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Molecular Docking Studies of Phytocompounds from *Phyllanthus niruri* against HER-2

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KEYWORDS

Phyllanthus niruri, Anti-cancer activity, Breast cancer, HER2, Docking studies

A B S T R A C T

Phyllanthus niruri is an important plant of Indian ayurvedic system of medicine. It is used for problems of the stomach, genitourinary system, liver, kidney, and spleen. It is a widespread tropical plant commonly found in coastal area, known by the common names gale of the wind, stone breaker or seed-under-leaf. It has wide variety of phytochemicals and pharmacological properties. Some of the most intriguing therapeutic properties include Anti-tumour, anti-hepatotoxic, anti-hypertensive, anti-HIV and anti-hepatitis B. The compounds with Anti-cancer activity in *Phyllanthus niruri* are Quercetin, Catechin Gallocatechin, Terpenes, Lupeol, Coumarins, Ethylbrevifolin carboxylate, Corilagin, β-sitosterol, phyllochrysine. Breast cancer is the most common invasive cancer in women. HER2 protein is one of the molecular abnormalities linked to the development of breast cancer. HER2-positive breast cancer accounts for 20–30% of hormone receptor-positive breast cancer that relates to the overexpression of HER2/neu protein. HER-2 positive treatment breast cancer is a breast cancer that tests positive for a protein called human epidermal growth factor receptor 2, which promotes the growth of cancer. The human epidermal growth factor receptor 2 (HER2) is a validated target in breast cancer therapy. The bioactive compounds from *Phyllanthus niruri* were docked against HER-2 protein using Auto Dock software. The docking scores and analysis of the interactions of the phytocompounds like corilagin, Quercetin, Estradiol and Ethylbrevifolin carboxylate with target protein suggest that HER2 may cure breast cancer.

Introduction

Phyllanthus niruri has a long history in herbal medicine systems such as Indian Ayurveda (Chopra *et al.*, 1986). The common name for *Phyllanthus niruri* is gale of the wind, stone breaker, or seed-under-leaf. It is a relative of the splurges, belonging to the *Phyllantus* genus of family *Phyllanthuceae*. Its grows 50-70 cm tall and bears ascending herbaceous branches. The bark is smooth and light green and bears numerous pale green flowers which are often flushed with red. The fruits are tiny,

smooth and capsules containing seeds. Among the *phyllanthus* species, *P.niruri* is a small erect annual herb growing up to 30-40cm in height and is indigenous to the amazon rainforest and other tropical areas (Girach *et al.,* 1994). *P.niruri* has been attributed to include lignans, tanins, coumarins, terpenes, flavonoids, alkaloids, saponins, and phenylpropanoids, which have been found in the leaves, stem and roots of this plant. Common lipids, sterols and flavonols also occur in the plant (Dhar *et al.,* 1968) shown in Figure: 1 Represent the plant *Phyllanthus niruri*

Subsequent studies revealed the preclinical pharmacological activity and therapeutic potential of phytochemicals isolated from *P.niruri*. The phytochemicals exhibited different structural characteristics with various pharmacological actions: lignans had excellent hepatoprotective and Anti-viral properties, whereas terpenes exhibited Anti-cancer, as well as Anti-microbial activity. Flavonoids from *P.niruri* showed Anti-oxidant activity and the alkaloids exhibited Anti-spasmodic activity.

The compounds with Anti-cancer activity in *Phyllanthus niruri* are Quercetin, Catechin Gallocatechin, Terpenes, Lupeol, Coumarins, Ethylbrevifolin carboxylate, Corilagin, β-sitosterol, phyllochrysine.

Breast cancer is a malignant tumor arising from the cells of the breast**.** Although breast cancer predominantly occurs in women, it can also affect men. After puberty, a women's breast consist of fat, connective tissues and thousands of lobules, tiny glands that produce milk for Breast feeding.

There are many types of breast cancer that differ in their capability of spreading to other body tissues. Showed in Figure: 2 Represent the Brest cancer.

HER2 protein is one of the molecular abnormalities linked to the development of breast cancer. The human epidermal growth factor receptor (HER) family of receptors plays a central role in the pathogenesis of several human cancers. They regulate cell growth survival, and differentiation via multiple signal transduction pathways and participates in cellular proliferation and differentiation. Its expressed in many tissues and its major role in this tissues is to facilitate uncontrolled cell growth and tumorigenesis. For about 20% women with breast cancer, the cancer cells test positive for HER-2. Its growth promoting protein located on the surface of some cancer cell. HER-2 positive breast cancers tend to grow more rapidly and spread more aggressively. Figure: 3 showed Mechanism of HER-2 Protein

The docking analysis of the target protein with the ligands was performed using AutoDock software. It used to dock the HER-2 protein extracellular domain.

