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IN VITRO ANTIOXIDANT ACTIVITY OF *CYPERUS BULBOSUS* VAHL

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ABSTRACT
In the present study the antioxidant potential of the tubers of *Cyperus bulbosus* was assessed. For this purpose the tubers of the plant was extracted by using different extraction solvents and evaluated for their antioxidant activity using different *in vitro* antioxidant assays like DPPH assay, hydroxyl assay, ABTS assay, superoxide assay and reducing power assay. The methanol extract showed high antioxidant activity for all assays. The radical scavenging abilities were found to be dose dependent. IC_{50} values of the methanol extracts of the species studied for DPPH, hydroxyl, ABTS and superoxide radical scavenging activity compared with ascorbic acid and were found to be 38.24 µg/ml, 44.96 µg/ml, 40.26 µg/ml and 41.92 µg/ml respectively. The results indicated that the plant sample studied have antioxidant property and these activities observed could be due to the synergistic effects of phytocompounds present in the plant.

KEYWORDS: Antioxidant activity, C*yperus bulbosus*, tubers.

INTRODUCTION
Plants are the potential source of natural antioxidants. It produces various antioxidative compounds to be counteract with reactive oxygen species (ROS) in order to survive. In recent years much attention has been given to natural antioxidant and their association with health benefits.[1] Last few decades screening for antioxidant property of food and medicinal plants have been carried out increasingly in hope of finding remedy for non communicable diseases (NCDs) and to delay aging.[2] There is also a huge demand for natural antioxidants in food industry for replacing the synthetic preservatives used to prevent fat rancidity or colour loss. Plants have many phytochemicals with various bioactivities, including antioxidant, antiinflamatory and anticancer activities. Various studies had reported that extracts from natural products such as fruits, vegetables and medicinal herbs have positive effects against cancer, compared with chemotherapy or recent hormonal treatments.[3] Therefore, many plants have been screened to identify new and effective antioxidant and anticancer compounds.[4]

The family of the Cyperaceae includes about 123 genera and 6000 species, is abundant in wetlands and in the water edge from Ecuador to the poles.[5] The genus *Cyperus* has a great importance in terms of their uses in traditional medicine due to its broad spectrum of biological activities, from the estrogenic activity of the ethanolic extract of the inflorescences of *C.alopecuroides*[6], analgesic activity of the decotion of the rhizomes of *C.articulatus*[7], hepatoprotective activity of the methanol extract of *C.scariosus*[8] and antioxidant activity of the extracts of *C.rotundus.*[9] There are many studies related to the phytochemical and bioactivity of *C.rotundus*. *Cyperus bulbosus* Vahl is a coastal sedge of this family that has not been investigated. It is widely distributed in tropical Africa, Asia and Australia. In Australia they are called as "Tubers of Nagloo". The plant has a slender, scaly creeping rhizomes, bulbous at the base and arising singly from the tubers which are about 1 – 3 cm long. The tubers are extremely blackish in colour and reddish white inside, with a characteristic odour. The culm grows about 20 – 40 cm tall and leaves are long as culms, 1 – 2 mm wide, midvein prominent on lower surface of leaf. Inflorescence much longer than the leaves, with 2 – 4 bracts consisting of tiny flowers with a red brown husk. The nut is trigonous, obovoid to ellipsoid, about two fifths as long as glume, 1.3 – 1.5 mm long, dark greyish to blackish with a very smooth testa. The tubers being edible and used as food and have no toxic element.[10] Perusal of previous literature revealed that there is no studies related to the phytochemical and bioactive potential of *C.bulbosus.* So the present study was designed to examine the antioxidant and radical scavenging potential of tubers of *Cyperus bulbosus*.

Preparation of powder and extract[11]
The tubers of the plants were shade dried and pulverized to powder in a mechanical grinder. The powder(100g)

was extracted successively with methanol, ethanol, petroleum ether, benzene and ethyl acetate, each 250ml in a soxhlet apparatus[11] for 48 hrs. All the extracts were filtered through Whatman no: 41 filter paper and concentrated in a rotary evaporator. The concentrated extracts were used for *in vitro* antioxidant activity.

