OPEN ACCESS

Saudi Journal of Medical and Pharmaceutical Sciences
Abbreviated Key Title: Saudi J Med Pharm Sci
ISSN 2413-4929 (Print) | ISSN 2413-4910 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article **Pharmacy**

Discovery of Novel *Heme oxygenase-1* Inhibitors from *Annona squamosa* Leaf Bioactives: Antioxidant Efficacy

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DOI: 10.36348/sjmps.2024.v10i05.006 | **Received:** 17.04.2024 | **Accepted:** 25.05.2024 | **Published:** 31.05.2024

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Abstract

***Background*:** The study of free radical chemistry has received a considerable lot of attention recently. Our bodies produce reactive oxygen and nitrogen species and free radicals as a result of a variety of endogenous processes, exposure to various physiochemical situations, or pathological conditions. For optimum physiological function, free radicals and antioxidants must coexist in balance. Oxidative stress results when the body's defences against free radicals are overpowered. As a result, free radicals damage lipids, proteins, and DNA and cause a variety of human disorders. Therefore, using antioxidants from an external source can help to manage this oxidative damage. In contrast to synthetic antioxidants, which are either added to food to increase its shelf-life or are synthesised by plants and found in the foods we consume, natural antioxidants are produced by plants (for example, vitamins and other naturally-occurring substances in our food) (e.g. BHT). Annona squamosa. (Annonaceae, Family). Hindi-speaking locals refer to it as "Sitaphal." Insecticidal, purgative, laxative, astringent, anti-inflammatory, antidiabetic, anti-ulcer, anti-oxidant, antimalarial, and antibacterial are only a few of the pharmacological effects of the plant. ***Method*:** In current study *HO-1* protein selected as target protein. The Auto Dock software used a grid-based docking algorithm to determine the bond. Using the Merck Molecular Force Field, 2D structures of compounds were created, transformed to 3D, and then energetically decreased up to an arms gradient of 0.01. (MMFF). ***Result*:** Flavonoids of *A.squamosa* found to be effective antioxidant component and effectively binds to be target protein *HO-1* with binding energy -6.18 & -5.26 kcalmol^{-1}for quercetin and rutin respectively. ***Conclusion*:** The finding of the *in-silico* molecular docking showed that both lead compound is effective binds & inhibitory action on target protein. The molecular docking of ligands like quercetin and rutin with human *HO-1* receptor revealed that it has exhibited the chemical interaction with the amino acids in the active pockets.

Keywords: *A.squamosa*, molecular docking, *HO-1* enzyme, quercetin and rutin.

INTRODUCTION

Antioxidant is short for "against oxidation." Antioxidant refers to any compound that, when present in small amounts compared to an oxidizable substrate, considerably slows down or stops that substrate from oxidising [1]. Antioxidants are essential for preserving food quality and sustaining human health. Depending on where the oxidation reaction occurs, different effects result. Food deteriorates if the food system is the site of the incident. In biological cell systems, oxidation results in cell damage or death. When fats and oils are present as a food ingredient, oxidative degradation causes them to lose flavour and aroma, which lowers nutritional value, sensory appeal, and safety [2]. This results from the auto-oxidation of unsaturated fatty acids through a free radical chain mechanism, which produces primary hydroperoxides and secondary potentially hazardous chemicals. Direct oxidation by oxygen in its ground triplet state, which has two free electrons in separate orbitals with the same spin direction, of unsaturated lipids with the double bond in a singlet state (no unpaired electrons, paired electrons in the same orbital, and opposite spin), is spin forbidden [3]. Cancer or cardiovascular disease are frequently brought on by oxidative stress and an imbalance in antioxidant qualities [4]. Natural anti-oxidants like ascorbic acid and tocopherols have been shown to guard against cancer and heart disease. Cereals, vegetables, fruits, oilseeds, legumes, cocoa products, beverages (tea, coffee, red wine, beer, fruit juices), herbs, and spices contain the most significant natural antioxidants [5]. The use of

antioxidants as functional food ingredients and nutritional supplements, both natural and synthetic, is increasingly gaining popularity [6]. Due to their accessibility and increased activity, synthetic antioxidants are used not only in the food business to stabilise fats, oils, and lipids, but also in the pharmaceutical sector and as preservatives in cosmetics [7-8]. "Custard apple" or *Annona squamosa* L. (Annonaceae). Due to its comprehensive pharmacological qualities and biological activities, such as their antioxidant, antibacterial, antidiabetic, antiviral, anticancer, and hepatoprotective capabilities, anona squamosa leaves (ASLs) have the potential to be valued [9].