From the six compounds that were docked to HER-2 protein, Ethylbreviflolin carboxylate show the least binding energy and show the best Anti-cancer activity.

Materials and Methods

Protein Preparation

PDB (Protein Data Bank)

The protein data bank is a crystallographic database for the three-dimensional structure data of large biological molecules, such as proteins and nucleic acid.

The data, typically obtained by X-ray crystallography, NMR, spectroscopy. The PDB is overseen by an organization called the worldwide Protein data Bank, (https://www.rcsb.org/).

UNIPORT

Uniport is a freely accessible database of protein sequence and functional information, many entries being derived from genome sequencing projects.

It contains a large amount of information about the biological functions of protein derived from the research literature.

Ligand Preparation

Pubchem

Pubchem is a database of chemical molecules and including chemical structure, name fragments, chemical formula, molecular weight, XLogP, and hydrogen bond donor and acceptor count. The system maintained by the national center of biotechnology information, a component of the national library of medicine, which is part of the united states National Institute of Health. Millions of compound structures descriptive datasets can be freely download via FTP. More than 80 database vendors contribute to growing pubchem database.

PyMol

PyMol is a computer software, a molecular visualization tool use in structural biology. Pymol can produce High-Quality 3D images of small molecules and biological macromolecules, such as protein.

ACD/Chemsketch

Chemsketch is a molecular modeling program used to create and modify images of chemical structures. There is a software allows molecules and molecular models displayed in two and three dimensional, to understand the structure of chemical bonds and the nature of the functional groups.

Open babel

Open babel is a computer software, a chemical expert system mainly used to interconvert chemical file formats. Due to the strong relationship to in f0ormat is this program belongs more to the category cheminformatics than to molecular modeling. It is available for windows, unix, linux, macos and android.

Rasmol

Rasmol is a computer program written for molecular graphics visualization intended and used mainly to depict and explore biological macromolecule structures, such as those found in the Protein Data Bank. It was originally developed by Roger Sayle in the early 1990s. Protein Data Bank (PDB) files can be downloaded for visualization from members of the Worldwide Protein Data Bank (https://www.rcsb.org/). These have been uploaded by researchers who have characterized the structure of molecules usually by X-ray crystallography, protein NMR spectroscopy, or cryo-electron microscopy.

Results and Discussion

Protein Structure Preparation

Sequence Retrieval

The sequence of HER-2 (Human Epidermal Growth Factor Receptor-2) is retrieved from UNIPORT database and sequence accession number is Q9NuP9 Homosapiens (Human).

Structural Retrieval

The Three Dimensional structure (Crystal Structure) of HER-2 is derived from PDB (Protein Data Bank) database. From the

PDB databank (Berman *et al.*, 2000), the PDB file was collected and the PDB ID was 3LRA.

The structure was visualized using Rasmol. White colour showing alpha helix, Pink colour showing beta sheets and turns.

Preparation of Ligands

The Six Compounds (Corilagin, Ethylbrevifolin Carboxylate, Estrodiol, Quercetin, Rutin, Catechin Gallocatechin) which shows Anti-cancer activity from *Phyllanthus niruri* is used as ligands and was retrieved from NCBI (National Center for Biotechnology Information) pubchem compound database (Bolton *et al.*, 2008) (http//pubchem.ncbi.nlm.nih.gov/).

For further docking analysis, the ligands were designed using Chemsketch and their 2D structure was converted to 3D structure. These data are saved as a molecular format file (MDL Mol format).

The molecular format converter tool (Open babel) is used to convert this file into the PDB format and is used to during Docking analysis.

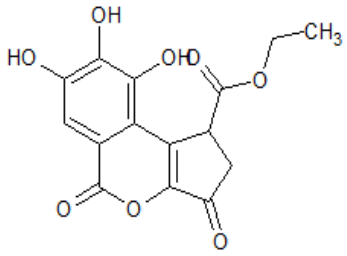
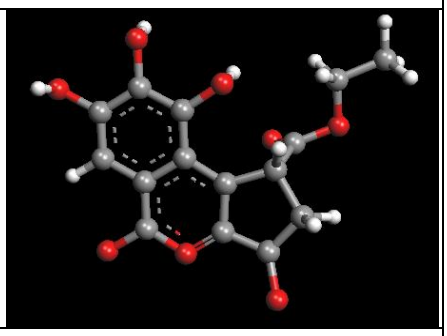
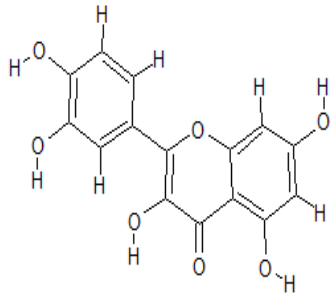
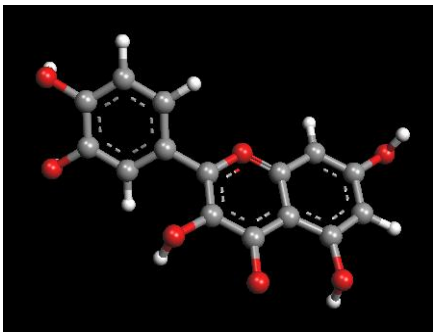
The 2D, 3D structure and molecular formula of Corilagin, Ethylbrevifolin Carboxylate, Estrodiol, Quercetin, Rutin, Catechin Gallocatechin compounds of *Phyllanthus niruri* shown in Table: 1

