WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 3, 715-722. Research Article ISSN 2277– 7105

ANTIOXIDANT POTENTIALS OF FRESH JUICE OF CYNODON DACTYLON L PERS (DURVA)

[1]Dr. Sharda Singh, [2*]Dr. Shashi Kant Pandey, [3]Dr. Kamal Nayan Dwivedi, [4]Dr. Yamini Bhushan Tripathi and [5]Amit Kumar Sharma

[1]Phd Scholar, Department of Anatomy, I.M.S, B.H.U, Varanasi, India.

[*2]Professor Department of Anatomy, Faculty of Medicine, IMS, BHU.

[3]Professor, Department of Dravya Guna, Faculty of Ayurveda, IMS, BHU.

[4]Professor, Department of Medicinal Chemistry, IMS, BHU.

[5]Phd Scholar, Department of Medicinal Chemistry, IMS, BHU.

Article Received on
22 Dec 2015,

Revised on 11 Jan 2016,
Accepted on 01 Feb 2016

***Correspondence for Author**
Dr. Shashi Kant Pandey
Professor Department of Anatomy, Faculty of Medicine, IMS, BHU.

ABSTRACT

Aim of the study was to analyze the free radical scavenging activity of fresh juice of *Cynodon dactylon*. Juice was extracted from fresh plant of *Cynodon dactylon*, which was then allowed to freeze drying at reduced pressure, in a lyophilizer, to prevent degradation of constituents of juice. Powder form of juice thus obtained was used to perform various tests for invitro free radical scavenging activity that was determined by superoxide radical scavenging, lipid peroxidation assay, nitric oxide radical scavenging using UV spectrophotometer. EC_{50} values for superoxide radical scavenging, lipid peroxidation assay, nitric oxide radical scavenging was 197.34 ± 4.36, 685.53 ± 7.04, 298.01±4.12 respectively with reference to standards having EC_{50} 41.70±2.40, 27.2±1.02, 63.11±3.67. The results show that fresh juice of *Cynodon dactylon* is a potential source of natural antioxidant.

KEYWORDS: free radical scavenging, fresh juice, spectrophotometer, antioxidant.

INTRODUCTION

Free radicals are reactive oxygen species (ROS) or reactive nitrogen species (RNS) which are generated in the body during normal metabolic activities or by environmental conditions.[1] At low or moderate levels, ROS and RNS exert beneficial effects on cellular responses and immune function. At high concentrations, free radicals are dangerous and can attack

biological molecules such as lipids, proteins, enzymes, DNA and RNA.[2-6] The human body has several mechanisms to counteract oxidative stress by naturally produced or externally supplied antioxidants. Research activities focusing on medicinal plants have been encouraging because of their high content of potent antioxidants, accessibility, economic viability and next-to-no side effects.[7] Various plant derived antioxidants are effective free radical scavengers which are used combinatorial for the treatment of various diseases as nutritional supplements.[8]

Cynodon dactylon has been used as an antiepileptic agent in traditional system of medicine in India.[9] Ethnomedicinally, plant juice is used in the treatment of wound healing,[10] blood clotting,[11] asthma,[12] dysentery,[13] skin diseases[14] etc. As there is no information pertaining to the antioxidant potential of fresh juice of Cynodon dactylon, the present study is designed to analyze the antioxidant capacity of *Cynodon dactylon* with different invitro models.

MATERIALS AND METHODS

Collection of Plant material

The whole plants of *Cynodon dactylon* (Durva) were collected during month of july from the Ayurvedic garden. Taxonomical identification and authentication was done in Deptt. of Dravya Guna, faculty of Ayurveda, BHU, Varanasi.

Preparation of fresh juice of plant

The aerial and underground parts of *Cynodon dactylon* were washed under running tap water. When the water evaporated, the drug was crushed with the help of pestle and mortar. Then it is squeezed through double layered muslin cloth. The juice thus obtained is called fresh juice. The juice thus formed was allowed to dry in lyophilizer (Christ, alpha 1-2, Central instrumental lab, Deptt. of Botany) at reduced pressure for freeze drying to prevent the degradation of constituents of juice, till all the water got evaporated and complete dry powder was formed. The dry juice was transferred to air tight container. This container was placed inside a vacuum container to avoid attack of moisture.

Chemicals and Reagents

Nitroblue tetrazolium (NBT), EDTA, Methionine, Riboflavin, Sodium nitroprusside, α-Napthyl-ethylenediamine, Sulphanilic acid were purchased from Sigma–Aldrich (Steinheim,

Germany). Ascorbic acid, Thiobarbituric acid, Ferrous sulphate, Phosphoric acid, Copper sulphate, Phosphate buffer were of analytical grade.

METHODS

Superoxide radical scavenging activity

Super oxide radical scavenging potential of the extract was reported in terms of its capacity to inhibit the formazan formation upon photochemical reduction of nitroblue tetrazolium (NBT).[15] In brief, each 3 ml reaction mixture (0.01M phosphate buffer (pH 7.8), 130 mM methionine, 60 µM riboflavin, 0.5mM EDTA, NBT (0.75mM) with 0.5ml extract/CuSO4 solution; positive Control). These tubes were kept in front of fluorescent light for 6 minutes and absorbance was taken at 560 nm.

The nonenzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system generates superoxide radicals, which reduce NBT to a purple formazan. The decrease in absorbance at 560 nm with the plant extract and the reference compound Quercetin indicates their abilities to quench superoxide radicals in the reaction mixture. Identical tubes were kept in the dark and served as blanks. The results were expressed in percent inhibition as compared to control.

Lipid peroxidation inhibition assay

For this assay, egg yolk homogenate was used as lipid source and free radicals were produced by Fenton reagent ($FeSO4/H_2O_2$), a modified thiobarbituric acid reactive substances (TBARS) assay.[16,17] In brief, 1 ml reaction mixture containing 0.5 ml Egg yolk homogenate (10% in distilled water, v/v), 0.1 ml. of extract was mixed with 0.05 ml FeSO4 (0.07 M) and incubated for 30 min to induce lipid per oxidation. Free radical ruptures the lipid bilayer to form malonaldehyde as a secondary product. Two molecule of thiobarbituric acid react with one molecule of MDA to form pink colored product showing maximum absorbance at 532 nm called TBARS. When the reaction mixture was mixed with different concentration of extract, it reduces the formation of TBARS product in concentration dependant manner in comparison to control.

Nitric Oxide Radical Scavenging Activity

Nitric oxide generated from aqueous Sodium nitroprusside (SNP) solution interacts with oxygen to produce nitrite ions at physiological pH, which may be quantified and determined according to Griess Illosvoy reaction.[18] The reaction mixture contained: 10mM SNP in 0.5M

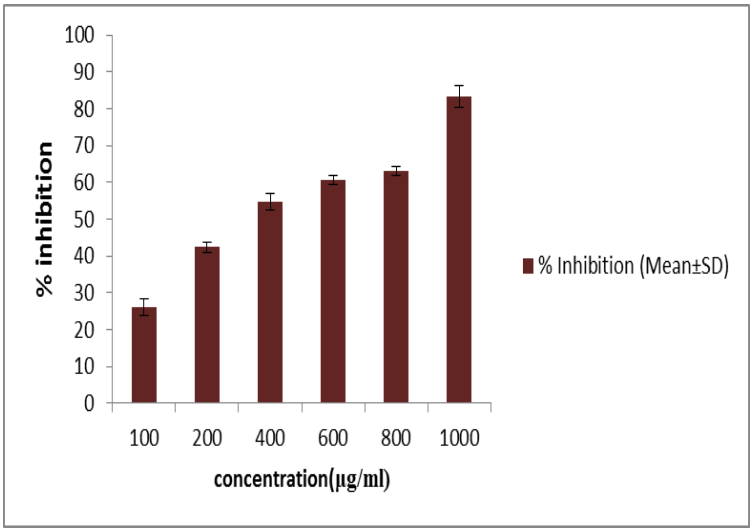
phosphate buffer (pH 7.4) and various concentrations (100–1000 μg/mL) of the extract in a final volume of 3mL. After incubation for 60 min at 37^{o}C, Griess reagent (0.1% α-napthyl-ethylenediamine in water and 1% sulphanilic acid in 5% H3PO4) was added. The pink chromophore generated during diazotization of nitrite ions with sulfanilamide and subsequent coupling with α-napthylethylenediamine were measured spectrophotometrically at 540 nm. Ascorbic acid was used as a positive control. Nitric oxide scavenging ability (%) was calculated by using above percent inhibition (%).

RESULTS AND DISCUSSION

Table 1: Effect of different fractions of Cynodon dactylon extract on Super oxide (SO) radicals.

Concentration (µg/ml)	% Inhibition (Mean±SD)
100	26.1±2.12
200	42.43±1.42
400	54.73±2.40
600	60.59±1.16
800	63.17±1.26
1000	83.38±2.98
EC_{50}	197.34±4.36

Trapping potential of Copper sulphate- EC_{50} 41.70±2.40.



Graph showing Effect of different fractions of Cynodon dactylon extract on Super oxide (SO) radicals.

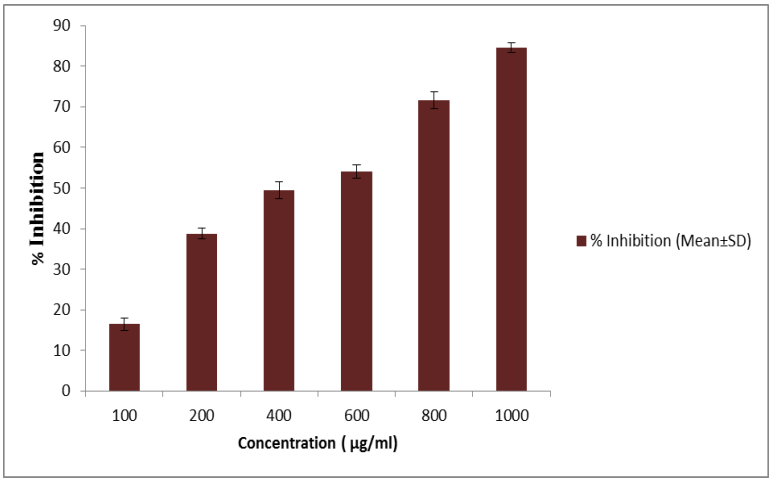
The ability to reduce NBT by PMS-NADH coupling can be measure the superoxide radicals generated from dissolved oxygen. The decrease in absorbance at 560 nm with the Cynodon

