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IN SILICO APPRAISAL OF LUPEOL AND BUTELINIC ACID AS COMPELLING ANTI-INFLAMMATORY MEDIATORS

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ABSTRACT

Background: Inflammation is a fundamental innate immune response that affects the tissue homeostasis and promotes tumor development and metastasis. Chronic inflammatory mediators exert pleiotropic effect in the development of inflammation. Some bioactive compounds possess therapeutic properties to suppress the stimulation of these chronic inflammatory mediators responsible for inflammation. Therefore, the current research work focusses on the anti-inflammatory role of two phytocompounds, butelinic acid and Lupeol against inflammatory mediators COX-2 and TNF-α.

Materials and Methods: The present study entails the plant derived active compounds which are potent anti-inflammatory bioactive constituents. Two proteins COX-2 and TNFα responsible for the inflammatory process were subjected as targeted proteins for the purpose of protein-ligand docking. These proteins were subjected to molecular docking with the selected phytoactive compounds Betulin and Lupeol. Three dimensional (3D) crystal structure of the enzyme was obtained from Protein Data Bank (PDB). Three dimensional structures of ligands i-e Lupeol, butelinic acid and standard celecoxib were drawn and optimized using ACD/Chem Sketch software. Molecular docking studies were performed by Auto Dock Vina.

Results: The selected model having satisfactory quality factors results which were COX-2 (90.34%) and TNF-α (73.61%). Post docking analysis revealed that ASN2039, GLN2042, ASN2043, ARG2044, LEU2152, LYS2468, ARG2469, SER2471, ASN2034, CYS2036, CYS2037, ASN2039, GLU2046, CYS2047, ET2048, SER2049, TYR2130, GLY2136, PRO2153, VAL2155, ALA2156, GLN2461, TYR2130, GLY2135, TYR2136, LYS2137, CYS2047, MET2048, SER2049 were observed as important common interactive residues and showed that effective binding with target, lower binding affinities and drug properties.

Conclusion: Results of present study concluded that phytoactive compounds (Lupeol and Butelinic acid) express significant binding interactions with COX-2 and TNF-α that are inflammatory proteins having major role in multiple organ inflammation. Anti-inflammatory property of aforesaid compounds illustrated significant anti-inflammatory action and might be used as anti-inflammatory therapeutic drugs in the near future.

Keywords: Inflammation induced cancer, Betulin, Lupeol, COX-2, TNF-α

INTRODUCTION

Flavonoids are naturally occurring polyphenolic compounds and are widely distributed among fruits and vegetables with wide pharmacological and biological importance [1]. Triterpenes are also widely distributed group of natural compounds. Lupeol has gained importance due to its anticancer and anti-inflammatory activities [2]. Butelinic acid also possesses various biological and pharmacological activities with higher efficacy in cancer treatment [3].

The molecular basis of drug binding to active site of enzyme or protein is elucidated by computer aided drug design to analyze the binding of molecules [4].

MATERIAL AND METHOD

STRUCTURE PREDICTION

Molecular docking studies were carried out using Auto Dock 4.2 [5]. Three dimensional (3D) crystal structure of the enzyme was obtained from Protein Data Bank (PDB). Three dimensional structure of ligand was drawn and optimized using ACD/Chem Sketch software. The site with lowest binding energy was further analyzed using Auto Dock 4.2 [6]. The reliability of docking program was validated by using re-docking method. In both cases the co-crystallize ligands were redocked in the active site of enzyme. Root mean square (RMSD) was then calculated and in all cases RMSD value of <2.0 Å is considered accurate in predicting binding orientation of ligand. The binding poses were further studied using discovery studio visualize [7].

