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ANTITUMOUR POTENTIAL OF *PASSIFLORA INCARNATA.*L AGAINST EHRLICH ASCITES CARCINOMA

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ABSTRACT

The present investigation aims at to evaluate the anti-tumour potential of the ethanolic extract of *Passiflora incarnata.L* (EEPI) on Ehrlich ascites carcinoma (EAC). Ethanol extract of *Passiflora incarnata.L* was subjected to preliminary phytochemical screening and the antitumor effect of EEPI was assessed by employing *in-vitro* methods. Compounds sspresent in the ethanol extract of the plant were identified using GC-MS and attempts were made to understand the mechanism of action using in silico methods.Present study revealed that *Passiflora incarnata.*L(EEPI) possessed significant antitumor activity against EAC.

Keywords: *Passiflora incarnata.L.*, Ehrlich ascites carcinoma, *In-vitro,* In silico.

INTRODUCTION

Worldwide about 20 million people per year are diagnosed with cancer and more than 6 million mortalities are recorded and rate of cancer incidences increases every year. The statistics released by the American Cancer Society in 2006 reveals that the total number of deaths due to cancer in 2005 was 7.6 million. By 2050, over 20 million new cancer cases and over 17 million cancer deaths are probable to occur in the world [1]. Ever since man existed, he had survived and prevented several diseases including cancer by using traditional herbs. Till date large number of herbal products has been screened for their anticancer potential through various experimental models. This has caused the discovery of the several drugs by the pharmaceutical and scientific communities. This prompted us to evaluate the antitumor activity of a common plant in hills and plain botanically equated as *Passiflora incarnata.*L

MATERIALS AND METHODS

Plant material

The leaves of *Passiflora incarnata.*L were obtained from in and around Ooty and the collected plant material was identified and authenticated by CARISM department, SASTRA University, Thanjavur.

Preparation of leaf powder

Leaves of *Passiflora incarnata.*L were collected and dried under shade. The dried materials were mechanically powdered after keeping them at room temperature for 48 hours. These coarse powder materials were used for further investigations. The ethanolic extract of *Passiflora incarnata* (EEPI) was filtered and solvent distilled off. The extracts were concentrated and were subjected to phytochemical analysis.

Extraction

The powdered leaves (600 g) were extracted with ethanol at room temperature. The extract was evaporated to dryness using a distillation set-up.

Preliminary phytochemical screening

EEPI was subjected to phytochemical screening test and the analysis of the extract disclosed the presence of alkaloids, carbohydrates, phytosterols, fixed oils and fats, phenolic compounds, tannins, flavonoids, proteins, amino acids, saponins, gums, mucilage and volatile oils in ethanolic extract of *Passiflora incarnata.*L[2].

Cells

Ehrlich Ascites Carcinoma (EAC) cells were acquired through the courtesy of Jiva Janthu Pariksha Kendra, Central animal facility at SASTRA University. They were maintained by intraperitoneal inoculation of 10^6 cells/mouse. Ehrlich ascites carcinoma is one of the commonest tumours. EAC is referred to as an undifferentiated carcinoma and is originally hyper - diploid, has high transplantable capability, no-regression, rapid proliferation, shorter life span, 100% malignancy and also does not have tumor-specific transplantation antigen. EAC resembles human tumors which are the most sensitive to chemotherapy due to the fact that they are undifferentiated and that they have a rapid growth rate.

Maintenance of cells

Ehrlich ascites carcinoma cells was maintained by the courtesy of central animal facility, SASTRA University and were maintained by weekly intraperitoneal inoculation of 1×10^6 cells/mouse[3].

Short term cytotoxicity assay

Short term *in-vitro* cytotoxicity was assessed using EAC cell lines by incubating the different concentration of drugs at 37ºC for 3 hours. The tumor cells were aspirated from peritoneal cavity of tumour 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 to 1×10^6 cells/ml. For the cytotoxicity assay, different concentrations of drug (200-1000µg/ml) were added to each and the final volume was adjusted to one ml with Phosphate buffer saline (PBS). Control tubes were kept with the solvent and without the solvent along with tumor cells. All the tubes were incubated at 37ºC 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[4]. The percent cytotoxicity (% dead cells) was calculated using the formula.

