

Anticancer Screening of the Phytochemicals Present in the Medicinal Plant *Vitex Negundo* against Mutant Anaplastic lymphoma Kinase (ALK) Protein: A *in-silico* Approach

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The lymphomas are a heterogeneous group of cancer of the lymphocytes and the lymphatic system and accounts for up to 3% of all malignancies [1]. Most of the drugs currently used for the treatment of lymphoma produce various side effects, hence in this study, we focus on natural compounds, obtained from the medicinal plant *Vitex negundo*, which exhibits selective toxicity against cancer cells. The objective of this research was to formulate the binding energies and interaction of selected phytochemicals present in the medicinal plant *Vitex negundo* [2] against anaplastic lymphoma kinase protein, which is overexpressed in an anaplastic large cell lymphoma [3, 4,5]. The structure of mutant human anaplastic lymphoma kinase protein was retrieved from the Protein Data Bank (PDB ID:4ANL) and the 3D chemical structure of the phytochemicals present in the medicinal plant *Vitex negundo* was obtained from the PubChem database. Molecular docking study was performed for these natural compounds to evaluate and analyze their anti-lymphoma-cancer activity. A total of 16 compounds present in *Vitex negundo*, based on a comprehensive literature survey was selected for this molecular screening. Molecular docking analysis was carried out by Molegro Virtual Docker software, to screen the 16 chosen compounds and rank them according to their binding affinity towards the site of interaction of the oncoprotein, anaplastic lymphoma kinase. Out of the 16 screened phytocompounds, only 4 compounds showed promising interactions against the oncoprotein ALK (4ANL). 6'-p-hydroxybenzoyl mussaenosidic acid exhibited a very good binding with a molecular docking score of -127.723 kcal/mol, ranking first among the compounds screened. This was followed by Betulinic acid, Viridiflorol and protocatechuic acid with molecular docking scores of -95.596 kcal/mol, -76.1648 kcal/mol and -63.0854 kcal/mol and - respectively. The docking scores from the above study shows that the phytocompounds present in *Vitex negundo* extract exhibits an effective inhibitory effect against anaplastic lymphoma kinase protein that is over expressed in lymphoma.

Keywords: In-silico, molecular docking, lymphoma, anaplastic lymphoma kinase, *Vitex negundo*, medicinal plant..

Anaplastic lymphoma kinase is an enzyme that is active during the embryonic nervous system development of humans, but slowly loses its activity over the course of postnatal life¹. This enzyme is prone to various kinds of mutations notably chromosomal fusion. There are twenty

such fusion proteins documented that are known to be involved predominantly in the pathogenesis of neuroblastoma, anaplastic large cell lymphomas and non- small cell lung cancer².

The fusion proteins of the produced from the mutated anaplastic lymphoma kinase gene

dimerize to activate the catalytic site of the ALK protein kinase domain, which leads to the activation of multiple cell proliferation pathways like Ras/Raf/MEK/ERK1/2 and the cell survival pathways like the JAK/STAT pathways³.

ALK was originally identified as a fusion oncogene nucleophosmin (NPM)-ALK resulting from a t(2,5) chromosomal translocation in ALCL. The fusion oncogene NPM-ALK is implicated in the pathogenesis of ALCL as it is detected in approximately 75% of all ALK-positive ALCL (6). Other ALK fusion genes, echinoderm microtubule-associated protein like 4 (EML4)-ALK is proven to be associated with non-small cell lung cancer (NSCLC)⁴.

A number of ATP-competitive ALK inhibitors have been developed and have proven oncolytic activity against ALK positive cancer cells both in vitro and in vivo. Biologicals like Crizotinib, developed as an ALK inhibitor have gained FDA approval for ALK positive cancer in EML4-ALK positive NSCLC and ALCL⁴.

