Exploring the anticancer potential of Vitex negundo L.: Cytotoxicity and gene regulation in PA1 ovarian cancer cells

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Abstract

Background

Ovarian cancer is a commonly occurring and significant health concern among women worldwide. There is an ongoing search for effective and natural therapeutic agents to combat this disease.

Objective

The study aimed to analyze the cytotoxic effect and gene expression in ovarian cancer (PA1) cell lines treated with methanolic leaf extract of *Vitex negundo*.

Methods

The cytotoxic effect of the methanolic leaf extract of Vitex negundo on the ovarian cancer cell line (PA1) was determined using the MTT assay. The IC50 value was calculated to assess the concentration required to inhibit 50% of cell growth. Gene expression analysis was performed using RT-qPCR to evaluate the mRNA levels of significant targets including PLEKHH3, STIP1, and ATP5F1A.

Results

The IC50 value of the methanolic leaf extract of Vitex negundo on PA1 cells was determined as 53.332 ± 1.449 µg/mL compared to vehicle control cells. RT-qPCR analysis revealed that among the three target genes analyzed, two targets (PLEKHH3 and ATP5F1A) were downregulated, while STIP1 was upregulated upon treatment with the plant extract compared with untreated control cells.

Conclusion

This study indicates that the methanolic leaf extract of Vitex negundo exhibits cytotoxic effects against ovarian cancer cells and modulates gene expression of key targets involved in cellular processes. These findings suggest potential therapeutic applications of Vitex negundo in the treatment of ovarian cancer.

Introduction

Ovarian cancer (OC) is one of the most common gynaecologic cancers that ranks third after cervical and uterine cancer. The high mortality rate of ovarian cancer is caused by asymptomatic and secret growth of the tumours, delayed onset of symptoms, and lack of proper screening that results in its diagnosis in the advanced stages [1]. Generally, ovarian cancer is classified into epithelial ovarian cancer, borderline tumours, germ cell tumours, and sex-cord stromal tumours. It has been observed that approximately 90% of all OC originate from epithelial cells, while the remaining cases are of a non-epithelial origin [2, 3]. Among epithelial OC, 3% are mucinous and others are non-mucinous. Non-mucinous are further found to have serous (70% of non-mucinous), endometrioid (10%), clear cell (10%), and unspecified subtypes (5%) [4]. Currently available drugs that have been used for cancer include cisplatin, carboplatin, olaparib, melphalan, and doxorubicin. Cisplatin is clinically proven to combat different types of cancers including sarcomas, cancers of soft tissue, bones, muscles, and blood vessels. Further, carboplatin is a chemotherapeutic drug used for cancers of the ovaries, lungs, head, and neck [5]. Relative to cisplatin, the greatest benefit of carboplatin is its reduced side effects, particularly the elimination of nephrotoxic effects [6]. Melphalan is second-line therapy after initial platinum therapy for epithelial ovarian cancer and doxorubicin (Dox) alone or in combination with other chemotherapy is a common first-line therapy for numerous cancers including breast, ovarian, bladder, and lung [7]. The side effects of chemotherapy used to treat OC are often severe, it may damage host cells and the immune system [8, 9]. Recently more attention has been paid to nature-based therapies to retain the host immune systems.

Vitex negundo, commonly called nochi is a frequently used medicinal herb in India from a long time ago and is still used widely. It belongs to the family *Verbenaceae* and is found in China, Japan, Taiwan, and India. The various parts of *Vitex negundo* L., extracts are exploited in Ayurveda, Unani, Siddha, Homeopathy, and Allopathy systems of medicine. It is habitually used in folk medicine in India, Bangladesh, China, the Philippines, Sri Lanka, and Japan (Ahuja et al., 2015). Several chemical constituents are present in *Vitex negundo*, and many of them were identified and reported as having therapeutic uses [10–12]. Among these, some compounds showed therapeutic effects against cancer cells more specifically with OC and still studies are ongoing to explore potential drugs to treat tumours. The vitex compounds showed their effects in tumour cells such as inducing apoptosis, controlling the damaged cell cycle, and several anti-cancer properties. Vitexin EVn-50 and its pure compound 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-7methoxy-3,4-dihydro-2-naphthaldehyde can induce apoptosis in tumour cells and this will upregulate Bax and downregulate the Bcl-2 level, resulting in the modulation of the signalling cascade. Therefore, these apoptotic signals produce cytotoxic effects on breast, prostate, and ovarian cancer cells [13, 14]. In addition, many more anti-proliferative compounds were reported from *Vitex negundo* plants such as vitexicarpin [15], chrysoplenetin and chrysophanol D [16], Even50 [17], vitexin compound 2 [18], vitetrifolin D [19], casticin [20], and vitexin 6 [21]. Given this, the present work was accomplished with drug-induced changes using *Vitex negundo* ethanolic extract against ovarian cancer (PA1) cell lines and analyzed the mRNA expression level of significant targets including PLEKHH3, STIP1, and ATP5F1A using RT PCR and ACTB were being used as control.

