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

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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 targeting cancer, which includes the discovery and condition of agents that would inhibit or kill cancer cells. The sensitivity of MCF-7, HT-29 and A549 cells to tamoxifen, vinblastine and fluorouracil [10-13].

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, Withania somnifera and Phyllanthus emblica revealed good binding affinity towards thymidylate synthase and p-glycoprotein respectively as compared to that of the standards.

Conclusion: Phyllanthus emblica 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 Withania somnifera 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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treated cells served as control. The cells were then treated with MTT reagent (20μl/well) for 4 h at 37°C 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 Phyllanthus emblica 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); withanolide E (-106.49); withanolide B (-102.595). The constituents of Withania somnifera were found to have a maximum affinity to thymidylate synthase when compared with standards, raltitrexed (-151.264) and tamoxifen (-129.451). Refer table 2.

| Mol dock score | Ligand             | Name        |
|----------------|--------------------|-------------|
| -141.817       | raltitrexed        | raltitrexed |
| -115.666       | tamoxifen          | tamoxifen   |
| -101.516       | vinblastine        | vinblastine |
| -60.7406       | ellagic acid       | ellagic acid|
| -57.7957       | gallic acid        | gallic acid |
| -57.1762       | curcumene          | curcumene   |
| -54.874        | phyllemblin        | phyllemblin |
| -54.6759       | fluorouracil       | fluorouracil|
| -49.0781       | alpha farnesene    | alpha farnesene |
| -44.6231       | phyllantidine      | phyllantidine |
| -44.5979       | withaferin A       | withaferin A |
| -37.4563       | withanolide A      | withanolide A |
| -33.4113       | withanolide E      | withanolide E |
| -32.6451       | withanine           | withanine   |
| -21.8885       | ascorbic acid      | ascorbic acid |
| -15.5545       | sesquiphellandrene | sesquiphellandrene |
| 10.5933        | withanolide B      | withanolide B |
| 54.6561        | gingerol           | gingerol    |
| 71.3498        | alpha zingiberin   | alpha zingiberin |

In vitro MTT assay

Screening of extracts of Withania somnifera, Phyllanthus emblica, Zingiber officinale 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.

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 Zingiber officinale at a concentration of 1000 μg/ml. Withania somnifera and Phyllanthus emblica 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.
Table 2: Thymidylate synthase-1HVY- Ranking based on MolDock Score

| MolDockScore | Ligand       | Name       |
|--------------|--------------|------------|
| -151.264     | raltrexed    | [01]raltrexed |
| -140.681     | withaferin A | [00]withaferin A |
| -140.656     | curcumene    | [00]curcumene |
| -129.451     | tamoxifen    | [00]tamoxifen |
| -106.49      | withanolide A | [00]withanolide A |
| -102.595     | withanolide E | [01]withanolide E |
| -101.426     | gingerol     | [00]gingerol |
| -95.4284     | vinblastine  | [00]vinblastine |
| -95.0899     | ellagic acid | [00]ellagic acid |
| -88.4583     | withanolone  | [00]withanolone |
| -88.0655     | alpha farnasene | [00]alpha farnasene |
| -86.0758     | phyllembalin | [00]phyllembalin |
| -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 |
| -69.0146     | 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

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

| Concentration (µg/ml) | 5-Florouracil | Phyllanthus emblica | Zingiber officinale | Withaniasomnifera |
|-----------------------|---------------|---------------------|--------------------|-------------------|
| 7.8                   | 23.2          | 0.9                 | 12.8               | 4                 |
| 15.6                  | 26.4          | 15.4                | 25.5               | 21.7              |
| 31.2                  | 36.2          | 17.9                | 38.2               | 45.5              |
| 62.5                  | 49.3          | 31.8                | 43.2               | 34.4              |
| 125                   | 54.9          | 43.2                | 55.9               | 53.3              |
| 250                   | 66.4          | 60.9                | 66                 | 60.9              |
| 500                   | 77.8          | 64.7                | 76.1               | 71.1              |
| 1000                  | 87.2          |                     |                    |                   |

A-549 cell lines, when subjected to different concentrations of Withaniasomnifera extract resulted in 87.3% inhibition at a concentration of 1000µg/ml. Similarly, Zingiber officinale and Phyllanthus emblica 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.
The present work aimed towards the evaluation of the cytotoxic and antiproliferative effects of the phytoconstituents in Phyllanthus emblica, Zingiber officinalis, and Withania somnifera, Phyllanthus emblica, Zingiber officinalis by docking analysis and MTT assay.

The phytoconstituents in the plant, Withania somnifera and Phyllanthus emblica 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 Phyllanthus emblica 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 Withania somnifera resulted in highest A549 cell death. Almost all the extracts were found to produce an excellent antiproliferative effect on breast cancer cell lines (MCF-7) when compared to the other plant extracts. Similarly, Zingiber officinalis and Withania somnifera 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 4: Percentage cell inhibition of plant extracts on A-549 cell lines

| Concentration (µg/ml) | Tamoxifen | Phyllanthus emblica | Zingiber officinalis | Withania somnifera |
|-----------------------|-----------|---------------------|----------------------|-------------------|
| 2.5                   | 31.2      | 15.6                | 7.8                  |
| 5                     | 31.2      | 15.6                | 7.8                  |
| 25                    | 31.2      | 15.6                | 7.8                  |
| 500                   | 31.2      | 15.6                | 7.8                  |
| 1000                  | 31.2      | 15.6                | 7.8                  |

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

| Concentration (µg/ml) | Tamoxifen | Phyllanthus emblica | Zingiber officinalis | Withania somnifera |
|-----------------------|-----------|---------------------|----------------------|-------------------|
| 2.5                   | 31.2      | 15.6                | 7.8                  |
| 5                     | 31.2      | 15.6                | 7.8                  |
| 25                    | 31.2      | 15.6                | 7.8                  |
| 500                   | 31.2      | 15.6                | 7.8                  |
| 1000                  | 31.2      | 15.6                | 7.8                  |

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### CONFLICT OF INTERESTS

Declare none

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