

STUDIES ON ANTICANCER AND ANTIMICROBIAL EFFICACY OF *ANISOCHILUS CARNOSUS* WALLICH - EXTRACT

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ABSTRACT

Cancer is a deadly disease resulting due to abnormal growth of cells in our body. Four most frequent serious cancers identified are lung, breast, colorectal & stomach. Existing modern lines of treatment for these cancers are causing serious side effects; hence there is a need to develop human friendly anti cancer drugs. In this connection traditional drug sources could be useful sources for developing safe efficacious human compatible anticancer drugs. Lamiaceous members are proven for the presence of anti cancer compounds such as terpenes, royleanones etc. With a view to develop an anti cancer drug a lamiaceous member botanically equated as *Anisochilus carnosus* is selected and subjected to chemical and invitro toxicity studies, besides scientific evaluation of the antimicrobial potential of the test drug is also carried out. GC MS Analysis was carried out to identify the compounds of the selected drug and In silico studies carried out to assess the anticancer potential. Preliminary phytochemical screening of ethanol extract of *Anisochilus carnosus* was carried out. Extract answered positively for the presence of alkaloids, sterol, and quinone. Ethanolic extract of the selected plant was evaluated for its antitumor efficacy against Ehrlich ascites carcinoma employing invitro methods. Data of the results obtained revealed high cell death rate in higher concentration. Compounds present in the ethanol extract of *Anisochilus carnosus* were analyzed using GC-MS. 45 compounds were identified. Compounds selected were subjected to antimicrobial activity studies and found to be more effective against *staphylococcus aureus* and *Pseudomonas aeuoroginosa*. Besides the compounds identified in the extract of the test drug were also docked with Bcl-2 protein using modeler software and pymol. Auto dock tool was used for analysis of docking stimulations.

Keywords: *Anisochilus carnosus* - Wallich, Ehrlich ascites carcinoma, Invitro methods, Anti tumor, In silico studies.

INTRODUCTION

Cancer is a neoplastic deadly disease that involves unregulated cell division and tissue invasiveness. Existing lines of cancer treatment include surgery, radiation, and chemotherapy. These modern lines of treatment produce serious side effects. Recent studies established that herbs and herbal medicine are free from serious side effects hence researchers started focusing towards the development of alternative anticancer drug from herbal sources.

With the view to identify a herbal drug source for the development of anti cancer drug in the present work *Anisochilus carnosus* Wallich (Karpuravalli) an aromatic annual herb, found in the Western Ghats, belonging to the family Lamiaceae is selected and studied.

Literature review suggested that Lamiaceae family plants are proven to suppress the formation of phosphatidylcholine hydro peroxide in the liver, lung & kidney and also possess strong anticancer and anti microbial activity^{1,2,3,4,5,6}.

METHODOLOGY

Plant collection

Fresh plants of the *Anisochilus carnosus* were collected from kodaikanal hills and Trichy Tamilnadu identified using floras and authenticated with the help of herbarium specimen kept in Rapinat herbarium of St. Joseph College at Trichy in Tamilnadu. [Specimen No: RHT 563,10567].

Extraction of plant material

Plant materials were shade dried and coarsely powdered. Measured amount of air-dried powdered plant materials was taken in an aspirator bottle and was soaked in hexane for 2 days at room temperature. On 3rd day the extract was distilled off and residue subjected to further analysis. Chloroform and alcohol were also added subsequently in the order of increasing polarity and extracts were obtained after distilling off the solvents. Then the extracts obtained were filtered and evaporated using a vacuum rotary evaporator at 40°C.

Preparation of ethanol extract

The whole plant was dried in the shade and pulverized. Ethanol extract was prepared using Soxhlet method by adding 200 ml of ethanol and incubated for 7 h; concentrated extract of 5 g was used for the study.

Antimicrobial activity

Pure bacterial culture were collected from the Microbial Type of Culture Collection (MTCC), MTCC 118 *E.coli*, MTCC 424 *Pseudomonas aeuoroginosa*, MTCC 426 *Proteus vulgaris*, MTCC 441 *Bacillus subtilis*, MTCC 737 *Staphylococcus aureus*, MTCC 1924 *Streptococcus pyogen*, and subjected for screening studies. Bacterial strains to be tested were streaked in nutrient agar plates to obtain the pure culture. The pure cultures were streaked on Luria agar slant and stored at 4°C. Luria broth (himedia) was prepared; 5 ml of broth taken in test tubes and sterilized at 121°C, at 151bs for 15 min. sterile broth were cooled and test tubes were labeled according to the type of bacterial culture to be inoculated. Tubes were inoculated with appropriate bacterial cultures under aseptic conditions and incubated at 37°C for 18 hours. Assay of antibacterial activity of plant extracts were done by disc diffusion method.