Materials and methods

Collection of plant

The *Vitex negundo* plant material (Leaf) was harvested from a plantation field in Tenkasi, Tamil Nadu, India. After harvesting, the material was cleaned and dried under shade. Subsequently, it was ground into a powder using a 40-mesh size and stored in an airtight container at 25 ℃.

Phytochemical analysis of *Vitex negundo* leaves extract

The powdered leaf material of *Vitex negundo* (25 g) was subjected to successive extraction in 250 ml of ethanol by using the Soxhlet apparatus. The collected extracts were evaporated to dryness in a rotary vacuum evaporated and stored for further analysis. Preliminary phytochemical analysis of *Vitex negundo* ethanolic extract was studied by using standard procedures [22, 23].

Ovarian cell line (PA1) culture and maintenance

Ten different concentrations (10 -100 µg/ml) of ethanolic extracts *V. negundo* were prepared with DMSO solvent. The ovarian cancer cell line PA1 was acquired from the cell repository of the National Centre for Cell Sciences (NCCS), Pune, India. The Dulbecco`s Modified Eagle Media (DMEM) was used for continuously maintaining the cell line, which was supplemented with 10% Fetal Bovine Serum (FBS). Penicillin (100 U/ml), and streptomycin (100 µg/ml) were added to the medium to prevent other microbial contamination. The medium with cell line was maintained in a humidified environment with 5% CO_2 at 37 °C.

Morphological study of ovarian cell line PA1 treated with *Vitex negundo* ethanolic extract

The ovarian cell line PA1 was treated with two different concentrations (50 µg and 100 µg) of *Vitex negundo* ethanolic extract for 24 h in a DMEM medium and without plant extract used as a control. After 24 h, significant morphological changes were spotted in the 20X photomicrograph of the cells.

MTT assay of *Vitex negundo* ethanolic extract

The cytotoxicity of the sample on ovarian cancer cell line PA1 was determined by the method of Mossman [24]. Ovarian cancer cell line PA1. the viable cells were harvested and counted using a haemocytometer diluted in DMEM medium to a density of 1×10^4 cells/ml seeded in 96 well plates and incubated for 24 h to allow attachment. Then each well cells were treated with different concentrations (10 to 100 µg/ml) of *Vitex negundo* ethanolic extract. All the treated cells were incubated at 37 °C in a humidified 95% air and 5% CO_2 incubator for 24 h. After incubation, the drug-containing cells were washed with fresh culture medium and the MTT (5 mg/ml in PBS) dye was added to each well, followed by incubation for another 4 h at 37 °C. The purple-precipitated formazan was dissolved in 100 µl of concentrated DMSO. The cell viability was measured by taking absorbance at 540 nm using a multi-well plate reader (Rononik-Readwell Touch). The results were expressed at the percentage of stable cells concerning the control with this formula;

$$\text{Proliferation Inhibition } (\%) = \frac{\text{Mean absorbence of the control} - \text{Mean absorbence of the sample}}{\text{Mean absorbence of the control}} \text{ X } 100$$

Half-maximal inhibitory concentration (IC_{50}) values were determined from the plant extract dose-responsive curve where inhibition of 50% cytotoxicity compared to control cells. The experiment was performed in triplicate.

Gene expression analysis

The expression of genes ACTB (Housekeeping gene), PLEKHH3, STIP1, and ATP5F1A in ovarian cancer cell lines after treating the cells with *Vitex negundo* ethanolic extract by RT-q PCR method. Primer pairs were designed with Primer Express 2.0 Abi Prism software (PE Applied Biosystem, USA) employing common design parameters. All amplicons' primers, except for those amplifying an intronic region of GAPDH (control for possible genomic DNA contamination), were designed to encompass exon-exon boundaries to avoid genomic DNA amplification. The specificity of amplicons and primer pairs was checked in silico using BLAT (UCSC Genome Browser) and BLAST (National Centre for Biotechnology Information) alignment tools. The primer sequences used for qPCR analysis are listed in Table 1.

