Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Asian Journal of Plant Science and Research, 2015, 5(6):88-90

ISSN : 2249-7412
CODEN (USA): AJPSKY

Free radical scavenging properties of the ethanol extract of *Cynodon dactylon*

G. P. Choudhary

School of Pharmacy, Devi Ahilya Vishwavidhyalaya, Ring Road, Indore, India

---

ABSTRACT

The in-vitro antioxidant activity of ethanol extract of has been investigated by DPPH free radical and nitric oxide scavenging methods. The ethanolic extract exhibited IC_{50} values of 72.43±4.46 and 68.52±3.82 respectively i n DPPH and nitric acid radical inhibition assay. Free radical scavenging activity might be due to the presence of flavonoids.

Key words: *Cynodon dactylon*, DPPH, Nitric oxide, Free radical scavenging.

INTRODUCTION

Reactive oxygen species are constantly formed in the human body by normal metabolic action and these are exert oxidative damaging effects by reacting with nearly every molecule found in living cells including nucleic acids, proteins, lipids or DNA and may involve in several chronic and degenerative diseases including gastritis, reperfusion injury of many tissues, atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others If excess Reactive oxygen species and free radicals are not eliminated by endogenous antioxidant system[1-2]. Antioxidant compounds in food play an important role as a health protecting factor. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds such as gallates which has strong antioxidant activity, while others such as the mono-phenols are weak antioxidants. The main characteristic of an antioxidant is its ability to trap free radicals[3].

The *Cynodon dactylon* (Family- Poaceae) commonly known as "*Doob*; Hindi" "*Aroogum pillo*; Tamil", "*Garike*; Telulgu" and "*Bermuda grass*; English" in India is a creeper. It is a weed and has been regarded to possess varied medicinal properties[4] . The aqueous fluid extract of the rhizome is used as anti-inflammatory, diuretic, antiemetic, antidiabetic and blood purifying agent[5]. Its high potential of hypoglycemic, hypolipidemic and antioxidant activities of both aqueous and ethanolic extracts have been reported[6-8].

MATERIALS AND METHODS

All chemicals and solvents were of analytical grade obtained from Ranbaxy fine chemicals Mumbai,India.1,1-diphenyl,2-picrylhydrazyl(DPPH)were obtained from sigma chemicals, India. Ascorbic acid and rutin were obtained from Hi-media Laboratories, Mumbai, India.

Plant material

Cynodon dactylon (CD) collected from plantation of Government. Agriculture College, Indore, were identified and authenticated by Prof. V. K. Mishra, Government Agriculture College, Indore. A voucher specimen is preserved in our laboratory for further reference.

Extraction
Dried grass of *Cynodon dactylon* were exhaustively extracted with 95% ethanol using a soxhlet apparatus for a period of 8 hrs. The residue was filtered and concentrated in vacuo(yield 3.5%).

Phytochemical analysis
phytochemical investigation on *Cynodon dactylon* have revealed the presence of glycoside, isoflavone, flavanones and flavonols[9].

Preparation of extract and standard
A weighted quantity of the extract was dissolved in distilled dimethyl sulphoxide (DMSO) and used. Solutions of ascorbic acid and rutin used as standards for these studies were prepared in distilled DMSO.

In-vitro antioxidant activity
1.*DPPH method*
The antioxidant activity of the plant extract and the standards were assessed on the basis of the radical scavenging effect of the stable DPPH free radical. Ten microlitre of extract(from5µg/ml to500µg/ml)was added to 200 µl of DPPH in methanol solution (100 µM)in a microtitre plate. After incubation at 37^0 c for 30 min., the absorbance of each solution was determined at 517nm using ELISA micro plate reader. The corresponding blank reading were also taken and the remaining DPPH was calculated[10] .IC_{50} value is the concentration of the sample required to scavenge 50% DPPH free radical.

2.*Nitric oxide radical inhibition assay*
Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be estimated by the use of Griess Illosvoy reaction[11].In the present investigation , Griess Illosvoy reagent is modified by using naphthyl ethylene diamine dihydrochloride (0.1%w/v) instead of 1-napthylamine(5%).Scavengers of nitric oxide complete with oxygen leading to reduced production of nitric oxide[12] .The reaction mixture containing sodium nitroprusside (10 mM, 2 ml) phoshate buffer saline (0.5ml) and extract or standard solution was (0.5ml) incubated at 25^0 c for 150 min. After incubation, 0.5ml. of the reaction mixture containing nitrite was pipetted and mixed with 1ml of sulfanilic acid reagent(0.33% in 20% glacial acetic acid)and allowed to stand for 5 min. for complete diazotization. Then, 1 ml. of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min.at 25^0c. A pink colored chromophore is formed in diffused light The absorbance of these solution was measured at 540 nm against the corresponding blank solutions in microtitre plate using ELISA reader.IC_{50} value is the concentration of sample required to inhibit 50% of nitric oxide radical.

Statistical Analysis- Linear regression analysis was used to calculate the IC_{50} values.

RESULTS

The qualitative phytochemical analysis indicates that *Cynodon dactylon* contained glycoside, isoflavone, flavanones and flavonols. Several concentration ranging from 5-500µg/ml of the ethanolic extract of *Cynodon dactylon* grass were tested for their antioxidant activity. It was observed that free radicals were scavenged by the extract in a concentration dependent manner.

