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Research Article

POTENT ANTIOXIDANT FROM A TRADITIONAL HERBAL HERITAGE

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ABSTRACT

In recent years, antioxidants have gained importance because of their potential prophylactic and therapeutic action in many stress related diseases. These are the molecules of defense that quench these hazardous free radicals and reduce their potential to attack the cells, thereby protecting the cells. A variety of antioxidant molecules have been isolated and analyzed. *Antidesma acidum* Retz. a shrub bearing sour foliage has been evaluated for antioxidant activity on different parameters. Present study showed that test drug has got strong antioxidant properties.

Keywords: antioxidants, free radicals, *Antidesma acidum* Retz., *in-vitro*

INTRODUCTION

For ill health, hope works sometimes, but herbs, all the time. Human beings, sought to alleviate his suffering from injury and disease by taking advantage of plants growing around him. It is increasingly being realized that majority of the present day disease are due to the shift in the balance of the pro-oxidant and the anti-oxidant homeostatic phenomenon in the body. Pro-oxidant condition dominate either due to the increased generation of the free radicals caused by excessive oxidation stress of the current life or due to the poor scavenging/quenching in the body caused by depletion of the dietary antioxidants[1]. These free radicals cause damage to cells and are responsible for aging and other ailments such as cancer etc which are life threatening and mostly fatal. Hence in recent years, antioxidants have gained importance because of their potential prophylactic and therapeutic action in many diseases. These are molecules of defense that quench these hazardous free radicals and reduce their potential to attack the cells, thereby protecting the cells[11]. A variety of antioxidant molecules have been isolated and analyzed. Studies show that antioxidant molecules in their isolated forms are not as efficacious as they are in their natural forms[2]. *Antidesma acidum* Retz., belonging to family Euphorbiaceae, is a large shrub bearing sour foliage, is being used by folklore practitioners for backache, myalgia and many other chronic debilitating disease especially in Udupi, Karnataka, India district.[3] The tender leaves and stem barks used in different vegetable preparations, as digestive, laxative and as a general tonic[3]. Hence with this background this drug has been selected for *In vitro* antioxidant activity.

MATERIALS AND METHODS

Antidesma acidum Retz. leaves were collected from its natural habitat, authentified through botanist, shade dried and deposited in department of biotechnology and microbiology, SDM Centre for research in Ayurveda and Allied Sciences, Udupi, Karnataka, India and voucher number (270920121) was taken. The sample was powdered and stored for further study.

Preparation of extract

1 g of this leaf powder was soaked in 10 ml of Methanol for 24 h and then filtered. Filtrate was used for further tests. Varying doses of the test drugs (10 – 100 µg/ml) were taken for the present study. The following models were used for the evaluation of *in vitro* antioxidant activity.

Nitric oxide radical scavenging activity

2 ml of 10 mM of sodium nitroprusside in 0.5 ml of phosphate buffer saline (pH-7.4) was mixed with the 0.5 ml of extract 10, 20, 40, 60, 80 and 100 µg/ml; the mixtures were incubated at 25°C for 2 ½ hours and from the incubated mixture 0.5 ml was added in to 1 ml of 0.33 % sulfanilic acid. And it is allowed to incubate in the room temperature for 5 minutes. Then 1 ml of 0.1 % of naphthyl ethylenediamide dichloride was added. Mixed the content and incubated at room temperature for 30 minutes. The absorbance of the mixture at 540 nm was measured with a Double beam UV-visible Spectrophotometer (SYSTRONICS 2201). Distilled water served as the blank. Nitric oxide radical scavenging activity was calculated according to the following formula[4],

$$\% \text{ inhibition} = \text{Absorbance of blank } (A_O) - \text{Absorbance of extract } (A_1) \times 100 / \text{Absorbance of blank } (A_O)$$

Hydroxyl radical scavenging activity

Two series of tubes were taken. In the first set 60 µl of 1 mM ferrous chloride, 90 µl of 1 mM 1, 10 phenanthroline, 2.4 ml of 0.2 M phosphate buffer saline (pH 7.4) were taken and 150 µl of 0.17 M hydrogen peroxide was added to initiate the reaction. This set was labeled as blank. In the second set before adding hydrogen peroxide, 1.5 ml of SG extract in varying doses (10,

20, 40, 60, 80 and 100 µg/ml) was added. After incubation at room temperature for 5 min, the absorbance of the mixture at 560 nm was measured with Double beam UV-visible Spectrophotometer (SYSTRONICS 2201). The hydroxyl radical scavenging activity was calculated as per the above formula[5].

