Lupeol from *Lycopersicon esculentum* as a Potential Antagonist of the Poly(ADP-ribose) polymerase-1 (PARP-1) for cancer chemotherapy: an *in silico* Study.

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Abstract

Inhibitors (antagonist) of Poly(ADP-ribose) polymerase-1 (PARP-1) are used as therapeutics in cancers chemotherapy. The current drugs, Niraparib and Talazoparib used to induce apoptosis by evading necrosis (the clinical tractability of these drugs) have been reported to potentiate adversely effect on human body resulting into thrombocytopenic condition. In view of this, there is an urgent need for the discovery of novel compounds from health-friendly source. The aim of this study is to out-source for best-in-class inhibitor for this druggable target via computational tools. The purpose of this study was to analyze the inhibitory potential of lupeol by computational docking studies. For this, sixteen (16) chemical compounds (phytochemicals) obtained from *Lycopersicon esculentum* and retrieved from literatures were screened for their inhibitory effects on poly(ADP-ribose) polymerase-1 (PARP-1). Lupeol was the lead compound with a binding energy of -11.0kcal/mol. Computational docking analysis was performed using PyRx, AutoDock Vina option based on scoring functions and the target was validated so as to ensure that the right target was used for this analysis. These results indicated that lupeol could be one of the potential ligands to treat cancer.

Key Words: Poly(ADP-ribose) polymerase-1, apoptosis, thrombocytopenia, lupeol

INTRODUCTION

As deficiencies in DNA repair processes are associated with cancer vulnerability as has been seen in cancer-prone syndromes such as Xeroderma pigmentosum, Ataxia Telangiectasia (AT), Nijmegen Breakage Syndrome (NBS), and Fanconi's anemia, the inhibition of DNA repair and other damage response proteins by small-molecule inhibitors can potentially be exploited to sensitize tumor cells when used in combination with chemo- and radiotherapy or in certain genetic backgrounds[2]. Poly(ADP-ribose)polymerases (PARPs) are a family of enzymes that mediate the catalysis of ADP-ribose transfer from nicotinamide adenine dinucleotide (NAD+) onto acceptor proteins. Among this family, which consists of at least 17 members, PARP-1 is the most widely investigated. It functions primarily as a DNA repair factor, especially in base excision repair (BER)[1]. Overactivation of PARP-1 has been implicated in the onset of several cardiovascular and neurological diseases such as stroke, myocardial infarction, neurodegenerative disorder, and several other inflammatory processes. Due to the response of PARP-1 to DNA damage and its involvement in necrotic cell death, pharmacological action on PARP-1 activity may constitute a useful tool to increase the activity of DNA-binding antitumor drugs[3]. Inhibition of PARP-1 results into the accumulation of DNA damage, caused by some anti-tumor drugs, by impairing single-strand DNA break repair (SBR) and trapping PARP-1 at single-strand break sites, leading to inhibition of DNA replication and thus results into programmed cell death[7].

The resistance of drugs in tumor cells is a common drawback of cancer chemotherapy. Resistance which is often a multifactorial process consisting of reduced accumulation of drug, detoxification of the drug within the cell, enhanced DNA repair/tolerance and failure of apoptotic pathways [4]. Considering breast cancer, one of the potential mechanisms of resistance is genetic reversion of BRCA1–2 mutations. Secondary mutations in BRCA-deficient cells may restore BRCA function and thus enable the translation of a functional BRCA protein which can repair DNA damage[5]. However, inhibition of PARP-1 in cells exposed to DNA-damaging drugs would decrease DNA repair and would induce apoptotic cell death, decreasing necrotic cell death and preventing the pathological side effects of necrosis. It is interesting to note that PARP-1 inhibitors might be more effective against tumor cells than against normal cells[3].

PARP inhibitors constitute a new set of anticancer drugs which have evolved swiftly since they were first developed in 2005. They target tumors that have deficits in homologous recombination repair (such as *BRCA* mutations) by a process known as synthetic lethality; therein, neither the deficiency in homologous recombination repair nor PARP inhibition alone is cytotoxic, but the combination of the two leads to cell death [6].

