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RESEARCH ARTICLE

A Comparative Analysis of Antioxidant Potentials of Aqueous and Ethanolic Extracts of *Cyperus Rotundus* (L.)

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ABSTRACT

This study deals with the evaluation of free radical scavenging capacity of aqueous and ethanolic extracts of rhizome of *Cyperus rotundus* by their ability to scavenge DPPH, nitric oxide, hydroxyl radical and exhibit reducing power. Both extracts showed effective free radical scavenging activity. Compared to aqueous extract, ethanolic extract has shown better activity. The results support its extensive usage in various diseases. The main basis for this use seems to be its role as a rich source of natural antioxidant which protects the body from various oxidative damage.

Keywords: *Cyperus rotundus*, DPPH assay, Nitric oxide scavenging assay, hydroxyl radical scavenging assay, reducing power assay.

1. INTRODUCTION:

Oxygen is necessary for living beings for various metabolic activities and the reactive oxygen species generated in the cells are essential in aerobic life and metabolism but excess of it can cause damage to the cell (1). The free radicals when formed in excess during the process of oxidation cause damage to the cell membrane, DNA, RNA and different enzymes of the cell. Environmental agents like toxicity of lead, pesticides, cadmium, ionizing radiation, alcohol, cigarette smoke, UV light and pollution may also initiate free radical generation (2-4). Antioxidants produced in the body neutralize the free radicals and protects the body from their harmful effects. This natural production of antioxidants is not sufficient always as in case of exposure to environmental hazards and in increasing age (5-6). The synthetic antioxidants may substitute the natural antioxidants, but their use is attendant with severe side effects (7). So there is a great need to identify and use natural antioxidants. Various fruits and vegetables were already proven for their various antioxidant contents (8). A large number of Indian medicinal plants have recently received a great attention in their antioxidant properties (9). Antioxidant-based drug formulations are used for the prevention and treatment of complex degenerative diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer (10).

Cyperus rotundus (L), commonly called "Musta" is a medicinal plant belonging to the *Cyperaceae* family grows all over India up to 2000 meters altitude, especially on the banks of streams and rivers. It commonly appears among Indian, Chinese, Japanese natural drugs used as home remedy (11). It is said to possess antidiarrhoeal, anti-inflammatory and antipyretic activities (12).As per Ayurveda the tubers have carminative and demulcent property, they are used to treat the abdominal disorders particularly diarrhea, indigestion and flatulence. They are used as analgesic, diuretic and for the treatment of cold and congestion, inflammation, wounds and sores, amenorrhea and dysmenorrhea (13-14).*C. rotundus* was to found produce protective effect in inflammatory bowel disease (15). The oil of *C. rotundus* showed a remarkable antibacterial activity and antimutagenic activity (16). In many of the above therapeutic effects free radical scavenging can play major role, hence

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in the present study a comparative analysis of free radical scavenging potential of both aqueous and ethanolic extracts of *C. rotundus* rhizome was carried out.

2. MATERIALS AND METHODS

2.1. Collection and Identification of the herb: Dried rhizomes of *C.rotundus* were collected from local Ayurvedic pharmacy in Mangalore. The plant material was authenticated by Dr.Sunilkumar, Senior Research officer, Department of Pharmacognosy, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi and sample voucher specimen (No.**11110101)** has been deposited in the above Laboratory's plant depository. The shade dried rhizomes of the test plant were coarsely powdered and preserved in freezer for further studies.

2.2. Preparation of the extracts: Extracts were prepared according to the procedure explained by Raaman (17).Aqueous extract was prepared by using 10 g of coarse powder of rhizomes by refluxing the powder in 100ml of distilled water for 24h and filtered. Filtrate was further diluted to suitable concentration (mg/ml solution). Working solutions were diluted to get 10-100 µg/ml concentration for antioxidant evaluation activity.For ethanolic extract**;** 10 g of coarse powder of rhizomes of *C. rotundus* were similarly processed as described above to obtain the test extract. These two extracts were subjected for following four assays to assess the antioxidant capacity.

2.3. DPPH SCAVENGING ASSAY (18)

2.3.1. Reagents: DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) was purchased from Sigma, USA. All the other chemicals used were of analytical grade.

