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IN SILICO MOLECULAR DOCKING STUDIES ON THE CHEMICAL CONSTITUENTS OF *CLERODENDRUM PHLOMIDIS* LEAF FOR ITS CYTOTOXIC POTENTIAL AGAINST LUNG CANCER MARKERS

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ABSTRACT

Lung cancer remains the leading cause of cancer morbidity and mortality worldwide and is the second cause of the death. In 2018, there were 234,030 newly diagnosed lung cancer patients, accounting for 13.5% of all types of malignant tumors. The vascular endothelial growth factor (VEGF) is the main mediator of angiogenesis and it contributes to cancer growth and metastasis directly targeting the tumor cells. *Clerodendrum phlomidis* is a shrub common in India and Sri-lanka with valuable medicinal properties. GC-MC analysis of the methanol leaf extract of *C. phlomidis* revealed the presence of 17 compounds. In the current study, we aimed to find out the binding effectiveness of the various chemical constituents present in the methanol extract of *C. phlomidis* leaf against lung cancer targets VEGFR-1 and VEGFR-2 and to identify the potent chemical moiety by *in silico* molecular docking studies. *In silico* docking studies were carried out using SYBYL package. The results indicated that the chemical constituents of *C. phlomidis* binds with the target VEGFR-1 and VEGFR-2 effectively with the total score value ranging from 3.0623 to 13.6396 and 0.2089 to 15.4975 respectively. Among the docked molecules compounds oleic acid eicosyl ester and DL-Alpha Tocopherol were found to have good total score value against VEGFR-1, oleic acid eicosyl ester and isopropyl linoleate were found to have good total score value against VEGFR-2. In future, designing of the chemical constituents having the pharmacophore similar to oleic acid, eicosyl ester, DL- Alpha Tocopherol and isopropyl linoleate or their derivatives with varying substituents in different position will be a good drug for the lung cancer target VEGFR-1 and VEGFR-2.

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INTRODUCTION

Cancer is a major public health problem worldwide and is the second leading cause of death. Lung cancer remains the leading cause of cancer morbidity and mortality worldwide. In 2018, there were 234,030 newly diagnosed lung cancer patients, accounting for 13.5% of all types of malignant tumors. In addition, lung cancer results in approximately 1,54,050 death cases each year, accounting for 25.3% of all cancer-related deaths, 80–85% of all lung cancer patients are diagnosed with non-small-cell lung cancer (NSCLC) subtype and 80% lung cancer-associated deaths are caused by NSCLC [1-4].

Vascular endothelial growth factor (VEGF) is a homodimeric glycoprotein with a molecular weight of approximately 45 kDa. It is the key mediator of angiogenesis (the formation of new blood vessels) and binds to VEGF receptors (VEGF receptor-1 and VEGF receptor-2), which are expressed on vascular endothelial cells. In healthy humans, VEGF promotes angiogenesis in embryonic development and is important in wound healing in adults. Angiogenesis is essential for cancer development and growth, before a tumor can grow beyond 1-2 mm, it requires blood vessels for nutrients and oxygen. The production of VEGF and other growth factors by the tumor results in the 'angiogenic switch', where new vasculature is formed in and around the tumor, allowing it to grow exponentially. Tumor vasculature formed under the influence of VEGF is structurally and functionally abnormal. Blood vessels are irregularly shaped, tortuous, have dead ends and are not organized into venules, arterioles and capillaries [5-7]. They are also leaky and hemorrhagic, which leads to high interstitial pressure. These characteristics mean that tumor blood flow is suboptimal, resulting in hypoxia and further VEGF production. This central role of VEGF in the production of tumor vasculature makes it a rational target for anticancer therapy.

Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site with overall minimum energy [8]. The small molecule, known as ligand usually fits within protein's cavity which is predicted by the search algorithm. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. The results are analyzed by a statistical scoring function which converts interacting energy into numerical values called as the docking score; and also the interacting energy is calculated. Predicting the mode of protein ligand interaction can assume the active site of the protein molecule and further help in protein annotation. Hence docking plays an important role in the rational drug designing and discovery [9].