From recent studies, it can be shown that diets rich in phytochemicals can significantly decrease the risk of cancer by as much as 20%[9,14,15]. Epidemiological data suggest that the phytosterols (such as lupeol) content of the diet is associated with a reduction in common cancers including cancers of the colon, breast, and prostate[12]. Lupeol was found to suppress tumor growth, impair cancer cell invasion by targeting NFκB signaling[11], sensitize cancer cells to cisplatin chemotherapy in an orthotopic metastasis. Lupeol treatment was shown to dramatically suppressed local metastasis and that this effect was more than cisplatin alone [12].

Niraparib and talazoparib induces apoptosis by inhibiting PARP-1 and thus useful in the treatment of cancer. Since thrombocytopenia is common with niraparib and talazoparib[17], it becomes imperative to research on an alternative drug-gable compound that offers better potency with little or no side effect.

Poly (ADP-ribose) polymerase 1 (PARP1) has an increased attraction as a target for anticancer therapeutics whether in preclinical studies or clinical trials. In this study, we utilized *in-silico* approach which provided a high-quality interaction between the ligand (lupeol) and the receptor (PARP-1). Lupeol was then channelled to Lipinski rule of five on ADMET (Adsorption, Distribution, Metabolism, Excretion and Toxicity) properties and was found to fulfill the rule of five on ADMET properties.

MATERIALS AND METHODS

Ligand selection and preparation

The chemical structures of Sixteen (16) phytochemicals were obtained from PubChem compound database (https://pubchem.ncbi.nlm.nih.gov). The MOL SDF format of these ligands were converted to PDBQT file using PyRx tool to generate atomic coordinates and energy was minimized by optimization using the optimization algorithm at force field set at mmff94 (required) on PyRx.

Accession and preparation of the target protein

The protein poly(ADP-ribose) polymerase-1 (parp-1) was prepared by retrieving the three-dimension crystal structure of Human constitutively active PARP-1 in complex with ligand olaparib (PDB: 5DS3) from RCSB PDB (http://www.rcsb.org/pdb/home/home.do)[8]. The protein was subsequently cleaned by removing the bound complex molecule, the non-essential water molecules and all the heteroatoms using Pymol tool. The crystallized ligand was extracted (not removed) from the active site so as to reveal the grid coordinate around the binding pocket when viewed on pymol.

Accession and Preparation of the Standards

The standard drugs used in the present study, Niraparib and Talazoparib, are the two potent well-known drugs; known for their inhibitory potential at the PARP-1 binding site which have been successfully used to induce apoptosis, though presented with life threatening adverse effect such as thrombocytopenia, anaemia e.t.c[18]. The structure of the standards (Niraparib and Talazoparib) were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov)

Molecular docking using PyRx

Subsequent to receptor and ligands preparation, molecular docking analysis was performed by PyRx, AutoDock Vina option based on scoring functions. For our analysis we used the PyRx, AutoDock Vina exhaustive search docking function. After the minimisation process, the grid box resolution was centered at 5.3607 ×37.1961 ×9.9918 along the x, y and z axes respectively at grid dimension of 25x 25 x 25 Å to define the binding site (figure 2). The standards were first docked within the binding site of PARP-1 and the resulting interaction was compared with that of lupeol into the similar active sites using the same grid box dimension.

Validation of docking results

The docking results obtained were validated with the blasting of the fasta sequence of the crystal structure of the human PARP-1 (ID: 5DS3) which was obtained from the protein data bank unto the online available ChEMBL Database (www.ebi.ac.uk/chembl/). The bioactivity generated by the database, having an activity of 24, IC50 value of 1919, and KI value of 404, was downloaded in txt format. The bioactivity was sorted out; missing or misplaced data were removed. Only 21 of the total 1919 drug-like compounds were recovered. The compiled compounds were split and converted to 2D (in sdf format) by Data Warrior software (version 2) and converted to pdbqt format by PyRx tool. The ligands were docked into the binding domain of PARP-1 using PyRx AutoDock vina scoring function. A correlation coefficient graph was plotted between the docking scores of the 13 compounds generated and their corresponding PCHEMBL_VALUE (experimentally determined) values. Spearman Rank correlation co efficient graph was plotted to obtain the correlation (R^2) between the dockings results of the ChEMBl's compounds and their corresponding experimentally generated results.

The docking protocol was validated by repeating molecular docking of the lead compound and standards within the PARP-1 binding pocket by adopting the ligands and

protein preparatory procedure as highlighted above using an online docking site (https://www.dockingserver.com/).

