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A Study on the Nutrients and Secondary Metabolites Composition of Two Varieties of Cynodon Available in Bangladesh and their Anti-Oxidant Activities

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ABSTRACT

Cynodon spp. is used as the most important traditional medicine in Bangladesh. The chlorophyll, carotenoids, moisture, ash, sugar, carbohydrate, crude protein, crude fiber, fat, alkaloid, saponin, tannin, steroid, glycoside, flavonoid and cardiac glycoside were analyzed and it was found that CA contained higher amounts of crude protein (11.38%), crude fiber (38.95%) and carotenoid (3.17 mg/g) while CD contained higher amounts of moisture (14.97%), ash (11.63%), total sugar (7.2%) and total fat (4.4%). Of these two samples, macro-minerals content of CA are estimated to be as follows- calcium (4.09 mg/100 g), phosphorus (2.14 mg/100 g), magnesium (2.01 mg/100 g), sodium (9.8 mg/100 g), potassium (11.7 mg/100 g) and chloride (8.3 mg/100 g) and for CD it was calcium (5.11 mg/100 g), phosphorus (2.29 mg/100 g), magnesium (2.65 mg/100 g), sodium (9.73 mg/100 g), potassium (12.1 mg/100 g) and chloride (9.1 mg/100 g), respectively. The phytochemical test showed that some important secondary metabolites like- alkaloids, saponins, tannins, steroids, glycosides, cardiac glycosides and flavonoids are also present. The total phenols and flavonoids have been investigated in different solvent extracts by spectrophotometric methods. The total phenolic contents were determined to be the highest and of same value (1.57 g%) for CD in 50% methanol as well as in 50% ethanol while in water 1000°C and 100% methanol for CA and it was 0.919 g% and 0.91 g%, respectively. Furthermore, all the experimental extracts showed low content of flavonoids and its contents were found to be 0.064 to 0.077 g% for CA and 0.065 to 0.088 g% for CD. The total antioxidant activity of two species was investigated by using phosphomolybdenum method. In each species 50% ethanol exhibited highest antioxidant activities and it was 2.50 g/100 g for CD and 1.50 g/100 g for CA. According to the result of anti-oxidant activity of the whole plants parts, it might be considered to be a potential source for use in pharmaceutical and food industry.

Keywords: Cynodon, Characterization of classes, Nutrition, Minerals, Secondary metabolites, Phenolic content, Antioxidant activity

INTRODUCTION

Herbals products are important source of traditional and modern medicine which is used widely to treat several diseases. Traditional plants are rich in several potential drugs. Cynodon is a genus of the family Gramineae (Poaceae) containing perennial, sod-forming, warm-season grasses distributed throughout the world. This grasses widely used for forage purposes and also has many therapeutic as well as decorative value and other explored potentials [1]. According to Harlan et al. [2] differentiated the genus into nine species and ten varieties. CA (*C. arcuatus*) is easily recognizable from other species of the genus by virtue of its distinct morphology, comprising broad lanceolate leaves and long slender flexuous racemes arranged in a single whorl [2,3]. CD (*C. dactylon* var *dactylon*) is the most widely distributed taxon of the genus occurring worldwide distribution between latitudes of about 45°N and 45°S [4].

CD is widely used in different conditions such as wound, hemorrhages, skin burning etc. It is traditionally used for diabetes, anti-inflammatory kidney problems, urinary disease, gastrointestinal disorder, constipation, and abdominal pain. Whole plants are used for diuretic, dropsy, syphilis, wounds infection and piles [1]. The juice of the plant is astringents and is applied externally to fresh cuts and wounds. It is used in the treatment of chronic diarrhea and dysentery. Leaf paste is also applied in traumatic wounds and piles, fresh juice of the plant is installed into eyes for catarrhal conditions and when used as nasal drops controls nasal bleeding [5]. The dried extracts of aerial parts of CD were examined for CNS activities in mice [6]. Anti-diabetic, antiulcer, analgesic, anti- pyretic, diuretic and antimicrobial activities have been also reported as its functions. CD is very effective in snakebite therapy and anti-snake venom from the plant extract is very effective to treat patients who are bite by a snake [7].

