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Sida Veronicaefolia as a Source of Natural Antioxidant

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ABSTRACT

Antioxidants play a major role in the management of diseases which are associated with oxidative stresses caused by excess free radicals and other reactive oxygen species. Antioxidant phytochemicals exert their effect by neutralizing these highly reactive radicals. The antioxidant activity of hexane, chloroform, hydro-alcoholic and aqueous extract of whole plant of *Sida veronicaefolia* (family *Malvaceae*) was evaluated using in-vitro models, DPPH free radical scavenging, scavenging of hydrogen peroxide and reducing power method. Dried powder of whole plant was extracted with hexane, chloroform, hydro-alcohol (50 %) and water using Soxhlet apparatus. Hexane, chloroform, hydroalcoholic and aqueous extracts showed 7.2 %, 8.4 %, 20.9 % and 14.1 % inhibition on DPPH, while 1.5 %, 2.5 %, 23.4 % and 11.5 % inhibition of hydrogen peroxide compared to 37.4 % and 40 % by standard (BHT) and increasing order of absorbance by reducing power method i.e. hexane (0.150) < chloroform (0.479) < aqueous (1.790) < hydro-alcoholic (1.906) < standard (2.082). The obtained results indicate that, the hydro-alcoholic extract of *Sida veronicaefolia* shows high scavenging activity. The antioxidant activity of the plant may be due to the presence of flavonoids, terpenoids and phenolic compounds of hydro-alcoholic extract of whole plant. In all the methods, the extract displayed its ability to scavenge free radicals in a concentration dependent manner.

Keywords: *Sida veronicaefolia,* Antioxidant, scavenging activity, DPPH, reducing method.

INTRODUCTION

Reactive oxygen species (ROS) such as singlet oxygen (O^2), superoxide anion (O^{2-}) and hydroxyl (.OH) radical and hydrogen peroxide (H_2O_2) are often generated as bye products of biological reactions or from exogenous factors. These reactive species exert oxidative damaging effects by reacting with nearly every molecule found in living cells including DNA, if excess ROS are not eliminated by antioxidant system. [1] They play important roles in aging and in the pathogenesis of age related disorders such as cancer, hypertension, atherogenesis, Alzheimers disease, and Parkinsons disease. [2-4] Recent investigations have shown that the antioxidants with free-radical scavenging properties of plant origins could have great importance as therapeutic agents in aging process and free radical mediated diseases including neuro degeneration. [5-6] Plant extracts and plant products such as flavonoids and other polyphenolic constituents have been reported to be effective radical scavengers and inhibitors of lipid peroxidation. [7-8]

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Many synthetic antioxidant compounds have shown toxic and/or mutagenic effects, which have stimulated the interest of many investigators to search natural antioxidant.

Sida veronicaefolia, family *Malvaceae*, is a straggling way side herb found very often growing in shady places. It grows mainly in clearing in the forest and as weeds in the over grown grass of public parks and gardens. [9] It is also known as *Rajbala, Bhumibala, Farid buti, Shaktibala*, etc. It has a capability to remove the three *doshas* from the body, and to provide strength and glow to the body. [10]

Sida veronicaefolia is very popular with rural womenfolk, especially in the areas where it grows in its natural habitat, and is used extensively in traditional medicine for shortening and reducing the pain of labour in childbirth. It is believed to render parturition almost painless and leads to shorter period of postpartum bleeding. Soup of this plant is taken in the last days of pregnancy. [11] Lutterodt reported that alcoholic extract of *Sida veronicaefolia* has abortifacient effect in pregnant rats. An oral dose produces abortifacient effect when administered from 15^{th}-17^{th} day of pregnancy. It is also reported that water soluble fraction from an alcoholic extract of *Sida veronicaefolia* has muscarine like active principle. [12] Literature survey revealed that no detailed phytochemical

work has been done on this plant and yet not being screened for its evaluation of antioxidant activity.

The aim of present study was to investigate antioxidant activity by using different antioxidant test including DPPH free radical scavenging, scavenging of hydrogen peroxide and reducing power method on various extracts of the plant.