Molecular Docking Studies

AutoDocking

AutoDock is a molecular modeling simulation software. It is especially effective for protein-ligand docking. AutoDock 4 is available under the GNU General Public License.

Table.1 Showing the compounds extracted from Phyllanthus niruri

S.No	COMPOUND NAME	MOLECULAR FORMULA	2D STRUCTURE	3D STRUCTURE
1.	Ethylbrevifolin carboxylate	$C_{15}H_{12}O_8$		
2.	Qurcetin	$C_{15}H_{10}O_7$		

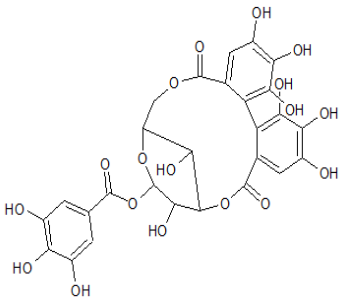
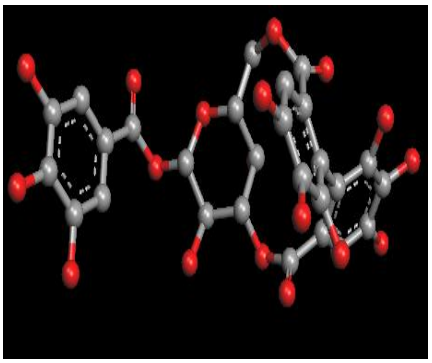
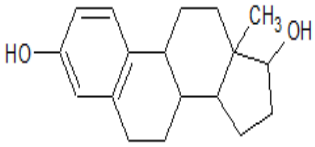
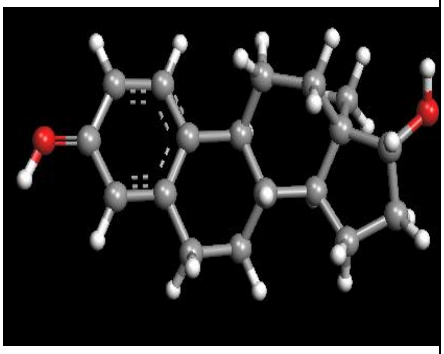
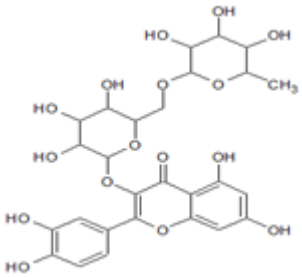
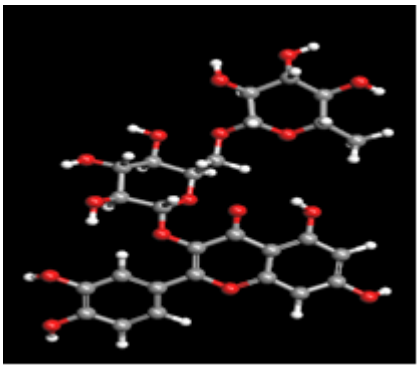
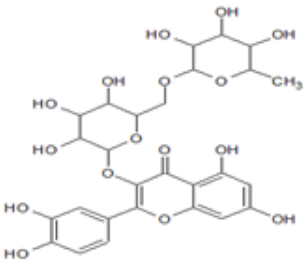
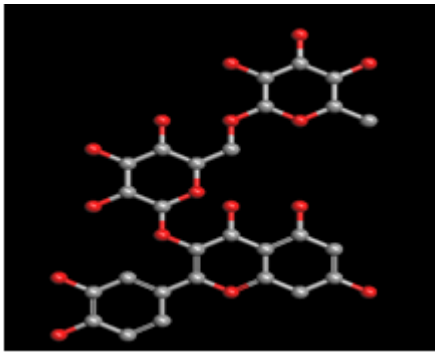
3.	Corilagin	$C_{27}H_{22}O_8$		
4.	Estradiol	$C_{18}H_{24}O_2$		
5.	Rutin	$C_{27}H_{30}O_{16}$		
6.	Catechin Gallocatechin	$C_{30}H_{26}O_{13}$		