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

GCMS 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) of 30m length was used. The oven program was fixed to 50ºC @ 8ºC/min to 220ºC (1min), @ 7º C/min to 280ºC (10min) and injector temperature was 290ºC. The carrier gas used was Helium at the flow rate of 1ml/min and the sample was injected [5] and the compounds obtained were matched using the library NIST 2005.

In silico analysis

To support the anticancer activity, the in silico approaches has been implemented in which the docking software Hex 6.3 was used. The target molecule[6] was chosen to be Bcl-2 as it is a major gene that codes for a large family of apoptosis regulating proteins[7].Compounds obtained from GCMS were docked with Bcl-2 and the results were found to be significant.

RESULTS

The percentage of dead cells was found to increase with increase in concentration of the EEPI against the EAC cell lines as shown in the Table 1 which proves that the plant extract has the capability to kill the cancer cells.

Table 1: Tryphan blue assay Cytotoxicity effect of ethanolic extract of *Passiflora incarnata.L* against EAC cell lines.

Concentration of the drug(µg/ml)	No. of viable cells	No. of Dead cells	% viability of the cells
200	36	0	100
400	32	1	96.96
600	30	3	90.9
800	6	18	25
1000	0	23	0

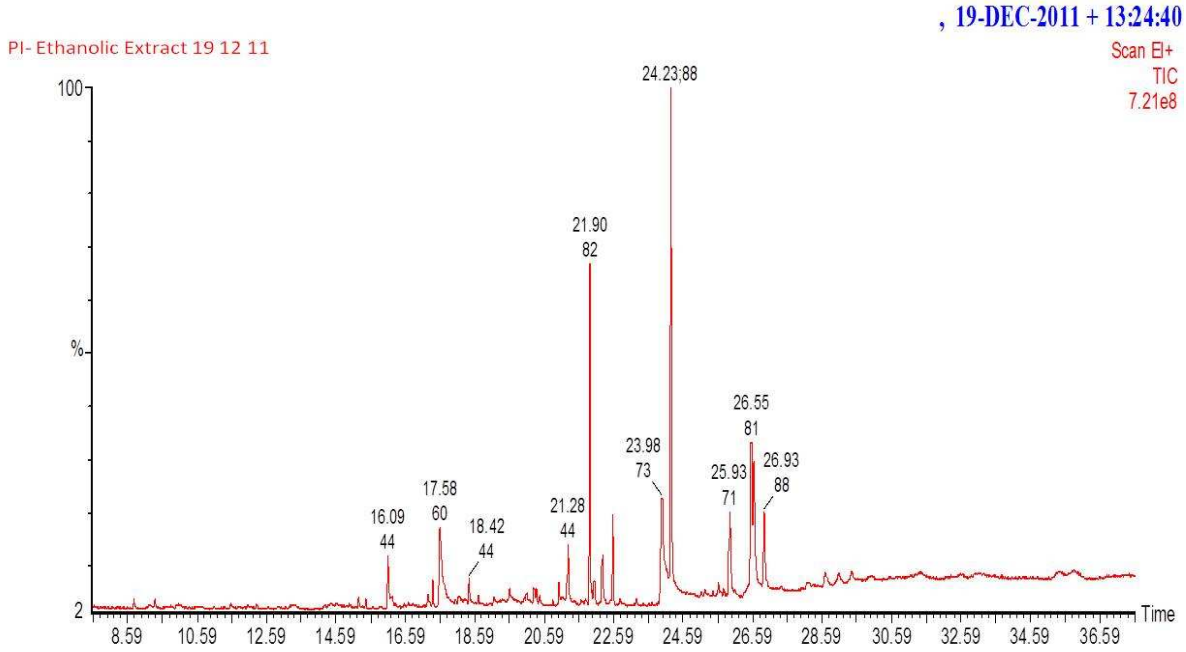


Fig. 1: GC-MS Profile of ethanolic extract of *Passiflora incarnata.L*

In silico analysis

