Screening of Phyto compounds in *Vitex negundo* and *Clerodendrum infortunatum* for anticancerous activity by *in silico* methods

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Abstract

Cancer remains a global and serious public health challenge. It is one of the biggest healthcare issues for the human race and demands a proactive strategy for cure. The existing medications towards cancer treatment have lots of side effects so the current researches are focused on developing plant based natural drugs. So the present study is focused on developing a lead compound by screening phytocompounds from plants which have high medicinal potential. For screening of phytocompounds two plants from Verbanaceae family, *Vitex negundo* and *Clerodendrum infortunatum* were chosen for the study. *In silico* molecular modeling studies were carried out for cancer drug design through a molecular approach. The computer aided drug discovery can reduce the time taken for drug development. For the present study molecular docking tools such as Autodock and MGL tools were used to find the best receptor ligand complex which has lower binding energy. As the result of screening of phyto compounds in *Vitex negundo* and *Clerodendrum infortunatum* a total of three compounds have been identified to have potential anti-cancerous activity that is better than the existing standard drug in terms of its affinity towards the drug target. So these Phytochemicals are most suitable candidates for anticancer drug development and it promises to act as a major milestone in cancer drug development after experimental validation.

Key words: Autodock, binding energy, MGL tools, receptor ligand complex.

1.INTRODUCTION

The basic function of a human body is based on the cells which are the basic unit of life. Any malfunctioning of cell system of the human body may lead to complete disorganization of the equilibrium of life. Usually cell divides and makes various tissues and tissues to organs. Cells die when they get too old or damaged and then new cells take their place in a normal human being. But in some conditions due to various reasons such as genetic changes, life style, Radiation exposure etc, may cause the rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other parts of the body and to other organs and this condition of a group of diseases are called cancer.

Considering the high profile nature of the disease, its treatment has been a constant struggle with relatively less success. Currently available options for cancer treatment involve surgical removal and radiation treatment of the large accumulated biomass of cancer, typically followed by systemic chemotherapy treatment for maintenance. The primarily available chemotherapeutic agents include antimetabolites (e.g., methotrexate), DNA-interactive agents (e.g., cisplatin, doxorubicin), anti-tubulin agents (taxanes), hormones, and molecular targeting agents (Nussbaumer et al., 2011). The major disadvantages of chemotherapy are recurrence of cancer, drug resistance, and toxic effects on non-targeted tissues that can restrain the use of anticancer drugs and thus impair patient's quality of life. To overcome the problems of present therapy, search for new promising anticancer agents with better efficacy and lesser side effects continues.

Phytochemicals and derivatives present in plants are promising options to improve treatment efficiency in cancer patients and decrease adverse reactions. A number of these phytochemicals are naturally occurring biologically active compounds with significant antitumor potential. The development of effective and side-effects free phytochemical based anticancer therapy begins with the testing of natural extracts (from dry/wet plant material) for potential anticancer biological activity followed by purification of active phytochemicals based on bioassay-guided fractionation and testing for *in vitro* and *in vivo* effects. In this study an attempt has been made to gather information specifically about the anti-cancer phytochemicals that are evaluated at preclinical and clinical levels as well as those available in the market, until now. In preclinical section, review of the phytochemicals have reported *in vivo* activity. This review proves that phytochemicals can be effective against cancer and further highlights phytochemicals which are assessed at preclinical level and also mentions some phytochemicals which are in the clinical trials along with the brief information on the presently used plant-based anticancer drugs.

Plants have been used to treat various disease aliments from time immemorial. Ayurveda, Traditional Indian Medicine (TIM), and the Traditional Chinese Medicine (TCM) remain the most ancient (4500 BC) yet living traditions. In the ancient period, the knowledge of selection of right plants, a specific time for their collection, method of drug preparation with their specific use was transferred verbally from one generation to the next generation. The folklore system has documented all parameters about the drugs and their specific uses in the disease conditions. These drugs were prepared as tinctures, teas, powders, poultices, decoctions, and other types of formulations (Ogbonna et al., 2012; Fridlender*et al*., 2015) which were the most common methods of drug preparation until 18th century. Unfortunately, none of them could fit into the modern scientific definition of a drug. With advances in organic chemistry and chemical analysis, an analytical investigation of active components of medicinal plants and herbal remedies was pursued in late 18th or early 19th centuries, which opened the doors toward the isolation/purification and characterization of numerous active principles of plants. This increased the pace of drug discovery and led to a miracle innovation in the medical field.

Clinical trials using phytochemicals against cancer are still in infancy through an overwhelming large number of anti-cancer compounds are currently under development. The clinical trials with phytochemicals focus on three major aspects of cancer research: 1) improving the response of cancer cells toward standard chemo- and radiotherapy, 2) reducing the severe adverse effects of standard cancer therapy, and 3) looking for unwanted interactions with standard therapy. Preclinical studies have shown the effectiveness of various phytochemicals such as berberine, curcumin, green tea, catechins including EGCG, lycopene, quercetin, resveratrol, and sulforaphane (AmitS. Choudhari, Pallavi C. Mandave, ManasiDeshpande, PrabhakarRanjekar, and Om Prakash et-al 2019).

1.1 MOLECULAR DOCKING

Molecular Docking is used to positioning the computer-generated 3D structure of small ligands into a receptor structure in a variety of orientations, conformations and positions. This method is useful in drug discovery and medicinal chemistry providing insights into molecular recognition. Docking has become an integral part of Computer-Aided Drug Design and Discovery (CADDD). Traditional docking methods suffer from limitations of semi-flexible or static treatment of targets and ligand. Over the last decade, advances in the field of computational, proteomics and genomics have also led to the development of different docking methods which incorporate protein-ligand flexibility and their different binding conformations. Receptor flexibility accounts for more accurate binding pose predictions and a more rational depiction of protein binding interactions with the ligand. Protein flexibility has been included by generating protein ensembles or by dynamic docking methods. Dynamic docking considers solvation, entropic effects and also fully explores the drug-receptor binding and recognition from both energetic and mechanistic point of view. Though in the fast-paced drug discovery program, dynamic docking is computationally expensive but is being progressively used for screening of large compound libraries to identify the potential drugs. So computational biology and molecular docking studies will help us to discover new drugs in a limited time period.(Ritujakhar, MehakDangi, AlkaKhichi and Anil Kumar Chhillar,et-al 2020).