Table 1
RT primers for selected targets

Primer	Primer sequence
ACTB F	GATGATGATATCGCCGCGCT
ACTB R	CCTCGTCGCCCACATAGGAA
PLEKHH3 F	CGGACATCGTTGTGAAAGGTT
PLEKHH3 R	ACACTGTGTTTCCGACCAGAC
STIP1 F	CCTTACAGTGCTACTCCGAAGC
STIP1 R	ATAGGCAGCAGAACGGTTGC
ATP5F1A F	AGGTCCAGGGGTATTGCAG
ATP5F1A R	TCCTCAGGGATCAGTCCATAAC

Extraction and reverse transcription of RNA

RNA from the ovarian Cancer cell line after being treated with plant extract was extracted as per the procedure given in the Quick Start protocol, RNeasy mini kit (Qiagen India Pvt. Ltd., India). Reverse transcription procedure was followed as per the Quick-Start Protocol, Quanti Tect Reverse Transcription kit, (Qiagen India Pvt. Ltd., India).

RT-qPCR

The amplification procedure was performed with Applied Biosystems 7500 and ViiA7 Real-Time PCR systems using the protocol given in Quick-Start Protocol, QuantiNova SYBR Green PCR Kit, (Qiagen India Pvt. Ltd., India) (Table 2).

Table 2
Cycling conditions

Steps	Time	T (°C)	Ramp rate
Initial step			
PCR initial activation	2.00 min	95	Maximal/fast mode
2nd step cycling			
Denaturation	5 sec	95	Maximal/fast mode
Combined (annealing/extension)	10 sec	60†	Maximal/fast mode
Number of cycles			
Melting curve analysis§		35–40‡	

In-silico analysis of Vitex negundo compounds with target proteins by molecular docking

In this study, molecular docking was performed using ten biologically significant compounds from *Vitex negundo* leaves, including Ursolic Acid, Luteolin, 5-hydroxy-7,4'-dimethoxy flavone, Chrysophenol D, Isovitexin, Casticin, Vitexin, Negundin B, Negundoside, and Agnuside reported earlier studies. These compounds served as ligands, while Pleckstrin homology domain-containing family H member 3 (PLEKHH3) and ATP synthase F1 subunit alpha from Homo sapiens (ATP5F1A) were chosen as target proteins. Protein structures were retrieved from the UniProt database, and ligand PDB files were obtained from the PubChem database. AutoDock tools graphical user interface facilitated the removal of ligands and water molecules from target proteins, and the addition of polar hydrogen bonds and Kollman charges to enhance binding affinity between ligands and proteins. Molecular docking calculations were performed using the Lamarckian Genetic Algorithm (LGA) in the AutoDock 4.0 software program [25].

Results

Phytochemical analysis of Vitex negundo extract

V. negundo is one of the important medicinal and anticancer enhancer herbs. This plant is commonly used in the preparation of traditional drugs to treat numerous diseases. Inspired by the medicinal properties of this plant, the current study aimed to investigate the antiproliferative potential and the primary molecular mechanisms of apoptotic induction against human ovarian cell line PA1. Further, the *Vitex negundo* extracts were evaluated for the antiproliferative potential against ovarian cancer cell line PA1 by cell viability assay and IC_{50} values respectively. Additionally, molecular docking was also conducted to rationalize cancer cell inhibition and to evaluate the ability of the compounds to be developed as good drug candidates for cancer treatment. The phytochemical analysis of *Vitex negundo* leaf extract revealed a rich profile of bioactive compounds with potential anticancer properties. The presence of flavonoids, alkaloids, phenolic compounds, terpenoids, sterols, glycosides, proteins, tannins, and saponins is present in Table 3.

Table 3
Phytochemical analysis of ethanolic extract of *Vitex negundo*

Compound	Inference
Flavonoids	+
Alkaloids	+
Phenolic Compounds	+
Terpenoids	+
Sterols	+
Glycosides	+
Carbohydrates	+
Proteins	+
Tannins	+
Fixed Oil and Fats	-
Saponin	+
(+)- Presence of Compound; (-) – Absence of Compound	

Morphological changes in ovarian cancer cell line PA1

This study treated the ovarian cancer PA1 cell lines with 50 µg/ml and 100 µg/ml of *Vitex negundo* ethanolic leaf extract for 24 h. The findings indicated that, at the 50 µg/ml treatment concentration, cells displayed characteristics such as detachment, shrinkage, membrane blebbing, and distorted shapes. Meanwhile, at the 100 µg/ml treatment concentration, cells exhibited a heightened level of morphological changes, resembling those observed with the 50 µg/ml concentration. The control group, in contrast, showed no discernible morphological alterations and maintained a normal, intact cell morphology as illustrated in Fig. 1.

