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ANTIOXIDANT ACTIVITY OF SEQUENTIALLY EXTRACTED SOLVENT EXTRACTS OF *CYNODON DACTYLON* GROWING IN COASTAL AREA OF BANGLADESH

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ABSTRACT

The present study was focused on the antioxidant activity of sequentially extracted different solvent extracts of *C. dactylon* growing in coastal area of Bangladesh. Powdered plant materials were continuously extracted with different solvents (petroleum ether, chloroform, methanol and water) and tested for antioxidant activity using reducing power assay, total antioxidant activity and reduction of ferric ions and also the extracts were tested to determine the available phytochemicals. The petroleum ether (PEE) extract showed appreciable antioxidant activity for reduction of ferric ions and methanol extract (ME) showed more effective total antioxidant activity. PEE, chloroform extract (CE) and aqueous extract (AE) also showed the moderate antioxidant activity.

KEYWORDS: *C. Dactylon*, Proximate Analysis, Sequential Extraction, Antioxidants.

INTRODUCTION

The oxidative stress, due to the presence or generation of free radicals, especially reactive oxygen and their activity plays a major role in human diseases. Alzheimer's disease, cancer, diabetes, sclerosis, Parkinson's disease, inflammation, stroke and other heart diseases are major diseases reported due to oxidative stress.[1] To maintain healthy biological system, maintenance of balance between oxidation and antioxidation is important.[2]

The weed *C. dactylon* commonly known as "Durva" in Bangladesh and belongs to the family of Poaceae. It possesses immense medicinal value and may be applied both externally as well as internally.[3] Decoctions of root are used in secondary syphilis and irritation of urinary

organs.[4] The plant possesses antimicrobial and antiviral activity.[5] The aqueous fluid extract of the rhizome is used as anti-inflammatory, diuretic, anti-emetic, purifying agent and also in dysentery.[6]

Medicinal plants are widely used in Bangladesh as alternatives to conventional medicines. As a part of the continuation of our research on bioactivity screening of Bangladeshi medicinal plants[7-11], present study was carried out to assess the antioxidant activity of sequentially extracted different solvent extracts of *C. dactylon* growing in coastal area of Bangladesh to justify its use in traditional treatments.

MATERIALS AND METHODS

Collection and Preparation of the Sample: *C. dactylon* were collected from the campus of Noakhali Science and Technology University, Noakhali, Bangladesh and identified by an expert. The plants were washed with distilled water followed by sun-drying for one week and the dried materials were then oven dried for 24 hours at considerably low temperature not exceeding 50 ^{o}C. The oven dried materials were then ground into coarse powder using high capacity grinding machine and were used for different investigation.

Proximate Analysis: Proximate analysis of a substance constitutes different classes of nutrients present in the samples such as moisture, total ash, acid insoluble ash, water soluble ash, and different solvents soluble extractive values according to the procedure describe in Afrose and Hossain (2015).[7]

Sequential Extraction: The method is based on the extraction of active constituents present in the drug using various solvents ranging from non-polar to polar. The solvents used are petroleum ether, chloroform, methanol and water. In this process, the substance which is soluble in a solvent with particular range of polarity was extracted in the solvent and remaining marc further extracted with next solvent. The powder (200 g) was extracted sequentially for 8 hours in petroleum ether, chloroform and methanol using a Soxhlet apparatus. After methanol extraction, the remaining dried marc was extracted with water to get water extract. For the preparation of aqueous extract, the above dried marc was macerated for 3 days with distilled water and the residue was removed by filtration and filtrate was concentrated to obtain aqueous extract. All the extracts were concentrated with a rotary evaporator and dried using oven dryer at 35-40 °C. Dried extracts were stored for further use.

Preliminary Phytochemical Screening

The preliminary phytochemical screening of the different extracts of studied plant were performed to detect the presence of active chemical constituents e.g. alkaloids, reducing sugar, flavonoids, saponins, and proteins as the methods described in Arefin et al. (2015).[12]

Antioxidant Activity

The antioxidant properties of the examined various extracts were determined through reducing power assay, total antioxidant capacity and reduction of ferric ions.

Reducing Power Assay

The reducing power of the prepared extracts was determined based on the ability to reduce ferric ions to ferrous ions, which was monitored by measuring the formation of Perl's Prussian blue at 700 nm. The extracts of *C. dactylon* (1 mL) at various concentrations (32-512 µg/mL) were mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of potassium ferricyanide (10 mg/mL). The mixture was left for 20 min at 50 °C followed by addition of 2 mL of trichloroacetic acid (100 mg/L). The mixture was centrifuged at 3000 rpm for 10 min and collected the upper layer (supernatant) of the solution. A volume of 0.5 mL from each of the mixture earlier mentioned was mixed with 1 mL of distilled water and 100 µL of 0.1% (w/v) fresh ferric chloride. After 10 min reaction, the absorbance was measured at 700 nm against a blank in a spectrophotometer. Increased absorbance of the reaction mixture indicated the increased reducing power. BHT was used as the reference standard.