So considering the current relevance of phytochemicals against cancer as a drug the relevance of molecular screening of phytochemicals in medicinal plants with drug targets have its own significance in the present scenario. So my study target upon finding active phytochemical in the selected plants of Verbanaceae family and analyzing its efficiency towards the drug target. The selected plants are *Clerodendrum infortunatum* and *Vitex negundo* which have been proved to have potential phytochemical activities against cancer by in-vitro cytotoxic test. This study has an immense scope for finding an active phytochemical which could be used as an effective drug in the future cancer treatment strategies.

2.MATERIALS AND METHODOLOGY

2.1. Retrieval of Target protein from PDB

The three dimensional structure of Janus- kinase Pdb id -2b7a was downloaded from Protein Databank in pdb format.

2.2. Retrieval of Phytocompounds from IMPPAT

All the phytocomponds present in *Vitex negundo* is retrieved by using IMPPAT database. From this database the PDB file of the ligands were downloaded.

2.3. Retrieval of Phytocompounds from PubChem

The details of the phytocompounds in *Clerodendrum infortunatum* was not available in IMPPAT database so the details of phytocompounds present in *Clerodendrum infortunatum* was mined from Literature and the compounds were from pubchem database in SDF format.

2.4. Conversion of SDF format to PDB format

Since docking requires compounds in PDB format SDF format was converted to PDB format by using online smiles translator.

2.5. Activity prediction using PASS

To identify the antineoplastic compounds the activity of all the compounds present in the plants were assessed using PASS server. So by using PASS the activity spectrum value is analysed for anticancerous property based on the Pa and Pi value. The compounds having higher spectrum of activity were chosen for docking.

2.6. Molecular docking using MGL Tools.

Molecular Docking was performes using MGL tools and Autodock by the following steps:

2.7. Preparation of Receptor

For the preparation of the receptor molecule, all the hetero atoms were deleted from the pdb format of the receptor molecule. And all the water molecules were removed and hydrogen is added to the receptor.Then the receptor molecule is saved in pdb format. Then by adding charges the pdbqt format of receptor is saved.

2.8. Preparation of Ligand.

Here the charges are added and then it is saved in pdbqt format.

2.9. Grid box generation

The grid box for receptor molecule was generated by analyzing the xyz coordinate value. In this study blind docking method was done in which the whole receptor was covered by the gridbox in which the whole molecule is screened to get the best docking site. After generating the grid box the grid parameter file is saved in the working directory.

2.10. Molecular docking by Autodock

Autogrid and Autodock commands were executed in the command prompt and the log files were generated for analysis. By performing molecular docking the best conformation of the receptor ligand complex molecule was obtained. Total 10 conformations were obtained and the one having the least binding energy and highest hydrogen bond was considered as the best ligand molecule. By performing molecular docking the informations such as pose, binding enery, ligand efficiency, IC mM , Electrostatic energy,Total internal energy, Tortional energy, Unbound energy, the number of hydrogen bonds and the hydrogen bond formed were shown.

2.11. Visualization using Pymol

By using pymol software the docked conformation was visualized. From pymol the image of the result is saved in PNG format. Two images were saved one showing the hydrogen bond and the one showing the cleft in which the molecule fit in the best position.

2.12. Comparison with a Standard Drug

Comparison with the standard drug is very important to know whether it acts better than the existing drug for the disease. For my study the existing standard drug Entrectinib is used for cancer treatment. So the binding energy and efficiency of the drug is compared with the existing drug.

3.RESULTS AND DISCUSSION

3.1 Antineoplastic phytocompounds in *Clerodendrum infortunatum.*

Based on the *In vitro* cytotoxicity study of *Clerodendrum infortunatum* and *Vitex negundo* in DAL cell lines and normal spleenocytes, *Clerodendrum infortunatum* is proved to destroy more cancerous cells without affecting the normal cells. The details of the phytochemicals present in the plant was collected from Literature and the activity prediction is done by analyzing the pa and pi values of the compound. The phytochemicals having highest pa value is choosen for doing molecular docking.

Table 1: List of Phytocompounds exhibiting anti-neoplastic activity in *Clerodendrum infortunatum*

S.No	Name of the compound	Pa	Pi
1	6-oxabicyclo[3.1.0]hexan-3-one	0.866	0.005
2	2,3,4,5,6,7,8-heptahydroxyoctanal	0.816	0.004
3	3,4,4a,5,6,7,8,8a-octahydrochromen-2-one	0.734	0.021
4	[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] (1E)-N-hydroxybut-3-enimidothioate	0.771	0.015
5	2-(3-acetyloxy-4,4,10,13,14-pentamethyl-2,3,5,6,7,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-17-yl)propanoic acid	0.758	0.017

3.2 Antineoplastic phytocompounds in *Vitex negundo*

The study of In vitro cytotoxicity study of *Clerodendrum infortunatum* and *Vitex negundo* in DAL cell lines and normal spleenocytes, *Vitex negundo* also shows potential anti-cancerous activity by killing more cells. So *Vitex negundo* is also considered for screening of phytochemicals. The phytoconstituents of this plant is collected from IMPPAT data base. The Phyto-constituents exhibiting anti-neoplastic activity was predicted using PASS server and the compounds with higher Pa values were chosen for molecular docking studies.

