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To Estimate the Amount of Total Phenolics and Antioxidant Activity in Water Extract of Centipedegrass *(Eremochloa Ophiuroides)*

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Abstract

The Poaceae (also called Gramineae or true grasses) are a large and nearly ubiquitous family of monocotyledonous flowering plants. With more than 10,000 domesticated and wild species, the Poaceae represent the fifth-largest plant family, following the Asteraceae, Orchidaceae, Fabaceae, and Rubiaceae. Some of the grass species have been proved to show therapeutic effect and have been effective in treatment of inflammation and sclerosis as they contain bioactive components called antioxidants which delay or prevent the oxidation of cellular substrates. These antioxidants exert their effect by scavenging reactive oxygen species (ROS) or preventing their generation. ROS are usually generated in physiological processes to produce energy and metabolites or to generate defenses against invasive microorganisms but can also cause oxidative damage associated with many degenerative diseases such as cardiovascular disease, cancer and neurodegenerative diseases such as Parkinson's and Alzheimer's diseases. Polyphenols like flavonoids and phenolic acids are one of the most important natural antioxidants present in this grass family. Examples of flavonoids are glycosides of apigenin, luteolin and tricin and examples of phenolic acids are ferulic acid, caffeic acid and p-hydroxybenzoic acid. These compounds show a wide spectrum of chemical and biological activities including radical scavenging activity. This paper will discuss the total content of phenolic compounds present in the sample of Centipedegrass (Eremochloa Ophiuroides) species of Poaceae family grass by Folin-Ciocalteu method. The paper will also highlight the total flavonoid content with the help of aluminium chloride colorimetric method. The results indicated that centipede grass is a good natural source of antioxidant compounds for use in food and pharmaceutical industry.

Abbreviations

TCP	Total phenolic content
TFC	Total flavonoid content
WEC	Water extract of Centipedegrass
GAE	Gallic acid equivalent
RE	Rutin equivalent
FW	Fresh weight.

Introduction

Natural antioxidants, particularly in fruits and vegetables have gained increasing interest among consumers and the scientific community because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer (Renaud *et al.*, 1998; Temple,2000). The defensive effects of natural antioxidants in fruits and vegetables are related to three major groups: vitamins, phenolics, and carotenoids. Ascorbic acid and phenolics are known as hydrophilic antioxidants, while carotenoids are known as lipophilic antioxidants (Halliwell, 1996).

Recent studies on cultured mammalian cells and animals indicate that polyphenolic compounds from numerous fruits and vegetables exert several health-promoting functions, including reducing the risks of cancer and heart and neurodegenerative diseases (Joseph *et al.*, 2005, Vita 2005). Besides that, epidemiological studies also show positive associations between intake of fruits and vegetables and reduced mortality rate from heart diseases, common cancers, and other degenerative diseases (Kaur and Kapoor, 2001; Art and Hollman, 2005; Scalbert *et al.*, 2005). The free-radical scavenging capability and consequent antioxidant properties of the phenolics play an important role in protecting the

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cells and tissues from oxidative stress and other biological effects associated with these chronic diseases (Rimbach *et al.*, 2005).

The contents of phenolic compounds and their antioxidant activity in selected grass species have been poorly investigated. Therefore, testing their antiradical properties is of interest, primarily in order to find new sources of natural antioxidants.

Mechanism: DNA is continuously attacked by reactive species that can affect its structure and function severely. Structural modifications to DNA mainly arise from modifications in its bases that primarily occur due to their exposure to different reactive species. Apart from this, DNA strand break, inter- and intra-strand crosslinks and DNA–protein crosslinks can also affect the structure of DNA significantly. These structural modifications are involved in mutation, cancer and many other diseases. As it has the least oxidation potential among all the DNA bases, guanine is frequently attacked by reactive species, producing a plethora of lethal lesions. DNA damage by reactive species has created profound interest in the medicinal fraternity because of the involvement of reactive species in different pathological conditions such as cancer, aging, neurodegenerative diseases, rheumatoid arthritis, etc. Reactive species such as free radicals, one-electron oxidants, different chemicals, etc., can react with different components of DNA to produce a plethora of DNA lesions. These reactive species can modify bases, induce inter and intra strand crosslinks, promote DNA– protein crosslinks and create strand break.