Antioxidants have capacity to counteract the free radical and avoid adverse effect cause by them before they damage proteins, DNA, lipids, enzymes and carbohydrates [8]. Antioxidant potential of oral feeding of aqueous extract of CD was evaluated on diabetes-induced oxidative stress of diabetic rats and the results showed that elevated level of lipid peroxide (LPO) came down significantly and decreased the activites of antioxidant enzymes in diabetic rats [9]. Plant materials are mainly focused as natural antioxidant sources due to its secondary metabolites which contain phenols and polyphenols. These phenolic compounds have conjugated ring structures and hydroxyl group which scavenge the free radicals.

Therefore information regarding nutrient values, antioxidant composition and antioxidant capacity of two different species of grasses available in our country might be useful for the preparation of herbal medicines. The objective of this study is to investigate the physicochemical characteristics including antioxidant capacities of the experimental grasses. The information obtained from the study will also be helpful to select the grass as alternative source of antioxidants and nutrients, as data on these parameters are not available on Bangladeshi grass.

MATERIALS AND METHODS

Collection plant

Fresh and disease free stem, leaves and roots of Cynodon samples (500 g) were collected from the cultivated paddy fields in Sagorika area near a sea beach of Chittagong in the month of March, 2015 at 24-26°C temperature in 60% humidity and brought to the ethno-botany lab for taxonomy. Identification of grass species Sample A, CA is a stoloniferous, creeping, perennial grass, culms slender 20-80 cm high, geniculate without rhizome, ligules membranous about 0.5 mm long, leaf blades linear-lanceolate to narrowly ovate 1-15 × 0.1-0.6 cm, rounded (Figure 1) and Sample-B, CD is a stoloniferous, mat forming, creeping, perennial grass, culms slender, up to 50 cm high, smooth underground rhizome, slender, ligules a short ciliolate rim about 0.3 mm long, leaf blades linear-lanceolate 0.5-12.0 × 0.2-0.5 cm , acuminate, blue green (Figure 2).

Figure 1: *C. arcuatus*

Figure 2: *C. dactylon*

Preparation of powder sample

The collected plants were separated from undesirable materials. Then washed with water and dried by sun for 15 days to ensure the active constituents free from decomposition and also to avoid any degradation. Again, they were dried for 24 h at 37°C in an incubator (Brand: Binder, Model: E 28, Country: Germany). Then fine powders from dry leaves including stem and root were prepared by using high speed blending machine (Brand: Miyako, Model: BL – 152 PF - AP, Speed: 25000 RPM) for 3 times until powder form and then again ground with mortar and pestle for getting fine powders. The powder was properly stored in an air tight clean plastic container and kept in cool, dark, dry and clean place until analysis is commenced.

Determination of pH

Dry powder (2 g) was homogenized well with 30 ml distilled water and then filtered through Whatman's No.1 filer paper. The filtrate was centrifuged for 10 min at 5000 rpm and the clear supernatant was collected. The pH of the extracted solution was determined by a Corning 215 – pH meter using standard buffer solution.

Determination of chlorophyll

Dry powder (5 g) was extracted with 80% acetone and then filtered. The filtrate was pooled and made up to 100 ml in a volumetric flask with 80% acetone. The chlorophyll content of Cynodon dry powder extract were calculated employing the formula using the specific absorption coefficient for Chlorophyll –a and Chlorophyll – b at 645 nm and 663 nm in 80% acetone, respectively [10].

Chemicals composition

Moisture was determined following the conventional procedure as reported ICOMR [11]. Ash and Crude fiber content were determined through the established procedure of AOAC [12]. Phenol Sulphuric Acid Method [13] was used for the determination of the total carbohydrate, whereas the total sugar was determined by anthrone method [14]. The following parameters were determined following the standard methods: Total reducing sugar by Di-Nitrosalicylic acid (DNS) method [15], Total protein by Micro-Kejeldahl Method [16] and total lipid content [17].

The dry powder was again dried till constant weight and then digested by HNO_3 and perchloric Acid ($HClO4$) for mineral determination. Analytical method was used to determine the important minerals content of Cynodon where Ca and Mg were examined by atomic absorption spectrophotometry, P and Cl by spectrophotometry, Na and K by flame photometry [18].