Table 2: List of Phytocompounds exhibiting anti-neoplastic activity in *Vitex negundo*

S. No	Name of the compound	Pa	Pi
1	3-epi-corosolic acid	0.874	0.005
2	Negundoside	0.737	0.020
3	Rapamycin	0.746	0.019
4	(+)-Sabinene	0.891	0.005
5	3-O-Acetyloleanolic acid	0.890	0.005
6	5-Demethylnobiletin	0.818	0.010
7	5,7-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-6,8-dimethoxy-4H-chromen-4-one	0.838	0.008
8	AC1NUKBO	0.849	0.007

9	Acerosin	0.838	0.008
10	Agnuside	0.757	0.017
11	Apigenin	0.774	0.015
12	Artemetin	0.831	0.008
13	Beta-Caryophyllene	0.915	0.005
14	Beta-Phellandrene	0.830	0.009
15	Betulic acid	0.925	0.005
16	Caryophyllone Oxide	0.950	0.004
17	Casticin	0.831	0.008
18	Celastrol	0.806	0.011
19	Chrysosplenol D	0.831	0.008
20	Corymbosin	0.836	0.008
21	Cynaroside	0.830	0.009
22	D-Glucose	0.833	0.008
23	Dipentene	0.812	0.010
24	Eupatin	0.827	0.009
25	Eupatorin	0.819	0.010
26	Gardenin	0.838	0.008
27	Geraniol	0.743	0.019
28	Myricetin	0.841	0.008
29	Nishindaside	0.830	0.009
30	Quercetin	0.797	0.012
31	SCHEMBL382085	0.830	0.009

3.3 Phyto- constituents selected for molecular docking

Fo r molecular docking study screening of phytochemicals based of pa and pi value is done. Table 3 below shows the phytocompounds selected for molecular docking based on highest Pa value and Conformation generated through docking.

Table 3: List of Compounds chosen for Docking

Compound no.	Phytochemical identifier	Plant	Phytochemical name
1	CID: 535532	*Clerodendrum infortunatum*	6-oxabicyclo[3.1.0]hexan-3-one
2	CID: 20487	*Clerodendrum infortunatum*	3,4,4a,5,6,7,8,8a-octahydrochromen-2-one
3	CID: 9601716	*Clerodendrum infortunatum*	[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] (1E)-N-hydroxybut-3-enimidothioate
4	CID: 631957	*Clerodendrum infortunatum*	2-(3-acetyloxy-4,4,10,13,14-pentamethyl-2,3,5,6,7,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-17-yl)propanoic acid
5	CID: 122383453	*Vitex negundo*	3-epi-corosolic acid
6	CID: 151202	*Vitex negundo*	3-O-Acetyloleanolic acid
7	CID: 5481244	*Vitex negundo*	AC1NUKBO
8	CID: 14350	*Vitex negundo*	Caryophyllone Oxide
9	CID: 261859	*Vitex negundo*	Gardenin
10	CID: 10887971	*Vitex negundo*	(+)-Sabinene

3.4 Drug Target

The macromolecule to which the drug binds and exhibits pharmaceutical response is called the drug target. The drug target chosen for this anti-neoplastic docking studies is Janus Kinase 2 (PDB ID:2b7a) whose 3D structure was downloaded from PDB. JAK2 is a member of Protein Tyrosine Kinase (PTKs) family which is an important signaling molecule in Cytokinesis. It is a well known drug target for anti-neoplastic activity, hence it was chosen as the drug target for the present study. The 3D structure of JAK2 is shown below.

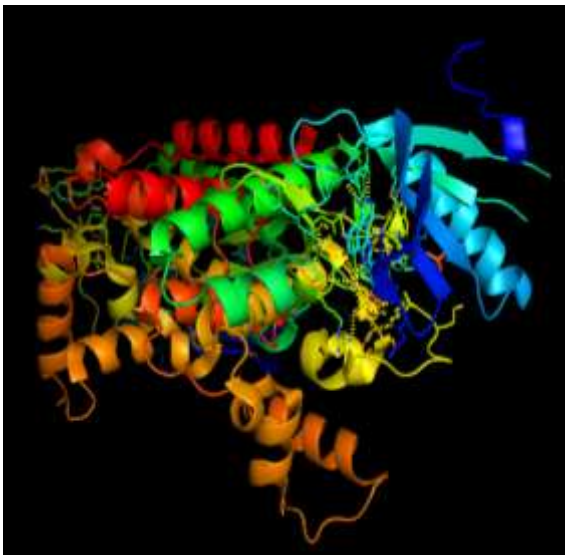

Fig.1 Structure of JAK2

3.5. Phytocompounds from *Clerodendrum infortunatum* docked with janus kinase 2 conformations

Compound 1: 6- Oxibicyclo(3.1.0) hexam-3-one

Docking of 6- Oxibicyclo(3.1.0) hexam-3-one with Janus Kinase 2

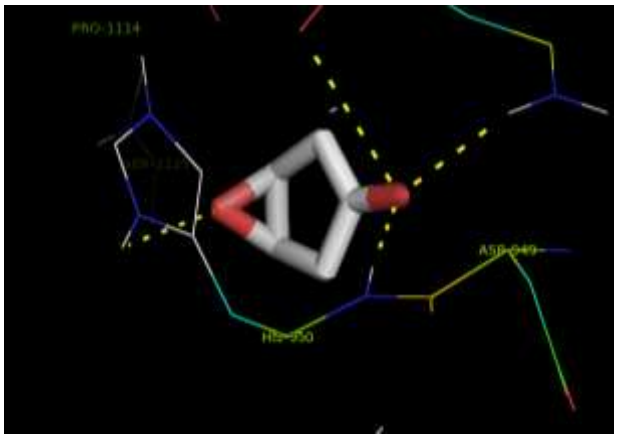

Fig 2: Docked conformation showing interaction between compound 1 and Janus kinase2

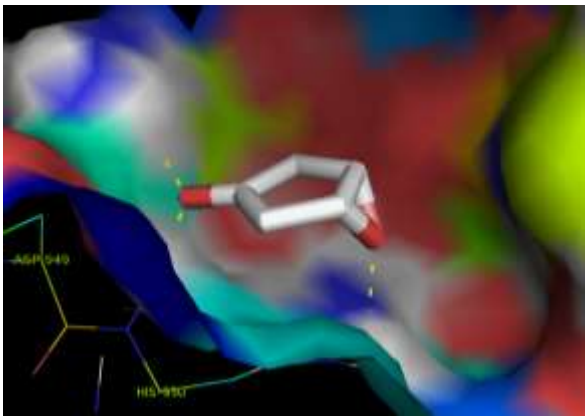

Fig 3: Docked conformation in solid surface showing interaction between Compound 1 and Janus kinase2.