2.3.2. Procedure: DPPH (1, 1-Diphenyl-2-picrylhydrazyl) is a stable free radical with purple color. If free radicals have been scavenged, DPPH will degenerate to yellow color. This assay uses this character to show free radical scavenging activity. The change in color from purple to yellow was measured at 517nm in a spectrophotometer (SSYTRONICS 2201). A 0.002% of DPPH in methanol was prepared and 1ml of this solution was added to 1ml of different concentrations of extracts. Methanol with extract served as blank and DPPH in methanol without the extracts served as positive control. The percentage inhibition of DPPH radical by the sample was calculated using the following formula.

$$\% \text{ inhibition} = [(A_0 - A_1) / A_0] \times 100$$

A_0 = Absorbance of control. A_1 = Absorbance of sample

The activity was compared with ascorbic acid, which was used as a standard antioxidant.

2.4. Nitric oxide radical scavenging assay (19)

2 ml of sodium nitroprusside (10µM) in 0.5 ml of standard phosphate buffer solution was incubated with 0.5 ml of different concentration of the test extracts (10µg/ml to 100µg/ml) dissolved in standard phosphate buffer (0.025M, pH 7.4) and the tubes were incubated at 25°C for 2½ h. To 0.5 ml of incubated mixture 1 ml of 0.33% sulfanilic acid was added and allowed to stand at room temperature for 5 min. After incubation 1 ml of 0.1% of naphthylethylenediamide dichloride was added, mixed the content and incubated at room temperature for 30 minutes. All tests were performed in triplicate. 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:

$$\% \text{ inhibition} = [(A_0 - A_1) / A_0] \times 100$$

Where, A_1 = Absorbance of control. A_1 Absorbance of sample.

The activity was compared with ascorbic acid, which was used as a standard antioxidant.

2.5. Reducing power assay (20)

A 0.75 ml of various concentrations of the extracts (10µg/ml- 100µg/ml) 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 20min. The reaction was stopped by adding 0.75 ml of 10%trichloroacetic acid, centrifuged at 800 rpm for 10minutes. 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 absorbances at 700 nm were measured with Double beam UV-visible Spectrophotometer (SYSTRONICS 2201). All tests were performed in triplicate. Higher absorbance of reaction mixture indicates the greater reducing power as compared to ascorbic acid (Standard). The percentage of radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = [(A_0 - A_1) / A_0] \times 100$$

Where, A_1=Absorbance of control, A_1 Absorbance of samples**.**

2.6. Hydroxyl radical scavenging activity (21)

Two series of tubes were taken. In the first set 60 µl of 1 mM ferrous chloride, 90 µl of 1mM 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 either the extracts or Vitamin C in varying concentrations such as 10, 20, 40, 60, 80 and 100µg/ml were 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 according to the following formula.

$$\% \text{ inhibition} = [(A_0 - A_1) / A_0] \times 100$$

Where, A=Absorbance of control, A_1 Absorbance of samples.

3. STATISTICAL ANALYSIS

The data obtained have been presented as Mean ± SEM. The difference between the control group and test extracts treated group was analyzed by employing one way ANOVA (Analysis of Variance) followed by Dennett's multiple't' test as post hoc test. P<0.05 was considered as statistically significant.

4. RESULTS

Yield of Aqueous extract of *C.rotundus* was 5.2% and that of ethanolic extract was 1.9%.

4.1. DPPH Assay:

Fig.No.1.shows the effect of *C.rotundus* related to the DPPH scavenging activity of aqueous and ethanolic extracts. Aqueous extract of *C.rotundus* showed moderate to good activity to scavenge free radicals and this activity was found to be dose dependent up to 80µg/ml. The difference between the control group and test drug group was found to be highly significant with respect to all the doses (p<0.001) except 10µg/ ml group in which the difference was found to be statistically non-significant (p>0.05). DPPH assay of ethanolic extract of *C.rotundus* also shows significantly high activity in a dose independent manner. But at very high concentrations of the extract the effect was found to be decreased. The highest effect is found in lowest dosage level (10 µg/ ml).The difference between the control group and test drug group was found to be very highly significant in all the test doses (p<0.001).

