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RESEARCH ARTICLE

Identification and Analysis of Natural Compounds as Fungal Inhibitors from *Ocimum sanctum* using *in silico* Virtual Screening and Molecular Docking

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ABSTRACT:

In ancient days, fungal infections were curable; now due the environmental changes the available synthetic drug is not able to cure the diseases. The day to day practice of using traditional plants as a medicine has been increased to cure various diseases. One of the most important Indian traditional plants was *Ocimum sanctum* and in Tamil, it is called as Thulasi. The previous pharmacological studies of the *Ocimum sanctum* were reported to possess anti-fertility, anticancer, antidiabetic, antifungal and antimicrobial actions. The 40 phytochemical compounds were identified from the plant *Ocimum sanctum* through literature survey. Virtual screening was carried out for these compounds and the result predicted that only 8 compounds were screened to be active drug molecules. The fungal protein Lanosterol 14-alpha demethylase was responsible for most of the fungal disease caused to human. Further 8 compounds were analyzed for its antifungal activity against Lanosterol 14-alpha demethylase using docking studies to explore the binding interaction between the compounds of *Ocimum sanctum* and the protein. The docking result revealed that only one compound Bornyl acetate exhibited the best binding interaction of -13.9783 Kcal/mol with binding site of the fungal protein through hydrogen bonding and the 4 compounds exhibited the good binding interaction of greater that -7 kcal/mol. Further *in vitro* studies on Bornyl acetate compounds can lead to discovery of novel potential drugs against fungal diseases.

KEYWORDS: *Ocimum sanctum*, Phytochemical compounds, Lanosterol 14-alpha demethylase, Virtual Screening, Docking.

INTRODUCTION:

Ocimum is a genus of annual and perennial herbs and shrubs belonging to family Labiatae. One of the most important species is *Ocimum sanctum* (holy basil) and it is also called as Thulasi or Tulsi[1]. It is commonly found in two varieties – one having green leaves called as Lakshmi Tulsi and the other with purple leaves called as Krishna Tulsi. It is cultivated throughout the world for both religious and medicinal purposes[2]. The compounds that are present in this plant is responsible for curing diseases and relieving pain[3].

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These compounds are called as phytochemical compounds. Phytochemical compounds are the naturally occuring chemical compounds present in a plant. It possesses anti-fertility, anticancer, antidiabetic, antifungal, antimicrobial actions[4-6].

From the literature survey it was reported that from the past 15 years, there has been rapid increase in the fungal infections in the immunosuppressed patients such as in HIV/AIDS, cancer and in transplant patients. So it has become a major cause of mortality in immunosuppresed patients throughout the world[7, 8]. The situation has exacerbated because of lack of number of effective antifungal drugs, problems of drug safety, side effects, resistance and effectiveness of drug. Therefore, there is an urgent need to improve currently used drugs and to design new antifungal drugs with no side effects[9, 10].

The protein that was found to cause most of the fungal

infections in human was Lanosterol 14-alpha demethylase[11, 12] .The biological role of this protein is the formation of cholesterol in humans, ergosterol in fungi, and other types of sterols in plants[13]**.** Ergosterol is a sterol found in cell membranes of fungi. Because many fungi cannot survive without ergosterol, the enzyme (Lanosterol 14-alpha demethylase) which is responsible for its formation can be an important drug target for drug discovery[14]. Now a day, drug researchers have begun targeting the 14 α-demethylase enzyme in fungi to destroy the fungal cell's ability to produce ergosterol causes a disruption of the plasma membrane, resulting in cellular leakage and finally the death of the pathogen.[15]

In virtual screening**,** large libraries of drug-like compounds that are commercially available are computationally screened against targets of known structure, and those that are predicted to bind well are experimentally tested[16]. Virtual Screening includes Lipinski's Rule of Five and Quantative Estimation of Drug Likeness (QED). In 2007, Lipinski proposed the "Rule of Five", the most famous drug-likeness filter, which provides four rules to determine whether a molecule could be orally absorbed or not[17]. In 2012, Hopkins proposed a concept of desirability for the drug (i.e) quantitative estimate of drug-likeness (QED) and results were generated by fitting the distributions of eight properties of the compound[18]. Docking technique is one of the most important and frequently used methods in structural-based drug designing, which predict the binding affinity of small molecules to their applicable target binding sites there by inhibiting the target functions[19].