COMPOUND 2: 3,4,4a,5,6,7,8,8a-octahydrochromen-2-one
Docking of 3,4,4a,5,6,7,8,8a-octahydrochromen-2-one with janus kinase 2.

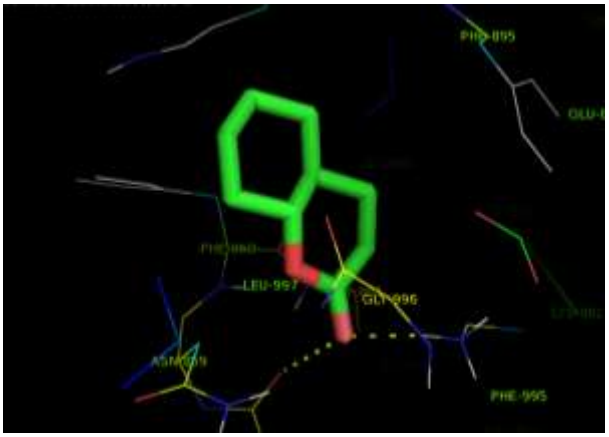

Fig 4: Docked conformation showing interaction between compound 2 and Janus kinase2

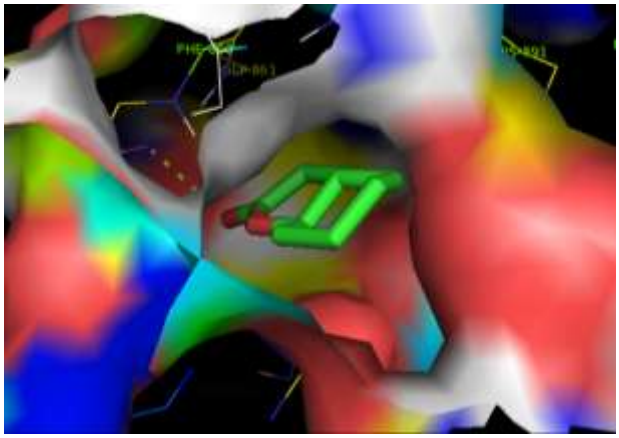

Fig 5: Docked conformation in solid surface showing interaction between compound 2 and Janus kinase2

COMPOUND 3: [3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] (1E)-N-hydroxybut-3-enimidothioate

Docked conformation of[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] (1E)-N-hydroxybut-3-enimidothioate with janus kinase 2.

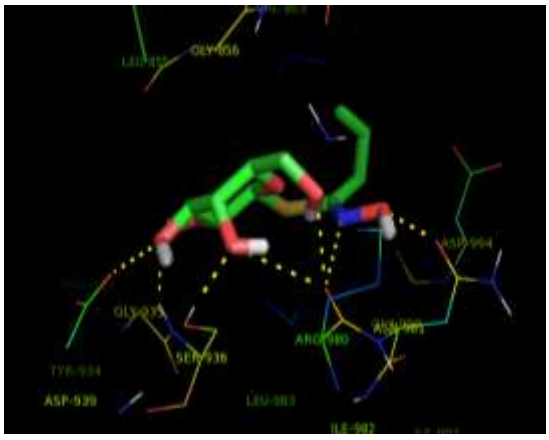

Fig 6: Docked conformation showing interaction between compound 3 and Janus kinase2

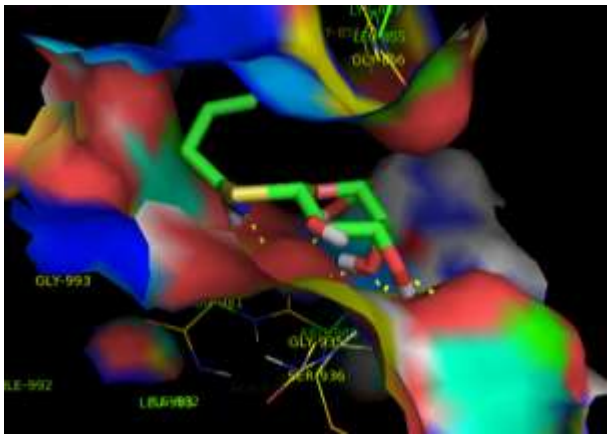

Fig 7: Docked conformation in solid surface showing interaction between compound 3 and Janus kinase2

COMPOUND 4 2-(3-acetyloxy-4,4,10,13,1 4-pentamethyl-2,3,5,6,7,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-17-yl)propanoic acid

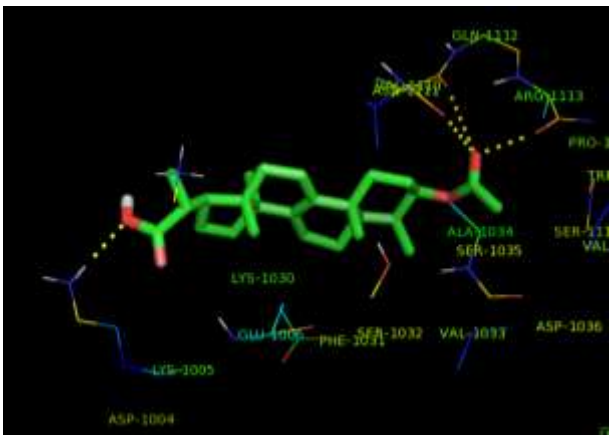

Fig 8: Docked conformation showing interaction between compound 4 and Janus kinase2