Few of these ALK inhibitors like Crizotinib (12) have advanced to the level of clinical trials^{5,6}; Crizotinib recently gained FDA approval for ALK-positive cancers. It is used in the treatment of EML4-ALK-positive NSCLC but unfortunately like any other drugs it is also known to cause side effects. Hence, there is a need for the development of more potent and specific ALK inhibitors to reduce the adverse effects of the current drugs. In recent years, a number of ALK antagonists have appeared of which a set of novel and potent tetracyclic derivatives (6, 6-Dimethyl-11-oxo-6, 11-dihydro-5-H-benzo (b) carbazoles) (13–15) have been chosen for our present studies⁷.

Vitex negundo, a plant native to South and Southeast Asia, is an erect shrub or small tree growing from 2 to 8 m (6.6 to 26.2 ft) in height. *Vitex* is a genus of flowering plants from the family Lamiaceae. It has about 250 species⁸. Species of *Vitex* are native throughout the tropics and subtropics and a few temperate regions⁹. Almost all parts of this plant has immense medicinal value, ranging from antimicrobial, anti-cancer, anti-inflammatory, anti-ulcer activity etc. It is one of the tropical plants with immense medicinal properties¹⁰.

METHODOLOGY

The molecular docking software used in this study is molegro virtual software.

Lead Identification & optimization

A comprehensive search of all possible studies on *Vitex negundo* compounds was made by searching the electronic literature (PubMed database) for relevant published reports and by manual searching of reference lists of articles on this topic. A total of 16 ligands with antineoplastic attribute were selected based on electronic literature study of the compounds present in *Vitex negundo* plant (Pub med database). The structures of these ligands were downloaded from the pubchem database¹¹.

ADMET Prediction

Once the compounds were selected, elementary physical descriptors like number of hydrogen bond acceptors and donors, log P, molecular weight were calculated for the selected compounds using molinspiration. Using these parameters the compounds were then scanned for "Lipinski's rule of 5". The shortlisted compounds that follow "Lipinski's rule of 5" were then chosen for further study and analysis¹¹.

Target Identification

Structure of G1269A Mutant Anaplastic Lymphoma Kinase (PDB ID: 4ANL) was chosen as our receptor target. This receptor protein was finalized based on literature study. ALK tyrosine kinase receptor is a single polypeptide chain protein (Chain A) obtained from Homo sapiens with a sequence length of 342 amino acids, which harbors a single genetically induced mutation. In its secondary structure the ALK tyrosine kinase receptor, has 36% alpha helix (16 helices; 126 residues) and 12 % beta sheets (12 strands; 42 residues).

Binding Site Identification

The site of interaction of the target protein, Anaplastic lymphoma kinase (4ANL) oncoprotein with the ligand was identified using SHARP. Potential protein-protein interaction sites on the receptor protein structures were predicted using SHARP software. Surfaced residue patches were defined and based on this a

maximum of 6 parameters like Solvation potential, hydrophobicity; Accessible surface area, residue interface propensity, protrusion and Planarity are calculated. Using these parameters the site of interaction of proteins with each ligands were calculated¹¹.

Docking studies

The selected compounds were docked against anaplastic lymphoma kinase protein (PDB ID:4ANL) using Molegro Virtual Docker (MVD). MVD employs MolDock scoring system (used in this study), which is based on guided differential evolution, a new hybrid search algorithm. The guided differential evolution algorithm combines the differential evolution optimization technique with a cavity prediction algorithm. The predicted cavities used during the search process, allows for a fast and accurate identification of potential binding poses¹¹.

The intact protein structure, obtained from the protein data bank (PDB) was loaded on to MVD platform for docking process. Potential binding sites (also referred to as cavities or active sites) has been identified using the built-in cavity detection algorithm of MVD. The search algorithm is taken as Moldock SE and numbers of runs are taken 10 and max iterations were 2000 with population size 50 with an energy threshold of 100¹².

At each step least 'min' torsions/translations/rotations were tested and the one giving lowest energy was chosen. After the docking simulation was over, the poses which were generated were sorted by rerank score.

The Rerank Score uses a weighted combination of the terms used by the MolDock score mixed with a few addition terms (the Rerank Score includes the Steric (by LJ12-6) terms which are Lennard-Jones approximations to the steric energy – the MolDock score uses a piecewise linear potential to approximate the steric energy)¹¹.

