Research Article

In vitro Assessment of Total Phenolic Content, Iron Chelating, Reducing Potential and DPPH Scavenging Activity of *Cynodon Dactylon* Hydroalcoholic Extract

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ABSTRACT

Cynodon dactylon is generally known as Druva grass exhibiting various medicinal properties and used in folk medicine. The current study we examined antioxidant properties along with estimation of total phenolic content, reducing property, Iron chelating capacity of hydro alcoholic extract *Cynodon dactylon* Modified method of Folin-Ciocalteu was used for Total phenolic content estimation of the plant extract. The capacity of Iron chelating by *Cynodon datylon* extract was studied by slightly customized procedure explained by Dinis. The ability of scavenging 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical was calculated in percentage by a reduction of absorbance at 517 nm as described by Hou comparing with standard Ascorbic acid and Ferric reducing antioxidant potential assay method described by Benzie and Strain. *Cynodon dactylon* displayed phenolic content ranging from 30 to140 mg GAE (Gallic acid equivalent)/g of extract. At 10 µg/ml concentration hydroalcoholic entire plant extract of *Cynodon dactylon* displayed 60.4 ±0.9% iron chelating capacity when compared to standard 89.77±0.33 %. The maximum reducing ability (absorbance 0.729±0.44) of plant extract showed at 1mg/ml and the standard Rutin showed (absorbance 2.983±0.55) at same concentration. The maximum scavenging ability (87.2%) of plant extract showed at 1mg/ml and the standard Ascorbic acid showed saturation at high concentration. The entire plant extract of *Cynodon dactylon* showed its scavenging capacity of free radicals in a concentration dependant method. The results of this studies shows that the hydro alcoholic extract of entire *Cynodon dactylon* plant is affluent in antioxidant components which might afford scientific basis for its use in phyto-medicine.

Keywords: *Cynodon dactylon*, DPPH, Antioxidant Gallic acid, Ascorbic acid.

INTRODUCTION

Antioxidants have capacity to counteract the free radical and avoid adverse effect cause by them before they damage proteins, DNA, lipids, enzymes and carbohydrates.[1] In current situation they are the key components in various field such as medication (cancer, cardiovascular disorder, and chronic inflammations), cosmetics (anti-ageing), food industry (preservative) etc.[2] Some synthetic antioxidant like Butylated Hydroxy Anisole (BHA), Butylated Hydroxy Toluene (BHT), and Tertiary Butyl Hydro Quinone (TBHQ) compounds are commonly used in food industries.[3] But it has been suggested that such compounds have toxic effects on organs and also cause mutagenesis.[4,5] Consequently, plant materials are mainly focused as natural antioxidant sources due to its availability and low toxicity. The efficacy of antioxidant is measured in terms of the inhibition of appropriate substrate oxidation. Presence of hydroxyl group in Phenolic components is known for their scavenging potential. Studies showed that several phenolic antioxidants such as Tannins, Couramins, Flavonoids and Xanthones act on radicals in concentration dependently and these are considered as therapeutic agent for free radical pathologies.[6] Some of the herbs have extremely high level of antioxidants that was confirmed by its action on reactive oxygen species and nitrogen species which are produced within the cell, by different mechanisms and also by many external factors such as chemical carcinogens, radiation and atmospheric pollution which is accountable for the etiology of diverse interlinked diseases.

Cynodon dactylon (Family: Poecea) known as Bermuda grass in English, Dhub in Hindi, is a hardy perennial spreading grass found in temperate weathers all over the earth. It forms spreading mats on the soil surface having rooting at nodes.

The entire plant juice is used for treatment of diuretic, dropsy, syphilis, wound infection and piles. It is used in the treatment of hysteria, antihyperlipedemic, ophthalmic, epilepsy, insanity, chronic diarrhea and reproductive disorders[8-10].

Therefore, this *in vitro* study is designed to establish Total Phenolic Content, Iron chelating capacity, Reducing Potential capacity and scavenging of free radical capacity of *Cynodon dactylon* hydro Alcoholic extract of entire plant.

