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# Study on *In-vitro* Antioxidant Potential of *Solanum Incanum* Fruit Extract

**T. Indhumathi*[1], Dr. S. Suja[2]**

[1]*PG and Research Department of Biochemistry, Dr. N.G.P Arts and Science College, Coimbatore-48, India*
[2]*PG and Research Department of Biochemistry, P.S.G College of Arts and Science, Coimbatore -14, India*

**A B S T R A C T**

Ethanolic extract of *Solanum incanum* fruit were tested for *in-vitro* free radical scavenging assays, such as hydroxyl radical, superoxide radical, 2,2'-diphenyl -1-picryl hydroxyl radical (DPPH) and reducing ability. *Solanum incanum* fruit extract effectively scavenged free radicals at all different concentrations and showed its potent antioxidant activity. These effects were in a dose dependent manner. Results were compared to standard antioxidant butylated hydroxy toluene (BHT). Further the study validates the therapeutic benefits of the Indian system of medicine.

**Keywords:** *Solanum incanum*, Antioxidant, DPPH.

**A R T I C L E I N F O**

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***Corresponding Author**
T. Indhumathi
PG and Research Department of Biochemistry, Dr. N.G.P Arts and Science College, Coimbatore-48, India
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## 1. Introduction

The health promoting benefits of antioxidants of plants are thought to be resulted from their potential effects against the reactive Oxygen/Nitrogen species. Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing such free radical-induced tissue injury. Hence, the development of antioxidants from natural origin has attracted considerable attention and is thought to be a desirable development. Moreover, several studies have indicated that medicinal plants contain a wide variety of natural antioxidants such as

phenolic acids, flavonoids and tannins, which possess antioxidant activity. *Solanum incanum* is both well-known and less popular medicinal herbs belong to the Solanaceae. Solanaceae plants have become increasingly important as the starting material for the production of steroidal hormones .Various species of Solanum like *Solanum khasianum, Solanum lyratum, Solanum xanthocarpum, Solanum nigrum, Solanum gracile, Solanum tuberosum, Solanum laciniatum* are being extensively used for the treatment of various ailments like asthma, liver diseases and inflammation in the traditional system of medicine.

## 2. Materials and Methods

**Plant Material**

*Solanum incanum* was collected from Sathyamangalam hills, Tamilnadu. The plant was identified by Dr.G.V.S. Murthy, Scientist'F'& Head of Office, Botanical survey of India, Southern Regional Centre TNAU Campus, Coimbatore-03 with the number BSI/SRC/5/2/2012-13/Tech312. The fruits were collected from the plant and it was washed with water thoroughly to free from debris. The fruits were sliced and shade dried for 20days. The dried fruit was ground coarsely and stored for further use.

**Preparation of Extract**

Using soxhlet extractor the dried fruit material was separately extracted with ethanol for 48 hours. The filtrate was evaporated to dryness at 40°C under vacuum. The extract was stored at 4°C for further use.

**Hydroxyl radical scavenging assay**

The hydroxyl radical scavenging activity of *S.incanum* was determined by the method of Halliwell *et al*. In this assay, hydroxyl radicals are produced by the reduction of $H_2O_2$ by the transition metal (iron) in the presence of ascorbic acid. The generation of hydroxyl radical is detected by its ability to degrade deoxyribose to form products which forms a pink colour chromogen when heating with TBA. Addition of *S.incanum* competes with deoxyribose for hydroxyl radicals and diminishes the colour formation. The incubation mixture in a total volume of 1 mL contained 0.1 mL of buffer, varying volumes of *S. incanum* (100, 200, 300, 400 and 500 µg), 0.2 mL of 500 µmol/L ferric chloride, 0.1 mL of 1mM ascorbic acid, 0.1 mL of 1 mmol/L EDTA, 0.1 mL of 10 mmo/L hydrogen peroxide and 0.2 mL of 15 mmol/L 2-deoxyribose. The contents were mixed thoroughly and incubated at room temperature for 60 min. Then 1 mL of 1% TBA (1 g in 100 mL of 0.05 N NaOH) and 1 mL of 28% TCA were added. All the tubes were kept in a boiling water bath for 30 min. The absorbance of the supernatant was read in a spectrophotometer at 535 nm with reagent blank containing water in place of extract. The percentage of scavenging was determined. The efficiency of *S. incanum* was compared with various concentrations (100, 200, 300, 400 and 500 µg) of standard BHT. Decreased absorbance of the reaction mixture indicated increased hydroxyl radical scavenging activity. The percentage of scavenging was calculated as shown below:

$$\% \text{ Scavenging } [OH^{\cdot}] = A_0 - A_1 / A_0 \times 100$$

Where $A_0$ was the absorbance of the control, and $A_1$ was the absorbance in the presence of the sample of *S. incanum* or standard.

