"In-silico Computational Docking Study of Curry Leaves Biomolecules (*Murraya koenigii*) : A Potent Anticancerous Agent"

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ABSTRACT

Due to advancement in the bioinformatics, there is a rapid increase in the computational method to predict the interaction between the interface of two biological origin molecules. Bioinformatics reduces the tedious task toperform the repeated analysing of various molecules interaction and gives the best interface interaction as anoutput. Prediction and experiment are the ways that undergo simultaneously and provides best route. It gives the promising result with a good precision value. The virtual screening method has been broadly acceptable as it omits the undesirable molecules from the compound repositories and gives a platform with a low cost and time-consuming process. In our present study we have carry out the computational approach to predict and find out the anticancerous protein from curry leaves. We have selected the target from PDB and the ligand from the PubChem data base. For the preparation of target, we have removed the water molecules and added the polar hydrogen group. And for the preparation of ligand, we have detected the torsion root where docking can be processed. All the files of target and ligand were saved in pdbqt format.

We have taken three breast cancerous protein into consideration for the molecular docking against the three anticancerous drugs obtained from PubMed are Oestradiol (PDB ID- 3HB5), HER2 (PDB ID- 1N8Z) and NUDT-5(PDB ID-5NQR). Oestradiol is a well characterizedsex hormone that stimulates breast cancer in female. HER2 protein, when inappropriately activated leads to proliferation and differentiation of breast cancer cells. NUDT 5 has the importance in the gene regulation and the proliferation of breast cancer cells. After the molecular docking via Auto dock Vina **Mahanine and Pyrayafoline D** showed least interaction energy with the breast cancerous protein. The cancerous protein taken into consideration is also responsible for the other types of cancer but we are mainly focused on the breast cancer.

Our present study concludes that the *Murraya koenigii* may serve as a potential source of bioactive compounds in the prevention of cancer. The potential for developing an anticancerous drugs from higher eukaryotic plants appears rewarding for the mankind as it leads to the development of new drugs that will be helpful for the cancer patient inpresent date.

Keywords- Anticancerous, Bioactive, Docking, Drugs, Repositories, Signalling etc.

1. INTRODUCTION

Breast cancer is one of the leading cause of deaths on women globally (Parkin DM et. al. 2000). Breast cancers contributed 12.3% of the total number of new cases diagnosed in 2020.There were nearly 2.26 million cases of breast cancer in 2020 in the world. In India for every 2 women newly diagnosed with breast cancer, one of them dies. Some common breast cancerous proteins are estradiol, HER2 and NUDT-5. Conventional and allopathic medication has a lot of side effects and are very expensive, so which makes herbal medicine a preferable alternative. Also there has been tremendous demand of drugs from natural sources. The plant *Murraya koenigii L.* belongs to the family Rutaceae, commonly called "curry leaf" in English and is native to Asia. Curry leaf tree was originally grown in India for its aromatic leaves. It slowly made a way to many Asian kitchens because of its amazing and distinct flavour. It is reported to possess a wide variety of medicinal importance such as anti-cancer, anti-inflammatory, anti-fungal, anti-bacterial and anti-oxidant (Makri and Kintzios,2007; Negi et al.,2011).

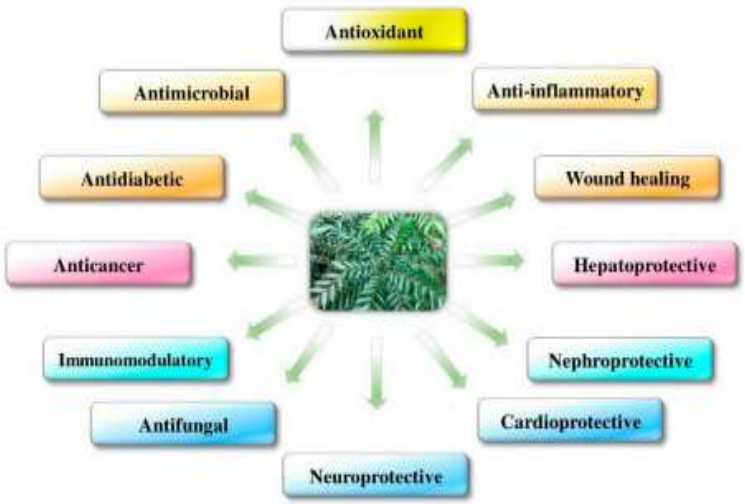
Recent studies found that curry leaf contains some carbazole alkaloids like Grinimbine, Mahanine, Pyrafoline-D, Mahanimbine are said to be possessed anti-cancerous and anti-oxidant activity (Iman V et al.,2016; Kumar VS. et. al. 1999). The curry leaf contains carbohydrates, fiber, magnesium, iron, copper and minerals. It also contains various vitamins such as nicotinic acid, vitamin A, vitamin C, vitamin B and vitamin E (Jain M. et. al. 2017). Curry leaf are a rich source of iron and folic acid. Folic acid is mainly responsible for carrying and helping the body absorb iron. Recently Syam et al. reported that girinimbine, a carbazole alkaloid isolated from this plant inhibited the growth and induced apoptosis in human hepatocellular carcinoma, hepg2 cells.

Different in vitro, in vivo and computational methods were employed to assess the anti-cancer potential of carbazole alkaloids. Among these methods, docking has been used widely in drug designing of breast cancer. Role of these carbazole alkaloids found in curry leaf is well studied by different scientists from time to time and their inhibition justifies their role in anti-cancer potential. The selected alakloids were Girnimbine(PubChem CID:96943), Mahanine(PubChem CID:36689305) and Pyrafoline-D(PubChem CID:375148).

Research on breast cancer treatment using curry leaves is limited and thus this study is important in providing information about breast cancer treatment by herbal medicine.

BOTANICAL DESCRIPTION OF MURRAYA KOENIGII

Murraya koenigii, also known as curry-leaf tree, is mainly grown in the Indian subcontinent, mostly found in the southern parts of India. A perennial tree, being handy as a flavoring agent for various food preparations, has a wide variety of medicinal importance such as Anti-bacterial, Anti-fungal, Anti-cancer, Anti-inflammatory, etc. (Makri and Kintzios, 2007; Negi et al., 2011). It belongs to the family Rutaceae and is native to Asia.



Taxonomy of plant

Kingdom-	Plantae
Sub-kingdom-	Tracheobionta
Superdivision-	Spermatophyta
Division-	Magnoliophyta
Class-	Magnoliospida
Subclass-	Rosidae
Order-	Sapindales
Family-	Rutaceae
Genus-	Murraya J.Koenig ex. L.
Species-	Murraya Koenigii L. Spreng.

MORPHOLOGY OF CURRY PLANT

A small spreading shrub, about 2.5 metres high; the main stem, dark green to brownish, with numerous dots on it; its bark can be peeled off longitudinally, exposing the white wood underneath; the girth of the main stem is 16 cm (Mhaskar et. al. 2000).

Leaves, exstipulate (with no stipules), bipinnately compound, 30 cm long, each bearing 24 leaflets, having reticulate venation; leaflets, lanceolate, 4.9 cm long, 1.8 cm broad, having 0.5-cm-long petiole.

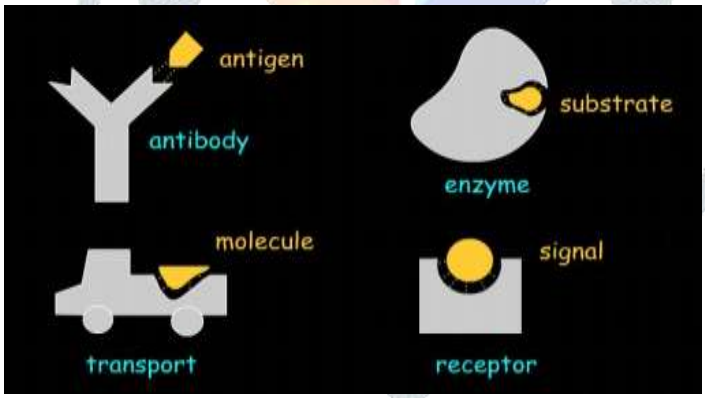
Flowers, bisexual, white, funnel-shaped, sweetly scented, stalked, complete, ebracteate, regular, actinomorphic, pentamerous, hypogynous, the average diameter of a fully opened flower being 1.12 cm; inflorescence, a terminal cyme, each bearing 60 to 90 flowers; calyx, 5-lobed, persistent, inferior, green; corolla, white, polypetalous, inferior, with 5 petals, lanceolate; length, 5 mm; androecium, polyandrous, inferior, with 10 stamens, densified, arranged into circles of five each; smaller stamens, 4 mm. long whereas the longer ones, 5 to 6 mm; gynoecium, 5 to 6 mm long; stigma, bright, sticky; style, short; ovary, superior.

Fruits, round to oblong, 1.4 to 1.6 cm long, 1 to 1.2 cm in diameter; weight, 880 mg; volume, 895 microlitres; fully ripe fruits, black with a very shining surface; pulp, Wistaria blue 640/2; the number of fruits per cluster varying from 32 to 80.

Seed, one in each fruit, 11 mm long, 8 mm in diameter, colour spinach green 0960/3; weight, 445 mg; volume, 460 microliters (Prajapati et. al. 2003).

