Antimicrobial and Anticancer Studies on *Euphorbia heterophylla*

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Abstract

*Euphorbia heterophylla* is a local medicinal plant used in ethno medicine for the treatment of constipation, bronchitis and asthma. *E. heterophylla* is medicinal plant with the common name “spurge weed”. In the present study selected plant drug was screened for its anticancer activity by In vitro and Insilico methods. Preliminary phytochemical screening revealed the presence of Terpenoids, Quinones, Alkaloids, Sterol, Coumarin, Starch, and Protein. Ethanol extract was tested for their antibacterial property against various organisms. The extract showed significant antimicrobial activity particularly in *Proteus vulgaris* and *Staphylococcus aureus*. Alcoholic extract of plant material showed significant in vitro cytotoxic activity on EAC (Ehrlich ascites carcinoma) cell lines. GC-MS was carried out in ethanol extract of *Euphorbia heterophylla*. 31 compounds were identified. Bioinformatics tools were employed in understanding the mechanism of action. 14 of the compounds were docked with the Bcl-2 protein, among them Allantoin acid was considered as best candidate as it is having least energy in docking.

Key words: Ehrlich ascites carcinoma, Docking studies, Bcl-2

Introduction

Herbs are now very popular in developing countries on account of improved knowledge about the safety, efficacy and quality assurance of ethno- medicine. In recent years, secondary plant metabolites (phytochemicals) have been extensively investigated as a source of medicinal agents. *Euphorbia* has been used for many years as a homeopathic remedy and has been reported to treat successfully some cancers. In homeopathy many varieties of the herb are made into homeopathic preparation and each one has a specific action.

The use of spurge (*Euphorbia* spp.) to treat tumours is well documented in Greek and Roman medical literature (J.L. Hartwell, 1969), and modern studies have shown that these plants are still employed to treat cancerous conditions in traditional medicine in many areas of the world. Traditionally, *Euphorbia* species have been used internally as laxatives and externally for rheumatism and skin conditions. *Euphorbia heterophylla* leaf is used in traditional medical practices as a laxative, anti gonorrheal, migraine and wart cures. The plant lattices have been used as fish poison, insecticide and ordeal poisons (Rodriguez et al., 1976, Falodun et al., 2003). The leaves of *E. heterophylla* have been reported to contain quercetin (Falodun and Agbakwuru, 2004).

Diterpenoids have also been reported in the root of *E. heterophylla* (Rowan and Orowuakeen, 2001). The skin irritant, tumor-promoting and anti-tumor/anticancer and recently anti-HIV activities of *Euphorbia* species have also been reported in *E.heterophylla* leaf Linn (Williams et al., 1995). *Euphorbia* is stated to possess antitussive, antifungal and antitumor properties. There is mixed evidence showing *Euphoria’s* effectiveness for chronic bronchitis, eczema, epilepsy and oral inflammation. In the present study selected plant drug was screened for its anticancer activity by In vitro and Insilico methods.

Materials and Methods

Plant material

The fresh plants of *E. heterophylla* were obtained from the in and around Trichy, in December, 2009. Collected plant material were identified and authenticated by Dr. P. Brindha., Dean, CARISM, SASTRA university, Thanjavur.

Phytochemical screening

The crude plant powdered sample was subjected to phytochemical, screening, testing for the presence of Alkaloids, Tannins, Flavonoids and Saponins using standard experimental procedure.

Docking studies

Bcl-2 protein, a anti apoptotic protein selected as target molecule. The 3D Extraction

The powdered leaves (600 g) were extracted first with n-hexane, chloroform, and ethanol at room temperature. The extracts were evaporated to dryness using an evaporator.

Assay of Antimicrobial Activity

The ethanolic extract of *Euphorbia heterophylla* was tested against various organisms such as *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus*, *Sregistrtococcus pyogenes*, *Psaelomonus aeruginosa*, *Bacillus subtilis*. Assay of antimicrobial activity was performed by disc diffusion method. (Bauer et al, 1966)

Cells

Ehrlich ascites carcinoma is a spontaneous murine mammary adenocarcinoma adapted to ascites form. EAC cells were obtained through the courtesy of AMALA cancer research center, Trichur, Kerala. They were maintained by intraperitoneal inoculation of 10^6 cells/mouse.

Short term *in vitro* cytotoxicity (Sheeja KR et al, 1997)

Short term *in vitro* cytotoxicity was assessed using EAC cell lines by incubating the different concentration of drugs at 37°C for 3 hours. The tumor cells were aspirated from peritoneal cavity of tumor bearing mice using a 10 ml syringe and transferred to a test tube containing isotonic saline. The cells were then washed in normal saline and cell number was determined using a haemocytometer and adjusted at 10 x 10^6 cells/ml. for the cytotoxicity assay , different concentrations of drug (50,75,100µg/ml) were added to each and the final volume was adjusted to one ml with normal saline. Control tubes were kept with the solvent and without the solvent along with tumor cells. All the tubes were incubated at 37°C for 3 hours. After incubation 0.1ml of 1% trypan blue dye in isotonic saline was added to each tube and the number of viable (unstained) and dead (stained) cells were counted using haemocytometer. The percent cytotoxicity (%dead cells) was calculated using the formula.

The dead cells were calculated by the formula,

\[
\text{%Dead cells} = \frac{\text{Total cells counted} - \text{total viable cells}}{\text{Total cells counted}} \times 100
\]

Gas chromatography and Mass Spectrometry

This is technique used to separate the compounds present in the given sample (solvent extraction), and this is combination of gas chromatography as well as mass spectrometry technique .We can get result a chromatogram graph in this GC-MS.