Phytochemical Screening of *Anisochilus Carnosus*

Various plant extracts obtained were subjected to preliminary phytochemical tests as per standard textual procedures⁷.

In-Vitro Cytotoxicity⁸

In- vitro Cytotoxicity was assessed using EAC by incubating the different concentration of the drugs at 37°C for 3 hours. After incubation 0.1ml of 1% trypan blue dye was added to each tube and the number of viable (unstained) and dead (stained) cells were counted using haemocytometer. The percentage Cytotoxicity (dead cells) was calculated using the formula.

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

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

Gas Chromatography And Mass Spectrometry (Gc-MS)

The sample is dissolved in the organic solvent till it dissolves completely. Gas chromatography condition is maintained at 100°C-280°C as 5°C/min. Then 2µl of sample is injected in to the column. The helium gas moves at 1ml/min through the column.

The compound splits in the ratio of 1:10. After the program is run, mass spectrometer scans the compounds separated. Then the peak area of the each peak is measured to detect the compounds present at the area with the structure.

Molecular Docking

Inhibitors identified from GC-MS techniques were converted to 3D structures using modeler software and pymol and docking process carried out. Auto dock tool is used to prepare run and analyze docking simulations.

RESULTS

Preliminary phytochemical screening of crude drug and various extract of *Anisochilus carnosus*

The preliminary phytochemical screening of drug powder and various extracts showed the presence of quinones, alkaloids, sterols, coumarins, and proteins. Flavones and lignin present in ethanol extract and terpenoids present in chloroform extract. The ethanol extract was subjected to microbiological screening using different concentrations like 200µg, 400µg, 800µg, 1000µg employing disc diffusion method, Diameter was measured (mm) & recorded.

In-Vitro Cytotoxicity Studies Of Ethanol Extract

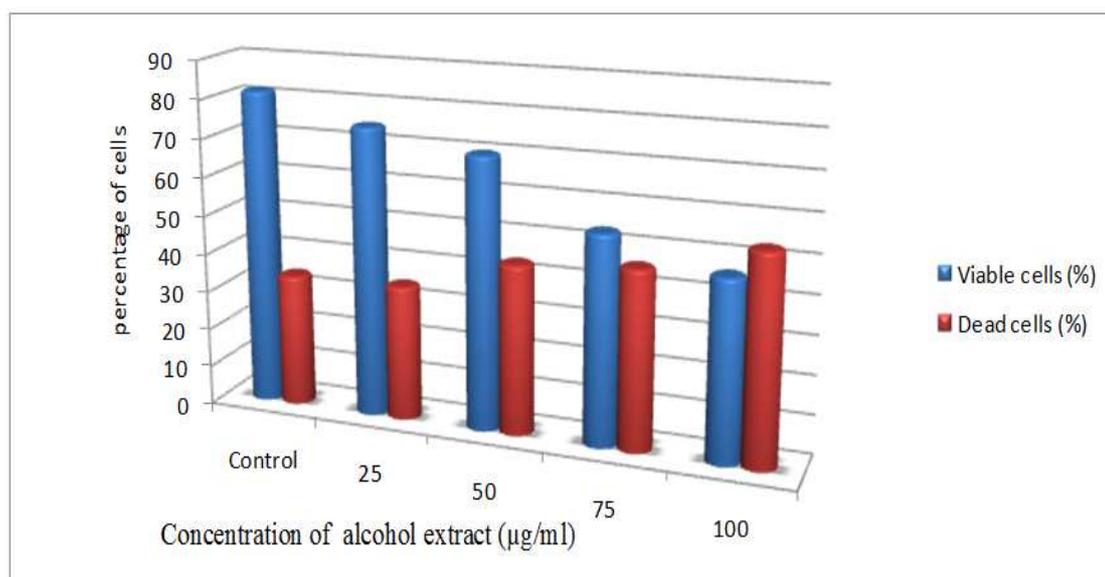
The in-vitro Cytotoxicity effect of ethanol extract of *Anisochilus carnosus* caused 53.61% of EAC cells death in higher concentration (100µg/ml) & 34.94% of death cell was noticed in lower concentration (25µg/ml). (Table-2)