INTRODUCTION TO MOLECULAR DOCKING

Molecular docking is the study of how two or more **molecular** structures (e.g., drug and enzyme or **protein**) fit together to form a stable complex with minimum overall energy. In a simple definition, **docking** is a **molecular** modelingtechnique that is used to predict how a **protein** (enzyme) interacts with small **molecules** (ligands).



2. **REVIEW OF LITERATURE**

2.1 Properties of *Murraya koenigii*

Murraya koenigii L. belongs to the Rutaceae family of the plant. It grows naturally in forests in India, Andaman Islands, Thailand, Cambodia, Vietnam, and Laos (Morton et. al. 1984; Ho et. al. 1999).

Many years from now, we are using traditional methods for curing the diseases by preparing medicines from different medicinal plants. The plants' part like bark, leaves, flowers, roots, fruits, and seeds are used to prepare medicines which have chemicals substance that produce a definite physiological action on the human body. These are used by 80% of the world's population as a traditional medicine because of its safety and their cost-effectiveness. The chemical substances present in the plant part are called the phytochemicals, and its extract is known as phytoextract have effective properties like anti-oxidant, anti-inflammatory, anti-microbial and also the anti-cancer properties. About 25% of the modern pharmaceutical drug have botanical origins (Ashokkumar K. et. al. 2013).

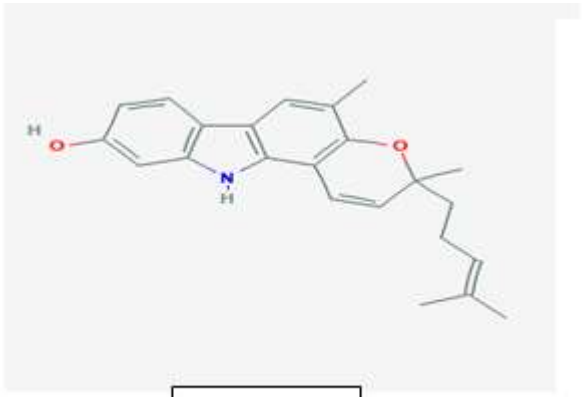
Curry leaves have been widely used as a main flavoring ingredient in chutney powders and pickles in India as well as in folk-medicines in Southern Asia. Consequently, Murraya koenigii L. is frequently referred to as the Indian curry leaf tree or just the curry leaf tree (Natarajan et. al.1974; Balaswamy et. al. 2004). The leaves are considered to be a good cure against dysentery and bites of poisonous animals, while the roots of these plants can be used as a pain-killer (Gupta et. al. 1970).

Phytochemical studies on the leaves stem bark, and root of this plant have shown the presence of large concentration of alkaloids, phenolic compounds and very high radical scavenging activity (Sharif et al. 2007; Tachibana et al. 2001).

2.2 Some of the alkaloids are given below as follows:

2.2.1 GIRINIMBINE

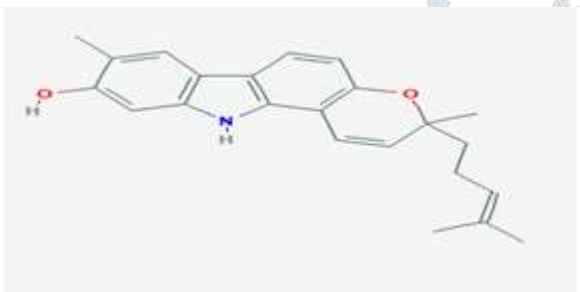
It is a secondary metabolite synthesized from curry plants. A 2011 study suggested that it helps in the apoptosis of cancerous cells.



GIRINIMBINE

2.2.2 MAHANINE

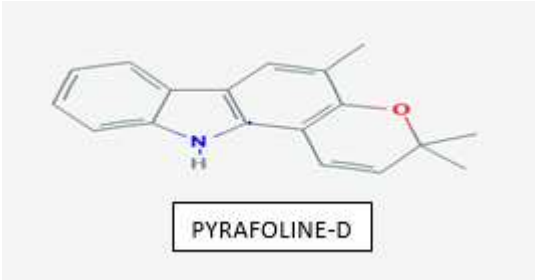
It is a carbazole alkaloid which is isolated from a curry leaf plant (*Murraya koenigii*) and has potentially inhibiting the growth of altered subtypes of breast cancer cells in vitro and also significantly reduces the mammary tumor burden in N-Methyl-N-nitrosourea (MNU) induced rat. (Momita Das et al. 2019).



MAHANINE

2.2.3 PYRAYAFOLINE-D

Pyrafoline D, also known as isomahanine, belongs to the class of organic compounds known as carbazoles. Carbazoles are compounds containing a three-ring system containing a pyrrole ring fused on either side to a benzene ring. Pyrafoline D is an extremely weak basic (essentially neutral) compound (based on its pKa). Pyrafoline D has been detected, but not quantified in, herbs and spices.



PYRAFOLINE-D

2.3 Breast-cancerous proteins which were used in molecular docking are as follows:

2.3.1 Oestradiol is a well-characterized sex hormone that stimulates breast cancer and other oestrogen-related diseases. 17 beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) catalyses the last step in the synthesis of oestradiol and androstenediol in breast tumour tissue. The enzyme's high expression and activity after simultaneousblockade of oestrogen receptors and inhibition of aromatase in the tumour shows the necessity for its inhibition as a requirement for breast cancer therapy. In the present paper, we report structures of the binary and ternary complexes of 17beta-HSD1 with a new inhibitor E2B {3- [3',17'beta- dihydroxyestra-1',3',5'(10')-trien-16'betamethyl] benzamide},and the enzyme inhibition by the later. The IC50 value for E2B was determined to be 42 nM in T47D cells.

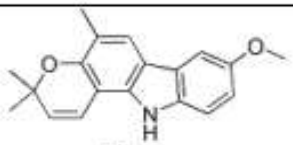
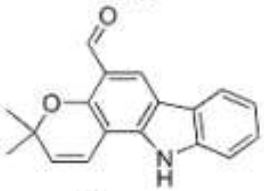
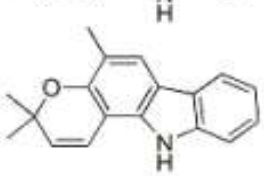
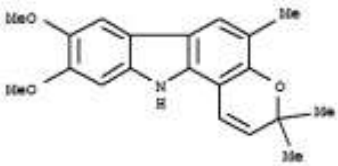
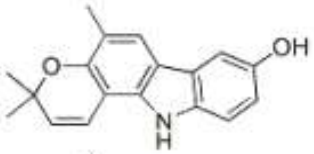
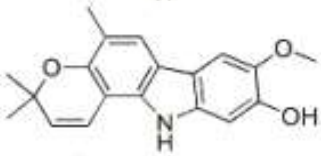
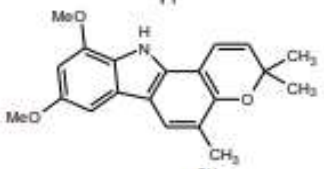
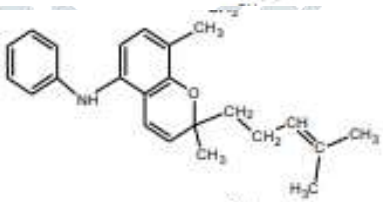
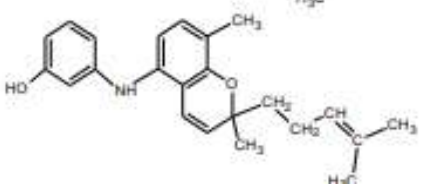
Multiple interactions between E2B and the enzyme include hydrogen bonds and hydrophobic interactions, as well as pi-piinteractions. A kinetic study demonstrated that E2B inhibits the enzyme's reduction forming oestradiol from oestrone, with a Ki of 0.9+/-0.15 nM. Such strong inhibition is in agreement with its extensive interaction with the enzyme, suggesting its potential as a lead compound for breast cancertherapy. In fact, this possibility is enhanced by its capacity for cell penetration similar to natural steroids. Such inhibitors that block oestrogen synthesis to suppress the sulfatase pathway producing oestradiol can be used in adjuvant therapies with oestrogen receptor blockade, opening a new orientation of breast cancer treatment.

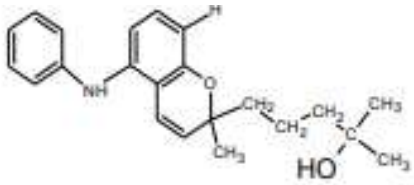
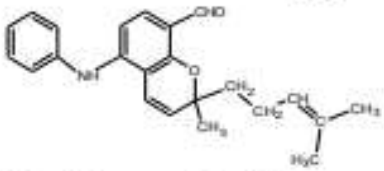
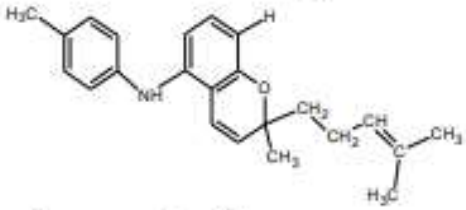
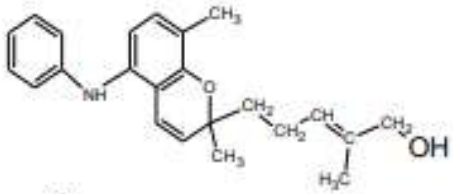
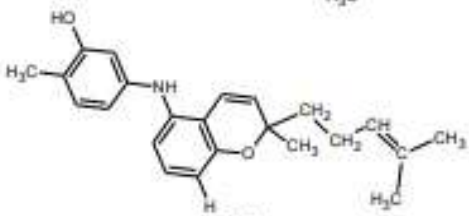
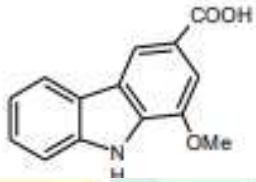
2.3.2 NUDT5 (also called NUDIX5) has been implicated in ADP-ribose and 8-oxo-guanine metabolism and was recently identified as a rheostat of hormone-dependent gene regulation and proliferation in breast cancer cells. Here, we further elucidate the physiological relevance of known NUDT5 substrates and underscore the biological requirement for NUDT5 in gene regulation and proliferationof breast cancer cells. We confirm the involvement of NUDT5 in ADP-ribose metabolism and dissociate a relationship to oxidized nucleotide sanitation. Furthermore, we identify potent NUDT5 inhibitors, which are optimizedto promote maximal NUDT5 cellular target engagement by CETSA.