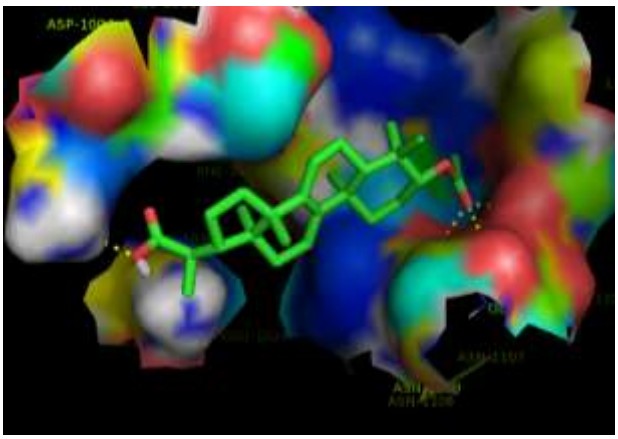

Fig 9: Docked conformation in solid surface showing interaction between compound 4 and Janus kinase2

4.6. Phytocompounds from *Vitex nedungo*

COMPOUND 5: 3-epi-corosolic acid

Docked conformation of 3-epi-corosolic acid with Janus kinase 2

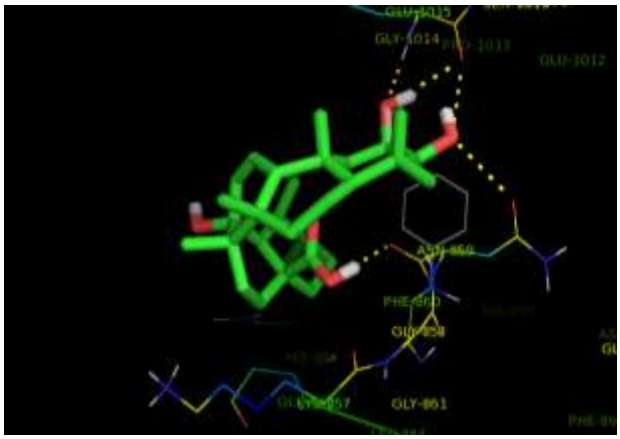

Fig 10: Docked conformation showing interaction between compound 5 and Janus kinase2

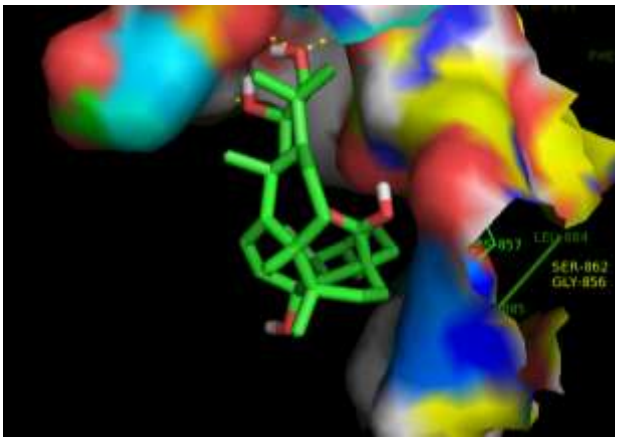

Fig 11: Docked conformation in solid surface showing interaction between compound 5 and Janus kinase2

COMPOUND 6 2.6.6 3-O-Acetyloleanolic acid

Docked conformation of 3-O-Acetyloleanolic acid with janus kinase 2

Fig 12 : Docked conformation showing interaction between compound 6 and Janus kinase2.

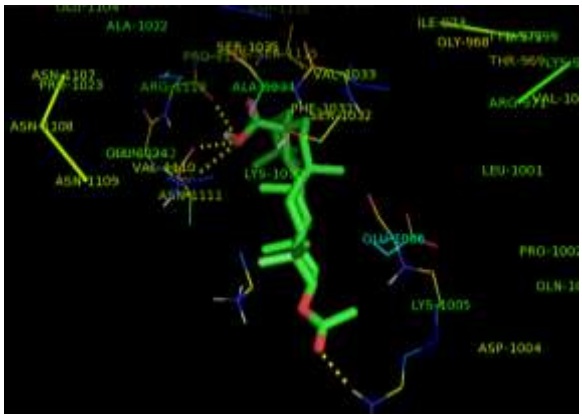

Fig 12: Docked conformation showing interaction between compound 6 and Janus kinase2

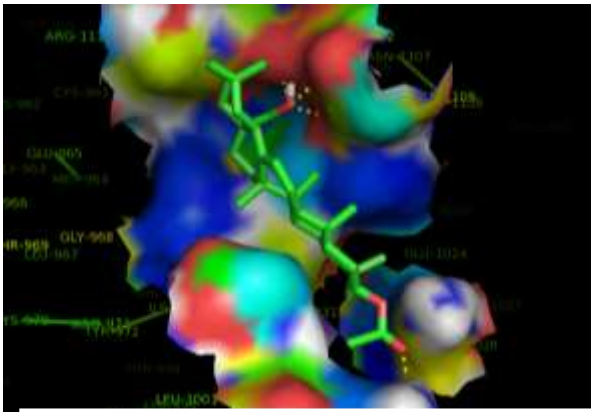

Fig 13: Docked conformation in solid surface showing interaction between compound 6 and Janus kinase2

COMPOUND 7:AC1NUKBO

Docked conformation of AC1NUKBO with Janus kinase 2

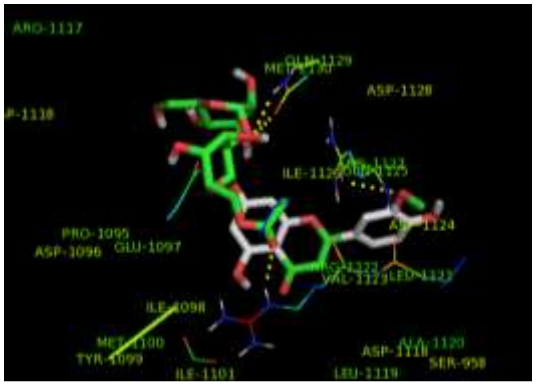

Fig 14: Docked conformation showing interactio between compound 6 and Janus kinase2

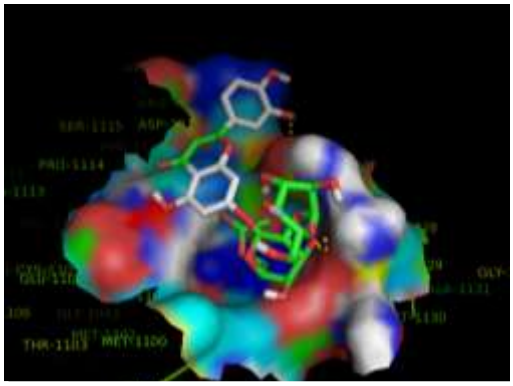

Fig 15: Docked conformation in solid surface showing interaction between compound 6 and Janus kinase2

COMPOUND 8: Caryophyllone Oxide
Docked conformation of Caryophyllone Oxide with janus kinase 2.