MTT assay

From the inhibition percentage values obtained, a line graph is constructed to get an idea of how the inhibition percentage fluctuates relative to the concentration of the *Vitex negundo* leaves extract. The concentration of *Vitex negundo* extract in µg/ml is given on the x-axis and the inhibition percentage of the ovarian cell line PA1 is given on the y-axis. The graph shows a gradual increase in the inhibition percentage, along with the increasing concentration of *Vitex negundo* extract, which indicates a linear relationship between the inhibition percentage of ovarian cell line PA1 cells and *Vitex negundo* extract concentration (Fig. 2). The IC_{50} value indicates how much drug is needed to inhibit a biological process by half. It is a widely used informative measure for determining the potency of an antagonist drug. The IC_{50} value of the *Vitex negundo* leaves extract was calculated using the inhibition (%) values of the ovarian cell line PA1 and the IC_{50} value was 53.332 ± 1.449 (Suppl. Table 1).

mRNA expression by quantitative real-time PCR (Δct Data Analysis)

Significant targets including PLEKHH3, STIP1, and ATP5F1A were analyzed at mRNA expression level using RT PCR. Among the three targets analyzed two targets including PLEKHH3 and ATP5F1A were downregulated and STIP1 was

upregulated upon treatment with plant extract compared with the untreated control samples. The complete data for all the targets were depicted and their respective ct values were represented in Suppl. Table 2.

Figure 3 shows the mRNA expression profiling of the target sequences. The relative m RNA expression of each target gene is compared with the control gene. The mRNA expression profile of ATP5F1A and PLEKHH3 shows that there is a decrease in their relative mRNA expression, indicating their down-regulation in the ovarian cancer cell PA1. This means the expression of both these genes was reduced within ovarian cancer cell PA1, resulting in decreased levels of their gene products. Meanwhile, the relative mRNA expression of STIP1 is higher when compared with the control gene. This indicates the up-regulation of STIP1 and increased production of its gene product in the ovarian cancer cell PA1. Ct value is specific to the sequence of interest (SOI) and corresponds to the number of cycles to reach a defined threshold. Delta Ct corresponds to the difference between Ct SOI and Ct of the reference sequence (RS), which is usually a housekeeping gene sequence. Delta Ct shows the difference of expression between 2 genes whereas Ct is specific to the expression of one gene. Delta Ct = Ct SOI – Ct RS.

Molecular docking analysis

Molecular docking analysis plays a critical role in drug discovery, particularly in evaluating the biological activities of natural compounds (Kitchen et al., 2004). This study investigates ten biologically significant compounds found in *Vitex negundo* leaves, including Ursolic Acid, Luteolin, 5-hydroxy-7,4'-dimethoxy flavone, Chrysophenol D, Isovitexin, Casticin, Vitexin, Negundin B, Negundoside, and Agnuside. These compounds were used as ligands, while Pleckstrin homology domain-containing family H member 3 (PLEKHH3) and ATP synthase F1 subunit alpha from Homo sapiens (ATP5F1A) were selected as target proteins. PLEKHH3 is recognized as a potential biomarker and therapeutic target in breast cancer due to its involvement in cell migration and invasion pathways critical for metastasis. ATP5F1A, vital for ATP synthesis in colorectal cancer cells, supports their high energy demands for growth and survival. Differential ATP5F1A expression in colorectal cancer tissues suggests its role in tumor progression and prognosis, motivating research into strategies targeting mitochondrial ATP synthesis to inhibit cancer cell growth and improve treatment outcomes. Figure 4 illustrates the lowest energy-docked poses of the investigated ligand compounds with their respective target proteins. It also shows the length of the hydrogen bonds formed between the ligands and targeted proteins, highlighting the specific amino acids involved in these interactions. Additionally, Table 4 provides a summary of the calculated binding affinities, inhibition constants, and intermolecular energies of the protein-ligand complexes.

