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Antioxidant activities and total flavonoids content of various extracts from aerial parts of *Saccharum spontaneum* (*Linn*.)

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ABSTRACT

The main aim of the present investigation was to evaluate the antioxidant activity of various extracts from aerial parts of *Saccharum spontaneum.* Determination of antioxidant properties evaluated by phosphomolybdic acid method and FRAP assay. And other phytochemicals such as tannins, saponins, flavonoids, terpenoids, glycosides, steroids and carbohydrates were revealed and confirmed by the phytochemical analysis of this extract. Our results indicate that petroleum ether extract of *Saccharum spontaneum* has IC_{50} value of 1010µg/mL and 410µg/mL respectively. The IC_{50} values of the ethyl acetate extract of *Saccharum spontaneum* has and ascorbate were found to be 590µg/mL and 410µg/mL respectively. The IC_{50} of the methanolic extract of *Saccharum spontaneum* and ascorate were found to be 300µg/mL and 410µg/mL respectively. 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 methanolic extract of *Saccharum spontaneum* was found higher content of flavonoids component among them. 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.

Keywords: Antioxidant, Ferric reducing antioxidant power assay (FRAP), Phosphomolybdic acid method, Total flavonoids.

1. INTRODUCTION

Oxidative stress is an important risk factor in the pathogenesis of numerous chronic diseases. Free radicals and other reactive oxygen species are recognized as agents involved in the pathogenesis of sicknesses such as asthma, inflammatory arthropathies, diabetes, Parkinson's and Alzheimer's diseases, cancers as well as atherosclerosis. Reactive oxygen species are also said to be responsible for the human aging [1, 2]. An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule[3]. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases[4]. Herbal plants considered as a higher resource of biologically active compounds known as phytochemicals. The phytochemicals have been found to act as an antioxidants by scavenging free radicals, and several have restorative potential for free radical related disorders[5,6]. Plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are affluent in antioxidant activity[7,8]. The uses of medicinal plants as traditional medicine is broad spread and signify a huge resource of natural anti-oxidants that might provide a lead for the improvement of the novel drugs. In recent times, much attention has been directed towards the progress of ethno-medicines with powerful antioxidant properties[9]. *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 aerial parts 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, respiratory troubles etc[10]. Leaves are employed for cathartic and diuretics[11]. However, the plant is reported to possess the activities like antidiarrhoeal[12], CNS depressant[13] and antiurolithiatic activity [14]. However, no data are available in the literature on the antioxidant activity of aerial parts of *Saccharum spontaneum*. Therefore we undertook the current investigation to examine the antioxidant activities of various extracts from aerial parts of *Saccharum spontaneum* by phosphomolybdic acid method and FRAP assay.

2. EXPERIMENTAL

2.1. Collection and Identification of Plant materials

The aerial parts 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 aerial parts of *Saccharum spontaneum* (Linn.), were dried out underneath shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve. It was then stored in a cool place until further use.

2.2. Chemicals

All chemicals used for the current study were of analytical grade and purchased from Sigma, USA and SD Fine, India.

2.3. Preparation of extracts

The *S.spontaneum* plant powered materials were successively extracted with petroleum ether (40-60˚C) by hot continuous percolation method in Soxhlet apparatus[15] for 24 hrs. Then the marc was subjected to ethyl acetate (76-78°C) for 24 hrs and then marc was subjected to methanol for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

2.4. Evaluation of in vitro antioxidant activity of various extracts of aerial parts of *saccharum spontaneum*

2.4.1. Total antioxidant activity (Phosphomolybdic acid method)

The antioxidant activity of the sample was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex[16]. An aliquot of 0.4 mL of sample solution was combined 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 95˚C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expresses relative to that of ascorbic acid.

2.4.2. Frap assay

A modified method of Benzie and Strain[17] 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 $Fecl_3$. $6H_2O$. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 ml TPTZ and 2.5 mL $Fecl_3$. $6H_2O$. The temperature of the solution was raised to 37˚C before using. Plant extracts (0.15 mL) were 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 taken at 593 nm. The standard curve was linear between 200 and 1000 µM FeSO4. Results are expressed in µM (Fe (II) /g dry mass and compared with that of ascorbic acid.