Chemical Constituents:

The bark and leaves of this plant were used to identify acetogenin, flavonoids, aporphine alkaloids, glycoside, and squamoline, among other bioactive compounds [10]. The presence of various uncommon elements like alkaloids, glycosides, flavonoids, resins, volatile oils, gums, and tannins, among others, is what gives this plant its medicinal benefits.

Therapeutic Uses:

Conventionally, the plant is used to cure a variety of conditions, including diarrhoea, epilepsy, cardiac issues, worm invasion, constipation, bleeding, bacterial infection, dysuria, fever, and ulcer. Additionally, it has anticancer, antifertility, and abortifacient effects [11–12]. As a poultice, leaves are used to cure boils and ulcers, and leaf infusion has been shown to be effective in treating prolapse in children. Cataplasm from bruised leaves that has been mixed with salt is used to extract guinea worms.

Experimental Work

Molecular Docking Studies

Ligand Preparation:

2D Structure of ligands like quercetin, and rutin were drawn using ChemSketch [13], the two-dimensional structures of the prepared ligands were converted into their 3-D structures optimized with 3D geometry. The optimized structures were saved in PDB format for AutoDock compatibility. The basic structures of the prepared ligands were given below:

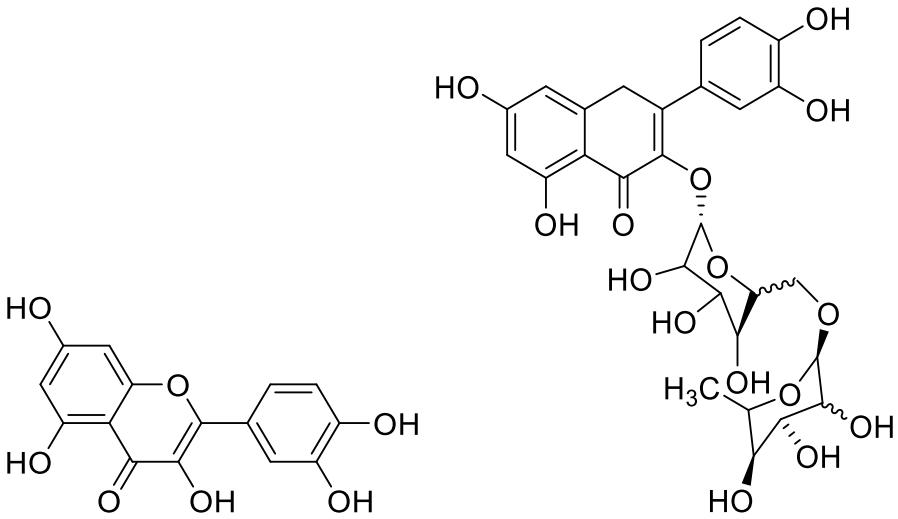


Figure 1: 2D structure of quercetin and rutin

Preparation of the grid file

The regions of interest used by Autodock were defined by considering grid area by making a grid box around the active sites. Grid box plays a central role in process of docking as it is made to cover all the amino acids present in active sites necessary for binding other than those present in receptor. Grid box has 3 thumbwheel widgets which let us change the number of points in the x, y and z dimensions. The spacing and grid points is given in table 1 [14, 15].