2.3.3 HER2 (also called Neu; ErbB2) is a member of the epidermal growth factor receptor (EGFR; also known as ErbB) family of receptor tyrosine kinases, which in humans includes HER1 (EGFR, ERBB1), HER2, HER3 (ERBB3) and HER4 (ERBB4). ErbB receptors are essential mediators of cell proliferation and differentiation in the developing embryo and in adult tissues, and their inappropriate activation is associated with the development and severity of many cancers. Overexpression of HER2 is found in 20-30% of human breast cancers, and correlates with more aggressive tumours and a poorer prognosis. Anticancer therapies targeting ErbB receptors have shown promise, and a monoclonal antibody against HER2, Herceptin (also known as trastuzumab), is currently in use as a treatment for breast cancer.

2.4 Chemical Constituents of *M. koenigii* with tested pharmacological activities table (Harish et. al. 2012):

Table 3: Chemical constituents of *M. koenigii* with tested pharmacological activities.

Sr no	Constituent	Constituent structure	Activity
1	Koenimbine		Anti- diarrhea
2	Murrayacine		Anti-microbial
3	Girinimbine		Anti-tumor
4	Koenimbidine/Koenidine/Koenigicine		Anti- diarrhea
5	Koenine		Anti-oxidant
6	Koenigine		Anti-oxidant
7	Mukonicine		Anti-oxidant
8	Mahanimbine		Cytotoxicity, Anti-oxidant, Anti-microbial, Anti- diabetic and Hyperlipidemic
9	Mahanine		Cytotoxicity, Anti- microbial, Anti cancer

10	Mahanimbinine		Anti-oxidant, Anti- microbial, Anti- diabetic and Hyperlipidemic
11	Murrayacinine		Anti-oxidant, Anti- microbial, Anti- diabetic and Hyperlipidemic
12	Isomahanimbine/ Mahanimbicine		Anti-oxidant, Anti- microbial, Anti- diabetic
13	Mahanimboline		Cytotoxicity, Anti-oxidant, Anti-microbial, Anti- diabetic and Hyperlipidemic
14	Isomahanine		Cytotoxicity, Anti-oxidant, Anti-microbial, Anti- diabetic and Hyperlipidemic
15	Mukoeic acid		Anti-oxidant

3. OBJECTIVE

3.1 Tools and Materials used

In our present study we took help of different biological databases such as PubChem, ZINC, RCSB-PDB (Protein Data Bank) and software's like Autodock vina, Discovery studio visualizer, PyMOL, Open Babel GUI and Avogadro.The PDB (Protein Data Bank) is the single worldwide archive of Structural data of Biological macromolecules, established in Brookhaven National Laboratories (BNL) in 1971[15]. It contains Structural information of the macromolecules determined by X-ray crystallography, NMR methods, etc. and provides access to 3D structure data for large biological molecules (proteins, DNA, and RNA). Autodock vina is an advanced version of Autodock 4.0 which is quite efficient and provides good accuracy and performance in comparison to Autodock 4.0. These both Autodock 4.0 and Autodock vina are currently maintained by The Scripps Research Institute, Florida, USA. Usage of AutoDock has contributed to the discovery of several drugs. Discovery studio visualizer is a software for performing simulations of small molecule, and analysing macromolecule systems. It includes tools for receptor-ligand docking. PyMOL is a software for visualization of macromolecules such as proteins. Open Babel GUI is a software mainly built for interconverting chemical file formats.

3.2 Methodology

3.2.1 Retrieval of protein files from major database (Target selection)

We retrieved protein.pdb files from major protein databases using following link.
https://www.rcsb.org/

Figure 3.1 Home page of RCSB-PDB

We then entered the name of protein that was docked (For example, Oestradiol or its pdb id:3HB5).

Figure 3.2 Oestradiol protein

We selected 'Download file from drop down list'.
Then we clicked PDB File (text) and downloaded it.
Then we opened this text file and deleted all the heteroatoms. Next step involved was deletion of X chains as all the chains are similar and ligand would bind to either of those chains.

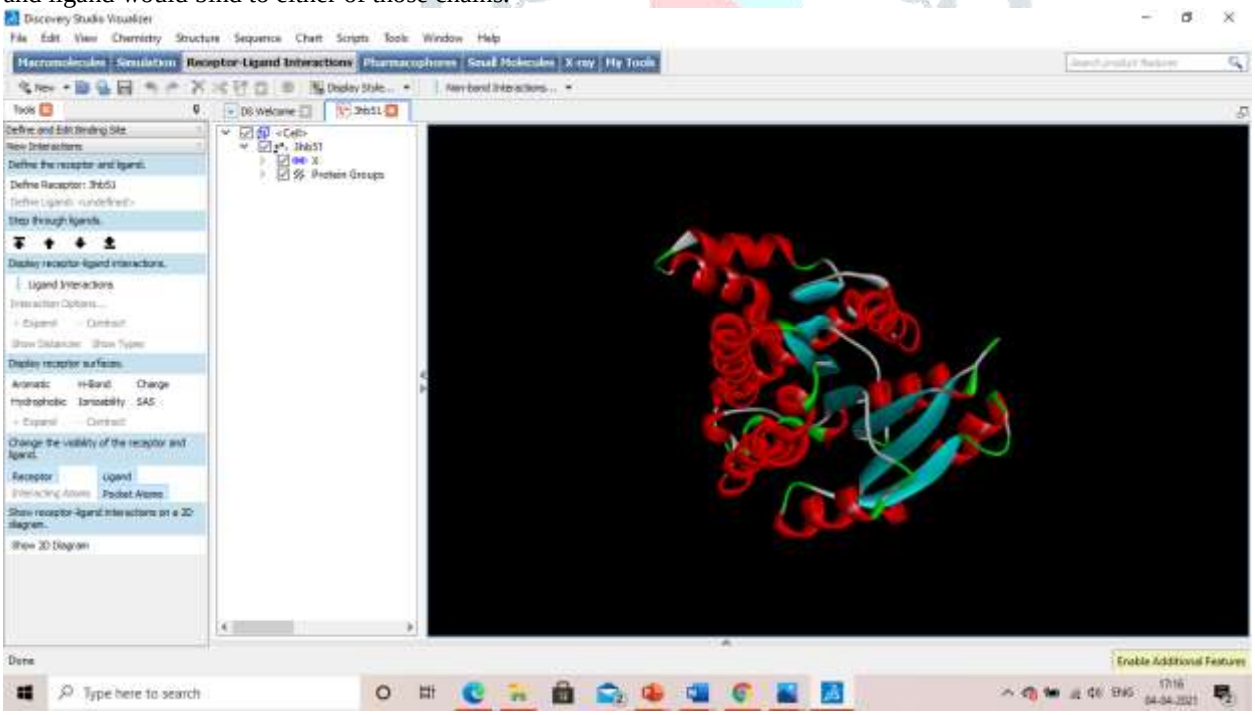


Figure 3.3 Prepared Oestradiol protein in Discovery Studio Visualizer

3.2.2 Retrieval of Ligand molecules from different databases https://zinc.docking.org/substances/home/ or https://pubchem.ncbi.nlm.nih.gov/
The above links are available to download the desired ligand molecules. Also, analogous structures of the ligand can be drawn with the help of ACD ChemSketch, Marvin Sketch, etc. However, it is more convenient to download the file from the database.