RESULTS AND DISCUSSION

PARP-1 belong to PARP family of enzymes. This enzyme is being considered for its DNA damage repair mechanism via poly ADP-ribosylation of proteins involved in DNA repair pathway during DNA damage, which can be caused by anti-tumor drugs. It is therefore reasonable to think that inhibiting PARP-1-dependent Double Stranded Break/Single Stranded Break repair, represents a sound pharmacological approach[7].

In the present study, sixteen (16) phytocompounds from *Lycopersicon esculentum* plant were docked into the binding pocket of PARP-1 for their PARP-1 inhibitory (antagonistic) properties. Lupeol was discovered as the lead compound with the binding energy of -11.0 kcal/mol (Table 4). The drug-likeness of lupeol was assessed by subjecting it to the Lipinski's rule of five, afterwards the lead compound, lupeol violated none of the rules, this describes its bioavailability and binding potential (Table 2).

Lupeol, the lead compound has a binding energy of -11kcal/mol, while the standard drugs Niraparib and Talazoparib have binding energies of -9.5 kcal/mol and -9.1 kcal/mol respectively (Table 1). The highest binding energy (-11kcal/mol) attributed to lupeol in this regard is believed to be as a result of the extensive high number of hydrophobic interactions (Sixteen hydrophobic interactions) of lupeol with certain residues at the active site of the PARP-1: HIS-862, LEU-877, ILE-872 and TYR-896, and hydrogen bonds, SER-904, (Table 4).While Niraparib and and Talazoparib showed six (6) and three (3) hydrophobic interactions respectively. However, lupeol, niraparib and talazoparib although showed difference in their binding affinities within the binding pocket (-11kcal/mol, -9.5kcal/mol and -9.1 kcal/mol respectively) yet shared the amino acid (LEU-877) in their respective hydrophobic interaction within the binding pocket of PARP-1 (Table 3). The average number of hydrophobic atoms in marketed drugs is 16, with one to two donors and three to four acceptors. This defines the importance of hydrophobic interactions in the design of drugs. Hydrophobic interactions can increase the binding affinity between target-drug interfaces[16].

We validated the accuracy of our docking protocol by redocking the co-crystallized ligand (Olaparib) back into the binding pocket of the PARP-1 (5DS3). As stated, the re-docked pose overlapped almost totally with the experimental orientation, indicating that Autodock vina on PyRx re-docked the co crystallized olaparib, with a very high accuracy, back into the binding pocket of the PARP-1, this reveals that our docking methodology was reliable and the docking scores obtained are correct (Figure 5). The reliability of our docking scores was further validated using the online available ChEMBL Database, the Fasta sequence of the crystal structure of the Human PARP-1 (ID: 5DS3) was blasted on www.ebi.ac.uk/chembl/. The compounds obtained from the search were docked into the binding site of the Human PARP-1, a correlation coefficient graph plotted between the docking scores of the compounds generated and their corresponding ChEMBL's Pchem values (experimentally determined IC50). This showed a strong correlation coefficient between the docking scores and the experimentally derived data in the present study which gave credence to the fact that computational experiment can replicate experimental data at least in this present study and that our docking scores, using PyRx AutoDock Vina algorithm is dependable (Figure 7). Furthermore, the validation of our docking protocol shows that lupeol, the lead compound has the highest binding affinity of -7.36kcal/mol as compared to the standard drugs, niraparib (-7.24) and talazoparib (-6.58kcal/mol) (Table 1). This further confirms that lupeol has a better binding potentials as compared to the standards and thus pose a better inhibitory effects on the target (PARP-1).

Natural PARP-1 antagonists such as lupeol have different binding modes (Figure 4) when compared with the Niraparib and Talazoparib antagonists (Figure 3), and these may be associated with their differences in binding potential within the binding pocket.