Table 4
The obtained docking parameters of the 10 important compounds on their rank calculated by Autodock

Ligand	Target protein (receptor)	Docking Parameters based on the rank								
		Binding energy (Kcal/mol)			Inhibition constant (μM/mM)			Intermolecular energy (Kcal/mol)		
		1	2	3	1	2	3	1	2	3
Ursolic Acid	PLEKHH3	-7.77	-6.99	-6.85	2.01	7.53	9.53	−8.67	−7.88	−7.74
	ATP5F1A	-6.45	-6.34	-5.97	18.63	22.38	42.03	−7.35	−7.24	−6.87
Luteolin	PLEKHH3	−5.79	−4.79	−4.34	57.35	310.38	657.34	−7.28	−6.28	−5.83
	ATP5F1A	−5.10	−4.70	−4.21	182.64	358.30	814.92	−6.59	−6.19	−5.71
5 hydroxy-7, 4' dimethoxy flavone	PLEKHH3	−4.71	−4.40	−4.34	355.10	593.58	656.98	−5.90	−5.59	−5.53
	ATP5F1A	−5.28	−5.28	−5.05	134.83	135.68	197.85	−6.47	−6.47	−6.25
Chrysophenol D	PLEKHH3	−4.23	−3.88	−3.76	794.35	1.44	1.75	−6.32	−5.97	−5.85
	ATP5F1A	−4.16	−3.99	−3.07	886.16	1.19	5.62	−6.25	−6.08	−5.16
Isovitexin	PLEKHH3	−3.18	−3.11	−2.84	4.63	5.26	8.32	−6.17	−6.09	−5.82
	ATP5F1A	−4.18	−2.51	−2.25	868.43	14.37	22.30	−7.16	−5.50	−5.24
Casticin	PLEKHH3	−3.94	−3.74	−3.55	1.29	1.81	2.52	−6.03	−5.83	−5.63
	ATP5F1A	−3.89	−3.52	−3.39	1.41	2.65	3.28	−5.98	−5.60	−5.48
Vitexin	PLEKHH3	−3.73	−3.04	−3.03	1.84	5.91	6.00	−6.71	−6.02	−6.01
	ATP5F1A	−2.54	−2.20	−1.98	13.84	24.45	35.44	−5.52	−5.18	−4.96
Negundin B	PLEKHH3	−3.54	−3.32	−2.86	2.56	3.66	8.06	−6.22	−6.01	−5.54
	ATP5F1A	−2.71	−2.63	−2.51	10.33	11.72	14.36	−5.39	−5.32	−5.20
Negundoside	PLEKHH3	−3.29	−2.38	−2.32	3.89	18.08	19.95	−7.17	−6.26	−6.20
	ATP5F1A	−2.46	−2.38	−1.69	15.74	18.07	57.67	−6.34	−6.26	−5.57
Agnuside	PLEKHH3	−1.02	−0.46	−0.25	180.23	462.12	660.27	−4.89	−4.34	−4.12
	ATP5F1A	−1.89	−0.88	−0.82	41.38	227.03	248.59	−5.76	−4.76	−4.70

Discussion

The phytochemical analysis of Vitex negundo leaf extract showed a diverse range of bioactive compounds that may possess anticancer properties (Table 3). It also suggested that this *Vitex negundo* might be a valuable source for developing novel anticancer therapies against various human cancers [26–29]. Further studies are necessary to isolate and characterize these compounds and to elucidate their specific mechanisms of action against ovarian cancer cell line PA1. The study highlights the antiproliferative potential of *Vitex negundo* extracts against human ovarian cancer cell line PA1. The observed morphological changes in treated cells, such as detachment, shrinkage, and membrane blebbing, are indicative of apoptotic induction. Previous studies have shown that compounds like chrysoplenetin and vitexicarpin from Vitex negundo exhibit anticancer properties [15, 16]. The downregulation of PLEKHH3 and ATP5F1A, along with the upregulation of STIP1, suggests that these genes play significant roles in the antiproliferative effects observed.

PLEKHH3 is involved in cell migration and invasion pathways critical for metastasis [30], while ATP5F1A supports high energy demands for cancer cell growth and survival [31]. Molecular docking analysis revealed that Ursolic acid has strong binding affinities for both PLEKHH3 and ATP5F1A, making it a promising inhibitor. The structure-activity relationship (SAR) analysis suggests that Ursolic acid's pentacyclic triterpenoid structure enables multiple interactions with the proteins' binding sites. Optimization strategies for Ursolic acid could involve modifying functional groups to enhance binding interactions, solubility, and bioavailability [32].