The entire above assays measures different characteristic of antioxidant activity of the plant extracts.

MATERIALS AND METHODS

Plant Collection: Plants were collected from the campus of Kasturba Medical College, Manipal University Mangalore. Plants were authenticated by its vernacular

name by Usharani S. Suvarna, Department of Botany, Mahatma Gandhi Memorial College Udupi Karnataka.

Preparation of *Cynodon dactylon* Extract (CDE)

The *Cynodon dactylon* plants were thoroughly washed with water removed all dirt. The plants were shade dried. The whole plants were powdered by an electrical mixer. Hydro alcoholic crude extract was prepared by extracting the 100 gm plant powder in 50% of methanol and 50% of water with total volume of 500 ml and refluxed for 72 hours at 50°C in soxhlet apparatus. Concentrating of extract has done by using rotary flash vacuum evaporator. Extract was stored at 4°C until use.

Chemicals and Reagents

Gallic acid, Rutin, DPPH, Folin-Ciocalteu reagent, Ferrozine are procured through Sigma Aldrich Chemicals and other chemicals and reagents used were of analytical grade.

Total Phenolic Content

For determination of Total phenolic content of hydroalcoholic plant extract the Folin Ciocalteu method was applied.[11] The Folin Ciocalteu gets reduced by polyphenols present in the plant extract which results in development of blue color complex. Gallic acid was used as reference standard for comparison of various concentration of plant extract (20-100 µg/ml) and was treated with 2 ml of (1:10) diluted Folin ciocalteu reagent. The mixture was neutralized by addition of 4ml sodium carbonate solution (7.5% w/v). The blue color developed in the mixture after incubation of 30 minutes in room temperature with constant shaking. The optical density was recorded at 765 nm using spectrophotometer. The phenolic content of plant extract was determined by plotting standard Gallic acid curve. Total phenolic content expressed in Milligram/gram Gallic acid equivalent (GAE) of dry extract. Formula was used to calculate the total phenol content is T =V×C/M

T = total phenolic content in mg GAE/gram dry weight of extract

V = quantity of plant extract in ml

C = concentration of Gallic acid mg/ml

M = weight of plant extract in grams

Iron Chelating Capacity

Modified method of Dinis was used for determination of Iron Chelating property of *Cynodon dactylon* extract.[12] The principle behind this estimation is that the capability of plant extract to decolorize iron-ferrozin compound. The colored complex develops quickly when Ferrozine reacts with iron.

1ml of different concentration (2-10µg/ml) of plant extract was mixed added to 0.2 ml of Fecl3 (1 mM) and 3.6 ml of distilled water was added.

The reaction was started by addition of 0.4 ml Ferrozine (5mM) and kept at room temperature for 25 minutes. The absorbance of solution was examined at 562 nm. Same concentration of Di sodium EDTA was used as a standard for evaluation.

Following equation was used for calculation of the percentage of Iron chelating capacity of plant extract.

Capacity of Iron Chelating (%) = $(A_O - A_1) / A_O) \times 100$

Where A_O =absorbance value of control and A_1 = absorbance value of standard or test.

Reducing Property of the Plant Extract

The reducing potential of *Cynodon dactylon* extract was determined by spectrophotometric method.[13] 2.5 ml of different concentration of plant extract was treated with 2.5 ml of phosphate buffer pH 6.6 (0.02 M). To the above mixture 1 % of 2.5 ml of potassium ferricyanide was added. The mixture kept in water bath at 50°C for 20 minutes. After boiling add 2.5 ml of 10 % trichloroacetic acid. Then centrifugation was done at 3000 rpm for 15 minutes. 5 ml of supernatant was treated with 1 ml of 0.1 % Fecl3. The absorbance was taken at 700 nm. Quercetin compound was used as standard for comparison.

The DPPH Free Radical Scavenging

The DPPH free radical scavenging activity of *Cynodon dactylon* plant extracts was determined by the method of Hou[14].

