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IN VITRO ANTIOXIDANT ACTIVITY AND PHENOLIC CONTENT OF *CYPERUS ROTUNDUS* LINN. RHIZOMES

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ABSTRACT

Cyperus rotundus is a plant, rich in alkaloids, phenolics and flavonoids as a potential source of compounds possessing beneficial biological activities. In this study the petroleum ether and ethyl acetate extracts of *Cyperus rotundus* rhizomes, were investigated to evaluate their antioxidant property. Antioxidant property was evaluated by using total antioxidant capacity, DPPH scavenging capacity, reducing spower assay, hydrogen peroxide scavenging assay. In DPPH assay, the percentage of inhibition of ethyl acetate extract (71±0.15at 125 µg/ml) showed higher activity than petroleum ether extract (63±0.04at 125 µg/ml). In hydrogen peroxide assay was found to be 3.09±0.006 at 125 µg/ml in ethyl acetate and 0.801±0.054 at 125 µg/ml in petroleum ether. The reducing power of ethyl acetate extract was 1.46±0.054 at 125 µg/ml and petroleum ether extract also was 0.86±0.054 at 125 µg/ml. In total antioxidant assay, the concentration range from 25-125µg/ml, the ethyl acetate extract showed strong dose than petroleum ether extract. Phenolic content was measured by Folin Ciocalteu assay and is expressed as Gallic acid equivalents. The results showed that *Cyperus rotundus* exhibits good antioxidant activity.

KEYWORDS: *Cyperus rotundus*, antioxidant, free radicals.

INTRODUCTION

Reactive oxygen species (ROS) generated in cells, are fundamental in modulating various physiological functions and represent an essential part of aerobic life and metabolism. Excessive generation of these radicals disrupts the antioxidant defense system of the body

which may lead to oxidative stress.[1] Oxidative stress plays a major part in the development of chronic and degenerative ailments such as cancer, autoimmune disorders, rheumatoid arthritis, aging, cardiovascular and neurodegenerative diseases.[2] Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection against infections and degenerative diseases. They can either directly scavenge or prevent generation of ROS.[3] The two most commonly used synthetic antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have begun to be restricted because of their toxicity and DNA damage induction.[4] The plant species have been investigated in the search for novel antioxidants, but generally there is still a demand to find more information concerning the antioxidant potential of plant species as they are safe and also bioactive. Therefore in recent years, considerable attention has been directed towards the identification of plants with antioxidant activity.[5]

C. *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.[6] It is said to possess antidiarrhoeal, anti-inflammatory and antipyretic activities.[7] 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 dysmenorrheal.[8-9] C. *rotundus* was to found produce protective effect in inflammatory bowel disease.[10] The oil of C. *rotundus* showed a remarkable antibacterial activity and ant mutagenic activity.[11] In many of the above therapeutic effects free radical scavenging can play major role, hence in the present study a comparative analysis of free radical scavenging potential of both extracts petroleum ether and ethyl acetate of C. *rotundus* rhizome was carried out.

MATERIALS AND METHODS

Plant material and extraction

The plant materials were collected and washed under running water, cut into pieces, air dried and pulverized into fine powder in a grinding machine. 100 g of the powder was then extracted with 500 ml of petroleum ether and ethyl acetate, filtered, squeezed off and evaporated under reduced pressure in a rotary evaporator to obtain crude extract.

Free radical scavenging activity

DPPH radical scavenging assay

The free-radical scavenging activities of these compounds were tested by their ability to bleach the stable radical DPPH. The antioxidant activity using the DPPH (1, 1-diphenyl-2-picryl hydrazyl) assay was assessed by the method of Blois.[12] The reaction mixture contained 100 µM DPPH in methanol and different concentrations (25-125µg/ml) of extracts. Absorbance at 517 nm was determined after 30 min at room temperature and the scavenging activity were calculated as a percentage of the radical reduction. Each experiment was performed in triplicate. Ascorbic acid was used as reference compound.