Table.2 Molecular Properties of the Ligands

S.No	Compounds	Molecular weight	Hydrogen bond donar	Hydrogen bond acceptor
1.	Corilagin	634.455 g/mol	11	18
2.	Ethylbrevifolin carboxylate	320.253 g/mol	3	8
3.	Estradiol	272.388 g/mol	2	2
4.	Quercetin	302.238 g/mol	5	7
5.	Rutin	610.521 g/mol	10	16
6.	Catechin Gallocatechin	594.525 g/mol	11	13

Table.3 Interaction between atoms of the ligands from *Phyllanthus niruri* and the amino acid residues of HER-2 protein along with the hydrogen bond distance and docking score

S.no	ligand	HER-2 protein		Ligand Atom	Distance (Å)	Docking Score Residue Atom (kcal/mol)
		Residue	Atom			
1.	Corilagin	ASN- 144 LEU-189 LEU-189 VAL-241	O O O O	H H H H	2.5 2.2 1.7 2.2	-6.03
2.	Ethylbrevifolin carboxylate	GLU-126 ASN-183 THR-178 GLU-126	OE2 N O OE2	H O O H	1.9 2.6 2.6 2.0	-5.85
3.	Estradiol	LEU 189 GLU 126	O NE2	H H	2.1 2.5	-7.15
4.	Quercetin	ASP-185 GLU-126 GLU-126	N NE2 OE2	O H H	3.4 1.9 1.9	-5.25
5.	Rutin	VAL-244 VAL-241 LEU-189 VAL-188 TYR-184 VAL-188	O O O O N O	O O O O O O	3.6 3.2 3.6 3.5 2.9 2.9	-9.56
6.	Catechin Gallocatechin	PHE-237 VAL-241 THR-178	O O O	H H H	2.7 3.3 2.8	-7.13