Principle

DPPH radical reacts with antioxidants and get converted 2, 2- Diphenylpicryl Hydrazine.

Decrease in the absorbance indicates the scavenging capacity of plant extract. Samples containing various concentrations (200–1000µg/ml) of Cynodon dactylon hydroalcoholic extract of 1 ml volume were mixed up with 1 ml of DPPH(0.05Mm), methanol used as control. Ascorbic acid (2-100µg/ml) was used as standard.

Reaction mixtures were kept in boiling water bath at 37°C for 20 min & the absorbance was noted at 517 nm. The ability of DPPH radical scavenging activity was calculated using the following formula

DPPH scavenging effect (%) = $(A_0-A_1)/A_0 \times 100$, where

A_0 is the absorbance value of the control and A_1 is the absorbance value of standard or test.

RESULTS AND DISCUSSION

Phytochemical analysis of hydroalcoholic plant extract done to check presence of tannins, steroids, flavonoids, saponins, glycosides and alkaloids qualitatively. The details of Phytoconstituents present are listed in Table 1.

Total Phenolic Content

The hydroalcoholic extracts of *Cynodon dactylon* was analyzed for their total phenolic content and it was

observed that whole plant extract of *Cynodon dactylon* displayed phenolic content ranging from 30 to140 mg GAE/g of extract. Plants produce secondary metabolites which contain phenols and polyphenols. These phenolic compounds have conjugated ring structures and hydroxyl group which scavenge the free radicals and also have metal chelating capacity[15].

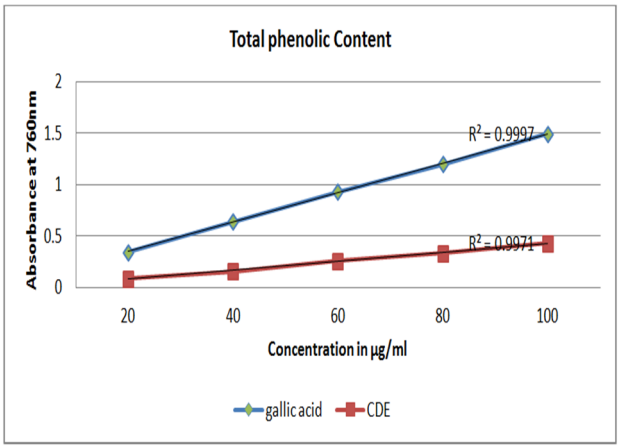

Figure 1: Total Phenolic content of *Cynodon dactylon* and standard Gallic acid.

Table 1: Phytochemicals detected in *Cynodon dactylon* extract (+ = present, - = absent). Qualitative test for Tannins, Flavonoids, Steroids, Saponins were showed positive in plant extract.

TEST	CDE
FLAVONOIDS	+
TANNINS	+
STEROIDS	+
SAPONINS	+
ALKALOID	-
GLYCOSIDES	-

Iron chelating capacity

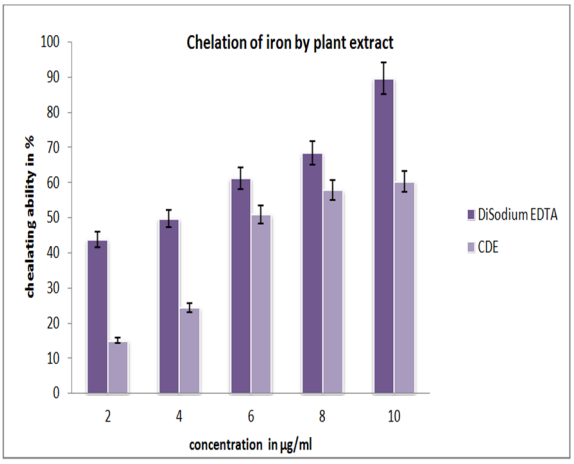

Figure 2: Iron chelating capacity of *Cynodon dactylon* plant extract and standard Di Sodium EDTA

Different concentration of (2-10 µg/ml) hydro alcoholic plant extract was tested for iron chelating capability.