Reducing power assay

0.75 ml of varying doses (10, 20, 40, 60, 80 and 100 µg/ml) of SG extract was mixed with 0.75 ml of phosphate buffer (0.2 M pH 6.6) and 0.75 ml of potassium ferricyanide (1 % v/v) and incubated at 50°C for 20 min. Reaction was stopped by adding 0.75 ml of 10 % trichlroacetic acid and centrifuged at 800 rpm for 10 minutes. 1.5 ml of supernatant was mixed with 1.5 ml distilled water and 0.1 ml ferric chloride (0.1 %). Incubated at room temperature for 10 minutes and the absorbance at 700 nm were measured with double beam UV-visible Spectrophotometer (SYSTRONICS 2201). Higher absorbance of reaction mixture indicates the greater reducing power[6].

DPPH scavenging assay

DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) was purchased from Sigma, USA. All the other chemicals used were of analytical grade. DPPH (1, 1-Diphenyl-2-picrylhydrazyl) is a stable free radial with purple color (absorbed at 517 nm). If free radicals have been scavenged, DPPH will degenerate to yellow color. This assay uses this character to show free radical scavenging activity. The test drug was in the doses of 1mg/ml which was then used to determine its antioxidant activity[7].

Control = Methanol + DPPH.
Test = DPPH + sample (Varying doses).
The percentage inhibition of DPPH radical by the sample was calculated using the following formula.

$$\% \text{ inhibition} = A_0 - A_1 \times 100 / A_0$$

A_0 = Absorbance of control, A_1 = Absorbance of sample

Hydrogen peroxide scavenging activity

1 ml of 10, 20, 40, 60, 80 and 100 µg/ml of SG extract and 1 ml of 0.1 M H_2O_2 was mixed followed by 2 drops of 3 % ammonium molybdate, 10 ml of 2M H_2SO_4 and 7 ml of 1.8 M potassium iodide. This reaction mixture was titrated with 509 mm of sodium thiosulphate until the disappearance of the yellow color. Percentage of scavenging of hydrogen peroxide was calculated using the following formula[8],

$$\% \text{ Inhibition} = \frac{V_0 - V_1 \times 100}{V_0}$$

Where, V_0 is volume thiosulfate used to titrate blank and V_1 is volume of thiosulfate used to titrate test sample

Statistical analysis

Statistical analysis performed using INSTAT Graph pad software.

Table 1: DPPH Assay for Anti oxidant Evaluation Activity

S. No.	Working sample Concentration	OD at 517 nm			Mean	Mean ± SEM	% Inhibition
1	Control	0.170	0.189	0.178	0.179	0.179 ± 0.00550	-
2	2 µg/ ml	0.073	0.083	0.076	0.077	0.077 ± 0.00296	56.98
3	4 µg/ ml	0.109	0.118	0.089	0.105	0.105 ± 0.00857	41.34
4	5 µg/ ml	0.098	0.103	0.125	0.108	0.108 ± 0.00829	39.66
5	6 µg/ ml	0.115	0.111	0.088	0.104	0.104 ± 0.00841	41.90
6	8 µg/ ml	0.150	0.127	0.147	0.141	0.141 ± 0.00721	21.23
7	10 µg/ ml	0.129	0.170	0.183	0.160	0.160 ± 0.01627	10.61

Table 2: Nitric oxide Scavenging Test

S. No.	Working sample Concentration	OD at 540 nm			Mean	Mean ± SEM	% Inhibition
1	Control	0.100	0.102	0.103	0.101	0.101 ± 0.00088	-
2	2 µg/ ml	0.074	0.074	0.078	0.075	0.075 ± 0.00133	25.74
3	4 µg/ ml	0.100	0.084	0.092	0.092	0.092 ± 0.00461	8.91
4	5 µg/ ml	0.085	0.099	0.098	0.094	0.094 ± 0.00450	6.93
5	6 µg/ ml	0.091	0.092	0.108	0.097	0.097 ± 0.00550	3.96
6	8 µg/ ml	0.086	0.093	0.079	0.086	0.086 ± 0.00404	14.85
7	10 µg/ ml	0.096	0.089	0.100	0.095	0.095 ± 0.00321	5.94

Table 3: Hydroxyl Radical Scavenging Activity

S. No.	Working sample Concentration	OD at 560 nm			Mean	Mean ± SEM	% Inhibition
1	Control	0.110	0.112	0.099	0.107	0.107 ± 0.00404	-
2	2 µg/ ml	0.067	0.060	0.059	0.062	0.062 ± 0.00251	42.06
3	4 µg/ ml	0.070	0.082	0.076	0.076	0.076 ± 0.00346	28.97
4	5 µg/ ml	0.075	0.078	0.077	0.077	0.077 ± 0.00088	28.04
5	6 µg/ ml	0.073	0.087	0.094	0.084	0.084 ± 0.00617	21.49
6	8 µg/ ml	0.089	0.091	0.090	0.090	0.090 ± 0.00057	15.89
7	10 µg/ ml	0.111	0.108	0.099	0.106	0.106 ± 0.00360	0.93

