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Research Article

IN VITRO ANTIOXIDANT ACTIVITY OF *SAUROPUS BACCIFORMIS* BLUME (EUPHORBIACEAE)

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ABSTRACT

The present study aims to evaluate the *in vitro* antioxidant activity of methanol extract of different parts of *Sauropus bacciformis* Blume. Among the investigated plant parts, highest amount of total phenolics and flavonoids were reported in the stem. The *in vitro* antioxidant activity was evaluated by DPPH radical scavenging activity, hydroxyl scavenging activity, superoxide scavenging activity and ABTS radical cation scavenging activity with reference standard ascorbic acid and trolox. The methanol extract showed highest *in vitro* antioxidant activities. These *in vitro* assays indicate that this plant extracts is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Keywords: *Sauropus bacciformis,* Total phenolics, DPPH, ABTS

INTRODUCTION

Plant and plant products are used as a source of medicine since long. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world due to their potent antioxidant activities[1]. Plants are a good source of biologically active compounds known as phytochemicals. The phytochemicals have been found to act as antioxidants by scavenging free radicals and many have therapeutic potential for free radical associated disorders[2].Free radicals are the major cause of various chronic and degenerative diseases such as coronary heart diseases , inflammatory stroke ,diabetes and cancer[3].

Free radicals are fundamentals to any biochemical process and represent an essential part of aerobic life and metabolism[4]. The most common reactive oxygen species (ROS) include superoxide anion, hydrogen peroxide (H_20_2), peroxyl (ROO^-) radicals, and reactive hydroxyl (OH^-) radicals. The nitrogen derived free radicals are nitric oxide (NO_2) and peroxynitrite anion (ONOO). Reactive oxygen species (ROS) are continuously generated inside the human body. The generated ROS are detoxified by the antioxidants present in the body. However, over production of ROS and inadequate antioxidant defense can easily affect and persuade oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA.

Recently there has been an upsurge of interest in the therapeutic potentials of plants as antioxidants in reducing free radical induced tissue injury. Although several synthetic antioxidants, such as butylatedhydroxyanisole (BHA) and butylatedhydroxytoluene (BHT) are commercially available but are quite unsafe and their toxicity is a problem of concern. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Natural antioxidants especially phenolics and flavonoids from tea, wine, fruits, vegetables and spices are already exploited commercially either as antioxidant additives or as nutritional supplements[5]. Also many other plant species have been investigated in the search for novel antioxidants but generally there is still a demand to find more information concerning the antioxidant potential of plant species as they are safe and also bioactive. Therefore, in recent years, considerable attention has been directed towards the identification of plants with antioxidant ability.

Genus *Sauropus* Blume includes about 56 species. *S.androgynus* (star gooseberry) is cultivated in India for consumption and commercial use. It is used as nutraceutical in health care and in prevention and treatment of diseases and it has anti-cancerous properties[6]. In view of the medicinal importance of genus *Sauropus,* the present study focused on the *in vitro* antioxidant activities of methanol extract of *Sauropus bacciformis* using different models viz., DPPH, hydroxyl, superoxide and ABTS radical cation scavenging activities. So far no reports are available *in vitro* antioxidant activity of this plant.

MATERIALS AND METHODS

Plant parts of *Sauropus bacciformis* Blume were freshly collected from Vallanadu, Tirunelveli District, Tamil Nadu. The plant specimen was identified and authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in Research department of Botany, St. Mary's College, Tuticorin, Tamil Nadu.

Preparation of Plant extract

Plant parts (root, stem, leaf and flower) were dried in shade for one week, powdered and extracted with methanol using cold extraction in shaker for 48h at room temperature. The methanol extracts were concentrated in a rotary evaporator to obtain concentrated methanol extract which was then used for the estimation of total phenolic, flavonoid and the assessment of antioxidant activity.

Estimation of Total Phenolics

Total phenolic contents were determined according to Lachman *et al* [7]. 1 ml of sample extract was transferred into a 50ml volumetric flask and diluted approximately with 5ml distilled water. Then, 2.5 ml Folin-Ciocalteau reagent and 7.5ml of 20% (w/w) Na_2Co_3 were added and made up to 50ml with distilled water. It was agitated and left to stand for 2hrs. Absorbance of the sample was measured on the spectrophotometry at 765nm against a blank prepared with distilled water. Gallic acid was used for calibration. The results were expressed as Gallic acid equivalent (GAE) in mg/100g.

Estimation of Flavonoids

The total flavonoid content was determined according to Eom *et al*, [8]. An aliquot of 0.5 ml of sample (1mg/ml) was mixed with 0.1 ml of 10% aluminium chloride and 0.1 ml of potassium acetate (1M). In this mixture, 4.3 ml of 80% methanol was added to make 5ml volume. The mixture was vortexted and the absorbance was measured spectrophotometrically at 415nm. The value of optical density was used to calculate the total flavonoid content present in the sample.

