International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

**Research Article** **CODEN: IJPNL6**

# *IN VITRO* ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACTS OF *SOLANUM ERIANTHUM* D. DON

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**ABSTRACT**

The present study involves assay of the *in vitro* antioxidant activity of methanolic extracts of leaf, stem, fruit and root of *Solanum erianthum*. The *in vitro* antioxidant activity was evaluated by using free radical scavenging studies: DPPH radical scavenging, hydroxyl radical scavenging, hydrogen peroxide, reducing power, phosphomolybdate, total phenol and total flavonoid assay. The methanolic extracts exhibited a dose-dependent scavenging activity of DPPH, hydroxyl radical and hydrogen peroxide. All the extracts showed significantly higher inhibition percentage and positively correlated with total phenolic content. In addition, the total antioxidant assay established the antioxidant property of the methanolic extracts. Fruit and leaf extracts proved to be effective for the said parameters.

**KEYWORDS**: Antioxidant activity, DPPH, hydrogen peroxide, phenol content, phosphomolybdate assay, *Solanum erianthum*.

## INTRODUCTION

Free radicals are known to be extremely hazardous and particularly reactive oxygen species can cause tissue injury and have been implicated in many diseases, including malaria, acquired immunodeficiency syndrome, heart disease, diabetes and cancer [1]. The synthetic antioxidants like BHA, BHT and gallic acid esters have been suspected to prompt negative health effects and therefore strong restrictions have been imposed on their usage [2, 3]. In recent years much attention has been devoted to natural antioxidants and their association with health benefits [4]. A large number of medicinal plants and their purified constituents have shown potential therapeutic benefits. Various herbs and spices have been reported to exhibit antioxidant activity. The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanins, coumarins, lignans, catechins and isocatechins [5]. Antioxidants help in dealing with oxidative damage caused by free radicals and hence natural antioxidants are gaining lot of importance. A number of *Solanum* species have previously been investigated for antioxidant activities. Plants such as *S. melongena***,** *S. pseudocapsicum*, *S. aculeastrum*, *S. trilobatum*, *S. grandiflora*, *S. torvum* and *S. nigrum* are known to exhibit strong antioxidant properties [6-11]. S. *erianthum*, a potential medicinal plant used by folklore for treating hemorrhoids, scrofula, leucorrhea, dysentery, fever, diarrhea, and digestive problems. It is also used as anti - inflammatory agent and to cure arthritis [12]. Leaves possess expectorant property [13]. Antioxidant profiling of *S. erianthum* leaf and stem sequential extracts has been carried out by Deepika and Sujatha (2013) using DPPH, superoxide, reducing power and TBARS assays [14]. However no such work has been carried out on methanolic extracts of *S. erianthum*. The objective of the present study was to investigate the antioxidant

activity of methanolic extracts of the *S. erianthum* using seven *in vitro* methods.

## MATERIALS AND METHODS

**Plant Material:** The plant specimen was collected from outskirts of Bangalore city and was identified as *Solanum erianthum* D. Don. (Syn *S. verbascifolium* non L) and subsequently authenticated by Regional Research Institute, Central Council for Research in Siddha and Ayurveda with the voucher specimen no RRCBI-4865. The voucher specimen is deposited in the herbarium of the same institute.

**Extraction:** The field grown fresh samples were washed with tap water followed by distilled water to remove the adhering dust particles. After blotting, samples were air dried in shade. The dried plant materials were ground to fine powder and stored in clean air tight containers. A sample of 30 g was placed in the soxhlet and run by using 250 mL of methanol at 40 °C for the extraction of bioactive compound. All the extracts were dried in vacuum rotary evaporator at 40 °C under reduced pressure. Each of these extracts were weighed and stored at 4 °C for further analysis.

***In vitro* antioxidant activity**

***Reducing power by Ferric chloride method:*** The reducing power was investigated by the $Fe^{3+}$- $Fe^{2+}$ transformation in the presence of the extracts as described by Fejes *et al* (2000) [15]. The $Fe^{2+}$ can be monitored by measuring the formation of perl's prussian blue at 700nm [16]. About 1 mL of the extract (100-500 $\mu gmL^{-1}$), 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of 1 % potassium ferricyanide were incubated at 50 °C for 30 min and 2.5 mL of 10 % trichloroacetic acid was added to the mixture and centrifuged for 10 min at 3000 rpm. About 2.5 mL of the supernatant was diluted with 2.5 mL of water and shaken with 0.5 mL of freshly prepared 0.1 % ferric chloride. The absorbance was measured at 700 nm. Butylated hydroxy toluene (100-500 $\mu gmL^{-1}$) was used as the standard.