Reducing power assay

The reducing power of extract was determined by the method of Yen and Duh.[13] Different concentrations of extracts (25-125 µg/ml) were mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of 1 % potassium ferricyanide. The mixtures were incubated at 50°C for 20 min. After incubation, 2.5 ml of 10% trichloroacetic acid were added to the mixtures, followed by centrifugation for 10 min. The upper layer (5 ml) was mixed with 5 ml of distilled water and 1 ml of 0.1 % ferric chloride and the absorbance of the resultant solution were measured at 700 nm.

Hydrogen peroxide radical scavenging assay

The ability of the extracts to scavenge hydrogen peroxide (H_2O_2) was determined according to the method of Nabavi.[14] A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer, pH 7.4. The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Extracts (25-125 µg/ml) in distilled water were added to a hydrogen peroxide solution at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as the standard.

Total antioxidant capacity assay

The total antioxidant capacity assay was determined as described by Prieto et al.[15] Different concentrations of the extract (25-125µg/ml) were taken and added 1.0 ml of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After cooling to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm

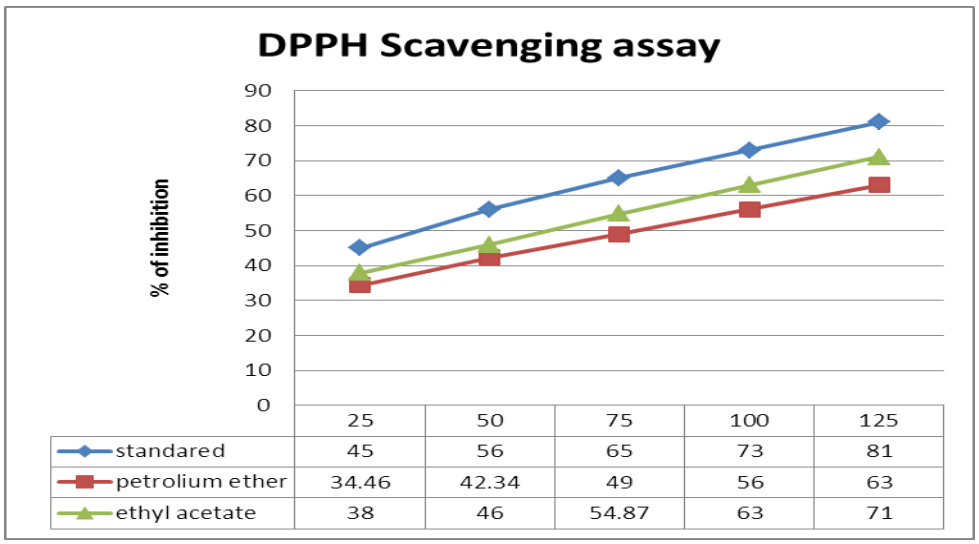
against a blank. Ascorbic acid was used as standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid.

Determination of Total Phenol

The content of total phenolic compounds in petroleum ether and ethyl acetate extracts of *C.rotundus* were determined by Folin–Ciocalteu Reagent. 1.0 ml of each plant extracts (10 µg/µl)or standard of different concentrations (250,200,150,100 and 50)µg/ml solution were taken in different test tubes. 5 ml of Folin –ciocalteu (Diluted 10 fold) and 4 ml of Sodium carbonate reagent solutions were added to the test tubes. The test tubes were incubated for 30 minutes at 200^0C to complete the reaction. The absorbance of the solutions was measured at 765 nm using a spectrophotometer against blank. Total content of phenolic compounds in plant petroleum ether and ethyl acetate extracts are expressed in terms of Gallic acid equivalent (mg/g of dry mass), which is a common reference compound.