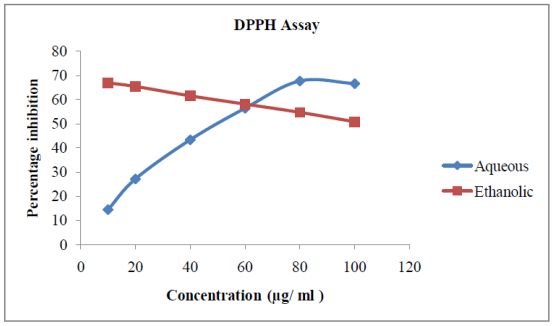

Fig.No.1. Effect of aqueous and ethanolic extracts of *C. rotundus* by DPPH assay

4.2. Nitric oxide scavenging activity:

Fig.No.2 depicts nitric oxide radical scavenging activity of aqueous and ethanolic extracts of *C.rotundus*. The observed effect is not dose dependent in case of aqueous extract. Two different dosage levels i.e 40 and 80µg/ml have produced similar effect. It has been observed that lower concentrations i.e 10 and 20 µg/ml was found be more effective compared to other dosage levels. Ethanolic extract produced a moderate inhibition of nitric oxide formation at the lower dose level tested but tended to decrease at higher dose level at which only weak effect was observed. The observed activity was not concentration dependent. Moderate inhibition of nitric oxide formation was observed at 10 and 20 µg/ ml. At higher concentration level the observed inhibition was less in comparison to the effect observed at lower concentration level. The difference between the control group and test drug group was found to be very highly significant in all the test doses (p<0.001).

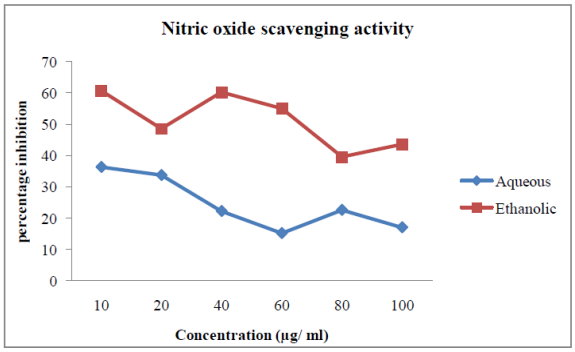

Fig 2: Nitric oxide scavenging activity of aqueous and ethanolic extracts of *C. rotundus*

4.3. Reducing power assay:

Fig.No.3 depicts data related to reducing power assay of aqueous and ethanolic extracts of *C.rotundus*. The aqueous extract produced moderate and dose dependent reducing activity till 60µg/ml. The test drug has showed greater activity of reducing power in lower dose (10µg/ml). The difference between the control group and test drug group (10µg/ml and 20µg/ml) was found to be highly significant (p<0.01). The effect observed at higher concentrations of the test extract (40-100 µg/ ml) was found to be non-significant in comparison to control (p>0.05).The ethanolic extract produced marked and dose independent increase in the reducing power. The test drug has showed significant activity against reducing power in all the six different dose levels tried. Higher absorbance of reaction mixture indicates the greater reducing power. Percentage of inhibition of the sample extract is comparatively higher in lower concentrations tried for the present study. The difference between the control group and test drug group was found to be very highly significant in 10-60µg/ ml group (p<0.001) and significant in 80µg/ ml and 100µg/ ml test doses (p<0.01).

