Research Article

Phytochemical Screening and *In Vitro* Antioxidant Activity of *Saccharum Spontaneum Linn.*

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ABSTRACT

Under most pathological conditions there is generation of reactive oxygen species and other free radicals. An increase in the antioxidant reserves of the organism can reduce oxidative stress and some of the plant-derived agents may help to reduce it. The aim of this study was to evaluate *in vitro* antioxidant activity inhibition of DPPH, FRAP and ABTs radicals scavenging activity. Results indicate that ethanolic root extract of *Saccharum spontaneum have* marked high content of secondary metabolites among all the solvents. At (100-500µg/ml) concentration the *S.spontaneum of* 500µg/ml concentration exhibited high DPPH radical scavenging capacity using ascorbic acid as positive control. *S.spontaneum at* the same concentration showed the dose dependent inhibition of FRAP, and ABTs radical scavenging activity. Collectively, our results indicate that the ethanolic root extract of *S.spontaneum has* the potential to scavenge free radicals and act as a good antioxidant for treating various diseases.

Keywords: Free radicals, reactive oxygen species, *Saccharum spontaneum*, antioxidant.

INTRODUCTION

An enormous variety of medicinal plants are used worldwide by about 80% of the world population, although in most cases no scientific studies have been done to prove the efficacy of these medicinal plants. Considering that most of the present-day western medicines are based on the traditional medicinal plants of European, Mediterranean and Arabic origin, the variety of plants in use around the world may very well represent an enormous treasure for drug[1].

Oxidation is a basic part of the aerobic life and our metabolism. During oxidation, many free radicals are produced which have an unpaired nascent electron. Atoms of oxygen or nitrogen having central unpaired electron are called reactive oxygen or nitrogen species[2-5]. This may be harmful to the body and may cause peroxidation of membrane lipids, aggression of tissue membranes and proteins or damage to DNA and enzyme[6]. These can be related to some pathology, such as arthritis, haemorrhagic shock, coronary artery diseases, cataract, cancer, AIDS as well as age-related degenerative brain diseases[7]. The immune system is vulnerable to oxidative stress. Oxidative stress refers to an imbalance between the production of free radicals and the antioxidant defense system. Reactive oxygen species (ROS) are various forms of activated oxygen which causes oxidative damage. Mechanisms responsible for the ROS-mediated injuries mainly include lipid peroxidation, oxidative DNA damage and protein oxidation[8, 9].

Antioxidants are compounds that detoxify ROS and prevent their damage through multi mechanisms. Synthetic antioxidants have been in use as food additives for a long time, but reports on their involvement in chronic diseases have restricted their use in foods. Therefore, international attention has been focused on natural antioxidants mainly from plant sources[10,11]. During certain diseased state, as well as during aging, there is a need to boost the antioxidant abilities, thereby potentiating the immune mechanism[12]. The antioxidants preserve and stimulate the function of immune cells against homeostatic disturbances[13].

In the human body the free radicals are continuously produced due to the oxygen utilization by the cells of the body. This generates a series of reactive oxygen species (ROS) like super oxide anion ($O2^{\cdot-}$) and hydroxyl ($HO\cdot$) radicals and non-free radical species such as H_2O_2, singled oxygen (O_2) and nitric oxide (NO)[14]. The free radicals are known to be scavenged by synthetic antioxidants, but due to their adverse side effects leading to carcinogenicity; search for effective and natural antioxidants has become crucial[15]. Free radical reactions have been implicated in the pathology of many human diseases like atherosclerosis, ischemic heart disease, diabetes and neurodegenerative disease etc. and disease conditions like aging process, inflammation, immuno suppression etc. A number of plants and plant isolates have been reported to protect free radical-induced damage in various experimental models.

Antioxidants may offer resistance against the oxidative stress by scavenging free radicals, inhibiting lipid peroxidation and thus prevent disease. In the present study, activity guided fractionation was adapted to identify the active fraction of this drug responsible for the antioxidant activity[16]. In recent times, focus on plant research has increased all over the world and a large body of evidences has collected to show immense potential of medicinal plants used in various traditional systems[17].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 rich in antioxidant activities[18]. A number of plants and plant isolates have been reported to protect free radical induced damage in variousexperimental models[19].

MATERIALS AND METHODS

Phytochemical studies of *Saccharum spontaneum* Linn.