As the concentration of plant extract increased the chelating activity also increased.

These results indicate that there is a concentration wise increase in the Iron chelating capability of plant extract.

At 10 µg/ml concentration the hydroalcoholic entire plant extract of *Cynodon dactylon* displayed 60.4 ±0.9% iron chelating capacity, where as the standard Disodium EDTA showed 89.77± 0.49 % iron chelating ability.

Reducing Potential capacity

The hydroalcoholic plant extract of *Cynodon dactylon* exhibit reducing property in dose dependent manner.

The maximum reducing ability (absorbance 0.729) of plant extract showed at 1mg/ml and the standard Rutin showed (absorbance 2.983) at same concentration.

Bioactive constituents of plant extract contribute an electron and reduce the oxidized intermediates[17].

In this assay reducing agents present in the leaf extract exchanges fe3+/ferricyanide to ferrous form; this gives the pearl Prussian blue color which determines fe3+ ion concentration.

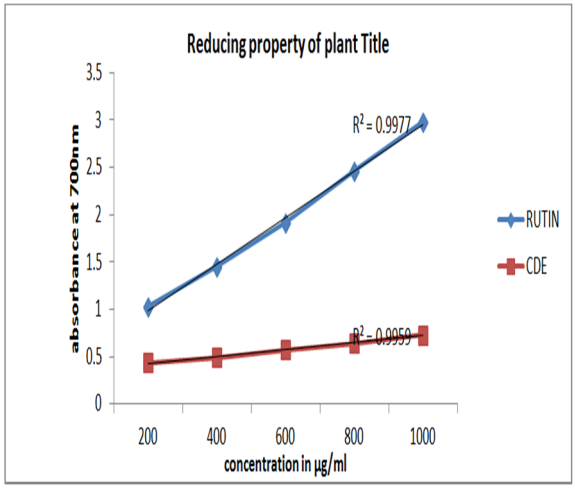

Figure 3: Reducing potential capacity of *Cynodon dactylon* and standard Rutin.

Free Radical Scavenging Capacity

DPPH method was followed to determination of radical scavenging activity and it is widely used.

Principle behind this method is that decrease of methanolic colored solution of free DPPH radical by antioxidant.

Disappearance of the DPPH radical by antioxidant present in the sample was detected by spectrophotometrically at 517 nm.

The highest scavenging ability (87.2%) of plant extract showed at 1mg/ml and the standard Ascorbic acid showed saturation at high concentration.

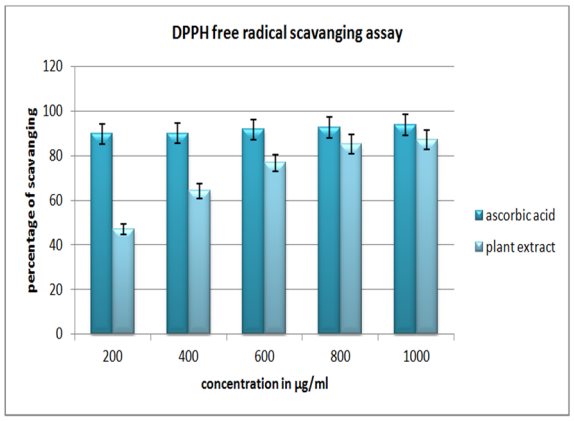

Figure 4: Graphical representation of percentage inhibition of DPPH free radical by *Cynodon dactylon* along with standard ascorbic acid.

CONCLUSION

In vitro studies of hydroalcoholic extract of entire plant *Cynodon dactylon* revealed that it has significant amount of phenolic content along with other phytochemical constituents. Combinations of these compounds attributes to radical scavenging, reducing potential and chelating activity of iron by which indicates that *Cynodon dactylon* plant can be used as potential antioxidant against different aspects in *in vivo* also.

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