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Research Article

IN-VITRO FREE RADICAL SCAVENGING AND ANTIOXIDANT ACTIVITY OF *JATROPHA CARCUS*

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ABSTRACT

To evaluate the *in vitro* free radical (FR) scavenging activity of leaves *Jatropha carcus* petroleum ether, ethanol, aqueous extracts of *J. carcus* 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 hydrazyl (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 *Jatropha carcus* had showed 71.8±0.69 % inhibition in superoxide scavenging model. Aqueous extract also showed almost similar activity (67.8±0.58 % compared to ethanolic extract), while petroleum ether extract showed poor inhibition of superoxide scavenging activity. The antioxidant capacity of various solvent fractions of *Jatropha carcus* was found to decrease in this order:ethanolic > aqueous > petroleum ether. All results showed antioxidant activity in dose dependent manner at concentration 10 to 30 μg/ml. Strong antioxidant activity of ethanolic extract statistically similar to ascorbic acid indicates strong antioxidants in this extract. All extracts showed dose and time dependent inhibition of superoxide scavenging activity.

Keywords: *Jatropha carcus*, 2, 2-diphenyl-1 picrylhydrazyl hydrazyl (DPPH), Phenolic content, Superoxide scavenging, Antioxidant activity.

1. INTRODUCTION

Jatropha carcus; 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. *Jatropha carcus* is one of the genera that are used in Chinese, Ayurvedic and Thai traditional medicine for the treatment of fever, pain and dysentery [1, 2].

Literature reveals that, the carbonyl groups are responsible for free radical scavenging activity [3]. Free radicals are atoms or groups of atoms with an odd number of electrons and can be formed when oxygen interacts with certain molecules. Once highly reactive free radicals are formed they can start chain reaction like dominoes. Their major threat comes from the damage they can do when they react important cellular components such as DNA, or cell membrane. Cells may function poorly or die if this occurs. To prevent free radical damage, the body has a defense system of antioxidants [4]. Antioxidants are able to give free radicals, which becomes a companion to their unpaired electron, thus eliminating the threat of gene alteration leading to cancer [5-6]. Medicinal plants have attracted attention of not only professionals from various systems of medicines, but also the scientific community belonging to different disciplines. Herbal drug, being generally harmless in prescribed doses, are becoming popular all over the world and WHO currently encourages, recommends and promotes inclusion of these drugs in national health care programme [7, 8]. In recent years, these have been a great interest in herbal remedies for the treatment of number of ailments. Plants are promising source of drugs. In continuation of search in potential free radical scavenging agents [9], the present investigation was aimed to determine free radical scavenging activity of *Jatropha carcus* Leave

(Linn.). Free radical scavenging properties help in strengthening the immune system of the body which helps to overcome cancer.

2. MATERIAL AND METHODS

2.1. Collection and preparation of extract

Bark of *Jatropha carcus* were collected from Bhopal (Madhya Pradesh). The authentication was done by Prof. Saba Naaz (Head Department of Botany Safia Science College, Bhopal (M.P.) India.

2.2. Preparation of Extracts

The bark of *Jatropha carcus* were collected and shade dried. The dried bark were coarse powdered and the powder was packed in to soxhlet column and extracted successively with petroleum ether (60-80°C), ethanol (64.5-65.5°C) and distilled water. The extracts were concentrated under reduced pressure (bath temp 50°C). The dried extracts were stored in airtight container in refrigerator below 10-20°C.

2.3. Preliminary Phytochemical Screening

The preliminary phytochemical screening was carried out on petroleum ether, ethanol and aqueous extracts of *Jatropha carcus* bark for the detection of various phytochemicals. Tests for common phytochemicals were carried out by standard methods [10-11].

2.4. Superoxide scavenging activity

Petroleum ether, aqueous and ethanolic extracts were screened for anti-oxidant activity using superoxide free radical scavenging activity in dose and time dependent manner [12].