MATERIALS AND METHODS:

Selection of small molecules:

Through literature survey, 55 phytochemical compounds were identified from the plant Ocimum sanctum (Thulasi) [20-22].For computational analysis, the compound should possess at least 2 dimensional structures. Using pubchem database 2 dimensional structures were identified and retrieved for 40 compounds and 2 dimensional structures for remaining compounds are not available till now.

Drug Likness prediction using Lipinski Rule of Five:

By using the Lipinski Rule of Five server, the oral drug likeness filter test was conducted for above 40 phytochemical compounds based on rule of Lipinski's. Christopher A Lipinski expressed four rules to determine whether a compound could be orally consummate or not: First rule – molecular weight ≤ 500, second rule - partition coefficient (logP) ≤ 5, third rule - number of hydrogen bond donors (HBD) ≤ 5, fourth rule - number of hydrogen bond acceptors (HBA) ≤ 10. He also stated that the compound should not violate more than 2 rules, if it violates then compounds fails in the absorption process during consumption.

Drug Likness prediction using Quantitative Estimation of Drug Likeness (QED):

The compound passes the Lipinski filter, were again analyzed for its biological properties using quantitative estimate of drug-likeness (QED) filter (http://crdd.osdd.net/oscadd/qed/). QED predict the 8 properties including molecular weight, LogP, HBA, HBD, polar surface area, number of rotatable bonds, number of aromatic rings and number of alerts for undesirable substructures. The compound which satisfies all the biological properties, the database predicts that the compound can be consumed as an oral druglike molecule and if it not satisfied, the compound cannot be resumed for further designing of drug molecules.

Selection of fungal Protein:

The protein Linasterol 14- alpha demethylase were found to be most important fungal protein. The three dimensional structure (3LD6) of the protein was retrieved using protein data bank (PDB) (http://www.rcsb.org/pdb/) which was determined by expermintal studies by X-Ray Diffraction[23].

Analyzing active sites of fungal protein:

The Binding sites of small molecules present in the protein were identified by PocketQuery (http://pocketquery.csb.pitt.edu/). PocketQuery server was developed by David Koes which explore not only hot spots and anchor amino acids in the target protein but also hot regions that interface during protein-protein interaction[24].

Prepreparation for docking:

The energy minimization of both small molecules and protein were carried using swiss-pdb viewer. The energy minimization was nothing removal of water molecule and addition on hydrogen bonds to their appropriate 3D structures. The binding interactions mainly occurred between the hydrogen bonds present in the structures.[25]

Molecular Docking Interactions:

After the preparation of the protein and ligand, molecular docking studies were performed to evaluate the interactions using ArgusLab 4.0.1. ArgusLab is a free molecular docking package that runs under windows. The protein was loaded and its 10 active sites (amino acid) were selected. Finally the small molecules were loaded as databases. Docking calculation was allowed to run using shape-based search algorithm and AScore scoring function. The scoring function is responsible for evaluating the energy between the ligand

and the protein target. The best docking model was selected according to the lowest AScore calculated by ArgusLab. The most suitable binding interaction was selected on the basis of hydrogen bond interactions between the small molecules and protein near the substrate binding site[26-28].

Visualization of docking interaction:

The best docking result were analysed using PyMOL which is an open source molecular visualization tool to view the hydrogen bond interactions between the protein and ligand. PyMOL is computer software and mainly used to visualize molecules. The bonding between the ligands and the protein can be clearly viewed and predict the distance of hydrogen formation. The predicted distance reveal that binding interaction was stable one and small molecule could inhibit the function of protein[29, 30].

