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Phytochemical screening, antioxidant activities and total phenolic content of ethanolic extract from whole plant of *Saccharum spontaneum* (Linn.)

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ABSTRACT

The aim of this study was to investigate phytochemical constituents, *in vitro* antioxidant activities and, total phenolic content of ethanolic extract from whole plant of *Saccharum spontaneum*. Physicochemical parameters like loss on drying, ash value, fibre content, foreign organic matter, fluorescence analysis and phytochemical screening of ethanolic extract was studied. Determination of antioxidant properties by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay, nitric oxide scavenging activity and iron chelating activity.Total phenolic content estimated by colorimetric assay. Presence of tannins, saponins, flavonoids, terpenoids, glycosides, steroids and carbohydrates were revealed and confirmed by the phytochemical analysis of this extract. Our results indicate that ethanolic extract of *Saccharum spontaneum* has IC_{50} value of 290 µg/ml,512 µg/ml,312 µg/ml of DPPH, nitric oxide, iron chelating activity and total phenolic content 5.38mg/g respectively.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, Phytochemical, Total phenol, *Saccharum spontaneum.*

1. INTRODUCTION

Oxygen is necessary for the survival of all on this earth. Approximately, 5% of oxygen gets univalently reduced to oxygen derived free radicals [1,2], recognized as reactive oxygen species (ROS) during the process of oxygen utilization in normal physiological and metabolic processes. Free radicals such as superoxide(O_2^-),hydroxyl (OH^-), nitric oxide (NO), peroxyl (RO_2^-), lipid peroxyl (LOO-), and non free radical species such as hydrogen peroxide (H_2O_2), singlet oxygen (O_2^{-2}), ozone (O_3), lipid peroxide (LOOH), are various forms of activated oxygen [3-5]. Excessive stimulation of NAD(P)H by cytokines or mitochondrial electron transport chain, xanthin oxidase and some exogenous sources such as UV radiation, pathogen invasion, herbicide action, oxygen lack can generate ROS, subsequently resulting in several diseases [6,7]. It is well known that free radicals are the chief source of a variety of chronic and degenerative diseases such as coronary heart disease, inflammation, stroke, diabetes mellitus and cancer [8]. Plants are 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 [9,10]. 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 [11,12]. 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[13]. In recent times, much attention has been directed towards the progress of ethno-medicines with powerful antioxidant properties [14].

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, respiratory troubles etc [15]. Leaves are employed for cathartic and diuretics [16]. However, the plant is reported to possess the activities like anti-diarrhoeal [17], CNS depressant [18] and antiurolithiatic activity [19]. However, no data are available in the literature on the antioxidant activity of whole plant of *Saccharum spontaneum*. Therefore we undertook the current investigation to examine the total phenolic content and antioxidant activities of ethanolic extract from whole plant of *Saccharum spontaneum* through various *in vitro* models.

2. EXPERIMENTAL

2.1. 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 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 extract

The *S.spontaneum* plant powered materials were extracted with ethanol (70-80^{0}c) by hot continuous percolation method in soxhlet apparatus [20] for 24 hrs. The extract was concentrated by using a rotary evaporator and subjected to freeze up drying in a lyophilizer till dry powder was obtained.

2.4. Physico-chemical and fluorescence analysis

Loss on drying, total ash, acid insoluble ash, water soluble ash, crude fiber content and foreign organic matter were performed as per Indian Pharmacopoeia [21]. Fluorescence analysis of the powder drug was carried out with different chemical reagents in day and UV light (254 nm & 365 nm). The dry powder drug was studied on glass slide whereas the extract was studied by adsorbing the extract on Whatmann filter paper[22].

2.5. Phytochemical screening of the plant extract

A small portion of the dry ethanolic extract of *S. spontaneum* was used for the phytochemical tests for compounds which consist of tannins, flavonoids, alkaloids, saponins, and steroids in accordance with the methods with slight modifications [23,24].

2.6. Scavenging ability towards 2, 2- diphenyl-1-picrylhydrazyl (DPPH) radical

The effect of DPPH radical on the extract was assayed using the following method [25]. A methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1 ml of the different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was read at 518 nm and converted into percentage radical scavenging activity as follows.

$$Scavenging activity(\%) = \frac{A_{518}\,\text{Control} - A_{518}\,\text{Sample}}{A_{518}\,\text{Control}} \times 100$$

Where A_{518} control is the absorbance of DPPH radical+ methanol; A_{518} sample is the absorbance of DPPH radical+ sample extract/ standard.