Screening for secondary metabolites

For phytochemicals screening, 100 g dry powder was soaked in 400 ml of 96% ethanol in a glass container for sixteen days with additional regular shaking and stirring. The extract was then separated from the debris by filtration using a piece of clean, white cotton material and it was repeated for two times. The filtrate was taken in a beaker and wrapped with a sheet of aluminum foil to which perforation was done for evaporation of ethanol. The concentrate was designated as a crude extract of ethanol and used for experimental purposes [19,20].

Total phenol content

The total phenol content was measured spectrophotometrically according to the Folin–Ciocalteu's method, as described by Scalbert et al. [21]. Dry powder was dissolved separately with hot water, methanol and ethanol, to a concentration of 200 µg/ml. Aliquots of these samples (0.5 ml) were mixed with 2.5 ml of the Folin–Ciocalteu's reagent (diluted 10 times with distilled water) and 2 ml of aqueous sodium carbonate solution (75 mg/ml).The final mixture was heated at 50°C for 10 min, thereafter the absorbance was read at 760 nm against a blank (solution with no extract addition).

Flavonoid content

The flavonoid content was determined spectrophotometrically according to the $AlCl_3$ method developed by Brighente et al. [22]. Dry extract was dissolved with methanol to a final concentration of 1 mg/ml. Aliquots of the sample (2 ml) was mixed with an equal volume of 2% w/v $AlCl_3.6H_2O$ solution. The mixture was vigorously shaken and absorbance was measured at 415 nm after 1 h of incubation at 20°C.

Determination of total antioxidant capacity (TAC)

The total antioxidant capacities of samples were evaluated by using the phosphomolybdenum method [23]. The assay is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and subsequent formation of a green phosphate/Mo (V) complex at acidic pH. Sample (300 µl) was mixed with 3 ml of reagent solution consisting of 28 mM sodium phosphate monobasic, 4 mM ammonium molybdate and 0.6 M sulfuric acid. The mixture was incubated at 95°C for 90 min and later the absorbance was taken at 695 nm. The total antioxidant capacities were expressed as mg ascorbic acid equivalent per g dry extract.

Statistical analysis

All experiments were performed in triplicate while the minerals were analyzed duplicate. The optical density of each sample was measured with the help of spectrophotometer and was plotted on a graph of respective standard used particularly for each biochemical's. From the graph, concentration of biomolecule/ml was calculated and then converted into 100 g and the results were the means of triplicate or duplicate ± Standard Deviation (SD).

RESULTS AND DISCUSSION

The pH of the grass extract at room temperature was found to be in the moderate acidic zones and varied between 4.4-4.7, indicating that acidic components are higher than neutral and alkaline components in the two types of grasses. The total chlorophyll and carotenoid content of CA and CD were found to be varied between (0.072-0.149) and (1.21-3.17) mg/g respectively, suggesting that CD is greener than the CA (Table 1).

The moisture and ash contents of the experimental grass from separate places were measured to be 12-15% and 10-12% respectively. Sultan et al. [24] reported ash and moisture content was varied between 6.6-10%, but Manzoor et al. [25] reported that the grass contained 13.7% ash which is very much related to the present results. Shah and Hussain [26] indicated in their report that grass contained 10.89%, moisture but our result is moderately higher than this value. The crude fiber, crude protein, and total sugar contents of CA were found to be 38.95, 11.38 and 6.8 and that of CD were 32.55, 11.03 and 7.2 g/100 g, respectively. The present value of crude protein is very similar to that reported by Manzoor et al. [25] but significantly less than that reported by Sultan (15.6-23.7%) [24]. Again Sultan et al. [27] reported that mature grasses contained 5.7% crude protein which is much less than the present findings. The present data clearly indicated that CD contained more than three times higher amount of fat in compare to that of CA. So on the basis of nutrient contents, CD is considered to be superior to CA due to its content of higher amount of moisture, ash, total sugar and total fat except crude protein which is found to be present in slightly less amount (Figure 3).