RESULTS AND DISCUSSION:

Preparation of small molecules:

The 40 phytochemical compounds were identified from plant *Ocimum sanctum* through literature survey. Using Lipinski Rule of Five only 17 compounds passes the filter test (Table1) and these 17 compounds were further tested for its Drug likness using QED (Table 2).The 8 compounds Eugenol, Carvacrol, Bornyl acetate, Vanillic acid, Vanillin, Methyl eugenol, Apigenin and Linalool showed drug likeliness properties whereas the other compounds showed non drug like properties.

Table 1: Lipinski Rule of Five of 40 phytochemical compounds

Compound	Pubchem.ID	Mol Wt.	LogP	HBA	HBD
1. Eugenol	3314	164.201	2.480	2	1
2. Luteolin-7-o-glucoside	5280637	448.377	-0.123	11	7
3. Carvacrol	10364	150.218	2.786	1	1
4. Cirsimaritin	188323	314.289	3.071	6	2
5. Luteolin	5280445	286.236	2.028	6	4
6. Isothymusin	630253	330.289	2.592	7	3
7. Apigenin-7-o-glucuronide	5319484	446.361	-0.101	11	6
8. Orientin	5281675	448.377	-0.140	11	8
9. Vicenin	13644663	564.492	-1.324	14	10
10. Molludistin	44258315	416.378	1.372	9	5
11. Bornyl acetate	6448	196.286	2.663	2	0
12. Camphene	6616	136.234	3.077	0	0
13. Campesterol	173183	400.680	6.633	1	1
14. Cholesterol	5997	386.654	6.398	1	1
15. Stigmasterol	5280794	412.691	6.862	1	1
16. Methyl chavicol/estragole	8815	148.202	2.965	1	0
17. Camphor	2537	152.233	2.855	1	0
18. Tannins	76419085	1701.198	-0.794	46	25
19. Triterpene	122724	450.610	5.387	4	2
20. Oleanolic acid	10494	456.700	5.850	3	2
21. Gallic acid	370	170.120	-0.202	5	4
22. Protocatechu-ic acid	72	154.120	0.260	4	3
23. Vanillic acid	8468	168.147	0.734	4	2
24. Vanillin	1183	152.147	1.492	3	1
25. 4-hydroxybenzaldehyde	126	122.121	1.522	2	1
26. Chlorogenic acid	1794427	354.309	-0.613	9	6
27. Methyl eugenol	7127	178.228	2.995	2	0
28. Apigenin	5280443	270.237	2.518	5	3
29. Ursolic acid	64945	456.700	5.456	3	2
30. Neral	643779	152.233	2.655	1	0
31. Stearic	5281	284.477	6.127	2	1
32. Palmitic	985	256.424	5.249	2	1
33. Oleic	445639	282.461	5.948	2	1
34. Linoleic	5280450	280.445	5.769	2	1
35. Linolenic acids	5280934	278.430	5.589	2	1
36. Linalool	6549	154.249	2.347	1	1
37. Methyl cinnamate	637520	162.185	2.200	2	0
38. Cirsilineol	162464	344.315	3.125	7	2
39. Beta-sitosterol	222284	414.707	7.042	1	1
40. Caffeic acid	689043	180.157	0.753	4	3

Note: Molecular Weight (Mol Wt), Hydrogen Bond Acceptor (HBA), Hydrogen Bond Donar (HBD)

Table 2: The Compounds predicted the druglikness properties

Compound	Pubchem.ID	PSA	ROTB	AROM	QED
1. Eugenol	3314	29.460	3	1	Drug like
2. Carvacrol	10364	20.230	1	1	Drug like
3. Bornyl acetate	6448	26.300	2	0	Drug like
4. Camphene	6616	0.000	0	0	Non drug like
5. Methyl chavicol	8815	9.230	3	1	Non drug like
6. Camphor	2537	17.070	0	0	Non drug like
7. Gallic acid	370	97.990	1	1	Non drug like
8. Protocatechuic acid	72	77.760	1	1	Non drug like
9. Vanillic acid	8468	66.760	2	1	Drug like
10. Vanillin	1183	46.530	2	1	Drug like
11. 4 – hydroxyl benzaldehyde	126	37.300	1	1	Non drug like
12. Methyl eugenol	7127	18.460	4	1	Drug like
13. Apigenin	5280443	90.900	1	3	Drug like
14. Neral	643779	17.070	4	0	Non drug like
15. Linalool	6549	20.230	4	0	Drug like
16. Methyl cinnamate	637520	26.300	3	1	Non drug like
17. Caffeic acid	689043	77.760	2	1	Non drug like