Binding Affinity analysis reveals that Ursolic acid demonstrates the strongest binding affinity for both PLEKHH3 and ATP5F1A, with binding energies ranging from − 7.77 to − 5.97 kcal/mol, suggesting it is the most promising inhibitor among the tested compounds. Luteolin exhibits moderate binding affinities, particularly for PLEKHH3, with energies between − 5.79 and − 4.34 kcal/mol, indicating lower inhibitory potential compared to Ursolic Acid [33]. 5-Hydroxy-7,4'-Dimethoxy Flavone and Chrysophenol D show weaker binding energies, suggesting reduced inhibitory capabilities relative to Ursolic acid and Luteolin. Negundoside and Agnuside display the least favorable binding energies, with high inhibition constants indicating weak or negligible binding affinity to both proteins. Inhibition constant analysis reveals that Ursolic acid exhibits inhibition constants ranging from 2.01 μM to 42.03 μM, highlighting potent inhibitory effects, particularly against PLEKHH3 [34]. Luteolin demonstrates higher inhibition constants, up to 814.92 μM for ATP5F1A, indicating weaker inhibition. Compounds like Casticin and Isovitexin show even higher inhibition constants, suggesting limited effectiveness as potential inhibitors. Intermolecular energy analysis shows that Ursolic acid consistently exhibits lower intermolecular energies, indicating stable and favorable binding interactions with both proteins. Luteolin and 5-Hydroxy-7,4'-Dimethoxy Flavone display intermediate intermolecular energies, suggesting moderate stability of their ligand-protein complexes [35]. Negundoside and Agnuside demonstrate higher intermolecular energies, indicative of less favorable binding interactions and potentially weaker complex stability. Structure-activity relationship (SAR) analysis indicates that Ursolic acid's strong binding affinities are attributed to its pentacyclic triterpenoid structure, enabling multiple interactions with the proteins' binding sites [36]. Luteolin, a flavonoid, exhibits lower binding affinity possibly due to fewer hydrophobic interactions and hydrogen bonds compared to Ursolic Acid. The weaker binding affinities of Chrysophenol D and other ligands may be attributed to suboptimal functional groups for interacting with key residues in PLEKHH3 and ATP5F1A binding sites [37]. Thus, ursolic acid is a lead compound for further development due to its strong binding affinity and favorable inhibition constants against both PLEKHH3 and ATP5F1A. Optimization strategies for Ursolic Acid and similar triterpenoids could involve modifying functional groups to enhance binding interactions, solubility, and bioavailability. Compounds with weaker binding affinities, such as Negundoside and Agnuside, may require significant structural modifications to improve interactions with target proteins. The findings of this study indicate that *Vitex negundo* extracts, particularly Ursolic acid, have potential therapeutic applications in the treatment of ovarian cancer. Further research is necessary to isolate and characterize these compounds fully and to elucidate their specific mechanisms of action against ovarian cancer cells. This approach could lead to the development of novel anticancer therapies using natural compounds from *Vitex negundo* [38].

Conclusion

In conclusion, ursolic acid emerges as the most promising candidate among the tested compounds, showing strong binding and potential inhibitory effects against both PLEKHH3 and ATP5F1A. Its inhibitory effects on PLEKHH3 are particularly significant in the context of breast cancer. While compounds like Luteolin and 5-Hydroxy-7,4'-Dimethoxy Flavone show moderate potential, further optimization is needed to enhance their efficacy. These findings provide a foundation for selecting lead compounds for advancing breast cancer drug design, emphasizing improvements in binding affinities and reduction of inhibition constants.

Declarations

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Author Contributions

Study concept and experiment design – Manivannan Govindasamy, Ganesh Kumar Anbazhagan, and Packia Lekshmi. Data collection and analysis – Premkumar R, Packia Lekshmi and Ponmurugan Karuppiah. The first draft of the manuscript –Premkumar R and Manivannan Govindasamy. Review and edit the manuscript – Ponmurugan Karuppiah and Abdurahman Hajinur Hirad.Project Administration -Manivannan Govindasamy and Ganesh Kumar Anbazhagan. All the authors approved the final version of the manuscript and submission.

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Data availability

All data were presented in the manuscript and no datasets were generated and analyzed.

Conflict of interest

The authors have not any relevant financial interests to reveal.

Ethical approval

Not applicable for this research work.