MOLECULAR MODELING STUDIES

The crystal structure of tumor necrosis factor alpha (TNF-α) and cyclo-oxygenase-2 (COX-2) enzymes were taken from RSC Protein Data Bank having PDB ID: 5M2J for anticancer activity and PDB ID: 1CVU for anti-inflammatory activity respectively. In our study triterpenes and flavonoids from acacia modesta and opuntia monocantha were tested for anticancer and anti-inflammatory activities against TNF-α and COX-2 enzymes respectively. For docking of triterpenes (lupeol, betulinic acid) the required three dimensional structures were generated by ACD/Chemsketch software and the resultant file was converted to auto dock file by Open Babel-GUI program. Docking studies were performed with Auto Dock 4.2. program. Polar and aromatic hydrogen were added with computing the gastesian charges on ligand atoms. Torsional degree of freedom was automatically defined in the software for ligand. The grid box was centered in the receptor and the dimension of grid was adjusted to include the entire protein fragment with the grid point spacing 0.375 Å. Grid potential maps were calibrated using the module autogrid 4.0. The Lamarckian genetic algorithm (LGA) was used to perform docking simulation, with an initial population size of 150 individuals, with a maximum number of 250000 energy evaluations, 150000 generations with a mutation rate of 0.02, and a cross-over rate of 2 points. The number of docking runs was chosen to be 100. The lowest binding energy conformation was taken from 10 different conformations for each docking simulation and the resultant minimum energy conformation was applied for further analysis.

RESULTS

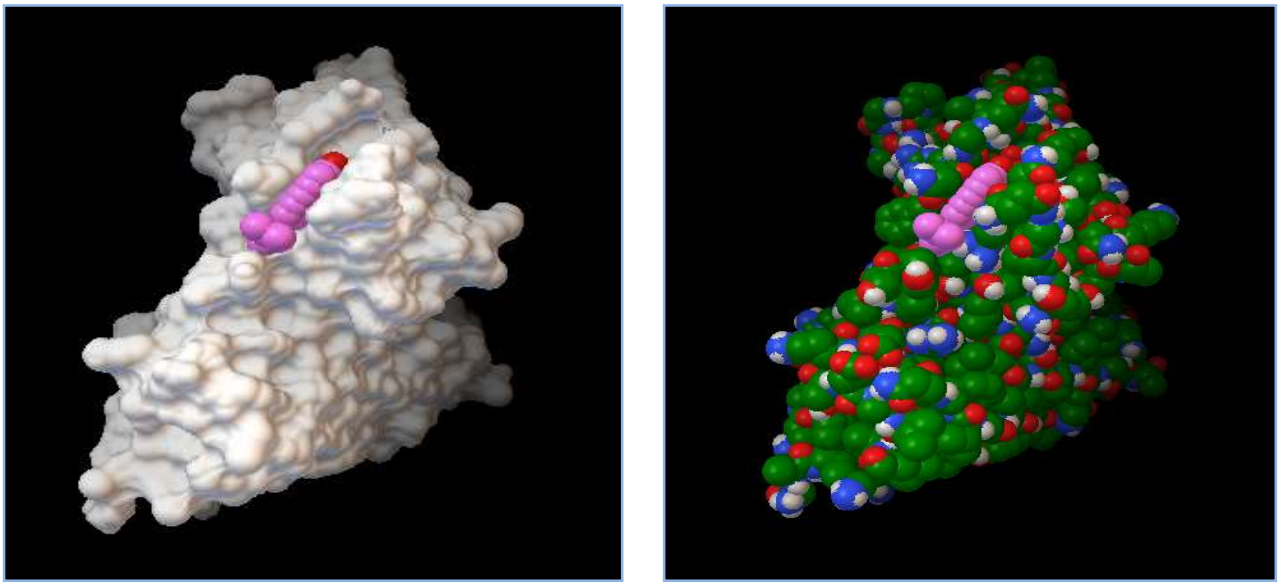
Molecular docking studies were used to estimate the enzyme inhibitor interacting geometries for selected compounds. Table-1 demonstrates the docking score of flavonoids and triterpenes with interacting TNF-α and the amino acid residues involved in binding of ligands with protein including hydrogen bonding, hydrophobic interacting forces and ionic bonding. It was discovered that the potential of lupeol and betulinic acid against TNF-α was linked with the binding energies and number of hydrogen bonds, hydrophobic bonds and ionic bonds formed in the catalytic site. The standard drug for TNF-α binding site was 5-flourouracil with amino acid involved in hydrogen bonding was found to be SER99, whereas, PHE103 shows hydrophobic interaction at the binding site, and ARG98 shows ionic interactions. As we compare flavonoids and