Table 1: Grid parameters used in current docking analysis of HO-1

S. No.	Receptor	x-axis	y-axis	z-axis	Spacing	x center	y center	z center
1	HO-1	40	40	40	0.375	17.868	0.697	4.707

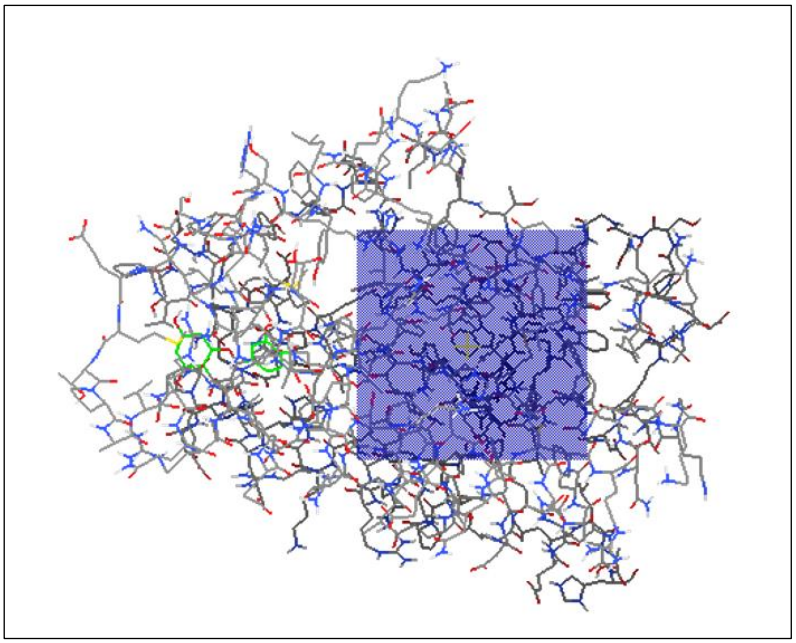


Figure 2: Grid box covering all active sites in HO-1 receptor

Preparation of the docking file

All the calculations were carried out by using Autodock 4.2 as docking tool. The visualization and other programs necessary for docking studies were performed out by means of Pymol, Chimera, DS visualizer, MMP Plus [16, 17].

Docking Study

Crystal structure

The crystal structure of the protein consisting of HO-1 receptor is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (1n45.pdb) registered in the Protein data bank was used [18, 19]. The complex ligand was separated by using Chimera software.

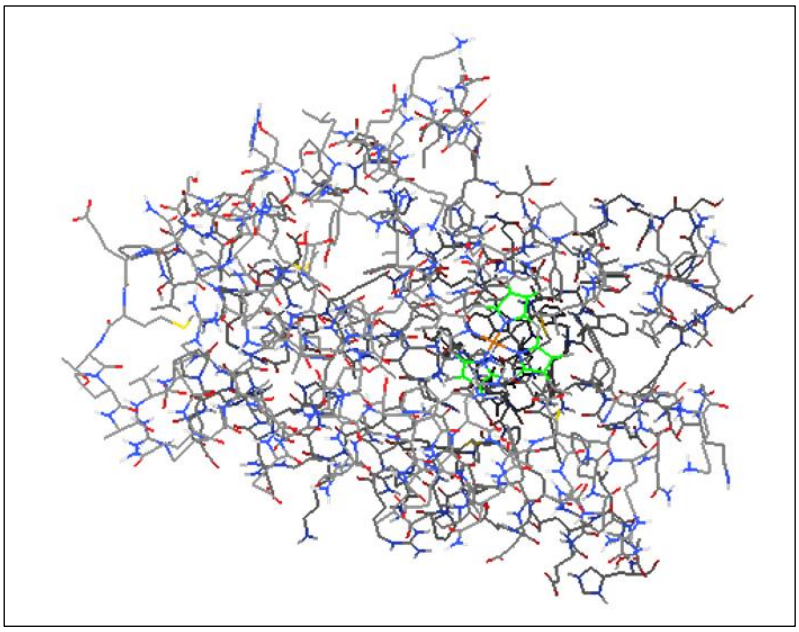


Figure 3: Crystal structure of HO-1 receptor (PDB ID-1n45)

Processing of Protein

The downloaded receptor protein is having two chains, i.e. chain A, and B. Out of these two chains, chain A was selected for experimental purpose and other chain B was removed from it. The bound ligand Heme was separated from the macromolecular complex by using software Chimera [20, 21].

Molecular Docking Simulation Studies

Docking of ligands like quercetin and rutin against human HO-1 receptor was performed by

Autodock. All the bonds of each ligand were kept flexible, while no residues in receptor were made flexible [22].

Toxicity & ADME-T Studies

The ligand molecules viz. quercetin and rutin were studied by online program OSIRIS, for prediction of presence of any toxic group as well as presence of any toxic group and ADME- T properties [23 & 25].