Material and methods

Sample preparations: 40 gm sample grass was weighed and mascinerated, boiled in distilled water at 60 degree Celsius for half an hour, the residue was filtered and mixed with 400 ml of distilled water.

Determination of total phenolic content: The determination of TPC of the grass extract was performed by using Folin-Ciocalteu reagent (Singleton and Rossi 1965). Briefly, 1ml of extract was prepared with 1.8 ml of Folin-Ciocalteu reagent (10 fold diluted) and kept for 5 min at 25 degree Celsius. Later 1.2 ml of 15% Sodium Carbonate was added to the reaction mixture and kept for 90 min at RT and the absorbance was measured at 765nm. The concentration of the TPC was determined as mg of gallic acid equivalents (GAE) per gm FW.

Determination of total flavonoid content: TFC of grass extract was determined by using the aluminium chloride colorimetric method (Chang et al. 2002). Briefly, 0.5 ml of the extract, 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water were mixed for 5 min by vortexing. Reaction mixture was kept at RT for 30 min and the absorbance was measured at 415 nm. The results were expressed as mg of rutin equivalents (RE) per gm FW.

Determination of reducing power: The reducing power assay can be determined by the method Athukorala et al (2006). 1 ml sample of different concentration were taken. 1ml 0.2M sodium phosphate buffer pH 6.6 was added to each sample. 1ml of 1% potassium ferricyanide was added and incubated at 50^0C for 20 mins. 1ml of 10% TCA (W/V) was added and then the samples were centrifuged at 2000 rpm for 10 mins, 2.5ml of upper layer was taken and mixed with 2.5 ml DW. 0.5 ml of 0.1% fresh ferric chloride was added and then the readings for their optical density were taken at 700 nm.

Results

Total phenolic content: The variation of the total phenolic content over time for various concentrations of WEC sample is presented in Table 1. TPC of the extract was expressed as mg GAE/g FW. Phenolic content GAE is increasing with increasing concentration of WEC sample concentration.

