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Full Proceeding Paper

ANTICANCER ACTIVITY OF ETHANOLIC EXTRACT OF *SOLANUM TORVUM* SW.

SWAYAMSIDDHA PANIGRAHI[1], MEENAKSHI SUNDARAM MUTHURAMAN[1*], RAVICHANDRAN NATESAN[2] AND BRINDHA PEMIAH[2]

[1]Department of Biotechnology, School of Chemical and Biotechnology, [2]CARISM, SASTRA University, Thirumalaisamudram, Thanjavur, TamilNadu, India. Email: msundar77@yahoo.com

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ABSTRACT

The present study aims to evaluate the cytotoxic effect of ethanolic extract of *Solanum torvum* (EEST) on Ehrlich's Ascites Carcinoma (EAC) cell lines. Ethanolic extract of *Solanum torvum* was subjected to preliminary phytochemical screening and cytotoxic effect of EEST was assessed by employing *in-vitro* method. Chemical compounds present in the ethanol extract of the *S. torvum* were identified using GC-MS and attempts were made to understand the mechanism of action of the bioactive molecule using *in-silico* methods. The study revealed that EEST showed the significant cytotoxic effect on EAC.

INTRODUCTION

Cancer is one of the most life-threatening diseases. It is a group of diseases characterized by the deregulated proliferation of cells that invade and disrupt surrounding tissues [1]. The limited success of clinical therapies, and the immense side effects of synthetic anticancer drugs, took into drug discovery from medicinal plants which have played an important role in the treatment of cancer [2].

Solanum torvum belongs to the family Solanaceae and is distributed throughout the Southern parts of India. This is a prickly shrub up to 5 m tall, cultivated in the tropics for its sharp-tasting, immature fruits [3]. It contains several pharmacologically active potential chemical compounds includes sapogenin steroid [4], chlorogenin, solasodine, solamargine and solanine. Extracts of the *S. torvum* are reported to be useful in the treatment of hyperactivity, colds and cough[5], pimples, skin diseases [6], and leprosy. In India, berries of this plant is commonly used in the treatment of various ailments and also used as vegetable [7]. In this study, *Solanum torvum* berry extract was screened for the presence of major phytochemical groups. Ethanol extract of berries of *S. torvum* was screened for anticancer activity. The maximum inhibition was observed in higher dose which proved the anticancer potential of berries of *S. torvum* against Ehrlich Ascites Carcinoma (EAC) [8] cell lines.

MATERIALS AND METHODS

Plant collection

The berries of *Solanum torvum* were procured from vegetable market, Thanjavur, Tamil Nadu, India. The berries were authenticated by Dr. P. Brindha, Associate Dean, CARISM, SASTRA University, Thanjavur.

Preparation of extract

The berries were dried in shade at room temperature. The dried berries were coarsely powdered and 1000 gm of coarsely powder was extracted by macerating with absolute ethanol (3000 mL) for 72 h. The extract was filtered and concentrated under vacuum and the concentrated syrupy mass was lyophilised and made as powder. The powder was used for the further experiments.

Preliminary phytochemical analysis

The ethanol extract of the *S. torvum* berries was subjected to preliminary phytochemical analysis [9] for the presence of tannins, terpenes, flavones, alkaloids, quinone, sterol, phenol, coumarin, proteins, sugar, saponin, and gum [10]

Maintenance of EAC cell line

Ehrlich Ascites Carcinoma cells [11] were obtained through the courtesy of Central Animal Facility, SASTRA University, Thanjavur and were maintained by weekly intraperitoneal inoculation of 1×10^6 cells/mouse.

In vitro cytotoxicity studies

Trypan blue assay

Short term *in vitro* cytotoxicity was assessed using Ehrlich Ascites Carcinoma cell lines by incubating with different concentrations of ethanol extract of *S. torvum* berries at room temperature for 3 h. The tumor cells were aspirated from peritoneal [12] 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 cytotoxicity assay, different concentrations of extract (100-1000 µL/mg) were added to each tube and the final volume was adjusted to 1 mL with normal saline. Control tubes were kept with the saline, tumor cell and without drug. All the tubes were incubated at 37°C for 3 h. After incubation 0.1 ml of 0.4% trypan blue dye in isotonic saline was added to each tube and the number of viable and dead cells were counted using haemocytometer [13]

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

Experimental Methods

Thin Layer Chromatography

The Thin-layer Chromatography procedure was performed using a slide coated with silica gel G with a thickness of 1mm, a mixture of chloroform: ethanol (9:1) was used as mobile phase. The sample was spotted on the slide, by using a capillary tube and placed in TLC chamber. The mobile phase was allowed to ascend to 3/4th of the TLC plate. After removal of the slide from the chamber, it was air-dried and then examined under ultraviolet light (366 nm), where a 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 appears as brown colored area in the plate.

