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RESEARCH ARTICLE

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INSILICO IDENTIFICATION OF POTENTIAL INHIBITORS FOR FARNESYL TRANSFERASE FROM *ALOE VERA* FOR CANCER

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ABSTRACT

Cancer is one of the major life threatening diseases worldwide. Many studies are in progress to inhibit the rate of distribution of the proteins involved in cancer. The *Aloe Vera* compounds were checked against the molecular targets of cancer by molecular docking studies. The compounds n-Hexadecanoic acid and tertadecanoic acid were found to interact more towards the target protein showing highest Dock score and more number of Hydrogen bonds. The study was further enlarged with cross reference confirmation with reference ligand (co-crystallized ligand) which has been bounded with selected molecular target protein. We concluded that *Aloe Vera,* with interesting biological properties and structural diversity, have often served as valuable lead drug candidate for the treatment of many diseases replacing the chemically synthesized drugs which causes side effects.

KEY WORDS

Cancer, *Aloe Vera*, docking, ligand

INTRODUCTION

Cancer is one of the most dreaded diseases of the 20th century and spreading further with continuance and increasing incidence in 21st century.[1]. Cancer is multifactorial, multifaceted and multi mechanistic disease requiring a multidimensional approach for its treatment, control and prevention. In recent years, great progress has been made in the treatment of cancer. Currently, great efforts have been put to the identification of novel anti cancer targets and so the life expectations of cancer patients have improved remarkably [2].

Carcinogenesis is a multi step process characterized by progressive changes in the amounts or activity of proteins that regulate cellular proliferation, differentiation and survival[3]. Approximately five decades of systemic drug discovery have established a respectable armamentarium of useful chemotherapeutic agents as well as a number of important successes in the treatment and management of human cancer. In fact, global efforts of sequencing human genome have provided us with an enormous number of potential targets associated with cancer therapy and now the focus is towards biological macromolecular targets [2].

Plant derived compounds are also of great significance to cancer therapy[4]. Medicinal plants are of great importance to the health of individuals and communities[5]. In many parts of the world, there is a rich tradition in the use of herbal medicine for the treatment of many infectious diseases. Throughout medical history, plant products have been shown to be valuable sources of novel anti-cancer drugs [6]. Photochemical are nonnutritive components of plants that are currently being studied in chemoprevention of various diseases for their pleiotropic effects and nontoxicity[7]. Perhaps, the drugs designed could inhibit the proliferation and differentiation of tumor cells and speed up their death.

In developing countries alone it is estimated that about 80% of the population rely on traditional medicines for their primary health care because of their wide biological activities, higher safety margins and cost effectiveness [8]. *Aloe Vera L* of Liliaceae is a cactus – like plant that grows readily in hot, dry climates and currently, because of demand, is cultivated in large quantities across the world. Numerous scientific studies on *Aloe Vera* demonstrate its analgesic, anti inflammatory, wound healing, immune modulating and antitumor activities as well as anti viral, antibacterial, and anti fungal properties [6].

Aloe Vera gel plays an important role in the treatment of tumors, ulcers and cancer [9]. The process of structure - based drug design started with the detailed analysis of binding site of the target protein, probably in its complex form with a ligand[5]. The enzyme protein farnesyl transferase, which catalyses the first step in the post-translational modification of *ras* protein along with number of other polypeptides, have emerged as an important targets for anticancer agents [10]. Protein farnesyltransferase (FTase) catalyzes the attachment of a farnesyl lipid group to the cysteine residue located in the C-terminal tetrapeptide of many essential signal transduction proteins, including members of the Ras superfamily. Farnesylation is essential both for normal functioning of these proteins, and for the transforming activity of oncogenic mutants. Consequently FTase is an important target for anti-cancer therapeutics. Several FTase inhibitors are currently undergoing clinical trials for cancer treatment[16]. An integral part of recent computational drug discovery research

focuses on the high- throughput screening of chemical databases to find inhibitors of specific protein targets [11].

MATERIALS AND METHODS

(i) Molecular Docking Study

Molecular docking continues to hold a great promise in the field of computer based drug design which screens small molecules by orienting and scoring them in binding site of a protein.