Collection of plant material

Saccharum spontaneum Linn. was collected from Koorappalayam, Erode district, Tamil Nadu, India during the month of September to November, 2011. The plant was identified and authenticated by taxonomist Dr.K. Arumugasamy, Assistant Professor, Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India. Voucher specimen was deposited in herbarium centre, Department of Botany, Kongunadu Arts and Science College, Coimbatore.

Qualitative phytochemical analysis of the root extract

The phytochemical screening with methanol, ethanol, petroleum ether, chloroform and aqueous extracts of *Saccharum spontaneum* was done by modern method,[20] to identify the presence of alkaloids, flavonoids, tannins, saponins, steroids, triterpenes and glycosides.

DPPH radical scavenging activity

The scavenging effect of root extract on DPPH radicals was determined according to the method of previously published.[21] Various concentrations of sample (4 ml) were mixed with 1 ml of methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2 mM. The mixture was shaken vigorously and left to stand for 30 min, and the absorbance was measured at 517 nm. The percentage inhibition was calculated according to the formula: (A0-A1)/A0]×100, where A0 was the absorbance of the control and A1 was the absorbance of the sample.

ABTs radical scavenging activity

The scavenging effect of root extract on ABTs radicals was determined according to the method of previously published.[22] ABTS decolourisation assay involves the generation of the $ABTS^{+}$ chromophore by the oxidation of ABTS with ammonium persulphate. It is applicable for both hydrophilic and lipophilic compounds. The scavenging activity of the plant extracts on ABTS radical cation were measured at 734 nm.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was used to estimate the reducing capacity of root extracts, according to the method.[23] The total antioxidant potential of sample was determined using ferric reducing ability of plasma FRAP assay as a measure of antioxidant power. FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue coloured Fe II-tripyridyl triazine compound from colourless oxidized Fe III form by the action of electron donating antioxidants.

Statistical analysis

Results were expressed as mean ± SD of six animals in each group. Statistical significance was determined by one-way analysis of variance (ANOVA) and post hoc least-significant difference (LSD) test.

RESULTS AND DISCUSSION

The present study carried out on the *Saccharum spontaneum* Linn. revealed the presence of medicinally active constituents. The results of the phytochemical screening with water, ethanol, methanol, petroleum ether and chloroform extracts of *S. spontaneum* Linn. are given in the table 1. Qualitative phytochemical analysis of the root extracts were done and it was found that the presence of phytochemicals were maximum in the ethanolic extract when compared to other extracts.

The phytochemical study with ethanolic extract of selected medicinal plant showed the presence of alkaloids, flavonoids, tannins, steroids, terpenoids, glycosides and phenolic constituents. Phytochemical analysis of various extracts reveals the presence secondary metabolites in the root extracts of *Saccharum spontaneum* thus providing knowledge of the phytochemical present in it. However, the presence of secondary metabolites was found to be higher in ethanolic extract when compared to other extracts. So this extract was selected for further investigations.

Table 1: Qualitative analysis of phytochemicals in the root extract of *S.spontaneum* Linn.

Chemical Constituents	Water	Ethanol	Methanol	Pet.Ether	Chloroform
Aklaloids	+	+	+	+	+
Flavonoids	+	++	++	+	_
Tannins	_	+	+	_	_
Saponins	_	_	_	_	_
Steroids	_	+	+	_	_
Terpenoids	+	++	+	+	+
Resins	_	_	_	_	_
Glycosides	+	++	+	_	_
Phenolic constituents	++	++	++	+	+

DPPH radical scavenging activity

DPPH radical scavenging is considered a good *in vitro* model widely used to assess antioxidant efficacy within a very short time. In its radical form, DPPH disappears on reduction by an antioxidant compound or a radical species to become a stable diamagnetic molecule resulting the colour change from purple to yellow, which could be taken as an indication of the hydrogen donating ability of the tested sample[24,25].

DPPH assay is a stable free radical method. It is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extract[26]. The free radical scavenging activity of the extracts was evaluated based on the ability to scavenge the synthetic DPPH. DPPH (1, 1-Diphenyl-2-picrylhydrazyl) is a stable nitrogen centred free radical which can be effectively scavenged by antioxidants and shows strong absorbance at 517 nm. DPPH radical accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The change in absorbance of DPPH radical caused by antioxidants is due to the reaction between the antioxidant molecules and the radical, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. Extent of DPPH radical scavenged was determined by the decrease in intensity of violet colour in the form of IC_{50} values[27].

