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Pharmacological evaluation of stem bark extract of *Jatropha Curcas* L. for anti-oxidant activity

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ABSTRACT

Current research on *Jatropha Curcas* L. is directed towards finding naturally-occurring antioxidants of plant origin that provided efficacy by additive or synergistic activities because antioxidants from plant origin are essential to prevent the progression of free radical mediated disorders. Antioxidant activity of *Jatropha curcas* L. bark extract (JCE) showed by using different in- vitro models. It includes Free radical scavenging activity of DPPH, Ferric reducing antioxidant power, Total flavonoid content and Total phenolic content. The plant contains much amount of Phenolic compounds and Flavonoids. Plant shows significant antioxidant activity.

KEYWORDS

Jatropha curcas;
Antioxidant;
Phenolic content;
Flavonoid content;
Scavenging.

INTRODUCTION

Jatropha curcas L. or physic nut, a drought-resistant shrub or tree, which is widely distributed in Central and South America, Africa, India and South East Asia[1]. It is a bush or small tree (up to 5 m height) and belongs to the Euphorbiaceae family and contains approximately 170 known species[2]. The plant has thick glorious branch lets with straight trunk and grey or reddish bark, masked by large white patches. The branches contain whitish latex, which causes brown stains. Inflorescences are formed terminally on branches. It has green leaves with a length and width of 6 to 15 cm, with 5 to 7 shallow lobes. The plant is monoecious and flowers are unisexual[3, 4]. After pollination, a trilocular ellipsoidal fruit is formed. The seeds are black and in the average 18 mm long and 10 mm wide ripe Jatropha fruits[5].

The wood and fruit can be used for numerous purposes including fuel. It is used against dermatomucosal diseases, arthritis, gout, jaundice, Toothache, gum inflammation, gum bleeding, diarrhoea, pyorrhea[6]. Plant extract used to treat Allergies, burns, cuts and wounds, inflammation, leprosy, leucoderma, scabies and small pox. Water extract of branches used in HIV, tumor, wound healing. The plant contains organic acids, cyclic triterpenes, stigmasterol[7], curcacycline A, curcin[8], a lectin phorbolesters esterases, sitosterol and its d-glucoside[9]. The leaf and bark have been shown to contain glycosides, tannins, phytosterols, flavanoids and steroidal sapogenins[6]. So this plant is studied for anti-oxidant activity using different models to evaluate its effect.

EXPERIMENTAL

Plant material

Fresh stem bark of *Jatropha curcas* L. collected from a local area of Jaipur was identified in the depart-

ment of botany, Rajasthan University, Jaipur. A voucher specimen number RUBL20844 was deposited in the department. The fresh stem bark was air-dried to constant weight, pulverized and stored in an air-tight container for further use. Dried stem bark was subjected to soxhlet extraction with methanol: acetone: water (70:20:10). The extract collected was evaporated and stored in a vacuum desiccator. The preliminary phytochemical investigations with the extract revealed the presence of alkaloids, carbohydrate, flavonoids, flavones, phenolic compound, tannins, volatile oil, saponins and glycosides[10, 11].

Chemicals

Chemicals namely methanol, ethanol, tri-chloro acetic acid, ascorbic acid, BHA, rutin, Folin-Ciocalteu's phenol reagent, potassium ferricynide, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), aluminium chloride, sodium nitrite, sodium hydroxide were used during the experimental study.

METHOD

Free Radical Scavenging Activity Measured by 1, 1-Diphenyl-2-picryl-hydrazil

The free radical scavenging activity of extract was measured by 1, 1-diphenyl-2- picryl-hydrazil (DPPH) using the method of Blois[12]. Briefly, a 0.1 mM solution of DPPH in ethanol was prepared and 1 ml of this solution was added to 3 ml of extract solution in water at different concentrations (20-60 μg ml^{-1}). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Lower absorbance values of the reaction mixture indicated higher free radical scavenging activity.

DPPH· scavenging effect (%) = [(Ao - A1/Ao) × 100]

Where,

Ao was the absorbance of the control reaction and

A1 was the absorbance in the presence of the sample of extract[13].

Ferric reducing antioxidant power (FRAP)

The total reducing power of the extract was determined according to the method of Oyaizu[14,15]. Briefly, different concentrations of extract (20, 40, and 60 μg ml-1) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. Trichloroacetic acid (2.5 ml, 10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm using a UV-Vis spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power.