(ii) Ligand preparation

To draw the three dimensional structures of the identified active principles from *Aloe vera* the SMILES notation were downloaded from PubChemdatabase. The drawn compounds were saved in Mol format, were prepared by adding hydrogen using Accelrys discovery studio software through add polar hydrogen option in the menu bar. The compounds energy was minimized using CHARMm force field. The molecular parameters of the compounds were shown in (Table 1).

Table 1
Lipinski properties of the compounds

S.No	Compounds	Molecular weight	H-Bond Donor	H-Bond Acceptor	Logp
1	n-hexadecanoic acid	256.42408	1	2	6.4
2	Tetradecanoic acid	228.37092	1	2	5.0
3	Oleic acid	282.46136	1	2	6.5
4	1,2 Benzene di-carboxylicacid diisooctyl ester	390.55612	0	4	8.5

(iii) Protein preparation

The target protein Human Farnesyl transferase (PDB Code: 1JCQ) was retrieved from protein Data Bank (www.rcsb.org) and crystallographic water molecules were removed from the protein. The chemistry of the protein was corrected for missing hydrogen followed by correcting the disorders of crystallographic structure by filling the valence atoms using alternate conformations and valence monitor options. Following the above steps of preparations, the protein was subjected to energy minimization using the CHARMm force field.

(iv) Docking

The interaction study was carried out in Ligandfit of Accelrys Discovery Studio software. The binding sites of the protein were predicted using find cavities from the receptor site parameter of the tool. Here site 1 was chosen as the binding site and the site sphere size was set to (4538.649 A° ^3,Partition level 1). The determination of the ligand binding affinity was calculated using LigScore and PLP1, JAIN and Dock score were used to estimate the ligand-binding energies. Apart from these, other input parameters for docking were set as default options.

RESULTS AND DISCUSSION

Molecular Docking continues to hold great promise in the field of Computer based drug design, which screens small molecules by orienting and scoring them in the binding site of a protein. As a result novel ligands for receptors of known structure were designed and their interaction energies were calculated using the scoring functions. Dock score was used to estimate the ligand-binding energies. Apart from these, other input parameters for

docking are also considered for evaluating the compounds inhibition efficacy. It is estimated that docking programs currently dock 70 – 80% of ligands correctly.

1. Dock scores

The Protein-Ligand interaction plays a significant role in structural based drug designing. In the present work, Human Farnesyl transferase was docked with retrieved compounds of *Aloe Vera* plant. As a result, ten different conformations were generated for each compound but only top ranked docked complex score was copied from the table browser view of Discovery studio for binding affinity analysis. The different score values represented in Table 2 included Ligscore 1 and 2 (Protein Ligand Affinity Energy)[12], PLP 1, PLP 2 (Steric and H-bonding intermolecular function, (Higher PLP score indicate stronger receptor-ligand binding)[13], JAIN (Sum of five interaction terms namely Lipophilic interactions, Polar attractive interaction, Polar repulsive interactions, Solvation of the protein and ligand, An entropy term for the ligand) [14], PMF (developed based on statistical analysis of the 3D structures of protein-ligand complexes, scores are calculated by summing pair wise interaction terms over all interatomic pairs of the receptor-ligand complex, a higher score indicates a stronger receptor-ligand binding affinity) [15] and DockScore (Candidate ligand poses are evaluated and prioritized according to the DockScore functions).

Table 2
Summary of docking information of the top ranked poses in each compound

S.No	Compounds	Dock score	PLP1	PLP2	JAIN	Lig Score 1	Lig Score2	PMF
1	Tetra decanoic acid	46.01	42.87	52.04	-0.58	4.07	3.44	81.38
2	Oleic acid	22.686	21.28	21.24	-3.58	1.86	3.08	32.57
3	n-hexa decanoic acid	46.628	35.23	42.61	-0.85	2.93	2.64	58.69
4	1,2 benzene dicarboxylic acid Diisooctyl ester	54.346	64.04	69.77	-2.22	3.37	4.3	83.45
5	Farnesyl diphosphate (Reference Compound)	44.102	3.57	4.83	71.16	80.03	-0.18	86.49

In this study the compounds isolated from *Aloe Vera* were screened for its anti cancer activity against Farnesyl transferase. In order to find the potential inhibitor against the selected target, the dock scores of the co-crystallized ligand (reference compound) was compared with the dock score of the plant compounds, where the co-crystallized ligand was run within the range of 44.102 kcal/mol for 1JCQ, among the plant compounds only four compounds have shown satisfactory results with dock scores and hydrogen bonds that are very near to reference compound. Therefore, re-scoring of best docked poses based on their interaction energies with respective protein active site residues was done using different scoring function. Two important parameters have been considered for selecting potential compounds among the given input: (i) Hydrogen bond details of the

top-ranked pose and (ii) prediction of Binding energy between the docked ligands and the enzyme using various score calculated using Discovery studio (Ligscore1 and 2, -PLP1 and2, JAIN, PMF and dockscore) scores were taken for the analysis.