Note: Polar Surface Area (PSA), No. of Rotatable Bonds (ROTB), No. of Aromatic Rings (AROM), Quantitative Estimation of Drug Likeness (QED)

Preparation of protein:

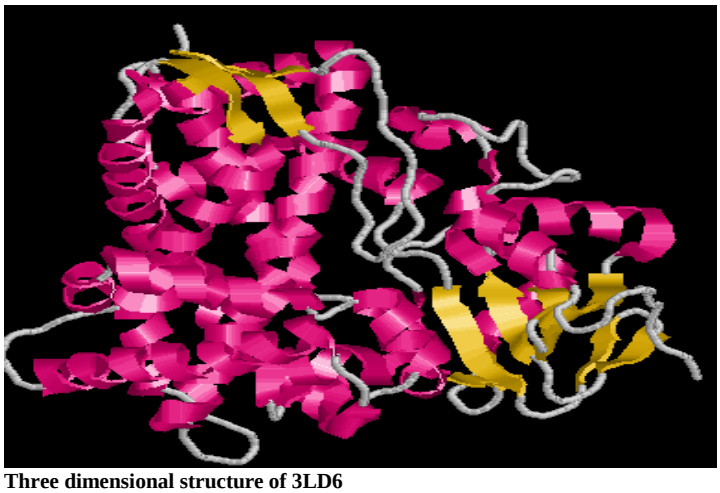
The structure and the sequence of protein were retrieved using pubchem Table3. The 10 active sites of the protein Lanosterol 14 – alpha demethylase (3DL6) were predicted using PocketQuery. The name, position and secondary structure of the amino acid present in the protein are given in Table 4

Docking Interaction:

Molecular docking was carried out for 8 compounds and the protein Lanosterol 14 – alpha demethylase using Arguslab.

Out of 8 compounds, one compound possesses least binding interaction with good hydrogen bond conformation. The four compounds linalool, methyl eugenol, eugenol, vanillic acid docked with the protein Linasterol 14- alpha demethylase exhibited the good binding interaction Table 5. Further the three compounds Carvacrol, Vanillin, Apigenin did not execute any binding interaction with the protein.

Table 3: The Three dimensional structure and the sequence of protein



Three dimensional structure of 3LD6

>3LD6:A|PDBID|CHAIN|SEQUENCE
MAKKTLPAGVKSPPYIFSPIPFLGH
AIAFGKSPIEFLENAYEKYGPVFSF
TMVGKTFTYLLGSDAAALLFNSKN
EDLNAEDVYSRLTTPVFGKGVAYD
VPNPVFLEQKKMLKSGLNIAHFKQ
HVSIIEKETKEYFESWGESGEKNVF
EALSELIILTASHCLHGKEIRSQLNEK
VAQLYADLDGGFSHAAWLLPGWLP
LPSFRRRDRAHREIKDIFYKAIQKRR
QSQEKIDDILQTLLDATYKDGRPLTD
DEVAGMLIGLLLAGQHTSSTTSAWM
GFFLARDKTLQKKCYLEQKTVCGEN
LPPLTYDQLKDLNLLDRCIKETLRLRP
PIMIMMRMARTPQTVAGYTIPPGHQ
VCVSPTVNQRLKDSWVERLDFNPDR
YLQDNPASGEKFAYVPFGAGRHRCIG
ENFAYVQIKTIWSTMLRLYEFDLIDGY
FPTVNYTTMIHTPENPVIRYKRRSTHH
HHHH