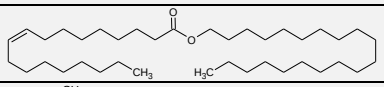
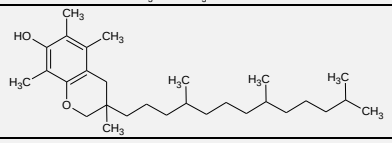
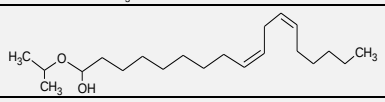
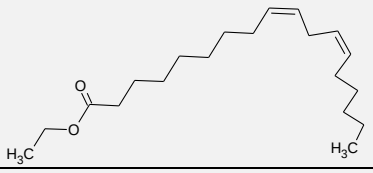
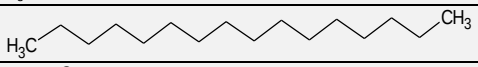
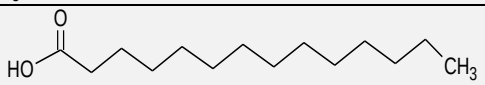
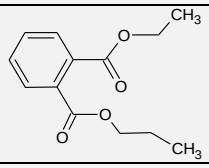
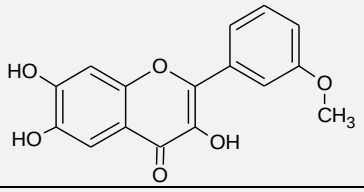
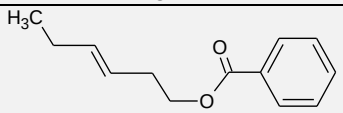
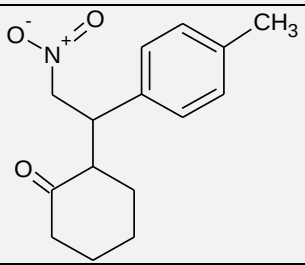
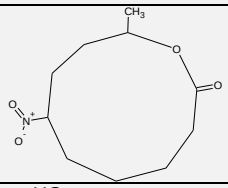
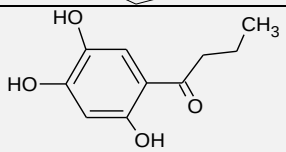
Clerodendrum phlomidis L., (Lamiaceae) is a shrub, generally found in south-east Asia. This genus has great ethno-medicinal importance in various indigenous systems of medicine like Indian, Chinese, Thai, Korean, Siddha, Unani and Japanese for the treatment of numerous diseases like syphilis, typhoid, cancer, jaundice, hypertension, constipation, gonorrhea, piles, urinary diseases, nervous disorders, inflammation and measles [10]. Previously the GC-MS analysis of the methanol leaf extract of *C. phlomidis* revealed the presence of seventeen compounds such as 2,3-dihydroxypropanal, 2-methoxy-4-vinylphenol, Isopropyl Linoleate, hexadecane, (11E)-trideca-1,11-diene-3,5,7,9-tetrayne, tetradecanoic acid, 3-Hexen-1-yl benzoate, 3,6,7-trihydroxy-2-(3-methoxyphenyl)-4*H*-chromen-4-one, 1-(2,4,5-trihydroxyphenyl)-1-butanone, 3-cyclohexen-1-ol, caryophyllene, 1,2-benzenedicarboxylic acid diethyl ester ethyl phthalate, DL-alpha tocopherol, oleic acid eicosyl ester, 2-(2-nitro-1-p-tolyl-ethyl)-cyclohexanone, ethyl linoleate, 8-nitro-11-dodecanolide [11]. Since *in-silico* molecular docking has been an efficient method for discovery and development of new drug candidates, the current investigation was focused on finding out the binding effectiveness of the chemical constituents present in the methanol extract of *Clerodendrum phlomidis* leaf and to identify the potent chemical moiety among the various chemical constituents of *Clerodendrum phlomidis* against the commonly used lung cancer markers by *in-silico* molecular docking studies.

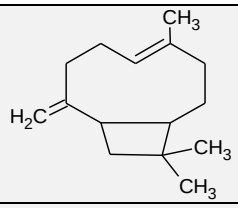
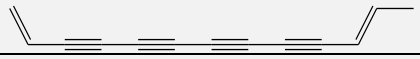
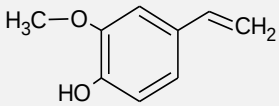
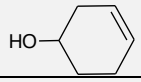
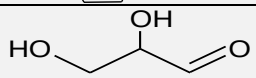
MATERIALS AND METHODS

