Cytotoxic and Antimicrobial Activity of *Ipomoea Obscura*

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**ABSTRACT**

**Background:** The present study was aimed to investigate cytotoxicity and antimicrobial activities of successive extracts of *Ipomoea obscura*. Cytotoxic activity of successive plant extracts were tested against three human cancerous and three normal cell lines by using MTT and SRB assays. The CTC50 (the concentration that kills cells growth by 50%) was calculated for each extract. Antimicrobial activity of *Ipomoea obscura* extracts was evaluated against four different bacterial and two fungal strains using cup plate method and the zone of inhibition was calculated for each microorganism. **Results:** Among the extracts tested, the successive methanol extract showed promising cytotoxic and moderate antimicrobial activities against human cancer cell lines and microbial strains, respectively. **Conclusion:** This study contributes to the knowledge of anticancer efficacy of *Ipomoea obscura* from southern India.

**INTRODUCTION**

About 650 species of morning glories (*Ipomoea* sp., family Convolvulaceae) are distributed across the world’s tropical and subtropical regions. Many species of *Ipomoea* were and often still are used in folk medicine in different parts of the world (Heacock, 1975). The main constituent of *Ipomoea* species is the tropane, indole and piperidine alkaloids which have a chemotaxonomic significance in this genus (Jenett-Siems et al., 2003). Chemical investigations of this plant have shown the presence of tropane alkaloids such as Calysteginine B-1, Calysteginine B-2, Calysteginine B-3, Calysteginine B-4 and Calysteginine C-1 and indole alkaloids such as Ipobscurine A, Ipobscurine B, Ipobscurine C and Ipobscurine D (Asano et al., 2001; Eich et al., 1986). This plant also showed the presence of other phytochemicals such as flavonoids, steroids and phenolic acids (Mungole et al., 2001). In Uganda, it is used for the treatment of diarrhea by traditional healers. Leaves of this plant are used as an application to aphtous affections after toasting, powdering and boiling with ghee and in admixture with the leaves of *Argyreia mollis* used for sores, hemorrhoids and swellings (Anokbonggo et al., 1990; Srinivasan et al., 2007). Various extracts of *Ipomoea obscura* have been reported to possess anti-inflammatory, nephroprotective, anti-angiogenic and immune modulatory activities (Mungole et al., 2001; Kirriik and Basu, 1999; Srinivasan et al., 2007). Previous studies in our laboratory have also shown that the whole plant extracts of *Ipomoea obscura* possess strong antioxidant activity against various free radicals (Anokbonggo et al., 1990). Plants belonging to *Ipomoea* species such as *Ipomoea aquatica* and *Ipomoea batatas* [L.] have shown significant antioxidant and anticancer activities (Prasad et al., 2005; Liu et al., 2005). However, there is no cytotoxic and antimicrobial activities are reported for *Ipomoea obscura* (L.) till date. Hence, in the present investigation different extracts of *Ipomoea obscura* (L.) were screened for their *in vitro* cytotoxic activity against three normal and three human cancerous cell lines and their antimicrobial activity using standard procedures.

**MATERIAL AND METHODS**

2.1. Collection of plant material:

A whole plant of *Ipomoea obscura* (L.) Ker-Gawl (Convolvulaceae) was collected from Masinagudi village of the Nilgiri district, Tamilnadu in the month of June 2004 and was authenticated at Medicinal plants Survey and Collection Unit, Government Arts College, Ootacamund, India. The voucher specimen (TIFAC 02) has been deposited for further reference at JSS College of Pharmacy, Ooty.


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2.2. Extraction:

The whole plant material was dried in shade. The dried plant material was powdered and passed through sieve no.20 and extracted (100 g) successively with 600 mL each of petroleum ether (60 – 80°C), methanol and water in a Soxhlet extractor for 18 – 20 h. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40 – 50°C). The petroleum ether extract yielded a yellowish green sticky semisolid, weighing 1.7 g (1.70%). The methanol and water extracts yielded dark green and dark brown semisolid residues, weighing 17.5 g (17.5%) and 2.58 g (2.58%), respectively. All the dried extracts were stored at -20°C until used. Each extract was then dissolved in 10% dimethyl sulfoxide (DMSO) for testing of antimicrobial and cytotoxic effects.

2.3. Cytotoxicity assays:

Cytotoxicity was determined with the MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium Bromide) and the sulforhodamine B (SRB) assay (Chiu and Wu, 2002; Scheers et al., 2001). Three normal cell cultures cells (Vero-African green monkey kidney, BRL-3A-normal rat liver and MDCK-normal dog kidney) and three cancerous cell lines (e HeLa-human cervical cancer cells, HEp-2-human larynx epithelial cancer cells and A-549-human small cell lung carcinoma cells) were used to determine cytotoxicity. Cytotoxicity is expressed as the concentration of test drug kills cells by 50% (CTC 50). All tests and analysis were run in triplicate and mean values recorded. The entire cell cultures were procured from National Centre for Cell Sciences, Pune. Dalton’s Lymphoma Ascitis (DLA) cells were procured from Amla Cancer Research Institute, Thrissur, India, propagated in peritoneal cavity of Swiss albino mice and maintained in J.S.S. College of Pharmacy, Ooty.

2.4. Antimicrobial assay:

Cup plate method using Mueller-Hinton agar medium was employed to study the preliminary antibacterial activity of various extracts of Ipomoea obscura (Alviano and Alviano, 2009). Bacterial cultures such as Escherichia coli NCIM 2065, Salmonella typhi NCIM 2501, Bacillus subtilis NCIM 2439, Bacillus steartothermophilus NCIM 5146, Candida albicans NCIM 3100 and Aspergillus flavus NCIM 650 were procured from National Collection of Industrial Microorganisms, Pune, and sub cultured and maintained at J.S.S. College of Pharmacy, Ooty, India. The diameter of inhibition zones was measured in mm and the results were recorded. The inhibition zones with diameter less than 12 mm were considered as having no antibacterial activity.

