Evaluation of *in-vitro* Antioxidant and Anthelmintic Activity of *Solanum indicum* Linn. Berries

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**ABSTRACT**

*Solanum indicum* Linn. (*Solanaceae*) is grown in waste places, road sides and in open scrublands in different parts of India and other Asian countries. Its roots and leaves are used in traditional system of medicine for a long time. Several phytochemicals like fatty acids, alkaloids, steroids, flavonoids and saponins have been reported from the different parts of the plant but their biological efficacies were not evaluated. Hence, the berries of the plants were collected, authenticated and extracted with MeOH for screening antioxidant and also anthelmintic activity. *In vitro* DPPH radical scavenging activity using BHT as a standard and anthelmintic activity on Indian earth-worm (*Pheretima posthuma*) using Albendazole as a standard of crude MeOH extract of *Solanum indicum* berries were evaluated. Nine different concentrations (200, 100, 80, 60, 40, 20, 10, 1, 0.5 µg/ml) of MeOH extract were studied for DPPH scavenging activity. The maximum DPPH radical scavenging activity with inhibition was found at the concentration 200 µg/ml and it was 70.007 ± 0.841% as comparable to that of BHT having 95.023 ± 0.091% inhibition at the same concentration. Three concentrations (25, 50, 100 mg/ml) of extract were studied for anthelmintic activity which involved the determination of time of paralysis and time of death of the worms. All the concentration of extracts exhibited significant dose dependent activity.

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INTRODUCTION

*Solanum indicum* Linn. syn *Solanum anguivi* Lam. (local name: Bekair, Phutibegun) belonging to the family Solanaceae is found throughout the tropical India, waste places, on road sides and in open scrublands. Different parts of this plant are used in traditional system of medicine for a long time. Roots are used for carminative, expectorant, asthma, cough, cataract affections, toothache, fevers, worm complaints, colic and in dysuria. Leaves juice mixed with ginger is taken to stop vomiting\(^1\). A literature survey indicated the fruits and roots of this plant contain wax, fatty acids, alkaloid solanine and solanidine. Disogenin, lanosterol, sitosterol, solasornine, solamargine and solasidine have been isolated from the plant\(^2\).

The cellular metabolisms such as mitochondrial respiration of living cells may results in the generation of free radicals and other reactive oxygen species as by products. Free radicals and other reactive oxygen species (ROS) are capable of damaging DNA, proteins, carbohydrates and lipids. As a results various chronic disease, such as cancer, diabetes, ageing and other degenerative disease are formed\(^3\). Antioxidants are one of such substance which has the capability to neutralize free radicals or their action. Plants are the important source of antioxidant phytochemicals having strong scavenging and oxidation-inhibiting properties.

Helminthiasis is a broadly class of disease, caused by parasitic worms. Different types of worms like Roundworm, hookworm, pinworm, tapeworm, flatworm etc. affect human beings and animals. Helminthiasis is the common life threatening infection in most of the tropical countries including the Asian countries\(^4\). Variety of medical complications like nausea-vomiting, diarrhoea, poor appetite, loss of weight, anaemia and several clinical symptoms arises due to this infection\(^5\). The restrictive application of terminology anthelmintic is invariably meant for such drugs exerting their action locally to expel parasites from GI tract exclusively. In a broader sense and prospective anthelmintics drugs are defined more appropriately as “Drugs used to combat any type of helminthiasis”. Anthelmintics are broadly categorized as ‘vermifuge’ which actually paralyses the worms and expel out and other is ‘vermicidal’ which solely kills the worms\(^6\). Several anthelmintic drugs like piperizine and bezoimidazole classes of drugs exhibits exclusive performances till now but the major drawback is the high cost, unusual side-effects and day by day drug resistance.

This paper deals with the evaluation of *in vitro* antioxidant and anthelmintic activity of MeOH extract of *Solanum indicum* Linn. berries using the modified procedure of Cottle *et al.*\(^7\) (for antioxidant) and Ajayieoba *et al.*\(^8\) (for anthelmintic).