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Figures

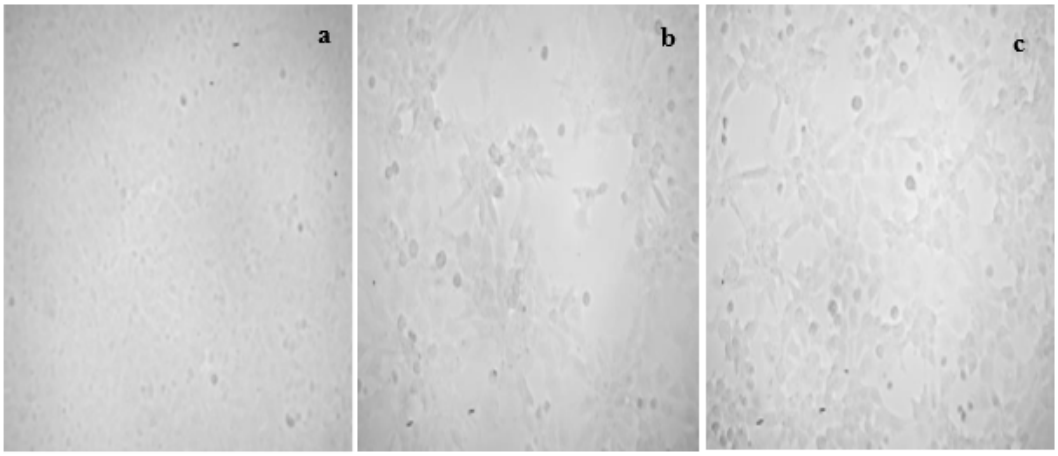

Figure 1

Morphological changes in control and Vitex negundo treated ovarian cancer cell line PA1 for 24 h; (a) control, (b) 50 µg/ml, (c) 100 µg/ml.

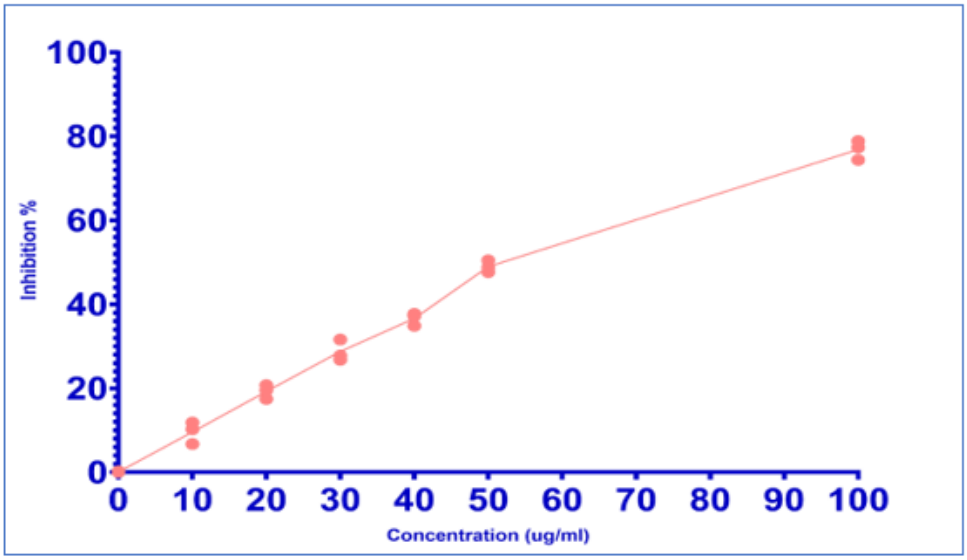

Figure 2

Percentage of inhibition against ovarian cancer cell line PA1 treated with *Vitex negundo* leaf extract