The solasodine in the extract 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:

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

High Performance Thin Layer Chromatography

The sample was prepared by taking about 0.108 gm of ethanolic extract dissolved in 5ml of ethanol. Silica Gel 60 F_{254} was taken as the stationary phase and chloroform: methanol in the ratio of 9:1 was chosen as the mobile phase. At a wave length of 366 nm a band corresponding to Solasodine is visible in test solution tracks.

Gas Chromatography – Mass Spectrometry Analysis

Perkin Elmer Clarus 500 GC-MS 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 290ºC. 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 [14].

***In silico* analysis**

To support the anticancer activity, the *in silico* 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 [15]. Compounds obtained from GCMS were docked with Bcl-2 [16] 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 carried out by standard procedure, and the presence of solasodine was confirmed. The obtained R_f value for test solution was found to be same as that of the standard R_f value for solasodine (0.8). The TLC plate containing spot of solasodine sample is shown in Fig.2.

High Performance Thin Layer Chromatography

As described earlier HPTLC was perfomed and the evaluation showed a band (R_f 0.81) corresponding to solasodine, visible in both test solution tracks (Track 1 and 2)**.** Fig. 3: shows the HPTLC fingerprinting profile of the extract at both 254nm and 366nm.

***In vitro* 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 torvum*

The ethanol extract of *Solanum torvum* was subjected to Gas chromatography/ mass spectrometry studies and the profile as shown in Fig.3

***In silico* analysis**

The major compounds obtained from the GC-MS analysis of ethanol extract of *S. torvum* berries were 4-Piperidinone, 2,2,6,6-tetramethyl-, Piperidin-4-one, 1-ethyl-2,3-dimethyl-, Phenol, 2,4-bis(1,1-dimethylethyl)-, Nonadecanol, Nonadecene, 1-Tetracosanol, 1-Tricosene- were docked with Bcl-2. In Fig.5, Solasodine 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 solasodine was found to be -6.16 as seen in Table 4 .

DISCUSSION

The results of the *in-vitro* cytotoxicity was revealed the anticancer potential of the ethanol extract of *Solanum torvum* berries. The concentrations of the extract ranging from 50µg/ml to 1000µg/ml, were tested for its % cytotoxicity, and the % cytotoxicity were obtained as 7.09% to 85.79% respectively. This indicates the cytotoxic effect was dose dependent.

Among the common anticancer agents are alkaloids, flavonoids and phenolic compounds [17]. The phenolic compounds have been used as an antioxidant [18] and anti-inflammatory agent. The plant extract showed the presence of many phenolic compounds from which compound having maximum % of peak area were identified using GC-MS. They were docked into the anti- apoptotic protein BCL2. The docking results show the binding energy of each of the compounds with the BCL2 protein. It was understood that the compound Solasodine with the least binding energy (-6.16 kcal/mol) was adjudged the best protein- ligand complex fit. This was because of the lowest amount of energy needed by Solasodine to bind with the BCL2 protein receptor and cause apoptosis thereby successfully killing EAC cell lines. Hence from the current study it is evident that the *Solanum torvum* extract can be developed as a potent anticancer drug.

Table 1: Phytochemicals analysed

S. No.	Phytochemical analyzed	Result
1.	**Phenols**	**+**
2.	**Reducing sugars**	
i	Fehlings test	**+**
ii	Benedicts test	**+**
3	**Carbohydrates**	**+**
4	**Flavones**	
i	Shinoda test	**+**
ii	Alkaline reagent test	**+**
iii	Ferric chloride test	**+**
iv	Lead Acetate Solution test	**+**
5	**Glycosides**	+
6	**Saponins**	+
7	**Steroids**	
i	Ferric chloride- Acetic acid test	+
8	**Alkaloids**	
i	Draggendorff test	+
ii	Meyers test	-
iii	Wagners test	+
iv	Hagers test	+
9	**Anthraquinone-Borntragers test**	+
10	**Quinones**	+
11	**Tannins**	+

Note : + Sign indicates presence of the compound; - Sign indicates absence of the compound

Table 2: *In Vitro* Cytotoxicity Assay

S. No.	Concentration	% Cytotoxicity
1.	1000 µg/ml	85.79
2.	800 µg/ml	72.87
3	600 µg/ml	57.71
4	400 µg/ml	31.83
5	200 µg/ml	17.15