As the electron became paired in the presence of free radical scavenging, the absorption vanishes on the resulting discoloration stoichiometrically, coincides with respect to the number of electrons taken up. The bleaching absorption of DPPH is representative of the capacity of the methanol and aqueous extracts to scavenge free radicals independently. Hence it has been widely used for rapid evaluation of the antioxidant activity of plant and microbial extracts relative to other methods.[28] DPPH is also considered as a good kinetic model for peroxyl radicals[29].

DPPH assay has been extensively used for screening antioxidant activity because it can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentration[30].When DPPH radicals encounter a proton donating substance such as an antioxidant, it would be scavenged and the absorbance is reduced. Thus, the DPPH radicals were widely used to investigate the scavenging activity of some natural compounds. In the present study, the ethanolic root extracts of *Saccharum spontaneum were* investigated in comparison with the known antioxidant ascorbic acid. The anti-oxidant effect of the test extract is however, less than that of standard (ascorbic acid) at all the concentrations of the extract. DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate anti-oxidant activity.

The radical scavenging activity of ethanolic root extract of *S.spontaneum* increased with increasing concentrations, with 35.14 %, 40.81%, 56.35%, 59.92% and 64.24% scavenging activity for 100, 200, 300, 400 and 500 µg /ml extract, respectively (Figure 1). The IC_{50} value was found to be 280 µg /ml.

These results indicated that ethanolic root extract of *Saccharum spontaneum* exhibited the ability to quench the DPPH radical, which indicated that extract was good antioxidant with radical scavenging activity. Radical scavenging activity of standard ascorbic acid increased with concentrations and IC_{50} value was found to be 230 µg /ml. The IC_{50} value indicates that ascorbic acid is better DPPH radical scavenging activity than that of the ethanolic root extract of *S.spontaneum*.

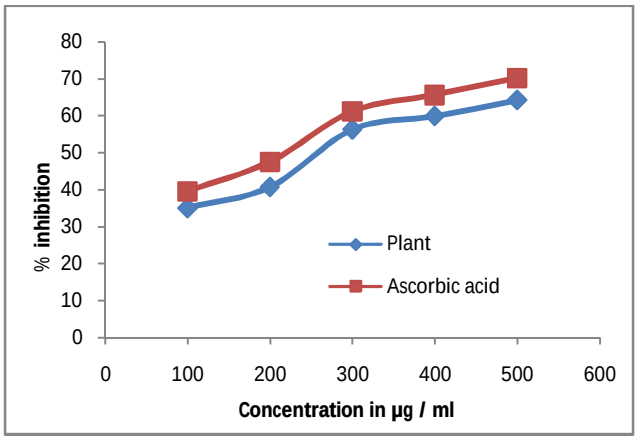

Figure 1: DPPH radical scavenging activity

The result of the present investigation is in accordance with the study of Bhuiyan *et al*.(2009)[31] who reported the *in vitro* studies on antioxidant and free radical scavenging activities of *Zizyphus mauritiana* fruit extract was showed potential free-radical scavenging activity.

ABTs radical scavenging activity

ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants and of chain-breaking antioxidants[32]. The extract efficiently scavenged ABTS radicals generated by the reaction between 2,2'-azinobis (3-ethylbenzothiazolin-6-sulphonic acid) (ABTS) and ammoniumpersulfate. Proton radical scavenging is an important attribute of antioxidants. ABTS, a protonated radical, has characteristic absorbance maxima at 734 nm which decreases with the scavenging of the proton radicals[33].

The activity was found to be increased in a dose-dependent manner from 36.56% to 74.76 % at concentrations from 100 to 500 µg /ml. The extract exhibited an IC_{50} value of 270 µg /ml. Therefore, the ABTS radical scavenging activity of ethanolic root extract of *S.spontaneum* indicates its ability to scavenge free radicals, thereby preventing lipid oxidation via a chain-breaking reaction.