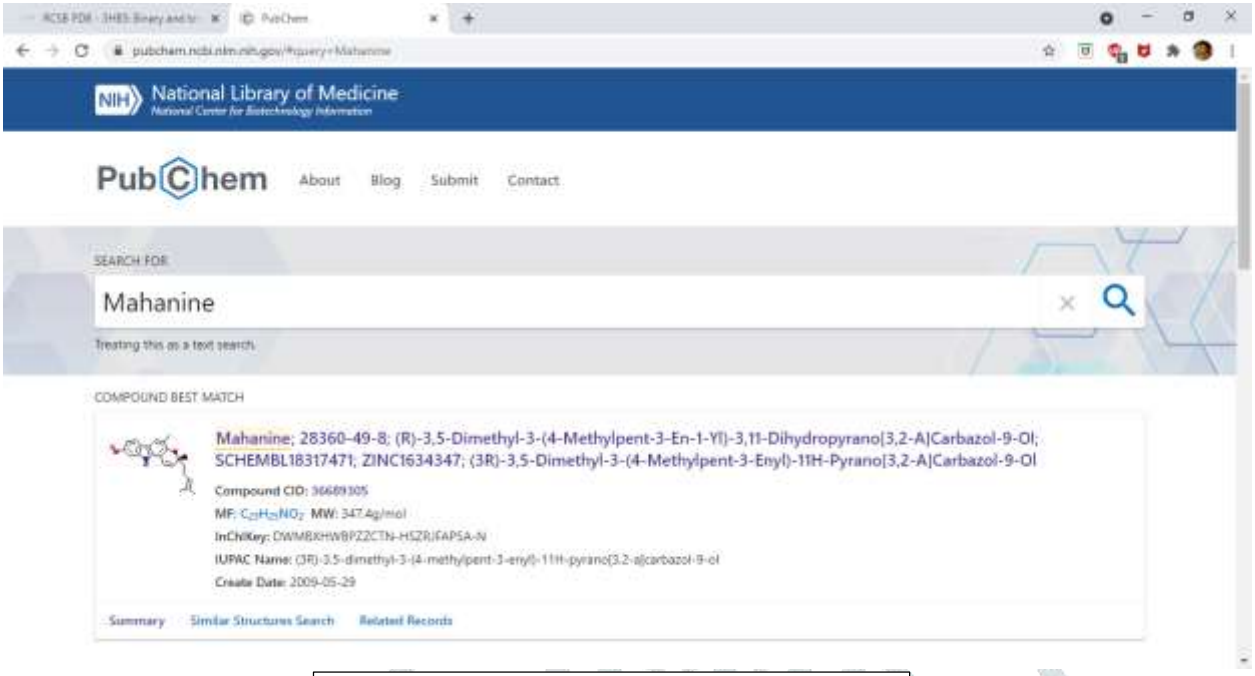


Figure 3.4 Ligand Mahanine in PubChem database

We clicked on "3D image and save Sdf".
Then the file was converted from Sdf file to pdb file with the help of Open Babel GUI as autodock can read only pdb files.

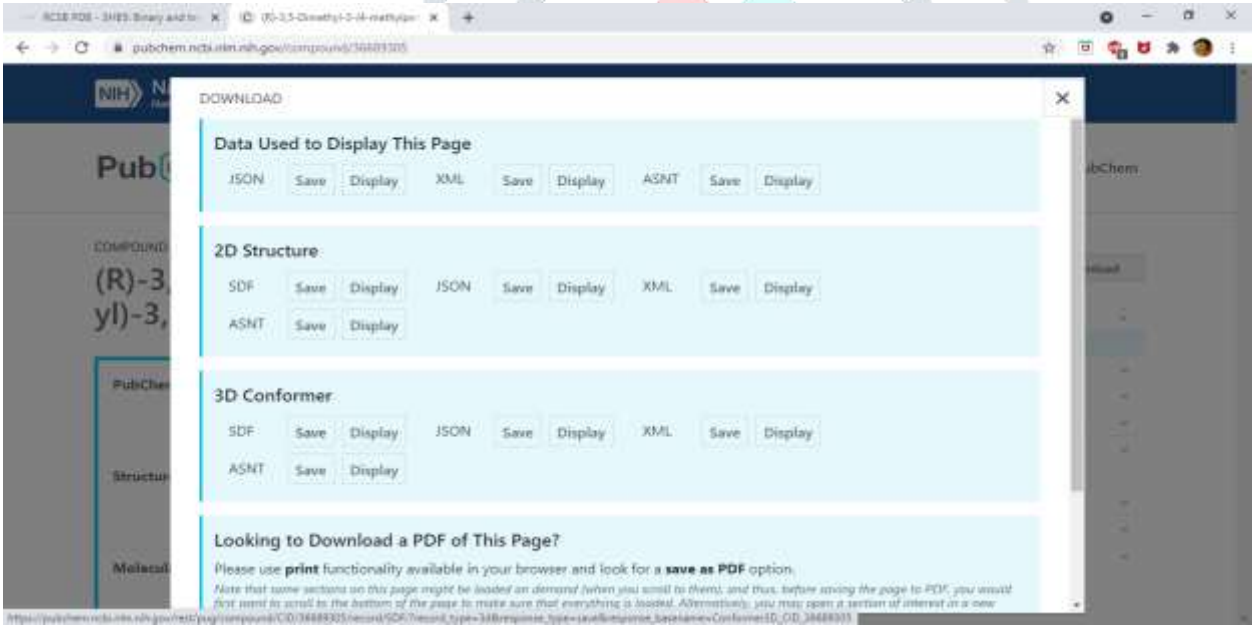


Figure 3.5 Click on Save [SDF format] under 3D conformer section to download ligand

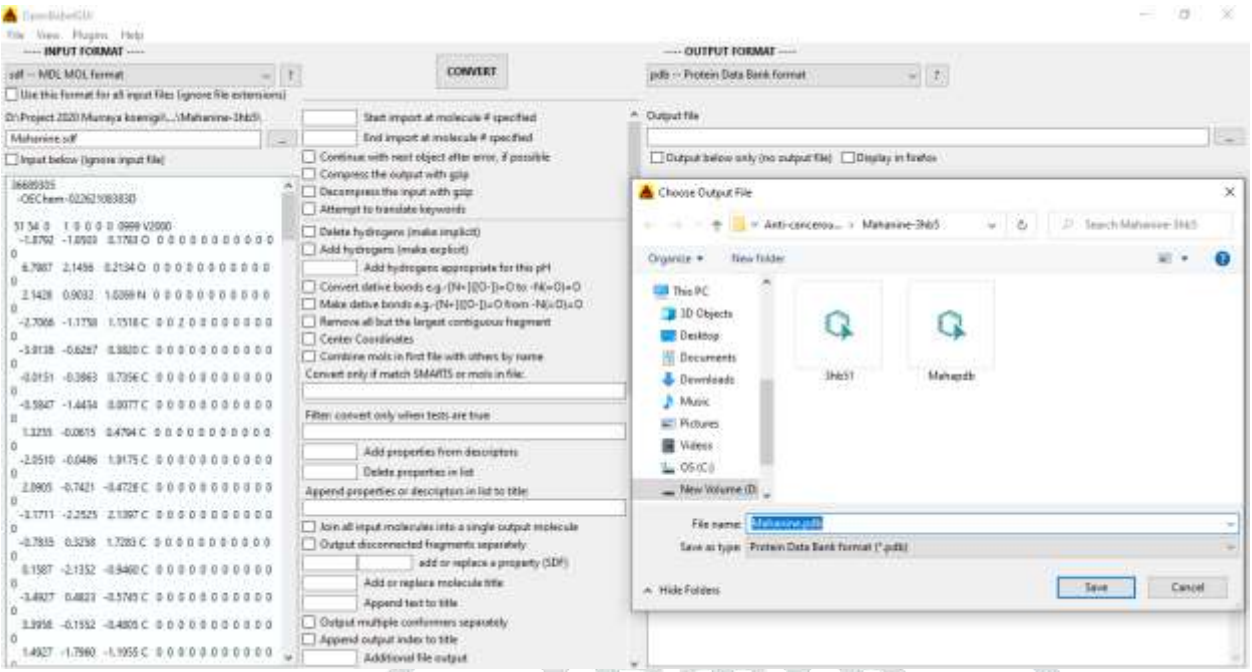


Figure 3.6 shows the conversion of Mahanine from sdf format to pdb format in Open Babel GUI application

3.2.3 Preparation of Pdbqt format for protein and ligand (protein.pdbqt and ligand.pdbqt)

The Pdbqt(s) of the protein and the ligand were prepared by using the Autodock tools software downloaded from MGL tools.

i) Preparation of Pdbqt file for Protein

- Remove the water molecule from the target receptor as it forms unwanted bond with other molecules (ligand) of interest.
- Add polar hydrogen group to the protein to stabilize it.
- Add the kollhman charges.
- Compute gasteiger charges to the protein.
- Save in pdbqt format.
- Know the X, Y, Z dimension of the active site for performing the site-specific docking.