**Superoxide anion scavenging activity**

Superoxide anion scavenging activity of *S. incaum* was determined by the method of Nishimiki *et al*. with modifications. The assay was based on the oxidation of NADH by PMS to liberate PMSred. PMSred converted oxidized nitroblue tetrazolium (NBToxi) to the reduced form NBTred, which formed a violet colour complex. In the PMS-NADH-NBT system, superoxide anion is derived from dissolved oxygen by PMSNADH coupling reaction and reduces NBT. The color formation indicated the generation of superoxide anion, which was measured spectrophotometrically at 560 nm. A decrease in the formation of colour after addition of the antioxidant was a measure of its superoxide scavenging activity. One millilitter of NBT (100 µmol of NBT in 100 mmol/ L phosphate buffer, pH 7.4), 1 mL of NADH (468 µmol in 100 mmol/L phosphate buffer, pH 7.4) solution and varying volumes of *S. incanum* (100, 200, 300, 400 and 500 µg concentration) were mixed well. The reaction was started by the addition of 100 µL of PMS (60 µmol of 100 mmol/ L phosphate buffer, pH 7.4). The reaction mixture was incubated at 30 °C for 15 min. The absorbance was measured at 560 nm in a spectrophotometer. Incubation without *S. incanum* was used as blank. BHT was used as standard for comparison. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage of scavenging was calculated as the previous equation.

**DPPH radical scavenging activity**

The radical scavenging activity of *S. incanum* against DPPH was determined spectrophotometrically by the method of Brand Williams's *et al*. DPPH reacts with an antioxidant compound that can donate hydrogen and it gets reduced. The change in colour (from deep violet to light yellow) was measured. DPPH is a stable free radical and accepts an electron, or hydrogen radical to become a stable diamagnetic molecule. The intensity of the yellow colour depends on the amount and nature of radical scavenger present. A reaction mixture containing 1 mL of 0.1 mmol/L DPPH, various concentration of *S. incanum* (100, 200, 300, 400 and 500 µg) were made up to 3 mL using water. Then the tubes were incubated for 10 min. The formed yellow colour chromophore was measured at 517 nm. BHT was used as a standard for comparison.

**Total antioxidant activity by ABTS radical cation decolorization assay**

The total antioxidant activity of *S. incanum* was measured by the method of ABTS radical cation decolourisation assay. The improved technique for the generation of ABTS+ described here involves the direct production of the blue/green ABTS chromophore through the reaction between ABTS and potassium persulphate. Addition of *S. incanum* and other antioxidants compete with ABTS diminish the color formation. ABTS was dissolved in water to a 7 mmol/L concentration. ABTS was produced by

reacting ABTS stock solution with 2.45 mmol/L potassium persulphate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. Because ABTS and potassium persulphate react stoichiometrically at a ratio of 1:0.5, this will result in incomplete oxidation of the ABTS+. The incubation mixture in a total volume of 5 mL contained 0.54 mL of ABTS, 0.5 mL of 100 mmol/L phosphate buffer pH 7.4 and varying concentrations of *S. incanum* (100, 200, 300, 400 and500 µg). The blank contains water in place of samples. The absorbance was read in a spectrophotometer at 734 nm and compared with standard BHT at various concentrations (100, 200, 300, 400 and 500 µg).

**Nitric Oxide scavenging assay**: The procedure described by Sreejayan, N & Rao, was followed for analysis of Nitric oxide scavenging activity. Sodium nitroprusside (5µM) in standard phosphate buffer solution was incubated with different concentration of the test extracts dissolved in standard phosphate buffer (0.025M, pH 7.4) and the tubes were incubated at 25°C for 5 hr. After 5 hr, 0.5 ml of incubation solution was removed and diluted with 0.5 ml Griess reagent (prepared by mixing equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in water). The absorbance of chromophore formed was read at 546 nm. The control experiment was also carried out in similar manner, using distilled water in the place of extracts. The experiment was performed (in triplicate) and the activity was compared with BHT, which was used as a standard antioxidant.