***DPPH radical scavenging assay:*** The free radical scavenging activity of the extracts was measured *in vitro* by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay [17]. About 1 mL of DPPH solution was added to 3 mL of the extract dissolved in ethanol at different concentrations (100-500 $\mu gmL^{-1}$). The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm. The percentage scavenging activity at different concentrations was determined and the $IC_{50}$ value of the extracts was compared with that of ascorbic acid, which was used as the standard.

Scavenging activity (%) = A* B / A x 100

Where A is absorbance of DPPH and B is absorbance of DPPH and extract combination.

***Hydroxyl radical scavenging assay:*** Hydroxyl radical scavenging activity was measured by the ability of the extracts to scavenge the hydroxyl radicals generated by the $Fe^{3+}$- ascorbate - EDTA - hydrogen peroxide system [18, 19]. The reaction mixture (1 mL) contained 100 µL of 2- deoxy -2 - ribose (28 mM in 20 mM $KH_2PO_4$ buffer, pH 7.4), 500 µL of the extracts at various concentrations (100 - 500 $\mu gmL^{-1}$) in buffer, 200 µL of 1.04 mM EDTA and 200 µM ferric chloride (1:1 v/v), 100 µL of 1.0 mM hydrogen peroxide ($H_2O_2$) and 100 µL of 1 mM ascorbic acid. Test samples were kept at 37 °C for 1 h. The free radical damage imposed on the substrate deoxyribose was measured using the thiobarbituric acid test. About 1 mL of 1 % thiobarbituric acid (TBA) and 1 mL 2.8 % trichloroacetic acid (TCA) were added to the test tubes and were incubated at 100 °C for 20 min. After cooling, the absorbance was measured at 532 nm against a blank containing deoxyribose and buffer. Quercetin (100-500 $\mu gmL^{-1}$) was used as a positive control.

***Hydrogen peroxide scavenging assay:*** Hydrogen peroxide solution (2 $mML^{-1}$) was prepared with standard phosphate buffer (pH 7.4). Various concentrations of the extracts (100-500 $\mu gmL^{-1}$) in distilled water were added to 0.6 mL of hydrogen peroxide solution. Absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging activity at different concentrations of the extracts was determined and the $IC_{50}$ values were compared with the standard, α-tocopherol [20].

***Phosphomolybdate assay:*** The total antioxidant capacity of the extracts was determined by phosphomolybdate method using α-tocopherol as the standard [21]. An aliquot of 0.1 mL of the extracts (100 µg) solution was combined with 1 mL of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated in boiling water bath at 95 °C for 90 min. The samples were cooled to room temperature and the absorbance was measured at 695 nm against the blank. The total antioxidant capacity was expressed as gallic acid equivalent per gram.

***Determination of Total Phenolic content:*** Total phenolic content was determined by the Folin-

Ciocalteau method. Stock solution (1 $mgmL^{-1}$) of gallic acid was prepared in distilled water [22]. Different aliquots of extracts ranging from 10 to 100 $\mu gmL^{-1}$ were prepared. Methanolic extract weighing 100 mg in 1 mL of distilled water, from the above stock 0.1 mL was pipetted out into test tube. A volume of 1.5 mL FC reagent was added in each test tube and kept aside for 5 min and 4 mL of 1 M sodium carbonate solution was added and made up to 10 mL with distilled water. The mixture was allowed to stand at room temp for 30 min and the absorbance was measured at 738 nm. Gallic acid was used as reference and the results were denoted as µg gallic acid equivalent.

***Estimation of total flavonoid content:*** Total soluble flavonoid content of the extracts was determined with aluminium nitrate using rutin as the standard [23]. 1 mg of the extract was added to 1 mL of 80 % ethanol. An aliquot of 0.5 mL of sample was added to test tubes containing 0.1 mL of 10 % aluminium nitrate, 0.1 mL of 1 M potassium acetate and 4.3 mL of 80 % ethanol. The absorbance of the supernatant was measured at 415 nm after incubation at room temperature for 40 min. The total flavonoid content in the extracts was determined as rutin equivalent per gram by using the standard rutin graph.