MATERIALS AND METHODS

Plant Material

The plants were collected in mid morning only during the rainy season by uprooting the whole herb from the grounds in the districts of Pratapgarh and Allahabad, India. The freshly gathered herb was identified and classified by National Botanical Research Institute, Lucknow.The meristem and leaves were separated, washed in water, air dried and placed in drying cabinet at 55-60°C. The dried material was then pulverized into fine powder and stored in a covered jar at room temperature

Preparation of Plant Extracts

Various extracts of *Sida veronicaefolia* were prepared by extracting 30 g of powdered herb with 500 ml of hexane, chloroform, hydro-alcohol (50 %) and water at 60-85^{o}C in a Soxhlet extractor. The extracts were subjected to evaporation, the yield being 214 mg. The dark greenish and sticky product was extracted with 10 ml of distilled water, water soluble portion separated off and used for different tests.

Antioxidant Activity

A) DPPH radical scavenging method [12]**:** Solution of DPPH (0.1 mM) in ethanol was prepared and 1ml of this solution was added to 1ml of different extracts and allowed to stand at room temperature for 30 min. the absorbance of resulting solution was measured at 517 nm and the percentage scavenging inhibition was determined and compared with that of butylated hydroxytoluene (BHT), used as a standard.

B) Hydrogen Peroxide scavenging Method [13]**:**

Extracts (1 ml) were added to 0.6 ml of hydrogen peroxide solution (40 mM, phosphate buffer pH 7.4) and the absorbance was measured at 230 nm using uv-visible spectrophotometer. The percentage inhibition of different extract was determined and compared with standard.

C) Reducing Power Method [12]: 1ml of different extracts were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1 % w/v) and incubated at 50^{o}C for 20 min, trichloroacetic acid (10 %, 2.5 ml) was added to the mixtures, which were then centrifuged for 10 min. The upper layer of solutions mixture (2.5 ml) were mixed with 2.5ml of distilled water and ferric chloride (0.5 ml, 0.1 %), and the absorbance was measured at 700 nm. The assay was carried out in triplicate and the results were expressed as mean ± S.D.

Calculation of % Inhibition:

Percentage Inhibition = (Ao-A / Ao) x100 …….. Eq 1

Where Ao was the absorbance of control and A was the absorbance of sample of *Sida extract*

RESULT AND DISCUSSION

Phytochemical screening

The phytochemical screening of plant extract showed positive reaction for psuedotannins, flavonoids, phenolic acid, choline and oxalic acid.

Table 1: Effects of different extracts on scavenging activity of hydrogen peroxide and DPPH (n=3) Dose of Ascorbic Acid- 0.3mg/100g, Dose of extract- 0.2mg/100g

S. No.	Treatment	DPPH Method (mean ± S.D.)		Hydrogen Peroxide Method (mean + S.D.)	
		Absorbance at 517nm	% Inhibition	Absorbance at 230nm	% Inhibition
1.	Control	1.602±0.077	0	0.813±0.0056	0
2.	Standard (BHT)*	1.002± 0.005	37.45±3.467	0.487±0.0011	40.09±0.1385
3.	Hydroalcoholic Extract	1.266±0.024	20.97±2.193	0.622±0.0005	23.49±0.0692
4.	Aqueous Extract	1.376±0.033	14.10±1.346	0.719±0.0186	11.56±4.761
5.	Chloroform Extract	1.470±0.026	8.42±1.027	0.792±0.0017	2.58±0.2078
6.	Hexane Extract	1.486± 0.014	7.24±0.975	0.800±0.0005	1.59±0.0692

