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INVITRO ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF *SACCHARUM SPONTANEUM*

M. Sai Krishna, K. Naga Sravanthi*, G. Sreenika, S. Chaithanya, M. Sudhakar

Malla Reddy College of Pharmacy, Maisammaguda, Secunderabad, Andhra Pradesh, India-500014.

***Corresponding author e-mail:** sravanthi_1521@yahoo.com

ABSTRACT

Biomolecules can be oxidized by free radicals which results in oxidative stress. This oxidative damage has an important etiological role in aging and development of diseases like cancer, atherosclerosis, and other inflammatory disorders. Synthetic antioxidants, like Butylated hydroxyl anisole, Butylated hydroxyl toluene are good free radical scavengers. However, synthetic antioxidants can be carcinogenic. Therefore, there is an increasing interest in searching for antioxidants of natural origin. We report here the *in vitro* antioxidant activity of methanolic extract of *S. spontaneum.* The activity of *S.spontaneum* has been tested using various antioxidant models viz., total phenolic and flavonoid content, estimation of DPPH, nitric oxide, superoxide and hydroxyl radical scavenging activity at different concentrations. This study indicates significant free radical scavenging potential of *S.spontaneum* which may be due to the presence of high Phenolic and Flavonoid content.

Keywords: *Saccharum spontaneum*, anti-oxidant, flavonoids, phenolic compounds.

INTRODUCTION

Antioxidants are the agents which scavenge free radicals and prevent the damage caused by reactive oxygen species (ROS). Antioxidants can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells and prevent damage to lipids, proteins, enzymes, carbohydrates and DNA. Antioxidants can be classified into two major classes i.e., enzymatic and non-enzymatic. The enzymatic antioxidants are produced endogenously which includes superoxide dismutase, catalase and glutathione peroxidase. The non-enzymatic antioxidants include tocopherols, carotenoids, ascorbic acid, flavonoids and tannins which are obtained from natural plant sources[1],[2]. A wide range of antioxidants from both natural and synthetic origin has been proposed for use in the treatment of various human diseases. There are some synthetic antioxidant compounds such as butylated hydroxyl toluene(BHT), butylated hydroxyl anisole(BHA) and tertiary butyl hydroquinone which are commonly used in processed foods. However, it has been suggested that these compounds have shown toxic effects like hepatic mutagenesis[3],[4]. Flavonoids and other phenolic compounds of plant origin have been reported as scavengers of free radicals. Hence, nowadays search for natural antioxidant source is gaining much importance [5].

Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties. Plant based natural constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, seeds i.e., any part of the plant may contain active constituents[6-8]. In addition to the above compounds found in natural foods, vitamins C and E, beta-carotene and tocopherol are known to possess antioxidant potential[9]. With the background of abundant source of unique active components harbored in this plant, the present study was taken up on the objective to investigate the antioxidant activity of *S.spontaneum in vitro*[10].

S.spontaneum commonly called Kans grass belongs to family Poaceae is native to South Asia. It is a perennial grass, growing up to three meters in height, with spreading rhizomatous roots[11]. According to

Ayurveda, roots are sweet, astringent, emollient, refrigerant, diuretic, lithotriptic, purgative, tonic, aphrodisiac and useful in treatment of dyspepsia, burning sensation, piles, sexual weakness, gynecological troubles, respiratory troubles etc.

MATERIALS AND METHODS

Collection and Identification of the plant

The plant material was collected from Karimnagar District of Andhra Pradesh, South India in the month of February 2012 and was identified as *Saccharum spontaneum.* Family Poaceae.It was authenticated by Dr. K. Rama Krishna, HOD, Department of Botany, Osmania University, Hyderabad voucher (No.0928)for further reference at Malla Reddy College of Pharmacy, Secunderabad, AndhraPradesh.

Preparation of plant extract

The whole *S. spontaneum* plant was shade dried and powdered. The coarse powder (65g) obtained was extracted with methanol in Soxhlet apparatus and filtered. The extract was concentrated under reduced pressureby using a rotary evaporator.It was stored in air tight container in a refrigerator. Percentage yield was calculated to be 10%. The extract was subjected to preliminary phytochemical investigation which confirmed the presence of alkaloids, carbohydrates, steroids, glycosides, saponins, flavonoids, tanins and protiens.