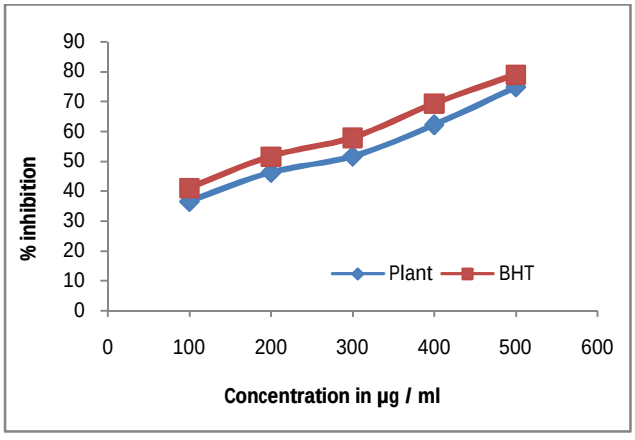

Figure 2: ABTs radical scavenging activity

The ABTs radical scavenging activity of standard BHT increased with increasing concentrations, 41.05%, to 78.96% scavenging activity for 100, 200, 300, 400, 500 µg /ml. IC_{50} values was found to be 190 µg /ml. The IC50 value indicates that BHT is better ABTs radical scavenging activity than that of the ethanolic root extract of *S.spontaneum*.

Our results coincides with that of Rana *et al*. (2010)[34] who show that the extract of *Medicago sativa* contains the highest amount of polyphenol compounds and exhibits the greatest antioxidant activity through the scavenging of free radicals which participate in various pathophysiology of diseases including ageing.

Ferric reducing antioxidant power (FRAP) assay

In FRAP assay the ability of plant extract to reduce ferric ions was determined. FRAP assay measures the changes in absorbance at 593 nm owing to the formation of blue colored Fe^{2+} tripyridyltriiazine compound from the colourless oxidized Fe^{3+} form by the action of electron donating antioxidants[35]. The FRAP (ferric reducing /antioxidant power) method assess the reducing potential of root extract and is based on the ability to reduce ferric ions.

Figure 3 represents the reductive capabilities of the *S.spontaneum* root extract, which was compared with butylated hydroxyl toluene (BHT) standard.

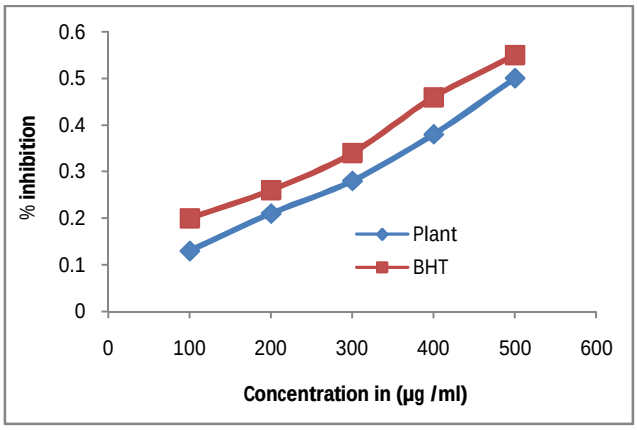

Figure 3: Ferric reducing antioxidant power (FRAP) assay

The activity was found to be increased in a dose-dependent manner from 0.13 to 0.50 nm at a concentration from 100 to 500 µg /ml. Therefore, the FRAP radical scavenging activity of ethanolic root extract of *S.spontaneum* indicates its ability to scavenge free radicals, thereby preventing lipid oxidation via a chain-breaking reaction. For the FRAP test, BHT was used as a standard were the activity was found to be 0.15 to 0.55 nm at a concentration of 100-500 µg /ml.

Our findings were similar to that of Surya *et al*. (2011)[36] who showed that the ethanolic extract of *Tabernaemontana coronaria* has the potential to scavenge free radicals and act as a good antioxidant for treating various diseases.

CONCLUSION

The free radical scavenging activity of *Saccharum spontaneum* root extract was evaluated based on the ability to scavenge the synthetic DPPH. This assay provided useful information on the reactivity of the compounds with stable free radicals, because of the odd number of electrons. The encouraging results of with the various *in vitro* antioxidant tests proved the *S.spontaneum* root as a reducing agent, its hydrogen donating ability. The results obtained in the present study indicate that *S.spontaneum* root extract exhibit potent free radical scavenging and antioxidant activity. This might be attributed to the presence of various phytoconstituents viz., alkaloids, flavonoids, tannins, steroids, terpenoids, glycosides and phenolic constituents. The findings of the present study suggest that *Saccharum spontaneum* root might be a potential source of natural antioxidant that could have great importance as therapeutic agents in disease prevention, health preservation and promotion of longevity promoter.

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