2.4.3. Estimation of total flavonoids[18]

0.2g of the plant material 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. H_2SO_4) was added and kept in a boiling water bath for 15 mins. The absorbance was read at 360 nm. A standard was run by using catechol (110 µg/mL).

3. RESULTS AND DISCUSSON

3.1. Total antioxidant activity (Phosphomolybdic acid method)

The percentage of total antioxidant activity of petroleum ether extract of *Saccharum spontaneum* presented in Table 1. The petroleum ether extract of *Saccharum spontaneum* exhibited a maximum total antioxidant activity of 51.33% at 1000µg/mL whereas for ascorbate (standard) was found to be 65.23% at 1000µg/mL. The IC_{50} values of the petroleum ether extract of *Saccharum spontaneum* and ascorbate were found to be 1010µg/mL and 410µg/mL respectively.

Table - 1: Total antioxidant activity of petroleum ether extract of *Saccharum spontaneum* by phosphomolybdic acid method

Concentration (µg/mL)	% of activity(±SEM)*	
	Sample (Pet. ether extract)	Standard (Ascorbate)
125	15.38 ± 0.032	26.87 ± 0.076
250	29.54 ± 0.044	30.30 ± 0.054
500	46.11 ± 0.022	60.64 ± 0.022
1000	51.33 ± 0.062	65.23 ± 0.014
	IC_{50} = 1010 µg/mL	**IC_{50} = 410 µg/mL**

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

Table - 2: Total antioxidant activity of ethyl acetate extract of *Saccharum spontaneum* by Phosphomolybdic acid method

Concentration (µg/mL)	% of activity(±SEM)*	
	Sample (Ethyl acetate extract)	Standard (Ascorbate)
125	20.56 ± 0.024	26.87 ± 0.076
250	30.48 ± 0.062	30.30 ± 0.054
500	48.72 ± 0.048	60.64 ± 0.022
1000	66.28 ± 0.064	65.23 ± 0.014
	IC_{50} = 590 µg/mL	**IC_{50} = 410 µg/mL**

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

The percentage of total antioxidant activity of ethyl acetate extract of *Saccharum spontaneum* presented in Table 2. The ethyl acetate extract of *Saccharum spontaneum* exhibited a maximum total antioxidant activity of 66.28% at 1000µg/mL whereas for ascorbate (standard) was found to be 65.23% at 1000 µg/mL. The IC_{50} values of the ethyl acetate extract of *Saccharum spontaneum* and ascorbate were found to be 590µg/mL and 410µg/mL respectively.

The percentage of total antioxidant activity of methanolic extract of *Saccharum spontaneum* presented in Table 3. The methanolic extract of *Saccharum spontaneum* exhibited a maximum total antioxidant activity of 72.26% at 1000 µg/mL whereas for ascorbate (standard) was found to be 65.23% at 1000 µg/mL. The IC_{50} of the methanolic extract of *Saccharum spontaneum* and ascorbate were found to be 300µg/mL and 410µg/mL respectively.

Table - 3: Total antioxidant activity of methanolic extract of *Saccharum spontaneum* by Phosphomolybdic acid method

Concentration (µg/mL)	% of activity(±SEM)*	
	Sample (Methanolic extract)	Standard (Ascorbate)
125	34.32 ± 0.032	26.87 ± 0.076
250	44.58 ± 0.065	30.30 ± 0.054
500	68.10 ± 0.024	60.64 ± 0.022
1000	72.26 ± 0.068	65.23 ± 0.014
	IC_{50} = 300 µg/mL	**IC_{50} = 410 µg/mL**

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

Based on the result clearly indicated the methanolic extract of *Saccharum spontaneum* was found to more effective than petroleum ether and ethyl acetate extract. But when compare all the extracts with standard the methanolic extract of *Saccharum spontaneum* was found strong antioxidant activity. The IC_{50} of the methanolic extract of *Saccharum spontaneum* and Ascorbate were found to be 300µg/mL and 410µg/mL respectively

3.2. 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 petroleum ether extract of *Saccharum spontaneum* and ascorbate at various concentrations (125, 250, 500, 1000 µg/mL) were examined and the values were presented in Table 4. The maximum reducing ability at 1000µg/mL for petroleum ether extract and ascorbate were

found to be 44.34% and 98.07% respectively. The IC_{50} values of petroleum ether extract and ascorbate were recorded as 1132µg/mL and 50µg/mL respectively.