Table 1: Antibacterial activity against various organisms

S. No	Name of the organisms	Concentration of ethanol extract(µg/Disc)/Zone of inhibition(in mm)					
		+ve control	-ve control	200µg/Disc	400µg/Disc	800µg/Disc	1000µg/Disc
1	<i>Escherichia coli</i>	10	-	11	11	10	11
2	<i>Proteus vulgaris</i>	13	-	10	10	10	14
3	<i>Staphylococcus aureus</i>	16	-	10	10	10	11
4	<i>Streptococcus pyogenes</i>	10	-	10	10	10	11
5	<i>Pseudomonas aeruginosa</i>	18	-	11	10	10	11
6	<i>Bacillus subtilis</i>	12	-	10	10	10	11

Table 2: In-vitro Cytotoxicity studies

S. No	Concentration of alcohol extract (µg/ml)	No. of	Viable cells (%)	No. of dead cells	Dead cells (%)
1	Control	499	81.93	210	34.48
2	25	242	74.92	130	34.94
3	50	240	70.175	150	43.85
4	75	202	53.86	173	46.133
5	100	314	46.38	363	53.61



Gc-MS Profile Of Ethanol Extract Of *Anisochilus Carnosus*

The compounds present in ethanol extract of *Anisochilus carnosus* was analysed by GC-MS technique and chromatogram obtained given in Fig 1.

There are 45 compounds identified in ethanol extract of *Anisochilus carnosus* and Some of the compounds showed the highest peak area in GCMS, they are styrene, thymol, 1, 3-Bis (cinnamoyloxymethyl) adamantane, Hexadecanoic acid, ethyl ester, (E)-9-Octadecenoic acid

ethyl ester, 1, 2-Benzenedicarboxylic acid, diisooctyl ester, 2-Benzyloxyethylamine.

Insilico Studies

In bioinformatics study, the compounds obtained from ethanol extract of *Anisochilus carnosus* were docked with Bcl2 Protein. Among 18 compounds selected only five compounds docked with Bcl2 protein and they are,

2-Benzyloxyethylamine, 2-Formylhistamine, Benzeneethanamine, 2-fluoro-a, 3-dihydroxy-N-methyl, Phenethylamine, p, a-diethyl, Imidazole, 2-amino5-[2carboxy] vinyl-

Out of five compounds Imidazole, 2-amino5-[2carboxy] vinyl- shows the least docking energy that is -7.21. (Fig-2) suggesting it's more anticancer effect.