The ligand preparation module of MVD was used to manually prepare the chosen ligands. Bond order and hybridization assign wherever missing and flexible torsion and the ligands were deducted^{13,14,15}.

The target protein structures were prepared after careful removal of hetero atoms and water molecules and its electrostatic surface was generated. The docking was subjected towards the

amino acid residues which were found to be part of interaction between HTSPs and VOPs.

The grid resolution was set at 0.3 Å. The maximum interaction was set at 1500 and maximum population size 50

RESULTS

Interaction site Prediction

The association of anaplastic lymphoma kinase (ALK) with anaplastic large cell lymphoma has been well characterized by various studies. There is a 60% association of anaplastic lymphoma kinase with the tumorigenesis process of anaplastic large cell lymphoma. It becomes quite imperative that their site of interaction be targeted in order to bring about a cure for Lymphoma. ALK has already been explored as a potential target for the treatment of Lymphoma².

The protein-ligand interaction between ALK and the selected compounds present in Vitex Negundo was analyzed and the precise site of interaction was identified by employing SHARP2. The amino acid residues of oncoproteins ALK that was interacting with the selected ligands were mapped out accurately.

Docking results

The natural compounds listed from the thorough literature survey were docked against mutant Anaplastic lymphoma kinase (4ANL) at the specific sites of interaction, as predicted from SHARP2 results. For each compound, out of the many docking poses, only those which possess the highest moldock score and relatively good Hydrogen bond interaction was chosen.

The best few compounds which exhibited very good affinity towards the interaction site of the selected receptor protein 4ANL were picked out and the best ligand binding pose were selected on the basis of the aforementioned criteria. The docking results were tabulated for compounds that exhibited negative binding energy when docked against the binding site of mutant ALK tyrosine kinase receptor.

Out of the 16 compounds, that were screened only 4 compounds showed favourable binding affinity with the receptor, the others had no binding at all with the receptor.

62 -p-hydroxybenzoyl mussaenosidic acid was found to possess the best binding affinity

towards the receptor mutant ALK(4ANL) and was found to form hydrogen bond interactions with the amino acid residues of the receptor. 62 -p-hydroxybenzoyl mussaenosidic acid was found to interact with the receptor protein through the amino acids ARG1120, ASP1203, GLY1202, GLU1210, SER1206 by forming hydrogen bonds. It forms five hydrogen bonds with high interaction energy of -127.723.

This is followed by betulinic acid with the binding score of -95.598 towards the binding site of the receptor. Betulinic acid interacts with



Fig. 1. Structure of the protein target ALK tyrosine kinase receptor (PDB ID:4ANL)depicted in secondary structure cartoon style

the receptor (4ANL) with the help of 3 amino acid GLY 1269, ASP 1270, LEU 1122. Following betulinic acid Viridiferol and Protocatechuic acid shows a promising moldock score of -75.1202 and -63.0854. Viridiferol binds with 3L9P predominantly at the MET1199 amino acid. Protocatechuic acid interacts with the protein 4ANL with the aid of the amino acids ASP 1160, ASP 1163, GLU1167, THR1151, LEU1152, PHE1164 present in the receptor protein.

DISCUSSION

Medicinal plants possess many bioactive phytochemicals, which are responsible for their specific medicinal properties. These phytochemicals can be identified with the help of numerous chromatographic separation techniques. The phytochemicals present in *Vitex negundo* were thoroughly identified by various chromatographic techniques and were documented in previous studies. Through a thorough electronic literature search of the Pubmed database, the phytochemicals present in *Vitex negundo* was identified and short listed. These selected compounds were then further shortlisted for their anticancer tendencies using PASS server.

In the *Vitex negundo* phytochemicals were docked against mutated anaplastic lymphoma kinase enzyme (PDB ID: 4ANL).

