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IN -SILICO AND *IN-VITRO* ANTICANCER ACTIVITY OF *EMBLICA OFFICINALIS, WITHANIA SOMNIFERA* AND *ZINGIBEROFFICINALE*

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ABSTRACT

The objective of the present study is to identify selected medicinal plants (*Emblica officinalis*, *Withaniasomnifera*&*Zingiberofficinale*) which possess anticancer activity according to their traditional claims. The phytoconstituents in the plant, *Withaniasomnifera* and *Emblica officinale* showed good binding affinity towards thymidylate synthase and p-glycoprotein respectively as compared to that of the standards. From the results of MTT analysis, it can be concluded that *Zingiber officinalis* was found to inhibit HT-29 cell lines to a greater extent Almost all the extracts were found to produce an excellent anticancer activity. The activity can be attributed either to the expression of the molecular targets having a maximal affinity to the chemical constituents present in these plants or might also be due to the higher penetration power of the active principles which might have resulted in cell inhibitions.

Key Words: *Withania somnifera, Zingiber officinale, Emblica officinale,* MTT assay

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INTRODUCTION

India is the largest producer of medicinal plants and is rightly called the “Botanical garden of the World”. Besides providing high quality of food and raw materials for livelihood, they also have natural therapeutic values against various diseases. Cancer is a major cause of death, and globally studies are being conducted to prevent cancer or to develop effective non-toxic therapeutic agents. It has been reported that medicinal plants may promote host resistance against infection by re- stabilizing body equilibrium and conditioning the body tissue. In recent years, the understanding of intracellular pathways in cancer cells discovery is focused on finding better strategies for cancer treatment, with fewer side effects and without impairment by drug resistance. Worldwide approximately 18% of cancer deaths are related to infectious diseases. This proportion ranges from a high of 25% in Africa to less than 10% in the developed world. Viruses are the usual infectious agents that cause cancer butcancer bacteria and parasites may also play a role. Oncoviruses (viruses that can cause cancer) include human papillomavirus (cervical cancer), Epstein–Barr virus (B-cell lymphoproliferative disease and nasopharyngeal carcinoma), Kaposi's sarcoma herpesvirus (Kaposi's sarcoma and primary effusion lymphomas), hepatitis B and hepatitis C viruses (hepatocellular carcinoma) and human T-cell leukemia virus-1 (T-cell leukemias). Bacterial infection may also increase the risk of cancer, as seen in *Helicobacter pylori*-induced gastric carcinoma. Parasitic infections

associated with cancer include *Schistosoma haematobium* (squamous cell carcinoma of the bladder) and the liver flukes, *Opisthorchis viverrini* and *Clonorchissinensis* (cholangiocarcinoma). Plants have been used as remedies and botanical literature has described the usage of plant extracts. Cancer is a dreadful disease and combating this disease is of great importance to public health. There is a necessity for search of new compounds with cytotoxic activity as the treatment of cancer with the available anticancer drugs is often unsatisfactory due to the problem cytotoxicity to the normal cells. Phytochemical examination has been making rapid progress and herbal products are becoming popular as sources of plausible anticancer compounds (1-2)

MATERIALS AND METHODS

In-vitro anticancer activity (3)

Cell line

HT29 cell lines were obtained from National centre for cell sciences Pune (NCCS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.

Molecular docking (In silico docking analysis) (4)

Preparation of Ligand

The major active constituents are identified from the selected medicinal plants namely *Withaniasomnifera*(withaferin A, withanoloide A, withanoloide B, withanoloide E ,withanone), *Emblicaofficinale*(gallic acid, phyllemblin, ascorbic acid, ellagic acid, phyllantidine), *Zingiberofficinale*(alpha farnesene, alpha zingiberene, sesquiphellandrene, gingerol, curcumene) which possess anti-cancer properties according to traditional claims. and the 3D structures of the various active constituents (ligands) are retrieved from PubChem chemical databases and saved in .mol format. The ligands and proteins are imported to the workspace and preparation of them is done. The results obtained are compared against the standards (raltitrexed, tamoxifen, vinblastine and fluorouracil).