The assay was based on the capacity of the samples to inhibit blue formazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system. The reaction mixture contained 50 mM phosphate buffer, pH 7.6, 20 µg riboflavin, 12 mM EDTA, O.1 mg/3 ml NBT, added in that sequence. The reaction was started by illumination the reaction mixture with different concentrations (5-100 µg/ml) of samples for 15, 30 and 45 min. Immediately after illumination, the absorbance was measured at 590 nm. Ascorbic acid was used as standard drug. Percentage inhibition and IC_{50} were calculated.

2.5. DPPH radical scavenging assay

Radical scavenging activity of plant extracts against stable DPPH (2, 2-diphenyl-2 picrylhydrazyl hydrate) was determined spectrophotometrically. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (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 (6x10.5 M) was prepared daily before UV measurements, 3 ml of this solution were mixed with 77µl extract solution in 1 cm path length disposable micro-cuvettes (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 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 [5]. The experiment was carried out in triplicate. Radical scavenging activity was calculated by the following formula:

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

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

2.6. Evaluation of total antioxidant activity by Phosphomolybdenum method

An aliquot of 0.5 mL of samples solution was combined with 4.5 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). In case of blank, 0.5 mL of 45% ethanol was used in place of sample. The tubes were incubated in a boiling water bath at 95°C for 90 min. After the samples were cooled to room temperature, the absorbance of the aqueous solution of each sample was measured at 695 nm against blank in UV-2450 spectrophotometer. The total antioxidant activity was expressed as the absorbance of the sample at 695 nm. The higher absorbance value indicated higher antioxidant activity [11].

2.7. Statistical analysis

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

3. RESULTS AND DISCUSSION

3.1. 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.

3.2. Free radicals scavenging activity

Ethanolic extract of *Jatropha carcus* had showed 71.8±0.69 % inhibition in superoxide scavenging model. Aqueous extract also showed almost similar activity (67.8±0.58 % 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.

3.3. DPPH Radical scavenging activity

The aqueous extract of *Jatropha carcus* 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} 10μg/ml). Compared to rutin, the extract exhibited a similar curve of antioxidant activity. This result demonstrated that *Jatropha carcus* extract has inhibitory activity against the DPPH radical.

3.4. Phosphomolybdate assay

The phosphomolybdate method is quantitative, since the total antioxidant capacity (TAC) is expressed as ascorbic acid equivalents. The antioxidant capacity of various solvent fractions of *Jatropha carcus* was found to decrease in this order:ethanolic > aqueous > petroleum ether. All results showed antioxidant activity in dose dependent manner at concentration 10 to 30 μg/ml. Strong antioxidant activity of ethanolic extract statistically similar to ascorbic acid indicates strong antioxidants in this extract and these could be attributable to the presence of phenolic compounds.

Table 1:IC_{50} of tested extracts

S. No.	Extracts	IC_{50} (μg/ml) Minutes		
		15	30	45
1.	Petroleum ether	97.5	24.5	50.0
2.	Ethanolic	25.0	18.0	39.0
3.	Aqueous	10.1	2.5	16.5