Table 1. Results of virtual screening of phytochemicals against target using Molegro Virtual Docker 4.5

S. No:	Compound Name	Compound Name	Moldock Score	H-Bonds	Interacting Amino Acids
1	23955877	62 -p-hydroxybenzoyl mussaenosidic acid	-127.723	-11.3025	ARG1120 ASP1203 GLY1202 GLU1210 SER1206
2	64971	Betulinic acid	-95.598	-6.432	GLY1269 ASP1270 LEU1122
3	11996452	Viridiflorol	-75.1202	-2.5	MET1199
4	CID_72	Protocatechuic acid	-63.0854	-11.755	ASP 1160 ASP1163 GLU1167 THR1151 LEU1152 PHE1164

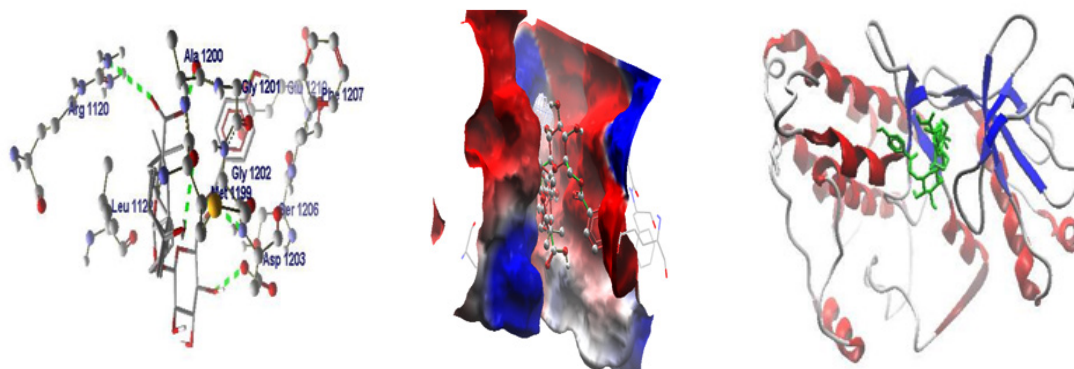


Fig. 3. (3A and 3B) Mussaenosidic acid interaction with target and positions of amino acids involved in the formation of H bonds (arg1120, asp1202, gly1202, glu1210,ser1206).(3C) Position of Mussaenosidic acid within the cavity of the selected protein

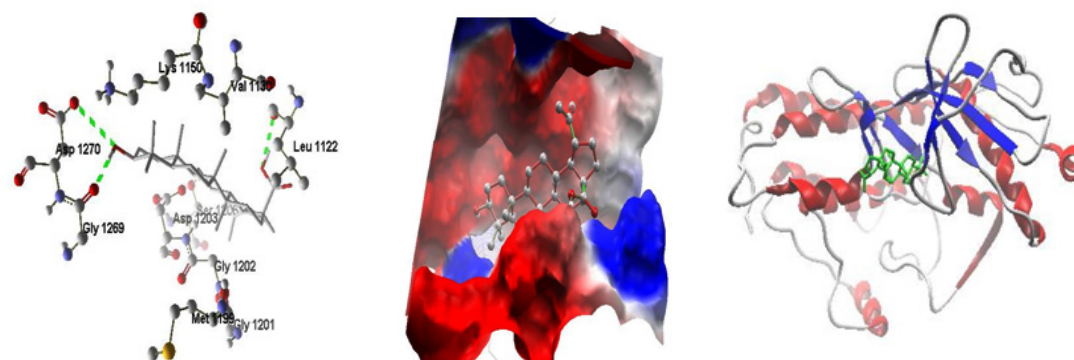


Fig. 4. (4A and 4B) Betulinic acid interaction with target and positions of amino acids involved in the formation of H bonds (gly 1269, asp1270, leu 1122).(4C) Position of Betulinic acid within the cavity of the selected protein