***Calculation of 50 % Inhibitory Concentration ($IC_{50}$)***
The concentration ($mgmL^{-1}$) of the extracts that was required to scavenge 50 % of the radicals was calculated by using the percentage scavenging activities at five different concentrations of the extracts.
Percentage inhibition (I %) was calculated using the formula,

$$I\ \% = (Ac-As) \times 100 / Ac$$

Where Ac is the absorbance of the control and As is the absorbance of the sample.

**RESULT**

***DPPH assay:*** All the methanolic extracts of *S. erianthum* demonstrated H-donor activity. The highest DPPH radical scavenging activity was detected in leaf extract ($IC_{50}$ 0.14 $mgmL^{-1}$), followed by fruit, stem and root extracts (Table 1). These activities were less than that of ascorbic acid ($IC_{50}$ 0.03 $mgmL^{-1}$).

***Reducing power ability:*** The reductive capabilities of different extracts of *S. erianthum* in comparison with standard BHT are evident (Fig. 1). The reducing power increased with increasing amount of the extracts. The leaf extract of *S. erianthum* demonstrated potent reducing ability than all the other extracts tested. However, the activity was less than the BHT. The fruit, stem and root extracts also showed significant activity indicating its reductive ability.

***Hydroxyl radical scavenging assay:*** Hydroxyl radical scavenging activity was quantified by measuring the inhibition of the degradation of deoxyribose by the free radicals generated by the Fenton reaction. The scavenging activity of leaf extract was equivalent to the standard, whereas the fruit extract activity ($IC_{50}$ 0.28 $mgmL^{-1}$) was higher than that of quercetin (0.30 $mgmL^{-1}$). The $IC_{50}$ values of the stem and root were 0.32 and 0.33 $mgmL^{-1}$ respectively (Table 1).

***Hydrogen peroxide scavenging assay:*** *S. erianthum* extracts scavenged hydrogen peroxide in a concentration - dependent manner. The fruit extract of *S. erianthum* exhibited hydrogen scavenging activity ($IC_{50}$ 0.19 $mgmL^{-1}$) whereas the standard, α-tocopherol had potent scavenging activity with 0.065 $mgmL^{-1}$. The leaf, stem and root extract showed moderate scavenging activities in comparison with standard (Table 1).

***Total phenolic content:*** Total phenolic content of the different extracts were expressed as µg gallic acid equivalent. The content of the total phenols in the extracts decreased in the order of leaf > fruit > stem > root (Table 1).

***Total antioxidant activity:*** The phosphomolybdate method is quantitative, since the total antioxidant capacity is expressed as gallic acid equivalents. Among the extracts tested, the fruit extract contained 40.83 mg GAE /g. The antioxidant activity increased in the order of fruit > leaf > stem > root (Table 2).

***Total flavonoid content:*** The total flavonoid content of *S. erianthum* extracts of leaf, fruit, stem and root were 53.85 mg, 35.62 mg, 13.43 mg and 2.31 mg - rutin equivalent per gram respectively (Table 2).

***Correlation of $IC_{50}$ values of antioxidant activities with DPPH, total phenolic content and total flavonoid content:*** The extracts showed a positive correlation with $R^2$ = 0.979 for DPPH radical scavenging and their total phenolic contents (Fig. 2). A positive and significant correlation existed between phenolic content and total antioxidant activity ($R^2$ = 0.888) (Fig. 3). However, a significant but marginal positive correlation ($R^2$ = 0.602) was found between flavonoid content and total antioxidant activity (Fig. 4).

## DISCUSSION

DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extracts [24]. The DPPH scavenging ability of the test extracts seem to be contributed by metabolites like phenol and flavonoids [25]. The DPPH scavenging activity of *S. erianthum* leaf and fruit were significantly higher when compared to *S. melongena* fruit, *S. pseudocapsicum* leaf, *S. aculeastrum* berries, *S. xanthocarpum*, *S. dulcamara*, *S. incanum* and *S. trilobatum* [6-9, 26, 27] . However *S. nigrum* leaf showed better DPPH scavenging activity with very low $IC_{50}$ value [28].

The reducing ability of a compound generally depends on the presence of reductones, which exert antioxidant activity by breaking free radical chain culminating in donating a hydrogen atom [18]. The antioxidant principle present in the extracts of *S. erianthum* caused the reduction of $Fe^{3+}$/ ferricyanide complex to the ferrous form, and thus proved the reducing ability [16]. The hydroxyl radical scavenging activity of *S. erianthum* was effectual than *S. trilobatum* [29].

