Cytotoxic Potential of various extracts of *Lepidium sativum* (Linn.) An In-vitro Evaluation

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**ABSTRACT**

Objective: To explore the cytotoxic activity of seeds of various extract of *Lepidium sativum* using different cell lines. Methods: The various extracts n-hexane, chloroform, ethyl acetate and methanol extracts of seeds of *Lepidium sativum* were tested against human cancer cell lines such as human neuroblastoma cell line (IMR-32), colon cancer cell lines (HT-15 & HT-29) and lung cancer cell line A-549. Results: The result showed that the methanolic extract of *Lepidium sativum* exhibited a very high degree of in-vitro cytotoxic activity than that of standard mitomycin-c. Similar results were not found in other three extracts. Conclusion: From the result it can be seen that the methanolic extract of *Lepidium sativum* has potent in-vitro cytotoxic activity.

**Keywords:** *Lepidium sativum*, IMR-32, HT-15, HT-29, A-549, Cytotoxicity.

**INTRODUCTION**

In recent years the popularity of complementary medicine has increased. Medicinal plants have played a key role in world health. Cancer is one of the most dangerous diseases in humans and presently there is a considerable scientific discovery of new anti-cancer agents from natural products [1]. It affects one-third of persons and is a major cause of deaths in the developed world during the year 2000 [2]. Available management options such as surgery, radiation therapy, immunotherapy and chemotherapy are either toxic, expensive or both. This lead to the search for alternative therapies in botanicals with anti-cancer activity. *Lepidium sativum* Linn. (Cruciferae) is commonly known as garden cress. It is a small, herbaceous annual plant, cultivated throughout India. The whole plant is administered in asthma and bleeding piles and the roots are used in secondary syphilis and tenesmus [3]. Seeds are considered to be aphrodisiac, galactogogue, emmenagogue and are used after boiling with milk to induce abortion. *Lepidium sativum* has been studied pharmacologically for its free radical scavenging [4], antidiarrheal, antispasmodic [5], hypoglycaemic [6], laxative [7], antibacterial [8], antioxidant and contraceptive effects [9] and in inflammatory bowel disease [10]. It has been evaluated for its fracture-healing [11] and diuretic activities [12]. *Lepidium sativum* is documented to possess alkaloids, riboflavin, α-tocopherol, β-carotenes, β-sitosterol, ascorbic, linoleic, oleic, palmitic and stearic acids. It is considered a good source of mono-unsaturated fatty acids and L-arabinose [13]. Moreover, cucurbitacins and cardenolides have also been identified as plant constituents [14]. As far as the pharmacological activities of *Lepidium sativum* are concerned, no previous reports have been demonstrated. Hence the present study was performed to investigate the cytotoxic effects of different solvents n-hexane, chloroform, ethyl acetate and methanol extracts of seeds of *Lepidium sativum* (Linn.) using A-549, HT-15, HT-29 and IMR-32 cell lines.

**MATERIAL AND METHODS**

**Collection and identification of the Plant materials**

The seeds of *Lepidium sativum* (Linn.), were collected from Tirunelveli District, Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medicinal Plants Unit Siddha, Government of India, Palayamkottai. The seeds of *Lepidium sativum* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

**Preparation of Extracts**

The dried powder was extracted sequentially by hot continuous percolation method using Soxhlet apparatus [15], using different polarities of solvents like n-hexane, chloroform, ethyl acetate and methanol. The dried powder was packed in Soxhlet apparatus and successively extracted with n-hexane for 24h. Then the marc was subjected to chloroform for 24h, ethyl acetate for 24h and then methanol for 24h. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

**Human cell lines:**

HT-15 and HT-29 human cancer cell lines of colon grown in RPMI medium were obtained from National Centre for Cell Sciences, Pune, India. IMR-32 neuroblastoma cell line was grown in Minimal Essential Media (MEM) obtained from Centre for Cellular and Molecular Biology, Hyderabad, India and A-549 which was grown in RPMI was obtained from National Cancer Institute, DTC, Frederick Cancer Research & Development Center, Madison, USA.

**Preparation of test material:**

**Stock solution:**

Stock solution of 20mg/ml was prepared. DMSO was used for 95% reconstitution of various extracts. Stock solutions were prepared one day in advance. Multiple aliquots of each sample were stored for initial tests and retests, if necessary. Stock solutions were filtered sterilized and microbial contamination was controlled by addition of gentamycin to the complete growth medium.

**Working test solution:**

On the day of assay, thaws an aliquot of frozen stock solution at room temperature. Prepared 100μg/ml concentration of the extract by serial dilution of stock solution using the complete growth medium containing 50mg/ml of gentamycin.

**Positive controls**

The positive control used was mitomycin-C.

**In-vitro Assay for cytotoxic activity**

The anti-cancer activity of n-hexane, chloroform, ethyl acetate and methanol extracts of *Lepidium sativum* was determined by the cytotoxic potential of the test material using human cancer cell lines which were allowed to grow on tissue culture plates in the presence of test material. The cell growth was measured on ELISA reader after staining with sulforhodamine B dye (SRB) which binds to basic amino acid residues in the trichloroacetic acid (TCA) fixed cells.

**Preparation of Cell suspension for assay:**

The desired human cancer cell line were grown in multiple TCFs at 37°C in an atmosphere of 5% in CO₂ and 90% relative humidity in complete growth medium to obtain enough number of cells as per requirement depending upon number of test samples. The flasks with cells at sub-confluent stage were selected. Cells were harvested by treatment with Trypsin-EDTA and added to complete growth medium to stop the action of trypsin. Cells were separated to single cell suspension by gentle pipetting action and the viable cells were counted in a haemocytometer using trypan blue. Cell viability at this stage should be >97%. Viable cell density was adjusted to 5,000-40,000 cells/100μl depending upon the cell line [16]. Cell suspension is ready for addition to tissue culture plates. 100μl of cell suspension together with 100μl of complete growth medium was added into each well. The plates were incubated at 37°C for 24h in an atmosphere of 5%CO₂ and 90% relative humidity in a CO₂ incubator. After 24h the test material, DMSO (vehicle control) and positive controls were added.

