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IN VITRO ANTIOXIDANT ACTIVITY OF FRUITS OF *RANDIA DUMETORUM* LAMK

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ABSTRACT

The Free radical scavenging potential of plant *Randia dumetorum Lamk* fruits was studied. It was determined in water extract, methanol extract and its fractions in different polarity solvents. The models used for the study were DPPH assay, nitric oxide scavenging activity and reducing power assay. Both the extracts and its fraction showed dose dependent free radical scavenging activity. High Nitric oxide scavenging activity was observed in chloroform and ethyl acetate fraction with IC_{50} values 350 µg/ml and 480 µg/ml respectively. Whereas these fractions showed moderate antiradical activity by inhibiting DPPH radical with IC50 value of 280µg/ml and 340 µg/ml respectively. In reducing power assay Chloroform and ethyl acetate fraction showed potent activity than the other group. The antioxidant potential of Chloroform fraction and Ethyl acetate fraction of methanolic extract of fruits of *R. dumetorum may* be due to presence of polyphenolic compounds.

Keywords: Antioxidant, Free radical, *Randia dumetorum*

INTRODUCTION:

Free radicals have been implicated in causation of ailments such as diabetes, liver cirrhosis, nephrotoxicity etc.[1] .Together with other derivatives of oxygen they are inevitable byproducts of biological redox reaction.[2] Reactive oxygen species (ROS) such as superoxide anions (O_2), Hydroxyl radical (OH) and Nitric oxide (NO) inactivate enzymes and damage important cellular components causing tissue injury through co valent bonding. This augments collagen synthesis and fibrosis. The increased production of toxic oxygen derivatives is considered to be universal feature of stress condition. [3]

As plant produces lot of chemical moieties as antioxidants to control oxidative stress caused by sunbeams and oxygen, these can serve as source of new antioxidant compounds. Experimental evidence suggests that free radicals (FR) and reactive oxygen species (ROS) can be involved in high number of diseases.[4]

Ayurveda, an ancient Indian system of medicine believes in medicinal plants to treat various which are considered active due to chemical constituents and are designated as 'Rasayana' as one of the clinical specialties. Rasayana is not only a drug therapy but is a specialized procedure practiced in the form of rejuvenating recipes, dietary regimen promoting good habits. The purpose of rasayana is two-fold prevention of disease and counteracting aging process, which result from optimization of homeostasis.[5] Sharma et al. reported the strong antioxidant activityshown by rasayana drugs.[6] Around 34 plants are identified as Rasayanas in the Ayurvedic system of medicine.[7] These plants are described to possess various pharmacological properties such as Immunostimulant, Tonic, Neurostimulant, Anti-ageing, Anti-bacterial, Anti-viral, Anti-rheumatic, Anti-cancer, Adaptogenic, Anti-stress etc. Among these plants enlisted, some have been specifically investigated for their well demonstrated antioxidant activity.

Randia dumetorum Lamk. (Rubiaceae) known as Madana (Sans), Mainphal (Hindi), Emetic nut (Eng). A large deciduous thorny shrub grows up to 5 meters of height. Leaves simple, obviate, wrinkled, shiny and pubescent. Flowers are white, fragrant, solitary, seen on at the end of short branches. Fruits are globes, smooth berries with longitudinal ribs; yellow when ripe. Seeds many, compressed, and embedded in the dark fruit pulp. Its fruits are considered to be tonic, demulcent, diuretic and restorative, the drug is claimed as a medical cure for piles, antidysenteric agent, asthma, jaundice, diarrhea, emetic and gonorrhea.[3] It also shows different activities such as antitumor, anti-inflammatory, anti-fertility, insecticidal, anthalmetics, immunomodulatory, analgesic.[8,9,10,11,12,] There is data available on in vitro antioxidant activity of methanol extract of *Randia dumetorum Lamk.* [13]

But present work aims at studying effect of water extract, methanol extract and its different fraction *In-vitro* antioxidant models.

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METHODS

Plant material

The fruits of *Randia dumetorum* were purchased from local market from Pune and authenticated From Botanical Survey of India, Pune.

All other reagents and chemicals required for the study were of AR grade.