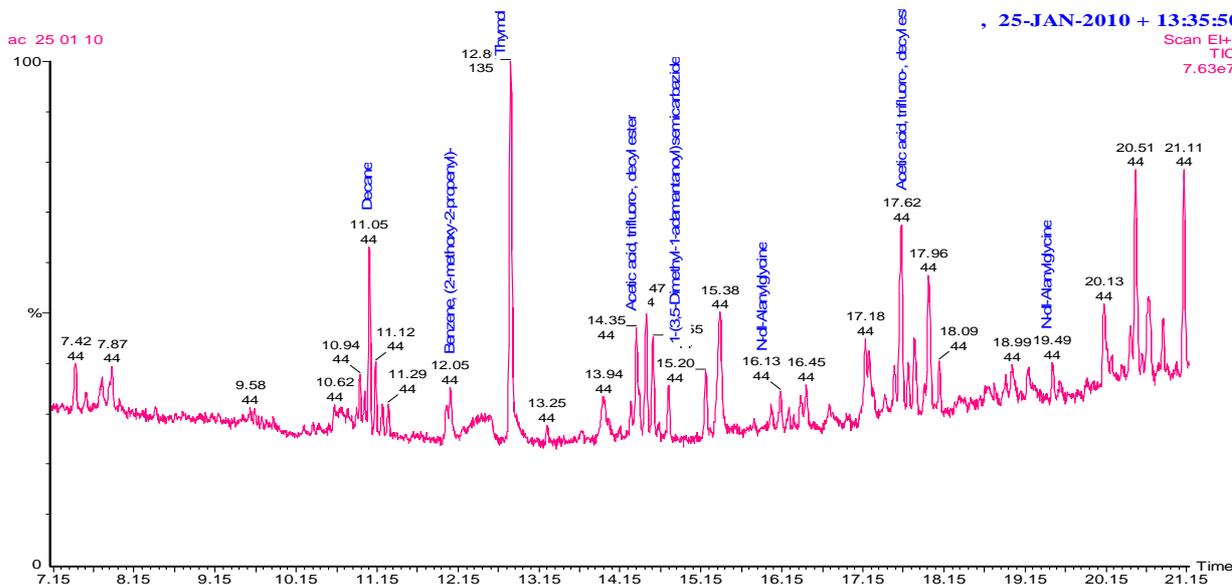


Fig. 1: GC-MS chromatogram for the ethanol extracts of *Anisochilus carnosus*

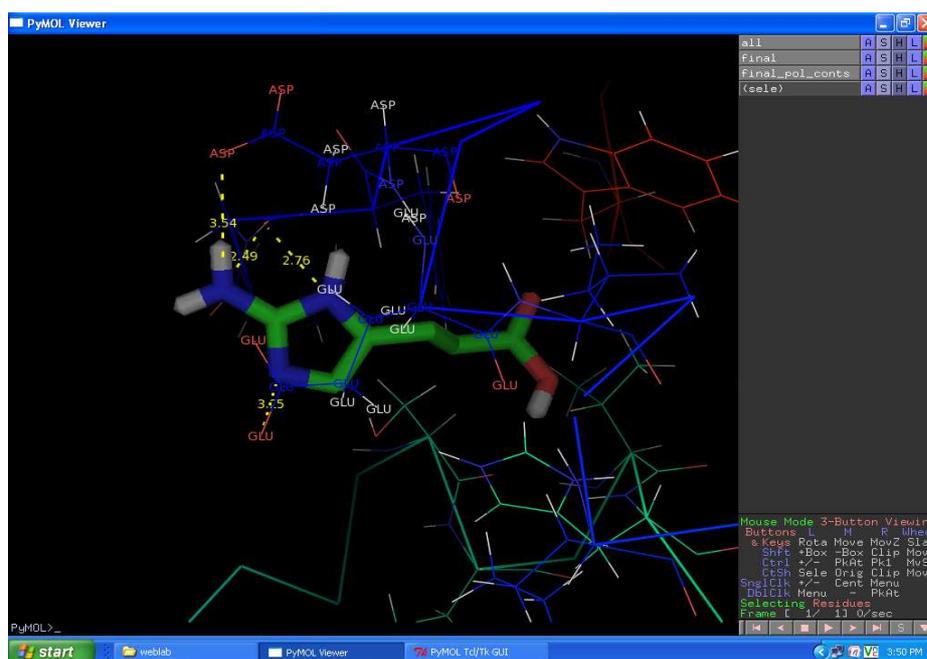


Fig. 2: Imidazole, 2-amino5-[2carboxy] vinyl- docked with Bcl2 protein

DISCUSSION

The lamiaceaeous members possess thymol, sesquiterpenes, flavanoids and aliphatic compounds. These compounds are known to act against cancer.

In the present investigation compounds present in the ethanol extracts of *Anisochilus carnosus* were identified through GC-MS analysis. There are 45 compounds. Some of the compounds showed highest peak area such as styrene, thymol, 1, 3-Bis (cinnamoyloxymethyl) adamantane, Hexadecanoic acid, ethyl ester, (E)-9-Octadecenoic acid ethyl ester, 2-Benzenedicarboxylic acid, diisooctyl ester, and 2-Benzyloxyethylamine.

In bioinformatics study, of the 18 compounds identified from ethanol extract of *Anisochilus carnosus* only five compounds docked with Bcl2 protein and they are, 2-Benzyloxyethylamine, 2-Formylhistamine, Benzeneethanamine, 2-fluoro-a, 3-dihydroxy-N-methyl, Phenethylamine, p, a-diethyl, Imidazole, 2-amino5-[2carboxy] vinyl-.

Out of five compounds Imidazole, 2-amino5-[2carboxy] vinyl- showed the least docking energy that is -7.21. So it was regarded as the best candidate in molecular docking process.

The anticancer activity of ethanol extract of *Anisochilus carnosus* was evaluated by in-vitro Cytotoxicity studies method. The results

showed 53.61% of EAC cells death in higher concentration. Besides the ethanol extract of selected plant also revealed good anti microbial activity against *staphylococcus aureus*, *Pseudomonas aeruginosa*, *E.coli*, *Proteus vulgaris*, *Bacillus subtilis*, *Streptococcus pyogenes*. These data suggested that this could be an effective safe antimicrobial agent.

CONCLUSION

The data of the results of the *In silico*, *in vitro* studies suggested the ethanol extract of selected plant *Anisochilus carnosus* possess potent anticancer activity against Ehrlich ascites carcinoma. Further in depth phyto chemical studies can result in the identification of a new anticancer compound leading to the development of a novel safe and efficacious anti cancer drug from this natural source. The data obtained on the anti microbial activity of the ethanol extract of selected drug suggested that this could be an effective safe antimicrobial agent especially for cancer patients in treating their microbial infections.

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