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Investigation on the Antioxidant Activity of Dheela Grass (*Cyperus rotundus*)

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Abstract: In the present study antioxidant activity of the plant was assessed. For this purpose *Cyperus rotundus* was extracted by using different extraction solvents and evaluated for their antioxidant activity using different *in vitro* antioxidant assays. Total flavonoids contents in methanol extracts of *Cyperus rotundus* (8.15-18.25 mg CE/g of dry matter) were higher as compared to ethanol extracts (6.44-13.77 mg CE/g of dry matter). Total phenolic contents in methanol extracts of *Cyperus rotundus* (27.40-37.85 mg GAE/g of dry matter) were also higher as compared to ethanol extracts (25.21-30.23 mg GAE/g of dry matter). Percent inhibition of linoleic acid system in methanol extracts of *Cyperus rotundus* (32.50-48.17%) was also higher as compared to ethanol extracts (25.20-45.53%). DPPH free radical scavenging capacity in methanol extracts of *Cyperus rotundus* (51.50-61.73%) were also higher as compared to ethanol extracts (38.37-47.86%). Reducing power in methanol extracts of *Cyperus rotundus* (0.754-1.112) were also higher as compared to ethanol extracts (0.711-0.837) at concentration of 2.5-10.0 mg/mL. We determine from above results that methanol extracts of *Cyperus rotundus* have higher antioxidant activity as compare to ethanol extracts. The data thus obtained was further analyzed by using t-test. The present investigation might lead to the exploitation of *Cyperus rotundus* extracts as a source of antioxidant.

Key words: *Cyperus rotundu* · Antioxidant activity · Phenols · Flavonoids

INTRODUTION

Plants are potential sources of natural antioxidants. It produces various antioxidative compounds to counteract reactive oxygen species (ROS) in order to survive. In recent years much attention has been devoted to natural antioxidant and their association with health benefits [1]. The screening studies for antioxidant properties of medicinal and food plants have been performed increasingly for the last few decades in hope of finding an efficient remedy for several diseases of the daya and means to delay aging symptoms [2].

There is also a huge demand for natural antioxidants in food industry, for replacing the synthetic preservatives used to prevent fat rancidity or color loss. Plants have many phytochemicals with various bioactivities, including antioxidant, anti-inflammatory and anticancer activities. Some studies have 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 examined to identify new and effective antioxidant and anticancer compounds [4].

Cyperus rotundus, vernacularly called "Dheela grass" is a medicinal plant belonging to plant family of *Cyperaceae* and used as home remedy against spasms, stomach disorders and irritation of bowel. This plant is used for treating fevers, digestive system disorders, dysmenorrhea and other maladies. Arabs of the Levant traditionally used roasted tubers to treat wounds, bruises and carbuncles, etc. And modern alternative medicine recommends using this plant to treat nausea, fever and inflammation; for pain reduction; for muscle relaxation and many other disorders [1]. In addition, the tubers are an important nutritional source of minerals and trace elements for migrating birds such as cranes. Although a lot of research work on various medicinal attributes of *Cyperus rotundus* has been conducted. However, little is known about the antioxidant activity of the said plant. Therefore, present research has been designed to investigate first time the antioxidant potential of this plant.

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MATERIALS AND METHODS

Collection of Samples: Fresh samples of roots, leaves and stems of dheela grass (*Cyperus rotundus*) were collected from the vicinity of University of Agriculture, Faisalabad, Pakistan. The samples were further identified and authenticated from Department of Botany, University of Agriculture, Faisalabad, Pakistan.

Pretreatment of Samples: The roots, leaves and stems were separated using sharp steel knife. Samples were dried and ground into fine powder and were subjected to extraction procedure.

Extraction: Cyperus rotundus sample (10g) was extracted separately with 100 mL of 80% methanol (methanol:water, 80:20 v/v) and 100 mL of 80% ethanol (ethanol:water, 80:20 v/v) and shaked for 24 hours at room temperature in an orbital shaker (Gallenkamp, UK). All extracts were separated from the residues by filtering through Whattman No. 1 filter paper. The residues were extracted twice with the same manner and extracts combined. The combined extracts were concentrated and freed of solvent under reduced pressure at 45°C, using a rotary evaporator. The dried, crude concentrated extracts was weighed to calculate the yield and stored in a refrigerator (- 4°C), until used for further analysis.