DPPH radical scavenging activity

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H [9].

The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method[9]. Briefly, an 0.1mm solution of DPPH in methanol was prepared, and 1ml of this solution was added to 3 ml of the solution of all extracts in methanol at different concentration (50, 100, 200 & 500 µg/ml).The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbances were measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10UV: Thermo electron corporation).Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following formula.

DPPH scavenging effect (% inhibition) = $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell *et al*. [10]. Stock solutions of EDTA (1mM), FeCl3 (10mM), Ascorbic Acid (1mM), H_2O_2 (10mM) and Deoxyribose (10 mM), were prepared in distilled deionized water.

The assay was performed by adding 0.1ml EDTA , 0.01ml of $FeCl_3$,0.1ml H_2O_2, 0.36ml of deoxyribose, 1.0ml of the extract of different concentration (50, 100, 200 & 500 µg/ml)dissolved in distilled water,0.33ml of phosphate buffer (50mM , pH 7.9), 0.1ml of ascorbic acid in sequence . The mixture was then incubated at 37^0C for 1 hour. 1.0ml portion of the incubated mixture was mixed with 1.0ml of 10%TCA and 1.0ml of 0.5% TBA (in 0.025M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532nm. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of deoxyribose degradation is calculated by using the following equation

Hydroxyl radical scavenging activity= $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Superoxide radical scavenging activity

The superoxide anion scavenging activity was measured as described by Robak and Gryglewski [11]. The superoxide anion radicals were generated in 3.0 ml of Tris – HCL buffer (16 mM, P^H 8.0), containing 0.5 ml of NBT (0.3mM), 0.5 ml NADH (0.936mM) solution, 1.0 ml extract of different concentration (50, 100, 200,5 & 500 µg/ml), and 0.5 ml Tris – HCl buffer (16mM, P^H 8.0). The reaction was started by adding 0.5 ml PMS solution (0.12mM) to the mixture, incubated at 25^oC for 5 min and the absorbance was measured at 560 nm against a blank sample, ascorbic acid. The percentage inhibition was calculated by using the following equation

Superoxide radical scavenging activity= $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the test were performed in triplicates and the results were averaged

Antioxidant Activity by Radical Cation (ABTS. +)

ABTS assay was based on the slightly modified method of Re *et al*. [12]. ABTS radical cation (ABTS) was produced by reacting 7mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS Solution was diluted with ethanol to an absorbance of 0.70+0.02 at 734 nm. After addition of 100µL of sample or trolox standard to 3.9 mL of diluted ABTS solution ,absorbance was measured at 734 nm by Genesis 10s UV-VIS (Thermo scientific) exactly after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC).

ABTS radical cation activity = $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Reducing Power

The reducing power of the extract was determined by the method of Singh *et al.*[13] with minor modification to Oyaizu[14]. 1.0ml of solution containing 50, 100, 200 & 500µg /ml of extract was mixed with sodium phosphate buffer (5.0 ml, 0.2 M, pH6.6) and potassium ferricyanide (5.0ml, 1.0%): The mixture was incubated at 50^oC for 20 minutes. Then 5ml of 10% trichloroacetic acid was added and centrifuged at 980gm (10 minutes at 5^oC) in a refrigerator centrifuge. The upper layer of the solution (5.0 ml) was diluted with 5.0ml of distilled water and ferric chloride and absorbance read at 700nm. The experiment was performed thrice and results were averaged.

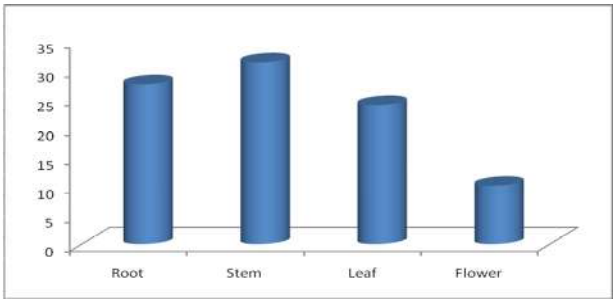

Figure 1: The total phenolic contents of methanol extract of different parts of *Sauropus bacciformis*

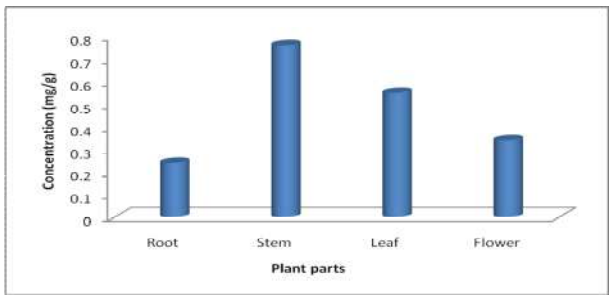

Figure 2: The total flavonoid contents of methanol extract of different parts of *Sauropus bacciformis*