Total Antioxidant Capacity

Total antioxidant activities of all extracts were evaluated spectrophotometrically by the phosphomolybdenum method. An aliquot of 100 µL of sample solution was combined in an Eppendorf tube with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated at 50°C for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695 nm against a blank. A typical blank solution contained 1 mL of reagent solution and the appropriate volume of the solvent used for the sample and it was incubated under the same conditions as the rest of the samples. Ascorbic acid (AA) was used as the standard and the total antioxidant capacity was expressed as equivalents of ascorbic acid.

Reduction of Ferric Ions

The reduction of ferric ions by the plant extracts was determined by *o*-phenanthroline method.[13] A reaction mixture containing 1 mL *o*-phenanthroline (5 mg in 10 mL methanol), 2 mL of 0.2 mM ferric chloride and 2 mL of various concentrations (32-512 µg/mL) of the extracts was incubated at ambient temperature for 10 min, then the absorbance was measured at 510 nm. AA and gallic acid (GA) were used as reference standards.

RESULTS AND DISCUSSION

Proximate Analysis

The proximate analysis of *C. dactylon* were performed to evaluate its moisture content, total ash, water soluble, acid insoluble ash content and solvent soluble extractive values. The results of proximate analysis are presented in the Table 1. Proximate analysis is generally performed to evaluate food components, which are important for product development, quality control or regulatory purposes in the food industry, and for purity and quality of crude drug. The moisture and ash contents reflects the mass content of the *C. dactylon*. The low moisture content (8.6%) of *C. dactylon* indicates that it may hinder the growth of microorganisms; therefore, its preservation period will be high. The ash content of 19.2% suggests that *C. dactylon* is comparatively rich in mineral elements and may contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. The extractive values for petroleum ether, chloroform, methanol and water were 1.64%, 2.33%, 4.55% and 7.17% respectively. It is inferable that highest amount of soluble bioactive compounds can be found by using water as solvent in contrast to other studied solvents.

Table. 1: Moisture content, ash value and extractive value of *C. dactylon*.

Moisture content	Ash value			Extractive value			
	Total ash	Acid insoluble ash	Water soluble ash	Petroleum ether	Chloroform	Methanol	Water
8.60%	19.20 %	8.84 %	5.28 %	1.64%	2.33%	4.55%	7.27%

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the different extracts was qualitatively analyzed to observe the presence of secondary metabolites such as alkaloid, reducing sugar, flavonoid, saponin and protein in the *C. dactylon*. Table 2 illustrates the results of preliminary phytochemical screening. ME contains all of the studied secondary metabolites while CE and PEE contains only alkaloids. AE contain only saponin. The phytochemical analysis is a

measure of the bioactive compounds which can be used to treat chronic as well as infectious diseases.

Table. 2: Qualitative chemical analysis of different solvent extracts of *C. dactylon*.

Tests	Extracts			
	PEE	CE	ME	AE
Alkaloids	+	+	+	–
Reducing sugars	–	–	+	–
Flavonoids	–	–	+	–
Saponins	–	–	+	+
Proteins	–	–	+	–
+: present; -: absent.				

Antioxidant activity

Reducing Power Assay: The reducing power abilities (absorbance at 700 nm) of different extracts of *C. dactylon*, and reference, BHT are shown graphically in Figure 1. The absorbance of all extracts and standard is a function of their concentrations, and generally, increases linearly with the increase in concentration. Absorbance by CE was highest compared to all other extracts at near 150 µg/mL concentrations. PEE and AE are almost in different to the absorbance at all concentration. In addition, absorbencies of all extracts were very low compared to standard BHT. The reducing power assay is based on single electron transfer reaction, which is a measure of the antioxidant capacity to reduce ferric ions to ferrous ions in a reaction mixture. The presence of reductants (i.e. antioxidant) causes the conversion of the Fe^{3+}/ ferricyanide complex (Perl's Prussian blue) to the ferrous form, where the intensity is dependent on the concentration of reductants.[14,15]