The concentrated extract was weighed and dissolved in distilled water to get the desired concentrations for the experiment.Different concentrations of the extract were obtained by dissolving 5mg of methanolic extract of *S. spontaneum* in 1ml of DMSO (5mg/ml) and from this further five concentrations were made (i.e. 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, 100 µg/ml).

Chemicals

Ammonium thiocyanate, Ferrous chloride, ferric chloride ($FeCl_2$), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), EDTA, butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA), α-tocopherol, ascorbic acid, rutin, catechin, pyrocatechol, nitrobluetetrazolium(NBT), thiobarbituric acid and trichloroacetic acid were purchased from Sigma Aldrich. All other chemicals and reagents were of analytical grade.

Estimation of total phenolic content

Total phenolic content in the *S. spontaneum* extract was measured according to the method suggested by Slinkard and Singleton[13]using Folin-Ciocalteau reagent.To 0.1 ml of *S.spontaneum* extract (i.e. 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml &100µg/ml) in Erlenmeyer flask, one ml Folin-Ciocalteu reagent was added. After three minutes, 3 ml 2% Na_2CO_3 was added. Subsequently, the mixture was shaken for 2 h at room temperature and absorbance was measured using a spectrophotometer at 760 nm. All tests were performed in triplicate. The concentration of total phenolic compounds in samples was determined as µg Pyrocatechol equivalents, using the following equation obtained from a standard Pyrocatechol graph.

Absorbance = 0.001 x pyrocatechol (µg) + 0.0033

Estimation of total flavonoid content

Total flavonoids content in the extract (*S. spontaneum*) was measured using Aluminum chloride hexahydrate reagent. To 0.1 ml of *S.spontaneum* extract (i.e. 20, 40, 60, 80& 100µg/ml) 0.1ml of Aluminum chloride hexahydrate reagent was added[14].After incubation for 10 minutes at room temperature, absorbance was measured using a Spectrophotometer at 450 nm. Quantification was performed with respect to the standard curve of Rutin. Results were expressed as mg of Rutin per 100g of the extract. All tests were performed in triplicates.

DPPH free radical scavenging activity

The free radical scavenging activity of the different fractions of *S. spontaneum* was measured using DPPH, employing the method of Blois.[12]One ml of *S. spontaneum*and the reference compound in various concentrations (20, 40, 60, 80 & 100 µg/ml) was added to one ml of 0.1 mM solution of DPPH in methanol. After 30 minutes, absorbance was measured at 517 nm, using a spectrophotometer. A 0.01mM solution of DPPH in methanol was used as control, whereas Ascorbic acid was used as a reference material. All tests were performed in triplicate. Percent inhibition was calculated using following equation.

$$\% \text{ Inhibtion} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}}$$

Estimation of nitric oxide

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacted with oxygen to produce nitrite ions, which were measured using the Griess reaction.[15]Three ml of 10 mM sodium nitroprusside in phosphate buffer was added to two ml of different concentrations(i.e.20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml &100µg/ml) of *S.spontaneum*and the reference compound in different concentrations (20, 40, 60, 80, and 100 µg/ml). The resulting solutions were then incubated at 25ºC for 60 minutes. A similar procedure was repeated with methanol as a blank, which served as control. To 5 ml of the incubated sample, 5 ml Griess reagent (1% sulfanilamide, 0.1% naphthyl

ethylenediaminedihydrochloride in 2% H_3PO_4) was added. The absorbance of the chromophore formed was measured using a spectrophotometer at 546 nm. All tests were performed in triplicate. Percent inhibition of the nitric oxide generated was measured by comparing the absorbance values of control and test preparations.Curcumin was used as standard.

