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Research Article

FREE RADICAL SCAVENGING ACTIVITY OF *RANDIA DUMETORUM* LAMK FRUITS

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ABSTRACT
Free radicals are implicated for many diseases including Diabetes mellitus, arthritis, cancer, ageing *etc*. In treatment of these diseases, antioxidant therapy has gained utmost importance. Antioxidants can also help to repair damage already sustained by cells. *Randia dumetorum* Lamk. is a plant of medicinal important belongs to the family Rubiaceae. Polyphenols are good antioxidant. Fruits consist of Phenolic in large amount. It is traditionally used to cure diseases but no scientific data is available. Thus the objective of present study was aimed to check the antioxidant potential of Aqueous extract of *Randia dumetorum* fruits using different models *viz.* DPPH radical scavenging, iron chelating activity and nitric oxide scavenging assay. The results were analyzed statistically by regression method. The % scavenging activity at different concentrations was determined and the IC_{50} value of the extracts was compared with that of standard, ascorbic acid. Its antioxidant activity was estimated by IC_{50} value and the values are 45.02 µg/ml (reducing power assay), 66 µg/ml, (DPPH scavenging assay) and 79.09 µg/ml (nitric oxide scavenging assay). In all the testing, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals, metal chelation. According to these results, it may be hypothesized that antioxidant effect of fruits could be related to presence of polyphenolic compounds. These results clearly indicate that *Randia dumetorum* is effective against free radical mediated diseases.

KEYWORDS: Antioxidant, Free radicals, *Randia dumetorum*, Polyphenols.

INTRODUCTION
The antioxidant prevents the risk of several aging related diseases including cancer, cardio vascular disorder, diabetes, neurodegenerative disorders and others Reactive oxygen species (ROS) have aroused significant interest among scientist in the past decade[1]. ROS are continuously produced during normal physiologic events, and are remove by antioxidant defence mechanism[2]. There is a balance between generation of ROS and antioxidant system in organism. In pathological condition, ROS are over produced and result in lipid peroxidation and oxidative stress. The imbalance between ROS and antioxidant defence mechanisms leads to oxidative modification in the cellular membrane or intracellular molecules[3]. In Indian system of medicine *Randia dumetorum* (Rubiaceae) is an important medicinal plant and popularly known as emetic nut. It is found in waste places & jungles all over India, extending northwest to the Bias river & ascending to outer Himalaya to 4000 ft. Ceylon, Java, & South China[4]. Literature survey reveals that the fruit is emetic, purgative, carminative, alexiteric, anthelmintic, abrortificient, antipyretic; cures abscess, ulcers, inflammations, wounds, tumors, skin diseases, piles and have antibacterial activity[5]. It contains triterpenoidal saponins (2-3%) in fresh & 10% in dried fruit. They are mostly concentrated in pulp. A mixture of two saponin Randia or neutral saponin (mp-289-90°) & Randia acid (mp-260°) and oleanolic acid which occurs at all stage of ripening. These all saponins yield oleanolic acid as sapogenin

on hydrolysis. It also contains essential oil, veleric acid, polyphenols and resin[6]. From the above traditional value, the plant was selected for evaluation of this study.

MATERIALS AND METHODS

All chemicals and solvents were of analytical grade and were obtained from Ranbaxy Fine Chemicals, Mumbai, India. The chemicals used were O-Phenanthroline, ferric chloride, ascorbic acid, sodium nitropruside, Folin Ciocalteu's reagent, Gallic acid, sodium carbonate, sodium hydroxide and potassium chloride. 1, 1- diphenyl, 2- picryl hydrazyl (DPPH) was obtained from Sigma Chemicals, USA.UV spectrophotometer (Shimadzu 1650), homogenizer (Remi, India), centrifuge (Remi, India), pH meter (Elico Ltd., India) were the instruments used for the study.

Plant material

Fruits of *Randia dumetorum* were collected during November from Botanical garden of M.S.U. Baroda and were identified by Head of Botany department, M. S. University, Baroda. A voucher specimen (PH-805) has been deposited in the museum of department of Pharmacognosy, M.S.U. Baroda.

Plant extract

The fruits were dried in sunlight and reduced to a coarse powder. The powdered materials were subjected to qualitative tests for the identification of various phytoconstituents like alkaloids, glycosides, steroids, terpenoids and flavonoids. About 500 g of the powder was taken and extracted with chloroform: water (1:1000) by maceration. The extract evaporated under vacuum gave a dry extract and was stored in a dessicator until further use.

Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of phytosterol, polyphenol, saponins, flavonoids and carbohydrates[7].

Determination of total polyphenolic compounds

Total polyphenolic compounds were determined according to a protocol similar to that of *Singleton* and *Rossi*[8]. 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 minutes 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 minutes. % of total phenolics was calculated from calibration curve of Gallic acid (50-250 µg) plotted by using the same procedure and total phenolics were expressed as % Gallic acid.

$$\frac{\text{Concentration of gallic acid (Cg)}}{\text{Concentration of sample (Cs)}} = \frac{\text{Absorbance of gallic acid (Ag)}}{\text{Absorbance of sample (As)}}$$

Reducing power ability

The reducing power was investigated by the Fe^{3+}-Fe^{2+} transformation in the presence of the extracts. The Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm[9]. Two ml of the extract (5-1000 µg/ml), 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide were incubated at 50°C for 30 min and 2.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged for 10 min at 3000 rpm. About 2.5 ml of the supernatant was diluted with 2.5 ml of water and is shaken with 0.5 ml of freshly prepared 0.1% ferric chloride. The absorbance was measured at 700 nm. Ascorbic acid (5-1000µg/ml) was used as the standard. All tests were performed in triplicate and the graph was plotted with the average of the three determinations.

DPPH radical scavenging assay

Different concentrations of standard and test samples (5-1000 µg/ml) were diluted with water up to 3 ml and 75 µl of DPPH was added. The absorbance was taken immediately after addition of DPPH solution at 516 nm using water as a blank at zero minute. The % scavenging activity at different concentrations was determined and the IC_{50} value of the extracts was compared with that of ascorbic acid which was used as the standard. Experiment was performed in triplicate[10-11].

Nitric oxide scavenging assay

Sodium nitroprusside 5mM was prepared in phosphate buffer pH 7.4. To 1 ml of various concentrations of test compound (5-1000 µg/ml), 0.3 ml of sodium nitroprusside was added. The test tubes were incubated at 25 ºC for 5 hr after which, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthyl ethylene diamine was read at 546 nm[10]. The experiment was performed in triplicate.

Statistical analysis

All results are expressed as mean ± S.E.M. Linear regression analysis (Origin 6.0 version) was used to calculate the IC_{50} values.

RESULTS

Several concentrations ranging from 5-1000 µg/ml of the Aqueous extract of fruits of *Randia dumetorum* Lamk. were tested for their antioxidant activity in different *in vitro* models. It was observed that free radicals were scavenged by the test compounds in a concentration dependent manner up to the given concentration in all the models. The percentage scavenging and IC_{50} values were calculated for all models. The maximum inhibitory concentration (IC_{50}) in all models viz. reducing power ability, DPPH, nitric oxide scavenging was reported in the **Table 1**. On a comparative basis the extract showed better activity in all in vitro antioxidant models. Total phenolic content was found to be 0.132 mg/ml.

DISCUSSION

Phenolic compounds are the principal antioxidant constituents of natural products and are composed of phenolic acids and flavonoids, which are potent radical terminators[12]. Many plants exhibit efficient antioxidant properties owing to their phenolic constituents[13]. Free radicals have been implicated in many disease conditions, the important ones being superoxide radical, hydroxyl radical, peroxyl radical and singlet oxygen. Herbal drugs containing radical scavengers are gaining importance in treating such diseases. The reducing power of aqueous extract of *R. dumetorum* fruits to reduce ferric ions was determined in this study. Extract had significant reducing power and also was in dose dependent manner (**figure -1**).

DPPH is one of the free radicals generally used for testing preliminary radical scavenging activity of a compound or a plant extract. DPPH forms a stable molecule on accepting an electron or a hydrogen atom and thus has applications in the determination of radical scavenging activity of natural products. In situ, free radicals like polyaromatic hydrocarbon cations have been linked with carcinogenesis[14]. Thus, products that will scavenge DPPH *in vitro* may also scavenge polyaromatic hydrocarbon cations *in vivo*. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm. In the case of aqueous extract it was concentration-dependent (**figure-2**).

Nitric oxide is a diffusible free radical which plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilation and antimicrobial and antitumor activities[13]. NO generated from sodium nitroprusside in aqueous solution at physiological pH reacts with oxygen to form nitrite ions[15]. Aqueous ext. of fruits of *R. dumetorum* significantly inhibited nitrite formation in concentration dependant manner. This may be due to antioxidant principles in the extract, which compete with oxygen to react with nitric oxide (**figure-3**).