Determination of total flavonoid content

The total flavonoid content was determined with aluminium chloride ($AlCl_3$) according to the known method of Zhishen[16] using Rutin as a standard. The plant extract (0.1 ml) was added to 0.3 ml distilled water followed by 0.03 ml $NaNO_2$ (5%) and incubated for 5 min at 25°C. Later 0.03 ml $AlCl_3$ (10%) was added and further after 5 min, the reaction mixture was treated with 0.2 ml (1mM) NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. All tests were performed six times. The flavonoid content was calculated from a Rutin standard curve .

Estimation of total phenolic content

The total phenolic content of the extract was estimated according to the method described by Singleton and Rossi[17]. From the stock solution (1 mg/ml) of the extract, suitable quantity was taken into a 25 ml volumetric flask and mixed with 10 ml of water and 1.5 ml of Folin Ciocalteu's reagent. After 5 min, 4 ml of 20% (w/v) sodium carbonate solution was added and volume was made up to 25 ml with double distilled water. The absorbance was recorded at 765 nm, after 30 min. Percentage of total phenolics was calculated from calibration curve of Gallic acid (50-250 μg) plotted by using same procedure and total phenolics were expressed as % Gallic acid.

RESULT

Free Radical Scavenging Activity Measured by 1, 1-Diphenyl-2-picryl-hydrazil

Free radical scavenging activity is given in the

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TABLE 1 and Figure 1, IC50 of Ascorbic acid against DPPH is 30.11µg/ml, IC50 of JCE against DPPH is 31.39 µg/ml.

TABLE 1: Free Radical Scavenging Activity (DPPH) of extract, compared with Ascorbic acid

S. No.	Group	Concentration (µg/ml)	Absorbance(517 nm)	% inhibition
	Control		0.9654±0.002	-
	STD (Ascorbic acid)			
1.		5	0.8241±0.001	14.64
2.		10	0.7336±0.001	24.01
3.		20	0.6135±0.001	36.45
4.		30	0.4991±0.001	48.30
5.		50	0.2137±0.003	77.86
	Test (Extract)			
1.		5	0.8672±0.001	10.17
2.		10	0.7967±0.002	19.24
3.		20	0.6312±0.001	34.62
4.		30	0.4469±0.002	53.80
5.		50	0.2613±0.001	72.93

Data presented in (MEAN ± SD), n=3

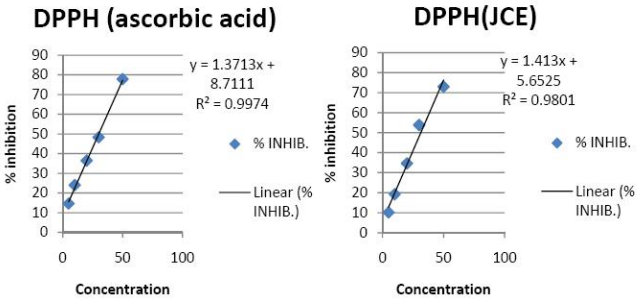

Figure 1: Free Radical Scavenging Activity (DPPH) of Extract, compared with Ascorbic

Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power given in the TABLE 2 and Figure 2, Ascorbic acid used as a reference drug and extract given significant activity.

Determination of total flavonoid content

Flavonoid content are given in the TABLE 3 and Figure 3, Rutin used as a reference drug and approximately 20 µg/ml rutin = 20 µg/ml extract.

Estimation of total phenolic content

Phenolic content are given in the TABLE 3 and Figure 3, gallic used as a reference drug and approximately 30 µg/ml gallic acid = 100 µg/ml extract.

Table 2: Ferric reducing antioxidant power (FRAP) of extract, compared with Ascorbic acid

S. No.	Group	Concentration (µg/ml)	Absorbance(700 nm)
	STD (Ascorbic acid)		
1.		10	0.0597±0.001
2.		20	0.1263±0.002
3.		30	0.2722±0.008
4.		40	0.3936±0.001
5.		50	0.5413±0.002
	Test (Extract)		
1.		50	0.0091±0.001
2.		100	0.1711±0.001
3.		200	0.2723±0.003
4.		300	0.5127±0.001
5.		500	0.8637±0.003

Data presented in (MEAN ± SD), n=3

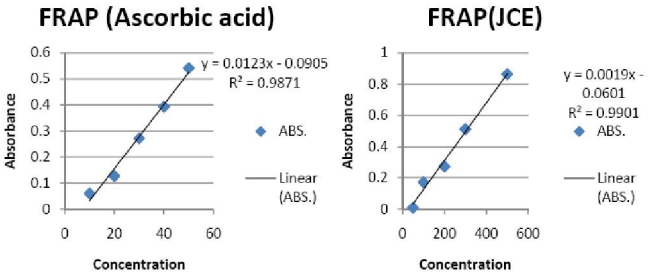

Figure 2: Ferric reducing antioxidant power (FRAP) of Extract, compared with Ascorbic acid