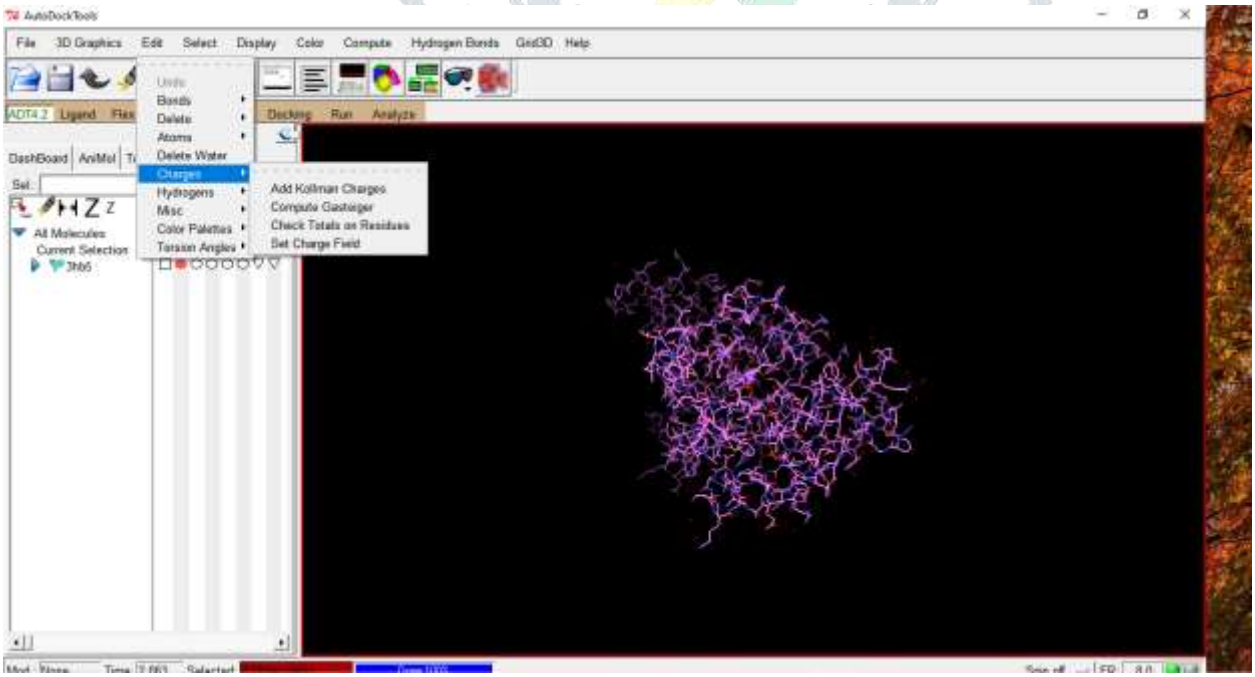


Figure 3.7 shows the addition of charges to the oestradiol protein in Auto dock Tools application

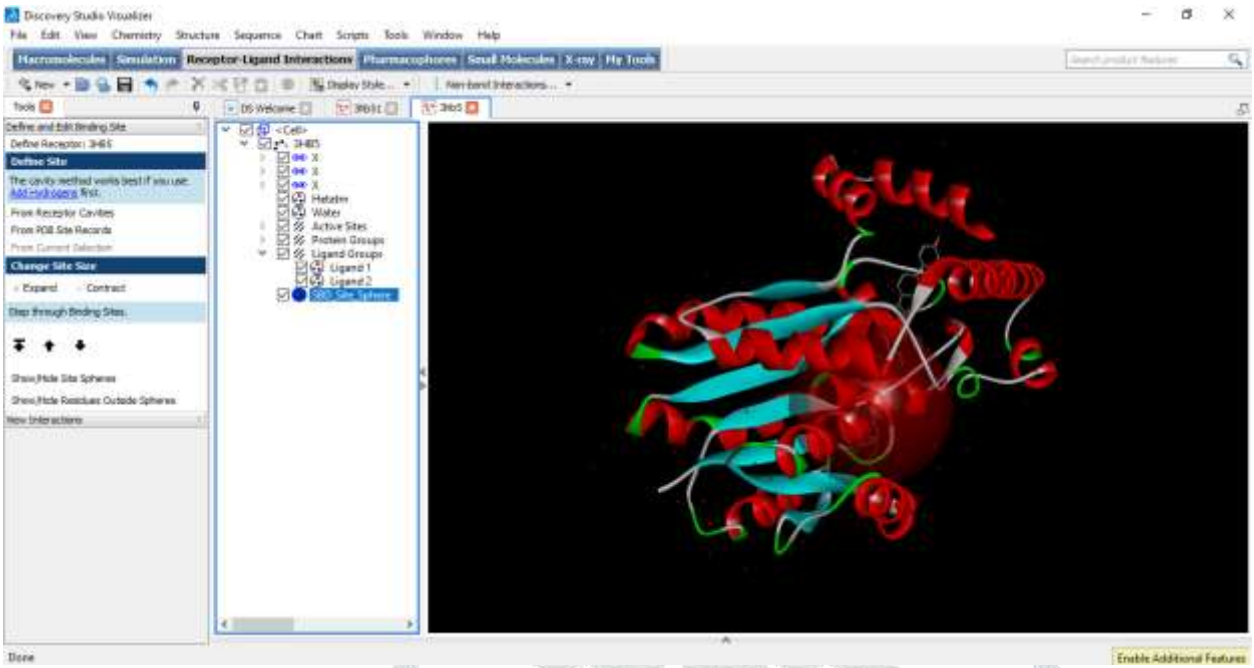


Figure 3.8 shows SBD Site Sphere inside which docking has to be processed in Discovery Studio Visualizer

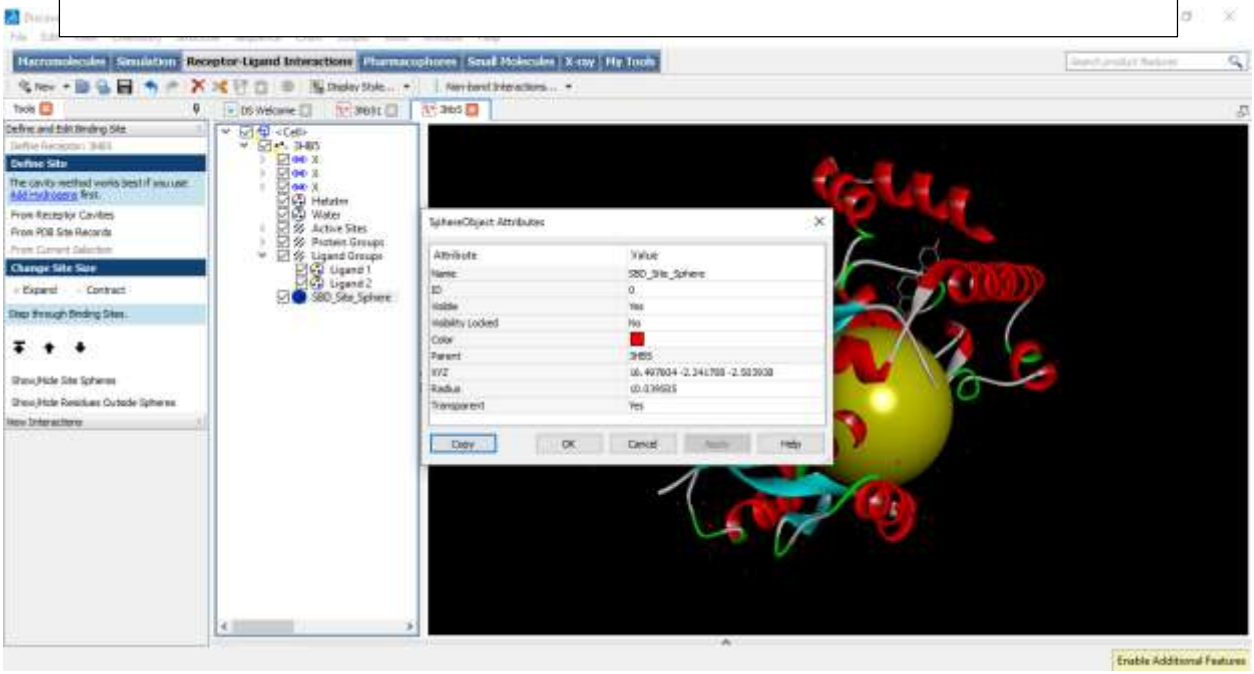


Figure 3.9 shows the Attributes of the site where Ligand was attached to the protein oestradiol

ii) Preparation of PDBQT file for Ligand

- Choose torsion root.
- Detect torsion root where docking has to be processed.
- Save in pdbqt format.

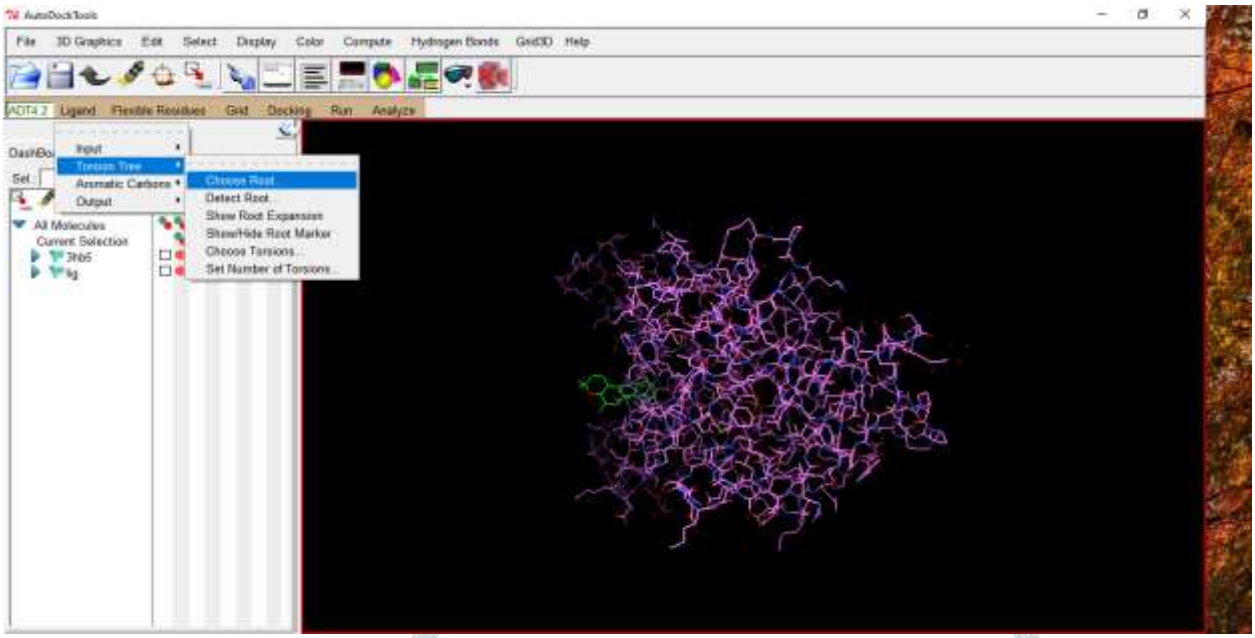


Figure 3.10 Choose and detect root where docking would be processed in the ligand