2. Hydrogen bond interaction

By enlarging this interaction analysis the hydrogen bond interaction is contributed as major parameter. The Hydrogen bonding interaction of the compounds (Fig 1, 2 and 3) was analyzed. Results were analysed using Hbond Monitor of Discovery studio.2.1 involved in hydrogen bond formation with aminoacids.

Hydrogen bond interaction between 1JCQ and n-hexadecanoic acid

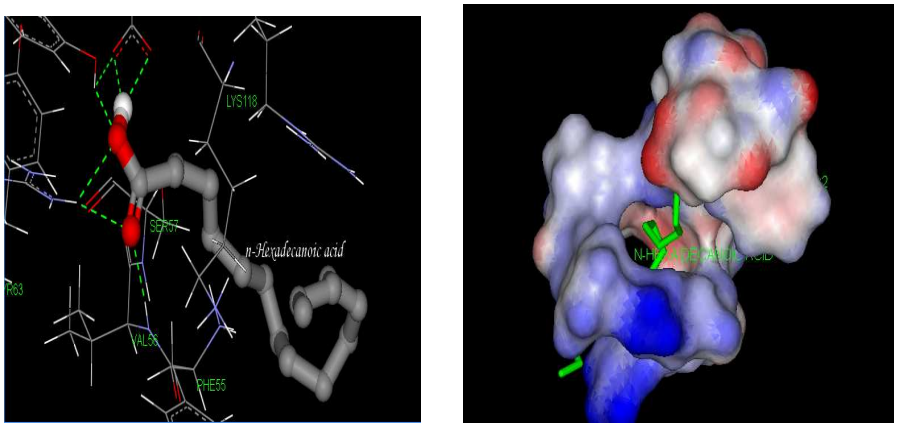

Figure 1

The green dotted lines represents the hydrogen bonds formations and green letters showing the amino acids involved in the bonding and n-hexadecanoic acid has been marked in green color.

In (Fig.1) the amino acid residues Val 56, Try 66, Arg69, Asp122 involved in hydrogen Bond interactions with enzyme Farnesyl transferase and the ligand n-hexadecanoic acid with a distance of 1.91265, 2.38895, 2.45658, and 2.41315 were analysed.

. **Hydrogen bond interaction between 1JCQ and tetradecanoic acid**

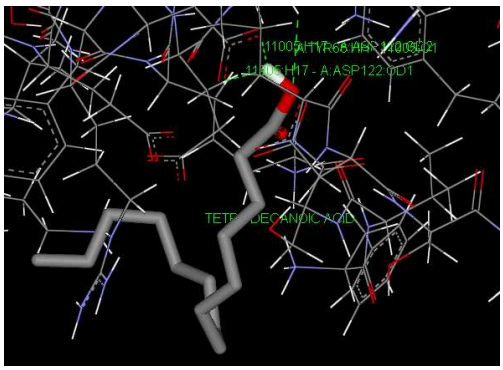

Figure 2

The green dotted lines represents the hydrogen bonds formations and green letters showing the amino acids involved in the bonding and tetradecanoic acid has been marked in green color.

Fig 2, shows the amino acid residues involved in hydrogen Bond interactions with enzyme Farnesyl transferase and the ligand tetradecanoic acid involved in hydrogen bond formation with aminoacids Val 56, Try 66, Asp122 with a distance of 1.98066, 1.73516 and 2.45429.