In silico docking studies were carried out using SYBYL package. The 3D structures of chemical constituents of *Clerodendrum phlomidis* leaf were generated using SYBYL package. The geometry optimization was done with the help of standard Tripos force field [12] using a distance dependent-dielectric function, energy gradient of 0.001 kcal/ mol and MMFF94 as the electrostatics. Conformational analysis of the constituents of *C. phlomidis* was performed using a repeated molecular dynamics-based simulated annealing approach as implemented in SYBYL-X 2.0. The molecule was heated up to 1000 K within 2000 fs, held at this temperature for 2000 fs and was annealed to 0 K for 10,000 fs using an exponential annealing function. By employing this procedure, 100 conformations were sampled out during 100 cycles to account the conformational flexibility to find the most likely conformations occurring most often in the resulting pool. All conformations were minimized with Tripos force field and atomic charges were calculated using MMFF94 method.

Molecular docking was used to get the information for further structural optimization by understanding the binding mode of the compounds. Surflex-Dock that adopted an empirical scoring function and a patented searching engine was employed for molecular docking. The crystal structures of VEGFR-1 in complex with *N*-(4-Chlorophenyl)-2-[(pyridin-4-ylmethyl)amino]benzamide [PDB ID: 3HNG] and Crystal structure of human VEGFR-2 kinase domain with a novel pyrrolopyrimidine inhibitor [PDB ID: 3VHE (1.55 Å X-Ray Diffraction Method)] were extracted from Brookhaven Protein Database. During the process of docking, water molecules were removed and ligand was extracted. The polar hydrogens as well as united atom AMBER7 FF02 were assigned for the protein. Then, "protomol" was generated by using ligand-based method, leaving the threshold and bloat parameters at their default values of 0.50 and 0 Å. Chemical constituents of *C. phlomidis* were docked within the prepared protein. The mode of interaction of the relative ligand in the crystal structure against PDB 3HNG & 3VHE were used as a standard docked model. The maximum number of poses per ligand was set to 17 with no constraints to perform the molecular docking. For comparative analysis of the designed molecules, D-score [13], PMF-score[14], G-score [15] and Chem-score[16] were estimated using the C-Score module of the Sybyl-X 2.1.1.

Table 1: Total scores value for the chemical constituents of methanol extract of *Clerodendrum phlomidis* leaf against VEGFR-1 and VEGFR-2 targets.

S.No.	Name of Compound	Structure	Total Score Value	
			VEGFR 1	VEGFR 2
1	Oleic Acid, Eicosyl ester		13.6396	15.4975
2.	DL- Alpha Tocopherol		12.2866	9.7767
3.	Isopropyl linoleate		11.22	12.1043
4.	Ethyl linoleate		10.3706	11.3532
5.	Hexa-decane		8.7271	8.8556
6.	Tetra-decanoic acid		8.0572	9.2849
7.	1,2-benzenedicarboxylic Acid,diethyl ester, Ethyl phthalate		6.7389	8.2701
8.	3,6,7-trihydroxy-2-(3-methoxyphenyl)-4H-chromen-4-one		6.6368	5.901
9.	3-Hexen-1-yl benzoate		6.4432	7.5154
10.	2-(2-nitro-1-p-tolyl-ethyl)cyclohexanone		5.9563	5.5144
11.	8-Nitro-11-dodecanolide		5.7024	0.2089
12.	1-(2,4,5-trihydroxyphenyl) -1-butaone		5.4875	6.9127

13.	Caryophyllene		5.3101	0.5116
14.	(11*E*)-trideca-1,11-diene-3,5,7,9-tetrayne		5.0826	4.1821
15.	2-methoxy-4-vinylphenol		4.6434	5.6991
16.	3-cyclohexen-1-ol		3.4009	3.9681
17	2,3-dihydroxypropanal		3.0623	3.475