**Addition of test materials**

Working solutions of the test materials 100μl and positives controls was added to equivalent complete growth medium into the wells in the tissue culture plate. It was prepared 24h in advance containing either cells or complete growth medium in a final volume of 100μl. The plates were incubated at 37°C for 48h in an atmosphere of 5% CO₂ and 90% relative humidity. The cell growth was determined after 48h by SRB assay.
Assay was carried out as described by Skehan et al (1990) using SRB dye [17]. The microtiter plates were taken out after 48h incubation of cells with test materials and gently layered with 50μl of chilled 50% TCA on top of the medium in all the wells to produce a final concentration of 10%. Tissue culture plates were incubated at 4°C for 1h to fix the cells attached to the bottom of the wells. All the contents of all the wells were pipetted out gently and the supernatant was discarded. The plates were washed five times with distilled water to remove TCA, growth medium, low molecular weight metabolites, serum proteins etc. For washing, the wells of tissue culture plates were filled with distilled water and then discarded the excess liquid in the wells by sharply flicking the plate over a sink. Plates were air dried and stored until use. 100μl of SRB solution was added to each well of the plates and incubated at room temperature for 30min. The unbound SRB was removed quickly (to avoid desorption of protein bound dye) by washing the wells five times with 1% acetic acid and then the plates were air dried. 100μl of Tris buffer (0.01M, pH=10.4) was added and shaken gently for 5min on a mechanical shaker. Optical density was recorded on ELISA reader at 515nm and then the data was recorded.

Calculations

Cell viability and growth in presence of test material was calculated as follows:

Percent growth in presence of test material = \( \frac{\text{Growth in presence of test material}}{\text{Growth in absence of test material}} \times 100 \)

Percent growth inhibition in presence of test material was calculated as under: 
100% growth in presence of test material

Criteria for Determination of Activity:

The test sample showing growth inhibition of >70% at 100μg/ml is considered to be active. Following table describes the results of in-vitro cytotoxicity studies carried out against human cancer cell lines in the present investigations.

RESULTS AND DISCUSSION

It has been known that plants have a long history of use in the treatment of cancer [18] and herbal medicines have a vital role in the prevention and treatment of cancer [19]. The use of plant derived natural compounds as part of herbal preparations and alternative sources of drugs continues to play major roles in the general wellness of people all over the world [20, 21]. Agents capable of inhibiting cell proliferation, inducing apoptosis or modulating signal transduction are currently used for the treatment of cancer. The use of multiple targets on cancer cells are considered to be more effective in cancer treatment. The search for anticancer agents that may inhibit cancer development is becoming an important objective for scientists. In this study we have explored the cytotoxic effect of n-hexane, chloroform, ethyl acetate and methanol extracts of seeds of Lepidium sativum using different human cell lines (Table 1). Cytotoxicity screening models provide important preliminary data to help selecting plant extracts with potential anti-neoplastic properties for future work [22, 23]. The n-hexane, chloroform, ethyl acetate and methanol extracts of seeds of Lepidium sativum were subjected to the following cell lines A-549, HT-15, HT-29 and IMR-32 for the evaluation of cytotoxic activity. The methanolic extract of Lepidium sativum showed a significant cytotoxicity against A-549, HT-15, HT-29 and IMR-32 cell lines whereas, the moderate cytotoxic activity has been exhibited by ethyl acetate extract of Lepidium sativum. Similar results were not seen in other two extracts.

Table 1: % growth inhibition of different cell lines having various extracts of Lepidium sativum with respect to the control

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment</th>
<th>Concentration (μg/ml)</th>
<th>Concentration (μg/ml)</th>
<th>Concentration (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A-549</td>
<td>HT-15</td>
<td>IMR-32</td>
</tr>
<tr>
<td>1.</td>
<td>n-HELs</td>
<td>100</td>
<td>53±0.05</td>
<td>56±0.11</td>
</tr>
<tr>
<td>2.</td>
<td>CELS</td>
<td>100</td>
<td>65±0.58</td>
<td>70±0.65</td>
</tr>
<tr>
<td>3.</td>
<td>EAELS</td>
<td>100</td>
<td>75±0.47</td>
<td>71±0.19</td>
</tr>
<tr>
<td>4.</td>
<td>MELS</td>
<td>100</td>
<td>90±0.88</td>
<td>95±0.24</td>
</tr>
<tr>
<td>5.</td>
<td>Mitomycin -C</td>
<td>1x10^-4</td>
<td>88±0.02</td>
<td>80±0.09</td>
</tr>
</tbody>
</table>

Values are means of three independent analyses ± standard deviation (n = 4)

n-HELs-Petroleum ether extract of Lepidium sativum; CELS-Chloroform extract of Lepidium sativum; EAELS-Ethyl acetate extract of Lepidium sativum; MELS-methanolic extract of Lepidium sativum.
CONCLUSION

In conclusion, the results obtained in this study indicate that the methanol extract of *Lepidium sativum* exhibited significant inhibitory activity against human cancer cell lines tested, the cytotoxic activity may be due to the presence in the flavonoid content of methanol extract that could probably have highly anti-growth effects. Furthermore, mechanistic work is essential to prove these compounds as one of the specific cancer drug.

REFERENCES