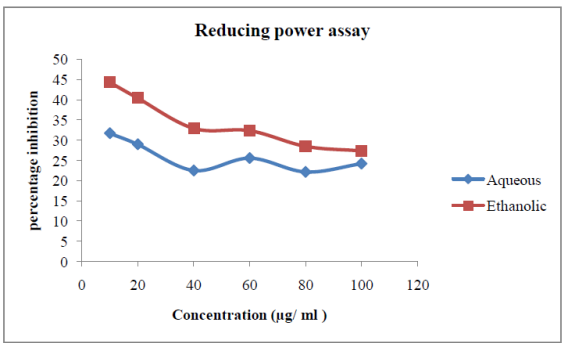

Fig 3: Reducing power assay of aqueous and ethanolic extracts of *C. rotundus*

4.4. Hydroxyl radical scavenging activity:

Fig.No.4 depicts the hydroxyl radical scavenging activity of aqueous and ethanolic extracts of *C.rotundus*. The aqueous extract exhibited moderate hydroxyl scavenging activity which was found to be dose independent. As observed with other tests, the higher concentrations were found to be less effective. The difference between the control group and test drug group was found to be highly significant in 10µg/ ml group ($p<0.01$) and significant in remaining test doses ($p<0.05$). The ethanolic extract exhibited moderate hydroxyl scavenging activity which was found to be dose independent. At the above dosage levels, the higher concentration was found to be less effective. Increased effect was observed from 10µg/ ml to-20µg/ ml concentration and then again activity is found to be higher at 60µg/ ml. The difference between the control group and test drug group was found to be significant only in 20µg/ ml group ($p<0.05$).

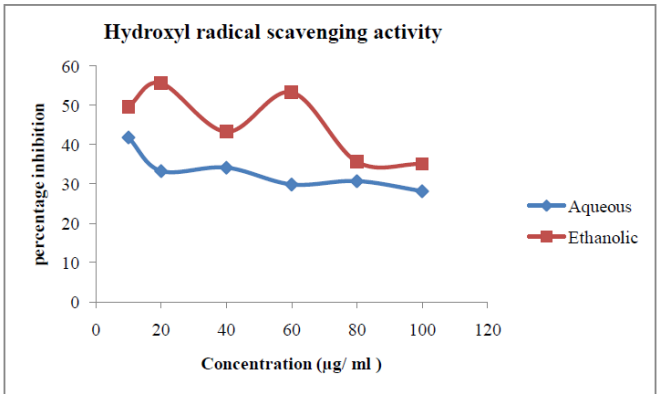

Fig 4: Hydroxyl radical scavenging activity of aqueous and ethanolic extracts of *C. rotundus*

5. DISCUSSION

Previous studies on the plant extracts have shown that antioxidant activity of any herb is likely to be due to the presence of phenolic compounds in them, since their hydroxyl groups have significant scavenging ability (22-23). The results of the present study show that both aqueous and ethanolic extracts possess good antioxidant potential against free radicals like reactive oxygen species and reactive nitrogen species.

DPPH assay is widely used for the screening of scavenging activity antioxidants because it is a rapid and sensitive method to detect hydrogen donating ability of plant extracts at low concentrations (24). DPPH radical is a model of lipophilic radical, and the scavenging activity of the extracts is due to their hydrogen donating nature (25). Aqueous extract of *C.rotundus* showed good activity to scavenge free radical in a dose dependent manner up to 80µg/ml. Ethanolic extract of *C.rotundus* shows significantly high activity in a dose independent manner.

Another free radical nitric oxide is required for various physiological process like, neurotransmission, vascular homeostasis, antimicrobial and antitumor activities (26). Aqueous extract of *C.rotundus* was found to be more effective at lower concentrations, i.e. 10 and 20µg/ml compared to other dosage levels. Ethanolic extract produced a moderate inhibition of nitric oxide formation at the lower dose level but its effectiveness decreased at higher dose.

The reducing power assay indicates the extracts ability to donate electron to react with free radicals and convert them into more stable metabolites and terminate the radical chain reaction (27). The aqueous extract produced moderate and dose dependent reducing activity till 60µg/ml. The ethanolic extract produced marked and dose independent increase in the reducing power.

Hydroxyl radical are the major active oxygen causing lipid peroxidation and enormous potential for biological damage. The highly reactive hydroxyl radical can cause oxidative damage to DNA, lipid and protein (28). The aqueous and ethanolic extract exhibited moderate hydroxyl scavenging activity which was found to be dose independent. The dose independent activity is quite common with plant extracts (29). This is likely due to presence of multiple phytochemical constituents in which some may have opposite effects.

6. CONCLUSION

The present investigation shows that both aqueous and ethanolic extracts of rhizomes of *C.rotundus* exhibits antioxidant and free radical scavenging ability which is more apparent in ethanolic extract. These differences in activities may be due to dissimilarity in the phytoconstituents present in each extracts. The present study is in agreement with the earlier investigations done by few researchers (16, 30). Based on the results obtained it can be suggested that this herb can be used as potent natural antioxidant which may be helpful to prevent various degenerative diseases. Detailed *in vivo* experiments may help to prove above results, which are in progress.

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Conflict of Interest: None Declared

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