Superoxide radical scavenging activity

The methoddescribed by Chang *et al*.[16]was used to investigate the superoxide anion scavenging activity of different concentrations of *S.spontaneum* extract. The reaction mixture was prepared by dissolving Na_2CO_3 (0.53 gm), EDTA (0.004 gm) and xanthine in 100 ml of distilled water,10ml of NBT solution (0.025 mM) was added to the reaction mixture. From this solution, 995 µl xanthine was further added to 5µl of different concentrations (i.e. 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml & 100µg/ml) of *S.spontaneum* extractand the reference compound, in different concentrations in distilled water. After 15 minutes, the absorbance was measured using a spectrophotometer at 560 nm. The reaction mixture with xanthine oxidase was used as a control, whereas BHT was used as a reference compound. All tests were performed in triplicate. Percent inhibition was calculated by comparing the results of control and test samples.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured comparing deoxyribose and *S.spontaneum*for hydroxyl radical generated by the Fe^{3+} -ascorbate-EDTA-H_2O_2 system (Fenton reaction), using the method of Kunchandy and Rao.[17] The reaction mixture containing (1.0 ml) 100 µl 2-deoxy-2-ribose (28 mM in 20 mM phosphate buffer, pH 7.4), 500 µl of different concentrations (i.e. 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml & 100µg/ml) of *S.spontaneum* extract and the reference compound in phosphate buffer (20 mM, pH 7.4), 200 µl 1.04 mM EDTA, and 200 µM $FeCl_3$ (1:1 v/v), 100 µl 1.0 mM H_2O_2 and 100 µl 1.0 mM ascorbic acid, was incubated at 37ºC for an hour. One ml of 1% thiobarbituric acid and one ml of 2.8% trichloroacetic acid were added and incubated at 100ºC for 20 min. After cooling, absorbance was measured using a spectrophotometer at 532 nm, against a control preparation containing deoxyribose and buffer. Catechin was used as a positive control. Reactions were carried out in triplicate. Percent inhibition was determined.

Statistical Analysis

Data are mean ± SD of three measurements. Statistical analysis was performed by the Student *t*-test followed by ANOVA.$P < 0.05$ was considered as significant.

RESULTS

Total phenolic content

Several studies report the linear correlation between the phenolic content present in the sample and their antioxidant capacity[18].The total phenolic content of different concentrations of the extract was estimated using Pyrocatechol as the reference standard and was expressed as Pyrocatecholequivalents. The different concentrations (20, 40, 60, 80 & 100µg/ml) of *S.spontaneum* extract were found to have 2.96, 7.23, 16.56, 28.3, 44.5µg Pyrocatechol equivalents of phenol.

Total flavonoid content

Flavonoids are polyphenolic compounds with antioxidant properties, and several studies have shown that a high intake of flavonoids is correlated to a decrease in heart diseases and many biological effects of this classof compounds have been described *in vivo* and *in vitro* studies. The total flavonoid content of different concentrations of the extract were estimated using Rutin as the reference standard and was expressed as Rutin equivalents. The different concentrations (20, 40, 60, 80 & 100µg/ml) of *S.spontaneum* extract were found to have 0.097, 0.21, 0.38, 0.85, 2.03µg Rutinequivalents of flavonoid.

DPPH free radical scavenging activity

The DPPH radical is considered to be a model of lipophilic radical. A chain reaction in lipophilic radicals was initiated by lipid autoxidation. The scavenging effects of *S.spontaneum*and BHA on the DPPH radical are illustrated in [Table 1]. *S.spontaneum*exhibited significant scavenging effect on the DPPH radical.

Nitric oxide radical scavenging activity

The percent inhibition of nitric oxide generation by *S.spontaneum*is shown in [Table 2]. The antioxidant activity of the extract was significant when compared to Curcumin, which was used as a reference compound.

Superoxide anion radical scavenging activity

*S.spontaneum*was found to possess scavenging effects on superoxide anions, in a concentration dependent manner as mentioned in [Table 3].*S.spontaneum* exhibited significant scavenging effect on superoxide radical.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and *S.spontaneum* for hydroxyl radical generated by Ferric $^{3+}$ascorbate-EDTA-H $_2O_2$ system (Fenton reaction). *S.spontaneum*was capable of reducing DNA damage at all concentrationssignificantlywhen compared with Catechin.