Table 3: Determination of total flavonoid content of extract, compared with Rutin

S. No.	Group	Concentration (µg/ml)	Absorbance(510nm)
	STD (Rutin)		
1.		5	0.0351±0.001
2.		10	0.0692±0.002
3.		20	0.0976±0.008
4.		30	0.1370±0.001
5.		50	0.1795±0.002
	Test (Extract)		
1.		5	0.0498±0.001
2.		10	0.0712±0.001
3.		20	0.0976±0.003
4.		30	0.1327±0.001
5.		50	0.1532±0.003

Data presented in (MEAN ± SD), n=3

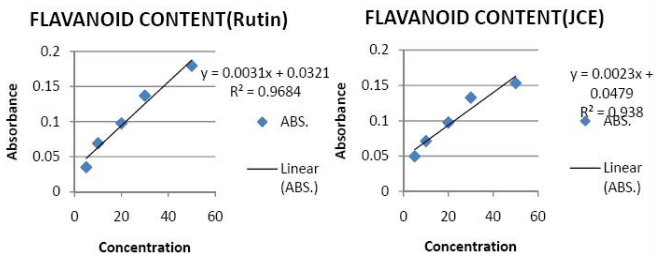

Figure 3: Determination of total flavonoid content of Extract, compared with Rutin

Table 4: Estimation of total phenolic content of extract, compared with Gallic acid

S. No.	Group	Concentration (µg/ml)	Absorbance(765nm)
	STD (Rutin)		
1.		10	0.0876±0.002
2.		20	0.1312±0.003
3.		30	0.1798±0.002
4.		40	0.2103±0.004
5.		50	0.3456±0.004
	Test (Extract)		
1.		50	0.1391±0.001
2.		100	0.1907±0.001
3.		200	0.3817±0.001
4.		300	0.5392±0.003
5.		500	0.7925±0.002

Data presented in (MEAN ± SD), n=3

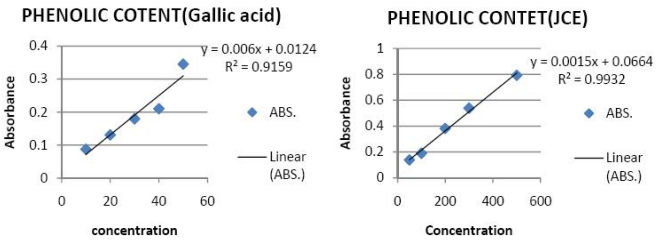

Figure 4: Estimation of total phenolic content of extract, compared with Gallic acid

DISCUSSION

2, 2-diphenyl-1- picrylhydrazyl (DPPH) is a stable radical that has been used widely to evaluate the antioxidant activity of various natural products[18]. In this study, DPPH scavenging activity has been observed in extract. DPPH radical scavenging activities of extract varied from 10.17 to 72.93%. All of the extracts tested possess radical scavenging activity. This activity was increased by increasing the concentration of the sample extract.

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides[19]. For the measurements of the reductive ability, it has been found that the Fe^{3+}-Fe^{2+} transformation occurred in the presence of extract samples which was postulated previously by Oyaizu[14] Tanaka have observed a direct correlation between antioxidant activities and reducing power of certain plant extracts[20]. The reducing properties are generally associated with the presence of reductones[21], which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom[22]. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. In this assay, depending on the reducing power of antioxidant compounds, the yellow color of the test solution changes into various shades of green and blue. Therefore, by measuring the formation of Perl's Prussian blue at 700 nm, we can monitor the Fe2+ concentration. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Extract indicates significant antioxidant activity.

Phenolic compounds are known powerful chain breaking antioxidants[23], important plant constituents because of their scavenging ability due to their hydroxyl groups and contribute directly to antioxidative action[24] Phenolic compounds are also effective hydrogen donors, which makes them good antioxidants[25] It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when ingested up to 1g daily with a diet rich in fruits and vegetables[26]. Further research on this plant helpful to find out more potent antioxidant.

CONCLUSION

The present study provide an evidence that extract even though having more amount of flavonoid and phenolic content, shows potential antioxidant and free radical scavenging activity. These in vitro assays demonstrate that plant extracts are important sources of natural antioxidants, which might be useful as preventive agents against oxidative stress. To elucidate the prime source of antioxidant properties further studies should

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be carried out with isolate active principles.

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