*BHT= Butylated Hydroxytoluene

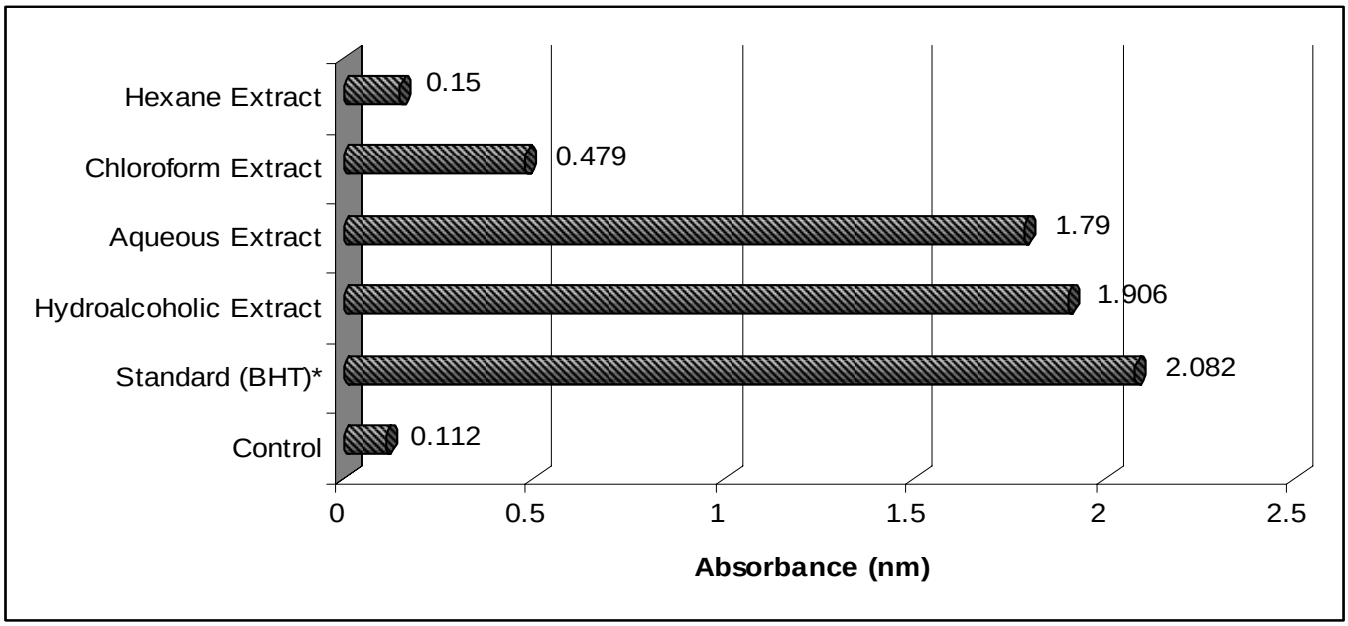

Fig. 1: Effects of different extracts on Reducing Power Method

DPPH radical scavenging method

The scavenging effect of different extract on DPPH radical decreased in the order of Std (BHT)> Hydroalcoholic extract (hydroalc)>Aqueous (Aq)>Chloroform extract (chl)>Hexane extract and was 37.45 %, 20.75 %, 14.1 0%, 8.42 %, 7.25 % respectively (Table 1). The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability on the DPPH radical is determined by the decrease in its absorbance at 517nm induced by antioxidants. The maximum absorption of DPPH radical in ethanol is 517 nm. The decrease in the absorption of DPPH radical is due to the reaction between antioxidant molecules and radical, which result in scavenging of the radical by hydrogen donation. This is visualized as discoloration from purple to yellow. [14-15]

Hydrogen Peroxide scavenging Method

The percentage of inhibition of BHT, hydro alcoholic extract, aqueous extract, chloroform extract and hexane extract were found to be 40 %, 23.49 %, 11.56 %, 2.58 %, 1.59 %. However, there was statistically a very significant correlation between those values and control ($P<0.05$) (Table-1). Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects. [16] Scavenging of hydrogen peroxide by plant extract may be attributed to their phenolics which could donate electrons to hydrogen peroxide, thus neutralizing it to water.

Reducing Power Method

In this method reducing power increase with increase in absorption. The reducing capacity of different extracts were decreased in the order of Std (BHT)> hydro alcoholic extract>Aqueous extract >Chloroform extract >Hexane extract. Fig. 1 shows the reducing power of the various extracts of *Sida*. In this assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. Presence of reducers causes the conversion of the Fe^{3+}/ferricyanide complex used in this method to the ferrous form. By measuring the formation of Perl's Prussian blue at 700 nm, it is possible to determine the Fe^{2+} concentration. The reducing capability of compound may serve as a significant indicator of its potential antioxidant activity.

From the above results it was found that among all the extracts hydro alcoholic extract displayed strong antioxidant activity and it is suggested that *Sida veronicaefolia* could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases.

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