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Research Article

FREE RADICAL SCAVENGING AND TOTAL PHENOLIC CONTENT OF *SACCHARUM SPONTANEUM* L. ROOT EXTRACTS

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ABSTRACT

Saccharum spontaneum L. known as Kasa (Family: Poaceae) is a traditional herb, it have excellence medicinal value; have been advocated in the treatment gynaecological troubles, respiratory disease. Roots are used as galactagogue and diuretic and in ayurveda system roots are also used as astringent, emollient, refrigerant, diuretic, purgative, tonic, aphrodisiac and useful in treatment of dyspepsia, burning sensation, piles and sexual weakness. Antioxidants thus play an important role of protecting the human body against damage by reactive oxygen species. The methanolic extract were prepared and screened for *in-vitro* antioxidant activities using 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) scavenging activity method. Also, extract were assessed for thiocyanate, reduction potential and nitric oxide scavenging activity. The total phenolic content was found to be 351.25 ± 1.31 $\mu g\ mL^{-1}$; however the flavonoid content was 48.60 ± 2.17 $\mu g\ mL^{-1}$ for *Saccharum spontaneum* root extract. From the research, it was concluded that the antioxidant played an important role of protecting the human body against free radicals.

Keywords: Antioxidant, *Saccharum spontaneum,* thiocyanate, galactagogue.

INTRODUCTION

The several types of plant materials such as vegetables fruits leaves oil seeds cereals crops bark and roots spices and herbs and crude plant drugs are potential sources of antioxidants compounds. Most of the isolated compounds with antioxidants activity are phenolic compounds (Odukoya et al., 2005). Reactive oxygen species singlet oxygen and hydrogen peroxide are often generated as by products of biological reaction or from exogenous factors (Kikuzaki and Nakatani, 1993). The reactive species play an important role in cell metabolism, phagocytosis and intercellular signaling (Ottolenghi, 1959). However, these reactive species produced by sunlight, ultraviolet rays, ionizing radiation, chemical reactions and metabolic processes have a wide variety of pathological effects such as DNA damage, carcinogenesis and various diseases such as cardiovascular diseases, aging and neuro-degenerative diseases (Osawa, 1994). In foods, the reactive species can cause lipid peroxidation, which leads to the deterioration of the food (Miller and Rice-Evans, 1997). The oxidative deterioration of the lipid-containing food is responsible for the rancid odours and flavours during processing and storage, consequently decreasing the nutritional quality and safety of foods, due to the formation of secondary, potentially toxic compounds. The addition of antioxidant is a method for increasing the shelf life of foods (Cook and Samman, 1996). The studies have been shown that a number of plant products containing polyphenols, flavonoids, terpenes and various plant extracts exerted an antioxidant action (Zhou and Zheng, 1991).

Saccharum spontaneum L. (*S. spontaneum*) (Family- Poaceae) locally known as Kasa is a tall erect reed-like perennial grass. It is distribute throughout India (Kirtikar and Basu, 2005) and tropical Asia (Parrotta, 2001) Leaves and stalks contain lignin, carbohydrates, proteins and amino acids (Ghani, 2003). Roots and root-stocks contain starch and polyphenolic compounds. Aerial parts possess laxative and aphrodisiac properties, and are useful in burning sensations, strangury, phthisis, vesical calculi, blood diseases, biliousness and haemorrhagic diathesis (Chopra et al., 1956). The stems are useful in vitiated conditions of pitta and vata burning sensation strongly and dyspepsia, haemorrhoids, menorrhagia dysentery, agalactia phthisis and general debility (Yoganarashimhan, 2002).

MATERIALS AND METHODS

Extract preparation

Saccharum spontaneum roots were air dried at room temperature for 3 weeks to get consistent weight. The dried roots were later ground to crude powder. Two hundred grams of crude powder plant material were shaken separately in ethanol for 24 hrs on an orbital shaker at room temperature. Extracts were filtered using a Buckner funnel and Whatman No. 1 filter paper. Each filtrate was concentrated to dryness under reduced pressure at 40°C through evaporator.

Chemicals

Ascorbic acid, gallic acid, 1, 1-Diphenyl-2-picrylhydrazyl (DPPH), ferric thiocyanate, nitric oxide, aluminium chloride and sodium nitrite rutin from purchased Qualigens. Folin-Ciocalteus's phenol reagent and sodium carbonate were from Merck chemical supplies (Damstadt, Germany). All the other chemicals used including the solvents, were of analytical grade purchased from SD fine and Qualigens manufacturing Ltd.