RESULT AND DISCUSSION

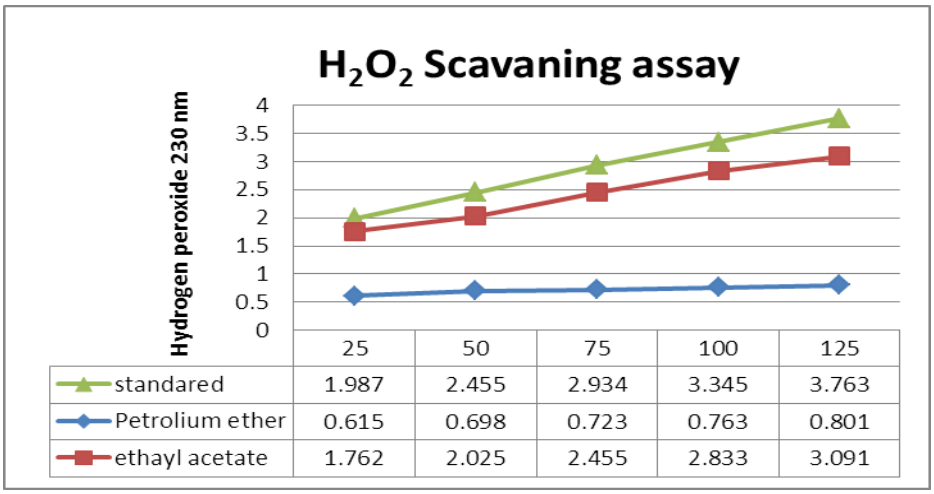
The antioxidant potential of petroleum ether and ethyl acetate extract of rhizome of the *C.rotundus* was studied by the DPPH assay, total antioxidant assay, hydrogen peroxide scavaning assay and reducing power assay at different concentrations, ranging from 25 to 125 µg/ml. The DPPH antioxidant assay based on the ability of DPPH a stable free radical, to decolorize in the presence of antioxidants. The percentage of inhibition were found to be higher in ethyl acetate extract than petroleum ether extract (Fig: 1). The IC50 value of ethyl acetate extract was found to be 62.5 µg/ml and petroleum ether 75 µg/ml.



	25	50	75	100	125
standared	45	56	65	73	81
petrolium ether	34.46	42.34	49	56	63
ethyl acetate	38	46	54.87	63	71

Figure 1: DPPH radical scavenging activity of ethyl acetate and petroleum ether extract of rhizome of *Cyperus rotundus.*

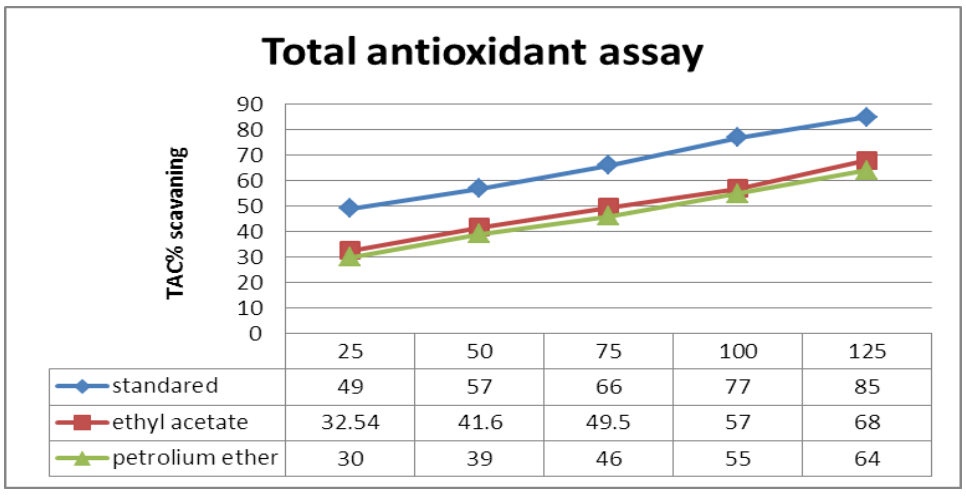
The hydrogen peroxide scavenging assay showed a marked increase with the increase in concentration of rhizome of *Cyperus rotundus.* The scavenging ability of the extract and standard are shown in(Fig:2) These result showed that ethyl acetate and petroleum ether extract had a potent hydrogen peroxide scavenging activity.



	25	50	75	100	125
standared	1.987	2.455	2.934	3.345	3.763
Petrolium ether	0.615	0.698	0.723	0.763	0.801
ethayl acetate	1.762	2.025	2.455	2.833	3.091

Figure 2: H_2O_2 Scavenging Results of ethyl acetate and petroleum ether extract of rhizome of *Cyperus rotundus.*

The total antioxidant capacity of the both extracts was determined by phosphomolybdenum with using ascorbic acid as standard. In phosphomolybdenum assay, the con concentration range from 25-125 µg/mL,the ethyl acetate extract showed higher dose dependent reducing activity than petroleum ether extract(Fig:3). The result obtained was confirmed by the high potency of the ethyl acetate extract towards the transition metal ions.