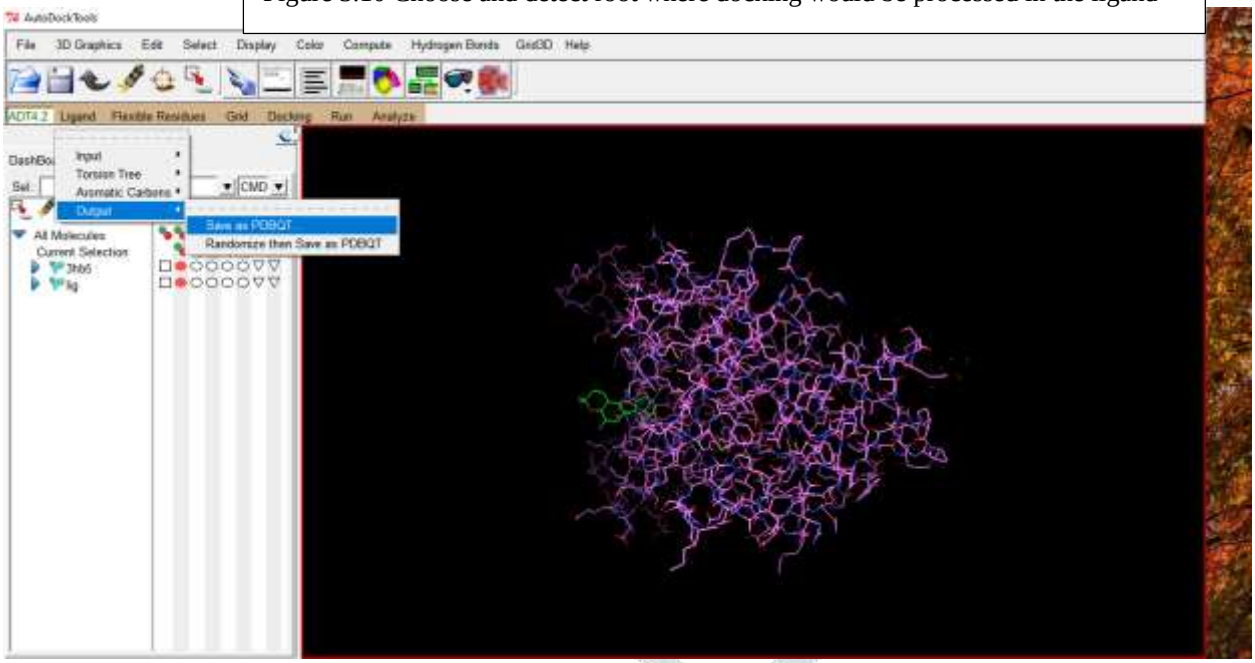


Figure 3.11 Then Save Ligand in pbdqt format shown above

Now we prepared the text file providing all the details about the pdbqt files of protein and its ligand and the attributes of SBD site sphere.

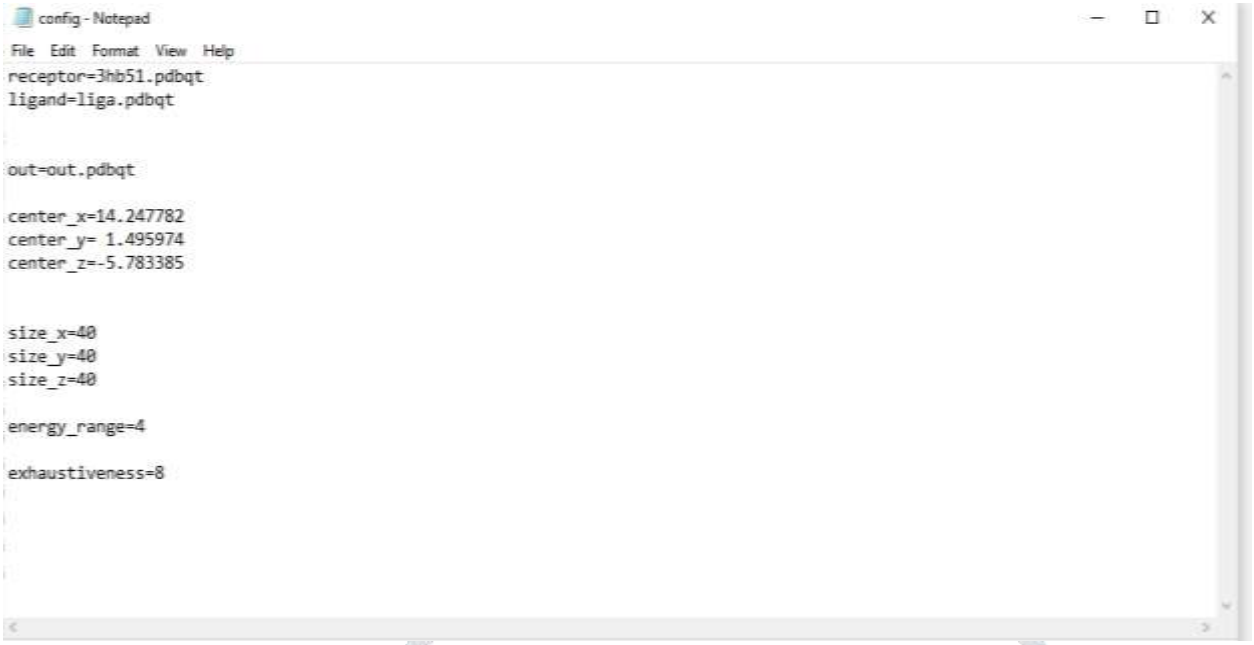


Figure 3.12 shows the configuration file which will be compiled and run

3.2.4 Molecular Docking using Autodock vina

- Set the path for auto-dock compilation where we placed the prepared file in the following manner.
- Give command as follows…
- vina.exe –config config.txt –log log.txt.