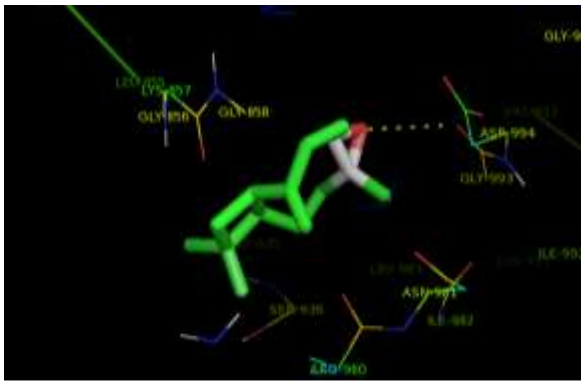

Fig 16: Docked conformation showing interaction between compound 8 and Janus kinase2

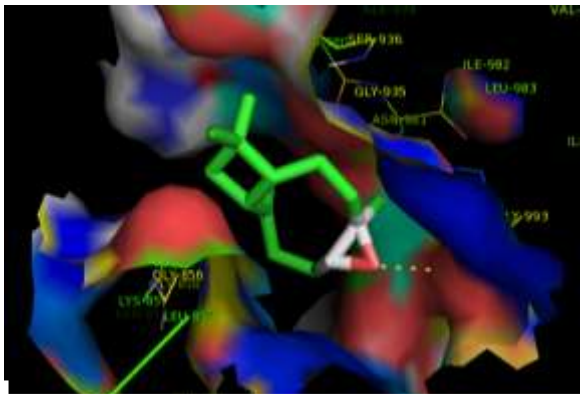

Fig 17: Docked conformation showing interaction between compound 8 and Janus kinase2

COMPOUND 9.Gardenin
Docked conformation of Gardenin with Janus kinase 2.

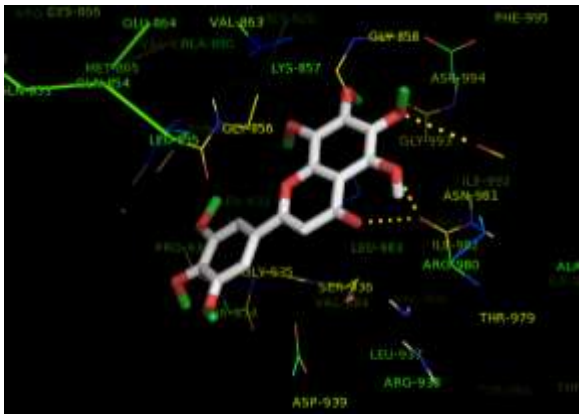

Fig 18: Docked conformation showing interaction between compound 9 and Janus kinase2

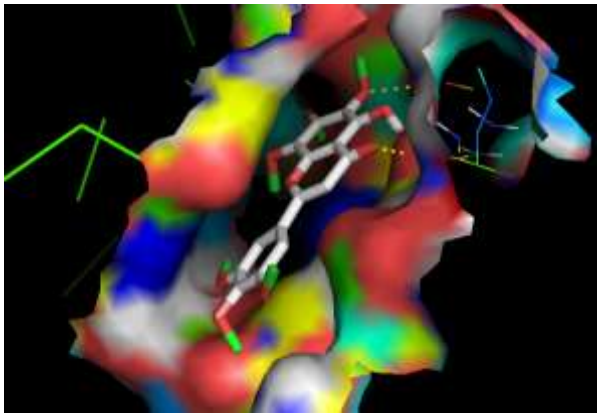

Fig 19: Docked conformation in solid surface showing interaction between compound 9 and Janus kinase2

COMPOUND 10: Sabinene
Docked conformation of Sabinene with Janus kinase 2

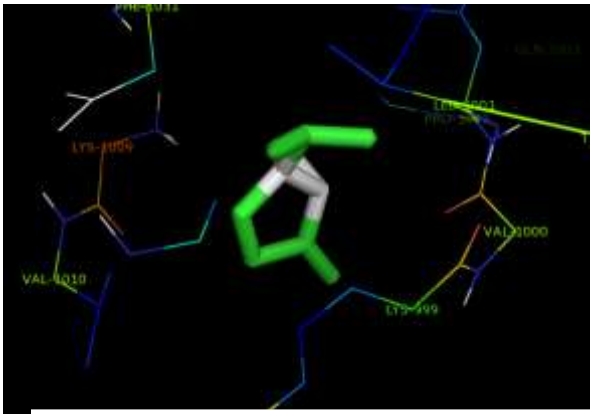

Fig 19: Docked conformation in solid surface showing interaction between compound 9 and Janus kinase2

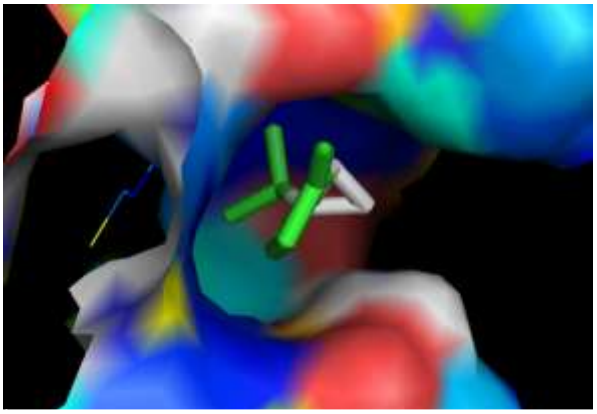

Fig 20: Docked conformation showing interaction between compound 10 and Janus kinase2

