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Antitumor Potential Of Ethanolic Extract Of Solanum trilobatum Against Ehrlich's Ascites Carcinoma

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Abstract: The present investigation aims to evaluate the anti-tumor potential of the ethanolic extract of *Solanum trilobatum* on Ehrlich's Ascites Carcinoma (EAC). Ethanolic extract of *Solanum trilobatum* was subjected to preliminary phytochemical screening and the antitumor effect was assessed by employing in-vitro methods. The maximum percentage of dead cells in short term invitro cytotoxicity assay was found to be 96%. Compounds present in the ethanol extract of the plant were identified using GC-MS and these compounds were subjected to docking using in-silico strategies. The results were found to be significant and confirmed that the *Solanum trilobatum* has significant antitumor activity.
Key words: *Solanum trilobatum*, anti-tumor, EAC, *invitro* and GC-MS.

Introduction

Cancer is the second leading cause of death in the United States and the Western World. There are millions of people worldwide who are living with cancer or have had cancer. Herbal treatment proves its efficacy in medicinal field without any side effects as synthetic medicines have; hence it's preferred to be more beneficial[1]. Plant extracts has the ability of activating the apoptotic pathway of cancer cells and doesn't have any ethical issues when it is used as drug formulations as it is purely herbal.

The use of herbal medicine dates back to several centuries and is still used mainly by above 75% of world's population. There is a growing interest in herbal remedies because of their effectiveness, minimal side effects and relatively low cost. However many of these inexpensive nutritive wild plants are yet to be adequately studied and utilized.

Cancer also called malignancy, is characterized by an abnormal growth of cells in which the cells exhibit an uncontrolled division, relatively in an autonomous fashion, leading to a progressive augmentation in the number of dividing cells[2]. Plant extracts as well as plant-derived compounds are found to be excellent medicinal agents for several cancers.

Solanum trilobatum, a thorny creeper with bluish violet flower, more commonly available in Southern India is a widely used plant in the Indian indigenous systems of medicine. It has been used traditionally in

Siddha system of medicines to treat various diseases[3]. It is used to treat respiratory disorders, especially bronchial asthma, arrest blood vomiting, reduce blood glucose level and bilious matter, phlegmatic rheumatism and several kinds of leprosy. It was reported that *S. trilobatum* possess antioxidant activity, hepatoprotective activity and protects UV induced damage and radiation induced toxicity in mice. Sobatum extract of *S. trilobatum* was reported to be very effective in tumor reduction[4].

Materials and Methods

Plant collection

The leaves of *Solanum trilobatum* was collected from the SASTRA university campus, Thanjavur, Tamil Nadu, India. The plant was identified and authenticated by Dr.P.Brindha, Associate Dean, CARISM, SASTRA university, Thanjavur.

Preparation of extract

The leaves of *Solanum trilobatum* was shade dried and pulverized. About 600gms of leaves of *Solanum trilobatum* dry powder was extracted using absolute alcohol by continuous cold percolation for 48hrs. The ethanolic extract was filtered and the solvent was distilled under reduced pressure at 40°C and evaporated to dryness. Ethanolic extract of *Solanum trilobatum* was used for the study.

Preliminary phytochemical analysis

The plant extract was subjected to preliminary phytochemical analysis for the presence of tannins, terpenes, flavones, alkaloids, quinone, sterol, phenol, coumarin, proteins, sugar, saponin, and gum[5].

Maintenance of EAC cell line

Ehrlich ascites carcinoma cells were obtained through the courtesy of Central Animal Facility, SASTRA University Thirumalaisamudram, Thanjavur and were maintained by weekly intraperitoneal inoculation of 1×10^6 cells/mouse[6].

Invitro cytotoxicity studies

Tryphan blue assay

Short term invitro cytotoxicity was assessed using Ehrlich ascites carcinoma cell lines by incubating with different concentrations of ethanol extract of *Solanum trilobatum* at room temperature for 3hours. The tumor cells were aspirated from peritoneal cavity of tumor bearing mice using an insulin syringe and transferred to a test tube determined using a Haemocytometer and adjusted to 1×10^6cells/ml. For the cytotoxicity assay, different concentrations of extract (100-1000µl/mg) were added to each tubes and the final volume was adjusted to 1ml with normal saline. Control tubes were kept with the saline and tumor cells but without drugs. All the tubes were incubated at 37°C for 3 hours. After incubation 0.1 ml of 0.4% tryphan blue dye in isotonic saline was added to each tube and the number of viable and dead cells were counted using haemocytometer[7].