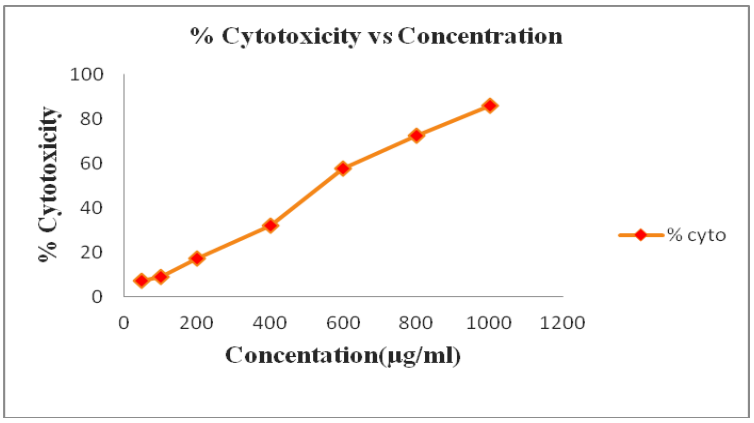

Fig. 1: Graph showing Cytoxicity vs Concentration of the drug

Fig. 2: TLC plate containing spots of Solasodine

Photo documentation under UV

AT 254nm AT 366nm

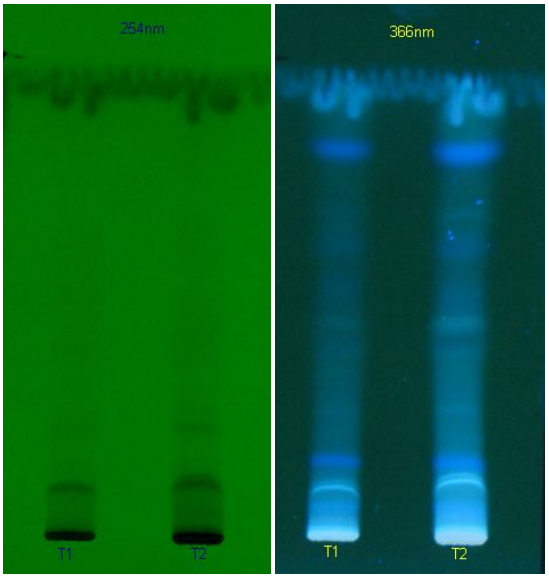

Fig. 3: HPTLC fingerprinting profile of *Solanum* ethanolic extract

TLC DETAILS

Track 1-5µl of Sample

Track 2-10µl of Sample

IDENTITY TEST

Sample preparation:About 0.108 gm of ethanolic extract was dissolved in 5ml of ethanol.

Stationary phase: Silica Gel 60 F_{254}

Mobile phase: Chloroform : Methanol (9:1)

Wave length: 366 nm

Evaluation: A band (R_f 0.81) corresponding to Solasodine is visible in both test solution tracks.(Track 1 ,2)

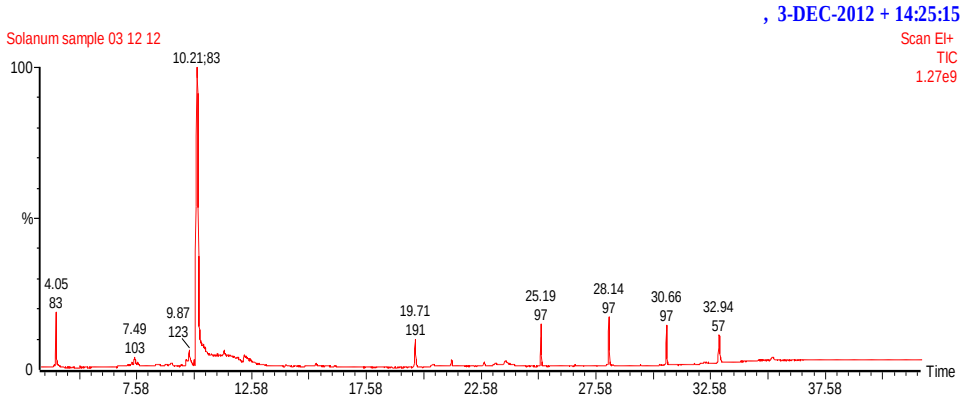

Fig. 4: GC-MS profile of ethanolic berry extract of *Solanum torvum*. Chromatogram (x-axis = Retention time; y-axis = % intensity/% abundance)