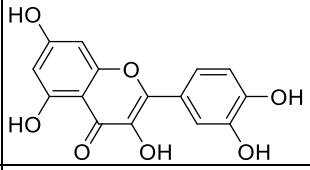
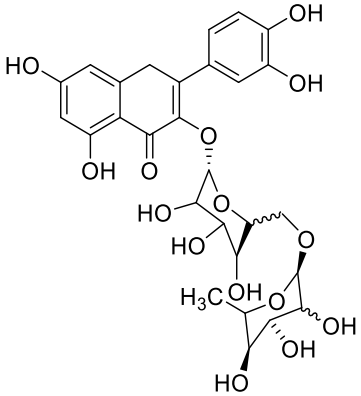
RESULT & DISCUSSION

Oxidative stress (OS) is an imbalance between the reactive oxygen species (ROS) formation and the antioxidant defense mechanisms. At their high concentrations, ROS can react with different macromolecules, therefore involved many disease processes. In our body, the cellular antioxidant defense systems including glutathione (GSH), and ROS scavenging enzymes, such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX) regulate the levels of ROS. Plants have conventionally provided a source of hope for narrative drug compounds, as plant herbal mixtures have made huge contributions to mankind and well-being. In the present work methanolic and aqueous extract of extract *A.squamosa* leaf were taken in consideration for evaluation of antioxidant efficacy. Flavonoids are polyphenolic chemicals found in a wide range of plant species. They are mostly used as a source of starting material in the pharmaceutical and food industries, and they exhibit a variety of biological activities of interest, such as antioxidant capacity, anti-inflammatory activity, wound healing properties, and immune system activation.

Heme oxygenase-1 (HO-1) is a cellular stress protein that plays an important role in the oxidative catabolism of heme leading to the formation of biliverdin (BV), free iron and carbon monoxide (CO). Whereby, BV formed is rapidly converted to the strong antioxidant bilirubin (BR), which is then converted back into BV through reacting with ROS allowing their neutralization. Therefore, HO-1 has its potential ability to regulate oxidative and inflammatory which contribute to an efficacy in controlling metabolic diseases and make it a target of several researches of validation of antioxidant potential per prious studied carried out by Brewer, M.S., 2011 [24].

The outcome of molecular modelling investigation revealed that both selected flavonoids of *A.squamosa* found to be effective antioxidant component and effectively binds to be target protein *HO-1* with binding energy -6.18 & -5.26 kcalmol^{-1}for quercetin and rutin respectively. The result was tabulated in table 2. The binding mode of quercetin and rutin showed in fig.4-5. The 2D and 3D interaction of selected compound displayed in fig.8-13. The interaction of quercetin showed that ligand binds specifically at TYR A:134, LYS A:18,PHE A:207,GLU A:29,THR A:26,LYS A:179,SER A:142 on *HO-1* protein through conventional hydrogen binding whereas rutin showed binding interaction at THR A:134,GLU A:29, THR A:26,LYS A:18,ARG A:183, SER A:142, LYS A:179.GLY A:139 on *HO-1* protein through conventional hydrogen binding with LEU A:138 & LYS A:22. Both lead compound showed interaction with LEU A:138 & LYS A :22 through Pi-sigma bonding along with binding at HIS A:25 site covalently on *HO-1* protein. The pharmacokinetic profile of ferulic acid reveals that it is having good pharmacokinetic profile but with the presence of any major toxic effects including mutagenicity, tumorogenicity and reproductive effects. The pharmacokinetic and toxicity profiling results of ligands like quercetin and rutin were shown in figure 6-7.

Table 2: Results of docking of ligands like quercetin and rutin against human *HO-1* receptor.

Sl. No	Compound Name	Structure	Binding Energy (Kcal/mole)
1	**Quercetin**		-6.18
2	**Rutin**		-5.26

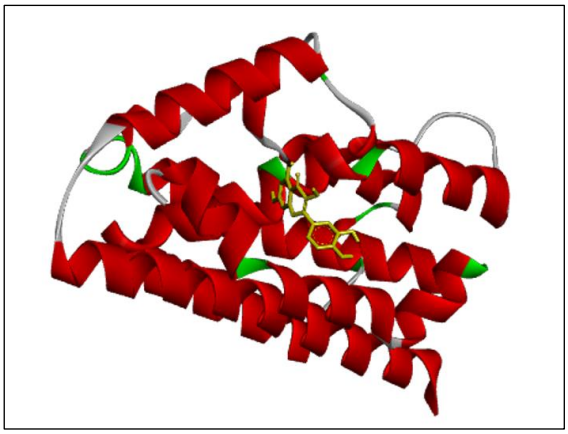


Figure 4: Binding mode of quercetin within the active site of human HO-1 receptor