RESULTS AND DISCUSSION

The cytotoxic and antimicrobial activities of successive extracts Ipomoea obscura are reported in Table 1 and 2. The results of the in vitro cytotoxicity studies on successive extracts of Ipomoea obscura reveal that the methanol extract has better cytotoxic nature over the other two extracts (water and petroleum ether) with CTC 50 values ranging from 122.11 µg/ml to 170.56 µg/ml. The water extract exhibits moderate cytotoxicity against all the cell lines with CTC 50 values ranging from 299.9 µg/ml to 368.06 µg/ml. The petroleum ether extract is nontoxic at test doses against all the cell lines. Among the three extracts tested, the methanol extract shows lesser toxicity towards normal cell lines such as Vero, BRL-3 A and MDCK when compared to cancerous cell lines. Hence, the methanol extract shows moderate specificity against cancer cell lines. In short term toxicity studies, all the three extracts do not show any toxicity even at 500 µg/ml, the highest concentration tested.

All the three extracts showed moderate antibacterial and antifungal activity against the six microbial strains tested. The possible antimicrobial mode of action of Ipomoea obscura is probably because of its ability to bind to the cell wall, thereby inhibiting its synthesis due to the presence of alkaloids.

Plants are promising source of anti-infective and anticancer chemotherapeutic agents (Bhahwal et al., 2007; Shanthy et al., 2011). Since India has a broad medicinal plant diversity and Ipomoea obscura is also one of the most commonly used plants in India to cure many diseases (Sripathi and Sankari, 2010). The Ipomoea obscura contains major pharmacologically active macrolactum type indole alkaloids such as ipobscurine-A, C and D. These indole alkaloids were reported to possess strong anti-inflammatory and anti-angiogenic properties (Hamsa and Kuttan, 2011).

In summary, the present study demonstrated that the successive methanol extract of Ipomoea obscura has shown good cytotoxic and moderate antibacterial activities. The possible mechanism involved in the cytotoxicity may be due to the induction of apoptosis pathways by indole alkaloids. These results indicate that Ipomoea obscura phytochemicals have distinct potentials for chemoprevention and chemotherapy strategies. However, further studies are required to isolate the active compounds and to determine the detailed and distinguishing features of intracellular pathway(s) involved in the mechanism of cytotoxicity.
**Table 1: Cytotoxicity of successive extracts of Ipomoea obscura on normal and cancer cell lines**

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Petroleum ether extract</th>
<th>Methanol extract</th>
<th>Water extract</th>
<th>MTT</th>
<th>SRB</th>
<th>Trypan blue exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vero</td>
<td>&gt; 500</td>
<td>-</td>
<td>&gt; 500</td>
<td>164.7 ± 5.36</td>
<td>142.38 ± 5.2</td>
<td>170.56 ± 9.37</td>
</tr>
<tr>
<td>BRL-3A</td>
<td>&gt; 500</td>
<td>-</td>
<td>&gt; 500</td>
<td>153.00 ± 8.28</td>
<td>148.32 ± 5.82</td>
<td>157.73 ± 11.36</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>&gt; 500</td>
<td>-</td>
<td>&gt; 500</td>
<td>142.38 ± 4.2</td>
<td>137.33 ± 3.10</td>
<td>153.33 ± 16.37</td>
</tr>
<tr>
<td>Hela</td>
<td>&gt; 500</td>
<td>-</td>
<td>&gt; 500</td>
<td>138.56 ± 7.57</td>
<td>129.39 ± 6.29</td>
<td>131.98 ± 4.92</td>
</tr>
<tr>
<td>Hep-2</td>
<td>&gt; 500</td>
<td>-</td>
<td>&gt; 500</td>
<td>138.56 ± 6.7</td>
<td>126.48 ± 7.92</td>
<td>122.11 ± 13.64</td>
</tr>
<tr>
<td>A-549</td>
<td>&gt; 500</td>
<td>-</td>
<td>&gt; 500</td>
<td>119.75 ± 6.13</td>
<td>114.08 ± 7.29</td>
<td>112.11 ± 13.64</td>
</tr>
<tr>
<td>DLA</td>
<td>-</td>
<td>-</td>
<td>&gt; 500</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* CTC50 (µg/ml) is the mean of three independent experiments, three replicates, Mean ± SE

**Table 2: Antimicrobial activity of successive extracts of Ipomoea obscura**

<table>
<thead>
<tr>
<th>Test microorganism</th>
<th>Petroleum ether extract</th>
<th>Methanol extract</th>
<th>Water extract</th>
<th>Standard antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>11.5</td>
<td>12</td>
<td>10.5</td>
<td>22</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>11.0</td>
<td>14</td>
<td>12</td>
<td>26</td>
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<tr>
<td>Bacillus subtilis</td>
<td>13.5</td>
<td>11.5</td>
<td>13</td>
<td>19</td>
</tr>
<tr>
<td>Bacillus stearotherophilus</td>
<td>12</td>
<td>13</td>
<td>14</td>
<td>21</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>12</td>
<td>15</td>
<td>14</td>
<td>19</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>11</td>
<td>13.5</td>
<td>12</td>
<td>18</td>
</tr>
</tbody>
</table>

Petroleum ether, methanol and water extract 1 mg/well
* Penicillin 10 IU/well; ** Streptomycin 10 µg/well; *** Amphotericin B 1 µg/well;

*average of two independent experiments, duplicate, values including well diameter (10mm).

**REFERENCES**


