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Original Article

COMPARATIVE STUDY OF *IN SILICO* AND *IN VITRO* ANTICANCER ACTIVITY OF TRADITIONAL INDIAN MEDICINAL PLANTS-A REVERSE PHARMACOLOGICAL APPROACH

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ABSTRACT

Objective: Cancer is one of the major deaths occurring worldwide and its prophylaxis demands the daily consumption of extracts or dietary supplements of traditional medicinal plants which possess anticancer activities. This study focuses on the evaluation of the chemo preventive and antiproliferative effects of the active constituents of Indian medicinal plants such as *Withaniasomnifera*, *Phyllanthusemblica* and *Zingiberofficinale* by *in silico* and *in vitro* studies.

Methods: *In silico* docking analysis is performed using Molegro Virtual Docker choosing the targets as p-glycoprotein and thymidylate synthase for the identified phytoconstituents. *In vitro* colorimetric cell metabolic activity assay is performed for the standardized extracts of these plants in various cell lines using the standards.

Results: The phytoconstituents in the plants, *Withaniasomnifera* and *Phyllanthusemblica* revealed good binding affinity towards thymidylate synthase and p-glycoprotein respectively as compared to that of the standards.

Conclusion: *Phyllanthusemblica* showed a maximal antiproliferative effect on breast cancer cell lines (MCF-7) when compared to the other plant extracts. *Zingiber officinalis* was found to inhibit HT-29 cell lines to a greater extent and *Withaniasomniferum* resulted in highest A549 cell death. A combination of these extracts in any dosage form could be used in the therapeutic efficacy in cancer.

Keywords: Molegro Virtual docker, MCF-7, HT-29, A549, MTT assay

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INTRODUCTION

India is rightly called as the "home" to all the medicinal plants. Colon, lung, breast, liver and stomach cancers are the most cause of deaths reported every year and the estimate is said to be around 7.9 million [1]. Use of tobacco and other drug abuses are the commonly occurring deaths worldwide [2]. Death due to cancer is projected to rise continually with an estimate of 13.1 million deaths in 2030 [3]. The consumption of these medicinal plants will promote the resistance to the host against any infection by conditioning the body tissues and by re-stabilizing body equilibrium [1]. Novel cancer drug discovery is mainly focussing on some of the better strategies for treatment of cancer by minimizing the side effects and without impairing the drug resistance [4].

This study aims at the evaluation of the cytotoxic and antiproliferative effects of the active constituents of medicinal plants such as *Withaniasomnifera*, *Phyllanthusemblica* and *Zingiber-officinale* by *in silico* and *in vitro* studies on the targets p-glycoprotein and thymidylate synthase [5-9].

MATERIALS AND METHODS

In silico docking analysis

The major active constituents were identified from the selected medicinal plants namely and are as follows:

- *Withaniasomnifera* (withaferin A, withanoloide A, withanoloide B, withanoloide E, withanone),
- *Phyllanthusemblica* (gallic acid, phyllemblin, ascorbic acid, ellagic acid, phyllantidine) and
- *Zingiberofficinale* (alpha-farnesene, alpha zingiberene, sesquiphellandrene, gingerol, curcumene).

These constituents were found to possess anti-cancer properties according to traditional claims. *In silico* docking, analysis is performed using Molegro Virtual Docker choosing the targets as p-glycoprotein and thymidylate synthase for the identified phytoconstituents in the plants and the results are compared against the standards raltitrexed, tamoxifen, vinblastine and fluorouracil [10-13]. 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 [14, 15]. Then 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 prepared for docking. The results thus obtained are compared against the standards [12, 13].

In vitro MTT assay

Cell lines were obtained from National Centre for Cell Sciences (NCCS), Pune. The cells were maintained in Minimal Essential Media supplemented with 10% Fetal Bovine Serum, penicillin (100U/ml) and streptomycin (100µg/ml) in a humidified atmosphere of 50µg/ml CO_2 at 37°C [16, 17].

Reagents–MEM (Minimal Essential Media) was purchased from Hi-Media Laboratories, Fetal Bovine Serum (FBS) was purchased from Cistron laboratories, MTT (3-(4,5 Dimethyl thiazole-2-yl)-2,5 Diphenyltetrazolium bromide) reagent and DMSO (Dimethyl sulfoxide) were purchased from Sisco research laboratory chemicals, Mumbai. Other chemicals were obtained from Sigma-Aldrich, Mumbai.

In vitro MTT assay was performed for the plant's standardized extracts in cell lines namely, A-549 (lung cancer cell line), HT-29 (colorectal cancer), MCF-7 (breast cancer cell line)using standards such as tamoxifen for MCF-7 and A-549 cell lines and 5-Florouracil for HT-29 cell line) [18].