Table 1: pH, TSS (Brix% content), TDS, Chlorophyll and Carotenoid contents of CA and CD

Content	CA	CD
pH	4.7	4.4
Total soluble solid (TSS)	1 °Brix	1.5 °Brix
Total Dissolved Solid (TDS)	>1990ppm	>1990 ppm
Cholrophyll a	0.0510 mg/g	0.11 mg/g
Cholrophyll b	0.021 mg/g	0.039 mg/g
Total chlorophyll	0.072 ± 0.016 mg/g	0.149 ± 0.009 mg/g
Carotenoid	3.17 ± 0.032 mg/g	1.21 ± 0.016 mg/g

*°Brix=1 g of sucrose in 100 g

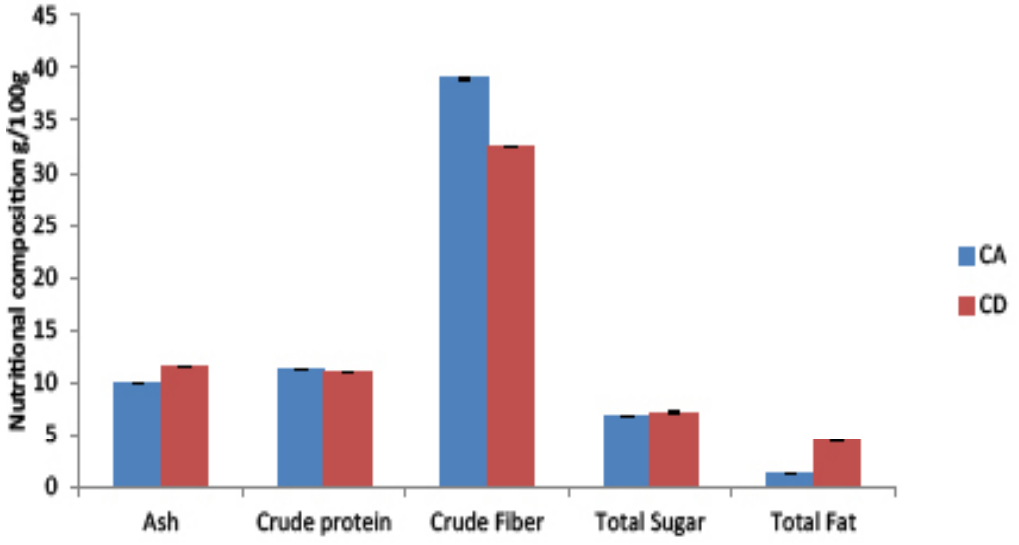

Figure 3: Nutritional composition of *Cynodon* spp. Values are expressed as mean ± SD

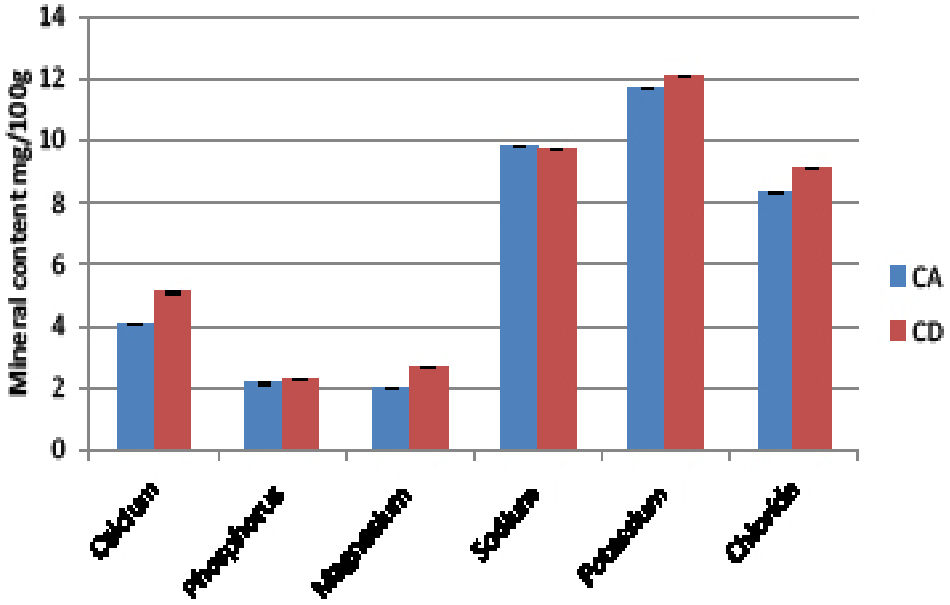

Figure 4: Mineral content of *Cynodon* spp. Values are expressed as mean ± SD

The phytochemical screening analysis clearly revealed that CD contained much higher amount of tested secondary metabolites than CA (Table 2). This analysis also suggesst that the whole part of the examined grasses are good sources of alkaloids, saponin, tannins, steroids, flavonoids, cardiac glycosides and glycosides.