Table 1: *Energy and RMSD values obtained during docking analysis of lupeol, Niraparib and Talazoparib as ligands molecules and PARP-1 as target protein*

S/N	Complex	Binding energy (From PyRx)	Binding energy (From dockingServer)	RMSD/ UB[a]	RMSD/ LB[b]
1	PARP-1_Lupeol	-11	-7.36	0	0
2	PARP-1_Niraparib	-9.5	-7.24	0	0
3	PARP-1_Talazoparib	-9.1	-6.58	0	0
	RMSD/UB: Root mean square deviation/upper bond; RMSD/LB: Root mean square deviation/lower bond; PARP-1: Poly(ADP-ribose) polymerase-1				

Table 2*: Lipinski's drug-like properties of lupeol: The rule describes drug candidate's pharmacokinetics in the human body which also including their absorption, distribution, metabolism, and excretion ("ADME") using an online server* (http://www.scfbio-iitd.res.in/)

Molecular Properties	Lipinski's rule of Five	Lupeol drug-like properties
Molecular Mass	<500	438
Hydrogen bond Acceptor	<10	1
Hydrogen bond Donor	<5	1
LogP	<5	4.4168
Molar Refractivity	Between 40-130	88.696396
Topological Polar surface .Area	<140Å^2	20.2

Table 3: *The decomposed interactions energies in kcal/mol obtained using DockingServer*

Complex	Hydrophobic Interaction	Hydrogen Bonds	Other
PARP-1_lupeol	HIS862 (-1.575) LEU877 (-0.6261) ILE872 (-0.4923) TYR896 (-0.4558)	SER904 (-0.46)	ASN868 (-0.449)
PARP-1_Niraparib	TYR907 (-1.971) LEU877 (-0.5995) ALA898 (-0.1684)	ARG878 (-0.7727)	SER864 (-0.3191) ASN868 (-0.1772)
PARP-1_Talazoparib	LEU877(0.0344)	GLY894 (-0.0877) GLY863 (3.0754)	ARG865 (-0.2841) ALA898 (-0.0555) HIS909 (-0.0304)

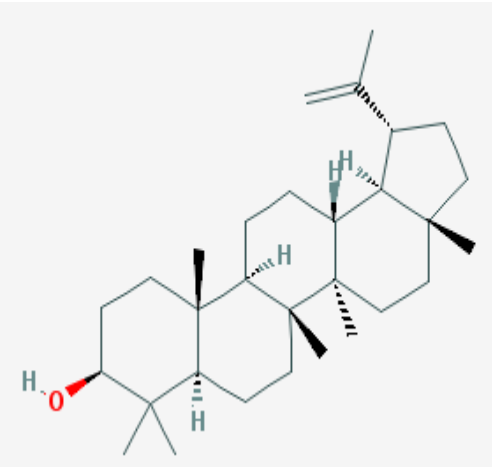
Table 4: *The hydrophobic interactions and Hydrogen bonds with the residues involved at the binding pocket of PARP-1. Obtained using DockingServer*

Complex	Hydrophobic Interaction	Hydrogen Bonds
PARP-1_lupeol	C3 – HIS862 C17 – HIS862 C5 – HIS862 C11 - HIS862 C12 – HIS862 C19 – HIS862 C30 – LEU877 C29 – ILE872 C8 – TYR896 C22 – TYR907	O1 - SER904

	C24 – TRY907 C10 – TYR896 C14 – TYR896 C16 – TYR896 C28 – TYR896 C27 – TYR907	
PARP-1_Niraparib	C19 – LEU877 C3 – TYR896 C4 – ALA898 C5 – ALA898 C1 – TYR907 C2 – TYR907	N4 - ARG878
PARP-1_Talazoparib	C1 – HIS862 C15 – HIS862 C15 – LEU877	N6 - GLY894 N1 - GLY863

Table 5: *Phytochemicals obtained from Lycopersicon esculentum with their respective binding energies in kcal/mol. Lupeol has the highest docking score as compared with others.*

Ligand	PUBCHEM ID	Docking Scores
Lupeol	259846	-11
Solasodine	442985	-9.9
campestero	173183	-9.7
Naringin	442428	-9.1
beta-sitosterol	222284	-9.1
cycloartenol	92110	-9
cholesterol	5997	-8.6
stigmaserol	5280794	-8.2
caffeic acid	689043	-7.1
Beta-damascenone	5366074	-6.6
Lycopene	446925	-6.6
Trigonelline	5570	-6.1
Adenine	190	-5.8
3-methylbutanol	31260	-4.2
3-methylbutanal	11552	-4.1
methional	18635	-3.4