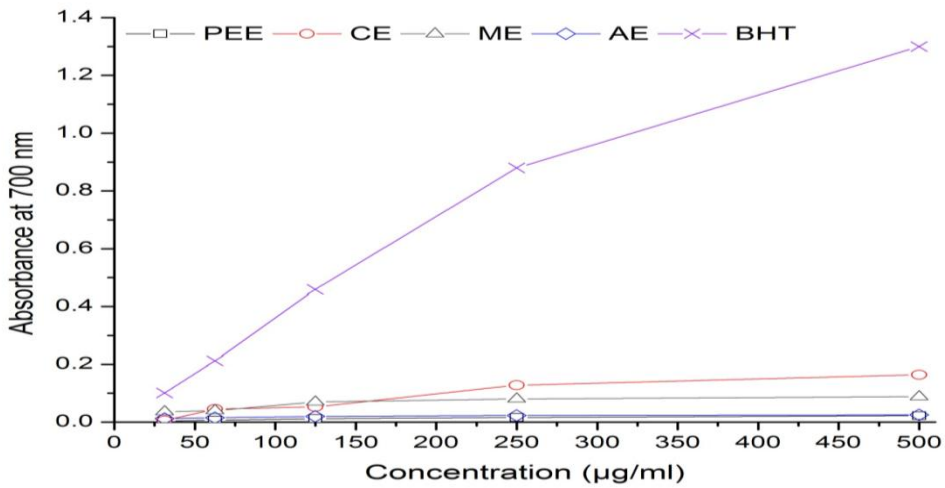

Figure 1: Reducing power assay of different extracts of *C. dactylon* with standard.

Total Antioxidant Capacity: The total antioxidant capacity (TAC) of various solvent extracts of *C. dactylon* were expressed quantitatively in ascorbic acid equivalents per gram of extracts (AAE/g extracts) and shown in Figure 2. ME shown significantly high (233 AAE/g) AAE/g extracts value over other extracts and that of lowest (143 AAE/g) shown by PEE. CE and AE show similar behavior which were in between PEE to ME. Total antioxidant activity mainly concentrates on the thermodynamic conversion and measures the number of electrons or free radicals donated or quenched by a given antioxidant molecule. It is based on the reduction of Mo (VI) to Mo (V) and subsequent formation of green phosphate/Mo (V) complex, which evaluates both water-soluble and fat-soluble antioxidant capacity. The present study reveals that the ME showed highest antioxidant activity in contrast to other extracts indeed. Furthermore, we can presumptively say that the higher TAC of ME may be due to presence of alkaloids, and flavinoids.

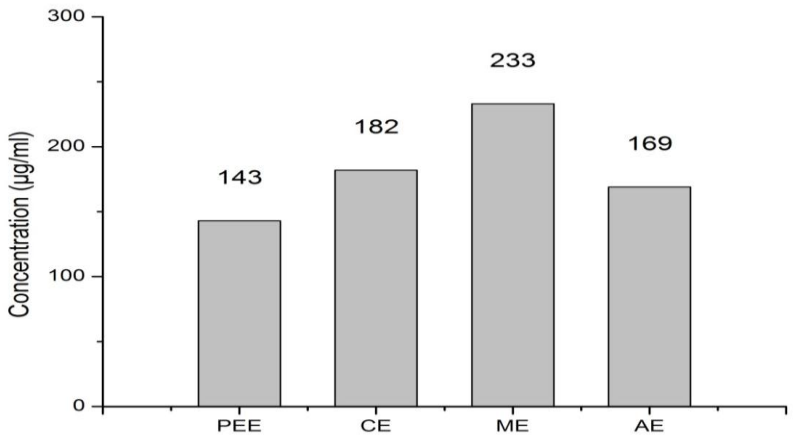

Figure. 2: Total antioxidant activity of extracts of *C. dactylon*.

Reduction of Ferric Ions: *o*-Phenanthroline acts as chelating agent and forms exceptionally stable red colored complex with the reduced Fe^{2+} ions, which intensity was measured by taking absorbance at 510 nm in the visible spectrum. The degree of coloration is a function of reduction potential to reduce Fe^{3+} to Fe^{2+}, which is also a function of absorbance. Therefore, the ability of a compound to transfer electron is a significant indicator of its potential as an antioxidant.[16] The measure of the reduction of Fe^{3+} to Fe^{2+} ions is another method to observe the antioxidant activity, which is depicted in Figure 3. CE, ME and AE showed disproportional absorbance at lower concentration (up to 256 µg/mL), while proportional at higher concentration (after 256 µg/mL). ME showed highest reducing potential at low concentration and showed most at higher concentrations. On the other hand, CE showed reverse behavior to ME. The absorbance by PEE was steadily increased linearly with the

increase of concentrations up to 200 µg/mL and sharply increased afterwards. The absorbance by all extracts showed higher absorbance than standards at all concentrations. It is assumable from the result that electron releasing rate increases steadily at lower concentration and sharply at higher concentration in case of CE.