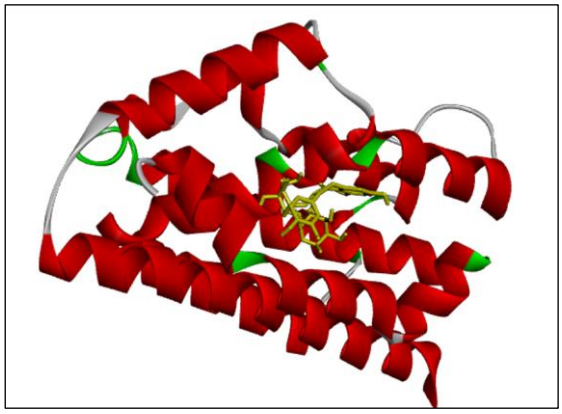


Figure 5: Binding mode of rutin within the active site of human HO-1 receptor

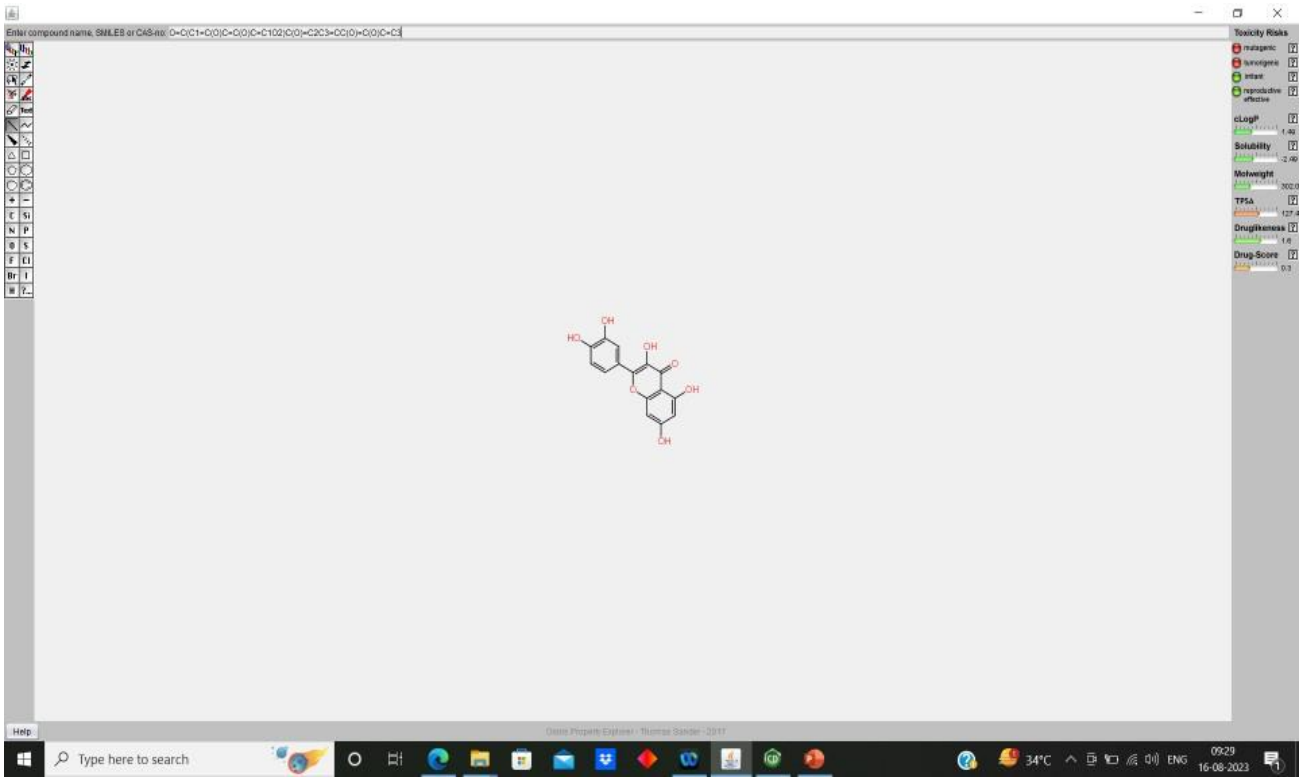


Figure 6: Pharmacokinetic and toxicity profiling of quercetin