Determination of total phenolic content

Total phenolic contents in the extracts were determined by the modified Folin-Ciocalteu method (Wolfe et al., 2003). Absorbance was measured at 765 nm using UV-VIS spectrophotometer. Total phenolic content was expressed as mg/g tannic acid equivalent (mg/g).

Determination of total flavonoid content

Total flavonoid contents were determined using the method (Ordonez at al., 2006). The absorbance was measured at 420 nm a yellow colour indicated the presence of flavonoids.

Determination of *in-vitro* antioxidant activities

DPPH free radical scavenging activity

The DPPH assay is based on the measurement of the scavenging ability of an antioxidant using the stable DPPH radical (Liyana-Pathirana and Shahidi, 2005). The absorbance is measured at 490 nm. DPPH radical scavenging activity (%) = {(Abscontrol – abssample)}/ (Abscontrol) × 100 where Abscontrol is the absorbance of DPPH radical + ethanol; Abssample is the absorbance of DPPH radical + sample extract/standard.

Nitric oxide scavengering activity

The ethanolic extract was dissolved in PBS in different concentration and sodium nitroprusside was at added in each test tube were incubated at 300C for 5h (Sreejayan and Rao, 1997). The absorbance was measured at 546 nm.

Reducing potential scavengering activity

The reducing capability of the sample extracts was measured by transformation of Fe^{+3} to Fe^{+2} in the presence of the extracts at 700 nm (Oyaizu, 1986). The absorbance was measured at 700 nm. Increase absorbance of the reaction mixture indicates increase reducing power. Gallic acid was used as standard.

Ferric thiocyanate scavenging activity

The ferric thiocyanate method in a linoleic acid emulsion was used (Haraguchi et al., 1992). The absorbance of the red-colour portion of the sample was measured at 500 nm.

Statistical analysis

The experimental results were expressed as mean ± standard error of mean (SEM) of three replicates. IC_{50} was calculated.

RESULTS

Preliminary phytochemical studies

Table 2 showed that the extractive value were found to be 2%, 0.35%, 0.45%, 4.5% and 5.2% in petroleum, chloroform, ethyl acetate, alcohol and aqueous extract respectively. Preliminary phytochemical analysis studied (Table 1) the absence (indicated by – mark) of

gums and mucilage in all solvents whereas carbohydrates, glycoside, flavonoids and amino acid were present (indicated by + mark) in alcoholic and aqueous extracts (Mukherjee, 200; Khandelwal, 2008).

Total phenolic and flavonoids contents

The total phenolic and flavonoids content in *S. spontaneum* root extract were showing higher level of phenolic and flavonoid compounds (351.25 ± 1.31 and 48.60 ± 2.17 $\mu g/mL$) in the methanolic extract. The maximum absorbance of 1.01 nm was observed at a concentration of 350 mcg/ml of extract equivalent to gallic acid which gave an absorbance of 0.99 μg/ml at a concentration of 350 nm and flavonoid observed in this plant 48 μg/ml which is equivalent to 400 μg/ml of rutin standard.

Effect of DPPH radical scavenging activity

DPPH radical scavengering activity of *S. spontaneum* root of methanolic extract compared with ascorbic acid. Fig 1 was showed that the *S. spontaneum* had DPPH inhibition free radical activity was 91.23 ± 1.12 at 1.50 mg/mL and IC_{50} was 0.17mg/ml of the methanolic extract.

Effects of Nitric oxide scavengering activity

The result indicated that the root extract might contain compounds able to inhibit nitric oxide and offers scientific evidence for the indigenous system in inflammatory condition. The nitric oxide scavengering activity of *S. spontaneum* root of methanolic extract was showed 90.17 ± 1.14 % at 150 mg/ml (Fig 2) and IC50 was 62.5 mg/ml.

Effect of reduction potential activity

The result shows that *S. spontaneum* root consist of poly phenolic compounds that showed the greater reducing power capacity. The reduction activity Fe^{+3} to Fe^{+2} transformations of ions were found to increase with increasing the concentration of the extracts. Fig 3 had shown that the *S. spontaneum* methanolic root extract maximum absorbance 0.314 nm was obtained at concentration 120mg/ml of extract. Gallic acid used as standard which give the maximum absorbance 0.319 nm was obtained at concentration 100 mg/ml.

Effect of ferric thiocyanate activity

The ferric thiocyanate method activity of methanolic plant extract studied. The FTC method measures the amount of peroxide value in the beginning of the lipid per oxidation. The percentage inhibitory potential of *S. spontaneum* methanolic root extract had maximum 90.72 ± 1.19 at 130 mg/ml (Fig 4) and IC_{50} was 72mg/ml.