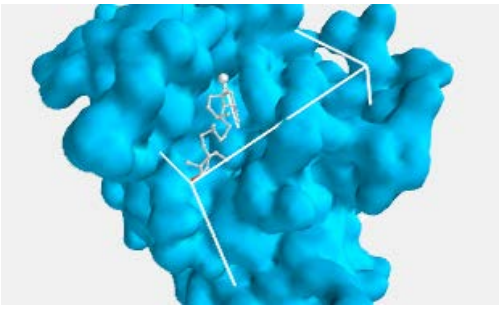


***Figure 1**: lupeol ligand*

***Figure 2:** Describing the grid box within which the binds into. 5.3607 ×37.1961 ×9.9918, along the X, Y, Z axis*

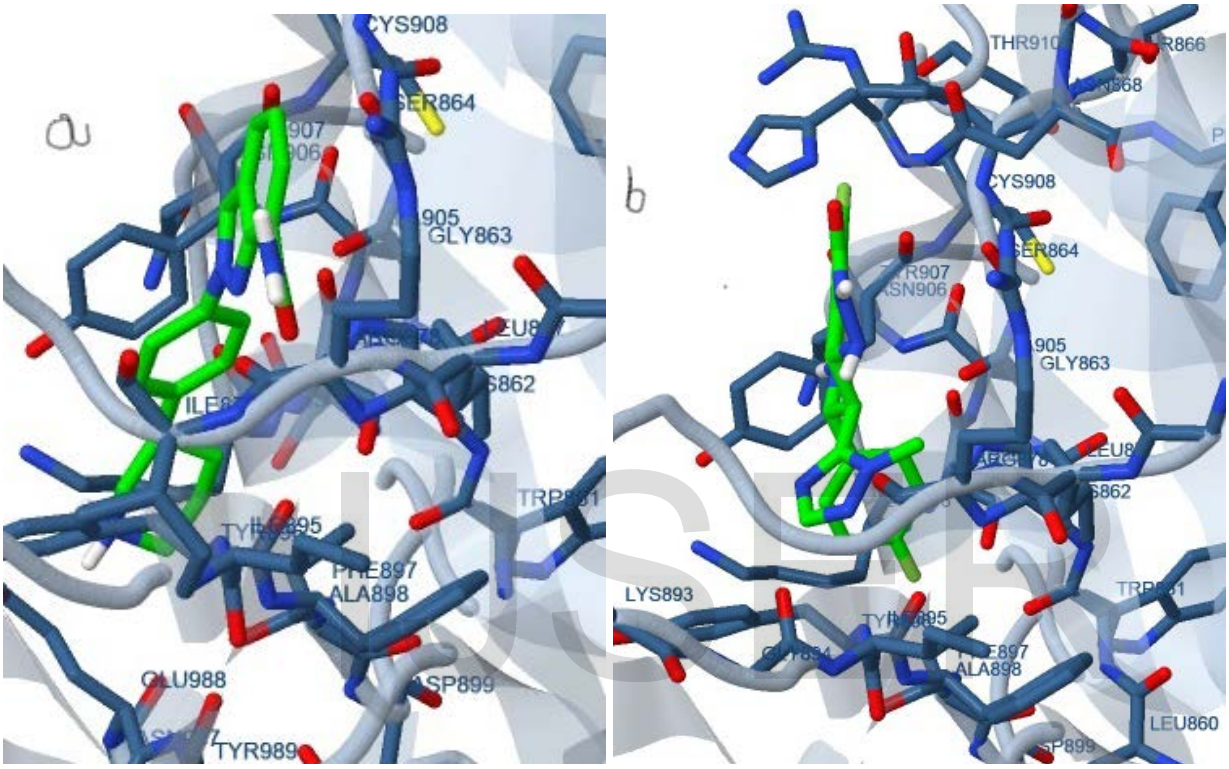


***Figure 3**: 3D dimensional structure of the interactions and binding pose of (a) Niraparib (green sticks) (b) Talazoparib (green sticks), within the binding pocket of PARP-1, Obtained on docking server*

