

Evaluation of *In-Vitro* free Radical Scavenging Potential of Whole Plant of *Saccharum spontaneum* (Linn).

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Abstract: The antioxidant activity of whole plant of *Saccharum spontaneum* (Linn) was investigated in various *in-vitro* methods. The antioxidant activity was evaluated by total antioxidant activity (Phosphomolybdic acid method), FRAP assay with reference standard ascorbate and total flavonoids content respectively. The ethanolic extract of *Saccharum spontaneum* was found to moderate effect in the total antioxidant activity. The IC₅₀ values of the ethanolic extract of *Saccharum spontaneum* and ascorbate were found to be 488µg/ml and 410µg/ml respectively. The ethanolic extract of *Saccharum spontaneum* was found moderate effective in FRAP assay. But when compare to the ethanolic extract with ascorbate (standard), the ethanolic extract of the *Saccharum spontaneum* showed the better result. The ethanolic extract of *Saccharum spontaneum* contains high amount of flavonoids. Moreover, the results were observed in a concentration dependent manner. All the above *in-vitro* studies clearly indicate that the ethanolic extract of *Saccharum spontaneum* has a better antioxidant activity. These *in-vitro* assays indicate that this plant extract is a better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Key words: *Saccharum spontaneum*, *In-vitro* antioxidant, Total antioxidant activity, FRAP assay, Total flavonoids.

Introduction

Oxygen free radicals are formed in tissue cells of our body by many endogenous and exogenous causes such as metabolism, chemicals, and ionizing radiation¹. These oxygen free radicals may attack lipids and DNA giving rise to a huge number of damaged products². Iron is known to be associated in the production of reactive oxygen species (ROS) and in the formation of highly toxic hydroxyl radicals from other active oxygen species such as hydrogen peroxide^{3,4}. The improved generation of ROS *in-vivo* could be quite deleterious, since they are associated in mutagenesis, apoptosis, ageing, and carcinogenesis⁴.

Antioxidant plays an essential role in the prevention of human diseases. Naturally occurring antioxidants in leafy vegetables and seeds, like ascorbic acid, vitamin E and phenolic compounds, possess the ability to diminish the oxidative damage associated with numerous diseases, including cancer, cardiovascular disease, cataracts, atherosclerosis, diabetes, arthritis, immune deficiency diseases and ageing^{5, 6, 7}. Current reports indicated that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases⁸. Therefore, many researchers have concentrated on natural antioxidants and in the plant kingdom indefinitely crude extracts and pure natural compounds were previously reported to have antioxidant properties.

Saccharum spontaneum (Linn).Synonyms, Ahlek, loa, wild cane, wild sugarcane, Family: Poaceae. In India, it is considered as valuable aromatic plant in traditional systems of medicine. It is popular folk medication. The whole plant used to treat diseases such as vomiting, mental diseases, abdominal disorders, dyspnoea, anaemia, and obesity. The rural public use the fresh juice of the stem of *Saccharum spontaneum* plant for the treatment of mental illness and mental disturbances. The stems are also useful for renal and vesicol calculi dyspepsia, haemorrhoids, menorrhagia dysentery, agalactia phthisis and general debility. The roots are sweet, astringent, emollient, refrigerant, diuretic, lithontriptic, purgative, tonic, aphrodisiac and useful in the treatment of dyspepsia, burning sensation, piles, sexual weakness, gynaecological troubles and respiratory troubles etc⁹. Leaves are employed for cathartic and diuretics¹⁰. However, the plant is reported to possess the activities like anti-diarrhoeal¹¹, CNS depressant¹² and antiurolithiatic activity¹³. However, no data are available in the literature on the antioxidant activity of whole plant of *Saccharum spontaneum*.

However, no data are available in the literature on the antioxidant activity of whole plant of *Saccharum spontaneum*. Therefore we undertook the present investigation to examine the antioxidant activities of ethanolic extract of whole plant of *Saccharum spontaneum* through various *in-vitro* models.

Material and Methods

Collection and Identification of Plant materials

The whole plant of *Saccharum spontaneum* (Linn), were collected from Cheranmahadevi, Tirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The whole plant of *Saccharum spontaneum* (Linn), were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extract

The above powdered materials were successively extracted with ethanol by hot continuous percolation method in Soxhlet apparatus¹⁴ for 24 hrs. The extract was concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Evaluation of Antioxidant activity by *in-vitro* Techniques:

Total antioxidant activity (Phosphomolybdic acid method)¹⁵

The antioxidant activity of the ethanolic extract was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex (Prieto et al., 1999)¹⁵. An aliquot of 0.4 ml of ethanolic extract solution was mixed in a vial with 4 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95⁰C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was read at 695 nm against a blank. The antioxidant activity was expressed relative to that of ascorbic acid.