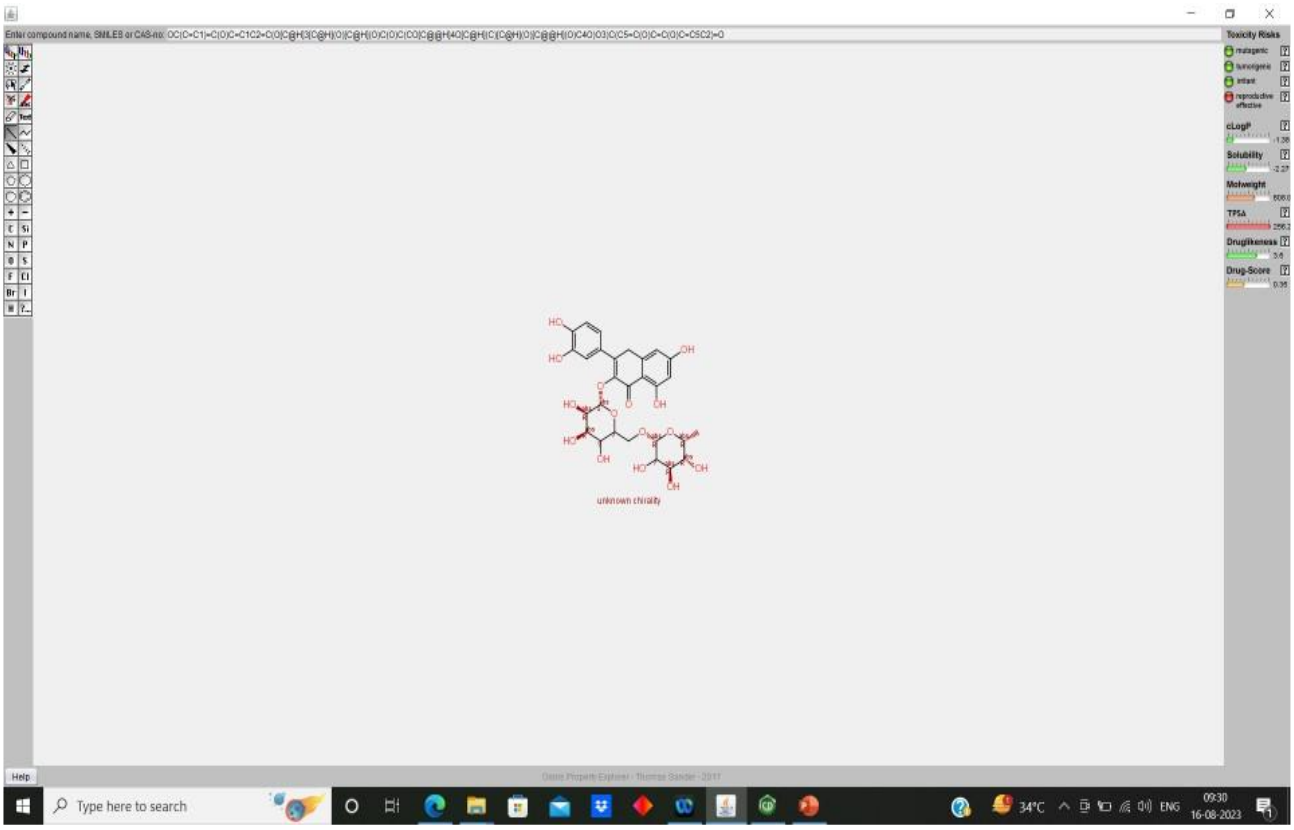


Figure 7: Pharmacokinetic and toxicity profiling of rutin

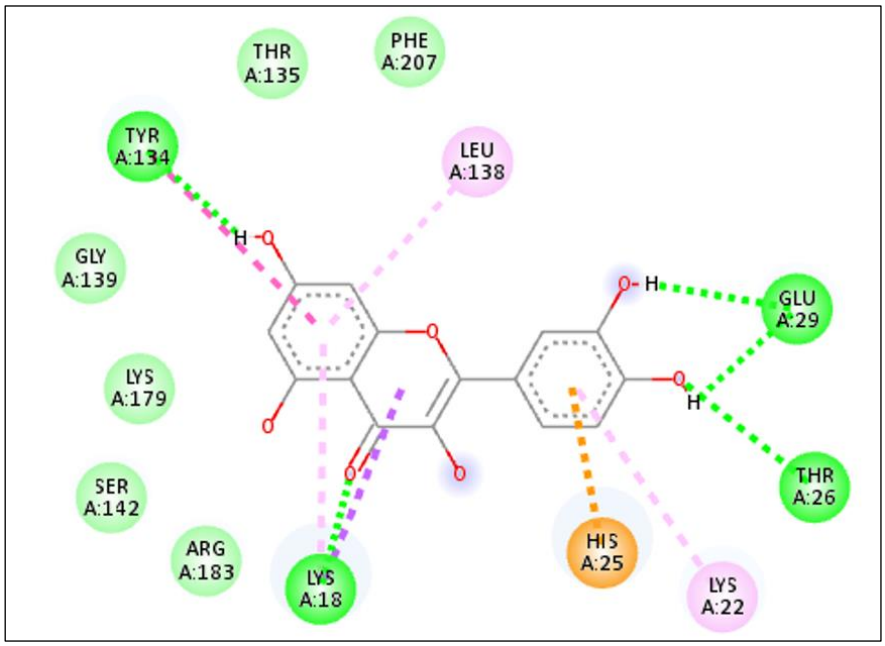


Figure 8: Two-dimensional binding