2.7. Nitric oxide radical scavenging activity

Nitric oxide generated from aqueous solution of sodium nitroprusside at physiological pH interacts with oxygen to produce nitrite ions, which was measured by the following method [26]. The reaction mixture (3ml) containing 2 ml of sodium nitroprusside (10mM), 0.5 ml of phosphate buffer saline (1M), 0.5 ml of ethanolic extract were incubated at 25^{0}C for 150 mins. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33%) and allowed to stand for 5 min for completing diazotization. Further, 1 ml of naphthylethylene diamine dihydrochloride (1% NEDA) was added to the above mixer and allowed to stand for 30 mins. Aqueous solution of Sodium nitroprusside at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess Illosvery reaction at 540 nm.

2.8. Iron chelating activity

The method [27] was adopted for the assay. The principle is based on the formation of *O*-Phenanthroline-Fe^{2+} complex and its disruption by the presence of chelating agents. The reaction mixture containing 1 ml of 0.05% O-Phenanthroline in methanol, 2 ml ferric chloride

(200µM) and 2 ml of various concentrations of extract ranging from 10 to 1000µg was incubated at room temperature for 10 min and the absorbance of the same was measured at 510 nm. EDTA was used as a classical metal chelator. The experiment was performed in triplicates.

2.9. Total phenolic content

Total phenolic content was estimated from the obtained extract using colorimetric assay procedures based on [28]. To 0.25g of sample, 2.5 ml of ethanol was added and centrifuged at 2°C for 10 mins. The supernatant was preserved. Then, the sample was re-extracted with 2.5 ml of 80% ethanol and centrifuged. The pooled supernatant was evaporated to dryness followed by the addition of 3 ml of water to the dried supernatant. 0.5 ml of Folin and Ciocalteu's phenol reagent and 2 ml of sodium carbonate (20%) was added to the above mixture and kept in boiling water bath for 1 min. The absorbance was measured at 650 nm in a spectrophotometer. Catechol was used for constructing the standard curve. The results were expressed in mg of catechol equivalents/g of extract.

2.10. Statistical analysis

Tests were carried out in triplicates. The mean values were calculated from the triplicate values. Values were expressed as the Mean ± SD (n=3) and differences between groups were considered to be statistically significant if $p<0.05$. Data from the test groups were compared with IC_{50} value of the standard which is the concentration of sample, required to scavenge 50% of DPPH, nitric oxide and iron free radicals.

3. RESULTS AND DISCUSSION

Physicochemical analysis of powdered drug like loss on drying, ash values, crude fibres and foreign organic matter of whole plant of *Saccharum spontaneum* were analysed. The percentage of all values in triplicate and their mean values ± SEM were calculated with reference to the air dried drug shown in figure 1 and Table1.

Table - 1: Physico chemical content of whole plant of *Saccharum spontaneum*

Parameters	Mean ± SEM
Loss on drying	11.78±1.52
Ash value	
i. Total ash value	4.26±0.62
ii. Water insoluble ash	1.89±1.28
iii. Acid insoluble ash	2.02±0.13
iv. Sulphated ash	0.67±0.62
Crude fiber content	5.52±1.87
Foreign organic matter	0.01±0.03

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

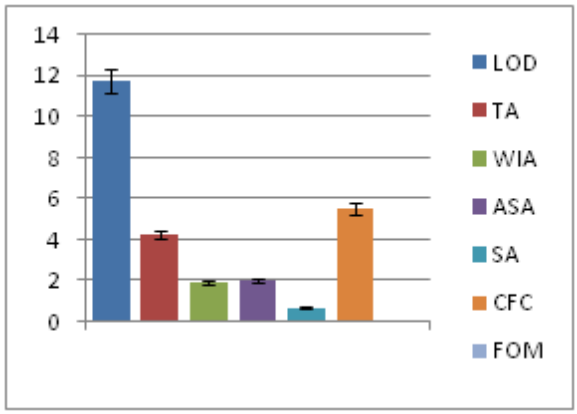

Figure - 1: Physico chemical content of whole plant of *Saccharum spontaneum.*

LOD- Loss on drying ; TA- Total ash; WIA- Water insoluble ash; ASA- Acid soluble ash; SA- Sulphated ash; CFC- Crude fibre content; FOM- Foreign organic matter.