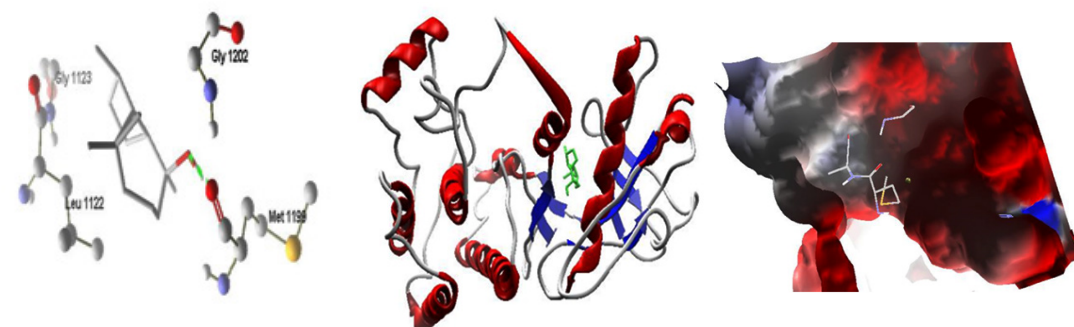


Fig. 5. (5A and 5C) Viridiflorol interaction with target and positions of amino acids involved in the formation of H bonds (met 1199).(5B) Position of Viridiflorol within the cavity of the selected protein

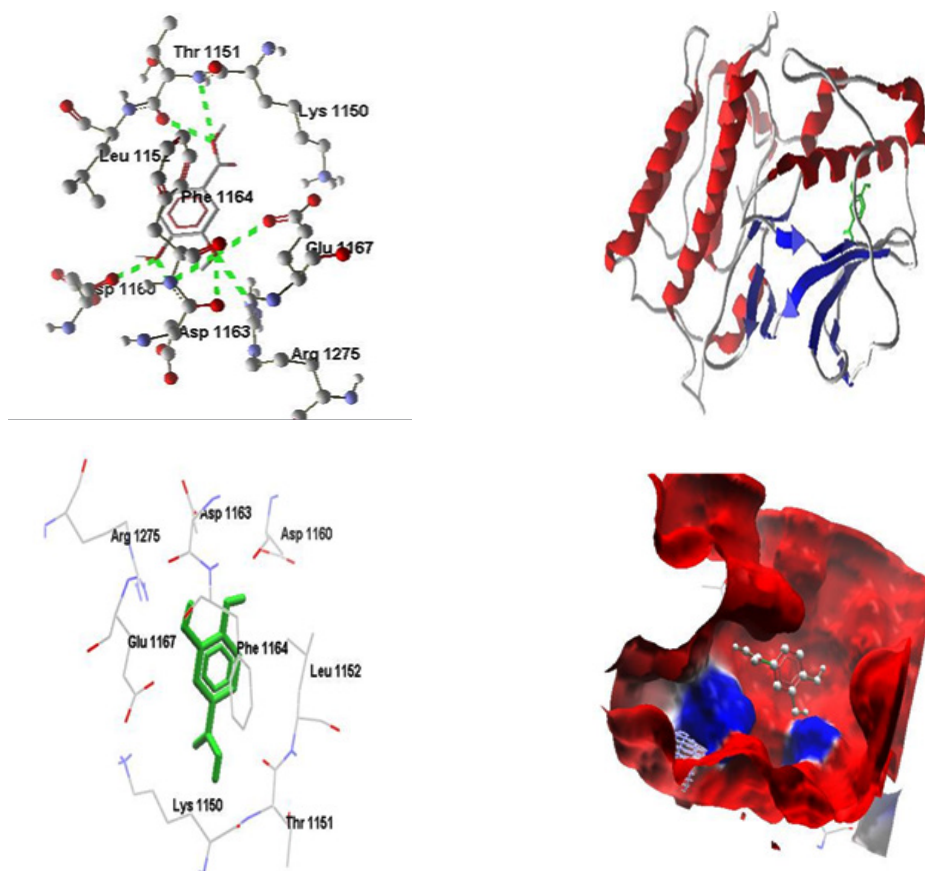


Fig. 4. (6A & 6C) Protocatechuic acid interaction with target and positions of amino acids involved in the formation of H bonds (asp1160, asp1163, glu1167, thr1151, leu1152, phe1164). (6B & 6D) Position of Protocatechuic acid within the cavity of the selected protein

Anaplastic lymphoma kinase is a receptor tyrosine kinase that belongs to the lymphocyte tyrosine kinase family. It is a monomer.