DPPH Radical Scavenging[12]

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H. The free radical scavenging activity of all the extracts are evaluated by 1, 1-diphenyl-2- picrylhydrazyl (DPPH). An 0.1 mm solution of DPPH in methanol is prepared, and 1 ml of this solution is added to 3ml of all extracts in methanol at different concentrations (125, 250, 500 and 1000 µg/ml). The mixtures are shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10 UV: Thermo electron Corporation). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging DPPH radical is calculated by using the following formula:

DPPH scavenging effect (% inhibition) = (A0 – A1) x 100/A0

Here, A0 is the absorbance of the control reaction and A1 is the absorbance in presence of all of the extract samples and reference.

All the tests are performed in triplicates and the results are averaged.

Hydroxyl radical scavenging activity[13]

The scavenging capacity for hydroxyl radical is measured according to the standard method. Stock solutions of EDTA (Ethylene diamine tetraacetic acid) (1mM), Fecl3 (10mM), Ascorbic Acid (1mM), H_2O_2 (10mM) and Deoxyribose (10 mM), are prepared in distilled deionized water. The assay was performed by adding 0.1ml EDTA, 0.01ml of FeCl3,1ml H2O2, 0.36mL of deoxyribose, 1.0 ml of the extract of different concentration (50,100,200,400 and 800µg/ml) dissolved in distilled water, 0.33ml of phosphate buffer (50m M, pH 7.4), 0.1ml of ascorbic acid in sequence. The mixture was then incubated at 37ºC for 1 hour. 1.0ml portion of the incubated mixture was mixed with 1.0 mL of 10%TCA and 1.0ml of 0.5% TBA (in 0.025M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532nm. The hydroxyl radical scavenging activity of the extract was reported as % inhibition of deoxyribose degradation was calculated by using the following equation.

Hydroxyl radical scavenging activity = {(A0 – A1)/A0)×100}

Where, A0 is the absorbance of the control reaction, and A1 is the absorbance in presence of all of the extract samples and reference.

All the tests are performed in triplicates and the results are averaged.

Superoxide radical scavenging activity[14]

The superoxide anion radicals are generated in 3.0 ml of Tris – HCl buffer (16 mM, PH 8.0), containing 0.5 ml of Nitro Blue Tetrazolium (NBT) (0.3mM), 0.5 ml NADH (0.936mM) solution, 1.0 ml extract of different concentration (50,100,200,400 & 800µg/ml), and 0.5 ml Tris – HCl buffer (16mM, pH 8.0). The reaction was started by adding 0.5 ml PMS (Phenazine methosulphate) solution (0.12mM) to the mixture, incubated at 25˚C for 5 min and the absorbance was measured at 560 nm against a blank sample, ascorbic acid. The percentage inhibition was calculated by using the following equation.

Superoxide radical scavenging activity= {(A0 – A1)/A0)×100}

Where, A0 is the absorbance of the control reaction, and A1 is the absorbance in presence of all of the extract samples and reference.

The entire test is performed in triplicates and the results are averaged.

Antioxidant activity by radical cation (ABTS +)[15]

ABTS assay is based on the slightly modified method of Huang *et al.,* 2011. ABTS radical cation (ABTS+) was produced by reacting 7mM ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6- sulphonic acid) solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS + solution are diluted with ethanol to an absorbance of 0.70+0.02 at 734 nm. After addition of 100µl of sample or trolox standard to 3.9 ml of diluted ABTS+ solution, absorbance was measured at 734 nm by Genesys 10S UV VIS (Thermo scientific) exactly after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC).

ABTS radical cation activity = {(A0 –A1)/A0)×100}

Where, A0 is the absorbance of the control reaction, and A1 is the absorbance in presence of all of the extract samples and reference.

All the tests are performed in triplicates and the results are averaged.

Reducing Power[16]

The reducing power of the extract is determined by standard methods. 1.0 ml of solution containing 50,100,200,400 and 800µg/ml of extract is mixed with sodium phosphate buffer (5.0 ml, 0.2 M, pH6.6) and potassium ferricyanide (5.0 ml, 1.0%). The mixture was incubated at 50˚C for 20 minutes. Then 5ml of 10% trichloroacetic acid was added and centrifuged at 980 x g (10 minutes at 5˚C) in a refrigerator centrifuge. The upper layer of the solution (5.0 ml) was diluted with 5.0

ml of distilled water and ferric chloride and absorbance read at 700 nm. The experiment was performed thrice and results are averaged.