Preparation of receptor

The targets for docking studies are selected as p-glycoprotein and thymidylate synthase. Docking analysis is done by initially selecting the target for the disease and followed by obtaining the 3D structure of thymidylate synthase (1HVY) and p-glycoprotein (3G61) protein data bank in pdb format. It is well known that PDB files often have poor or missing assignments of explicit hydrogens, and the PDB file format cannot accommodate bond order information. Therefore, proper bonds, bond orders, hybridization and charges were assigned using the MVD. The potential binding sites of both the targets were calculated using the built-in cavity detection algorithm implemented in MVD. The search space of the simulation exploited in the docking studies was studied as a subset region of 25.0 Angstroms around the active side cleft. The water molecules are also taken in to consideration and the replaceable water molecules were given a score of 0.50.

MVDs docking search algorithms and scoring functions

Ligand docking studies were performed by MVD, which has recently been introduced and gained attention among medicinal chemists. MVD is a fast and flexible docking program that gives the most likely conformation of ligand binding to a macromolecule. MolDock software is based on a new heuristic search algorithm that combines differential evolution with a cavity prediction algorithm. It has an interactive optimization technique inspired by Darwinian Evolution Theory (Evolutionary Algorithms - EA), in which a population of individuals is exposed to competitive selection that weeds out poor solutions. Recombination and mutation are used to generate new solutions. The scoring function of MolDock is based on the Piecewise Linear Potential (PLP), which is a simplified potential whose parameters are fit to protein-ligand structures and a binding data scoring function that is further extended in GEMDOCK (Generic Evolutionary Method for molecular DOCK) with a new hydrogen bonding term and charge schemes (5-8).

In vitro assay for anticancer activity (MTT assay)

The anticancer activity of samples on HT29 was determined by the MTT assay (3). Cells (1×10^5/well) were plated in 0.2 ml of medium/well in 96-well plates. Incubate at 5 % CO_2 incubator for 72 hours.

Then, add various concentrations of the samples in 0.1% DMSO for 48h at 5 % CO_2 incubator. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 20µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) in phosphate-buffered saline solution was added. After 4hrs incubation, 1ml of DMSO was added. Viable cells were determined by the absorbance at 540nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The effect of the samples on the proliferation of HT29cells was expressed as the % cell viability.

RESULTS AND DISCUSSION

In-silico docking results

The ability of the phytoconstituents to bind with the targets is given in terms of Mol dock score. The mol dock score is used as the parameter for analysing the docking results. The phytoconstituents are ranked according to their mol dock score. The ligand possessing the highest mol dock score shows a strong affinity towards its target. *In-silico* docking analysis of phytoconstituents from *Emblica officinalis*, *Withaniasomnifera*&*Zingiberofficinale* on thymidylate synthase (PDB ID: 1HVY) are tabulated in table-1 and represented in figure-1 and p-glycoprotein (PDB ID: 3GP1) are tabulated in table-2. The top 5 ligands which were found to have greater affinity to thymidylate synthase are withaferin A(-140.681); curcumene(-140.656); withanoloide A(-109.302); withanoloide E(-106.49); withanoloideB(-102.595) (Figure-3-6). The constituents of *Withaniasomnifera* was found to have a maximum affinity to thymidylate synthase when compared to that of the standard, raltitrexed(-151.264) and tamoxifen(-129.451). The top 5 ligands for the target p-glycoprotein are ellagic acid(-60.7406); gallic acid(-57.7957); Curcumene (-57.1762); Phyllemblin(-54.874); alpha farnesene (-49.0781). The constituents of *Emblicaofficinale*was found to have moderate affinity to p-glycoprotein when compared to that of the standard, raltitrexed(-141.817) and tamoxifen(-115.666).

Table 1*In-silico* docking analysis of phytoconstituents from *Emblica officinalis*, *Withaniasomnifera*&*Zingiberofficinale* on thymidylate synthase (PDB ID: 1HVY)

Name	Ligand	MolDock Score	Rerank Score	HBond
[02]Ralitrexed	Ralitrexed	-161.507	-74.7499	-5.81313
[04]curcumene	Curcumene	-123.972	-109.282	-4.27874
[03]withaferinA	Withaferin A	-122.301	-47.2819	-3.66324
[01]withanoloideA	Withanoloide A	-118.385	-103.061	-1.55906
[00]ellagic acid	ellagic acid	-107.212	-101.07	-8.55675
[02]withanoloideB	Withanoloide B	-101.754	-86.1358	-3.01209
[00]alphafarnesene	alpha farnesene	-98.9771	-81.5346	0
[02]Vinblastine	Vinblastine	-95.3642	87.9539	-1.72455
[01]withanone	Withanone	-92.5521	-24.1122	-3.38431
[00]alphazingiberin	alpha zingiberin	-88.7164	-75.9188	0
[04]gingerol	Gingerol	-87.0432	-62.5049	-7.66442
[00]phyllantidine	Phyllantidine	-86.7644	-73.7242	-3.22853
[00]sesquiphellandrene	sesquiphellandrene	-85.3651	-72.3351	0
[02]phyllemblin	Phyllemblin	-81.1621	-73.5355	-9.05635
[00]gallic acid	gallic acid	-80.9971	-76.4823	-3.1345
[02]withanoloideE	Withanoloide E	-73.6466	18.2038	-2.95644
[02]ascorbic acid	ascorbic acid	-67.1024	-37.7178	-4.68475