The macro-minerals profiles of grasses, *Cynodon* spp. is presented in Figure 4. Some minerals such as Ca, Mg, P, K, Na and Cl were determined in CA and CD. Among the minerals examined Potassium content is found to be higher (11.7 and 12.1 mg/100 g) in both the types, followed by sodium (9.8 and 9.73 mg/100 g) and chloride (8.3 and 9.1 mg/100 g).On the other hand, calcium, phosphorus, magnesium, sodium, potassium and chloride were found to be less than 4.5 mg/100 g.

Phenolic compounds are ubiquitous constituents of plants and their major sources in human diet are fruit, vegetables and various beverages. The bioactive polyphenol such as flavonoids are also available in forest trees and consequently from the residues of industrial wood transformation. In order to evaluate the potential antioxidant capacity of the extracts from grasses their polyphenol content were determined in different solvents methanol, ethanol and hot water extracts [28].

Total phenolic contents of methanol and ethanol extracts at different concentration in mature grasses are shown in Figure 5. Sample CD contain higher amounts of polyphenol and 50% methanol as well as ethanol is found is the best solvents for extraction of phenolic compound from CD (1.57 g%) while water at 100°C and 100% methanol are found to be best for CA (0.919-0.902 g%). All the extracting solvents are found to be good for isolating phenolic compounds of from CD. Pellegrini et al. [29] also reported the *Cynodon dactylon* using hot water, acetone and ethanol and their result were found to be agreed to our result.

Flavonoid contents of the experimental grasses are presented graphically in Figure 6. All the above mention solvents were found to be suitable for extraction of flavonoids from the samples and its concentration was found to be varied between 0.01 to 0.10%. Further among the solvents 50% ,70% and 100% methanol are the best solvents for extracting flavonoids from the samples and the amount was estimated to 0.064 to 0.077 g% for CA and 0.065 to 0.088 g% for CD which is good agreement to the findings of Melinda et al. [30].

The antioxidant activity of extract from CD and CA was evaluated using phosphomolybdenum method [23]. This assay is based on the reduction of Mo (VI) to Mo (V) complex at acidic pH, which is measured at 695 nm. The highest potency in CD and CA were found by ethanol is found to be the best solvent for showing highest antioxidant activity as shown in Figure 7, that was found to be 2.50 ± 0.040 and 1.50 ± 0.040 g/100 g respectively. Similar results were also reported by Melinda et al. [30]. In our study it was found that among the solvents use water showed lowest antioxidant activity that may be due to the thermal effect.

Table 2: Phytochemical screening of CA and CD

Sample	Alkaloids Test	Saponin Test	Tannins Test	Steroids Test	Glycosides Test	Flavonoids' Test	Cardiac glycosides Test
CA	+	++	+	+	+	++	+
CD	+++	+++	+++	+++	+++	+++	+++

Here, (+++) indicate high, (++) indicate medium, (+) indicates low, as based on color intensity