The sensitivity of MCF-7, HT-29 and A549 cells to *Withaniasomnifera*, *Phyllanthusemblica, Zingiberofficinale* was determined individually by the MTT colorimetric assay. Cells were seeded in a flat bottomed 96-well plate and were incubated for 24 h at 37 °C and 50% CO_2. Both the cell lines were exposed to all three plant extracts mentioned above. The solvent (Dimethyl sulfoxide)

treated cells served as control. The cells were then treated with MTT reagent (20µl/well) for 4 h at 37C and then DMSO (200µl) was added to each well to dissolve the formazan crystals. The optical density was recorded at 540 nm in a microplate reader. The percentage of cell inhibition was determined as [1-(OD of treated cells/OD of control cells]*100 [19-21].

RESULTS

In silico docking analysis

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 analyzing 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.

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 *Phyllanthusemblica* was found to have a moderate affinity to p-glycoprotein when compared to that of the standard, raltitrexed (-141.817) and tamoxifen (-115.666). Refer table 1.

The top 5 ligands which were found to have a greater affinity to thymidylate synthase were withaferin A (-140.681); curcumene (-140.656); withanolide A (-109.302); withanoloide E (-106.49); withanoloide B (-102.595). The constituents of

Withaniasomnifera were found to have a maximum affinity to thymidylate synthase when compared with standards, raltitrexed (-151.264) and tamoxifen (-129.451). Refer table 2.

Table 1: P-GLYCOPROTEIN-3G61-ranking based on mol dock score

Mol dock score	Ligand	Name
-141.817	raltitrexed	[00] raltitrexed
-115.666	tamoxifen	[00] tamoxifen
-101.516	vinblastine	[00] vinblastine
-60.7406	ellagic acid	[02] ellagic acid
-57.7957	gallic acid	[02] gallic acid
-57.1762	cucurmene	[00] curcurmene
-54.874	phyllemblin	[02] phyllemblin
-54.6759	flourouracil	[00] flourouracil
-49.0781	alpha farnesene	[01] alpha farnesene
-44.6231	phyllantidine	[01] phyllantidine
-44.5979	withaferinA	[01] withaferinA
-37.4563	withanoloide A	[01] withanoloide A
-33.4113	withanoloide E	[01] withanoloide E
-32.6451	withanone	[00] withanone
-21.8885	ascorbic acid	[02] ascorbic acid
-15.5545	sesquiphellandrene	[02] sesquiphellandrene
10.5933	withanoloideB	[02] withanoloideB
54.6561	gingerol	[00] gingerol
71.3498	alpha zingiberin	[00] alpha zingiberin

***In vitro MTT* assay**

Screening of extracts of *Withaniasomnifera, Phyllanthusemblica, Zingiberofficinale* resulted in impotent anticancer activities against A-549, MCF-7 and HT-29 cell lines. The inhibitory properties of these extracts are compared with the standards, tamoxifen for MCF-7 and A549 cell lines and 5-flourouracil for the HT-29 cell. The percentage cancer cell inhibition profiles were found to be concentration dependent. The maximum concentration (µg/ml) used in the study was 1000µg/ml.

The inhibitory properties of plant extracts are compared with standard 5-Fluorouracil for HT-29 cell line and tamoxifen for A-549 and MCF-7 cell lines and are represented in the tables 3, 4 and 5 respectively.

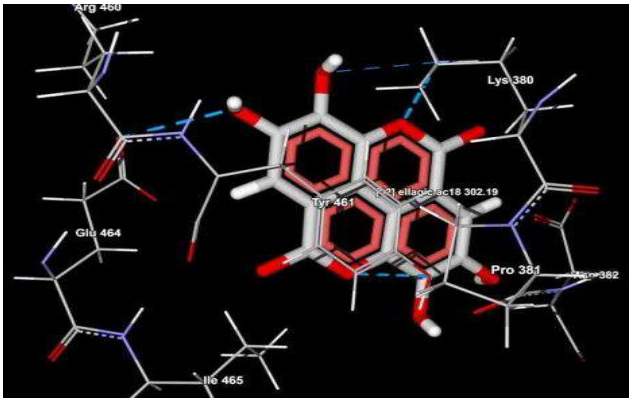

Fig. 1: 3D View of 3G61 docked with raltitrexed

HT-29 cancer cell line, when subjected to various concentrations of fluorouracil, resulted in 87.2% of cell death. A maximum cell inhibition of 76.1% was observed with *Zingiberofficinale* at a concentration of 1000 µg/ml. *Withaniasomnifera* and *Phyllanthusemblica* extracts resulted in 71.1% and 64.7% of HT-29 cell inhibition respectively. Refer fig. 5 and table 3.