MATERIALS AND METHOD

General

All the chemicals and solvent used were analytical grade. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and di-tert-butyl hydroxyl toluene (BHT) were purchase from Sigma-Aldrich chemicals, Germany. Methanol was obtained from Merck chemicals, Mumbai, India. Carboxy Methyl Cellulose (CMC) was obtained from Rankem Laboratory, New Delhi, India. Albendazole suspension (Zentel) was purchased from GSK pharmaceutical Ltd., Bangalore, India.

Plant materials

The fresh berries of *Solanum indicum* Linn. were collected from the local market (Lake Chowmuhani Bazar, Agartala, West Tripura) in the month of February 2012 and authenticated by Prof. B.K.Datta, Taxonomist, Department of Botany, Tripura University. A voucher specimen of dried sample
Preparation of plant extract

The fresh berries of *S. indicum* were dried in sun shine and crushed into coarse powder in a hand mill. Dried coarse powder (1.2 kg) was extracted with MeOH (3L × 3) at room temperature for 72 h. The MeOH extract was concentrated under reduced pressure in vacuo to get a semisolid residue (75 g). These semisolid residues have been used for evaluation of *in vitro* antioxidant and anthelmintic activity.

DPPH radical scavenging activity

The free radical scavenging activity of MeOH extract of *S. indicum* was evaluated with the DPPH stable radical according to the method of Cotelle *et al.* with slight modification. In brief, 0.1 mM stock solution of DPPH in methanol was prepared and 2mL of this solution was added to 1.5 ml of different concentrations of fractions (0.5 µg/ml—200 µg/ml) and allowed to react at room temperature. After 30 minutes the absorbance values were measured at 515 nm using Perkin Elmer Lambda 25 spectrophotometer. Using these absorbance values of the solutions the radical scavenging activity (percentage of inhibition) was calculated using the following equation.

\[
\text{Percentage of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100\%
\]

Where test means tested extract or BHT solution. All the tests were run in triplicates and the average values were calculated.

**IC₅₀ Value**

The amount of extract/BHT required decreasing the absorbance of DPPH by 50%, called IC₅₀ and it was evaluated.

**Statistical analysis**

All mean values (± SD) of measured activities of the extracts and control shown in the Tables 1 and 2 were evaluated by unpaired Student’s t test. Values of p<0.05 were considered as significant.

**Selection of Experimental Animal for Anthelmintic activity**

Indian adult earthworms (*Pheretima posthuma*) were used to carry out the experiment. The earthworms were collected from the local supplier. Worms were washed with normal saline water to remove all faecal matter. The earthworms of 8-10 cm in length and 0.3-0.4 cm in width were used for all the experimental protocol. Ready availability, anatomical and physiological resemblance of *Pheretima posthuma* made it to be used initially for *in-vitro* evaluation of anthelmintic activity.

**Experimental Design**

Indian earthworm (*Pheretima posthuma*) of nearly equal size, six in each group was taken for the experiment. Each concentration of dried extract was suspended in 1% w/v CMC (Carboxy Methyl Cellulose) solution prepared in normal saline water in three different concentrations (25, 50, 100 mg/ml). Albendazole...
suspension of same concentration was taken as standard and normal saline water with 1% CMC was taken as a control. Worms were placed in Petri dishes containing 15 ml of sample solution. Time for paralysis was noted either when any movement could not be observed except when the worms were shaken vigorously or when dipped in warm water (50°C). Death was included when the worms lost their motility followed by white secretions and fading away of their body colour\textsuperscript{8,11,12}.

RESULTS AND DISCUSSION

DPPH radical scavenging activity of crude MeOH extract of \textit{S. indicum} berries and also synthetic antioxidant BHT are shown in Table-1 and IC\textsubscript{50} values are shown in Table 2. The observed scavenging activity of crude MeOH extract at the concentration 200 µg/ml was found to be 70.007 ± 0.841%, where as BHT was found 95.023 ± 0.091%. The remaining concentrations i.e. 100 µg/ml, 80 µg/ml, 60 µg/ml, 40 µg/ml, 20 µg/ml and 10 µg/ml the crude MeOH extract showed scavenging activity 59.613 ± 0.747%, 45.896 ± 0.681%, 41.806 ± 1.036, 31.790 ± 1.464%, 14.936 ± 0.850% and 8.7 ± 1.103%, respectively and in case of BHT it was found to be 94.523 ± 0.089%, 94.116 ± 0.097%, 92.426 ± 0.788%, 82.773 ± 0.301%, 66.883 ± 0.106% and 49.4 ± 0.334%, respectively. At the concentration 1 and 0.5 µg/ml, the crude MeOH extract does not showed any positive scavenging activity, where as BHT showed 16.89 ± 0.435% and 8.533 ± 0.120%. Out of nine concentrations of the crude MeOH extract, concentration 200 µg/ml has showed maximum antioxidant activity.