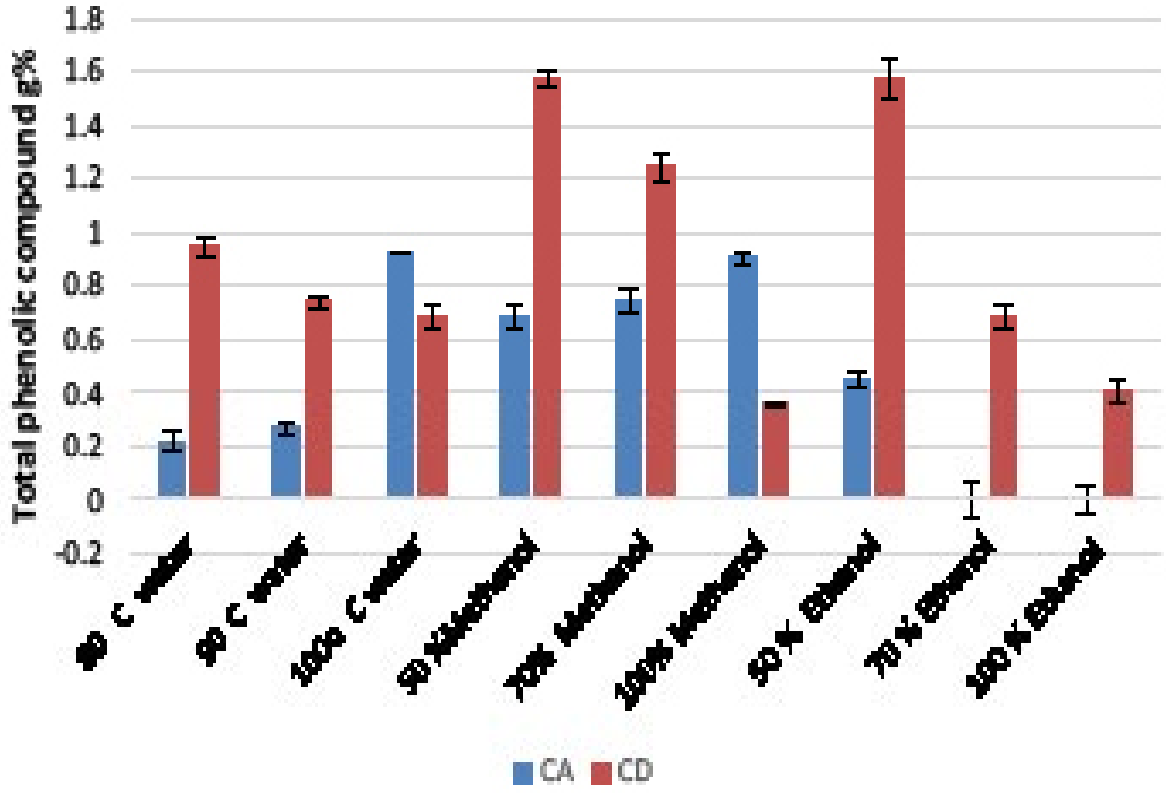

Figure 5: Determination of total phenolic content (Polyphenol). Values are expressed as mean ± SD