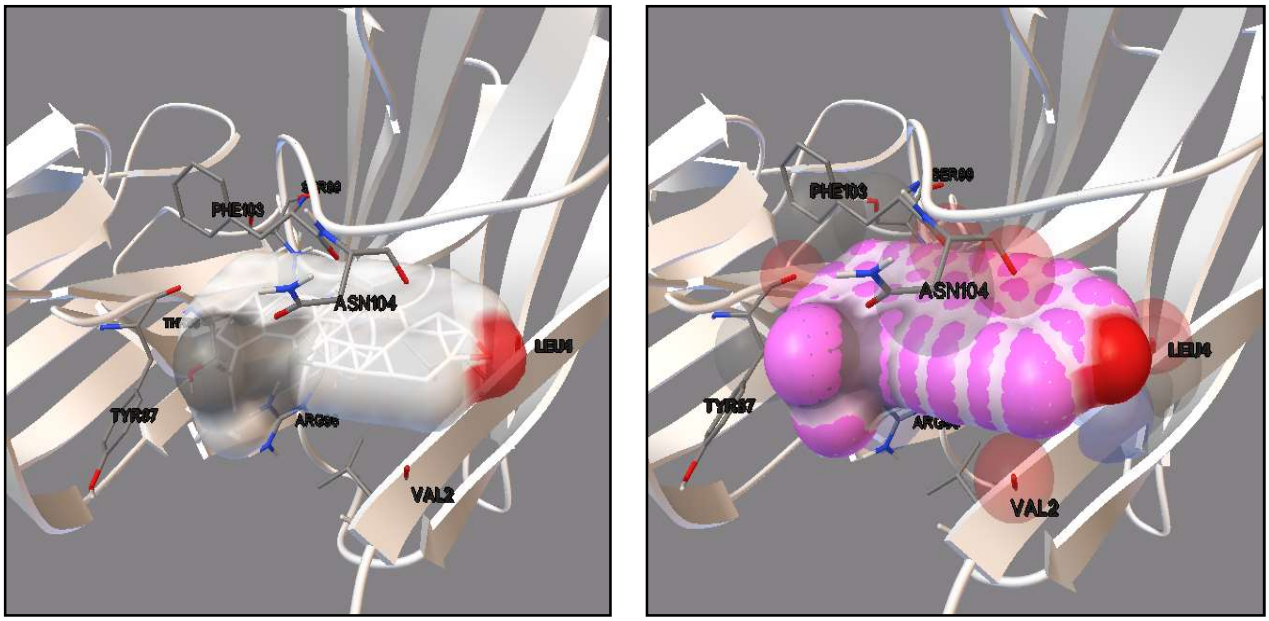
triterpenes with standard drug, lupeol was found to be the most potent with amino acid residue involved in hydrogen bonding at the binding site were THR89 and SER99, whereas, VAL2, LEU4, TYR87, and PHE103 were involved in hydrophobic interaction. ARG98 and ASN104 were found to for ionic bonds. Table-2 illustrates the docking score of flavonoids and triterpenes with interacting COX-2 and the amino acid residues involved in binding of ligands with protein including hydrogen bonding and Vander Waals interacting forces. It was discovered that the potential of lupeol and betulinic acid against COX-2 inhibitor was related with the binding energies and number of hydrophobic interactions, hydrogen bonds and ionic bonds formed at the catalytic site. The drug used to standardize the results was celecoxib a potent COX-2 inhibitor. The amino acids involved in hydrogen bonding with celecoxib at the binding site were CYS2047, and SER 2049, while, the amino acids involved in hydrophobic interaction were TYR2130, GLY2135, TYR2136, and MET 2048, whereas, amino acid involved in ionic interaction was found to be LYS2137. We compare the binding energies of different flavonoids and triterpenes with standard drug celecoxib and we found betulinic acid to be the most active COX-2 inhibitor. The amino acid residues involved in hydrogen bonding of betulinic acid at the binding site of COX-2 were CYS2036, CYS2037, CYS2047, and SER2049, whereas, TYR2130, GLY2136, TYR2136, MET2048, PRO2153, VAL2155, and ALA2156 were found to show hydrophobic interactions, while, ASN2034, ASN2039, GLU2046, and GLN2461 were involved in ionic interactions.