Table 1: DPPH Scavenging Activity (% of inhibition) of crude MeOH Extract of \textit{Solanum indicum} Linn. Berries and BHT (control).

<table>
<thead>
<tr>
<th>Conc. of Samples, µg/ml</th>
<th>Inhibition (%) (3 repeats ± SD) each of 3 experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MeOH extract</td>
</tr>
<tr>
<td>200</td>
<td>70.007 ± 0.841</td>
</tr>
<tr>
<td>100</td>
<td>59.613 ± 0.747</td>
</tr>
<tr>
<td>80</td>
<td>45.896 ± 0.681</td>
</tr>
<tr>
<td>60</td>
<td>41.806 ± 1.036</td>
</tr>
<tr>
<td>40</td>
<td>31.790 ± 1.464</td>
</tr>
<tr>
<td>20</td>
<td>14.936 ± 0.850</td>
</tr>
<tr>
<td>10</td>
<td>8.7 ± 1.103</td>
</tr>
<tr>
<td>1</td>
<td>------</td>
</tr>
<tr>
<td>0.5</td>
<td>------</td>
</tr>
</tbody>
</table>
Figure 1: Scavenging activity of crude MeOH extract and BHT (control) on DPPH at the concentration range 0.5 µg/ml – 200 µg/ml.

Table 2: *In vitro* antioxidant activity of crude methanolic extract and BHT.

<table>
<thead>
<tr>
<th>Extract / Standard</th>
<th>Antioxidant activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DPPH scavenging effect (%)</td>
</tr>
<tr>
<td></td>
<td>at 100 µg/ml conc.</td>
</tr>
<tr>
<td>BHT</td>
<td>94.523 ± 0.089</td>
</tr>
<tr>
<td>MeOH extract</td>
<td>59.613 ± 0.747</td>
</tr>
</tbody>
</table>

In the experiment of anthelmintic activity, all the tested doses of extract exhibit dose dependent activity. The peak activity showed by the highest concentration 100 mg/ml. Potency of the extract was inversely proportional to the time for paralysis (vermifuge) and death (vermicidal) of the worms. Literature report evident the presence of steroids, flavonoids, saponin etc. Probably the saponin and flavonoids (phenolics) are responsible for the exhibited activity.
Table 3: *In vitro* Anthelmintic activity of crude MeOH Extract of *Solanum indicum* Linn. Berries and Albendazole (standard).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Conc. (mg/ml)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Paralysis</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>31.22 ± 0.32</td>
</tr>
<tr>
<td>ME (Methanolic extract)</td>
<td>25</td>
<td>22.25 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>9.16 ± 0.12</td>
</tr>
<tr>
<td>Standard (Albendazole)</td>
<td>25</td>
<td>8.43 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>5.28 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.33 ± 0.21</td>
</tr>
</tbody>
</table>

ME: Methanol Extract, Alb: Albendazole. Values are expressed as mean ± SEM (n = 6). Diameters of bars are inversely proportional to the activity.

Figure 2: Anthelmintic activity of crude methanolic extract and standard Albendazole at the concentration range 25 mg/ml – 100 mg/ml.

**CONCLUSION**

The results obtained in the present investigation indicates that the berries of *Solanum indicum* is the rich source of antioxidant phytochemicals and this MeOH extract may used for herbal drug formulation. The MeOH extract of *S. indicum* berries has also showed significant anthelmintic activity at all the tested doses when compared to control as vermifuge and vermicidal, while highest activity exhibited by the higher conc.

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which assures the ethno-medicinal claim. Hence, we can think about this plant as alternate source of antioxidant and anthelmintic drugs.

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Authors’ Statements

Competing Interests

UGC-NERO, Guwahati, India
REFERENCES