Determination of Total Phenolic Contents (TPC): Amount of TPC was assessed using Folin-Ciocalteu reagent [5]. Briefly, 50 mg of dry mass of *Cyperus rotundus* extract was mixed with 0.5 mL of Folin-Ciocalteu reagent and 7.5 mL deionized water. The mixture was kept at room temperature for 10 min and then 1.5 mL of 20% $NaCO_3$ (w/v) added. The mixture was heated on a water bath at 40°C for 20 min and then cooled in an ice bath. The absorbance was measured at 755 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). Amounts of TPC were calculated using gallic acid calibration curve within range of 10-50 ppm. The results were expressed as gallic acid equivalents (GAE) mg/100g of dry plant matter. The results are reported on dry weight basis.

Determination of DPPH Free Radical Scavenging Assay: Free radical scavenging activity of *Cyperus rotundus* extracts was assessed using procedure described by [6]. The samples (from 0.2 to 500µg/mL) were mixed with 1 mL of 90 µM DPPH solution and filled up with 95% methanol, to a final volume of 4 mL. The absorbance of the resulting solutions and the blank were recorded after 1h at room temperature. Butylated hydroxytoluene (BHT) was used as a positive control. For each sample, three replicates were recorded. The disappearance of DPPH was read spectrophotometrically at 515nm using spectrophotometer. (U-2001, Hitachi Instruments Inc., Tokyo, Japan). Inhibition of free radical by DPPH in percent (%) was calculated in the following way:

$$I\% = 100 - (A_b - A_s) / A_b$$

Where A_b is the absorbance of the control reaction mixture excluding the test compounds and A_s is the absorbance of the test compounds.

Determination of Reducing Power: The reducing power of the extracts was determined according to the procedure described by [7]. 1.0 mL of solution containing 2.5, 5.0, 7.5 and 10.0 mg of extract was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 mL, 1.0%); the mixture was incubated at 50°C for 20 min. Then 5 mL of 10% trichloroacetic acid was added and centrifuged at 5°C for 10 min in a refrigerated centrifuge. The upper layer of the solution (5.0 mL) was diluted with 5.0 mL of distilled water and ferric chloride (1.0 mL, 0.1%) and absorbance read at 700 nm. The experiment was performed thrice and results were averaged.

Determination of Total Flavonoid Contents (TFC): Amount of TFC was determined following the procedure described by [8]. 1 mL of aqueous extract containing 0.1 g/mL of dry matter was placed in a 10 mL volumetric flask, then 5 mL of distilled water added followed by 0.3 mL of 5% $NaNO_2$. After 5 min, 0.6 mL of 10% $AlCl_3$ was added. After another 5 min 2 mL of 1M NaOH was added and volume made up with distilled water. The solution was mixed and absorbance measured at 510 nm. TF amounts were expressed as catechin equivalents per dry matter. All samples were analyzed thrice and results averaged.

Determination of Antioxidant Activity in Linoleic Acid System: The antioxidant activity of *Cyperus rotundus* extracts was also determined by measuring%age of oxidation of linoleic acid system [6]. Extracts, 5 mg of dry matter, were added to a solution of linoleic acid (0.13 mL), 99.8% ethanol (10 mL) and 10 mL of 0.2 M sodium phosphate buffer (pH 6.6). The mixture was made up to 25 mL with distilled water and incubated at 40°C up to 72 h. Extent of oxidation was measured by peroxide value applying thiocyanate method as described by [7]. Briefly,

10 mL of ethanol (75% v/v), 0.2 mL of aqueous solution of ammonium thiocyanate (30% w/v), 0.2 mL of sample solution and 0.2 mL of ferrous chloride ($FeCl_2$) solution (20 *m*M in 3.5% HCl; v/v) added sequentially. After 3 min of stirring, the absorption was measured at 500 nm using a Spectrophotometer. A control contained all reagents with exception of extracts.

Statistical Analysis: Three samples of each part of *Cyperus rotundus* were taken. Each sample was analyzed individually in triplicate and data are reported as mean (n = 3×3)±SD (n = 3×3). And t-test was performed by using Minitab 2000 Version 13.2 statistical software (Minitab Inc.USA).

RESULTS AND DISCUSSION

Percent Yield of Extracts: In the present investigation to get the yield of extract of stem, leaves and roots of *Cyperus rotundus*, aqueous methanol (80:20) and aqueous ethanol (80:20) were used as solvent. The maximum yield (13.4%) was achieved with 80% methanol from leaves of *Cyperus rotundus,* followed by methanolic extract of roots (11.4%), methanolic extract of stem (10.4%), ethanolic extract of leaves (9.6%), ethanolic extract of roots (9.5%) and ethanolic extract of stem (7.3%). This is in agreement with the findings of [9] who reported that methanol and hot water are more efficient to extract antioxidant compounds from *Phellinus baumii*.