DISCUSSION

Most of the mammals have inherent mechanisms to prevent and neutralize the free radical induced damage. In biochemical system, superoxide radical and H_2O_2 react together to form a singlet oxygen and hydroxyl radical, which can attack and destroy biochemicals.[19] The hydroxyl radical produced may cause sugar fragmentation, base loss and leakage of DNA strand.[20] Hydroxyl radicals are the major ROS, causing lipid oxidation and enormous biological damage[21]. It was already reported that naturally occurring phenolic compounds have free radical scavenging properties, due to thepresence of hydroxyl groups.[22]Phenolic compounds are effective hydrogen donors, which make them antioxidant.[23]FurtherPhenolic compounds are known as powerful chain breaking antioxidants.

The activity of polyphenols could be due to their ability to adsorb, neutralize and quench free radicals[24-27]. In the present study, it was found that the extract of *S. spontaneum* contains high level of phenol content that might account for the strong activity observed against H_2O_2 radicals. This scavenging activity may be due the presence of hydroxyl groups attached to the aromatic ring structure which helps to quench the radicals. Flavonoids are important secondary metabolites of plant modulating lipid peroxidation involved in atherogenesis, thrombosis and carcinogenesis. It has been confirmed that pharmacological effects of flavonoids is correlating with their antioxidant activities[28-31].

The DPPH left provides information on the reactivity of compounds with a stable free radical. DPPH gives a strong absorption band at 517nm in visible region. When the odd electron becomes paired off in the presence of a free radical scavenger the absorption reduces and the DPPH solution is decolorized as color changes from deep violet to light yellow. The degree of reduction in absorbance is reflection of the radical scavenging power of the compound. [32,33] The experimental data of the extract revealed that the extract is likely to have the effects of scavenging free radicals. From the result, in the present study a dose dependent relationship in the DPPH radical scavenging activity

CONCLUSION

The results indicate *S. spontaneum* possesses high phenolic and flavonoid contents which may attribute to antioxidant activity. It is also apparent from the present study that *S.spontaneum* scavenges the free radicals significantly. The presence of high phenolic, flavonoid compounds and potent DPPH, superoxide, nitric oxide, hydroxil radical scavenging activity attributed to *in vitro* antioxidant activity of *S.spontaneum*. From the above study it may be concluded that extract of *S.spontaneum* is a potential source of antioxidants of natural origin.

Table 1: 1,1-diphenyl-2-picryl-hydrazol (DPPH) radical scavenging activity of different concentrations of methanolic extract of *S.spontaneum* and butylatedhydroxy anisole.

Concentration used (µg/ml)	% Inhibhition	
	S.spontaneum	BHA
20	35.23±2.54*	65.22±2.24
40	45.68±4.12*	44.68±4.48
60	48.87±5.18*	78.94±5.78
80	57.27±2.24*	80.29±5.02
100	62.23±2.96*	82.59±4.12

Table 2: Inhibition % of nitric oxide radicals by different concentrations ofmethanolic extract of *S.spontaneum* and curcumin.

Concentration used (µg/ml)	% Inhibhition	
	S.spontaneum	Curcumin
20	30.24±5.12*	52.33±5.02
40	38.95±2.51*	70.19±2.54
60	43.44±2.96*	76.47±1.32
80	48.52±4.58*	80.81±8.95
100	52.36±5.11*	84.27±4.26

Table 3: Superoxide anion scavenging activity of different concentrations ofmethanolic extract of *S.spontaneum* and butylatedhydroxy toluene

Concentration used (µg/ml)	% Inhibhition	
	S.spontaneum	BHT
20	21.45±2.54*	63.56±5.11
40	35.23±3.98*	70.95±4.17
60	46.22±2.24*	75.45±5.02
80	58.52±4.12*	80.00±4.12
100	62.23±2.54*	82.12±3.21

Table 4: Hydroxyl radical scavenging activity of different concentrations ofmethanolic extract of *S.spontaneum*andcatechin on deoxyribose damage

Concentration used (µg/ml)	% Inhibhition	
	S.spontaneum	Catechin
20	38.95±4.12*	52.04±3.98
40	46.22±2.24*	64.45±2.54
60	57.45±4.19*	68.06±2.48
80	61.02±3.28*	70.22±4.28
100	64.45±2.46*	75.45±2.24