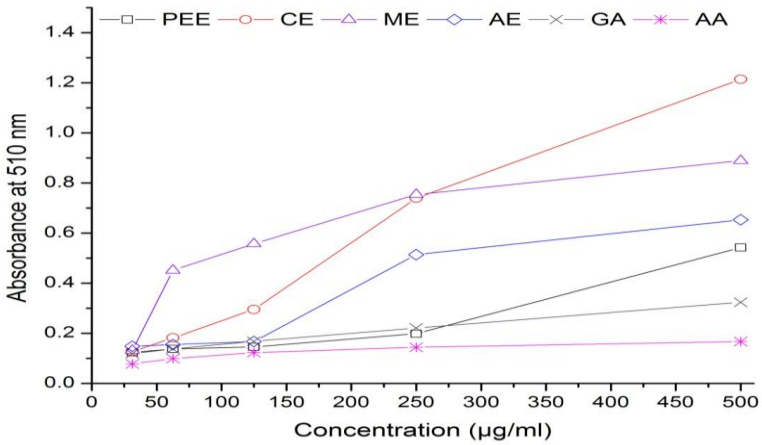

Figure 3: Reduction of ferric ions assay of different extracts of *C. dactylon* with standards.

REFERENCES

1. Ozgen, U., Mavi, A., Terzi, Z., Yildirim, A., Coskun, M., Houghton, P.J. Antioxidant properties of some medicinal Lamiaceae species. Pharmacological Biology., 2006; 44: 107-112.
2. Tiwari, A.K. Imbalance in antioxidant defense and human diseases: Multiple approach of natural antioxidant therapy. Current Science., 2001; 8: 1179–1187.
3. Rita, P., Aninda, M., Animesh, D.K. An updated overview on Cynodon dactylon (L.) Pers. International Journal of Research in Ayurveda and Pharmacy., 2012; 3: 11-14.
4. Auddy, B., Ferreira, M., Blasina, F., Lafon, L., Arredondo, F., Dajas, F., Tripathi, P.C., Seal, T., Mukherjee, B. Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. Journal of Ethnopharmacology., 2003; 84: 131–138.
5. Dhar, M.L., Dhawan, J.T., Melhrotra, M. Screening of Indian plants for biological activity Part I. Indian J. Exp. Biol., 1968; 16: 232–247.
6. Ahmed, S., Reza, M.S., Jabbar, A. Antimicrobial activity of Cynodon dactylon. Fitoterapia., 1994; 65: 463–464.

7. Afroze, F., Hossain, M.T. Proximate analysis, phytochemical screening and antioxidant activity of Psidium guajava leaves growing in coastal area of Bangladesh. World J. Pharm. Pharm. Sci., 2015; 4: 140-151.
8. Dev, U.K., Hossain, M.T., Islam, M.Z. Phytochemical investigation, antioxidant activity and anthelmintic activity of Mikania micrantha leaves. World J. Pharm. Res., 2015; 4: 121-133.
9. Hossain, M.T. Antioxidant, cytotoxicity, membrane stabilization and anthelmintic activity of ethanolic extract of Sarcochlamys pulcherrima leaves. Int. J. Green Herbal Chem., 2015; 4: 274-283.
10. Islam, M.Z., Hossain, M.T., Hossen, F., Akter, M.S., Mokammel, M.A. In-vitro antioxidant and antimicrobial activity of Bougainvillea glabra flower. Res. J. Med. Plant., 2016; 10: 228-236.
11. Islam, M.Z., Sarkar, M., Hossen, F., Mukkharjee, S.K., Akter, M.S., Hossain, M.T. Phytochemical and biological study of Quisqualis indica leaves extracts. Journal of Noakhali Science and Technology University., 2017; 1: 9-17.
12. Arefin, S., Islam, M.T., Hossain, M.T. Proximate analysis, phytochemical screening and antioxidant activity of Tagetes erecta flower growing in coastal area of Bangladesh. Journal of Global Biosciences., 2015; 4: 2060-2066.
13. Akhter, S., Islam, M.T., Hossain, M.T. Proximate analysis, phytochemical screening and antioxidant activity of Tagetes erecta leaves. World J. Pharm. Res., 2015; 4: 1856-1866.
14. Ibrahim, G.I., Jalal, A.F., Ibrahim, B.M. Evaluation of antioxidant activity, phenolic, flavonoid and ascorbic acid contents of three edible plants from Erbil/Kurdistan. Tikrit Journal of Pure Science., 2013; 18: 46-51.
15. Amel, B., Seddik, K., Shtaywy, A., Saliha, D., Mussa, A.Z., Assia, B., Saliha, D., Abderahmane, B., Smain. A. Phytochemical analysis, antioxidant activity and hypotensive effect of algerian azarole (Crataegus azarolus L.) leaves extracts. RJPBCS., 2014; 5: 286-305.
16. Sudha, G., Priya, M.S., Shree, R.I., Vadivukkarasi, S. In vitro free radical scavenging activity of raw pepino fruit (Solanum muricatum). Int. J. Curr. Pharma. Res. 2011; 3: 137-140.