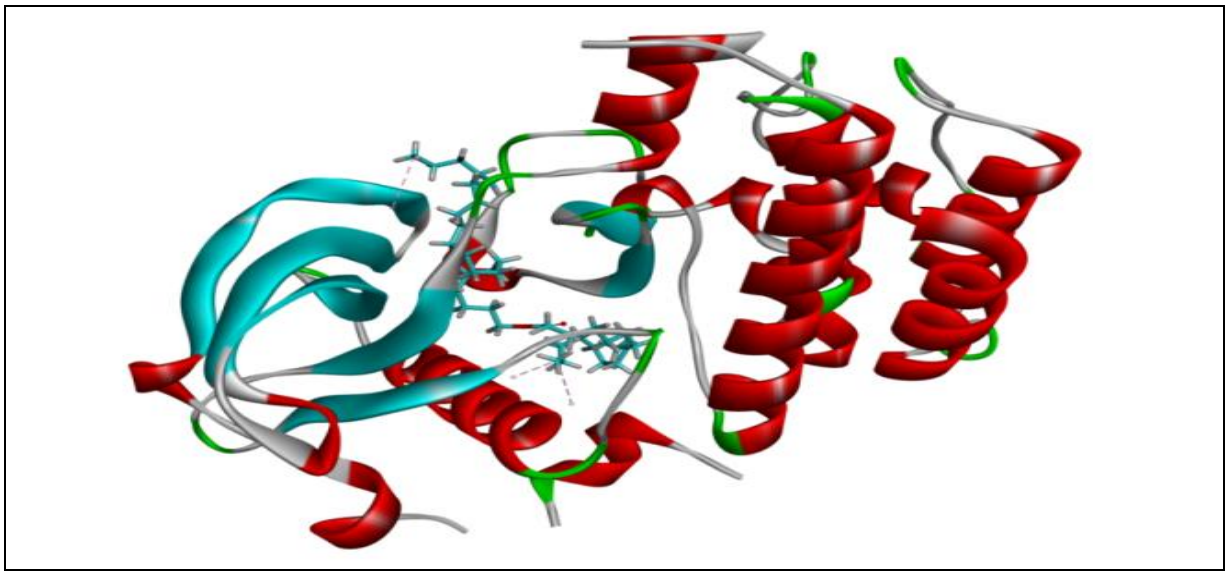


Figure 1: Interaction of Oleic acid eicosyl ester at the active site of VEGFR-1 target (PDB ID: 3HNG).

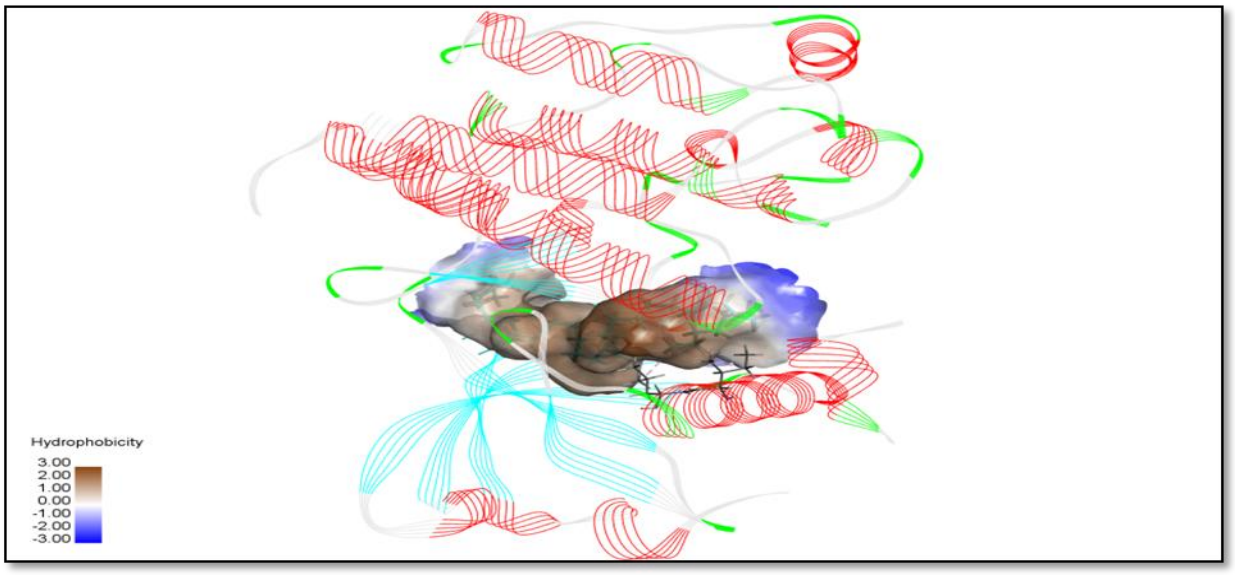


Figure 2: Hydrophobic Interaction of Oleic acid, eicosyl ester at the active site of VEGFR-1 (PDB ID: 3HNG)