Docking Studies

Bcl-2 protein, a anti apoptotic protein selected as target molecule. The 3D
structure of the compounds present in the sample was drawn in chemskeet tool. The 3D structure of the compounds was converted into PDB file by using molecular converter and was taken as ligand molecule. The active site in the target protein was predicted by using the online tool Q-site finder. The PDB file of target protein, the PDB file of the ligand and text file of amino acids in the active site were taken as input files and autodocking was carried out. The 3D structure of the docked molecules and the bonds, residues involved the docking was viewed by using Pymol viewer.

RESULTS

Phytochemical screening

The Preliminary phytochemical tests answered positively for the presence of Terpenoids, Quinones, Alkaloids, Sterol, Coumarin, Starch, and Protein in case of raw drug powder. Phytochemical screening of Hexane extract shows positive result for Terpenoids, Sterol, Coumarin. Phytochemical screening of Chloroform extract shows positive result for Quinine, Sterol, Coumarin, starch. Phytochemical screening of Ethanol extract shows positive result for Alkaloid, Sterol, Coumarin, protein.

Antimicrobial activity

The ethanolic extract of Euphorbia heterophylla was tested against various organisms such as Escherichia coli, Proteus vulgaris, Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa & Bacillus subtilis. The extract showed significant antimicrobial activity particularly in Proteus vulgaris and Staphylococcus aureus.

In vitro cytotoxic assay

Ethanolic extract of Euphorbia heterophylla was subjected to the in vitro cytotoxic assay. Euphorbia heterophylla showed significant in vitro cytotoxic activity against Ehrlich Ascites carcinoma cell lines. At the dose level of 100µg/ml 46.49% of cell death noticed. (Table1)

Insilico studies

Anticancer activity of Euphorbia heterophylla was studied by insilico method. Bioinformatics tools were employed in understanding the mechanism of action. 14 of the compounds were docked with the Bcl-2 protein, among them Allantoic acid seems to be best candidate because it had least energy among 8 compounds. Allantoic acid was carried as best candidate as it is having least energy in docking. (Table 2&Figure 1)

GC-MS analysis

GC-MS was carried out in ethanol extract of Euphorbia heterophylla. 31 compounds were identified in the analysis. among them 6 of the compounds had high percentage peak area that are

- Acetacacid,3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a octahydropthalalen-2-yl ester.
- Bicyclo[4.2.0]octa-1,3,5-triene.
- I.2-Benzenedicarboxylic acid, diisooctyl ester.
- 2-Benzoyloxylamine.
- Styrene.
- 3-Trifluoracetoxycododecane

Table 2: Dock score for the compounds

<table>
<thead>
<tr>
<th>No.</th>
<th>Compounds</th>
<th>Docking Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2-Benzoyloxylamine</td>
<td>-7.11</td>
</tr>
<tr>
<td>2</td>
<td>2-Fomylhistamine</td>
<td>-7.90</td>
</tr>
<tr>
<td>3</td>
<td>Allatopic acid</td>
<td>-8.16</td>
</tr>
<tr>
<td>4</td>
<td>Benzeneethanamine, 2-thoro-4,3-dihydroxy-N-methyl-</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>3-Trifluoroacetoxycododecane</td>
<td>-8.14</td>
</tr>
<tr>
<td>6</td>
<td>Pterin-6-carboxylic acid</td>
<td>-7.51</td>
</tr>
<tr>
<td>7</td>
<td>Imidazole, 2-amino-5-(2-carboxyvinyl)-</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydropthalalen-2-yl ester</td>
<td>-7.72</td>
</tr>
</tbody>
</table>

DISCUSSION

The plant was commonly used in treating constipation, bacterial and inflammatory disease conditions (arthritis and rheumatism). Traditionally the leaf of the plant is used to treat ear pain (otitis media or externa) while the poultice induces milk flow from breast. In the present study selected plant drug was screened for its anticancer activity by In vitro and Insilico methods.

Phytochemical screening helps to reveal the chemical nature of the constituents of the plant extract and the one that predominates over the others. It may also be used to search for bioactive agents that could be used in the synthesis of very useful drugs (Yakubu et al., 2005). Phytochemical screening of the leaves of the selected plant revealed the presence of Terpenoids, Quinones, Alkaloids, Sterol, Coumarin, Starch, and Protein as the major phytochemical components.

Bcl-2 (Codes for a protein that blocks cell suicide mechanism) Suppresses apoptosis in a variety of cell systems including factor-dependent lymphohematopoietic and neural cells. The compounds isolated from ethanolic extract of Euphorbia heterophylla were docked with the Bcl-2 protein. Their docking structure view in pymol. 14 of the compounds were docked with the Bcl-2 protein; only 8 compounds were produce hydrogen bond with Bcl-2. Allatotic acid seems to be best candidate because it had least energy among 8 compounds.

CONCLUSION

To conclude Preliminary phytochemical screening revealed the presence of Terpenoids, Quinones, Alkaloids, Sterol, Coumarin, Starch, and Protein. The extract showed significant antimicrobial activity particularly in Proteus vulgaris and Staphylococcus aureus. Ethanolic extract of plant material showed significant invitro cytotoxic activity on EAC cell lines. Insilico studies were employed in understanding the mechanism of action. Thus present study revealed that ethanolic extract of Euphorbia heterophylla possessed significant antitumour activity.

REFERENCES


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Figure1: Allantoic acid docked with BCL2


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