Hydrogen bond interaction between 1JCQ and 1,2Benzene dicarboxylicacid diisooctyl ester

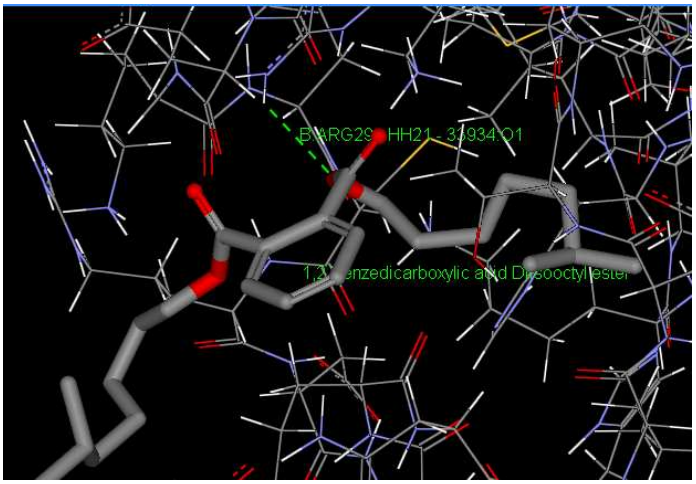

Figure 3

The green dotted lines represents the hydrogen bonds formations and green letters showing the amino acids involved in the bonding and 1,2Benzene dicarboxylicacid diisooctyl ester has been marked in green color.

The amino acid residues in (Fig 3) Phe 55:HT1, Phe55:HT3 involved in hydrogen Bond interactions of distance 1.8673 and 2.49863 with enzyme Farnesyl transferase and the ligand 1,2Benzene dicarboxylicacid diisooctyl ester.

The compounds that have highest dockscore with Farnesyl transferase were taken for H-Bond analysis. Table 3,4 and 5 shows, the hydrogen bonds formed and also the distance of H-Bonds formed between the ligand and receptor. The compounds n-Hexadecanoic acid had formed five Hydrogen bonds and tetradecanoic acid which had four and 1, 2 Benzene dicarboxylic acid diisooctyl ester had 2 bonds. Results were analyzed using Hbond Monitor of Discovery studio.2.1.

Hydrogen Bond interaction between the enzyme Farnesyl transferase and the ligand n-hexa decanoic acid are in Table 3. The first column in table 3 describes about the amino acids and atoms participated in the Hydrogen bonding A:Val 56 HN- n-hexa decanoic acid:O2, A:Try 66 HH- n-hexa decanoic acid :O1, A:Arg69 HH22- n-hexa decanoic acid:O1, n-hexa decanoic acid:H19-A:Asp122:OD1, n-hexa decanoic acid:H19-Asp122:OD2 and followed by the ligand molecule position in the bond sharing. Second column represents the distance between the atoms of amino acids and atom of ligand molecules which shares their position for hydrogen bonding.

Table 3
Hydrogen bond interaction of n-Hexadecanoic acid

Amino acids involved in H-Bonding with Ligand Atoms	Distance of H-Bonds
A:Val 56 HN- n-hexa decanoic acid:O2	1.91265
A:Try 66 HH- n-hexa decanoic acid :O1	2.38895
A:Arg69 HH22- n-hexa decanoic acid:O1	2.45658
n-hexa decanoic acid:H19-A:Asp122:OD1	2.41315
n-hexa decanoic acid:H19-Asp122:OD2	0.935038

Table 4 explains about the hydrogen bond interaction between the enzyme Farnesyl transferase and the ligand Diisooctyl ester. The table describes the amino acids Phe 55 and atoms HT1- Diisooctyl ester:O4, HT3- Diisooctyl ester:O4 participated in the Hydrogen bonding and followed by the ligand molecule position in bond sharing. The distance between the atoms of amino acids and atom of ligand molecules were 1.8673 and 2.49863, shares their position for hydrogen bonding.

Table 4
Hydrogen bond interaction of Diisooctyl ester

Amino acids involved in H-Bonding with Ligand Atoms	Distance of H-Bonds
A:Phe 55:HT1- Diisooctyl ester:O4	1.8673
A:Phe55:HT3- Diisooctyl ester:O4	2.49863

Hydrogen Bond interactions between the enzyme Farnesyl transferase and the ligand tetra decanoic acid (Results were analysed using Hbond Monitor of Discovery studio.2.1). The first column in table 5 describes the amino acids and atoms participated in the Hydrogen bonding, followed by the ligand molecule position, in bond sharing. Second column represents the distance between the atoms of amino acids and atom of ligand molecules which shares their position for hydrogen bonding.