Sequence of the 3LD6

Note- Pink colour – helix, Yellow colour – Sheets, White colour – loops

On further analyzing the best interaction with PyMol. Bornyl acetate posses the least binding interactions with the fungal protein -13.9783 kcal/mol by forming 4 hydrogen bonds conformation. The atom OH present in the amino acid Isoleucine 79 formed hydrogen bond with O_2D atom of bornyl acetate by the bond length of 2.74 Å. Followed by the amino acid lysine 827 formed two hydrogen bonds. The atoms NH_1, NH_2 of the protein bounded to the atoms O_1A, O_2A present in the compound by forming the bond length of 3.00 Å and 2.62 Å. The amino acid Asparagine 87 formed hydrogen bond between NZ atom and O_{10} atom of the ligand with a bond length of 3.31 Å[31]. All the four binding interaction were observed in the helical structure of the

protein which confirm the strong inhibitory activity and the stability of the compound[32].

Table 4: Active Sites of Protein Lanosterol 14 – alpha demethylase

Sr. No.	Active Sites	Position	Secondary structure
1.	GLU	492	Loop
2.	ILE	75	Helix
3.	PRO	67	Loop
4.	ALA	76	Helix
5.	ILE	68	Loop
6.	LYS	79	Helix
7.	HIS	73	Helix
8.	GLU	83	Helix
9.	ASN	87	Helix
10.	LYS	91	Helix

Table 5: Molecular Docking between Phytochemical Compounds and the Protein

S. No.	Molecular Docking	Binding Interaction between Protein and the Phytochemaical Compounds
1.	3LD6 -Bornyl Acetate	-13.9783 kcal/mol
2.	3LD6 – Linalool	-8.55655 kcal/mol
3.	3LD6 - Methyl Eugenol	-10.9225 kcal/mol
4.	3LD6 – Eugenol	-7.9641 kcal/mol
5.	3LD6 – Vanillic Acid	-7.30071 kcal/mol
6.	3LD6 – Carvacrol,	No binding pose
7.	3LD6 – Vanillin,	No binding pose
8.	3LD6 – Apigenin	No binding pose

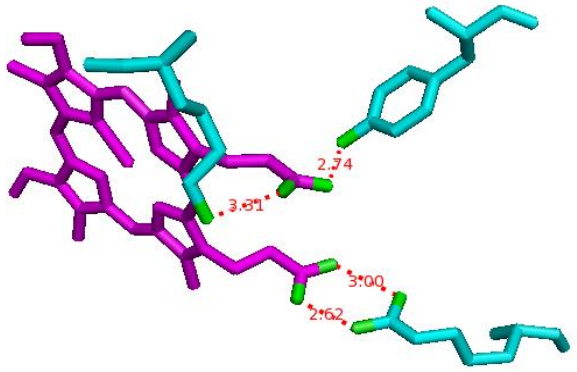


Figure1 : Best docking interactions and its hydrogen distance between Bornyl acetate and Linasterol 14- alpha demethylase

CONCLUSION:

Docking studies play an vital role in the designing and development of rational drugs In this work, the secondary metabolites of *Ocimum sanctum* are the potential leads to progress as novel antifungal drugs From the above virtual screening and docking results, it was revealed that out of 40 phytochemicals present in the plant *Ocimum sanctum* only one compounds bornyl acetate exhibited best fungal inhibitory activity. The current work strongly recommends the compound Bornyl acetate from the *Ocimum sanctum* for further *in vitro* and *in vivo* studies to explore the functions and molecular mechanisms of the compound toward the fungal proteins which lead to the discovery and development of potential drugs for fungal diseases.

CONFLICT OF INTEREST:

The authors declare they have no competing interests.