Fig.1 *Phyllanthus niruri*

Fig.2 Breast cancer

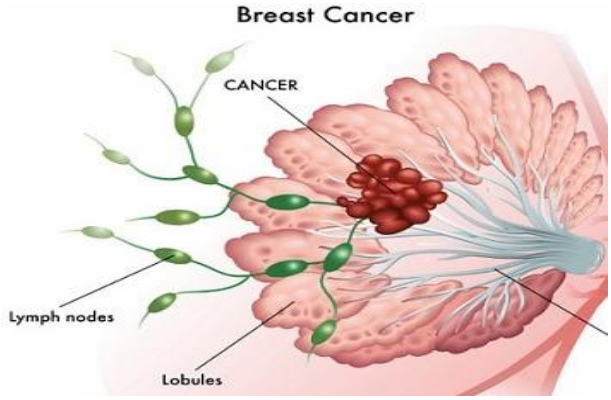


Fig.3 Mechanism of HER-2 Protein

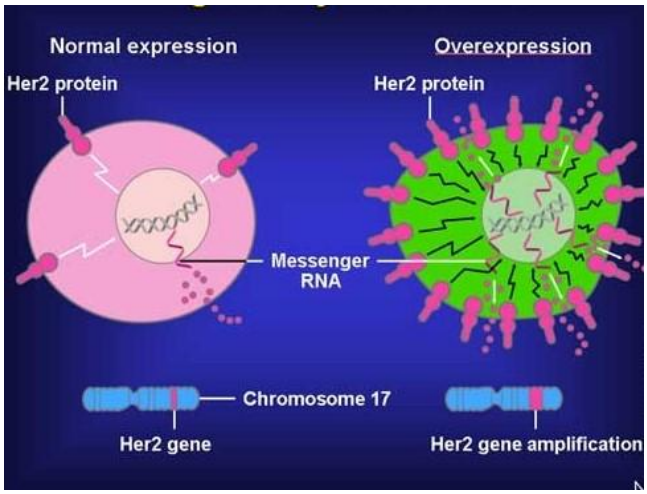


Fig.4 Crystal Structure of HER-2 Protein

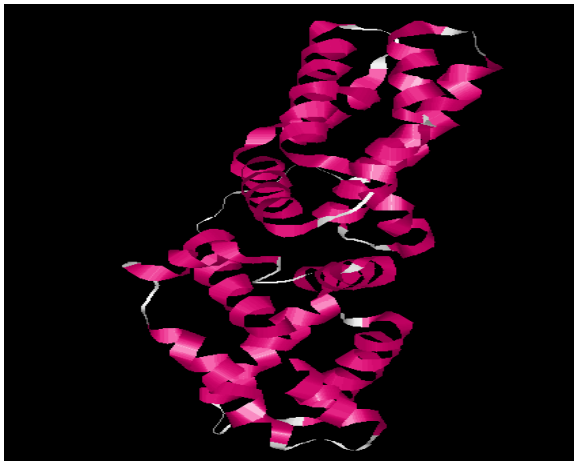
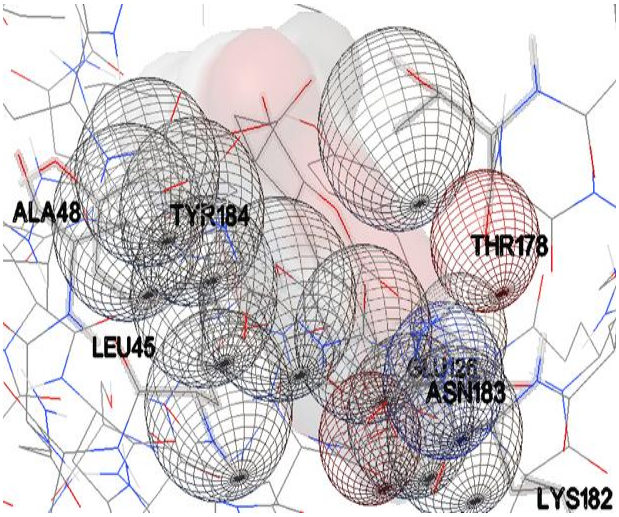


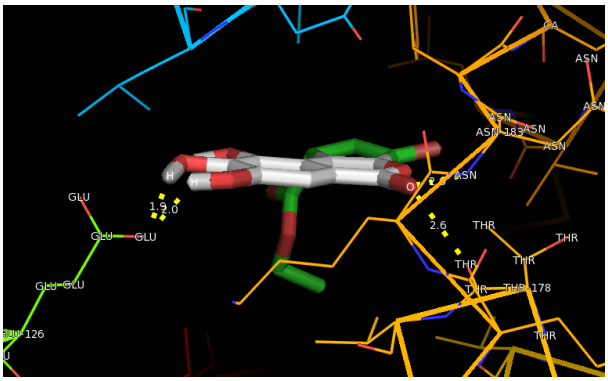
Fig.1 (a) Docking score (b) Interactions between HER-2 and Ethylbrevifolin carboxylate visualized using Autodock and (c) Visualization of hydrogen interaction between HER-2 and Ethylbrevifolin carboxylate using PyMol

binding_energy=-5.85
ligand_efficiency=-0.25
inhib_constant=51.72
inhib_constant_units=uM
intermol_energy=-7.64
vdw_hb_desolv_energy=-7.2
electrostatic_energy=-0.43
total_internal=-2.26
torsional_energy=1.79
unbound_energy=-2.26
filename=best.dlg
clRMS=0.0
refRMS=75.42
rseed1=None
rseed2=None
2 hydrogen bonds formed:
ethylbio-3:A:MOL0:H : 3lra_2:A:GLU126:OE2
ethylbio-3:A:MOL0:H : 3lra_2:A:GLU126:OE2

(a)

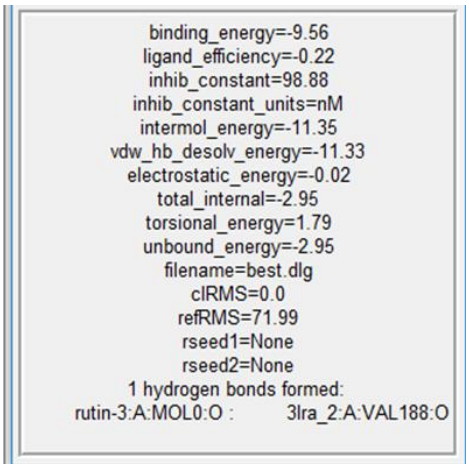


(b)

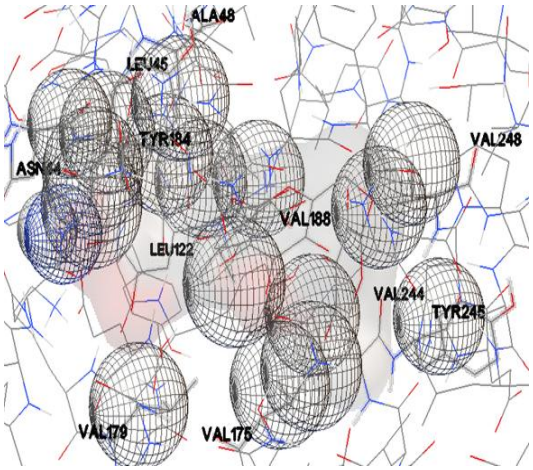


(c)

Fig.2 (a) Docking score (b) Interactions between HER-2 and Rutin visualized using Autodock and (c) Visualization of hydrogen interaction between HER-2 and Rutin using PyMol



(a)