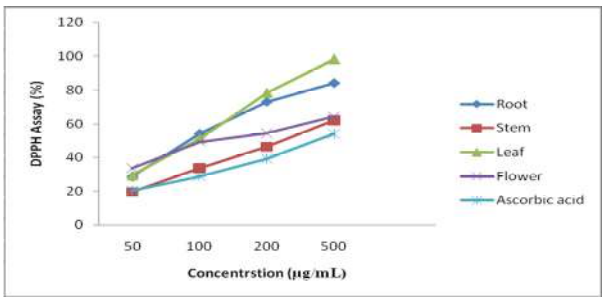

Figure 3: DPPH radical scavenging activity of methanol extract of different parts of *Sauropus bacciformis*

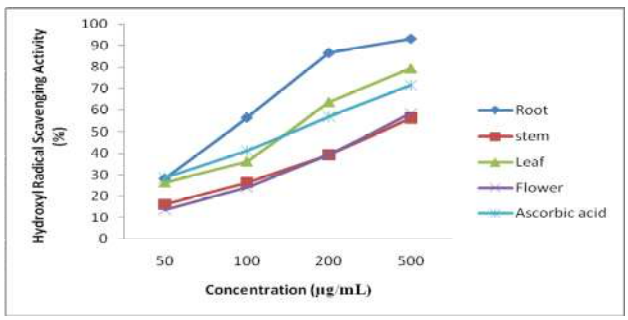

Figure 4: Hydroxyl radical scavenging activity of methanol extract of different parts of *Sauropus bacciformis*

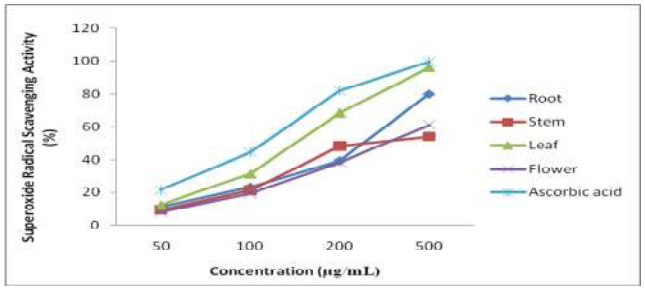

Figure 5: Superoxide radical scavenging activity of methanol extract of different parts of *Sauropus bacciformis*

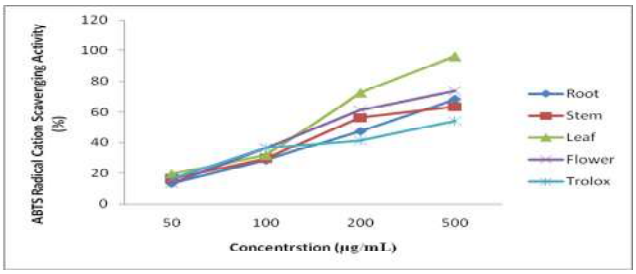

Figure 6: ABTS radical cation scavenging activity of methanol extract of different parts of *Sauropus bacciformis*

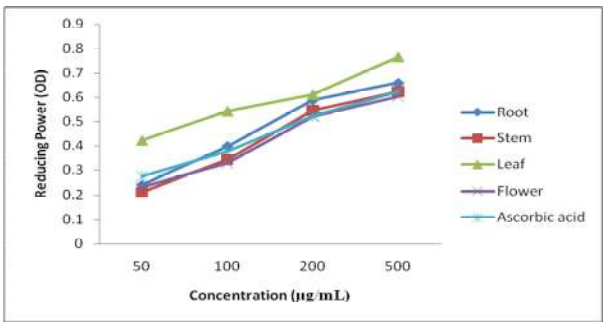

Figure 7: Reducing power ability of methanol extract of different parts of *Sauropus bacciformis*

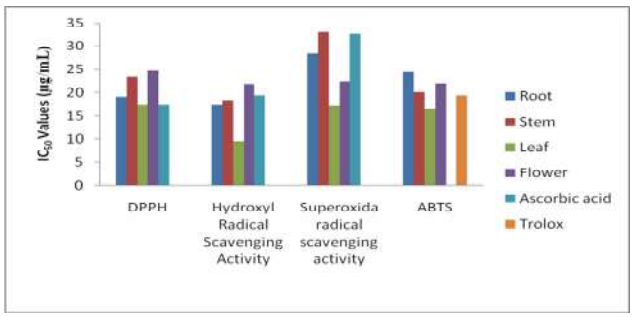

Figure 8: IC_{50} values of methanol extract of different parts of *Sauropus bacciformis*

RESULTS AND DISCUSSION

The total phenolic and flavonoid contents of methanol extract of different parts of *Sauropus bacciformis* were presented in Figure 1 and 2. The highest amount of phenol was found to be observed in stem of (31.3 mg $100g^{-1}$) *S .bacciformis*, whereas lowest content was to be observed in flower (10.1 mg $100g^{-1}$). Flavonoid content varied from 0.24 mg $100g^{-1}$ to 0.76 mg $100g^{-1}$. High amount of flavonoid content was observed in the stem (0.76 mg $100g^{-1}$) when compared to that of other parts.

