitory concentration (IC_{50}) were calculated (Figure 4).

2, 2-Diphenyl-1 picrylhydrazyl hydrate (DPPH) radical scavenging assay

Radical scavenging activity of plant extracts against stable DPPH (Sigma-Aldrich Chemie, Steinheim, Germany) was determined spectrophotometrically. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour (from deep violet to light yellow) were measured at 520 nm on a UV/visible light spectrophotometer. Radical scavenging activity of extracts was measured by slightly modified method of Brand-Williams et al. (1995). Extract solutions were prepared by dissolving 0.025 g of dry extract in 10 ml of methanol. The solution of DPPH in methanol was prepared daily, before UV measurements 3 ml of this solution were mixed with 77 µl extract solution in 1 cm path length disposable microcuvettes (final mass ratio of extracts with DPPH was approximately 3:1, 1.5:1, 0.75:1). Similar concentrations of rutin were used as reference standard. The samples were kept in the

Table 1. Percentage inhibition of superoxide free radical scavenging activity of petroleum, ethanolic and aqueous extracts.

S/N	Concentration (µg/ml)	Percentage inhibition								
		15 min			30 min			45 min		
		Petroleum ether	Ethanolic	Aqueous	Petroleum ether	Ethanolic	Aqueous	Petroleum ether	Ethanolic	Aqueous
1.	5	26.8 ± 0.28	37.0 ± 0.32	33.6 ± 0.22	32.9 ± 0.34	40.4 ± 0.38	38.7 ± 0.39	39.4 ± 0.44	51.4 ± 0.49	43.1 ± 0.33
2.	10	31.5 ± 0.31	44.8 ± 0.49	38.4 ± 0.27	39.7 ± 0.39	54.9 ± 0.45	42.4 ± 0.47	47.6 ± 0.45	62.8 ± 0.58	48.2 ± 0.51
3.	25	38.6 ± 0.32	47.8 ± 0.53	42.7 ± 0.39	48.3 ± 0.54	57.5 ± 0.55	48.3 ± 0.49	53.6 ± 0.57	61.8 ± 0.66	52.8 ± 0.57
4.	50	45.0 ± 0.52	57.6 ± 0.62	55.3 ± 0.48	54.9 ± 0.53	61.6 ± 0.59	50.9 ± 0.52	60.4 ± 0.63	68.4 ± 0.65	60.0 ± 0.63
5.	100	50.6 ± 0.47	61.2 ± 0.51	59.7 ± 0.53	59.7 ± 0.59	68.6 ± 0.61	60.5 ± 0.64	68.5 ± 0.67	70.8 ± 0.69	67.8 ± 0.58

Data are mean ± SD of three measurements. Statistical analysis was performed by the Student's *t*-test and by ANOVA.

kept in the dark for 15 min at room temperature and then the decrease in absorption was measured. Absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured daily. The experiment was carried out in triplicate. Radical scavenging activity was calculated by the following formula:

$$\text{Percentage inhibition} = [(AB - AA) / AB] \times 100$$

Where: AB, Absorption of blank sample (t = 0 min); AA, absorption of tested extract solution (t = 15 min).

RESULTS

Phytochemicals investigation

It was found that petroleum ether extract contained steroids, fat and fixed oils; aqueous extract contained carbohydrates, amino acids, steroids, flavonoid, alkaloids, glycosides and tannins; ethanolic extract also showed almost similar phytochemicals as compared to aqueous extract.

Total phenolic content of the extracts

Studies on total phenolic content had been published in several papers. Yen and Hsieh (1998) reported that the total phenolic content in Du-Zong (*E. ulmoides*) ranged from 8700 to 21000 mg gallic acid equivalent (GAE) / dry weight. Total phenolic content of *J. gossypifolia* in three different climates (India, Nicaragua and Niger) ranged from 2940 to 4250 mg GAE / dry weight (Siddhuraju and Becker, 2003) and water plants extracts studied by Noriham et al. (2004) ranged from 257 to 3234 mg TAE /100 g dry weight. In addition, Jerez et al. (2007) evaluated the total phenolic from the bark of two kinds of pine, *Pinus pinaster* and *Pinus radiate*. Different levels reported in these studies may be attributed to the different plants, procedures and standards used to express as total phenolic contents used by individual groups of investigator. The usage of Folin-Ciocalteu reagent also was measured based on the colour measurement which was non-specific on phenol. Perhaps, there were other components that can react with the reagent such as ascorbic acid (Shahidi and Naczk, 1995). Besides, various phenolic compounds have different response to this assay (Singleton and Rossi, 1965). However, the measurement of colour changes after 2 h storage could be used to determine the existence of phenol in samples. This may due to the antioxidant properties of plant extract that react as reductant agent which known as redox action.