mode of quercetin within the active site of human HO-1 receptor

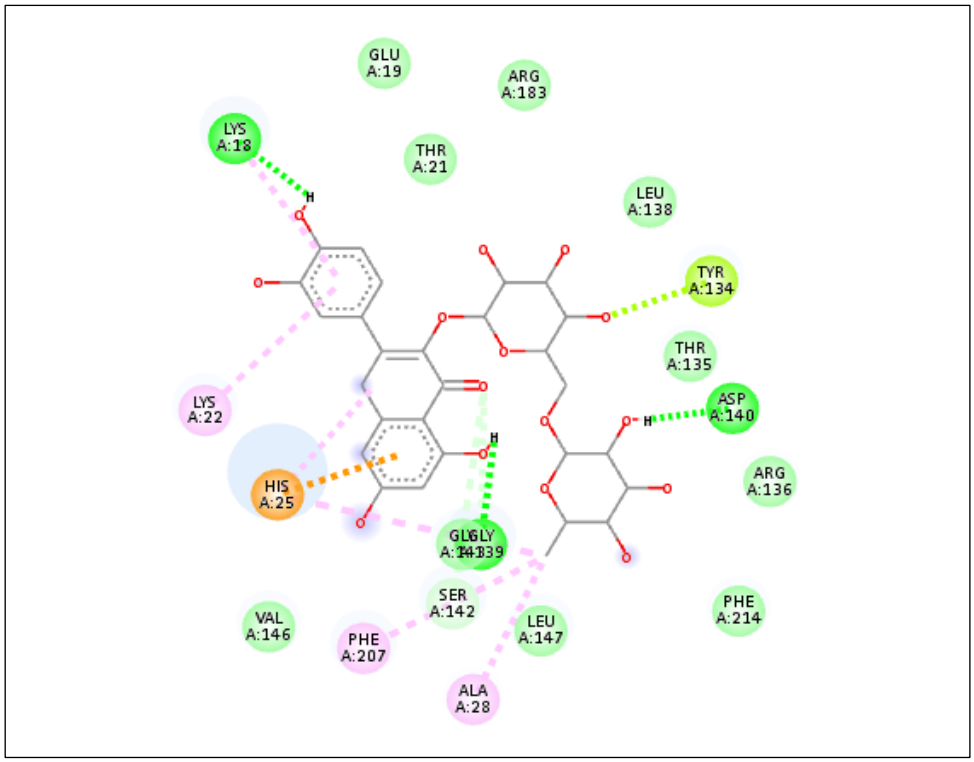


Figure 9: Two-dimensional binding mode of rutin within the active site of human HO-1 receptor