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REFERENCES:

1. Bhateja S, Arora G. Therapeutic Benefits of Holi Basil (Tulsi) in General and Oral Medicine: A Review. International Journal of Research in Ayurveda and Pharmacy. 2012; 3(6): 761-764.
2. Priyabrata P, Pritishova B, Debajyoti D, and Sangram KP. *Ocimum sanctum* Linn. A Reservoir Plant for Therapeutic Applications: An Overview. Pharmacognosy Reviews. 2010; 4(7): 95–105.
3. Subramani P, Subramani B, Parayil VC, Rajendran RP, Wong YY, Jeyabalan S and Chockalingam V. Evaluation of Antidiabetic and Antihyperlipidemic Effects of Hydroalcoholic Extract of Leaves of *Ocimum tenuiflorum* (Lamiaceae) and Prediction of Biological Activity of its Phytoconstituents. Pharmacognosy Research. 2015; 7(2): 156–165.
4. Siva M., Shanmugam KR, Shanmugam B, Venkata SG, Ravi S, Sathyavelu RK, Mallikarjuna K. *Ocimum sanctum*: A Review on the Pharmacological Properties. International Journal of Basic and Clinical Pharmacology. 2016; 5(3): 558-565.
5. Babita Labh Kayastha. Queen of Herbs Tulsi (*Ocimum sanctum*) Removes Impurities from Water and Plays Disinfectant Role. Journal of Medicinal Plants Studies. 2014; 2(2): 1-8.
6. Londhe AM, Kulkarni AS, Lawand RV. In-Vitro Comparative Study of Antibacterial and Antifungal Activities: A Case Study of *Ocimum kilimandscharicum, Ocimum tenuiflorum* and *Ocimum gratissimum*. International Journal of Pharmacognosy and Phytochemical Research. 2015; 7(1): 104-110.
7. Premjanu N, Jaynthy C, Diviya S. Antifungal Activity of Endophytic Fungi Isolated from *Lannea Coromandelica*–an Insilico Approach. International Journal of Pharmacy and Pharmaceutical Sciences. 2016; 8(5): 207-210.
8. Chian-YL and Coleman R.. Emerging Fungal Infections in Immunocompromised Patients. F1000 Medicine Reports. 2011; 3(14): 1-8.
9. Akansha J, Shubham J, and Swati R. Emerging Fungal Infections among Children: A Review on its Clinical Manifestations, Diagnosis, and Prevention. Journal of Pharmacy and Bioallied Sciences. 2010; 2(4): 314–320.
10. Xiangpeng Z, Guozhen Z. Imaging Pulmonary Infectious Diseases in Immunocompromised Patients. Radiology of Infectious Diseases. 2014; 1(1): 37-41.
11. Joel DAT, Manya S, Alia AS, Mikhail VK, Jacopo N, Rajni KW, Matthew AW, Klaus T, Brian CM. Structural and Functional Elucidation of Yeast Lanosterol 14α-Demethylase in Complex with Agrochemical Antifungals. PLOS ONE. 2016; 11(12): 1-18.
12. Galina IL and Michael RW. Sterol 14α-Demethylase Cytochrome P450 (CYP51), A P450 in all Biological Kingdoms. Biochimica et Biophysica Acta. 2007; 1770(3): 467–477.
13. Pravesh V, Navneet KY, Kunwar S, Srivastava AK. In-Silico Characterization of 14-alpha Sterol Demethylase of Aspergillus Fumigatus. Journal of Recent Advances in Applied Sciences. 2015; 29(1): 1-8.
14. Mahmoud AG and Louis BR. Antifungal Agents: Mode of Action, Mechanisms of Resistance, and Correlation of these Mechanisms with Bacterial Resistance. Clinical Microbiology Reviews. 1999; 12(4): 501–517.
15. Russell EL, Pierluigi V and Dimitrios PK. The Potential Impact of Antifungal Drug Resistance Mechanisms on the Host Immune