Table 2 *In-silico* docking analysis of phytoconstituents from *Emblica officinalis*, *Withaniasomnifera*&*Zingiberofficinale* on p-glycoprotein (PDB ID: 3GP1)

Name	Ligand	MolDock Score	Rerank Score	HBond
[00]Ralitrexed	Ralitrexed	-136.199	-60.6256	-3.08834
[00]curcumene	Curcumene	-111.437	-78.5286	-4.99344
[00]Vinblastine	Vinblastine	-101.239	-41.496	-3.95618
[00]gingerol	Gingerol	-88.7964	-72.666	-4.71772
[00]withaferinA	Withaferin A	-88.4887	-73.6559	-5.38396
[00]withanoloideB	Withanoloide B	-87.685	-72.5747	-6.14196
[00]withanoloideA	Withanoloide A	-83.3521	50.6954	-2.19803
[00]alphazingiberin	Alpha zingiberin	-83.1694	-62.3781	0
[00]withanoloideE	Withanoloide E	-77.8661	-70.1261	-1.98091
[01]ellagic acid	Ellagic acid	-77.4644	-67.2545	-10.5944
[00]alphafarnesene	Alpha farnesene	-73.7184	-58.7687	0
[00]sesquiphellandrene	Sesquiphellandrene	-73.546	-57.7826	0
[00]phyllemblin	Phyllemblin	-69.321	-60.9125	-7.2956
[00]phyllantidine	Phyllantidine	-68.2984	-55.3783	-2.07708
[00]withanone	Withanone	-68.0238	-64.8553	-3.68629
[00]ascorbic acid	Ascorbic acid	-64.3326	-55.3118	-8.61592
[00]gallic acid	Gallic acid	-61.939	-52.8576	-6.59955

Screening of standardised extracts of *Withaniasomnifera*, *Zingiberofficinale*and *Emblicaofficinale* resulted in moderate anticancer activities against HT-29 cell lines. The inhibitory properties of these extracts are compared with standard 5-Fluorouracil for HT-29 cell line (Table-3) respectively. The Percentage cancer cell inhibition profiles were found to be concentration dependent (Table-3). The maximum concentration (µg/ml) used in the study was 1000µg/ml.

Table- 3 *In-vitro* anticancer activity of standardized extract of *WithaniasomniferaZingiberofficinale*, *Emblicaofficinale*& 5 Fluorouracil on colorectal cell line (HT29) by % cell inhibition

S.NO	Concentration (µg/ml)	% Cell Inhibition			
		Withaniasomnifera	*Zingiberofficinale*	*Emblicaofficinale*	5 Fluorouracil
1.	7.8	4	12.8	0.9	23.2
2.	15.6	12.8	15.4	9	26.4
3.	31.2	21.7	25.5	15.4	36.2
4.	62.5	45.5	38.2	17.9	49.3
5.	125	34.4	43.2	31.8	54.8
6.	250	53.3	55.9	43.2	66.4
7.	500	60.9	66	60.9	77.8
8.	1000	71.1	76.1	64.7	87.2

CONCLUSION

The results of the present study supports the anticancer properties of medicinal plants used in the traditional Indian medicine system and further evaluation of the selected medicinal plants for an effective anticancer therapy with minimal side effects. It can also be recommended that daily consumption of some of the medicinal herbs in the form of extracts or dietary supplements are promising therapy for the prophylaxis of cancer. The rate at which cancer is progressing seems to have an urgent and effective effort for improving the health of humans and animals as well. Plant-derived compounds have been an important source of several clinically useful anti-cancer agents. These include vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, etoposide, derived from epipodophyllotoxin, and paclitaxel (taxol®). A number of promising new agents are in clinical development based on selective activity against cancer-related molecular targets, including flavopiridol and combretastin A4 phosphate, while some agents who failed in earlier clinical studies are stimulating renewed interest.

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