S. No.	Sample conc. (gm/ml)	Phenolic content GAE (gm/gm)
1	.1	2.1
2	.08	1.8
3	.06	1.65
4	.04	1.2

Table 1: Total phenolic content of WEC

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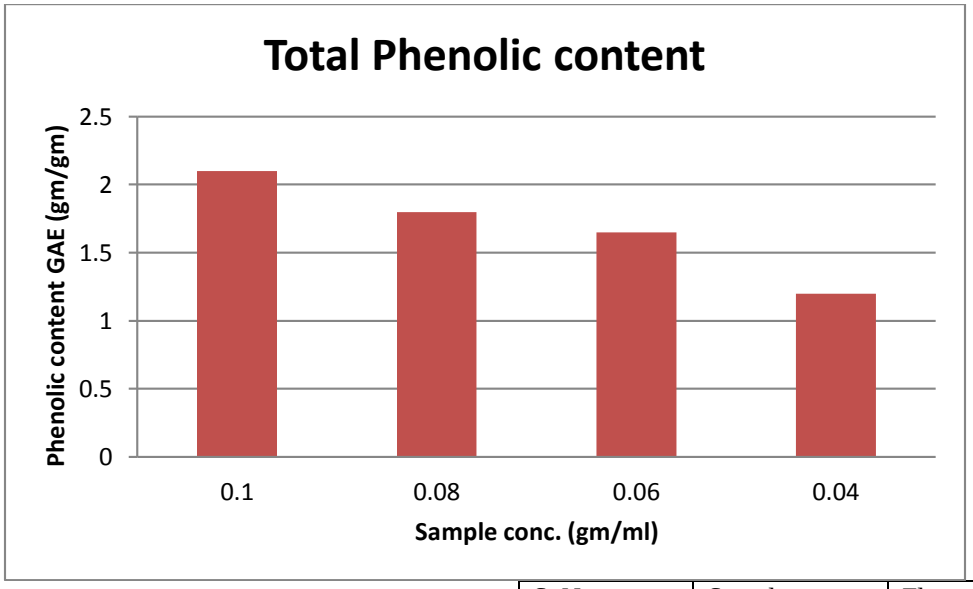
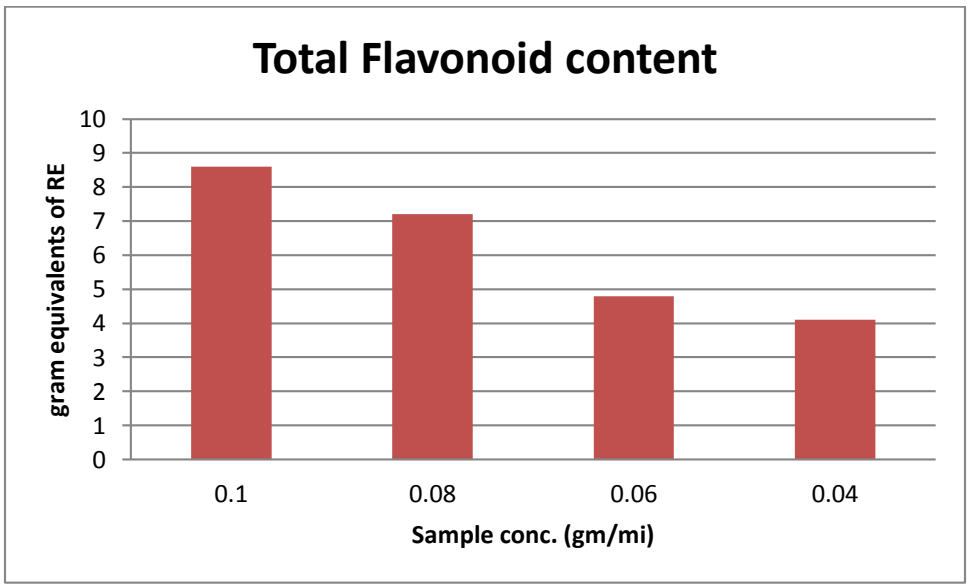


Total flavonoid content: The variation of flavonoid content over time for various concentration of WEC sample is shown in table 2. TPC of the extract was expressed as mg RE/g FW. The TFC in different concentrations of the grass sample varied considerably, and occurred to be maximum at .04 gm/ml concentration of WEC sample. Flavonid content RE is increasing with increasing concentration of WEC sample concentration.

S. No.	Sample conc. (gm/ml)	Flavonoid content RE (gm/gm)
1	.1	8.6
2	.08	7.2
3	.06	4.8
4	.04	4.1

Table 2: Total flavonoid content of WEC



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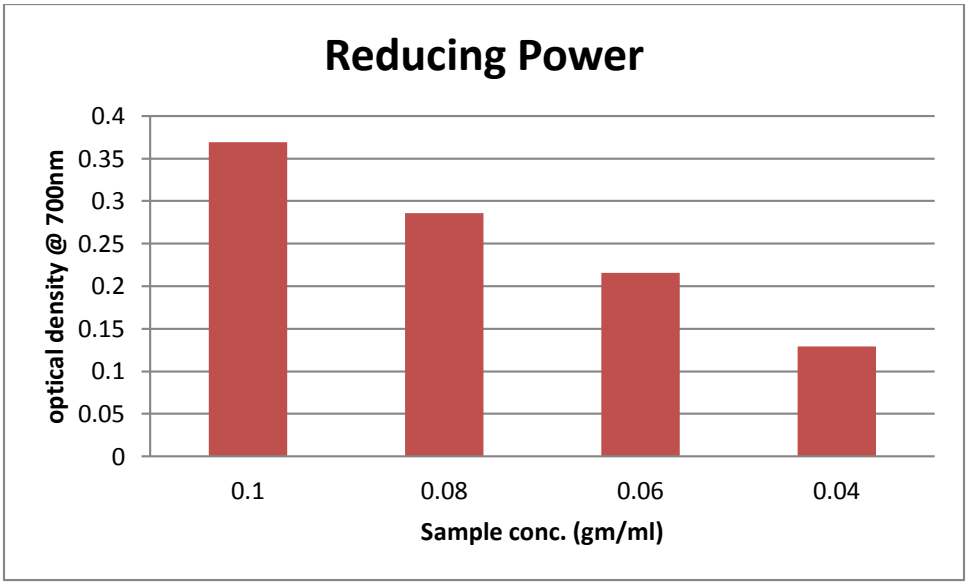
Reducing power assay: The variation in reducing activity for various concentrationes of WEC samples is shown in table 3. Reducing activity increases with increasing concentration of WEC sample.