Free radicals scavenging activity

Ethanolic extract of *J. gossypifolia* had showed 57.6 ± 0.62% inhibition in superoxide scavenging model. Aqueous extract also showed almost similar activity (55.3 ± 0.48% compared to ethanolic extract), while petroleum ether extract showed poor inhibition of superoxide scavenging activity. All extracts showed dose and time dependent inhibition of superoxide scavenging activity. The results are reported in Table 1 and in Figures 1, 2 and 3.

DPPH radical scavenging activity

The aqueous extract of *J. gossypifolia* exhibited a significant dose dependent inhibition of DPPH activity, with a 50% inhibition (IC_{50}) at a concentration of 11.4 µg/ml. The IC_{50} value of the extract was found to be close to that of the standard; rutin (IC_{50} 10 µg/ml). Compared to rutin, the extract exhibited a similar curve of antioxidant activity. This result demonstrated that *J. gossypifolia* extract has inhibitory activity against the DPPH radical (Figure 5).

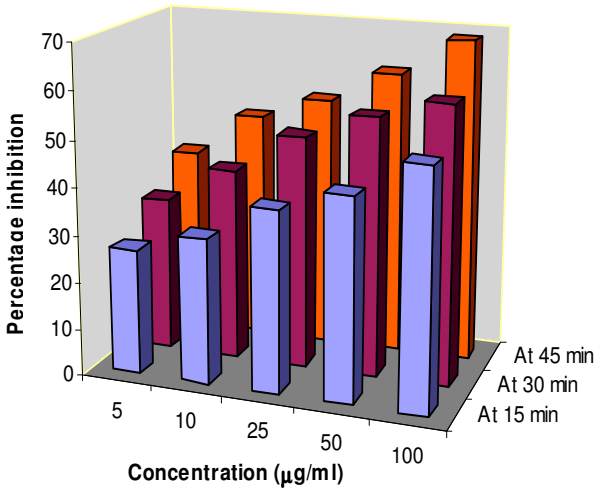

Figure 1. Effect of petroleum ether extract on superoxide free radicals.

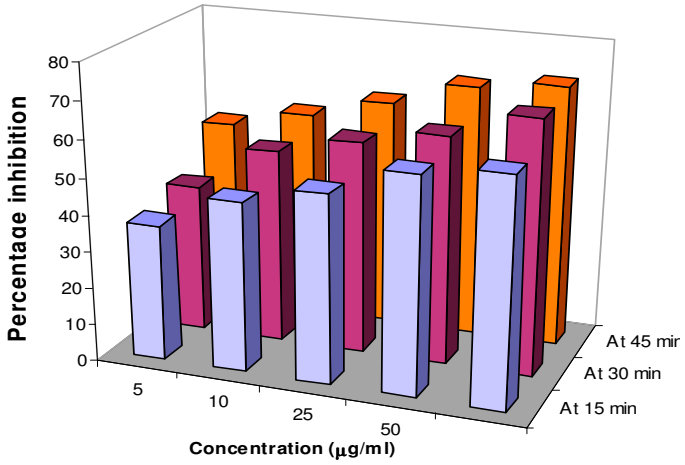

Figure 2. Effect of ethanolic extract on superoxide free radicals.

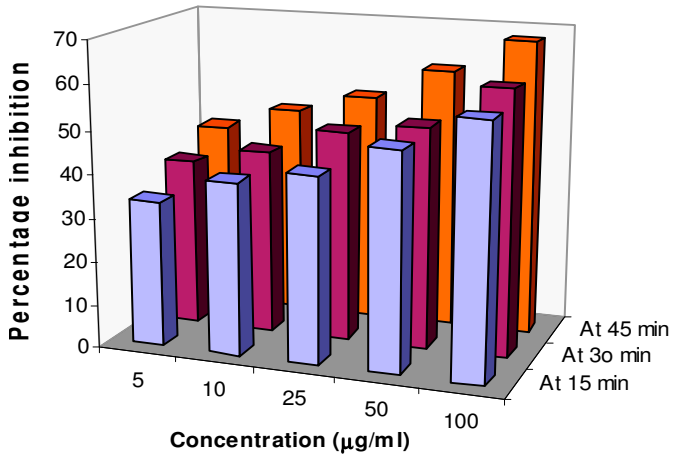

Figure 3. Effect of aqueous extract on superoxide free radicals.