The Docking of 6- Oxibicyclo(3.1.0) hexam-3-one from *Clerodendrum infortunatum* with the drug target Janus Kinase 2 resulted in 4 conformations with the binding energy in the range of -3.42 Kcal to -2.89 Kcal. All the Conformations except Conformation 3 had formed one Hydrogen bond between the compound, 6- Oxibicyclo(3.1.0) hexam-3-one and receptor JAK2. But, the docking of the compound 3,4,4a,5,6,7,8,8a-octahydrochromen-2-one with the drug target Janus Kinase 2 showed lesser binding energy than the compound 6- Oxibicyclo(3.1.0) hexam-3-one. A total of 10 conformations were formed while docking the compound 3,4,4a,5,6,7,8,8a-octahydrochromen-2-one with the drug target and the binding energy were in the range of -6.09 Kcal to -4.27 Kcal. All the Conformations except Conformation 2,6,9 and 10 had formed one or two Hydrogen bonds between the compound 6- Oxibicyclo (3.1.0) hexam-3-one and receptor JAK2.

On the other hand the compound 3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] (1E)-N-hydroxybut-3-enimidothioate from *Clerodendrum infortunatum* showed lesser affinity to the drug target Janus Kinase 2 as the binding energy was more. A total of 10 conformations were formed and the binding energy were in the range of -2.44 Kcal to -0.11 Kcal. All the Conformations except Conformation 9 and 10 had formed one to four Hydrogen bonds between the compound [3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] (1E)-N-hydroxybut-3-enimidothioate and receptor JAK 2. While docking the compound 2-(3-acetyloxy-4,4,10,13,14-pentamethyl-2,3,5,6,7,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-17-yl)propanoic acid from *Clerodendrum infortunatum* Only one conformation was formed and the binding energy was -4.46 Kcal. There was no Hydrogen bond between the compound 2-(3-acetyloxy-4,4,10,13,14-pentamethyl-2,3,5,6,7,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-17-yl)propanoic acid and receptor JAK 2.

While docking the compound 3-epi-corosolic acid from *Vitex negundo* with the drug target, Only one conformation was formed and the binding energy was -4.5 Kcal. The conformation had formed two Hydrogen bonds between the compound 3-epi-corosolic acid and receptor JAK2. But the compound3-O-Acetyloleanolic acid from *Vitex negundo* has shown better affinity with drug target Janus Kinase2 as the

binding energy was low. A total of 10 conformations were formed and the binding energy were in the range of -7.52 Kcal to -5.47 Kcal. All the Conformations except Conformation 4,6,7,9 and 10 had formed one Hydrogen bond between the 3-O-Acetyloleanolic acid and receptor JAK2.

A poor binding was seen in the docking of the compound AC1NUKBO from *Vitex negundo* with the drug target as the binding energy was -0.6 Kcal to -0.11 Kcal with a total of 4 conformations. All the Conformations had formed one to three Hydrogen bonds between the compound AC1NUKBO and receptor JAK2. But the compound Caryophyllone Oxide from *Vitex negundo* had better affinity for the drug target as a total of 10 conformations were formed with the binding energy in the range of -5.73 Kcal to -4.83 Kcal. All the Conformations except Conformation 9 and 10 had formed one Hydrogen bond between the Caryophyllone Oxide and receptor JAK2.

Higher binding energy and lesser affinity was seen in the docking of the compound Gardenin from *Vitex negundo* with the drug target. A total of 3 conformations were formed and the binding energy were in the range of -4.03 Kcal to -2.54 Kcal. All the Conformations except Conformation 3 had formed one Hydrogen bond between the compound Gardenin and receptor JAK2.Similar affinity was seen in the compound sabinene from *Vitex negundo* with the drug target.A total of 10 conformations were formed and the binding energy were in the range of -4.2 Kcal to -3.81 Kcal. None of the conformation shows Hydrogen bond between the compound sabinene and receptor JAK2.

Table 4. Comparison of Docking results of Compounds from *Clerodendrum infortunatum*

S no	Name of the compound	Binding energyKcal	IC µM	No of hydrogen atom	Hydrogen bond
1	6-Oxabicyclo[3.1.0]hexan-3-one	-3.42	3.1	1	PHE860:HN::0:O
2	3,4,4a,5,6,7,8,8a-Octahydrochromen-2-one	-6.09	34.07	1	A:LYS882::0:O
3	[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] (1E)-N-hydroxybut-3-enimidothioate	-2.44	16.3	2	B:SER936:OG::0:H B:ASN981:OD1::0:H
4	2-(3-acetyloxy-4,4,10,13,14-pentamethyl-2,3,5,6,7,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-17-yl)propanoic acid	-4.46	537.84	0	

By analyzing the result of docking study the binding energy, inhibition constant and the number of hydrogen bonds are plotted above. From this data 3,4,4a,5,6,7,8,8a-Octahydrochromen-2-one from *Clerodendrum infortunatum* with drug likeliness score of -1.54 shows better binding affinity than the standard drug. So by this study it was found that 3,4,4a,5,6,7,8,8a-Octahydrochromen-2-one can act as a lead compound from natural source that can be better than the existing standard drug.

Table 5.Comparison of Docking results of Compounds from *Vitexnegundo*

S. No	Name of the compound	Binding energyKcal	IC µM	No of hydrogen atom	Hydrogen bond
1	Acetylolenoic acid	-7.52	3.06	1	B:ARG897:HH12::UNL1:O
2	Ac1nukbo	-0.6	350.73	1	A:val878:HN::UNL1:O
3	Caryophyllone oxide	-5.73	63.42	1	A:HIS950:HN::UNL1:O
4	Corosolic acid	-4.5	761.97	2	A:GLU1015:O::UNL1:H A:GLU1015:O::UNL1:H
5	Gardenin	-4.03	1.11	1	B:ARG980:O::UNL1:H
6	Sabinene	-4.2	837.84	0	

By analyzing the result of docking study the binding energy, inhibition constant and the number of hydrogen bonds are plotted above. From this data it is shown that Acetylolenolic acid and Caryophyllone oxide with drug likeliness score 0.57 and -1.74 respectively from *Vitex negundo* shows better binding energy than the existing standard drug. So the phytocompounds promise to act as a better lead compound than the currently existing one.