Table 1: Lowest binding energy of flavonoids and triterpenes with amino acids residues involved in drug binding at TNF-α enzyme

No	Drug with TNF-α	Lowest binding energy	Amino acid residues involved in binding
1.	Lupeol	-12.11	VAL2, LEU4, TYR87, THR89, ARG98, SER99, PHE103, ASN104
2.	Betulinic acid	-8.3	GLN25, LEU26, TRP28, ASN46, SER133, ALA134, GLU135, ILE136, PRO139

(a) **(b)**

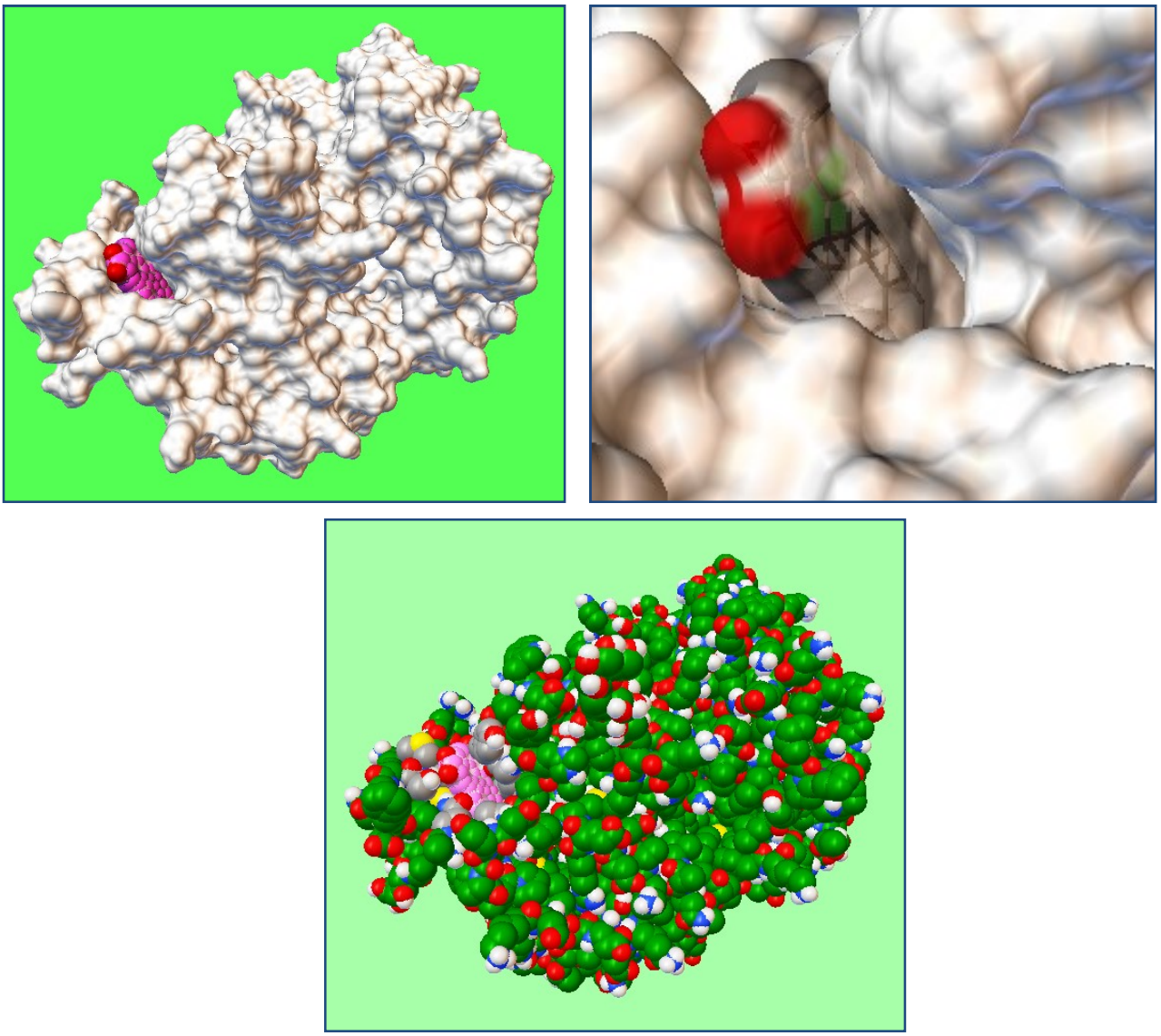




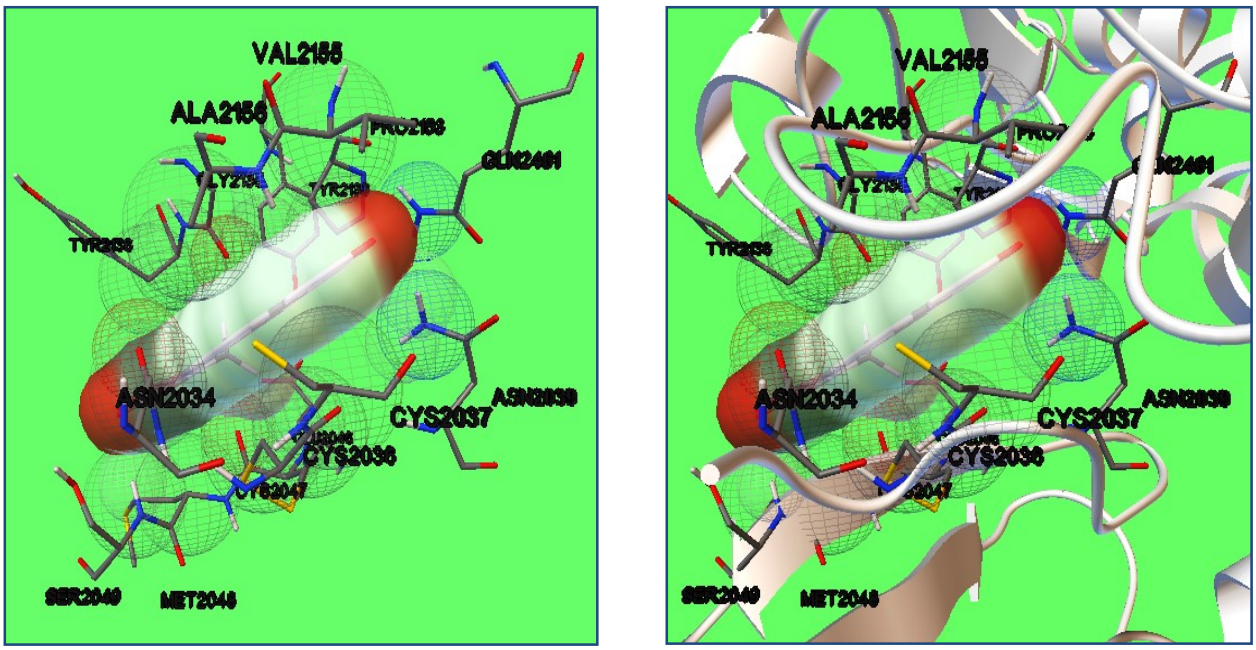
(c) **(d)**

Figure-1: (a) Ligand (pink) present on the molecular surface of protein (white); (b) the atomic surface of protein shown in green with the drug shown in pink color at the binding site of protein; (c) the amino acid residues involved in the binding of Lupeol with TNF-α in the binding pocket with least binding energy; (d) the atomic surface of ligand (pink) Lupeol in interaction with secondary structure of protein at the binding site