FRAP assay¹⁶

A modified method of Benzie and Strain (1996)¹⁶ was adopted for the FRAP assay. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM FeCl₃. 6H₂O. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml FeCl₃. 6H₂O. The temperature of the solution was raised to 37⁰ C before using. Ethanolic extract (0.15 ml) was allowed to react with 2.85 ml of FRAP solution for 30 min in the dark condition. Readings of the colored product (Ferrous tripyridyltriazine complex) were measured at 593 nm. The standard curve was linear between 200 and 1000 μM FeSo₄. Results were expressed in μM (Fe (II) /g dry mass and compared with that of ascorbic acid.

Total flavonoids¹⁷

0.2g of the ethanolic extract was ground with ethanol-water in 2 different ratios namely 9:1 and 1:1 respectively. The homogenate was filtered and these 2 ratios were combined. This was evaporated to dryness until most of the ethanol has removed. The resultant aqueous extract was extracted in a separating funnel with hexane or chloroform. The solvent extracted aqueous layer was concentrated 0.5 ml of aliquot of extract was

pipette-out in a test tube. 4 ml of the vanillin reagent (1% vanillin in 70% conc. H₂SO₄) was added and kept in a boiling water bath for 15 mins. The absorbance was measured at 360 nm. A standard was run by using catechol (110 µg/ml).

Results and Discussion

Antioxidant compounds may act as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation¹⁸. Most of the plants are reported to possess antioxidant and free radical scavenging activity due to presence of phenolic compounds and flavonoids as major constituents¹⁹. Therefore, the importance of search for natural antioxidants has increased in the current years so many researchers concentrated the same²⁰.

Total antioxidant activity (Phosphomolybdic acid method)

The percentage of total antioxidant activity of ethanolic extract of *Saccharum spontaneum* presented in Table 1. The ethanolic extract of *Saccharum spontaneum* exhibited a maximum total antioxidant activity of 69.67 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 % at 1000 µg/ml. The IC₅₀ of the ethanolic extract of *Saccharum spontaneum* and ascorbate were found to be 488µg/ml and 410µg/ml respectively.

Table 1: Total antioxidant activity of Ethanolic extract of *Saccharum spontaneum* (Linn).

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethanolic extract)	Standard (Ascorbate)
1	125	21.48 ± 0.044	26.87 ± 0.076
2	250	34.60 ± 0.065	30.30 ± 0.054
3	500	49.18 ± 0.046	60.64 ± 0.022
4	1000	69.67 ± 0.024	55.23 ± 0.014
		IC₅₀ = 488 µg/ml	IC₅₀ = 410 µg/ml

*All values are expressed as mean ± SEM for three determinations

Based on the results clearly indicated the ethanolic extract of *Saccharum spontaneum* was found to moderate effective. But when compare the extract with standard the ethanolic extract of *Saccharum spontaneum* was found moderate antioxidant activity. The IC₅₀ of the ethanolic extract of *Saccharum spontaneum* and ascorbate were found to be 488µg/ml and 410µg/ml respectively.

FRAP assay

The antioxidant potential of *Saccharum spontaneum* was ascertained from FRAP assay based on their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II). The reducing ability of the ethanolic extract of *Saccharum spontaneum* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values are presented in Table 2. The maximum reducing ability at 1000µg/ml for ethanolic extract and ascorbate were found to be 75.34% and 98.07% respectively. The IC₅₀ values of ethanolic extract and ascorbate were recorded as 245µg/ml and 50µg/ml respectively.

Table 2: FRAP assay of Ethanolic extract of *Saccharum spontaneum* (Linn).

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethanolic extract)	Standard (Ascorbate)
1	125	34.78 ± 0.034	72.04 ± 0.014
2	250	49.83 ± 0.038	82.05 ± 0.034
3	500	62.23 ± 0.042	86.04 ± 0.026
4	1000	75.34 ± 0.054	98.07 ± 0.041
		IC₅₀ = 245 µg/ml	IC₅₀ = 50 µg/ml

*All values are expressed as mean ± SEM for three determinations

Based on the above results indicated, the ethanolic extract of *Saccharum spontaneum* was found to moderate effective when compare to the ethanolic extract with standard ascorbate.

Total flavonoids

Flavonoids present in food of plant origin are also potential antioxidants^{21,22}. Most beneficial effects of flavonoids are attributed to their antioxidant and chelating abilities²³. The total amount of flavonoids content of ethanolic extract of whole plant of *Saccharum spontaneum* is presented in Table 3.

Table 3: The total flavonoids content of ethanolic extract of whole plant of *Saccharum spontaneum* (Linn).

S.No	Extract	Total flavonoids content (mg/g) (\pm SEM)*
1	Ethanolic extract of <i>Saccharum spontaneum</i>	3.279 \pm 0.032

*All values are expressed as mean \pm SEM for three determinations

Based on the result the ethanolic extract of *Saccharum spontaneum* was found higher content of flavonoids.

Conclusion

The present study was clearly indicated the ethanolic extract of *Saccharum spontaneum* showed moderate antioxidant activity by total antioxidant activity and FRAP assay when compared with standard ascorbate. In addition, the ethanolic extract of *Saccharum spontaneum* was found to contain a noticeable amount of flavonoids, which play a major role in controlling antioxidants. Therefore, further inquiries need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

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