Nitric oxide radical scavenging that is calculated by the Formula: **% Nitric oxide radical scavenging =**
(Control OD - Sample OD) × 100 /Control OD

## 3. Results and Discussion

### DPPH Radical Scavenging Activity

The DPPH radicals were widely used to investigate the scavenging activity of some natural compounds. The scavenging ability of *S.incanum* on DPPH is shown in Table 1 and compared with that of BHT. Table 1 shows the results of scavenging DPPH radical ability of *S.incanum* at various comparisons with same doses of BHT. In DPPH scavenging assay the IC50 value of the extract was 313µg/ml. *S.incaum* showed dose-dependent DPPH radicals scavenging activity. The decrease in absorbance of DPPH caused by antioxidant molecules and radical, which results in the scavenging of the radical by hydrogen donation (Saha *et al*., 2008, Nazin Ara *et al*., 2009).

The antioxidant activity has been attributed to various mechanisms, among which the prevention of chain initiation, the binding of transition metal ion catalysts, decomposition of peroxides, the prevention continued hydrogen abstraction and the reductive capacity of a compound may serve as a significant indicator of its potential antioxidant (Bhalodi *et al*., 2005). The antioxidant activity may be due to the presence of secondary metabolites. The difference in the $IC_{50}$ value can be due to the distribution of secondary metabolites that may fluctuate between different plant organs (Lisiewska *et al*., 2006).

**Table 1:** DPPH Radical Scavenging Activity of *Solanum incanum* fruit extract

| **Concentration (µg/ml)** | **Percentage inhibition** | |
|---|---|---|
| | ***Solanum incanum* fruit extract** | **BHT(Standard)** |
| 100 | 33.21±1.38 | 58.30±1.51 |
| 200 | 40.47±0.89 | 68.30±1.01 |
| 300 | 49.55±0.96 | 71.28±0.96 |
| 400 | 56.61±1.13 | 80.33±1.11 |
| 500 | 61.40±1.06 | 93.50±1.14 |
| | $IC_{50}$ = 313.7 µg/ml | $IC_{50}$ = 79.62 µg/ml |

### Hydroxyl Radical Scavenging Activity

The scavenging ability of *S.incanum* on hydroxyl radical is shown in figure 2 and compared with BHT. *S.incanum* fruit extract exerted inhibition of hydroxyl radical formation during incubation period. Among the oxygen radicals, hydroxyl radicals are the most reactive and induce severe damage to the adjacent bio molecules. It can abstract hydrogen atoms from biological molecules, including thiols, leading to the formation of sulfur radicals capable to combine with oxygen to generate oxysulfur radicals, a number of which damage membranes, DNA strand breakage, and finally inducing cytotoxicity, carcinogenesis (Babu *et al*., 2001).

**Table 2:** Hydroxyl Radical Scavenging Activity of *Solanum incanum* fruit extract

| **Concentration (µg/ml)** | **(Percentage Inhibition)** | |
|---|---|---|
| | ***Solanum incanum* fruit extract** | **BHT(Standard)** |
| 100 | 34.78±0.57 | 58.45±0.84 |
| 200 | 40.88±1.58 | 64.48±1.85 |
| 300 | 48.54±0.94 | 71.29±1.05 |
| 400 | 55.65±2.21 | 76.34±1.02 |
| 500 | 62.77±1.54 | 84.43±1.02 |
| | $IC_{50}$ = 325.6 µg/ml | $IC_{50}$ = 67.98µg/ml |

**Superoxide Radical Scavenging Activity:**

Superoxide radical is also another harmful reactive oxygen species as it damages cellular components in biological systems. This species is produced by a number of enzyme systems in auto-oxidation reactions and by non-enzymatic electron transfers that univalently reduce molecular oxygen. It can also reduce certain iron complexes such as cytochromes (Mohan *et al*., 2013). The present study showed potent superoxide radical scavenging activity for *S.incanum* fruit extract. The highest percentage activity 50.51±1.20 was found for ethanol extract of *S.incanum* fruit. The IC50 value was found to be 497.6µg/ml