$$\% \text{ Dead cells} = \frac{\text{Total cells counted} - \text{Total viable cells} \times 100}{\text{Total cells count}}$$

Experimental Methods

Thin Layer Chromatography

The Thin-layer Chromatography procedure was performed using a glass slide precoated with silica gel G, a mixture of Toluene:Ethyl acetate:Diethylamine in the ratio 7:2:1 was chosen as the perquisite mobile phase. Sample was spotted simultaneously on the slide, before placing the slide in the chromatographic chamber. The sample was allowed to ascend to the top of the slide through capillary action. After removal of the slide from the chamber, it was air-dried and then examined under ultraviolet light (366 nm), where blue fluorescent spot was observed. In order to get a clear picture and to enhance its observation using naked eye , the slide was later kept in a bottle containing iodine for a period of about 5-10 minutes. The sample spot now appear as brown colored area in the plate.

The sobatum in the extract (from the spots) was clearly identified by comparing the measured R_f value with that of the standard R_f value. The formula used for the calculation is given as:

$$R_f \text{ value} = \frac{\text{Distance moved by the sample}}{\text{Distance moved by the solvent front}}$$

Gas Chromatography – Mass Spectrometry Analysis:

Perkin Elmer Clarus 500 GCMS instrument was used for analyzing the compounds present in plant extract under study. Capillary column made of Elite- 5MS (5% phenyl 95% dimethyl polysiloxane) was used. The oven program was fixed to 50ºC @ 8ºC/min to 220ºC (2min) @ 7ºC/min to 280ºC (10min) and Injector temperature was 280ºC. The carrier gas used is Helium at the flow rate of 1ml/min. Sample was injected and the compounds obtained were matched using the library NIST 2005.

Insilico analysis

To support the anticancer activity, the insilico approaches has been implemented in which the docking software Autodock was used. The target molecule was chosen to be Bcl-2 as it is a major gene that codes for a large family of apoptosis regulating proteins[19]. Compounds obtained from GCMS were docked with Bcl-2 and the results were found to be significant.

Results

Phytochemical Analysis

The qualitative tests revealed the presence of compounds such as saponins, tannins, alkaloids and phenolic compounds (Table 1).

Thin Layer Chromatography

TLC was performed and presence of sobatum was confirmed. The obtained R_f value for sobatum was found to be same as that of the standard R_f for sobatum being 0.60. (Fig 1).

Table 1 - Phytochemical Screening Of Ethanolic Extract Of *Solanum trilobatum*

S.No	Phytochemical	Result
1	Saponins	+
2	Tannins	+
3	Test for Phenolic compounds	
	i) Ferric chloride test	+
	ii) Gelatin test	+
	iii) Lead acetate test	+
4	Carbohydrate tests Molish's test	-
5	Anthraquinones	-
6	Alkaloids	+
7	Protein and aminoacids	
	i) Millon's test	-
	ii) Ninhydrin test	-
8	Flavonoids Alkaline reagent test	+
9	Glycosides Borntragers test	-
10	Fixed oils and fats Saponification	-

Note : + sign indicates presence of the compound
- sign indicates absence of the compound

Fig.1 TLC plate containing spot of sobatum

Invitro cytotoxicity assay

Invitro cytotoxicity assay showed significant effect against EAC cell lines as shown in Table 2 which makes it evident that the plant extract has capability to kill the cancer cells.

GC-MS Profile of ethanolic extract of *Solanum trilobatum*

The ethanol extract of *Solanum trilobatum* was subjected to Gas chromatography/ mass spectrometry studies and the profile as shown in Fig.2.

Table 2 - Invitro Cytotoxicity Assay

Concentration of ST drug («g/ml)	Count of dead cells	Count of viable cells	Count of total number of cells	% cytotoxicity (%)
50	44	137	181	24.30
100	36	65	101	35.64
200	75	45	120	62.50
400	80	8	88	90.90
600	81	5	86	94.18
800	72	4	76	94.73
1000	50	2	52	96.15