Response to Candida. Virulence. 2012; 3(4): 368–376.
16. Brian KS. Virtual Screening of Chemical Libraries. Nature. 2004; 432(7019): 862–865.
17. Sheng T, Junmei W , Youyong L, Dan L, Lei X, Tingjun H. The Application of in Silico Drug-Likeness Predictions in Pharmaceutical Research. Advanced Drug Delivery Reviews. 2015; 86: 2-10.
18. Prerana BJ, Akshay RY And Megha GG. Concept of Drug Likeness in Pharmaceutical Research. International Journal of Pharma and Bio Sciences. 2015; 6(4): 142 – 154.
19. Douglas BK, Hélène D, John RF and Jürgen B. Docking and Scoring in Virtual Screening for Drug Discovery: Methods and Applications. Nature Reviews Drug Discovery. 2004; 3: 935-949.
20. Abhay KP, Pooja S, Nijendra NT. Chemistry and Bioactivities of Essential Oils of Some Ocimum Species: An Overview. Asian Pacific Journal of Tropical Biomedicine. 2014; 4(9): 682–694.
21. Hanaa AY, Edwin CP, Nitin M, and Margaret AD . Antimicrobial Activity of Tulsi (*Ocimum Tenuiflorum*) Essential Oil and their Major Constituents against Three Species of Bacteria. Frontiers in Microbiology. 2016; 7: 681.
22. Ali KB, Ehsan HS. In silico Molecular Docking of Lavandula Angustifolia Mill's Compounds along with a Number of Antianxiety Drugs with GABAA Receptor for Reduce Stress. Oriental Journal of Chemistry. 2014; 30(2): 889-894.
23. Rani SK, Ashwini HP, Dr.Anwar RS. Homology Modelling and Docking Studies for Lanosterol 14-Alpha Demethylase of *Candida Albicans* and 1,2,4-Triazole Clubbed 1,3,4-Oxadiazole Derivatives. International Journal of Clinical and Biological Sciences. 2015; 2(6): 24 -30.
24. David RK and Carlos JC. PocketQuery: Protein–Protein Interaction Inhibitor Starting Points from Protein–Protein Interaction Structure. Nucleic Acids Research. 2012 ; 40: 387-392.
25. Vinita H, Prasada BG, and Paramageetham C. Sequence Analysis and Homology Modeling of Peroxidase from Medicago Sativa. Bioinformation. 2012; 8(20): 974–979.
26. Ambreen H, Zafar SS, Afshan N, Farzana Y and Naheed A . Molecular Docking Study on the Interaction of Riboflavin (Vitamin B_2) and Cyanocobalamin (Vitamin B_{12}) Coenzymes. Journal of Computational Medicine. 2013; 2013: 1-5.
27. Pasupuleti SK, Yellapu NK, Uppu VP, Sthanikam Y, Vimjam S, Gopal S, Katari V, Lokanathan S, Valasani KR and Potukuchi VGKS. *In silico* Designing and Molecular Docking of a Potent Analog against *Staphylococcus aureus*porphobilinogen Synthase. Journal of Pharmacy and Bioallied Sciences. 2014; 6(3): 158–166.
28. Krishnamoorthy M and Reena B. Docking Studies for Screening Anticancer Compounds of Azadirachta Indica using Saccharomyces Cerevisiae as Model System. Journal of Natural Science, Biology and Medicine. 2014 ; 5(1): 108–111.
29. Lill MA, Danielson ML. Computer-Aided Drug Design Platform using PyMOL. Journal of Computer-Aided Molecular Design. 2011; 25: 13–19.
30. Daniel S and Bert LDG. Ligand Docking and Binding Site Analysis with Pymol And Autodock/Vina. Journal Of Computer - Aided And Molecular Design. 2010; 24(5): 417–42.
31. Subramani Parasuraman, Ramasamy Raveendran, Balakrishnan Vijayakumar, Devadasan Velmurugan, Subramani Balamurugan. Molecular docking and ex vivo pharmacological evaluation of constituents of the leaves of *Cleistanthus collinus* (Roxb.) (Euphorbiaceae). Indian Journal of Pharmacology. 2012; 44(2): 197-203.
32. Anne Rietz, Dino P. Petrov, Matthew Bartolowits, Marsha DeSmet, V. Jo Davisson and Elliot J. Androphy. Molecular Probing of the HPV-16 E6 Protein Alpha Helix Binding Groove with Small Molecule Inhibitors. PLoS ONE. 2016; 11(2): e0149845