**Table 3:** Superoxide Radical Scavenging Activity of Solanum incanum fruit extract

| **Concentration (µg/ml)** | **Percentage inhibition** | |
|---|---|---|
| | ***Solanum incanum* fruit extract** | **BHT(Standard)** |
| 100 | 29.56±1.30 | 36.67±1.53 |
| 200 | 34.37±0.99 | 42.49±2.05 |
| 300 | 38.46±0.80 | 49.59±0.96 |
| 400 | 45.40±1.01 | 56.04±1.40 |
| 500 | 50.51±1.20 | 64.22±1.60 |
| | $IC_{50}$ = 497.6µg/ml | $IC_{50}$ = 315.2 µg/ml |

**Nitric oxide Scavenging Activity**

Nitric oxide radical (NO) act as a chemical mediator and is involved in the regulation of physiological activities. It is found to be generated by endothelial cells, macrophages and neurons (Forstermann, 2010). Nitric oxide radicals play a vital role in vascularisation and metastasis of tumor (Jayakumar and Kanthimathi, 2011).

**Table 4:** Nitric oxide Radical Scavenging Activity of *Solanum incanum* fruit extract

| **Concentration (µg/ml)** | **Percentage inhibition** | |
|---|---|---|
| | ***Solanum incanum* fruit extract** | **BHT(Standard)** |
| 100 | 28.38±1.07 | 42.35±1.05 |
| 200 | 33.45±1.00 | 45.52±1.21 |
| 300 | 48.31±0.95 | 49.46±1.12 |
| 400 | 51.50±1.21 | 53.30±1.01 |
| 500 | 54.41±1.16 | 57.23±1.01 |
| | $IC_{50}$ = 373.9 µg/ml | $IC_{50}$ = 315.2 µg/ml |

Nitric oxide radical (NO) generated from sodium nitro prusside at physiological pH was found to be inhibited by *S.incanum* fruit *extract at different concentration*. *S.incanum fruit* extract and standard BHT exhibited IC50 value of 373.9 µg.ml and 315.2 µg.ml respectively which was shown in table 4.

**ABTS Radical Scavenging Activity**

Total antioxidant capacity of *S.incanum* fruit extract was determined by ABTS radical cation decolourisation assay by measuring the reduction of the radical cation as the percentage inhibition. The *S.incanum* fruit extract was subjected to ABTS radical cation scavenging activity and the results was presented in table 5. The scavenging effect of *S.incanum* and BHT was observed to be linear increase in ABTS radical scavenging activity with increasing concentration. The fruit extract exhibited potent ABTS radical cation scavenging activity in concentration dependent manner. At 500µg/ml concentration the fruit extract possessed 54.41±1.16 percentage of scavenging activity on ABTS.

**Table 5:** ABTS Radical Scavenging Activity of *Solanum incanum* fruit extract

| **Concentration (µg/ml)** | **Percentage Activity** | |
|---|---|---|
| | ***Solanum incanum* fruit extract** | **BHT(Standard)** |
| 100 | 28.39±1.07 | 42.35±1.05 |
| 200 | 33.45±1.00 | 45.52±1.23 |
| 300 | 48.30±0.95 | 49.46±1.12 |
| 400 | 51.50±1.21 | 53.42±1.06 |
| 500 | 54.41±1.16 | 57.23±1.01 |
| | IC50= 395.6 µg/ml | IC50= 312.9 µg/ml |

## 4. Conclusion

In very recent years, potent free radical scavenging has attracted a tremendous interest as possible therapeutics against free radical mediated diseases. Free radicals are constantly generated *in vivo* both by accidents of chemistry and for specific metabolic purposes. When an imbalance between free radical generation and body defense mechanism occurs, oxidative damage will spread over all the cell targets (DNA, lipids and proteins). It has been reported that a series of human illness such as cancer, atherosclerosis, cardio and cerebrovascular diseases and as

well as premature body aging can be linked to the damaging action of extremely reactive free radicals. From the results of the present study ethanolic extract of *S. incanum* fruit posses antioxidant activity which may be used as natural antioxidants replacing the synthetic antioxidants.

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