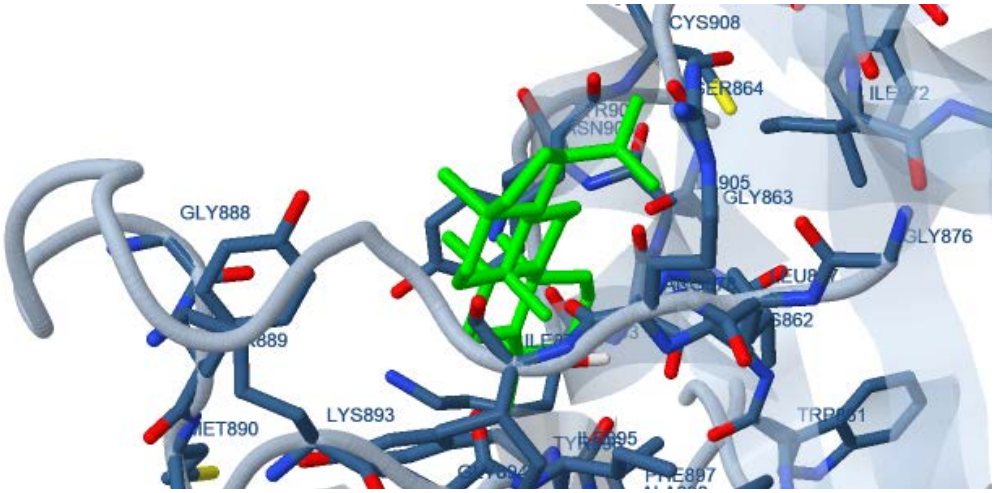


Figure 4: *3D dimensional structure of the interactions and binding pose of Lupeol (green stick), within the binding pocket of PARP-1. Obtained on docking server.*

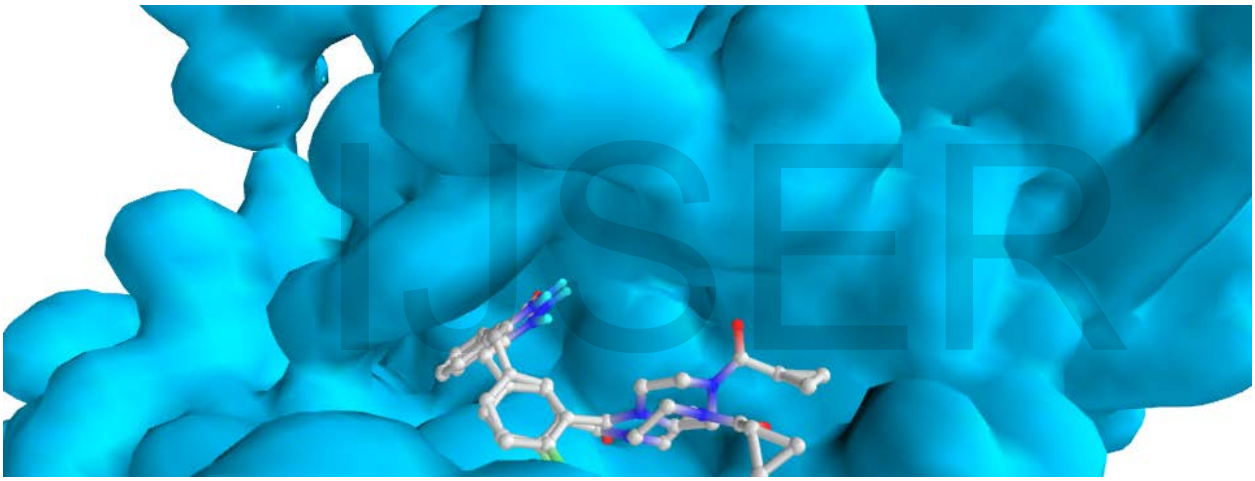


Figure 5: *Validation of docking: Comparability of the re-docked binding mode and the co-crystallized pose of olaparib with the accompany residues of PARP-1 binding pocket. A snapshot from PyRx*