The antioxidant activity of the extract is close and identical in magnitude, and comparable to that of standard antioxidant compounds used. IC_{50} value for different assays was calculated and reported.

CONCLUSION

In conclusion, the results of the present study show that the extract of fruits of *R. dumetorum* exhibits the better *in vitro* antioxidant effect. From the *in vitro* studies, the antioxidant activity may be due to inhibiting the formation of radicals or scavenge the formed radical and it may be due to the presence of the phenolics compounds. Overall, the plant extract is a source of natural antioxidants, justifies their application in nutrition. *R. dumetorum* could therefore provide a useful source of antioxidants in oxidative stress related disorders, but in vivo studies are needed to confirm this action.

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Table 1: IC_{50} value for different assays

Sr. No.	Assay method	Aqueous ext. (µg/ml)	Ascorbic acid (µg/ml)
1	Reducing power assay	45.02	11.12
2	DPPH scavenging assay	66	9.93
3	Nitric oxide scavenging assay	79.09	28.43

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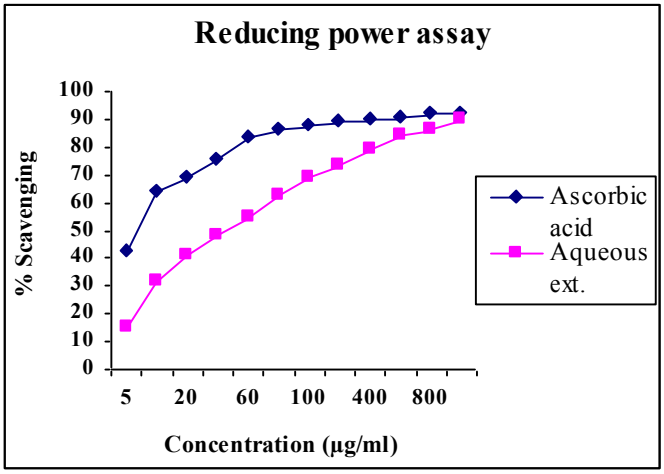

Figure 1: Antioxidant activity of different concentrations of aqueous extract and ascorbic acid in reducing power method. Each value represents mean ± SEM

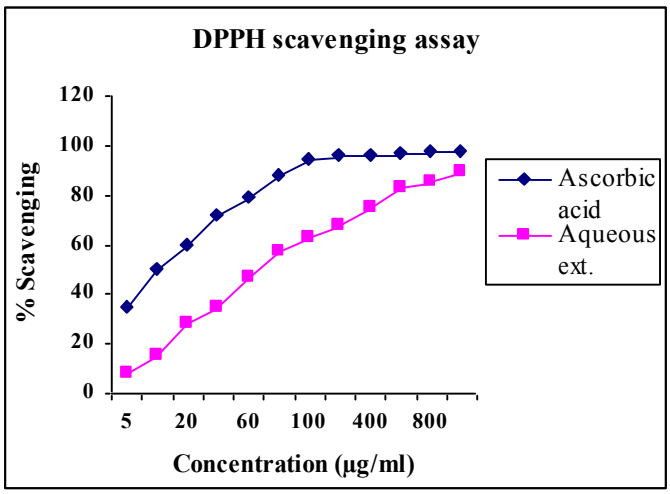

Figure 2: Antioxidant activity of different concentrations of aqueous extract and ascorbic acid in DPPH scavenging assay. Each value represents mean ± SEM.

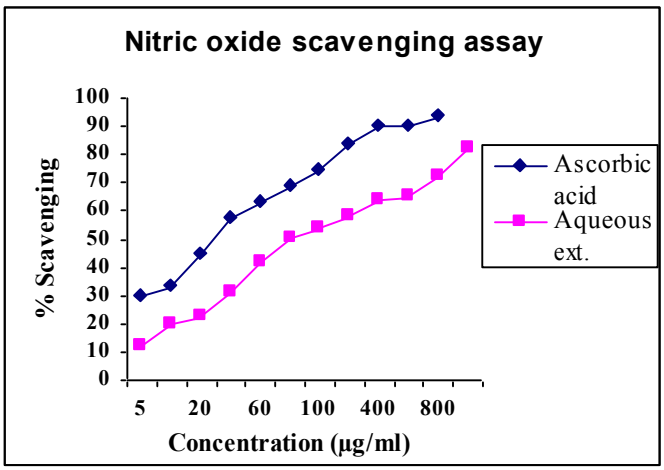

Figure 3: Antioxidant activity of different concentrations of aqueous extract and ascorbic acid in nitric oxide scavenging method. Each value represents mean ± SEM.

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