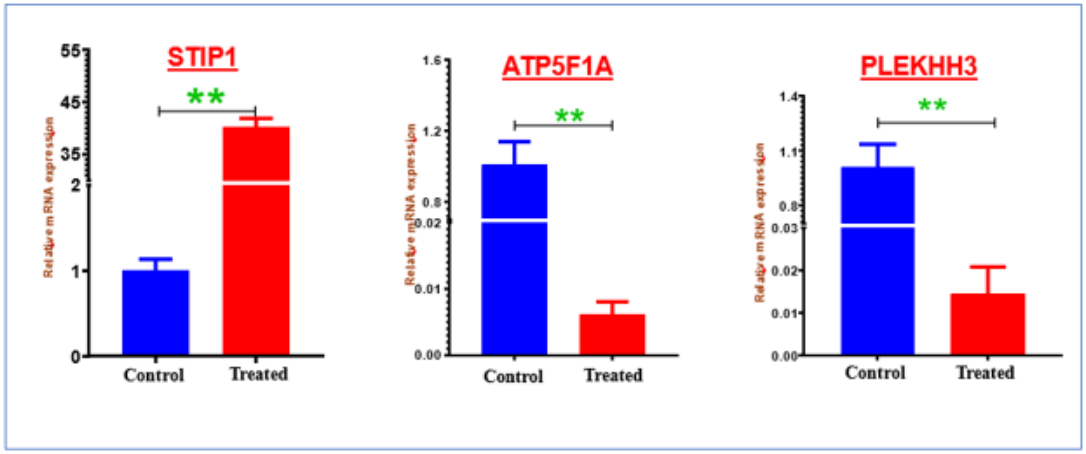

Figure 3

mRNA Expression profiling for the candidate targets which are down and up-regulated upon treatment using RT-PCR

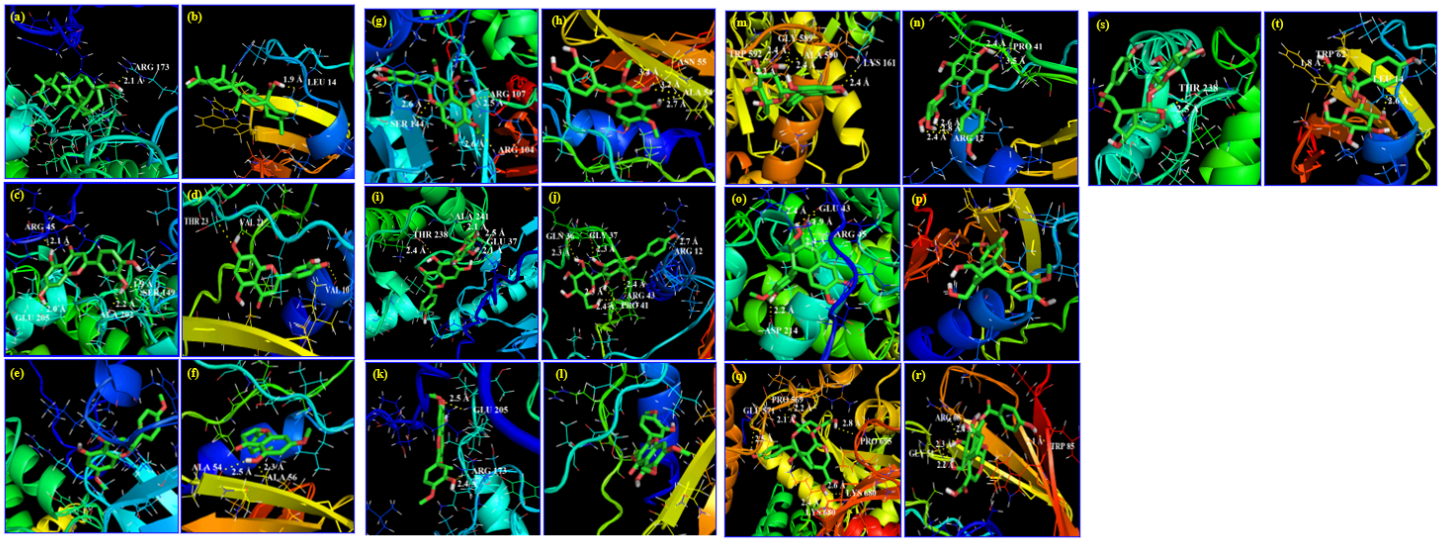

Figure 4

Lowest energy docked poses of the (a) Ursolic Acid-PLEKHH3 complex, (b) Ursolic Acid-ATP5F1A complex, (c) Luteolin-PLEKHH3 complex, (d) Luteolin-ATP5F1A complex, (e) 5-hydroxy-7,4'-dimethoxy flavone-PLEKHH3 complex, (f) 5-hydroxy-7,4'-dimethoxy flavone-ATP5F1A complex, (g) Chrysophenol D-PLEKHH3 complex, (h) Chrysophenol D-ATP5F1A complex, (i) Isovitexin- PLEKHH3 complex, (j) Isovitexin-ATP5F1A complex, (k) Casticin-PLEKHH3 complex, (l) Casticin-ATP5F1A complex, (m)Vitexin-PLEKHH3 complex, (n) Vitexin- ATP5F1A complex, (o) Negundin B-PLEKHH3 complex, (p) Negundin B- ATP5F1A complex, (q) Negundoside-PLEKHH3 complex, (r) Negundoside-ATP5F1A complex, (s) Agnuside-PLEKHH3 complex and (t) Agnuside- ATP5F1A complex.

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