Hydrogen peroxide itself is not particularly reactive with most biologically important molecules, but it is an intracellular precursor of hydroxyl radicals which is very toxic to the cell [25]. The methanolic extracts of *S. erianthum* scavenged hydrogen peroxide, which may be attributed to the presence of phenolic group that donate electrons to hydrogen peroxide there by neutralizing it into water, as opined by Miyake and Shibamoto (1997) [30] .

The antioxidant activity by phosphomolybdenum method exhibited higher antioxidant activity of *S. erianthum* fruit extract in comparison with *S. muricatum*, *S. xanthocarpum* and *S. trilobatum* [26, 29-33]. Only *S. nigrum* leaf revealed better antioxidant activity than *S. erianthum* [28].

The total phenol content was relatively higher than those reported in *S. melongena* fruit, *S. dulcamara*, *S. inacum*, *S. nigrum* leaf, *S. trilobatum*, *S. diphyllum* and *S. torvum* leaf [6, 11, 27-29]. The species *S. guaraniticum* possessed very high total phenolic content than other *Solanum* species [34]. As antioxidants, phenols improve the cell survival and as pro - oxidants, they induce apoptosis and prevent tumor growth [35]. The role of phenols in the prevention of degenerative diseases, particularly cardiovascular diseases and cancers are well established [36-37].

The role of flavonoids as antioxidants has been well established and there have been numerous reports on structure - activity relationships in the last decade [38]. Flavonoid compounds are also responsible for effective free radical scavenging and antioxidant activities [39]. The flavonoid content of *S. erianthum* leaf was significantly higher than *S. muricatum*, *S. dulcamara*, *S. incanum*, *S. trilobatum*, *S. nigrum* and *S. grandiflorum* [10, 27- 29, 31].

The strong correlation between antioxidant activity and the total phenolic content shows that phenol compounds largely contribute to the antioxidant activity of this species. The marginal correlation of antioxidant capacity and total flavonoid content suggests that flavonoids weakly contributed for the oxidation of free radicals [40].

## CONCLUSION

The *in vitro* assays indicate that *S. erianthum* is a significant source of natural antioxidants, which might be helpful in preventing the progress of various oxidative stresses. Further investigation on isolation and identification of the phyto constituents responsible for antioxidant activity is desirable.

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**Table 1: Antioxidant activities of methanolic extracts of *S. erianthum***

| Extracts | DPPH $IC_{50}$ mg/mL | Hydroxyl Radical Scavenging $IC_{50}$ mg/mL | Hydrogen Peroxide Scavenging $IC_{50}$ mg/mL |
|---|---|---|---|
| Leaf | $0.14 \pm 0.43^{a}$ | $0.28 \pm 0.03^{a}$ | $0.19 \pm 0.03^{b}$ |
| Fruit | $0.17 \pm 0.03$ | $0.30 \pm 0.03$ | $0.49 \pm 0.03$ |
| Stem | $0.21 \pm 0.03^{b}$ | $0.32 \pm 0.03^{c}$ | $0.71 \pm 0.03^{a}$ |
| Root | $0.26 \pm 0.03$ | $0.33 \pm 0.03$ | $0.81 \pm 0.03$ |
| Ascorbic acid | $0.03 \pm 0.01^{e}$ | - | - |
| Quercetin | - | $0.308 \pm 22.04^{b}$ | - |
| α-tocopherol | - | - | $0.065 \pm 0.83^{e}$ |

n=18, $P < 0.05$. Values within a column followed by different letters are significantly different

**Table 2: Total phenol, total flavonoid and total antioxidant assay of *S. erianthum* methanolic extract**

| Extracts | Total Phenol (mg GAE/g)[A] | Total flavonoid (mg RE/g)[B] | Total antioxidant assay (mg GAE/g)[A] |
|---|---|---|---|
| Leaf | 30.68 ± 0.68 | 53.85 ± 0.35 | 31.66 ± 0.83 |
| Fruit | 33.62 ± 1.02 | 35.62 ± 0.85 | 40.83 ± 0.47 |
| Stem | 18.36 ± 0.75 | 13.43 ± 0.56 | 20.33 ± 0.95 |
| Root | 7.67 ± 0.82 | 2.31 ± 0.41 | 16.89 ± 0.54 |

n =18, P < 0.05. [A]GAE - Gallic acid equivalents; [B]RE- Rutin equivalents.