Table 4: Reducing Power Assay

S. No.	Working sample Concentration	OD at 700 nm			Mean	Mean ± SEM	% Inhibition
1	Control	1.896	1.857	1.855	1.869	1.869 ± 0.01335	-
2	2 µg/ ml	0.264	0.261	0.211	0.245	0.245 ± 0.01719	86.89
3	4 µg/ ml	0.269	0.288	0.280	0.279	0.279 ± 0.00550	85.07
4	5 µg/ ml	0.303	0.317	0.300	0.306	0.306 ± 0.00523	83.63
5	6 µg/ ml	0.323	0.344	0.343	0.337	0.337 ± 0.00683	81.97
6	8 µg/ ml	0.410	0.405	0.410	0.408	0.408 ± 0.00166	78.17
7	10 µg/ ml	0.496	0.476	0.477	**0.483**	0.483 ± 0.00650	74.16

Figure 1: *Antidesma acidum* Retz. (Picture taken during Fruiting)

RESULT AND DISCUSSION

The model for scavenging the stable DPPH radical is widely applied to evaluate antioxidant activities in a relatively short time as compared to other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability[12]. Table 1 depicts the data related to the antioxidant activity of Methanolic extracts of test drug by DPPH assay. The test drug has showed activity in dose dependant manner to certain levels. Effect/ inhibition is found to be high at lower dosage level tried (2 µg/ ml) and very low at higher dosage level. Nitric oxide is a free radicals produced in mammalian cells, involved in the regulation of various physiological processes. Nitric oxide is implicated in diseases such as cancer and inflammation. It also mediates smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated cytotoxicity. Sodium nitroprusside spontaneously generates nitric oxide at physiological pH, in aqueous solutions. The nitric oxide, generated is converted into nitric and nitrous acids on contact with dissolved oxygen and water. The liberated nitrous acid was estimated using a modified Griess-Illosvoy method. Nitrous acid reacts with Griess reagent, to form a purple azo dye. In presence of antioxidants, the amount of nitrous acid will decrease and the degree of decrease in the formation of purple azo dye will reflect the extent of scavenging. Table 2 shows the data related to *in vitro* antioxidant activity of test drug by nitric oxide scavenging test. The effect is found to be dose dependant to certain level (2 µg/ ml-6 µg/ ml). Lower dose level (2 µg/ ml) has induced comparatively higher activity than all other higher doses were used. Marginal decrease in activity was observed from 8 µg/ ml to 10 µg/ ml. Hydrogen peroxide is a weak oxidizing agent which inactivates enzymes by oxidation of the essential thiol (SH-) groups. It rapidly transverses cell membranes and once inside the cell interior, interacts with Fe^{2+} and Cu^{2+} to form hydroxyl radicals, which is harmful to the cell[8]. Table 3 depicts the data of *in vitro* antioxidant activity of test drug by hydroxyl radical scavenging activity. Test drug has showed effect in dose dependant manner, but is inversely proportional to the dosage level tried. As the dosage level increases the effect is found to be decreased. Lower dose level (2 µg/ ml) has induced higher activity, where as higher dose level (10 µg/ ml) has induced less activity in terms of percentage of inhibition. Reducing power assay measures the electron donating capacity of an antioxidant. The reducing properties are generally associated with the presence of reductones, which have been shown to exhibit antioxidant action by breaking the chain reactions by donating a hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. Table 4 depicts the *in vitro* antioxidant activity related to reducing power assay of test drug. The test drug exhibited good reducing power activity at lower dose level (2 and 6 µg/ ml). The reducing activity was found to be statistically high for the test drug.

CONCLUSION

Leaves of *Antidesma acidum* Retz having sour taste, which in the vicinity of Udupi district, India used to cure chronic debilitating disease like arthritis could be used to scavenge free radicals produced in the biological system was evident from the results obtained from the present study. Oxidative stress has been implicated in the pathology of many diseases and conditions including diabetes, cardio vascular diseases, inflammatory conditions, cancer and aging[10]. Research in finding a natural antioxidant from the plant source is therefore important as plants are potential source of immense chemicals for the treatment of number of ailments[11]. Present study showed that test drug has got strong antioxidant properties, hence this drug could be used as an antioxidant agent and further studies can be conducted to evaluate in different *in vivo* models.

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