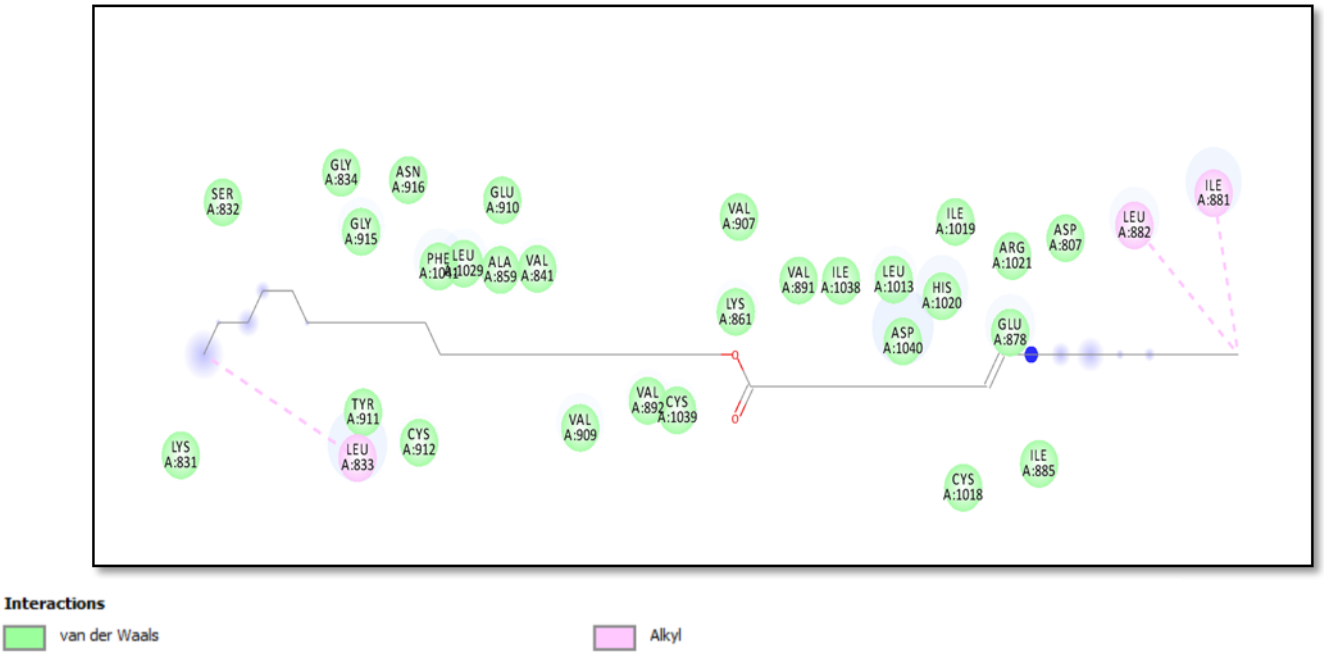


Figure 3: 2D structure of Oleic acid, eicosyl ester at the active site of VEGFR-1 (PDB ID: 3HNG).