Plant material and extract preparation:-

Maceration of air-dried powdered fruits of *R. dumetorum* afforded water extract (30%w/w) methanol extract (17w/w). Methanol extract so obtained was then fractioned by maceration into different polarity solvents like petroleum ether, chloroform, ethyl acetate and methanol. All fractions were concentrated under vacuum. Phytochemical screening was done for water extract, methanol extract and its fraction.[14]

In-vitro antioxidant activity

Assay for Nitric oxide scavenging activity

Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction.
Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions which can be estimated by use of Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. Sodium nitroprusside (5mM) in phosphate buffered saline was mixed with different concentrations of test extracts dissolved in methanol and incubated at room temperature for 150 minutes. Blank without test extract but equivalent amount of methanol was conducted in an identical manner. After incubation solutions were removed and equal amount of Griess reagent (1% sulphanilamide,2% H3PO4 and 0.1% naphthylehylenediamine dihydrochloride). The absorbance of the chromophore formed was read at 546nm. IC50 was calculated as 50% reduction in absorbance brought about by sample compared with blank.[15] Curcumin was used as Standard.

Assay for antiradical activity with DPPH

Antiradical activity was measured by a decrease in absorbance at 516 nm of a methanolic solution of colored 1, 1, diphenyl picryl hydrazine brought about by sample.[16] A stock solution of DPPH was prepared by dissolving 4.4 mg in 3.3 ml methanol. Test medium included 150µl of DPPH solution along with different concentration of samples in 3 ml methanol. Blank was prepared in the same way without addition of sample. The decrease in absorbance caused by the presence of sample was noted after 15 minutes. IC_{50} was calculated as the 50% reduction in absorbance brought about by sample compared with blank.

Determination of reducing power

The reducing power of extracts and fractions was determined according to the method of Oyaizu.[17] Samples were mixed with 5 ml phosphate buffer (2M, pH 6.6) and 5 ml potassium ferricyanide (1%). The mixture was then incubated at 50°C for 20 minutes. 5 ml trichloroacetic acid (10%) was added and the mixture was centrifuged at 4000 rev/ min. The upper 5 ml solution was then mixed with 5 ml distilled water and 1 ml ferric chloride (0.1%). The absorbance was then measured at 700 nm. An increase in absorbance of the reaction mixture indicated an increase in the reducing power. Ascorbic acid (0.3 mg) was used as standard.

RESULTS

Phytochemical screening:-

Table 1: Table showing Qualitative chemical test

Test	Methanol	Pet. ether	Chloroform	Ethyl acetate	Methanol	Water extract
Carbohydrate	+	-	-	-	+	+
Protein/amino acid	-	-	-	-	-	+
Fats/ waxes	-	+	-	-	-	-
Glycoside	-	-	-	-	-	+
Flavonoides	+	-	+	+	+	-
Alkaloids	+	-	-	-	+	-
Terpenes	+	+	+	+	-	-
Steroids	+	+	+	+	-	-
Saponins	+	-	-	-	+	-
Phenolics /Tannins	+	-	+	+	-	-

Table 2: Antioxidant activity of different extract and fraction in Nitric oxide scavenging assay

Sample	Concentration (μg/ml)	% inhibition	IC_{50} (μg/ml)
Methanol extract	100	38.18±0.8027	600
	200	48.64±0.6504	
	400	59.65±0.6766	
	800	67.40±2.330	
Water extract	100	19.73±0.46	500
	200	25.65±0.2187	
	400	40.76±0.5493	
	800	47.06±0.9303	
Pet.ether fraction	100	26.83±0.9739	570
	200	32.51±0.4478	
	400	34.58±1.000	
	800	39.42±1.327	
Chloroform fraction	100	31.14±0.4664	350
	200	49.28±0.6658	
	400	57.63±0.6974	
	800	66.58±0.6447	
Ethyl acetate fraction	100	27.65±2.051	480
	200	36.82±1.741	
	400	41.7±0.4386	
	800	47.18±0.8103	
Methanol fraction	100	35.96±1.383	590
	200	56.45±1.759	
	400	62.77±1.107	
	800	69.51±1.757	

Table 3: Antioxidant activity of ascorbic acid

Test μg/ml	**% Inhibition**	IC_{50} (μg/ml)
10	53.47±1.397	13.5
15	63.15±0.550	
20	70.91±0.5033	
25	83.15±6.473	

All Values represents Mean± SEM

It was observed that free radicals were scavenged by the test compounds in concentration dependent manner. The nitric oxide was inhibited by water extract, methanol extract and its fractions. Both the extracts and fractions showed a concentration dependent antiradical activity. The results are as displayed in Table 2. The Chloroform fraction and ethyl acetate fraction showed high Nitric oxide scavenging activity with IC_{50} values 350 μg/ml and 480 μg/ml respectively. The difference in Nitric oxide scavenging activity due to small difference in their proton and electron transferring energy among the constituent or may be due to the higher amount of polyphenols.