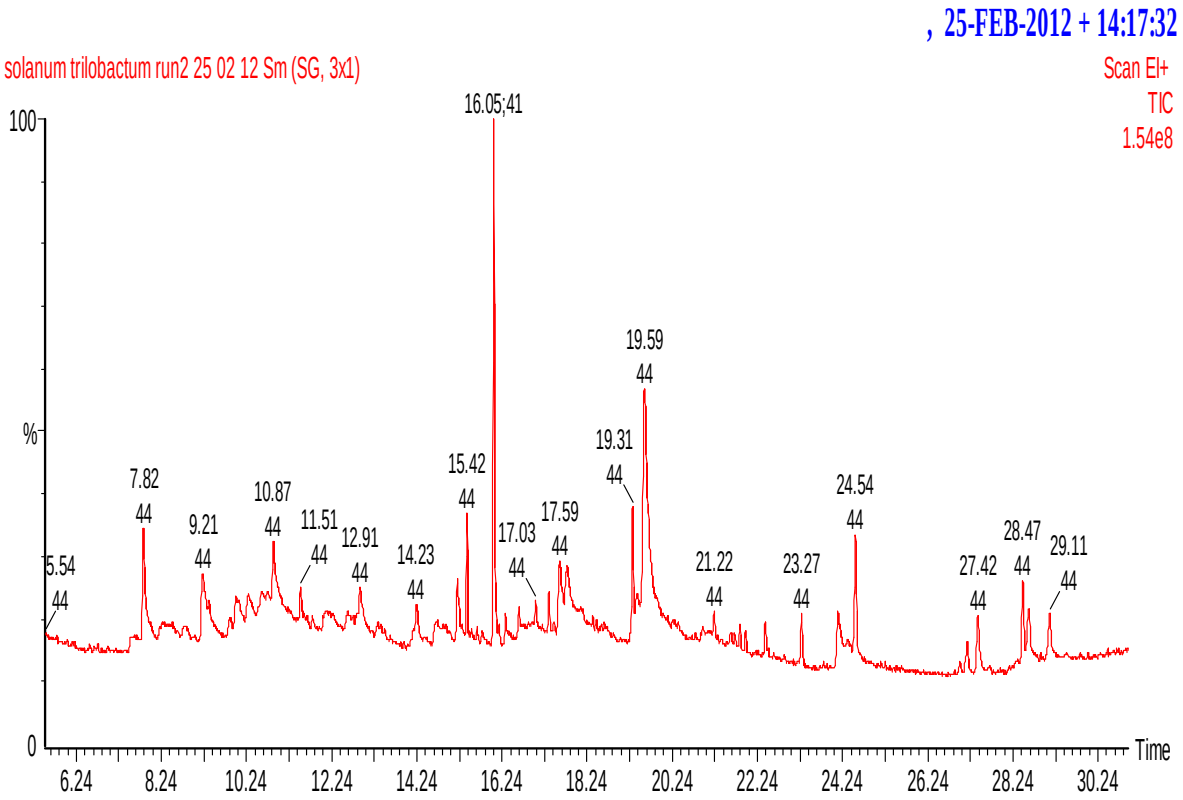

Fig.2 GC-MS profile of ethanol extract of *Solanum trilobatum*

Insilico analysis

The major compounds obtained from the GCMS analysis of *Solanum trilobatum* were (R)-(+)-1-(p-Tolyl)ethylamine, 1,2-Cyclohexanediol, 1-methyl-, trans-, 1-[4-(hydroxymethyl)phenyl]ethanone, 1-cyclohexene-1-acetonitrile, 2,5-Dimethyl-2,3-dihydro-5H-1,4-dioxepine, 2-ethoxy-3,4-dihydro-2H-pyran, 3,5-dihydroxy-2-methyl-4H-pyran-4-one, 3,5-dihydroxy-6-(hydroxymethyl)oxan-2-one, Bicyclo[4.1.0]-3-heptene, 2-isopropenyl-5-isopropyl-7,7-dimethyl-, Caryophyllene, Phenol, 2,4-bis(1,1-dimethylethyl)- were docked with Bcl-2.

In Fig.3, Caryophyllene was docked with BCL2 protein was shown. The docking was by means of hydrophobic bonds interaction with phenylalanine and tryptophan residues. The Binding energy (kcal/mol) for caryophyllene was found to be -7.5.