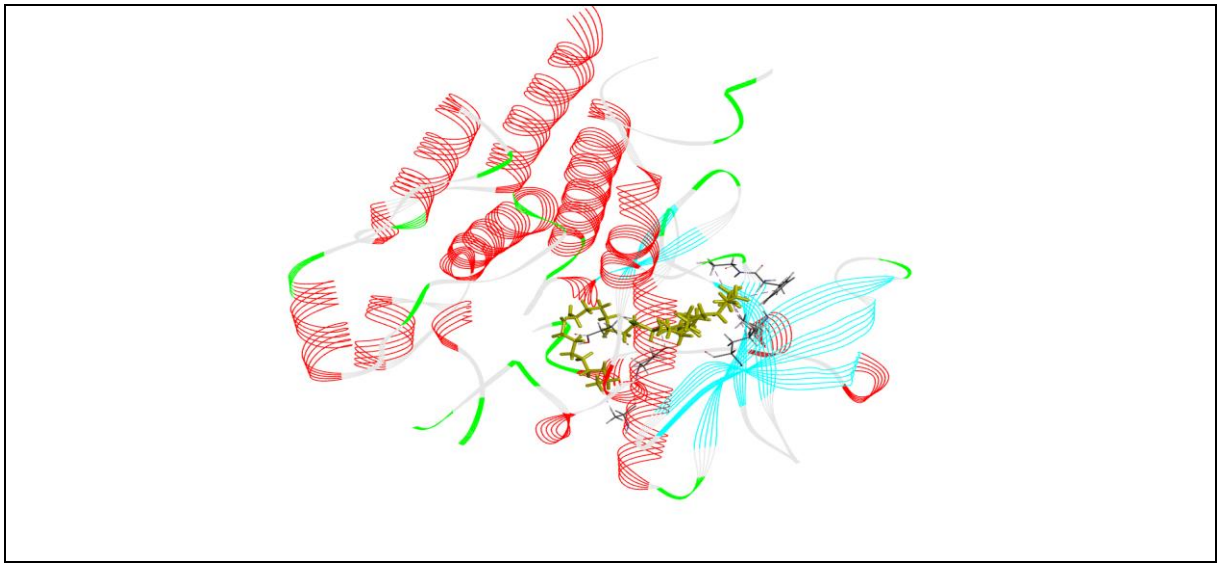


Figure 4: Interaction of Oleic acid eicosyl ester at the active site of VEGFR-2 (PDB ID 3VHE).

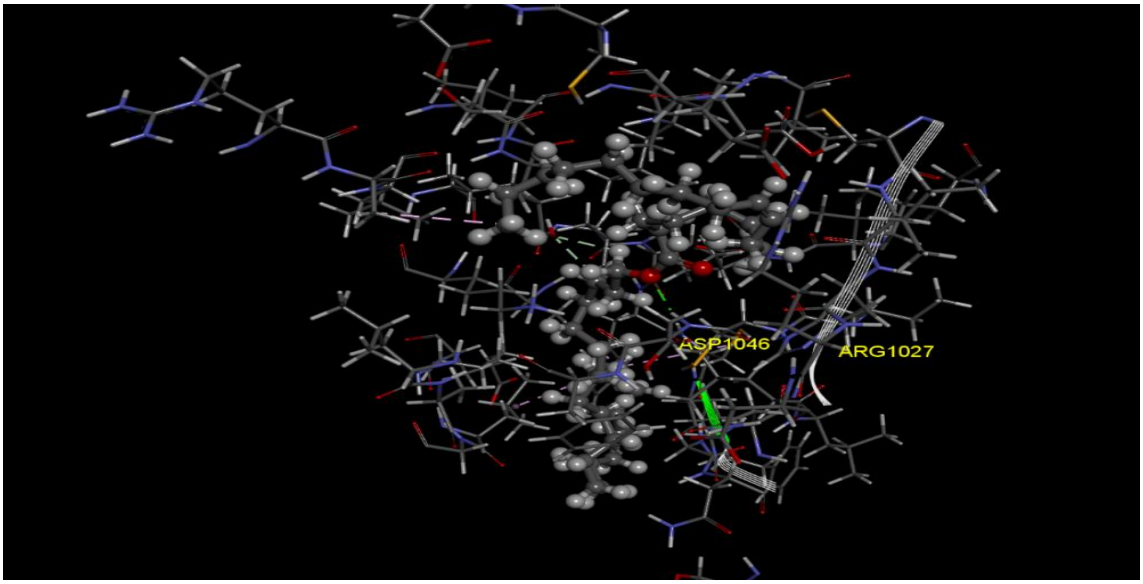
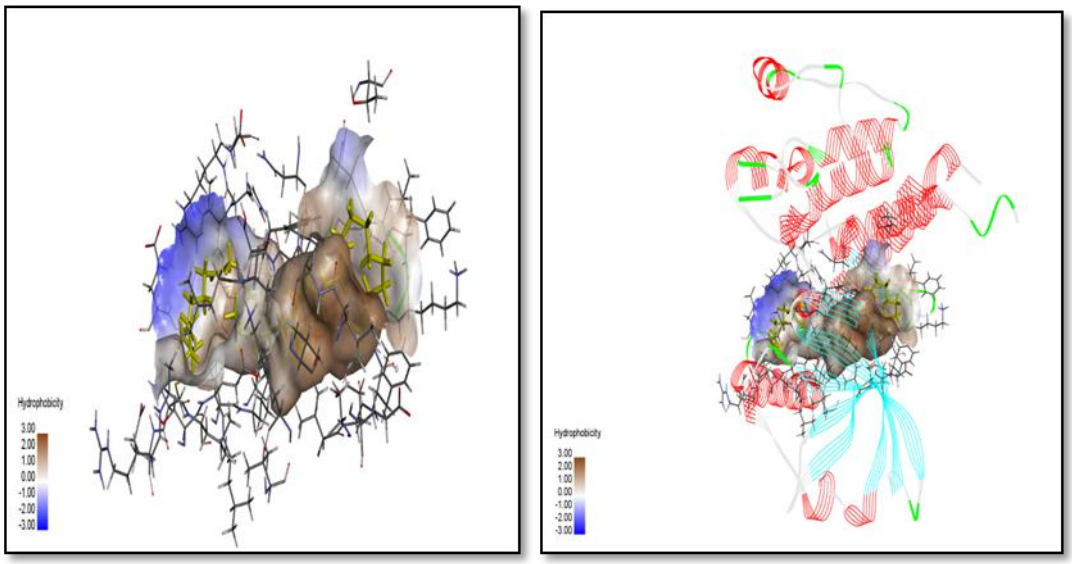