S. No.	Sample conc.	Reducing activity
1	0.1	0.369± 0.019
2	0.08	0.286± 0.009
3	0.06	0.216±0.012
4	0.04	0.129±0.014

Table 3: Reducing activity of WEC



Discussion

WEC showed high polyphenolic and flavonoid content as well as strong antioxidant potential. The grass extract contains lower amount of phenolics as compared to the flavonoids. The raw material i.e Centipedegrass being inexpensive and easily available should be regarded as potential nutraceutical resource, capable of offering significant nutritional dietary supplements. Also the natural antibiotics in the form of phenols and flavonoids can be easily extracted and thus it offers opportunities to formulate value added products in nutraceutical and food applications to enhance health benefits.

Reference

1. Kirkinezosa IG and Moraesa, CT 2001 Reactive species and mito- chondrial diseases. Sem. Cell Dev. Biol. 12 449–457
2. Jena NR and Mishra PC 2005 Mechanisms of formation of 8- oxoguanine due to reactions of one and two OH radicals and the H_2O_2 molecule with guanine: a quantum computational study. J. Phys. Chem. B 109 14205–14218
3. Jena NR and Mishra PC 2006 Addition and hydrogen abstraction reactions of an OH radical with 8-oxoguanine. Chem. Phys. Lett. 422 417–423
4. Jena NR and Mishra PC 2007 Formation of 8-nitroguanine and 8-oxoguanine due to reaction of peroxynitrite with guanine.J. Comput. Chem. 28 1321–1335
5. Jena NR and Mishra PC 2012 Formation of ring-opened and rearranged products of guanine: mechanisms and biological significance. Free Radical Biol. Med. 53 81–94
6. Jena NR, Kushwaha PS and Mishra PC 2008 Reaction of hypo- chlorous acid with imidazole: formation of 2-chloro and 2-oxoimidazoles. J. Comput. Chem. 29 98–107
7. Bauer GB and Povirk LF 1997 Specificity and kinetics of inter- strand and intrastrand bifunctional alkylation by nitrogen

ISSN: 2277-9655
Scientific Journal Impact Factor: 3.449
(ISRA), Impact Factor: 2.114

mustards at a G-G-C sequence. Nucleic Acid Res. 25 1211–1218

8. Johansen ME, Muller JG, Xu X and Burrows CJ 2005 Oxida- tively induced DNA-protein cross-linking between single stranded binding protein and oligodeoxynucleotides containing, 8-oxo-7,8-dihydro-2'-deoxyguanosine. Biochemistry 44 5660–5671
9. Yermilov V, Yoshie Y, Rubio J and Ohshima H 1996 Effects of carbon dioxide/bicarbonate on induction of DNA single strand breaks and formation of 8-nitroguanine, 8-oxoguanine and base-propenal mediated by peroxynitrite. FEBS Lett. 399 67–70
10. Chang C, Yang M, Wen H, Chern J (2002) Estimation of total flavonoid content in prepolis by two complementary methods. J Food Drug Anal 10:178-182m
11. Chavan J.J, Jagpat U.B, Gaikwad N.B, Dixit G.B, Bapat V.A (October-December 2013) Total phenolics, flavonoids and antioxidant activity of Saptarangi (Salacia chinensis L.) fruit pulp. J. Plant Biochem. Biotechnol. 22(4):409-413