Total Phenolics Contents (TPC): The TPC of different part of *Cyperus rotundus*, using two solvent systems: methanol and ethanol is shown in Table 2. Among the different plant materials, methanolic extract of *Cyperus rotundus* roots offered the highest TPC (37.86 mg GAE /g of dry weight), followed by methanolic extract of leaves (34.29mg GAE/g of dry weight), ethanolic extract of roots (30.23 mg GAE /g of dry weight), ethanolic extract of leaves (29.92 mg GAE/g of dry weight), methanolic extract of stem (27.45 mg GAE /g of dry weight) and ethanolic extract of stem (27.21 mg GAE /g of dry weight).

Overall result showed that higher amount of TPC was found in roots followed by leaves and stem. These results are in good agreement with the previous report of [10] who denoted greater TPC in leaves as compared to stem. Antioxidant activities appeared to depend on their content of total phenolic compounds, which contained greater amount in the roots, was more active in the methanolic extract [11]. Achakzai *et al*., [12] also revealed that old leaves contained high level of TPC as compared to old stem, while young leaves and stem showed mix trend towards the TPC. Kayani *et al*., [13] further explained that out of 37 plant species, the TPC of shoot (62-524µg g^{-1}) were comparatively greater that the root (37-112µg g^{-1}) of the same plant species. They also noted that phenolic contents were present in root of only 7 plant species.

Table 1: Percent yield of extracts of *Cyperus rotundus* (g/100g of dry weight)

	Cyperus rotundus		
Solvent	Roots	Leaves	Stem
80% methanol	11.4±0.35	13.4±0.44	10.4±0.31
80% ethanol	9.5± 0.32	9.6±0.38	7.3±0.43

Table 2: Total phenolic contents of extracts of *Cyperus rotundus* (mg GAE/g of dry weight)

	Cyperus rotundus		
Solvent	Roots	Leaves	Stem
80% methanol	37.86±0.73	34.29±0.67	27.40±0.81
80% ethanol	30.23±0.66	29.92±0.47	25.21±0.66

Table 3: Total flavonoids contents of extracts of *Cyperus rotundus*(mg CE/g of dry weight)

	Cyperus rotundus		
Solvent	Roots	Leaves	Stem
80% methanol	18.25±0.62	13.69±0.56	8.15±0.72
80% ethanol	13.77±0.48	11.71±0.68	6.44±0.63

Table 4: Percent DPPH free radical scavenging activity of extracts of *Cyperus rotundus*

	Cyperus rotundus		
Solvent	Roots	Leaves	Stem
80% methanol	61.73±0.63	56.71±0.59	51.50±0.48
80% ethanol	47.86±0.53	43.98±0.61	38.37±0.46

Table 5: Percent inhibition of linoleic acid lipid peroxidation of extracts of*Cyperu rotundus*

	Cyperus rotundus		
Solvent	Roots	Leaves	Stem
80% methanol	48.17±0.48	39.02±0.64	32.52±0.46
80% ethanol	45.53±0.71	34.96±0.59	25.20±0.57

Table 5(a): Reducing power of ethanolic extracts of *Cyperus rotundus*

	Concentration (mg/mL)							
	Ethanol				Methanol			
Sample	2. 5	5. 0	7. 5	10. 0	2. 5	5. 0	7. 5	10. 0
Roots	0.805±0.38	0.821±0.65	0.837±0.46	0.851±0.57	0.856±0.43	0.887±0.52	0.899±0.34	1.112±0.54
Leaves	0.728±0.67	0.745±0.54	0.767±0.71	0.782±0.63	0.805±0.51	0.826±0.45	0.846±0.43	0.865±0.39
Stem	0.711±0.66	0.728±0.49	0.749±0.44	0.764±0.56	0.754±0.42	0.777±0.54	0.793±0.38	0.816±0.63

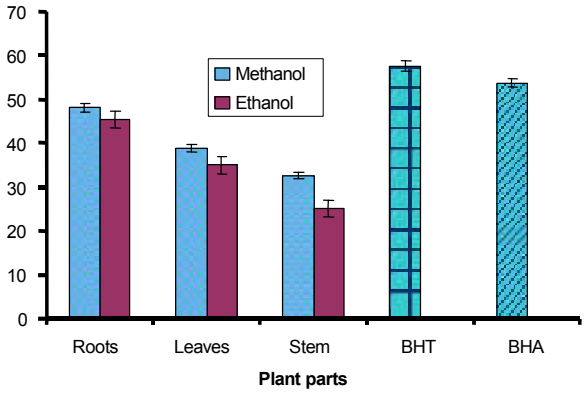

Fig. 1: Maximum percent inhibition of lipid peroxidation activity

Total Flavonoids Contents (TFC): The Total flavonoids contents of dry mass of *Cyperus rotundus* using two solvents, ranged from 6.24-18.26 mg CE /g of dry weight. However, TFC of methanloic extracts was higher as compare to ethanol extracts. Among different organ materials, methanolic extract of roots offered the highest TFC (18.26 mg CE/g of dry weight) and minimum was offered by ethanolic extract of stem (6.44 mg CE/g of dry weight).