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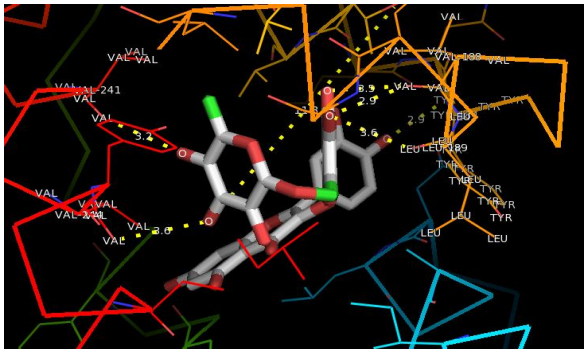
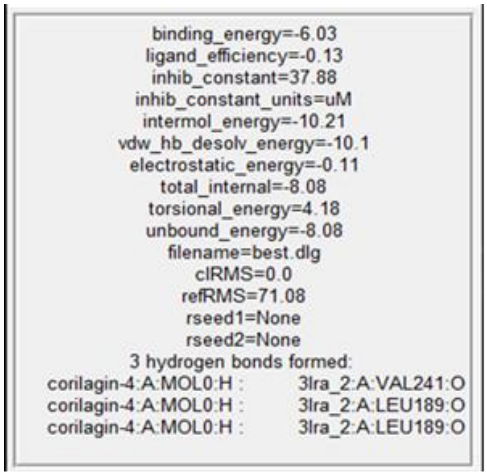
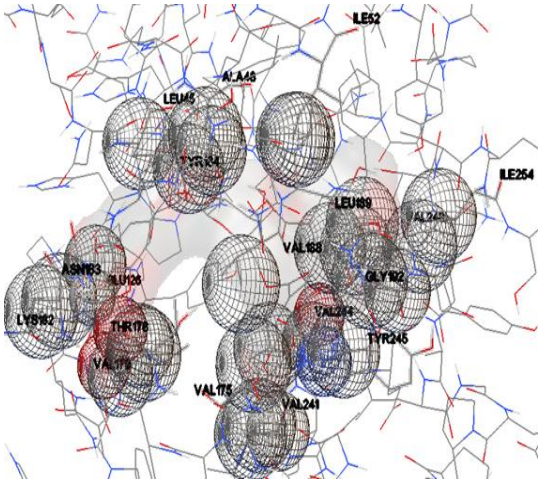


(c)

Fig.3 (a) Docking score (b) Interactions between HER-2 and Corilagin visualized using Autodock and (c) Visualization of hydrogen interaction between HER-2 and Corilagin using PyMol



(a)



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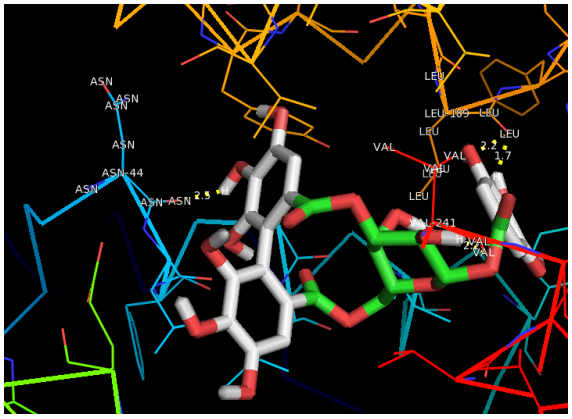
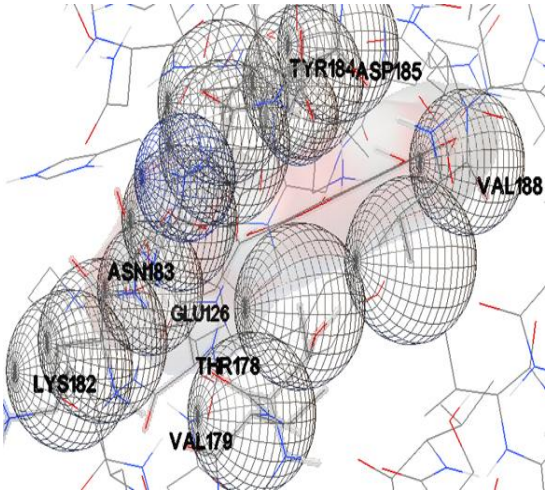


(c)