REFERENCES

1. Darshan Shankar: Field tested participatory methodology for rapid assessment of the community's therapeutic use of medicinal plants,(FRLHT), Bangalore, India: 2006. http://www.fmrindia.org/Workshop%20Proceedings.pdf
2. Approaches towards evaluation of medicinal plants prior to clinical trials: Organized by The Foundation of Medical Research at Yashada,Mumbai:2006:Pg6-13.
3. WHO: In progress Report by the Director General: 1991. Document no. A44/20, World Health Organization, Geneva.
4. Kamboj V P: Herbal Medicine. Current science: 2000; Pg:35-39.
5. Ballabh B, Chaurasia OP, Ahmed Z. Current science, 2007; l. 92(12): 1664-1665.
6. Famsworth: 1989; Harvey: 1999.
7. Akah PA, Nnaeto O, Nworu C S, Ezike AC. Res J of pharmacology.
8. Berliner JA,Heinecke JW. Free Radical Biology and Medicine. 1996; 20: 707- 727.
9. Shetgiri PP, D′Mello PM. Indian Drugs, 2003;40:567-569.
10. GurpreetKaur, Saqrwar AM, Zoobi J, Kaleem J,Mohemmad A. J Ethnopharmacology,2006;108:340-348.
11. Suresh kumar CA, Varadharajan R,Muthumani P. Int J PharmTech Research,2010; 2(1): 319-321.
12. Yen GH, Chen HY. J Agr Food Chem, 1995;43:27-32.
13. Slinkard K, Singleton VL. Am J Enol Viticulture, 1977;28:49-55.
14. Huang D, Ou B, Prior RL. J. Agric. Food Chem, 2005; 53: 1841-1856.
15. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JK, Tannenbaum SR. Anal Biochem, 1982;126:131-8.
16. Chang WS, Lin CC, Chuang SC, Chang HC. Am J Clin Med, 1996;24:11-7.
17. Kunchandy E, Rao MN. Int J Pharmacog, 1990;58:237-40.
18. Hatano T, Edamatsu R, Hiramatsu M, Mori A, Fujita Y, Yasuhara D.ChemPharma Bull, 1989;37:2016-21.
19. Ko KM, Yick PK, Chiu TW, Hui TY, Cheng CH, Kong YC. Fitotherapia, 1993;L14:539-544.
20. Aurand LW, Boonme NH, Gidding GG. J Dairy Sci, 1977;60:363-369.
21. Diplock AT. Free Radic Res, 1997;27:511-532.
22. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. Free Radic Res, 1995;22:375-383.
23. Gonzalez-Barrios J. A, Escalante B, Valdes J and Leon-Chavez BA. Brain Research,2002; 945 (1):114-122.
24. Asima C, Satyesh CP. The treatise of Indian medicinal plants. New Delhi; Publication and information directorate CSIR: 1992, 2: pp 24-26.
25. Perry LM. Medicinal Plants of East and Southeast Asia-Attributed Properties and Use. The Massachusetts Institute of Technology Press. 1980; 80—81.
26. Cheng HY, Lin TC, Yu KH, Yang CM , Lin CC. Biol. Pharm. Bulletin,2003; 26[9]:1331- 1335.
27. Toren Finkel , Nikki J Holbrook. Nature,2000; 408: 239-247.

28. Huy Lap, He H ,HuyCP. Int J Biomed Sci ,2008; 4(2): 89-96.
29. ZhengW , Wang SY. J. Agric. Food Chem, 2001;49:5165-5170.
30. Miliauskas G, Venskutonis PR , Van-Beek TA. J. Agric. Food Chem, 2004;85:231-237.
31. Yu L, Haley S, Perret J, Harris M, Wilson J and Qian M, J. Agric. Food Chem, 2002;50:1619-1624.
32. Zahin M, Aqil F, Ahmad I.Int J Pharm Pharm Sci,2009;1(1):88-95.
33. Chakraborti S, Naik AA, Reddy GR. BiochimBiophysActa, 1990;1028:89-94.