Figure 5: Hydrogen bond interaction of Oleic acid eicosyl ester at the active site of the VEGFR-2 target (PDB ID 3VHE).



(a) **(b)**

Figure 6: Hydrophobic interactions of Oleic acid eicosyl ester at the active site of VEGFR-2 (PDB ID:3VHE).

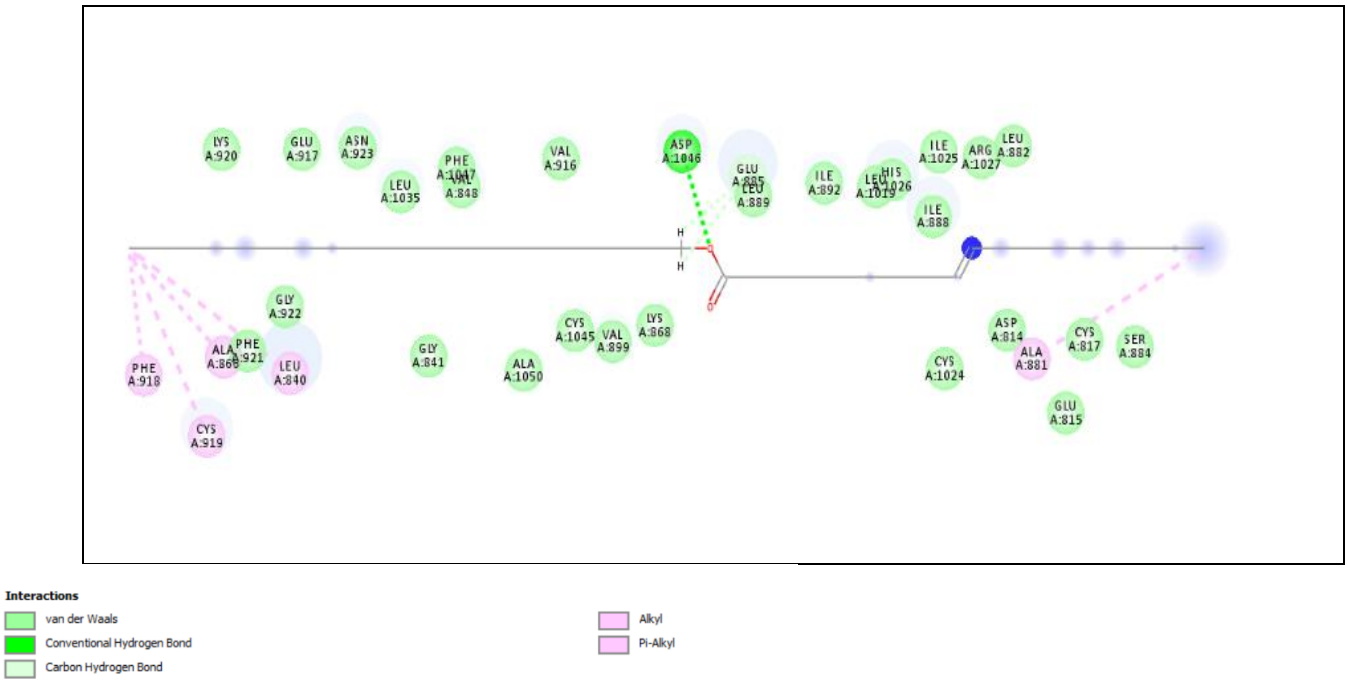


Figure 7: 2D structure of Oleic acid, eicosyl ester at the active site of VEGFR-2 (PDB ID 3VHE).