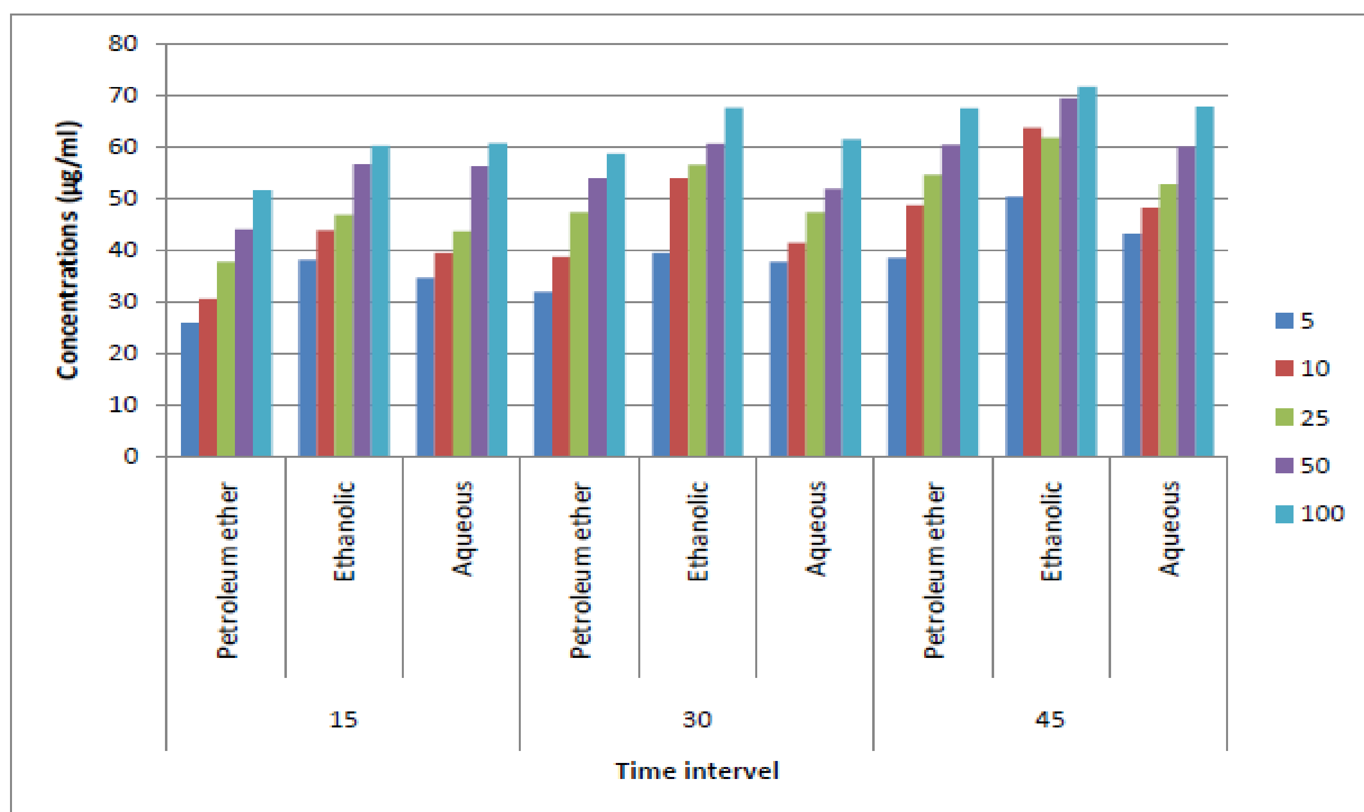

Fig. 1:Percentage inhibition of superoxide free radical scavenging activity of petroleum, ethanolic and aqueous extracts.

Table 2:Percentage inhibition of superoxide free radical scavenging activity of petroleum, ethanolic and aqueous extracts

Concentrations (μg/ml)	% Inhibition								
	Minutes								
	15			30			45		
	Petroleum ether	Ethanolic	Aqueous	Petroleum ether	Ethanolic	Aqueous	Petroleum ether	Ethanolic	Aqueous
5	25.8±0.28	38.0±0.22	34.6±0.23	31.9±0.14	39.4±0.35	37.7±0.39	38.4±0.44	50.4±0.49	43.1±0.33
10	30.5±0.31	43.8±0.49	39.4±0.22	38.7±0.29	53.9±0.44	41.4±0.47	48.6±0.45	63.8±0.58	48.2±0.51
25	37.6±0.32	46.8±0.43	43.7±0.31	47.3±0.44	56.5±0.53	47.3±0.49	54.6±0.57	61.8±0.66	52.8±0.57
50	44.0±0.52	56.6±0.62	56.3±0.48	53.9±0.43	60.6±0.54	51.9±0.52	60.4±0.63	69.4±0.65	60.0±0.63
100	51.6±0.47	60.2±0.21	60.7±0.52	58.7±0.49	67.6±0.60	61.5±0.64	67.5±0.67	71.8±0.69	67.8±0.58