(a) **(b)**

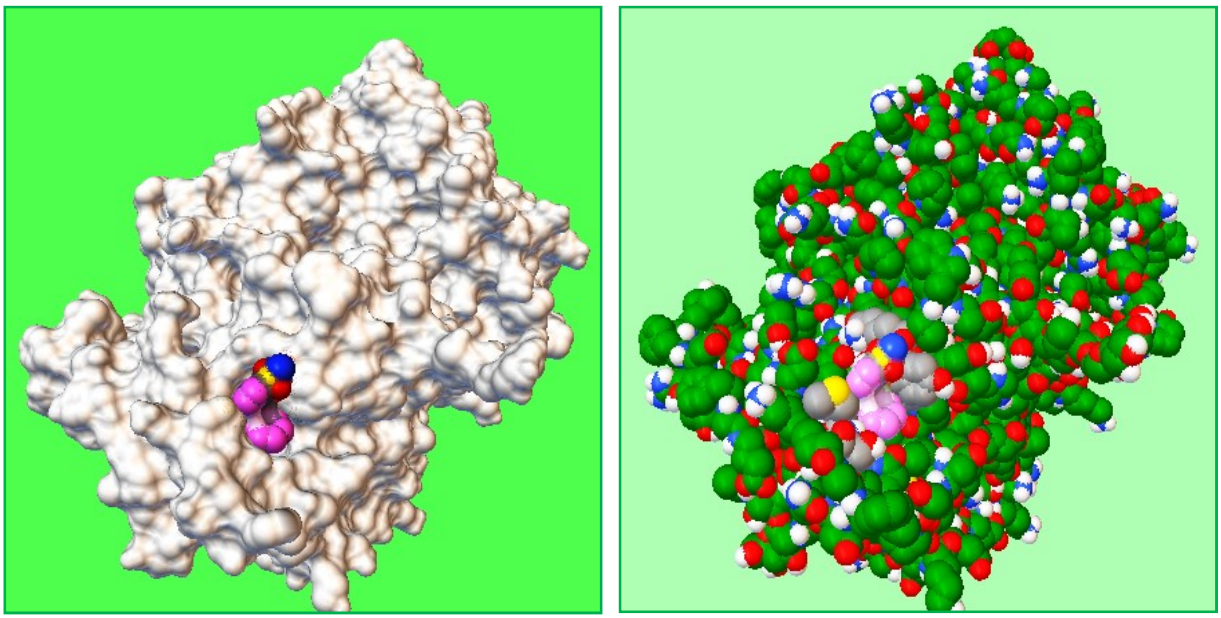


(c)