Table 3: GC-MS results

S. No.	Peak Name	Retention time	Peak area	%Peak area
1.	Name: 3-Penten-2-one, 4-methyl- Formula: C6H10O MW: 98	4.05	7364015	3.2052
2.	Name: Pyridine, 2,4,6-trimethyl- Formula: C8H11N MW: 121	7.61	817133	0.3557
3.	Name: 2,5-Heptadien-4-one, 2,6-dimethyl- Formula: C9H14O MW: 138	9.86	2418008	1.0524
4.	Name: 4-Piperidinone, 2,2,6,6-tetramethyl- Formula: C9H17NO MW: 155	10.21	159102640	69.2487
5.	Name: Piperidin-4-one, 1-ethyl-2,3-dimethyl- Formula: C9H17NO MW: 155	12.25	6397074	2.7843
6.	Name: Phenol, 2,4-bis(1,1-dimethylethyl)- Formula: C14H22O MW: 206	19.71	7206650	3.1367
7.	Name: Methyl 6-O-[1-methylpropyl]-á-d-galactopyranoside Formula: C11H22O6 MW: 250	20.47	3595918	1.5651
8.	Name: 9-Eicosene, (E)- Formula: C20H40 MW: 280	21.30	2887065	1.2566
9.	Name: 1-Nonadecanol Formula: C19H40O MW: 284	25.19	6920269	3.0120
10.	Name: 1-Nonadecene Formula: C19H38 MW: 266	28.14	8949107	3.8951
11.	Name: 1-Tetracosanol Formula: C24H50O MW: 354	30.66	7563971	3.2922
12.	Name: 4-tert- Butylaniline Formula: C10H15N MW: 149	9.12	1098093	0.4779

Table 4: Docking of Compounds in Ethanolic extract of *Solanum Torvum* with Bcl2 Protein

Ligand	Binding energy
Solasodine	-6.16
4-piperidinone-2,2,6,6-tetramethyl-	-5.19
piperidin-4-one,1-ethyl-2,3-dimethyl-	-3.84
phenol-2,4-bis(1,1-dimethylethyl)-	-5.21
methyl-6-O-[1-methylpropyl]-a-d-galactopyranoside	-3.13
4-tert-butylaniline	-5.56
3-penten-2-one,4-methyl-	-3.67

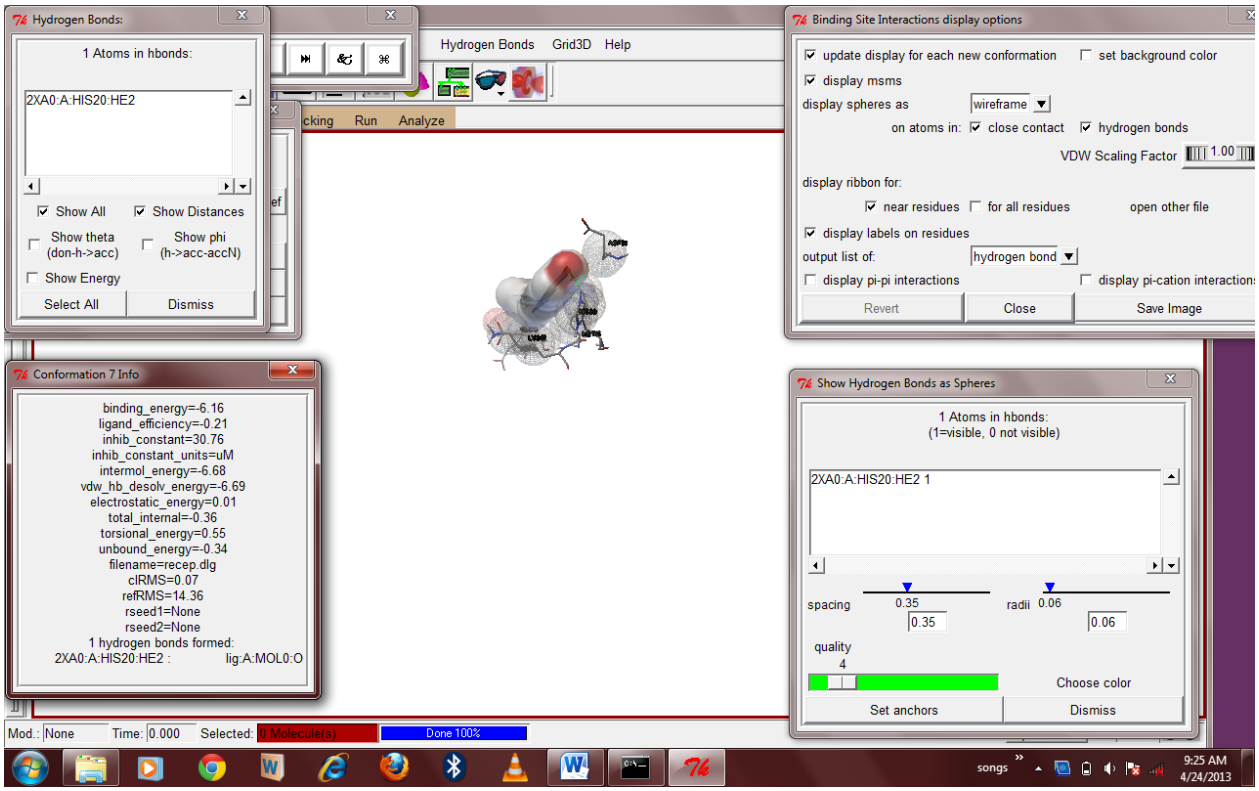

Fig. 5: Pictorial representation of docking of Solasodine with BCL2 protein

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