vina_split.exe –input out.pdbqt

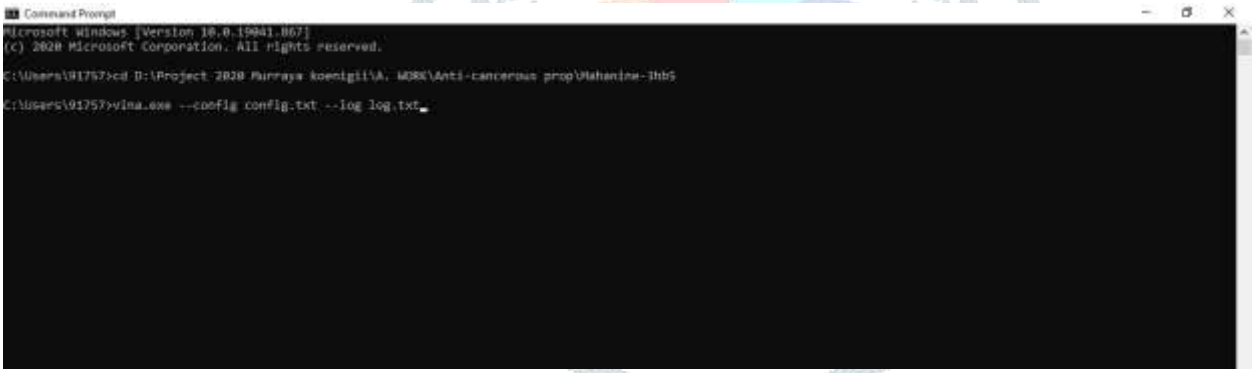


Figure 3.13 shows the Command Prompt dialog box

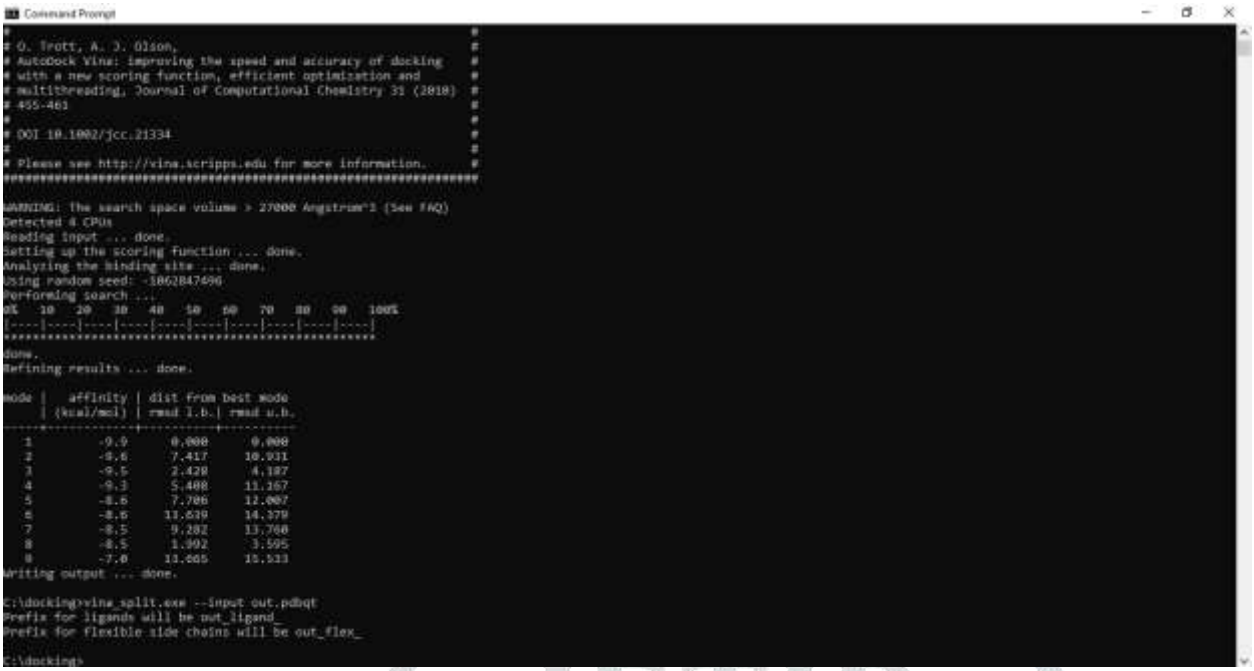


Figure 3.14 shows the Docking results of Ligand Mahanine and Protein oestradiol.

3.2.5 Analysis of molecular docking with Discovery Studio Visualizer to have information about the various conformations of the ligand on the protein.

- See the different interactions between the protein and the ligand.
- Calculate the distance between the amino acid residues and the ligand.
- Find out the different bonds involved in the interaction between the ligand and the protein.
- Find the best pose of the ligand during the interactions between the ligand and the protein.
- See the 2-D interaction between the ligand and the protein.
- Look for the amino acids' residues involved in the bond formation between ligand and the respective protein.

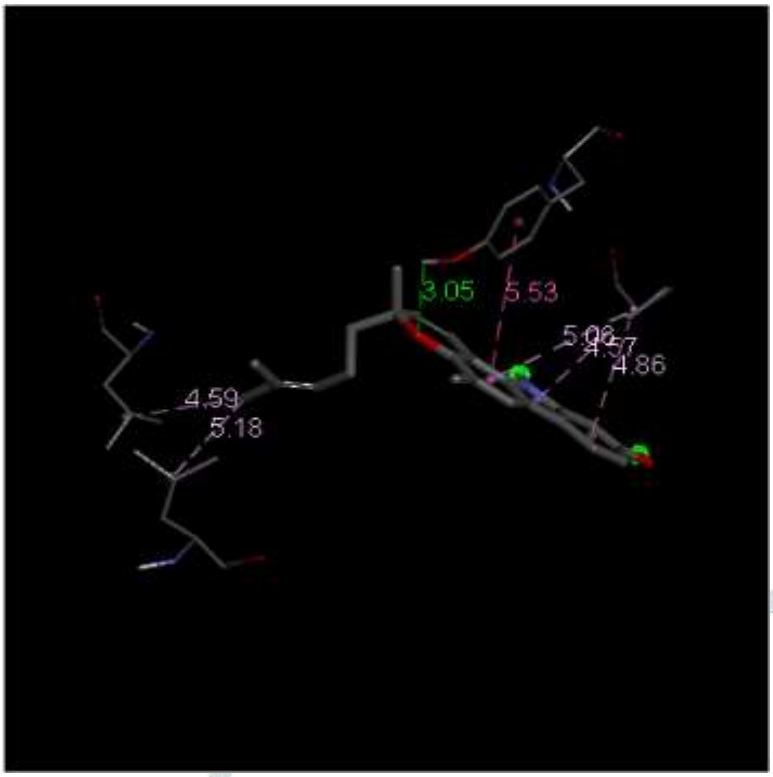


Fig 3.15 This figure shows the distance of the bond lengths between the mahanine molecule and and amino acid residues of protein HER-2.

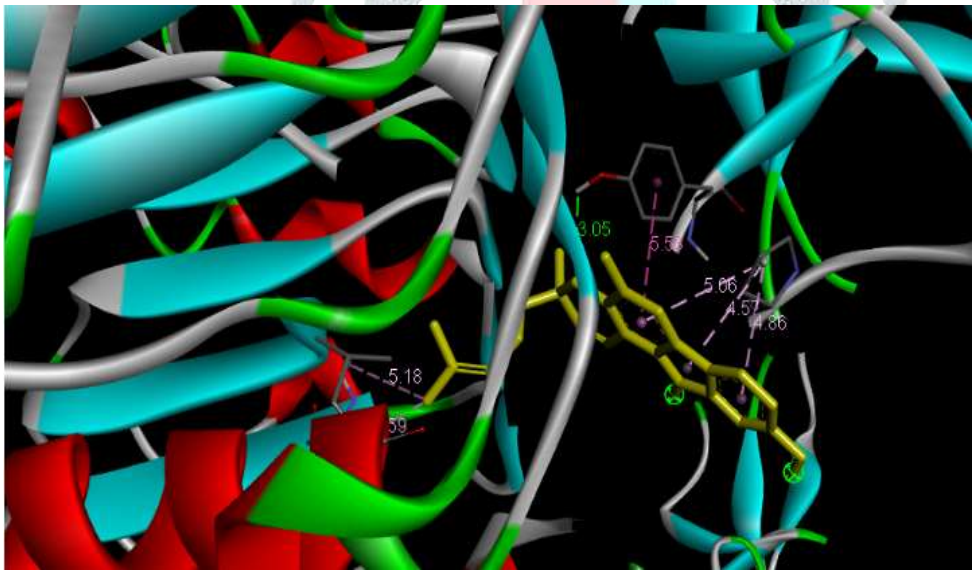


Fig 3.16. Shows the interaction of Mahanine and HER-2 protein.

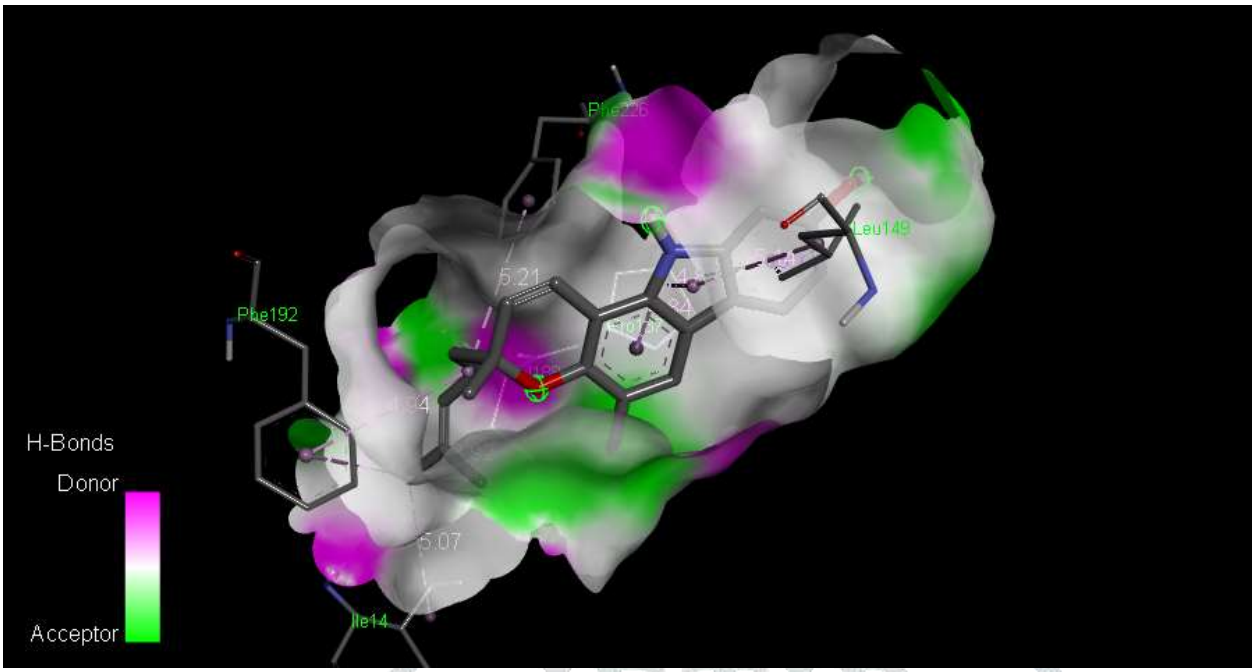


Figure 3.17 shows the H-Bonds of the docked Girinimbine and Oestradiol.

4. RESULTS AND DISCUSSIONS

All of these compounds have been shown to be potent anti-cancerous properties. Docking studies were performed for breast cancerous proteins with three marker compounds. The interaction of protein and ligands for the docked ligands with least binding energy was calculated.