The fluorescence analysis of the powdered *S.spontaneum* shows changes in the colour under UV radiation & day light in the presence or absence of chemical constituents. The change in the colour is the due to the presence of different chemical reagents (Table 2).

The phytochemical analysis from ethanolic extract of *S.spontaneum* (Table 3) revealed the presence of tannins, saponins, flavonoids, alkaloids, proteins, glycosides, steroids, terpenoids and carbohydrates.

Free radicals can cause oxidative damage to lipids, proteins and DNA, ultimately leading to many chronic diseases, such as cancer, diabetes, aging, and other degenerative diseases in human beings [29]. Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen development [30]. Flavonoids are a group of polyphenolic compounds with well-known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes [31]. Some verification suggests that the biological actions of these compounds are correlated to their antioxidant activity [32]. Therefore, in the recent years the search for natural antioxidants has increased among researchers [33].

DPPH is a stable free radical at room temperature often used to evaluate the antioxidant activity of several natural compounds. The reduction capacity of DPPH radicals were determined by the decrease in its absorbance at 518 nm, which is induced by antioxidants. The percentage of DPPH radical scavenging activity of ethanolic extract of *Saccharum spontaneum* presented in Table 4. The ethanolic extract of

Saccharum spontaneum exhibited a maximum DPPH scavenging activity of 74.63% at 1000 µg/ml whereas for Rutin(standard) it was found to be 69.83% at 1000 µg/ml. The IC_{50} for the ethanolic extract of *Saccharum spontaneum* and Rutin were found to be 290µg/ml and 480µg/ml respectively.

Table - 2: Fluorescence analysis of powder of *Saccharum spontaneum*

Experiments	Colour in day light	Colour in shorter UV	Colour in longer UV
Drug powder	White	Light yellow	Brown
Drug powder + Aqueous 0.1N HCl	Light yellow	White	Black
Drug powder + Alcoholic. HCl	Faint green	Light green	Black
Drug powder + Aqueous 1N NaOH	White	Greenish yellow	Black
Drug powder +Alcoholic. NaOH	Light green	Green	Blackish brown
Drug powder + 5% NaOH	Yellow	Green	Blackish brown
Drug powder + 50% H_2SO_4	Faint green	Light green	Brown
Drug powder + 50% HNO_3	Brown	Greenish yellow	Black
Drug powder + Picric acid	Yellow	Green	Blackish brown
Drug powder + Acetic acid	Faint yellow	Light yellow	Brown
Drug powder + Ferric chloride	Brown	Greenish brown	Black
Drug powder + HNO_3 + NH_3	Brick red	Greenish brown	Black
Drug powder + 5% Iodine	Yellowish brown	Greenish yellow	Brown

Table - 3: Phytochemical screening from ethanolic extract of *Saccharum spontaneum*

Name of the test	Ethanolic extract
Alkaloids	+
Carbohydrates	+
Coumarins	+
Flavonoids	+
Glycosides	+
Phenol	+
Proteins	+
Saponins	+
Steroids	+
Tannins	+
Terpenoids	+
Gum mucilage	-
Resin	-

Nitric oxide free radical scavenging activity for the ethanolic extract of *Saccharum spontaneum* presented in Table 5. The free radical scavenging potential shown maximum activity of 75.42% at 1000µg/ml and Standard (ascorbate) was found to be 62% at 1000 µg/ml. The IC_{50} for the ethanol extract of *Saccharum spontaneum* and standard (ascorbate) were found to be 512µg/ml and 410µg/ml respectively. The extract showed a better antioxidant activity than that of the standard.