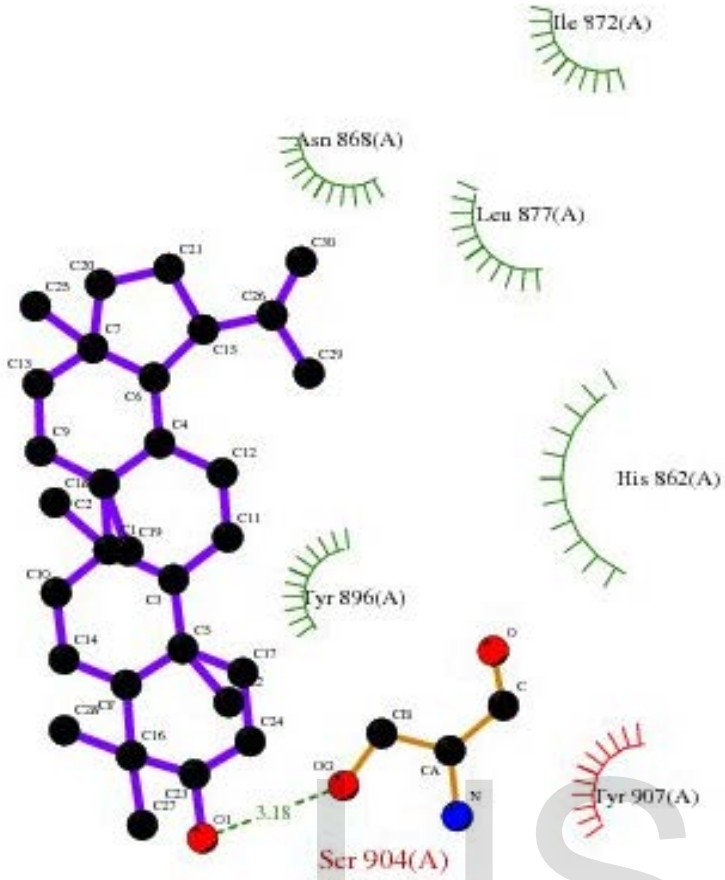


Figure 6: *Ligplot molecular interaction of lupeol and amino acids residues at the binding pocket of Poly(ADP-ribose) polymerase-1 (PARP-1), obtained on docking server.*

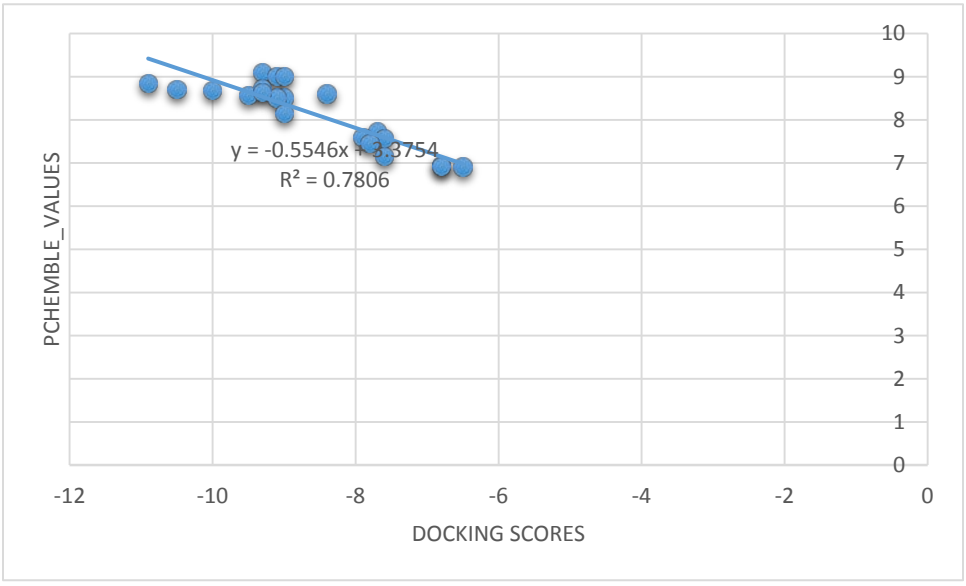


Figure 7: *Correlation coefficient graph of docking scores of various antagonists of the PARP-1 and their corresponding experimental pIC50 (pchembl_values) values. The antagonists (compounds) and their corresponding pIC50 (experimentally derived IC50) were downloaded from the ChemBL database, the strong correlation (0.7806) between the docking scores and pIC50 shows that computer can reproduce experimental values and this gives credence to the docking scores generated, in the present study.*

CONCLUSION

Docking studies and ADMET evaluation of lupeol with PARP-1 showed that this ligand is a drug-gable molecule which docks well with PARP-1 target. Therefore, lupeol molecule plays an important role in inhibiting PARP-1 and thus should be implicated as a potential agent in cancer therapy.

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