Fig. 1 and 2 represent the 3d view of the protein 3G61 docked with raltitrexed and the ligand having the highest affinity respectively.

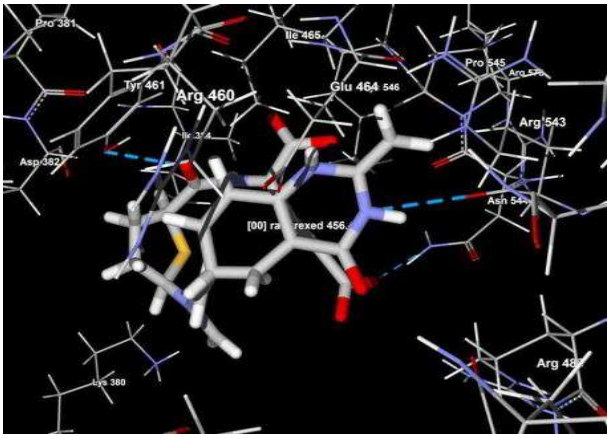

Fig. 2: 3D view of 3G61 docked with the ligand having the highest affinity, vinblastine

Table 2: Thymidylate synthase-1HVY- Ranking based on MolDock Score

MolDockScore	Ligand	Name
-151.264	ralitrexed	[01]ralitrexed
-140.681	withaferin A	[00]withaferin A
-140.656	curcumene	[00]curcumene
-129.451	tamoxifen	[00]tamoxifen
-109.302	withanoloide A	[00]withanoloide A
-106.49	withanoloide E	[01]withanoloide E
-102.595	withanoloide B	[00]withanoloide B
-101.426	gingerol	[00]gingerol
-95.4284	vinblastine	[00]vinblastine
-95.0899	ellagic acid	[00]ellagic acid
-88.4583	withanone	[00]withanone
-88.0655	alpha farnasene	[00]alpha farnasene
-86.0758	phyllemblin	[00]phyllemblin
-85.5623	alpha zingiberene	[00]alpha zingiberene
-84.6785	sesquiphellandrene	[00]sesquiphellandrene
-77.1248	phyllantidine	[00]phyllantidine
-75.5379	ascorbic acid	[00]ascorbic acid
-70.5988	gallic acid	[02]gallic acid
-59.0146	florouracil	[00]florouracil

Fig. 3 and 4 shows the 3d view of the protein 1HVY docked with raltitrexed and the ligand having the highest affinity respectively

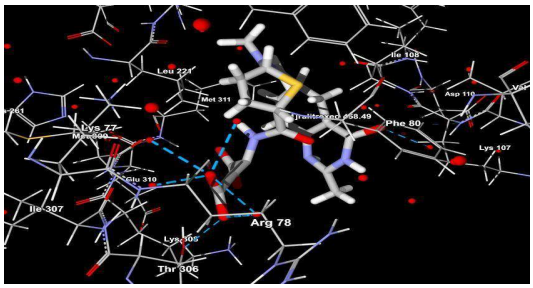

Fig. 3: 3D view of 1HVY docked with raltitrexed

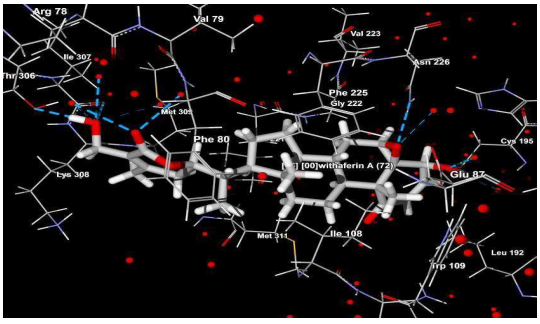

Fig. 4-3D: View of 1HVY docked with the ligand having the highest affinity, withaferin a

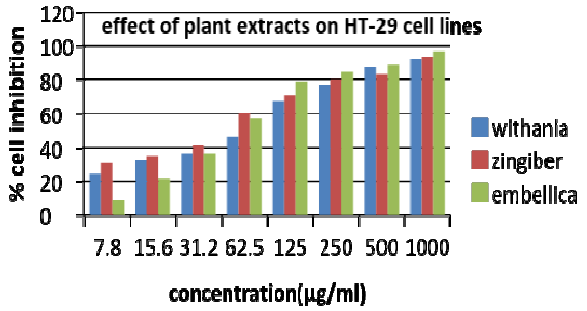

Fig. 5:

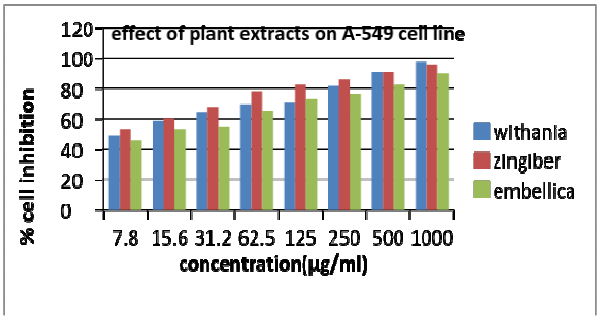

Fig. 6:

Table 3: Percentage cell inhibition of plant extracts on HT-29 cell lines

% cell inhibition				Concentration (µg/ml)
5-Florouracil	Phyllanthus emblica	Zingiber officinale	Withaniasomnifera	
23.2	0.9	12.8	4	7.8
26.4	9	15.4	12.8	15.6
36.2	15.4	25.5	21.7	31.2
49.3	17.9	38.2	45.5	62.5
54.8	31.8	43.2	34.4	125
66.4	43.2	55.9	53.3	250
77.8	60.9	66	60.9	500
87.2	64.7	76.1	71.1	1000

A-549 cell lines, when subjected to different concentrations of *Withaniasomnifera* extract resulted in 87.3% inhibition at a concentration of 1000µg/ml. Similarly, *Zingiberofficinale* and *Phyllanthusemblica* resulted in 85.5% and 80% of A-549 cell death respectively. Comparison with tamoxifen showed 96.4% of cell inhibition at the maximum concentration. Refer fig. 6 and table 4.

Table 4: Percentage cell inhibition of plant extracts on A-549 cell lines

% cell inhibition				Concentration (µg/ml)
Tamoxifen	**Phyllanthus emblica**	**Zingiberofficinale**	**Withaniasomnifera**	
46.4	35.9	43.3	38.7	7.8
56.6	43.3	50.6	48.8	15.6
65.2	45.1	57.9	54.3	31.2
68.9	55.2	68	59.8	62.5
71.3	63.4	72.6	60.9	125
82.6	56.2	76.3	71.7	250
91.3	72.6	80.9	80.9	500
96.4	80	85.5	87.3	1000

MCF-7 cell lines, when subjected to different concentrations of *Phyllanthusemblica* extract resulted in 87% inhibition at a concentration of 1000µg/ml. Similarly, *Zingiberofficinale and Withaniasomnifera* resulted in 83.9% and 82.4% of MCF-7 cell death respectively. On the other hand, comparison with tamoxifen showed that 95.6% MCF-7 cell line inhibition at the same tested dose (1000 µg/ml).

Refer fig. 7 and table 5.

Table 5: Percentage cell inhibition of plant extracts on MCF-7 cell lines

% cell inhibition				Concentration (µg/ml)
Tamoxifen	**Phyllanthusemblica**	**Zingiberofficinale**	**Withania somnifera**	
11.4	9.3	20.8	14.7	7.8
22.4	11.6	25.4	22.4	15.6
39	27	31.6	27	31.2
56.4	47	50	36.2	62.5
76.4	68.5	60.8	57.7	125
86.7	74.7	70	67	250
88.4	79.3	73.1	77.7	500
95.6	87	83.9	82.4	1000

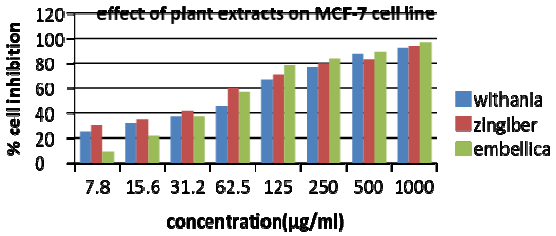

Fig. 7:

DISCUSSION

The present work aimed towards the evaluation of the cytotoxic and antiproliferative effects of the phytoconstituents in *Withania-somnifera*, *Phyllanthusemblica, Zingiberofficinale* by docking analysis and MTT assay.

The phytoconstituents in the plant, *Withaniasomnifera* and *Phyllanthusemblica* revealed good binding affinity towards thymidylate synthase and p-glycoprotein respectively as compared to that of the standards.

From the results of MTT analysis, it is concluded that *Phyllanthusemblica* showed a maximal antiproliferative effect on breast cancer cell lines (MCF-7) when compared to the other plant extracts. *Zingiber officinalis* was found to inhibit HT-29 cell lines to a greater extent and *Withaniasomnifera* resulted in highest A549 cell death. 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. Overall, it can be stated that all the extracts contain potential compounds or active principles which might render the plants with anticancer proliferative activities against the various cell lines.

The results of the present study support the anticancer properties of medicinal plants used in the traditional Indian medicine system. It can also be recommended that daily consumption of some of the medicinal herbs in the form of extracts or dietary supplements are a 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.

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CONFLICT OF INTERESTS

Declare none

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