Table - 4: DPPH Scavenging activity of rutin, ethanolic extract of *Saccharum spontaneum*

Concentration (µg/ml)	% of activity(±SEM)*	
	Sample (Ethanolic extract)	Standard (Rutin)
125	34.25±0.065	18.85± 0.076
250	46.30±0.110	22.08 ± 0.054
500	68.43±0.167	52.21 ± 0.022
1000	74.63±0.071	69.83 ± 0.014
IC_{50}	**290 µg/ml**	**480 µg/ml**

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

Iron is necessary for life because it is requisite for oxygen transport, respiration and activity of many enzymes. However, iron is an enormously reactive metal and catalyzes oxidative changes in lipids, proteins and other cellular components [34,35]. It causes lipid peroxidation via the Fenton and Haber-weiss reaction [36] and decomposes the lipid hydroxide into peroxyl and Alkoxyl radicals that can be responsible for the chain reactions [37]. Iron binding capability of the ethanolic extract of *Saccharum spontaneum* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in table 6. Maximum

chelating of metal ions at 1000µg/ml for plant extract and EDTA was found to be 79.46% and 97.90% respectively. The IC_{50} value for ethanolic extract of *Saccharum spontaneum* and EDTA was recorded as 312µg/ml and 65µg/ml respectively.

Table - 5: Nitric oxide free radical Scavenging activity of ascorbate, Ethanolic extract of *Saccharum spontaneum*

Concentration (µg/ml)	% of activity (±SEM)*	
	Sample (Ethanolic extract)	Standard (ascorbate)
125	23.30±0.06	27.63±0.076
250	36.26±0.08	49.53 ±0.054
500	48.78±0.22	55.12±0.022
1000	75.42±0.05	62.00±0.014
IC_{50}	**512 µg/ml**	**410 µg/ml**

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

Table - 6: Iron chelating activity of EDTA, Ethanolic extract of *Saccharum spontaneum*

Concentration (µg/ml)	% of activity (±SEM)*	
	Sample (Ethanolic extract)	Standard (EDTA)
125	28.34 ± 0.13	58.68 ± 0.007
250	40.44 ± 0.17	65.87 ± 0.018
500	70.44 ± 0.12	83.83 ± 0.012
1000	79.46 ± 0.06	97.90 ± 0.019
IC_{50}	**312 µg/ml**	**65 µg/ml**

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

The results indicated that the plant extract possess iron binding capability which might be due to the occurrence of polyphenols that averts the cell from free radical damage by reducing the transition metal ions [38,39]. A variety of plant extracts were proved to be good chelators[40] and correlation exists between phenols, flavonoids and chelating activity.

Phenolic compounds are well-known as potent chain breaking antioxidants [41]. Phenols are very essential plant constituents because of their scavenging capacity due to their hydroxyl groups [42]. The total amount of phenolic content for ethanolic extract of whole plant of *Saccharum spontaneum* (Table 7) was found to possess higher content of phenolic components.

Table - 7: Total phenolic content of ethanolic extract of *Saccharum spontaneum*

Extract	Total phenol content (mg/g of Catechol) (±SEM)*
Ethanolic extract of *Saccharum spontaneum*	5.38 ± 0.023

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

The physiochemical parameter like loss on drying, ash value, fibre content, foreign organic matter and florescence analysis is helpful in detection of the plant material [43,44]. The fluorescence analysis of powdered drug play a very vital role in the determination of quality and purity of drug. The information obtained from the preliminary phytochemical screening will expose the useful finding about the chemical nature of the drug. The phytochemical evaluation of *S.spontaneum* can afford useful information for the identification and authentification of the plant.

The antioxidant capability of crude drugs broadly used for evaluating the medicinal bioactive components. The current study has confirmed the free radical scavenging activity of S. spontaneum. The antioxidant activity in the plant extract may mostly be due to polyphenols [45]. Phenolics are the huge group of phytochemicals and most of the anti oxidant activity of plants or plant products accredited to them. Various studies have been reported that the natural antioxidants are able to reduce DNA damage, mutagenesis, carcinogenesis. These events are frequently associated with the termination of free radical proliferation in biological systems [46,47]. Ethanolic extract from the whole plant of *S.spontaneum,* contains a large amounts of phenolic compounds, exhibits high antioxidant and free radical scavenging activities. These *in vitro* assays point out that this plant extracts is a considerable source of natural antioxidant, which might be useful in preventing the evolution of various oxidative stresses. Therefore, further investigations require to be carried out to isolate and identify the antioxidant compounds responsible for the *in vivo* antioxidant activity of this plant extract for further assessment earlier to clinical use.

In conclusion, ethanolic extract from the whole plant of *S.spontaneum* exhibited a significant free radical scavenging effect on DPPH, nitric oxide and iron chelating methods at various concentration. The result of this study revealed that the use of the plant in traditional medicine is potential for drug development.

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