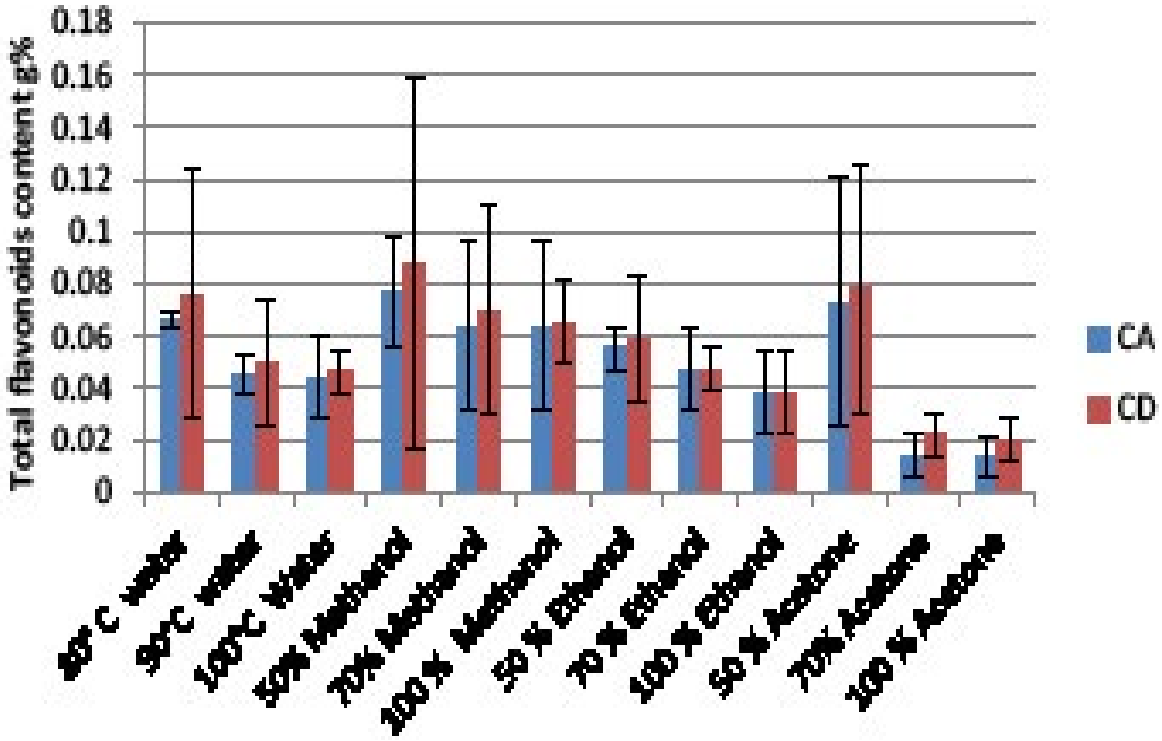

Figure 6: Flavonoids content in *Cynodon* spp. Values are expressed as mean ± SD

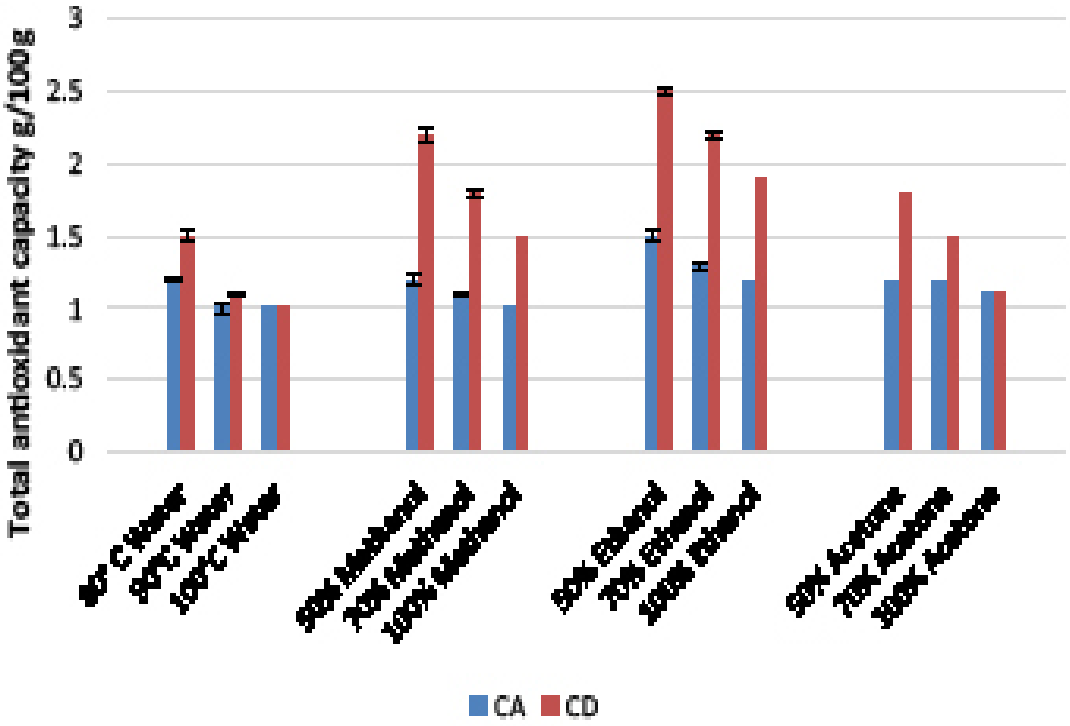

Figure 7: Total antioxidant activity of CA and CD. Values are expressed as mean ± S

CONCLUSION

Cynodon spp. has widely been used in traditional medicine in ancient times for curing several human diseases. This plant has abundant medical and clinical application which can be made only after large-scale research on its pharmacological activity, mechanism, bioactive and extensive safety studies [31]. Further study is needed to confirm the pharmacological and biological effect. The present study clearly demonstrates that the experimental grasses contain anti-oxidant capacity so it may be considered as a source of antioxidant in pharmaceutical food industry.

COMPETING INTERESTS

The authors have no conflict of interests.

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