The ethanolic extract of *Cynodon dactylon* scavenged the DPPH with IC_{50} value of 72.43±4.46. Incubation of solution of sodium nitroprusside in PBS at 25^0c for 150 min resulted in linear time dependent nitrite production, which was maintained by the extract with IC_{50} value 68.52±3.82 (Table 1). These values were found to be slightly more than those obtained for the reference standards.

Table1. *In vitro* antioxidant activity of the ethanolic extract of *Cynodon dactylon*

S. No.	Tested Material	IC_{50}(µg/mL)±S.E.*	
		DPPH Method	Nitric oxide Method
1.	Ethanolic extract	72.43±4.46	68.52±4.08
2.	Ascorbic acid	30.18±2.54	41.72±3.39
3.	Rutin	16.44±2.10	48.27±3.76

** Average of six determinations.*

DISCUSSION

Free radical oxidative stress has been implicated in the pathogenesis of a wide variety of clinical disorders, resulting usually from deficient natural antioxidant defences[7]. Antioxidant activity using DPPH method, DPPH is a relatively

stable free radical. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH· DPPH radicals react with suitable reducing agents, the electrons become paired off and the solution loses colour stoichometrically depending on the number of electrons taken up[13]. From the present results it may be postulated that CD reduces the radical to the corresponding hydrazine when it reacts with the hydrogen donors in the antioxidant principles [14].

Nitric oxide is a free radical produced in mammalian cells, involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases[15-16]. In the present study the nitrite produced by the incubation of solutions of sodium nitroprusside in standard phosphate buffer at 25°C was reduced by the ethanolic extract of CD. This may be due to the antioxidant principles in the extract which compete with oxygen to react with nitric oxide[17] thereby inhibiting the generation of nitrite. Grasses of CD are rich in flavonoids. Flavonoids are natural products, which have been shown to possess various biological properties related to antioxidant mechanisms [18-21].

CONCLUSION

In conclusion, CD has the potential to be a rich source of flavonoids. Consideration of the antioxidant properties of ethanolic extract of CD reported here and the potential disease preventive properties, suggests that it is appropriate for further work with this, to be directed at exploration of its chemopreventive properties.

REFERENCES

[1] Narendra Vyas, Mukul Tailang, Narayan Prasad Gavatia (**2010**) *Int. J. PharmTech Res* ; 2: 417-419.
[2] Nayan R. Bhalodia, Pankaj B. Nariya, R. N. Acharya, V. J. Shukla (**2011**) *Int. J. PharmTech Res* ; 3: 589-599.
[3] Rumit Shah, Heena Kathad, Rajal Sheth & Naveen Sheth(**2010**) *Int J Pharmacy Sci*; 2: 30-33.
[4] Kritikar KK, Basu BD(**1980**) *Indian Medicinal Plants*, 2nd ed. India: 2650pp.
[5] Ahmed S, Reza MS, Jabbar A. (**1994**) *Fitioterapia* ; 65: 463-64.
[6] Singh SK, Kesari AN, Gupta RK, Jaiswal D, Watal G(**2007**) *J Ethnopharmacol*; 114: 174-9.
[7] Singh SK, Rai PK, Jaiswal D, Watal G(**2007**) *Evid Based Complement and Alt Med* 10,44.
[8] Hwang BY Kim HS, Lee JH, & Hong YS (**2001**).*Jour. of Natural Products*.64,82-84.
[9] Kokate C.K., Purohit A.P., Gokhale S.B.(**2002**)In; *Pharmacognosy*,22nd Edition; Nirali Prakashan; Pune , 258-259.
[10] Garrat DC (**1964**) *The Quantitative Analysis of Drugs* ,III edition. Chapman and hall ltd. Tokyo, Japan 456-458.
[11] Marcocci L Packer L Sckatki A & Albert GM, (**1994**). *Methods enzymology*, 234,462-475.
[12] Hellwell B & gutteridge JMC (**1989**) *Free Rad. BioI. Med.* II ed, Clerendon Press, Oxford.
[13] Blois MS. (**1958**) *Nature.* 26:1199-1200.
[14] Sanchez-Moreno C. (**2002**) *Food. Sci .Tech. Int.* 8: 122-137.
[15] Ialenti A Moncada S & Di Rosa M. (**1993**) *Br. J. Pharmacol.* 110: 701-706.
[16] Ross R.(**1993**) *Nature.* 362: 801-809.
[17] Marcocci L Packer L Droy-Lefaiz MT Sekaki AGardes-Albert M. (**1994**) *Meth. Enzymol.* 234: 462-475.
[18] Krewson CF & Naghski J (**1953**) *Amer.J Pharm*.125,19-21.
[19] Glyglewski RJ Korbut R Robak J & Swies J. (**1987**) *Biochem. Pharmacol*.36:317-322.
[20] Middleton E. (**1984**) *Trends Pharmacol. Sci*. 5:335-338.
[21] Robak J & Glyglewski RJ (**1988**), *Biochem.pharmacol*.37;837-841.