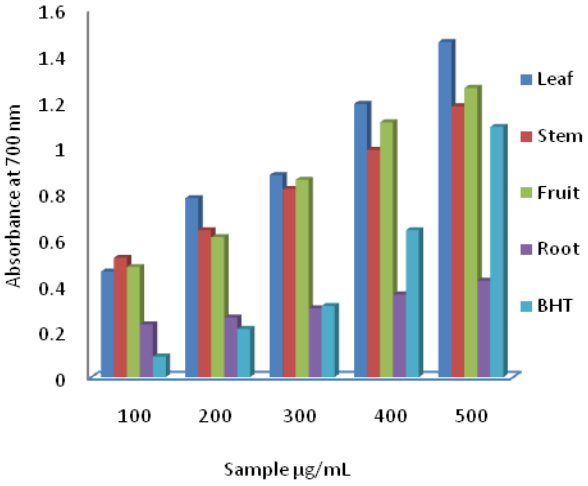


Figure: 1 Reducing power of methanolic extracts of *S. erianthum*

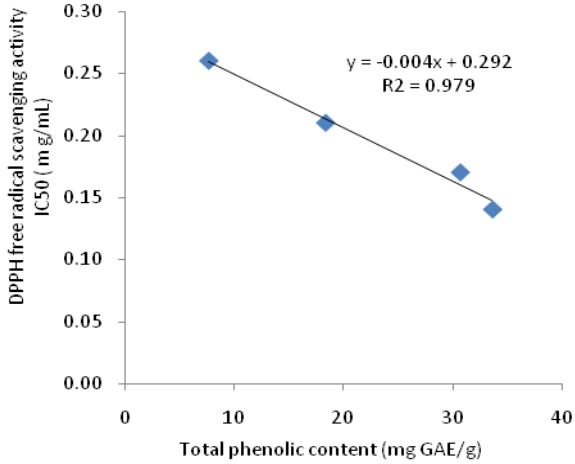


Figure 2: Linear correlation between DPPH radical scavenging activities of the different extracts of *S. erianthum* and their total phenolic contents

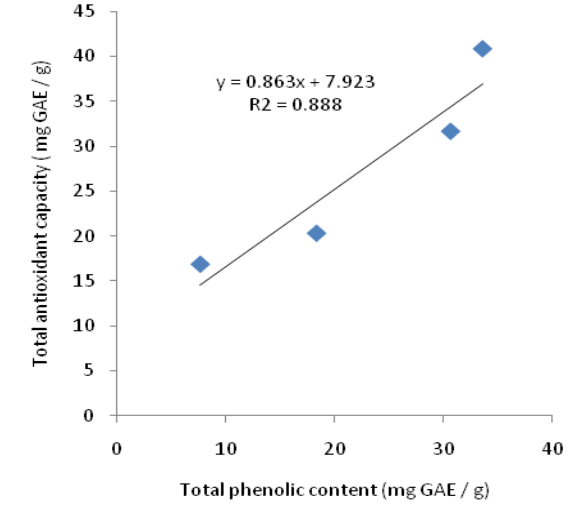


Figure 3: Linear correlation between the total antioxidant capacity of the different extracts of *S. erianthum* and their total phenolic contents.

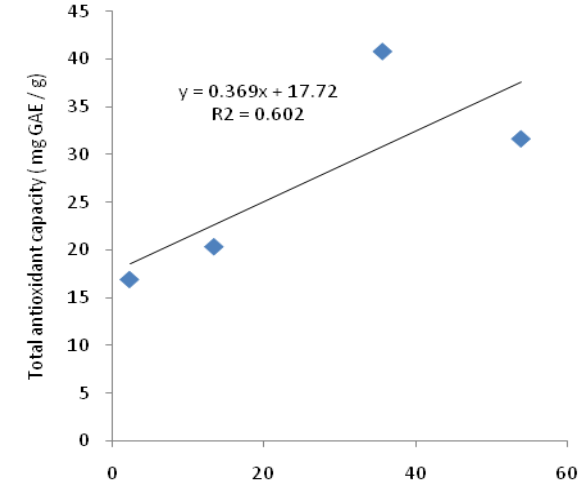


Figure 4: Linear correlation of flavonoid content with antioxidant activity

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