Table - 4: Reducing ability of pet.ether extract of *Saccharum spontaneum* on FRAP assay

Concentration (µg/mL)	% of activity(±SEM)*	
	Sample (Pet. ether extract)	Standard (Ascorbate)
125	20.98± 0.040	72.04 ± 0.014
250	25.32± 0.066	82.05 ± 0.034
500	35.67± 0.042	86.04 ± 0.026
1000	44.34± 0.038	98.07 ± 0.041
	IC_{50} = 1132 µg/mL	**IC_{50} = 50 µg/mL**

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

The reducing ability of the ethyl acetate extract of *Saccharum spontaneum* and ascorbate at various concentrations (125, 250, 500, 1000 µg/mL) were examined and the values were presented in Table 5. The maximum reducing ability at 1000µg/mL for ethyl acetate extract and ascorbate were found to be 60.09% and 98.07% respectively. The IC_{50} values of ethyl acetate extract and ascorbate were recorded as 422µg/mL and 50µg/mL respectively.

Table - 5: Reducing ability of ethyl acetate extract of *Saccharum spontaneum on* FRAP assay

Concentration (µg/mL)	% of activity(±SEM)*	
	Sample (Ethyl acetate extract)	Standard (Ascorbate)
125	38.84 ± 0.034	72.04 ± 0.014
250	44.96 ± 0.022	82.05 ± 0.034
500	54.68 ± 0.046	86.04 ± 0.026
1000	60.09 ± 0.058	98.07 ± 0.041
	IC_{50} = 422 µg/mL	**IC_{50} = 50 µg/mL**

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

The reducing ability of the methanolic extract of *Saccharum spontaneum* and ascorbate at various concentrations (125, 250, 500, 1000 µg/mL) were examined and the values were presented in Table 6. The maximum reducing ability at 1000µg/mL for methanolic extract and ascorbate were found to be 76.26% and 98.07% respectively. The IC_{50} values of methanolic extract and ascorbate were recorded as 228µg/mL and 50µg/mL respectively.

Table - 6: Reducing ability of methanolic extract of *Saccharum spontaneum* on FRAP assay

Concentration (µg/mL)	% of activity(±SEM)*	
	Sample (Methanolic extract)	Standard (Ascorbate)
125	35.02 ± 0.028	72.04 ± 0.014
250	53.54 ± 0.042	82.05 ± 0.034
500	64.48 ± 0.066	86.04 ± 0.026
1000	76.26 ± 0.050	98.07 ± 0.041
	IC_{50} = 228 µg/mL	**IC_{50} = 50 µg/mL**

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

Based on the above results indicated, the methanolic extract of *Saccharum spontaneum* was found to most effective than that of petroleum ether & ethyl acetate extract. But when compare to the all the three extracts with ascorbate (standard), the ethyl acetate extract of the *Saccharum spontaneum* showed the moderate result.

3.3. Total flavonoids

Flavonoids present in food of plant origin are also potential antioxidants. Most beneficial effects of flavonoids are attributed to their antioxidant and chelating abilities. The total amount of flavonoids content of various extract of aerial parts of *Saccharum spontaneum* was presented in table 7.

Table - 7: The total flavonoids content of various extracts of aerial parts of *Saccharum spontaneum*

Extracts	Total flavonoids content (mg/g)(±SEM)*
Petroleum ether extract of *Saccharum spontaneum*	0.032 ± 0.044
Ethyl acetate extract of *Saccharumspontaneum*	1.015 ± 0.036
Methanolic extract of *Saccharum spontaneum*	3.458 ± 0.028

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

4. CONCLUSION

The percentage of various extracts of aerial parts of *Saccharum spontaneum* were 5.23% w/w, 7.95% w/w and 10.35% w/w respectively. The various extracts of *Saccharum spontaneum* on *in vitro* antioxidant activities were estimated by total antioxidant activity (Phosphomolybdic acid method) and FRAP assay. Methanolic extract of *Saccharum spontaneum* and standard drugs was found more antioxidant activity among them with IC_{50} values of 300µg/mL, 410µg/mL, and 228µg/mL, 50µg/mL respectively. These values were compared with synthetic antioxidant agent Ascorbate IC_{50} values.

The total flavonoids content were estimated in various extracts of *Saccharum spontaneum*. Methanolic extract of *Saccharum spontaneum* was found higher content of flavonoids component among them.

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