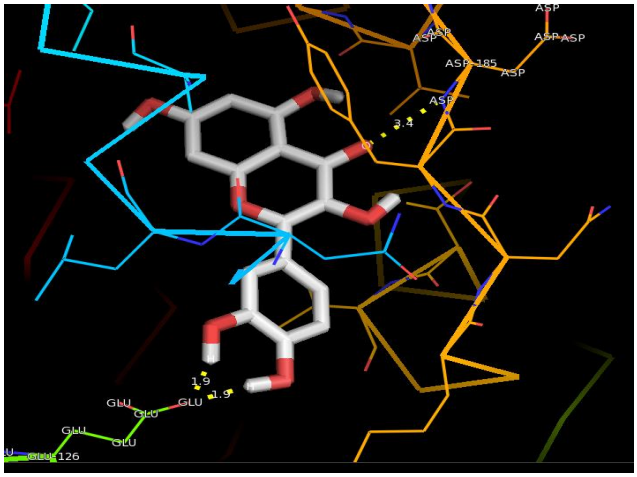
Fig.4 (a) Docking score (b) Interactions between HER-2 and Quercetin visualized using Autodock and (c) Visualization of hydrogen interaction between HER-2 and Quercetin using PyMol

binding_energy=-5.25
ligand_efficiency=-0.24
inhib_constant=142.93
inhib_constant_units=uM
intermol_energy=-7.04
vdw_hb_desolv_energy=-6.57
electrostatic_energy=-0.46
total_internal=-2.64
torsional_energy=1.79
unbound_energy=-2.64
filename=best.dlg
clRMS=0.0
refRMS=74.4
rseed1=None
rseed2=None
2 hydrogen bonds formed:
Quercetincompound-3:A:MOL0:H : 3lra_2:A:GLU126:OE2
Quercetincompound-3:A:MOL0:H : 3lra_2:A:GLU126:OE2

(a)



(b)



(c)

Fig.5 (a) Docking score (b) Interactions between HER-2 and Estradiol visualized using Autodock and (c) Visualization of hydrogen interaction between HER-2 and Estradiol using PyMol

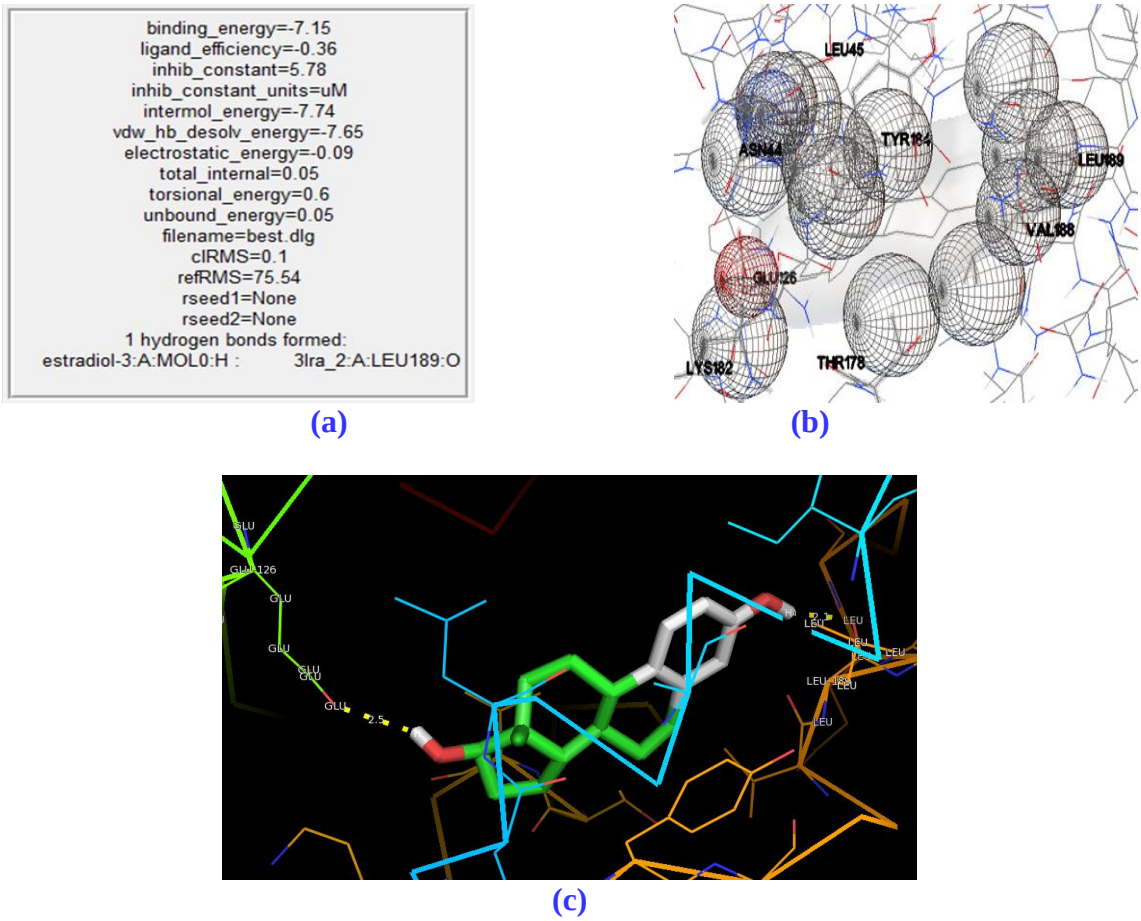
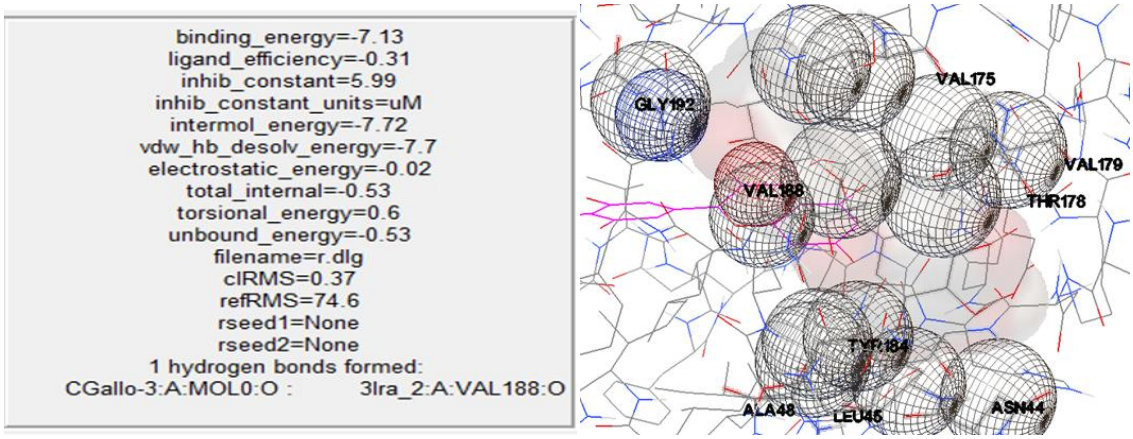
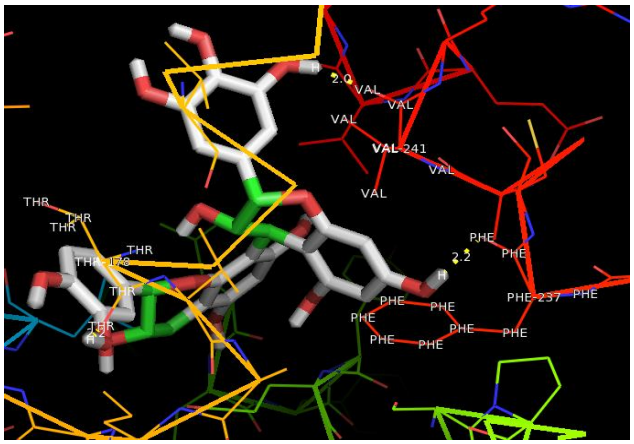