In our investigation total flavonoids were higher than [14], who reported the total flavonoids in different parts of *Z. officinale* (1.3 - 7.05). The reason for the higher value of TFC may be due to the fact that phenolics and flavonoids were often extracted extracts of different parts of plant materials. And higher TFC in the roots were due to the presence of polyphenolic compounds [15].

% DPPH Free Radical Scavenging Activity: In the present study the maximum% DPPH free radical scavenging activity was found in methanolic extract of roots (61.30%), followed by methanolic extract of leaves (56.71%), methanolic extract of stem (51.50%), ethanolic extract of roots (47.86%), ethanolic extract of leaves (43.98%) and ethanolic extract of stem (38.37%).

The present data was compared with that of the synthetic antioxidant BHT (reference compound), which exhibited DPPH free radical scavenging at a level of 71.3%. Our findings were also comparable with the results provided by [16] who reported that% DPPH free radical scavenging activity of methanolic extract of roots of *D. hamiltoni was* 69.77. Our results are slightly greater than selected Indian brown seaweeds (*Sargassum marginatum, Padina tetrastomatica and Turbinaria conoides*) that were reported by [17]. The results of the present study indicated that the methanol extract reduces the radical to the corresponding hydrazine when it reacts with the hydrogen ions released from the samples which contain antioxidant principles [18].

Percent Inhibition of Linoleic Acid Lipid Peroxidation: In the present investigation of *Cyperus rotundus,* the higher% inhibition of linoleic acid lipid peroxidation was achieved by methanol extracts as compared to ethanol extracts. Further more the maximum inhibition was noted by methanolic extract of roots (48.17%), followed by ethanolic extract of roots (45.53%), methanolic extract of leaves (39.02%), ethanolic extract of leaves (34.96%), methanolic extract of stem (32.52%) and ethanolic extract of stem (25.2%). The present data was compared with that of the synthetic antioxidant BHT (reference compound), which exhibited inhibition of linoleic acid oxidation at a level of 57.67% and also compared with that of the BHA which exhibited inhibition of linoleic acid oxidation at a level of 53.76%. The maximum% inhibition of linoleic acid lipid peroxidation achieved from roots of *Cyperus rotundus* may be due to the reduction of hydroperoxides, inactivation of free radicals, chelation of metal ions or combinations thereof.

Reducing Power: The reducing power of methanol extracts and ethanol extracts of *Cyperus rotundus* is shown in Table 5. The reducing power of different parts i.e., leaves, roots and stem of methanol extracts of

Cyperus rotundus was found to be in the range of 0.754-1.112. Whereas the ethanol extracts exhibited in the range of 0.711-0.851 at the concentration of 2.5-10 mg/mL. The higher antioxidant activity was achieved at the concentraction of 10 mg/mL. The result of reducing power increased in the following order:- methanolic extract of roots > methanolic extract of leaves > methanolic extract of stem > ethanolic extract of roots > ethanolic extract of leaves > ethanolic extract of stem.

Our results are comparable with the results reported by [19] that the reducing ability of the extracts increased with the concentration. Our results are also comparable with the results reported by [20] that the methanolic extract has the maximum value of reducing power than ethanolic extracts. In our study, methanolic extract of sample showed much higher activity (0.805-1.112) as compared to ethanolic extract (0.711-0.816) that were comparable with [21], who the reported that the reducing power of methanolic extract of 18 different species of ginger ranged from 0.340 to 1.600 nm.

MeOH extract had higher reducing activity and this fact might be associated with the relationship between the antioxidant activity and the reducing power of extracts [22].

CONCLUSION

The results obtained in the present study indicated that methanol extracts exhibit a higher level of antioxidant activity as compare to the ethanol extracts. It is further observed that methanol extract of roots of *Cyperus rotundus* exhibited the maximum antioxidant activity. The findings of the present study suggest that *C. rotundus* could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases.