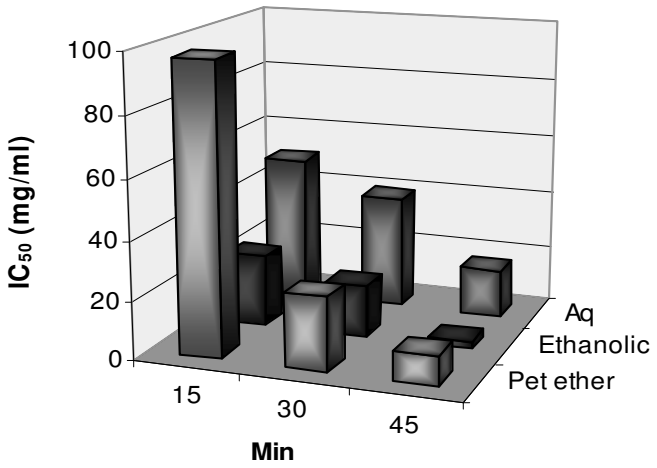

Figure 4. IC_{50} of tested extracts.

Statistical analysis

All analyses were run in triplicates. Data were analyzed by an analysis of variance (ANOVA). Statistical analysis was performed by the Student's *t*-test and by ANOVA.

DISCUSSION

The traditional medicine all over the world is nowadays revalued by an extensive activity of research on different plant species and their therapeutic principles. Experimental evidence suggests that FR and reactive oxygen species (ROS) can be involved in a high number of diseases (D'Mello et al., 2000). As plants produce many antioxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity.

In the present study, aqueous and ethanolic extract were selected as they contain alkaloids, glycosides, saponins, tannins, flavonoids and phenolic compounds. This may have active constituents for producing the FR scavenging effect. FR are produced under certain environmental condition and during normal cellular function in the body. These molecules are missing an electron, giving them an electric charge. To neutralize this charge, FR try to withdraw an electron from, or donate an electron to, a neighboring molecule. Other antioxidants work against the molecules that form FR, destroying them before they can begin the domino effect that leads to oxidative damage (Matill, 1947). For example, certain enzymes in the body, such as superoxide dismutase, work with other chemical to transfer FR into harmless molecules. Vitamin C is an antioxidant that may prevent cataracts and cancers of the stomach; throat, mouth, and pancreas (D'Mello et al., 2000). It may also prevent the oxidation of LDL cholesterol, lowering the risk of heart disease. Literature reveals that, the carbonyl groups present

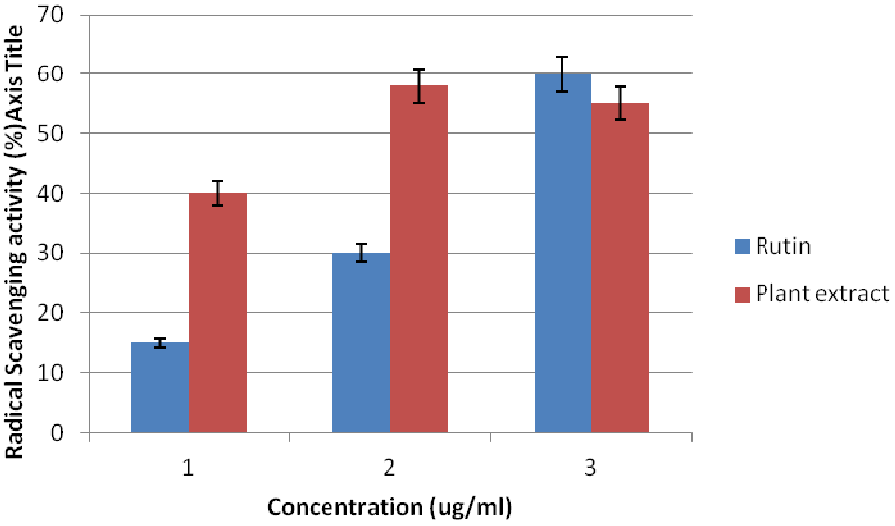

Figure 5. DPPH radical scavenging activity of *J. gossypifolia* extract added to an ethanolic solution of DPPH and radical scavenging activity was measured at 520 nm as compared to rutin.

in the flavonoids and phenolic compounds were responsible for FR scavenging activity (Nicholls and Budd, 2000). This investigation revealed that the *J. gossypifolia* contains pharmacologically active substance (s) such as alkaloids, glycosides, saponins, tannins, flavonoids and phenolic compounds, which are responsible for the superoxide scavenging activity.

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