Plant phenolic constitutes one of the major groups of compounds acting as primary antioxidant or free radical terminators. Phenolic compounds such as flavonoids, phenolic acids and tannins are considered to be the major contributor to the antioxidant activity of vegetables, fruits or medicinal plants. The antioxidant activity of the phenolic compounds were attributed to the redox properties, which allow them act as reducing agents, hydrogen donators, singlet oxygen quenchers and have also metal chelating properties[15]. These compounds have been effective in many health related properties such as anticancer, antiviral, anti-inflammatory activities, effects on capillary fragility and ability to inhibit human platelet aggregation[16,17].

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 was determined by the decrease in its absorbance at 517nm, which is induced by antioxidants. The percentage of DPPH radical scavenging activity of methanol extract of different parts of *Sauropus bacciformis* were presented in Figure 3.The

methanol leaf extract of *Sauropus bacciformis* exhibited a maximum scavenging activity of 98.27% at 500 µg/mL whereas for ascorbic acid (standard) was found to be 54.26% at same concentration. The IC_{50} values of methanol extract of different parts such as root, stem, leaf, flower of *Sauropus bacciformis* and ascorbic acid were found to be 19.02µg/ mL, 23.45 µg/mL, 17.45 µg/mL, 24.78 µg/mL and7.46 µg/ mL respectively (Figure 8).

In biochemical systems, superoxide radical can be converted into hydrogen peroxide by the action of superoxide dismutase and the H_2O_2 can subsequently generate extremely reactive hydroxyl radicals in the presence of certain transition metal ions or by UV photolysis. Hydroxyl radicals can attack DNA molecules to cause strand scission[18]. Figure 4 showed the hydroxyl radical scavenging activity of methanol extract of *Sauropus bacciformis* and compared with ascorbic acid. It was observed that methanol extract of root and leaf had more activity than ascorbic acid whereas methanol extract of stem and flower had less activity than ascorbic acid. At a concentration of 500 ug /mL, the hydroxyl scavenging activity of methanol extract of root reached 98.11%. The IC_{50} values of methanol extract of different parts such as root, stem. leaf, flower of *Sauropus bacciformis* and ascorbic acid were found to be 17.45 µg/ mL, 18.34 µg/mL, 9.56 µg/mL, 21.77 µg/mL and 19.26 µg/ mL respectively (Figure 8).

Superoxide is a highly reactive molecule that reacts with various substances produced through metabolic processes. Superoxide dismutase enzyme present aerobic and anaerobic organisms catalyses the breakdown of superoxide radical[19]**.** Superoxide radical scavenging activity of methonal extracts of different parts of *Sauropus bacciformis* were presented in Figure 5. The maximum scavenging activity of leaf extract and ascorbic acid at 500 ug /mL were found to be 96.31% and 99.74% respectively. Superoxide scavenging ability of plant extract might primarily due to the presence of flavonoids[20]. IC_{50} values of methanolic extract of different parts such as root. Stem, leaf, flower of *Sauropus bacciformis* and ascorbic acid were found to be 28.44µg/ mL, 33.05µg/mL, 17.34 µg/mL, 22.45 µg/mL and 32.68 µg/mL respectively(Figure 8).

The ABTS radical cation scavenging potential of the methonal extract of different parts of *Sauropus bacciformis* were presented in Figure 6. The highest percentage activity of leaf extract and trolox (standard) were found to be 96.31 µg/mL and 54.26 at 500 µg/ mL. IC_{50} values of methanolic extract of different parts such as root, stem, leaf, flower of *Sauropus bacciformis* and trolox were found to be 24.45 µg/ mL, 20.12 µg/mL, 16.54 µg/mL, 2.98 µg/mL and 19.27 µg/ mL respectively (Figure 8).

Figure 7 showed the reducing capacities of the plant extract compared to ascorbic acid. The reducing power of methanol extract of leaf of *Sauropus bacciformis* was very potent and the reducing power of the extract was increased with increasing concentration. At a concentration of 500ug/mL, reducing power of methanol extract of leaf was 0.767.

From the results obtained in the present study, it is concluded that methanolic extract of different parts of *Sauropus bacciformis* which contains high amount of phenolic compounds, exhibits high antioxidant and free radical scavenging activities. These *in vitro* assays indicate that this plant extracts are a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract. Furthermore, the in vivo antioxidant activity of this extract needs to be assessed prior to clinical use.

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