REFERENCES

1. Talukdar, A.D., R.G. Tarafdar, M.D. Choudhury, D. Nath and S. Choudhury, 2011. A Review on Pteridophyte Antioxidants and their Potential Role in Discovery of New Drugs. Sci. and Tech., 7(I): 151-155.
2. Halliwell, B., 2008. Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and *in vivo* studies? Arc. Biochem. Biophy, 476: 107-112.
3. Wu, J., Y. Wu and B.B. Yang, 2002. Anticancer activity of Hemsleya amabilis extract. Life Sci., 71: 2161-2170.
4. Swamy, S.M.K. and B.K.H. Tan, 2000. Cytotoxic and immunopotentiating effects of ethanolic extract of Nigella sativa L. seeds. Ethnopharmacology, 70: 1-7.
5. Chaovanalikit, A. and R.E. Wrolstad, 2004. Total anthocyanins and total phenolics of fresh and processed cherries and their antioxidant properties. J. Food Chem. and Toxic., 69: 67-72.
6. Iqbal, S., M.I. Bhanger and F. Anwar, 2005. Antioxidant properties and components of some commercially available verities of rice bran in Pakistan. J. Food Chem., 93: 265-272.
7. Yen, G.C., P.D. Duh and D.Y. Chuang, 2000. Antioxidant activity of anthraquinones and anthrone. Food Chemistry, 70: 307-315.
8. Dewanto, X., K.K. Adom and R.H. Liu, 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Agri. and Food Chem., 50: 3010-3014.
9. Shon, M.Y., S.D. Choi, G.G. Kohng, S.H. Nam and N.J. Sung, 2004. Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate extracts from white, yellow and red onion. Food and Chemical Tox., 42: 659-666.
10. Ismail, H.I., K.W. Chan, A.A. Mariod and M. Ismail, 2010. Phenolic content and antioxidant activity of cantaloupe (*cucumis melo*) methanolic extracts. J. Food Chem., 119: 643-647.
11. Jakmatakul, R., R. Suttisri and P. Tengamnuay, 2009. Evaluation of antityrosinase and antioxidant activities of *Raphanus sativus* root: comparison between freeze-dried juice and methanolic extract. Thai J. Pharma. Sci., 33: 22-30.
12. Achakzai, A.K.K., P. Achakzai, A. Masood, S.A. Kayani and R.B. Tareen, 2009. Response of plant parts and age on the distribution of secondary metabolites on plants found in Quetta. Pak. J. Bot., 41(5): 2129-2135.
13. Kayani, S.A., A. Masood, A.K.K. Achakzai and S. Anbreen, 2007. Distribution of secondary metabolites in plants of Quetta-Balochistan. Pak. J. Bot., 39(4): 1173-1179.
14. Ghasemzadeh, A., H.Z.E. Jaafar and A. Rahmat, 2011. Effects of solvent type on phenolics and flavonoids content and antioxidant activities in two varieties of young ginger (*Zingiber officinale* Roscoe) extracts. Medicinal Plants Research, 5(7): 1147-1154.

15. Sasikumar, J.M., U. Jinu and R. Shamna, 2009. Antioxidant Activity and HPTLC Analysis of *Pandanus odoratissimus* L. Root. Eu. J. Bio. Sci., 1(2): 17-22.
16. Srivastava, A., S.R. Harish and T. Shivanandappa, 2006. Antioxidant activity of the roots of *Decalepis hamiltonii* (Wight and Arn.). Food Sci. and Tech., 39: 1059-1065.
17. Chandini, S.K., P. Ganesan and N. Bhaskar, 2008. *In vitro* antioxidant activities of three selected brown seaweeds of India. J. Food Chem., 107: 707-713.
18. Sanchez, M.C., 2002. Review: Methods used to evaluate the free radical scavenging activity in foods and biological systems. Food Sci. and Tech., 8: 121-137.
19. Premanath, R. and N. Lakshmidevi, 2003. Studies on Anti-oxidant activity of *Tinospora cordifolia* (Miers.) leaves using *in vitro* models. American Sci., 6(10): 739-739.
20. Perez, M.B., N.L. Calderon and C.A. Croci, 2007. Radiation-induced enhancement of antioxidant activity in extracts of rosemary (*Rosmarinus officinalis* L.). J. Food Chem., 104: 585-592.
21. Chen, I., C. Chang, C. Ng, C. Wang, Y. Shyu and T. Chang, 2008. Antioxidant and antimicrobial activity of Zingiberaceae plants in Taiwan. Plant Food for Human Nut.J., 63(1): 15-20.
22. Yin, J., S. Heo and M. Wang, 2008. Antioxidant and antidiabetic activities of extracts from *Cirsium japonicum* roots. Nut. Research and Practice, 2(4): 247-251.