dactylon extract and the reference compound Copper sulphate indicates their abilities to quench superoxide radicals in the reaction mixture. Superoxide free radicals showed maximum inhibition of 83.38 ± 2.98 at concentration of 1000 µg/ml plant extract with EC_{50} value of 197.34 ± 4.36 proving again the better antioxidant activity**.** The EC_{50} values of the Copper sulphate on superoxide scavenging activity was 41.70 ± 2.40 µg/ml.

Table 2: Effect of different fractions of Cynodon dactylon extract on $FeSO_4$ induced lipid per oxidation in egg yolk homogenate.

Concentration (µg/ml)	% Inhibition (Mean±SD)
100	16.42±1.45
200	38.77±1.24
400	49.46±2.12
600	54.01±1.65
800	71.62±2.15
1000	84.46±1.19
EC_{50}	685.03±7.04

Ascorbic acid EC_{50} 27.2±1.02 µg/ml.



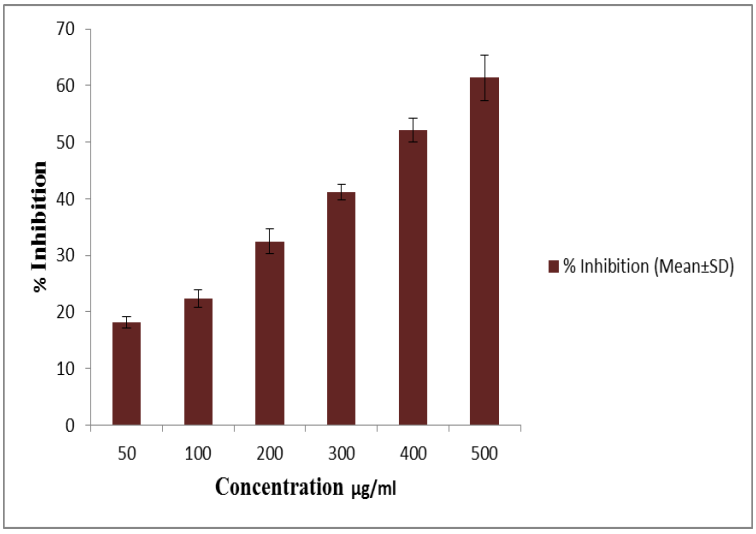
Graph showing Effect of different concentration of Cynodon dactylon extract on $FeSO_4$ induced lipid per oxidation in egg yolk homogenate.

Result of anti-lipidperoxidation free radicals of Cynodon dactylon extract to prevent peroxidation showed maximum trapping potential for LPO radicals is 84.46 ± 1.19 at the concentration of 1000 µg/ml with EC_{50} 685.53 ± 7.04. Oxidative stress in cells and tissues can be best monitored by its lipid per oxidation assay, a well established mechanism both in plants and animals.

Table 3 Effect of different fractions of Cynodon dactylon extract on Nitric Oxide activity.

Concentration(µg/ml)	% Inhibition (Mean±SD)
50	18.11±1.01
100	22.41±1.54
200	32.48±2.11
300	41.18±1.35
400	52.11±2.05
500	61.39±3.98
EC_{50}	298.01±4.12

(Trapping potential of ascorbic acid- EC_{50} 63.11±3.67).



Graph showing Effect of different fractions of Cynodon dactylon extract on nitric oxide scavenging activity.

The phytochemical screening reveals the presence of secondary metabolites such as phenolics, flavonoids, glycosides in the crude extract of Cynodon dactylon[19] and the free radical scavenging activity inherent in the plant species. The high antioxidant activity may relate to the plants' curative and/or management potential of many ailments claimed in its ethno-medicine. In vitro experiments on antioxidant compounds in higher plants show how they protect against oxidation damage by inhibiting or quenching free radicals and reactive oxygen species.[20] Phenolic compounds may contribute directly to the antioxidant action because of their hydroxyl groups.[21]

CONCLUSION

On the basis of the results obtained in the present study, it was concluded that the fresh juice of Cynodon dactylon possess significant antioxidant activity. Presence of adequate amount of

phenolic and flavonoid compounds may account for this fact. So the findings of present study suggest that this plant have a potential source of natural antioxidant.

ACKNOWLEDGEMENT

The authors wish to acknowledge the support received from the laboratory members of medicinal chemistry, IMS, BHU.

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