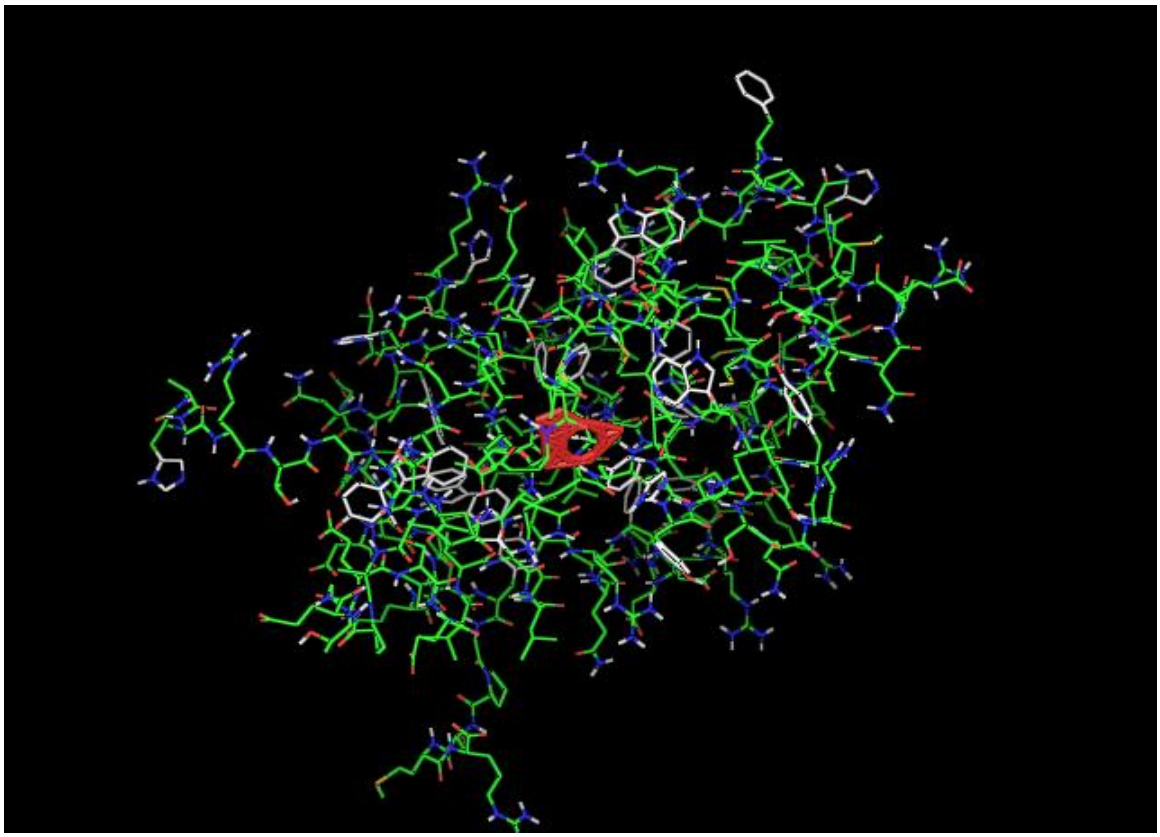

Fig.3 Pictorial representation of docking of Caryophyllene with BCL2 protein

Table 3 - Docking Of Gcms Compounds Of *Solanum trilobatum* With BCL2 Protein

Ligand	Binding energy (kcal/mol)
(R)-(+)-1-(p-Tolyl)ethylamine	-6.8
1,2-Cyclohexanediol, 1-methyl-, trans-	-6.1
1-[4-(hydroxymethyl)phenyl]ethanone	-6.8
1-cyclohexene-1-acetonitrile	-6
2,5-Dimethyl-2,3-dihydro-5H-1,4-dioxepine	-5.6
2-ethoxy-3,4-dihydro-2H-pyran	-5.5
3,5-dihydroxy-2-methyl-4H-pyran-4-one	-5.1
3,5-dihydroxy-6-(hydroxymethyl)oxan-2-one	-5.5
Bicyclo[4.1.0]-3-heptene, 2-isopropenyl-5-isopropyl-7,7-dimethyl-	-7.2
Caryophyllene	**-7.5**
Phenol, 2,4-bis(1,1-dimethylethyl)-	-7.4

Discussion

The results of the *in-vitro* cytotoxicity tests reveal the level of anticancer potential of ethanolic extract of *Solanum trilobatum*. The increasing percentage cytotoxicity of the cells with increasing concentration of extract indicates the significant cytotoxic effect against EAC.

Among the common anticancer agents are alkaloids, flavonoids and phenolic compounds. The phenolic compounds have been used as an antioxidant and anti-inflammatory agent. The plant extract showed the presence of many phenolic compounds from which about 10 cyclic compounds were identified. They were docked with the anti-apoptotic protein BCL2. The docking results show the binding energy of each of the compounds with the BCL2 protein (Table 3). It was understood that the compound Caryophyllene with the least binding energy (-7.5kcal/mol) was adjudged as the best protein- ligand complex fit. This was because of the lowest amount of energy needed by

caryophyllene to bind with the BCL2 protein. From the study it is evident that the *Solanum trilobatum* extract possess significant anticancer activity and it can be developed as a potent anticancer drug.

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