RESULT AND DISCUSSION

In the present study the antioxidant activity of methanol, ethanol, petroleum ether, benzene and ethyl acetate fraction were assessed using DPPH assay, hydroxyl assay, ABTS assay, superoxide anion radical assay and reducing power assay. IC50 values were shown in the (Table1). There are many different experimental methods to assess free radical scavenging activity. The IC50 value 38.24 µg/ml in methanol solvent and this was higher than that of other solvents. 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.[17] In the methanol, ethanol, petroleum ether, benzene and ethyl acetate extracts of *Cyperus bulbosus* the scavenging effect increased with concentration of the standard and sample. Among the solvent tested, the methanol extract (800 µg/ml) exhibited highest DPPH radical scavenging activity (129.16%) than the standard ascorbic acid whose scavenging effect was 98.36%. DPPH radical is a model of lipophilic radical, and the scavenging activity of the extracts is due to their hydrogen donating nature.[18] Methanolic extract of *Cyperus bulbosus* showed significantly high activity in a dose dependent manner. 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.[19] The methanolic extract exhibited highest hydroxyl scavenging activity which was found to be dose dependent. The dose dependent acitivity is quite common with plant extracts.[20] The antioxidant potential is due to the presence of multiple phytochemical constituents in which some may have opposite effects. The methanolic extracts of the selected plant scavenged hydroxyl radical more 137.11% than other extracts (Table 2).

Table-1 DPPH radical scavenging activity of different solvent extract of tubers of *Cyperus bulbosus.*

Concentration (µg/ml)	Ethanol (%)	Methanol (%)	Petroleum (%)	Benzene (%)	Ethyl Acetate (%)	Standard (Ascorbic acid) (%)
50	28.36±0.16	26.31±0.81	20.31±0.16	18.26±0.18	23.18±0.31	28.13±0.11
100	41.36±0.22	40.26±0.67	36.18±0.24	27.13±0.11	38.13±0.26	40.22±0.26
200	63.28±0.84	66.88±0.31	52.81±0.18	43.86±0.26	65.13±0.13	62.16±0.31
400	82.84±0.91	92.81±0.36	73.16±0.11	70.16±0.21	74.12±0.27	80.26±0.36
800	112.16±0.86	129.16±0.22	66.88±0.31	52.81±0.18	93.18±0.13	98.36±0.22
IC 50	**36.54**	**38.24**	**32.16**	**30.16**	**33.16**	**34.88**

Each value is expressed as percentage of activity mean ± standard deviation (n=3)

Table 2: **Hydroxyl radical scavenging activity of different solvent extract of tubers of *Cyperus bulbosus*.**

Concentration (µg/ml)	Ethanol (%)	Methanol (%)	Petroleum Ether (%)	Benzene (%)	Ethyl Acetate (%)	Standard (Ascorbic acid) (%)
50	29.16±0.76	34.16±0.27	21.88±0.54	20.92±0.26	26.13±0.23	22.66±0.21
100	42.88±0.26	57.13±0.18	41.33±0.16	37.92±0.13	40.16±0.18	37.84±0.16
200	73.91±0.15	78.26±0.13	52.86±0.26	55.16±0.22	71.92±0.22	54.88±0.26
400	92.88±0.13	104.92±0.18	68.13±0.31	76.31±0.18	87.66±0.18	84.13±0.15
800	126.89±0.76	137.11±0.65	81.16±0.36	93.16±0.26	101.31±0.26	102.88±0.16
IC 50	**41.36**	**44.96**	**28.16**	**37.16**	**40.16**	**41.98**

Each value is expressed as percentage of activity mean ± standard deviation (n=3)

ABTS (2,2' – azino – bis (3 – ethylbenzothiazoline – 6 – sulfonic acid) assay is an excellent tool for measuring antioxidant activity of phytochemical products. The reduction capability of ABTS radical was determined by the decrease in its absorbance at 734 nm which is induced by antioxidant. The methanolic extracts of the selected taxon showed potent antioxidant activity. The antioxidant activity was dose dependent and more in methanolic extract (121.66%) (Table 3). Superoxide radical is very harmful to cellular compound and it was considered as a major biological source of reactive oxygen species.[21] All solvent extracts exhibited superoxide scavenging activity but the methanolic extract has effective capacity of scavenging for superoxide radical and it be correlated with flavonoid content and suggesting its antioxidant potential as observed by Saeed *et al.*[22] (Table 4).