DISCUSSIONS

Total phenolic and flavonoids contents

The antioxidant activity mainly due to the redox properties (Zheng and Wang, 2001) which showed an important activity in adsorbing and neutralizing free radicals, entrapments of singlet and triplet oxygen or oxidising peroxides. The results from this study suggested that phenolic and flavonoid contents (351.25 ± 1.31 and 48.60 ± 2.17 $\mu g/mL$) are important components of these plants.

DPPH radical scavenging activity

DPPH scavenging activity between total phenolic and reductive potential and due to the donating ability of hydrogen (Miliauskas, et al., 2004) Which could serve as free radical inhibitors Although the DPPH scavenging activity of the extracts were significantly lower than those of ascorbic acid but it was evident that the extract did show the proton-donating ability and could serve as free radical inhibitors possibly as primary antioxidants. The solubility of the extract in different testing system has been reported to affect the capacity of extracts to react and entrap different radicals (Yu et al., 2002). The antioxidant potential on plants has been found a correlation between the phenolic content and the antioxidant activity (Zahin, et al., 2009). The antioxidant potential of plants have the lower DPPH scavenging activity, percentage inhibition of *S. spontaneum* have maximum DPPH scavenging (91.23 ± 1.12 % at 1.50 mg/ml) activity. Study showed that the capability of the extracts to different scavengering free radicals in different systems, indicating that they may be useful therapeutic agents for treating radical-related pathological damage.

Nitric oxide scavengering activity

Nitric oxide showed a potent mediator in physiological process mainly smooth muscle relaxant, inhibition of platelet aggregation and regulation of toxicity through cell. It is a diffusible free radical which showed activity as an effectors molecule in biological systems

including vasodilatation, antimicrobial and antitumor activities (Miller et al., 1993). Although nitric oxide free radicals are involved in defence mechanism over production of these free radicals contributes to the pathogenesis of some inflammatory diseases (Guo et al., 1999). Fig 2 showed that the maximum NO % inhibition scavenging potential was 90.17 ± 1.14 % in *S. spontaneum* root extract than other NO % inhibition activity in plants. This result indicated that the root extract might contain compounds able to inhibit nitric oxide and offers scientific evidence for the indigenous system in inflammatory condition.

Reducing power activity

Reducing power was measured the reductive ability of antioxidant, and transformation of Fe^{+3} to Fe^{+2} in the presence of the extract (Gulcin et al., 2003). The activity of antioxidants has been indicate to various mechanisms such as inhibition of chain initiation, binding of ion catalysts, decomposition of peroxides, reductive capacity and radical scavenging (Yildirim et al., 2000). The various studied done on plants showed lesser concentration of peroxide whereas Fig 3 had shown that the *S. spontaneum* methanolic root extract maximum absorbance 0.314 nm was obtained at concentration 120 mg/ml 0f extract. Gallic acid used as standard which give the maximum absorbance 0.319 nm was obtained at concentration 100 mg/ml.

Ferric thiocyanate scavenging

The ferrous chloride reacts with peroxide molecule to produced ferric chloride, which is reacting with ammonium thiocyanate to form ferric thiocyanate reddish colour pigment. *S. spontaneum* root extract had maximum thiocyanate inhibition activity (90.72 ± 1.19) (Fig. 4). The changes in absorbance of extract observed the reduction of peroxide at the initial stages of linoleic acid oxidation. The phenolic compounds donate H^+ ion and can cease the free radical reaction of stable compounds (Farag et al., 1989).

CONCLUSION

The present study showed that the antioxidant activities of the extracts of *S. spontaneum* root showed approximate similar activity as those of the standard drugs used in this experiment; the present results indicate that the *S. spontaneum* root extracts possess antioxidant properties and could serve as free radical scavenging activity, acting possibly as primary antioxidants. *S. spontaneum* root extract showed maximum inhibitory concentration than other research on antioxidant scavenging potential. This study has to some extent validated the medicinal potential of the root extract of *S. spontaneum.*

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Table 1: Qualitative Chemical analysis of *S. spontaneum* root extract

Test	Pet ether extract	Chloroform extract	Ethyl acetate extract	Alcoholic extract	Aqueous extract
Carbohydrate	-	-	-	+	+
Glycoside	-	-	-	+	+
Tannin	-	-	-	+	+
Flavonoid	-	-	-	+	+
Protein	-	-	-	+	+
Gum	-	-	-	-	-
Mucilage	-	-	-	-	-