The primary step was to enumerate the amino acid residues involved at the site of interaction. A comprehensive list of all the amino acid residues which would be a part of the vital part of the interaction with the ligand was obtained through SHARP2 analysis.

Our study results showed that amino acids residues ARG1120, ASP1203, GLY1202, GLU1210 and SER1206 of mutant anaplastic lymphoma kinase were involved in the binding of 62 -p-hydroxybenzoyl mussaenosidic acid with the highest binding score of -127.723. p-hydroxybenzoyl mussaenosidic acid was identified and characterized from the ethanolic

extracts of the leaf of *Vitex negundo* plant. P-hydroxybenzoyl mussaenosidic acid from *vitex negundo* plant has a proven hepatoprotective activity. N.Tiwari et al (2013) has demonstrated ant tubercular effect of p-hydroxybenzoyl mussaenosidic acid^{17,18}.

Betulinic acid had the next highest binding energy of -95.598 with the mutated anaplastic lymphoma kinase receptor. GLY1269, ASP1270, LEU1122 are the amino acids of the receptor proteins mediating this interaction. Betulinic acid a pentacyclic terpenoid was identified from *Vitex negundo* leaves. Apart from *vitex negundo* leaves Betulinic acid is present in various other plants as well. Our results were consistent with the findings of Kumar et al, who established the anticancer activity of Betulinic acid from *Dillenia indica* L.

fruits in leukemic cell lines. Apart from anticancer activity Betulinic acid from other plant sources have proven anti-HIV, anti-malarial, anti-bacterial actions as well. Thus, betulinic acid is one of the interesting phytochemicals from *Vitex negundo* leaves which can be further explored for their wide range of therapeutic effects^{19,20}.

Following Betulinic acid it was the phytochemical Viridiflorol that demonstrated good moldock score with our target protein. Viridiflorol interacts with the aminoacid anaplastic lymphoma kinase through the amino acid MET1199. Viridiflorol is a phytochemical present in the oil extracted from *Vitex negundo* leaves. Viridiferol is an essential oil which is present in other plants apart from *Vitex negundo*. Lucas et al demonstrated the antioxidant activity, anti-inflammatory and antituberculous activity of Viridiflorol from *Allophylus edulis* plant^{21,22}.

Protocatechuic acid exhibited a binding score of -63.0854 when docked against our target receptor 4ANL. Protocatechuic acid is present in the seeds of the vitex negundo plant. Protocatechuic acid interacts with the target receptor mutant ALK through the following amino acids ASP1160, ASP1163, GLU1167, THR1151, LEU1152, PHE1164²³. Protocatechuic acid is widely distributed in many plants and natural products like green tea and honey. Cytotoxicity, apoptosis and antigenotoxicity of Protocatechuic acid was demonstrated by Anter et al²⁴.

CONCLUSION

Anaplastic lymphoma kinase is an important tyrosine kinase receptor. The mutant form of anaplastic lymphoma kinase is involved in pathogenesis of various tumors like neuroblastoma, anaplastic large cell lymphoma etc. Thus, making this enzyme an attractive target for various therapeutic interventions. Among the 16 selected phytochemicals screened from *Vitex negundo* extracts in this study, only four compounds have emerged as promising candidates for their anti-cancer potential, evaluated by their binding affinity towards their target protein. The docking results revealed that p-hydroxybenzoyl mussaenosidic acid exhibit the highest binding affinity towards the site of interaction. Thus our study indicates that p-hydroxybenzoyl mussaenosidic acid as a

potential candidate against the mutant anaplastic lymphoma kinase enzyme that induces tumors such as neuroblastoma and anaplastic large cell lymphoma. Further in-vitro and in-vivo analysis is needed to follow up and substantiate the anti-cancer activities of these compounds.

Limitations of the study

Only selected compounds were shortlisted and screened for their anti cancer activity against mutated anaplastic lymphoma kinase protein. Lack of validation of the results with in-vitro and in-vivo studies.

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