Table 3: ABTS radical scavenging activity of different solvent extract of tubers of *Cyperus bulbosus.*

Concentration (µg/ml)	Ethanol (%)	Methanol (%)	Petroleum Ether (%)	Benzene (%)	Ethyl Acetate (%)	Standard (Ascorbic acid) (%)
50	26.93±0.73	31.67±0.13	20.26±0.16	13.84±0.31	21.63±0.22	22.84±0.18
100	42.16±0.92	49.31±0.86	31.67±0.34	23.15±0.26	38.16±0.92	40.65±0.36
200	64.88±0.26	73.18±0.92	48.26±0.13	40.36±0.16	63.66±0.76	61.30±0.26
400	79.31±0.92	92.08±0.36	67.84±0.92	58.27±0.34	82.92±0.13	82.66±0.16
800	101.16±0.36	121.66±0.26	87.33±0.16	78.27±0.73	99.36±0.26	109.22±0.73
IC 50	**36.18**	**40.26**	**30.16**	**26.84**	**34.18**	**38.13**

Each value is expressed as percentage of activity mean ± standard deviation (n=3)

Table 4: Superoxide anion radical scavenging activity of different solvent extract of tubers of *Cyperus bulbosus.*

Concentration (µg/ml)	Ethanol (%)	Methanol (%)	Petroleum Ether(%)	Benzene (%)	Ethyl Acetate (%)	Standard (Ascorbic acid) (%)
50	34.13±0.26	31.65±0.84	25.16±0.18	21.96±0.31	25.84±0.15	24.08±0.18
100	56.18±0.16	48.36±0.16	38.26±0.54	32.16±0.34	39.16±0.24	40.36±0.15
200	74.88±0.93	70.88±0.34	64.88±0.26	51.67±0.16	67.36±0.26	59.16±0.18
400	101.36±0.26	106.54±0.86	79.33±0.86	67.16±0.26	82.16±0.18	87.16±0.36
800	131.66±0.84	138.16±0.83	95.16±0.13	90.16±0.31	101.65±0.24	106.15±0.84
IC 50	**40.13**	**41.92**	**36.18**	**34.28**	**36.16**	**38.16**

Each value is expressed as percentage of activity mean ± standard deviation (n=3)

The reducing power assay indicated the extracts ability to donate electron to react with free radicals and convert them into more stable metabolites and terminate the radical chain reaction[23], so that they can act as primary and secondary antioxidant. Table 5 showed the reducing ability of different extracts of *Cyperus bulbosus* compared to ascorbic acid. The results clearly indicated that the reducing power of the selected plant increased in a dose dependent manner. Among extracts tested methanol extract exhibited the higher reducing activity (0.543 %).

Table 5: Reducing power (OD) of different solvent extract of tubers of *Cyperus bulbosus.*

Concentration (µg/ml)	Ethanol	Methanol	Petroleum Ether	Benzene	Ethyl Acetate	Standard (Ascorbic acid)
50	0.349±0.026	0.319±0.026	0.286±0.020	0.236±0.037	0.301±0.026	0.301±0.041
100	0.396±0.018	0.340±0.013	0.306±0.092	0.284±0.016	0.348±0.016	0.336±0.026
200	0.446±0.025	0.403±0.081	0.358±0.026	0.326±0.037	0.390±0.022	0.367±0.081
400	0.498±0.021	0.489±0.011	0.391±0.016	0.368±0.084	0.434±0.084	0.391±0.065
800	0.554±0.026	0.543±0.086	0.426±0.093	0.391±0.086	0.488±0.056	0.443±0.026

Each value is expressed as percentage of activity mean ± standard deviation (n=3)

CONCLUSION

Based on the results obtained it is suggested that the tubers of *Cyperus bulbosus* can be used as potent natural antioxidant which may be helpful to prevent various degenerative diseases. Detailed *in vitro* experiments may help to prove above results, which are in progress.

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