Table 4: Antioxidant activity of different extract and fraction in DPPH

Sample	DPPH of different concentration μg/ml **IC_{50} (μg/ml)**				IC_{50} (μg/ml)
	100	200	400	800	
Methanol extract	16.518±3.447	28.802±3.417	36.841±3.416	57.821±4.021	760
Water extract	15.602±1.352	25.802±1.356	32.021±1.414	61.821±1.021	630
Pet. ether fraction	6.001±0.341	18.213±1.061	25.721±1.321	51.241±1.025	780
Chloroform fraction	38.563±1.082	45.213±1.413	62.760±1.421	85.620±1.041	280
Ethyl acetate fraction	26.002±3.447	39.312±2.410	56.021±2.410	63.542±3.412	340
Methanol fraction	15.512±0.052	27.219±1.012	33.231±1.019	55.312±1.082	730

All Values represents Mean± SEM.

Curcumin was used as standard, giving a reading of IC_{50} value 52.72μg/ml

Water extract, methanol extract and its fraction showed concentration dependent antiradical activity by inhibiting DPPH radical. While Chloroform fraction and ethyl acetate fraction show better activity with an IC50 value of 280µg/ml and 340 µg/ml (Table 4)

Table 5: Antioxidant activity of different extract and fraction in Reducing power assay

Sample	Reducing power of different concentration µg/ml			
	100	200	400	800
Methanol extract	0.2066±0.008	0.2373±0.007	0.2863±0.004	0.3143±0.020
Water extract	0.1232±0.003	0.1352±0.002	0.1936±0.007	0.2020±0.008
Pet. ether fraction	0.1024±0.09	0.1263±0.008	0.1573±0.0032	0.1666±0.012
Chloroform fraction	0.2513±0.055	0.2983±0.088	0.3573±0.0269	0.3646±0.016
Ethyl acetate fraction	0.2423±0.013	0.3338±0.126	0.3762±0.014	0.4041±0.061
Methanol fraction	0.1863±0.0052	0.2195±0.012	0.2313±0.019	0.3126±0.082

All Values represents Mean± SEM.

Ascorbic acid (0.3 mg) was used as standard, giving a reading of 0. 4386 at 700nm. In reducing power assay, reduction and consequent formation of the ferrous product occurs at 700 nm. An increasing absorbance is indicative of potent antioxidant activity. Chloroform and ethyl acetate fraction showed potent activity than the other group. (Table 5). The greater reducing power means phytoconstituent in extract are electron donor and can react with free radical and then convert it into more stable metabolite.

DISCUSSION

Free radicals are chemical entities that can exist separately with one or more unpaired electron. The propagation of free radical can bring about thousands of reaction and thus may cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radicals. [17 18] Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macro phases, neurons etc. are involved in regulation of various physiological process.[19]

DPPH is a relatively stable free radical. The assay determines the ability of extract and fraction to reduce DPPH radical to the corresponding hydrazine by converting the unpaired electron to pair once. Antioxidants can act by converting the unpaired electron to pair one. [20,21.] The measurement of reductive ability was done by Fe^{3+}-Fe^{2+} transformation in the presence of water extract, methanol extract and its fraction and standard antioxidant, ascorbic acid. The reducing power is associated with antioxidant activity[22] Preliminary phytochemical analysis indicates the presence of Phenolic, flavonoids, and tannins in Chloroform fraction and Ethyl acetate fraction. Polyphenols particularly flavonoids and tannins are well known natural antioxidants.[22.23,24]. Thus antioxidant potential of Chloroform fraction and Ethyl acetate fraction of methanolic extract of fruits of *R. dumetorum may* be due to presence of polyphenolic compound and exact mechanism of action, however, can only be unfolded after detailed characterization of active moieties from tested fraction which needs further analysis.

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