The major compounds obtained from the GC-MS analysis of *P. incarnata.L* were Cyclohexanol, 1-methyl-, acetate (Formula: $C_9H_{16}O_2$ MW: 156), D-Allose (Formula: $C_6H_{12}O_6$ MW: 180 CAS), 6-Hepten-2-one, 7-phenyl-(Formula: $C_{13}H_{16}O$ MW: 188), Bicyclo[2.2.1]heptane, 1,3,3-trimethyl-(Formula: $C_{10}H_{18}$ MW: 138), 4H-1-Benzopyran-4-one, 5,7-dihydroxy-2-(4-hydroxyphenyl)-(Formula: $C_{15}H_{10}O_5$ MW: 270) ,(3E,5E,7E)-6-Methyl-8-(2,6,6-trimethyl-1-cyclohexenyl)-3,5,7-octatrien-2-one (Formula: $C_{18}H_{26}O$ MW: 258) and Phenol, 2,4-bis(1,1-dimethylethyl)-(Formula: $C_{14}H_{22}O$ MW: 206) were docked with Bcl-2.

In the Fig 2, Phenol 2,4-bis(1,1-dimethylethyl)-(Formula: $C_{14}H_{22}O$ MW: 206) from *Passiflora incarnata.L* was docked with Bcl-2 was shown. The binding energy for Phenol 2,4-bis(1,1-dimethylethyl)-was found to be -2.709588e+02eV.

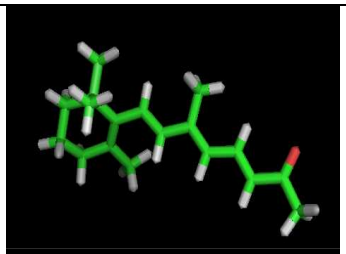
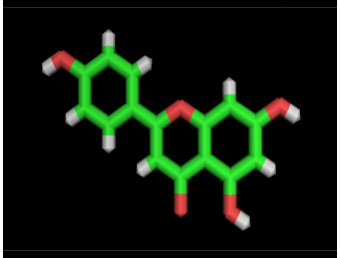
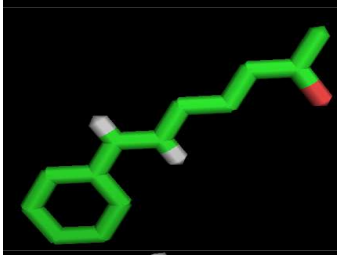
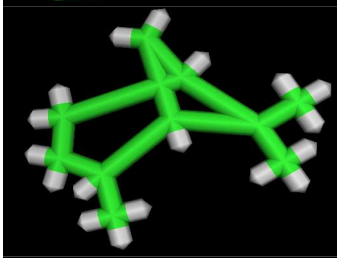
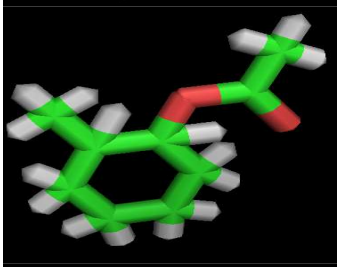
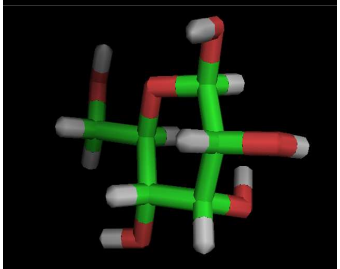
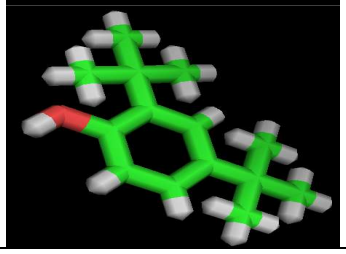
DISCUSSION

Cancer being the second fatal disease in India, The United States and The Western World. There is anomalous number of plant derived anticancer drugs of diverse structural types both in present clinical use and as malignant drug undergoing clinical trials. This suggests that plants will continue to be an imperative and prized resource for anticancer drug discovery. Herbal treatment proves its efficacy in medicinal field without producing any serious side effects as synthetic medicines do; hence it's preferred to be more beneficial. 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.