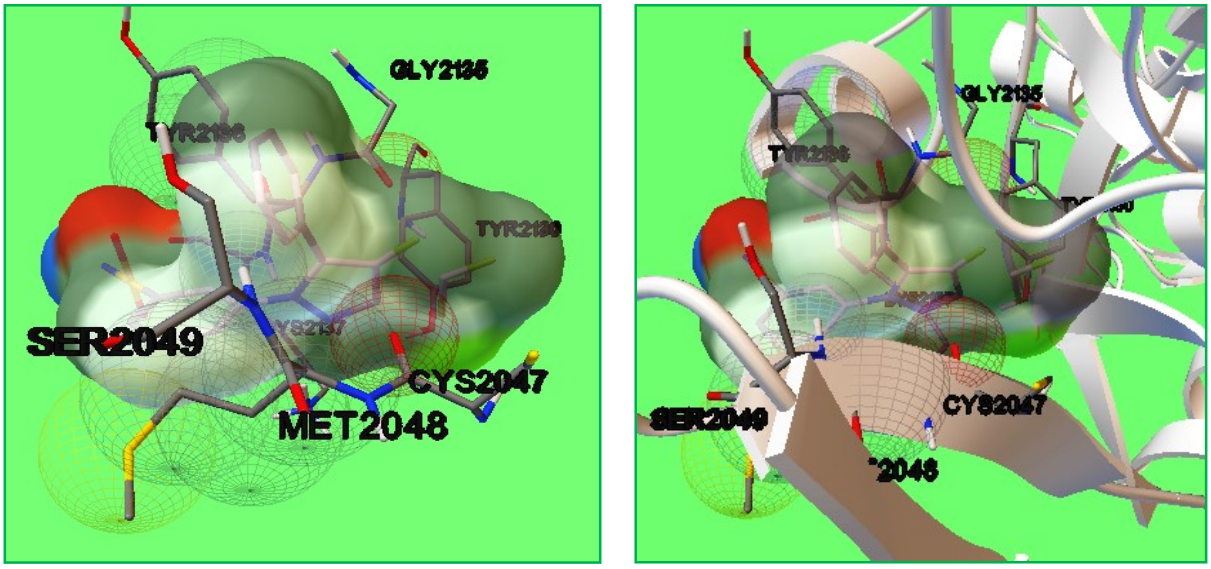


(d) **(e)**

Figure-2: (a) Ligand present on molecular surface of protein showing close interaction in the binding pocket of protein; (b) ligand with atomic surface (pink) present on molecular surface of protein; (c) showing molecular surface of protein (green) with ligand (pink); (d) amino acid residues in close interaction with betulinic acid in the binding pocket of COX-2; (e) betulinic acid showing interaction with the secondary structure of protein at the lowest binding site



(a) **(b)**



(c) (d)

Figure-3: (a) Standard drug (Celecoxib) for COX-2 inhibitor present on the molecular surface shown in pink color; (b) atomic surface of protein (green) with ligand (pink) in the binding pocket of protein; (c) wire frame view of ligand with interacting amino acids at the lowest binding site of protein; (d) secondary structure of protein-ligand interaction in the binding pocket of protein with lowest binding energy

Table 2: Lowest binding energies of Lupeol and Betulinic acid with amino acid residues involved in drug binding at COX-2 enzyme

No	Drug with COX-2	Lowest binding energy	Amino acids involved in binding
3.	Lupeol	-12.03	ASN2039, GLN2042, ASN2043, ARG2044, LEU2152, LYS2468, ARG2469, SER2471
4.	Betulinic acid	-13.4	ASN2034, CYS2036, CYS2037, ASN2039, GLU2046, CYS2047, ET2048, SER2049, TYR2130, GLY2136, PRO2153, VAL2155, ALA2156, GLN2461

DISCUSSION

The present work involves the molecular docking simulation studies in which we visualize the reaction between protein (macromolecule) and ligand (small molecule) at molecular level. By such reactions we can predict the behavior of small molecules in the binding site of targeted proteins which ultimately interpret the biochemical process. Given the goal of molecular docking we will be able to predict the binding affinities [7-11]. Based on docking energies and good interactions with active site residues, the docked ligand molecules were selected. The three dimensional docking of flavonoids and triterpenoids at TNF-α and COX-2 sites were carried out and generate binding energies from which we select lowest binding energy with high binding affinity. The lower the binding score the greater is the binding capacity of ligand [12-14]. Hence, the docking scores and binding interactions of flavonoids and triterpenoids obtained from Acacia modesta and Opuntia

monocantha were expressively associated with their capability to inhibit the activity of TNF-α and COX-2 [15-21]. The binding energy of Lupeol was lowest as compared to other flavonoids and terpenes at TNF-α binding site, hence Lupeol show greater affinity with TNF-α. The binding energy of Lupeol was found to be -12.11Å, with the residues involved are VAL2, LEU4, TYR87, THR89, ARG98, SER99, PHE103, ASN104 in the binding pocket of protein as shown in figure-1c.

The binding energy of butelinic acid was found to be lowest as compared to Lupeol at COX-2 binding site, hence show greater binding affinity at receptor site [7]. The residues involved in binding of butelinic acid at COX-2 site involve ASN2034, CYS2036, CYS2037, ASN2039, GLU2046, CYS2047, ET2048, SER2049, TYR2130, GLY2136, PRO2153, VAL2155, ALA2156, GLN2461 as shown in figure-2d. The binding energy of standard COX-2 inhibitor (celecoxib) was 9.4Å and amino acid residues involved in its binding at the binding site are TYR2130, GLY2135, TYR2136, LYS2137, CYS2047, MET2048, SER2049 as shown in figure-3c. These in-silico docking results were in good agreement with the in-vitro experimental data. In particular, this study is the first report of TNF-α and COX-2 inhibitory activity of lupeol, and betulinic acid derived via molecular docking simulation.

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