Table 3:Total antioxidant capacity of different plant extracts at different concentrations

Plant extract	Time interval Minutes	Concentration (μg/ml)	Total antioxidant capacity
Ascorbic acid	15	10	8.0±0.1
	30	20	8.3±0.2
	45	30	8.7±0.3
Petroleum ether	15	10	236±1*
Ethanolic		20	212±4
Aqueous		30	247±3*
Petroleum ether	30	10	135±2
Ethanolic		20	196±1
Aqueous		30	237±4
Petroleum ether	45	10	201±5
Ethanolic		20	193±2
Aqueous		30	217±6

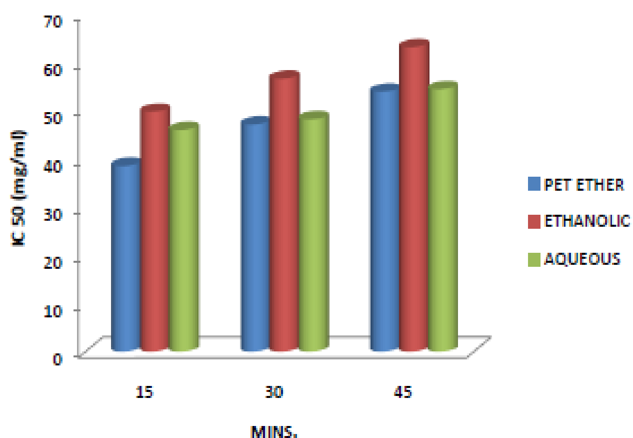

Fig. 2:IC$_{50}$ of tested extracts

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 free radicals (FR) and reactive oxygen species (ROS) can be involved in a high number of diseases [13]. As plants produce a lot of 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 free radical scavenging effect.

Free radicals 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, free radicals try to withdraw an electron from, or donate an electron to, a neighboring molecule. The newly created free radicals, in turn, looks out for another molecule and withdraw or donates an electron, setting off a chain reaction that can damage hundreds of molecules. Antioxidants halt this chain reaction. Some antioxidants are themselves free radicals, donating electrons to stabilize the dangerous free radicals. Other antioxidants works against the molecules that form free radicals, destroying them before they can begin the domino effect that leads to oxidative damage [14].

The antioxidant capacity of the fractions was measured spectrophotometrically through phosphomolybdenum method, based on the reduction of Mo (VI) to Mo (V) by the test sample and the subsequent formation of green phosphate/Mo (V) compounds with a maximum absorption at 765 nm. The present study demonstrated that ethanolic extract ar maximum concentration exhibited the highest antioxidant capacity for phosphomolybdate reduction. Recent studies have shown that many flavonoid and related polyphenols contribute significantly to the phosphomolybdate scavenging activity of medicinal plants.

4. CONCLUSION

Antioxidants work to control the levels of free radicals before they do oxidative damage to the body. For example, certain enzymes in the body, such as superoxide dimutase, work with other chemicals to transfer free radical into harmless molecules. Vitamin C; an antioxidant may prevent cataracts and cancers of the stomach, throat, mouth, and pancreas. It may also prevent the oxidation of LDL cholesterol, lowering the risk of heart disease. Literature reveals that, the carbonyl groups present in the flavonoids and phenolic compounds were responsible for free radical scavenging activity. This investigation revealed that the *Jatropha carcus* contains pharmacologically active substance such as alkaloids, glycosides, saponins, tannins, flavonoids and phenolic compounds, which are responsible for the superoxide scavenging activity.

Conflict of interest

None declared

5. REFERENCE

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