	25	50	75	100	125
standared	49	57	66	77	85
ethyl acetate	32.54	41.6	49.5	57	68
petrolium ether	30	39	46	55	64

Figure 3: Total antioxidant activity of ethyl acetate and petroleum ether extract of rhizome of *Cyperus rotundus.*

The reducing power assay was found to be 0.86 at 125 µg/mL in ethyl acetate and petroleum ether extract was 0.234 at 125 µg/mL. This result showed that the extract of ethyl acetate and petroleum ether of *Cyperus rotundus* showed excellent reducing power activity (Fig: 4). In the concentration range from 25-125µg/mL, the ethyl acetate extract showed strong dose dependent reducing activity. The results obtained were confirmed by the high potency of the ethyl acetate extract towards the transition metal ions.

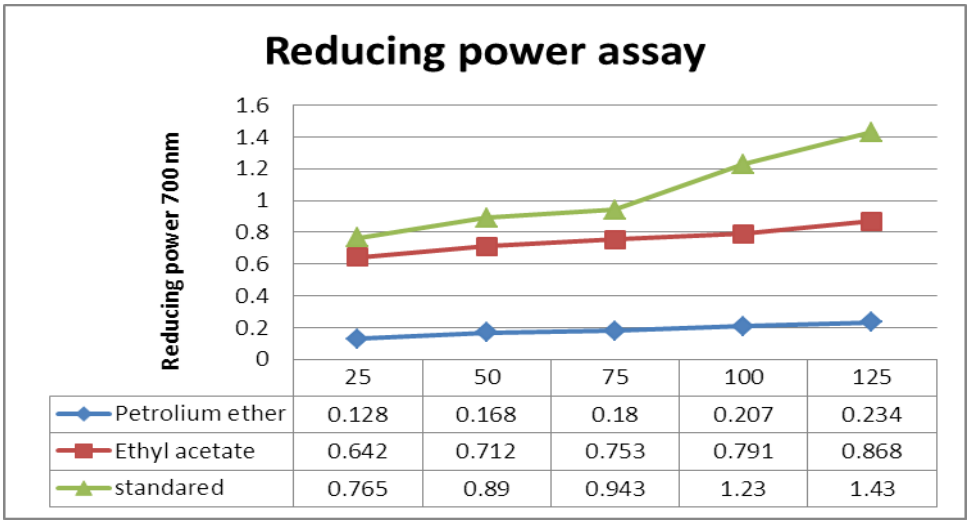


Figure 4: Reducing power assay of ethyl acetate and petroleum ether extract of rhizome of *Cyperus rotundus.*

Total phenolic content was estimated by using Folin Ciocalteu reagent. Phenolic compounds are a class of antioxidant agents which acts as free radicals terminators. The total phenolic content results were expressed as mg Gallic acid equivalent as this compound represents the most simple form of phenolic compound. The total phenolic contents of two extracts were calculated using the standard curve of Gallic acid. In comparison with standard Gallic acid, ethyl acetate extract showed highest amount of phenol than petroleum ether extract(Table :1).

Table: 1 Total phenolic content of *Cyperus rotundus* of petroleum ether and ethyl acetate

Rhizome extracts	Total phenol content (mg/g Gallic acid equivalent)
Petroleum ether	7.34±0.026
Ethyl acetate	11.76±0.044

CONCLUSION

In this study it was concluded that the radical scavenging activity of various assay showed that the ethyl acetate extract is better radical scavenger. The result obtained in the present study, indicates the both extract of *Cyperus rotundus* exhibits free radical scavenging, reducing power, total antioxidant activity. Presence of significant quantity of antioxidant as reflected from our studies may prove the pharmacological importance of this plant. Significant correlation between antioxidant and phenol content further indicate that the antioxidant activity is due to polyphenolic component present in this plant.

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