(-): Absent; (+): Present

Table 2: Successive extraction of *S. spontaneum* root extract

Fractions	Extractive value (%)
Petroleum ether	0.40
Chloroform	0.44
Ethyl acetate	0.24
Alcohol	5.04
Aqueous	4.16

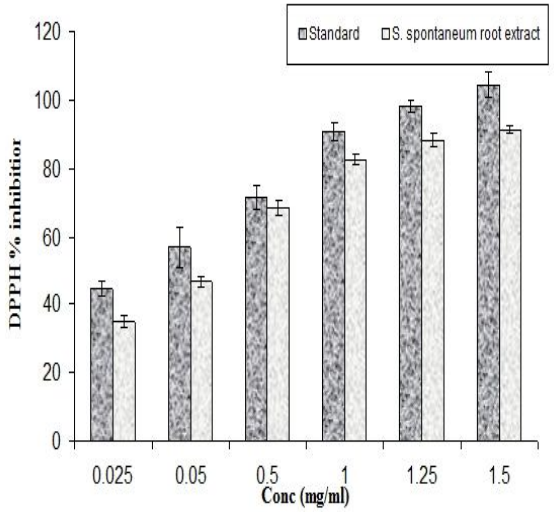

Fig. 1: DPPH scavenging activity of *S. Spontaneum*

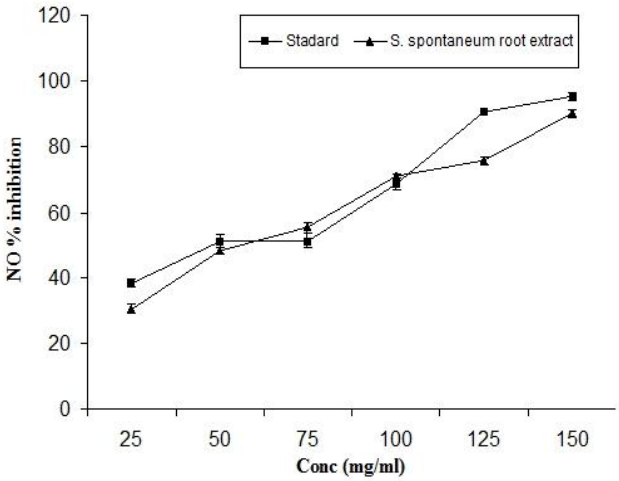

Fig. 2: NO scavenging activity of *S. spontaneum*

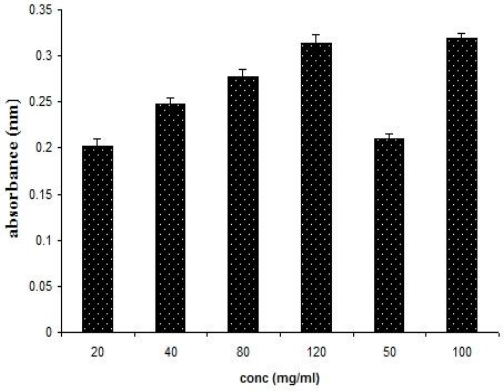

Fig. 3: Reducing Potential of *S. Spontaneum*

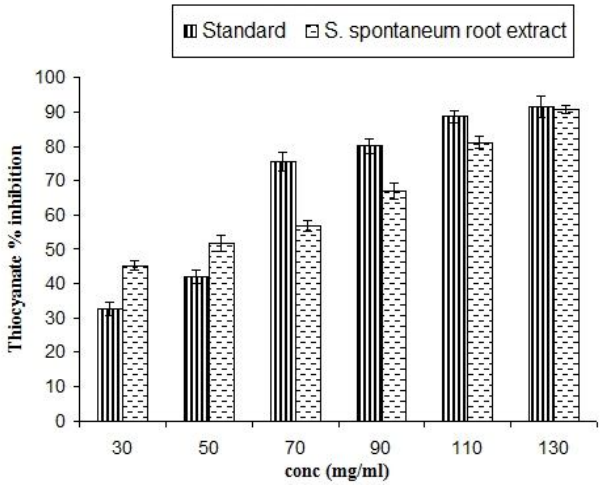

Fig. 4: Thiocyanate scavenging activity *S. spontaneum*

REFERENCES

1. Odukoya OA, Jenkins MO, Ilori MOO and Sofidiya MO. The use of selected Nigerian natural products in management of environmentally induced skin damage. Pak J Biol Sci. 2005a;8:1074-1077.
2. Kikuzaki H and Nakatani. Antioxidant effects of some ginger constituents. J Food Sci. 1993;58(6):1407-1410.
3. Ottolenghi A. Interaction of ascorbic acid and mitochondria lipids. Arch Biochem Biophy. 1959;79:355-363.
4. Osawa T. Postharvest biochemistry. In: Uritani I, Garcia VV, Mendoza, EM, editors. Novel neutral antioxidant for utilization in food and biological systems. Japan: Japan Scientific Societies Press, 1994:241-251.
5. Miller NJ and Rice-Evans CA. The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidants to the activity of orange and apple fruit juices and black currant drink. Food Chem. 1997;60:331-337.
6. Cook NC and Samman S. Flavonoids-chemistry, metabolism, cardioprotective effect and dietary sources. J Nutr Biochem. 1996:7:66-76.
7. Zhou YC and Zheng RL. Phenolic compounds and an analogue as superoxide anion scavengers and antioxidants. Bioch Pharm. 1991;2:1177-1179.
8. Kirtikar KR and Basu BD. Indian medicinal plants. International Book Distributor, Dehradun, India. 2005;2668.
9. Parrotta JA. Healing Plants of Peninsular India. CABI publishing, USA. 2001:591.
10. Ghani A. Medicinal plants of Bangladesh with chemical constituents and uses, 2nd ed, The Asiatic society of Bangladesh, Dhaka. 2003;369
11. Chopra RN, SL Nayar and Chopra IC. Glossary of Indian Medicinal Plants. CSIR, New Delhi. 1956;1-259
12. Yoganarashimhan SN. Medicinal Plants of India. Vol. 2, 2002;474-475.
13. Wolfe K, Wu X and Liu R. Antioxidant activity of apple peels. J Agric Food Chem. 2003;51:609-614.