Standard Drug: Entretinib

The standard drug for cancer Entrectinib was docked against the target protein for cancer treatment, Janus kinase and the results are tabulated in Table 4.

Table 6: Docking result showing Standard drug Entrectinib with Janus kinase 2.

Pose	B.E	IC µM	No. of H bonds	Hydrogen bond
1	-5.35	119.73	1	A:SER1016:O::UNK0:N:
2	-4.84	282.04	0	-
3	-4.72	347.95	2	A:GLN853:HE21::UNK0:N A:TYR931:HH::UNKO:N

BE – Binding Energy; LE – Ligand Efficiency; IC – Inhibitory Constant; Int.E – Intermolecular Energy; Vdw-VanderWaals Energy; Elec.E – Electrostatic Energy; Total.IE – Total Intermolecular Energy; Tor.E – Torsional Energy; Unb.E – Unbound Energy

Table 4 shows the Docking results of standard drug Entrectinib with the drug target Janus Kinase 2. A total of 3 conformations were formed and the binding energy were in the range of -5.35 Kcal to -4.72 Kcal. Conformation 1 and Conformation 3 had one and two Hydrogen bonds respectively between the standard drug Entrectinib and receptor JAK2. The Docked Conformation showing interactions is displayed in Figure 22 and its surface representation is shown in Figure 23 below.

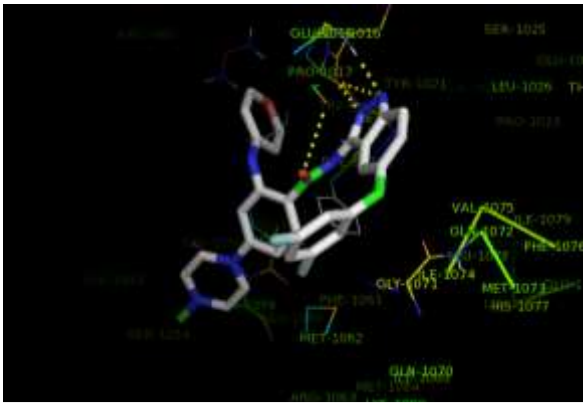

Fig 22: Docked conformation showing interactic between Standard drug and Janus kinase2

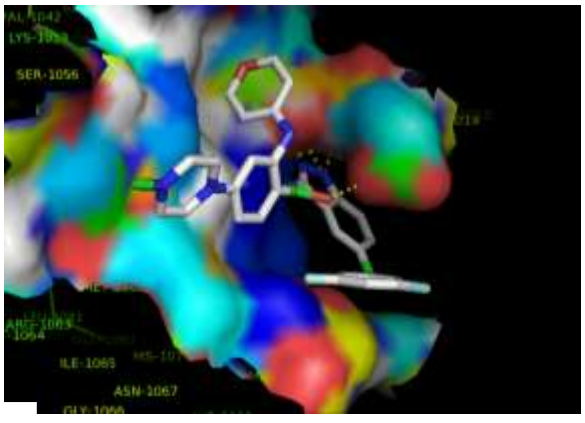

Fig 23: Docked conformation in solid surface showing interaction between Standard drug and Janus kinase2

Table 7. Drug Likeliness of the compounds

Compound	Volume (A3)	TPSA (A2)	HBA	HBD	MW (Da)	Log*P*	Lipinski's Violation	BBB	Drug likeliness constant
6-Oxabicyclo[3.1.0]hexan-3-one	102.86	22.18	2	0	98.04	-3.37	0	3.63	-1.26
3,4,4a,5,6,7,8,8a-Octahydrochromen-2-one	166.49	21.15	2	0	154.10	2.08	0	4.22	-1.54
[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] (1E)-N-hydroxybut-3-enimidothioate	248.71	102.58	8	5	278.08	-1.40	0	1.90	-0.51
2-(3-acetyloxy-4,4,10,13,14-pentamethyl-2,3,5,6,7,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-17-yl)propanoic acid	539.51	49.17	4	1	430.31	5.52	1	3.43	0.74
3-epi-corosolic acid	587.55	74.56	5	4	488.35	4.57	0	1.82	0.32
3-O-Acetyloleanolic acid	637.59	48.73	4	1	498.37	7.17	1	3.33	0.57
AC1NUKBO	596.71	210.24	17	8	666.18	-0.23	3	1.00	0.61
Caryophyllone Oxide	305.51	8.34	1	0	220.18	3.97	0	3.94	-1.74
Gardenin	427.37	83.08	9	1	418.13	3.56	0	2.59	-0.34
(+)-Sabinene	190.75	0.00	0	0	136.13	3.15	0	0.98	-0.87

Table 5 shows the drug likeliness of the Compounds taken for the study. It is clear from the Table that the Compound AC1NUKBO showed Lipinski violation of three and very poor drug likeliness. Similarly compounds 3-O-Acetyloleanolic acid and 2-(3-acetyloxy-4,4,10,13,14-pentamethyl-2,3,5,6,7,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-17-yl)propanoic acid showed one violation and all the other compounds had good drug likeliness without any violation.

Conclusion

Molecular docking has become an increasingly important tool for drug discovery. The molecular docking approach can be used to model the interaction between a small molecule and a protein at the atomic level, which allows us to characterize the behavior of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes. In this study Autodock tools for doing molecular docking and by analyzing the drug likeliness score , binding energy and number of hydrogen bonds and other important values it is found that Acetylolenolicacid, Caryophyllone oxide and 3,4,4a,5,6,7,8,8a-Octahydrochromen-2-one shows to have better antineoplastic activity than existing standard drugs. Since it is a natural compound derived from plant based phytochemicals it have less side effects and more activity towards cancer cells. So the study concluded to be successful by finding three active phytochemicals against cancer which can contribute a big asset for drug research area for cancer prevention. Since, it is an *in silico* study, it needs validation experimentally.

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