RESULTS AND DISCUSSION

As it was mentioned earlier Vascular Endothelial Growth Factor Receptor (VEGFR-1 and VEGFR-2) were found in high concentration in various cancer diseases and known as targets for anticancer agents. Molecular docking was conducted on both VEGFR 1 & VEGFR 2 along with their ligands, to elucidate their interactions and to obtain additional information in molecular binding mode with selected targets (3HNG and 3VHE). The results of molecular docking include appropriate interactions of the chemical constituents with the main amino acid residues at the active site of the enzymes.

The docking results of the ligands with the total score value are given in the Table 1 and the interaction images of Oleic acid eicosyl ester against the targets VEGFR-1 and VEGFR-2 are shown in the figure 1-7. Among the docked molecules, compounds oleic acid eicosyl ester, DL-alpa tocopherol, isopropyl linoleate and ethyl linoleate was found to bind effectively with the target VEGFR-1 with the total score value of 13.6396, 12.2866, 11.22 and 10.3706 respectively. As depicted in the figures 1-3, the terminal methyl group of the oleic acid eicosyl ester shows hydrophobic interactions with amino acid residues like LEU 833, LEU 882, ILE 881. Hydrogen bonding was also observed between the oxygen of hydroxy group of Oleic acid eicosyl ester with LYS 861, CYS 1039, VAL 892. Among the docked molecules, the compounds oleic acid eicosyl ester, isopropyl linoleate and ethyl linoleate was found to bind effectively with the target VEGFR-2 with the total score value of 15.4975, 12.1043 and 11.3532 respectively. As depicted in the figures 4-6, the chemical constituent oleic acid eicosyl ester showed hydrogen bonding interaction with GLU885, LEU889, ASP1046. It also showed hydrophobic interactions at the active site of the enzyme with amino acid residues PHE918, CYS919, ALA868, PHE921, ALA881. All the figures indicate that the Oleic acid eicosyl ester molecule is embedded in the hydrophobic region of subdomains of selected targets (Fig. 1-7). The thermodynamic studies also indicated that hydrogen bonding and vanderwaals forces contributed to the interaction of Oleic acid eicosyl ester with VEGFR-1 and VEGFR-2 respectively. Oleic acid eicosyl ester showed better binding energy among the various constituents of *C. phlomidis* such as the affinity of ligand towards protein (C-score), penetration capacity of ligand in to the target (Crash score), hydrogen bonding and free energies of interactions ie., potential of mean force (PMF) in selected targets. From the results of the molecular docking studies, it can be analyzed that the long chain hydrocarbon of oleic acid, eicosyl ester is responsible for the hydrophobic interaction with the amino acid of target protein VEGFR. Presence of esteric oxygen molecule is essential for the formation of conventional hydrogen bond which increases the binding affinity towards VEGFR-2.

CONCLUSION

With the help of the extensive literature studies, we have selected VEGFR-1 and VEGFR-2 as the lung cancer targets for finding out the binding effectiveness of the chemical constituents of *C. phlomidis*. By performing molecular docking study on various chemicals constituents of *C. phlomisdis*, we have understood that one chemical entity (i.e, oleic acid eicosyl ester) was found to have good binding affinity towards both the target VEGFR-1 and VEGFR-2. To conclude we can say that the chemical constituents oleic acid eicosyl ester and DL- Alpha Tocopherol were found to have good total score values against the target VEGFR-1, which indicates that the compounds with long hydrocarbon chain will have good interaction with the target VEGFR-1. Chemical constituents oleic acid eicosyl ester and isopropyl linoleate were found to have good total score values against VEGFR-2, which also indicates that the compounds with long hydrocarbon chain will have good interaction with the target VEGFR-2. In future, designing of the chemical constituents having the pharmacophore similar to oleic acid eicosyl ester, DL- Alpha Tocopherol and isopropyl linoleate or their derivatives with varying substituents in different position will be a good drug for the lung cancer targets VEGFR-1 and VEGFR-2. This study will be the basement support for the synthesis of more substituted compounds or molecule with similar structure towards the lung cancer therapy.

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Competing Interest:
The authors declare no conflict of interest.

List of Abbrevation

AMBER - Assisted Model Binding with Energy Refinement
D score - Docking score
G score - Glide score
GC-MC - Gas Chromatography Mass Spectrometry
NSCLC - Non Small Cell Lung Cancer
PDB - Protein Data Bank
PMF - Potential of Mean Force
VEGF - Vascular Endothelial Growth Factor Receptor

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