14. Ordonez AAL, Gomez JD, Vattuone MA and Isla MI. Antioxidant activities of *Sechium edule* (Jacq.) Swart extracts. Food Chem. 2006;97:452-458.
15. Liyana-Pathirana CM and Shahidi F. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. J Agric Food Chem. 2005;53:2433-2440.
16. Sreejayan and Rao MN. Nitric oxide scavenging by curcuminoids. J Pharm Pharmacol.49:105-107.

17. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Jpn J Nutr. 1986;44:307-315.
18. Haraguchi H, Hashimoto K and Yagi A. Antioxidative substances in leaves of *Polygonum hydropiper*. J Agric Food Chem. 1992;40:1349-1351.
19. Mukherjee PK. Quality control of herbal drugs. Business horizons Pharmaceutical Publishers, (New Delhi. India). 2007;186.
20. Khandelwal KR. Practical Pharmacognosy. Techniques and experiments, Nirali Prakashan. 2008;149.
21. Zheng W and Wang SY. Antioxidant activity and phenolic compounds in selected herbs. J Agric Food Chem. 2001;49:5165-5170.
22. Miliauskas G, Venskutonis PR, and Van-Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem. 2004;85:231-237.
23. Yu L, Haley S, Perret J, Harris M, Wilson J and Qian M. Free radical scavenging properties of wheat extracts. J Agric Food Chem. 2002;50:1619-1624.
24. Zahin M, Aqil F and Ahmad I. The *in vitro* antioxidant activity and total phenolic content of four Indian medicinal plants. Intern. J Pharmacy and Pharma Sci. 2009;1(1):88-95.
25. Miller MJ, Sadowska-krowicka H, Chotinaruemol S, Kakkis JL and Clark DA. Amelioration of chronic ileitis by nitric oxide synthase inhibition. J Pharmacol Exper Ther. 1993;264:11-16.
26. Guo X, Wang WP, Ko JK and Cho CH. Involvement of neutrophils and free radicals in the potentiatimg effects of passive cigarette smoking on inflammatory bowl disease in rats. Gastroenterology. 1999;117(4):884-892.
27. Gulcin I, Oktay M, Kıreçci E and Küfrevio_lu I. Screening of antioxidant and antimicrobial activities of anise (*Pimpella anisum* L.) seed extracts. Food Chem. 2003;83:371-382.
28. Yildirim A, Mavi A, Oktay M, Kara AA, Algur OF and Bilaloglu V. Comparison of antioxidant and antimicrobial activities of tilia (*Tilia argentea* Desf ex DC), sage (*Salvia triloba* l.), and black tea (*Camellia sinensis*) extracts. J Agric Food Chem. 2000;48:5030-5034.
29. Farag RS, Badei AZMA, Hawed FM and El-Baroty GSA. Antioxidant activity of some spice essential oils on linoleic acid oxidation in aqueous media. J Amer Oil Chem Soc. 1989;66:793-799.