Fig.6 (a) Docking score (b) Interactions between HER-2 and Catechin Gallocatechin visualized using Autodock and (c) Visualization of hydrogen interaction between HER-2 and Catechin Gallocatechin using PyMol





(c)

Molecular Docking study of the *Phyllanthus niruri* against HER-2 Protein. The *Phyllanthus niruri* Compounds (Corilagin, Ethylbrevifolin Carboxylate, Estrodiol, Quercetin, Rutin, Catechin Gallocatechin) are docked against HER-2 Protein.

The Graphical User Interface program ‘‘AutoDock Tools’’ was used to prepare, run, and analyse the docking simulations. Koll man atom charges, salvation parameters and polar hydrogens were added into the receptor PDB file for the preparation of protein in docking stimulation.

Auto Dock (Good sell, 1999; Jones, 1997; Rarey, 1996) required precalculated grid map, one for each atom type present in the flexible molecules being docked and its stores the potential energy arising from the interaction with rigid macromolecules. This grid must surround the region of interest in the rigid macromolecule. The grid box size was set at 126, 126, and 126A°(X, Y, and Z) to include all the amino acid residues that present in rigid macromolecules. Auto Grid 4.2 program, supplied with Auto Dock 4.2 was used to produce grid maps.

The docking study between Corilagin, Ethylbrevifolin Carboxylate, Estrodiol, Quercetin, Rutin, Catechin Gallocatechin compounds from *Phyllanthus niruri* against HER-2 receptor and ligand complex.

The docked structure were analysed and the interaction were seen. Hydrogen bond interactions and the binding distance between the donors and acceptors were measured foe the best conformers.

From the binding energy values, the Anti-cancer activity of ligand to the corresponding receptor was predicted. The Rutin is having least binding score (-9.56 Kcal/mol) than the Corilagin, Estrodiol, Quercetin, Catechin Gallocatechin, Ethylbrevifolin Carboxylate.

Therefore, this compound shows Good inhibion against HER-2 protein act as Anti-cancer activity agent.

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