Table 5

Hydrogen bond interaction of Tetradecanoic acid

Amino acids involved in H-Bonding with Ligand Atoms	Distance of H-Bonds
A: Val 56: HN- tetra decanoic acid :O2	1.98066
A:Try 66:HH- tetra decanoic acid : O1	1.73516
Tetra decanoic acid: H17-A: Asp122:OD1	2.45429
tetra decanoic acid:H17-A:Asp 122:OD2	1.08151

From the analysis, it is evident that extracted *Aloe Vera* compounds exhibit anti-

cancer activity. The best compounds are analyzed from docking and scoring runs that resulted in few hits. The major interacting compounds are n-Hexadecanoic acid and tetradecanoic acid from *Aloe Vera*. It represented the best compound which has shared more hydrogen bonds towards Farnesyl transferase protein, when compared to 1, 2 Benzene dicarboxylic acid diisooctyl ester, which showed two hydrogen bonds even though having higher dock score than above said compounds.

CONCLUSION

Screening methods are routinely and extensively used to reduce cost and time of drug discovery. It has been clearly demonstrated that the approach utilized in this study is successful in finding novel anti-cancer inhibitors from plants. The plant compounds that targeted the Farnesyl transferase protein were screened and ranked based on their dock score. The Lipinski prediction helped in the identification of more suitable ligand towards target protein. The dock score and other scores (JAIN, Ligscore1, 2, PLP1, 1 and PMF) were observed out of which 1, 2 Benzene dicarboxylic acid diisooctyl ester had highest dock score. In spite of having good binding score for 1, 2 Benzene dicarboxylic acid diisooctyl ester, it rendered unsatisfactory results in drug likeness (higher logp = 8.5,normal logp = >5)parameter. Lipinski's rule of 5 is used as a first step filter to perform virtual screening of compound libraries, in an effort to quickly eliminate lead candidates that have poor physicochemical properties for oral bioavailability. However, the compound tetradecanoic acid having the dock score (46.01) was not higher than the 1,2 Benzene dicarboxylic acid diisooctyl but it qualified all the important parameters for being a good inhibitor for Farnesyl transferase protein. A good drug score & drug likeness score are two properties that are important for becoming a successful drug. The compound n-hexadecanoic acid having good dock score which had similar action like tetradecanoic which had five hydrogen bonds towards the target protein but n-hexadecanoic also had higher logp value.

These few compounds predicted to inhibit the Farnesyl transferase protein are not effective in all forms. The 28 compounds (Table 6) were screened by molecular docking and drug likeness parameters were evaluated. This analysis involves a comparative raking of compounds based on their binding scores and diverse strategies. It is clear that tetradecanoic acid satisfied almost all parameters and thus can be treated as a good plant based inhibitor for treating cancer.

Table 6
Activity of Phyto components identified from the plant extract- Aloe vera

No	RT	Name of the compound	Molecular formula
1	3.06	p-Xylene	C_8H_{10}
2	3.78	1,5-Heptadien-4-one, 3,3,6-trimethyl-	$C_{10}H_{16}O$
3	5.44	Undecane	$C_{11}H_{24}$
4	7.03	1-Heptanol, 2-propyl-	$C_{10}H_{22}O$
5	8.25	Tridecane	$C_{13}H_{28}$
6	9.47	7-Tetradecene, (Z)-	$C_{14}H_{28}$
7	10.87	Tetradecane	$C_{14}H_{30}$
8	12.14	Hexadecane	$C_{16}H_{34}$

9	13.49	Eicosane	$C_{20}H_{42}$
10	13.64	12,15-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$
11	13.96	(4,7-Dinitronaphthalen-1-yl)-(4-methoxyphenyl)diazene	$C_{17}H_{12}N_4O_5$
12	14.44	Tetradecanoic acid	$C_{14}H_{28}O_2$
13	14.93	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$
14	15.14	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	$C_{28}H_{44}O_4$
15	15.87	1,2-Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$
16	16.58	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4Si_2$
17	16.73	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
18	17.28	1,2-Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$
19	17.42	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
20	19.38	11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$
21	19.47	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$
22	20.22	Oleic Acid	$C_{18}H_{34}O_2$
23	26.29	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$
24	27.15	Eicosane	$C_{20}H_{42}$
25	28.71	Heptacosane	$C_{27}H_{56}$
26	29.07	Octacosane	$C_{28}H_{58}$
27	31.20	Squalene	$C_{30}H_{50}$
28	35.43	Hentriacontane	$C_{31}H_{64}$

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