In vitro cytotoxicity studies employing tryphan blue assay was carried out to assess the cytotoxic potential of the plant extract (EEPI). The principle is based on the fact that the live cells with rigid cell membrane would not uptake the tryphan blue dye whereas the dead cells with disrupted cell membrane would uptake tryphan blue. When different concentrations of EEPI were assayed, the dead cells were found to increase with increase in concentration of the extracts. The dead cells of EEPI against EAC the number of dead cells at 1000µg/ml observed to be 100% (Table 1) which proves that the cancer cells were totally destroyed by the EEPI.

To support the antitumor activity, the in silico analysis was done by means of docking study using the ligands identified with GC-MS analysis. The structures given by GCMS analysis were imported to Hexlog server and constructed to 3D structure which was docked with Bcl-2 which showed the interaction between the compounds and Bcl-2. Among all the compounds Phenol, 2,4-bis(1,1-dimethylethyl)-was found to be the best candidate that was bound to Bcl-2 by means of hydrophobic interaction with energy level as -2.709588e+02eV.

Table 2: The binding energy and no. of hydrogen bonds interacted with the ligand.

S. No.	Structure	Ligand's Name	Energy (in eV)	No. of Hydrogen Bonds
1		(3E,5E,7E)-6-Methyl-8-(2,6,6-trimethyl-1-cyclohexenyl)-3,5,7-octatrien-2-one)	-1.529424e+02	18
2		4H-1-Benzopyran-4-one,5,7-dihydroxy-2-(4-hydroxyphenyl)-	-2.456945e+02	10
3		6Hepten-2-one,7-phenyl-	-2.262737e+02	2
4		Bicyclo[2.2.1]heptane,1,3,3-trimethyl-	-2.177857e+02	18
5		Cyclohexanol,1methyl-acetate	-2.105562e+02	16
6		D-allose	-2.595229e+02	12
7		Phenol,2,4-bis(1,1-dimethylethyl)	-2.709588e+02	22

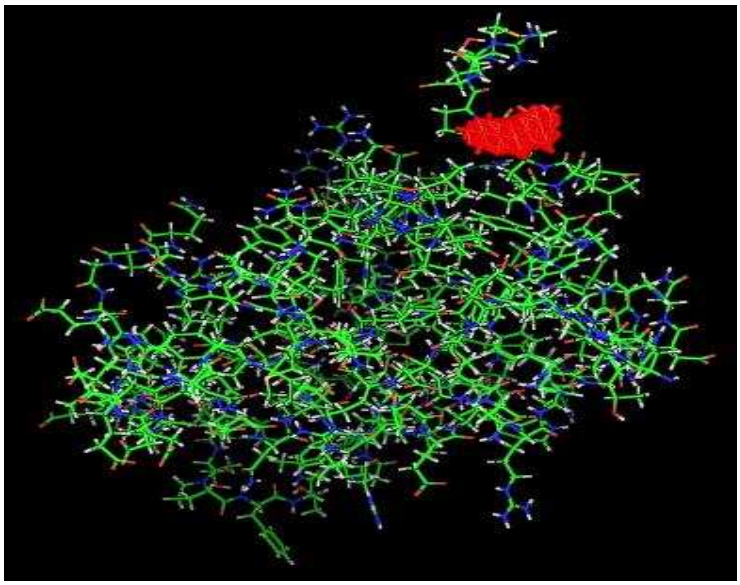


Fig. 2: Docking of Phenol 2,4-bis(1,1-dimethylethyl)- with Bcl- 2 protein

CONCLUSION

Data obtained in the present work through invitro and in silico approach depicted that the Ethanolic extract of *Passiflora incarnata.L* possess antitumor potential. After carrying out in depth pre-clinical and clinical trials, can be developed as a safe, efficacy, human compatible anticancer drug and can be useful in combating this second killing disease.

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