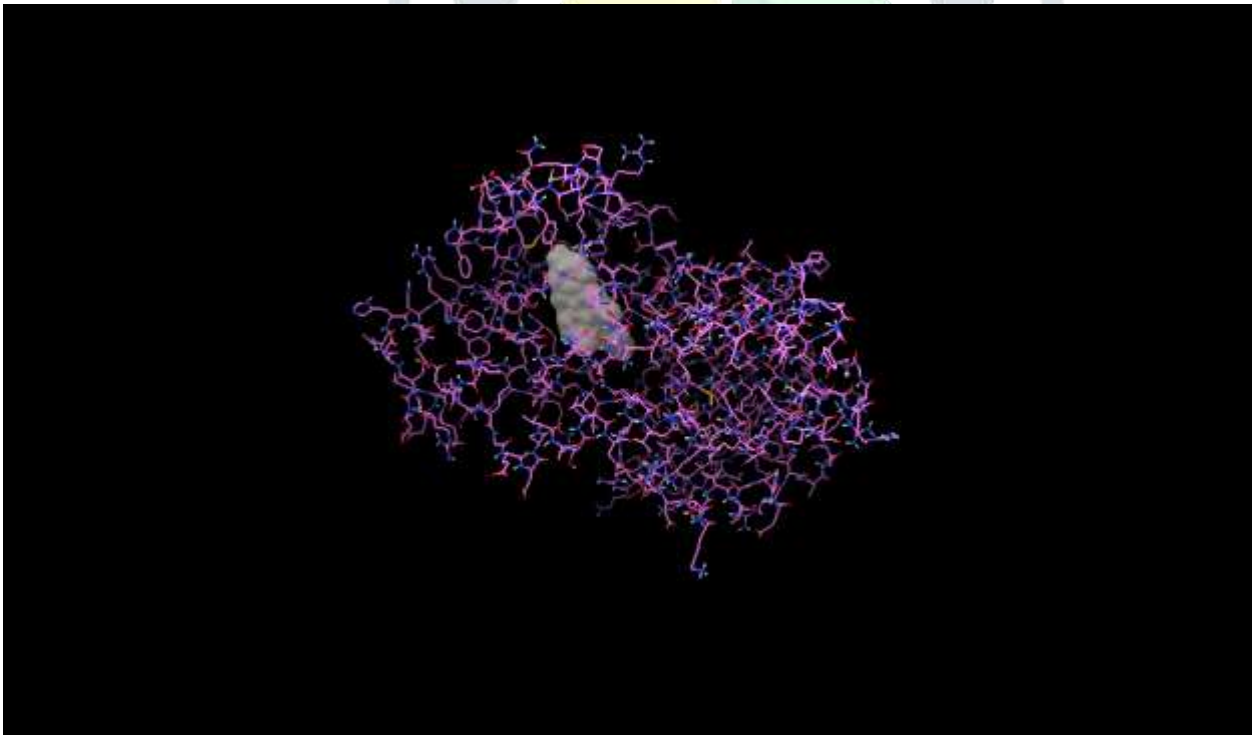


Figure 4.1 shows the Molecular docking between Oestradiol and Girinimbine

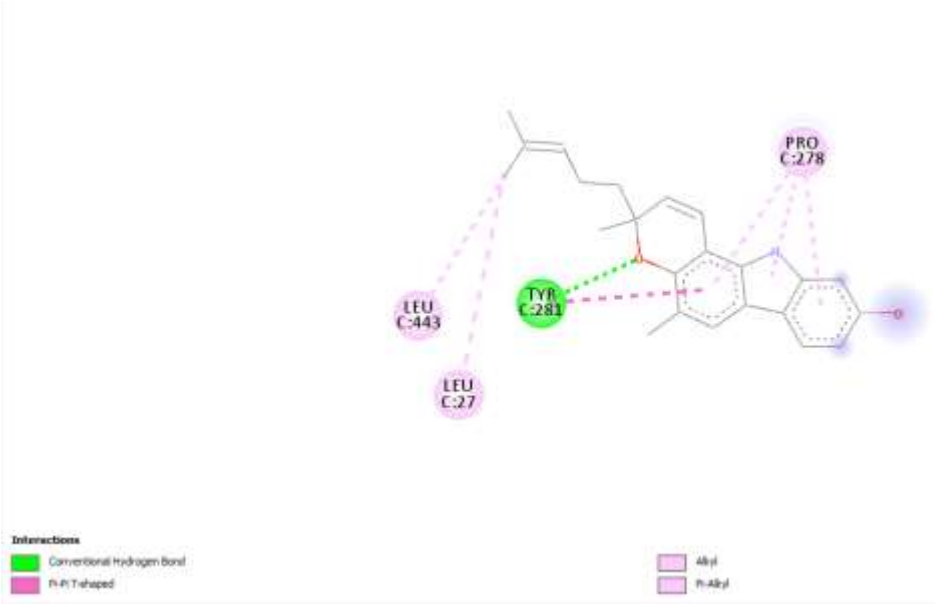


Figure 4.2 shows the 2-D interaction of Ligand Mahanine and HER2 protein

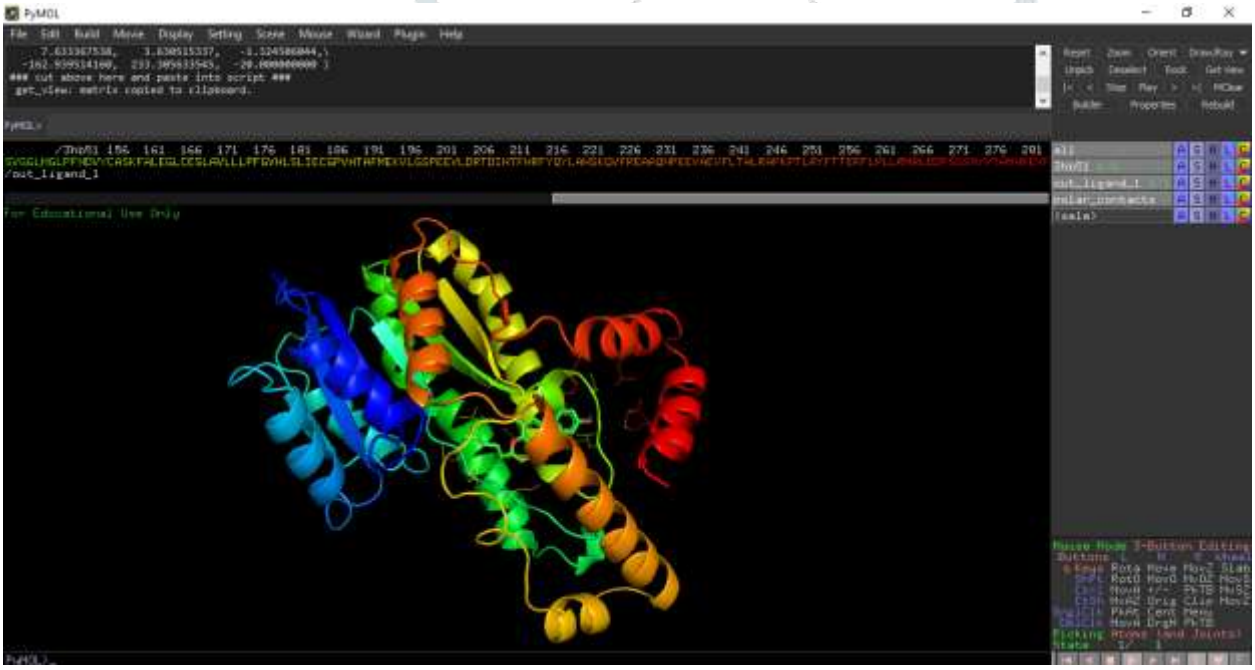


Figure 4.3 shows the molecular docking between the ligand Girinimbine and oestradiol protein

TABLE

S. NO.	COMPOUNDS	PROTEINS	BINDING ENERGY(kcal/mol)
1.	Girinimbine (PubChem CID:96943)	Oestradiol (PDB ID:3HB5)	-9.1
		NUDT5 (PDB ID:5NQR)	-9.5
		HER2 (PDB ID:1N8Z)	-8.6
2.	Mahanine (PubChem CID:36689305)	Oestradiol(PDB ID:3HB5)	-9.9
		NUDT5(PDB ID:5NQR)	-8.2
		HER2 (PDB ID:1N8Z)	-9.6
3.	Pyrayafoline D (PubChem CID:375148)	Oestradiol(PDB ID:3HB5)	-9.9
		NUDT5(PDB ID:5NQR)	-7.3
		HER2 (PDB ID:1N8Z)	-9.2

Table 1. This table depicts the binding energy between proteins and their respective ligands.

5. CONCLUSION

Mahanine and Pyrayafoline D showed least binding energy with the breast cancerous proteins. The present study concludes that the Murraya koenigii may serve as a potential source of bioactive compounds in the prevention of cancer. The potential for developing anticancerous drugs from higher plants appears rewarding as it leads to the development of new drugs which is required today.

In future research work the molecular modelling and molecular dynamic simulations of the protein-ligand complex can be performed and the ADME/T (Absorption, Distribution, Metabolism, Excretion/Toxicity) properties of these compounds can be tested in wet lab and research can be proceeded for clinical trials. In future, research work can be used further in clinical trials to test its effectiveness and for social benefit thus reducing the time and cost in drug discovery process.

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