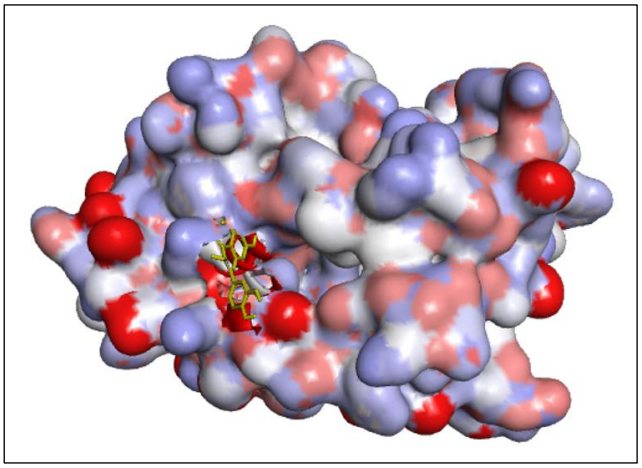


Figure 10: Three-dimensional binding conformation of quercetin within the active site of human HO-1 receptor

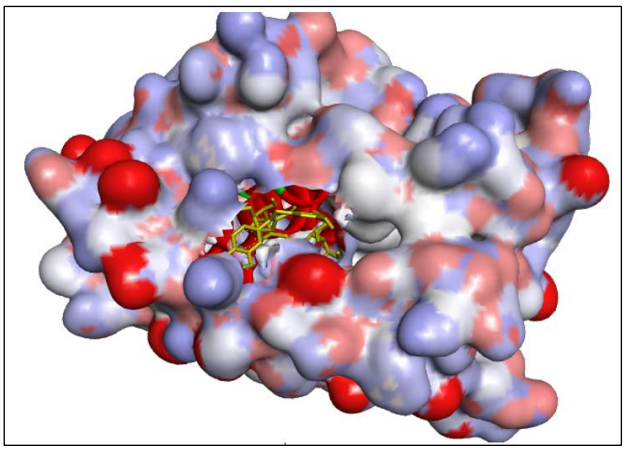


Figure 11: Three-dimensional binding conformation of rutin within the active site of human HO-1 receptor

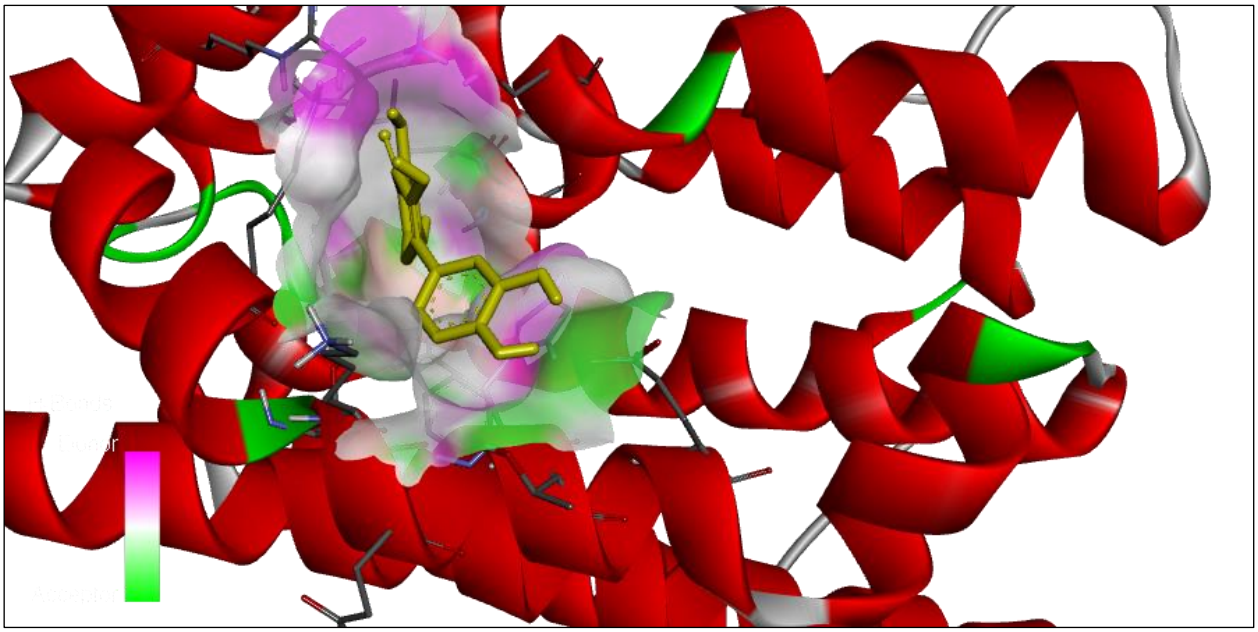


Figure 12: Three-dimensional binding mode of quercetin within the active site of human HO-1 receptor

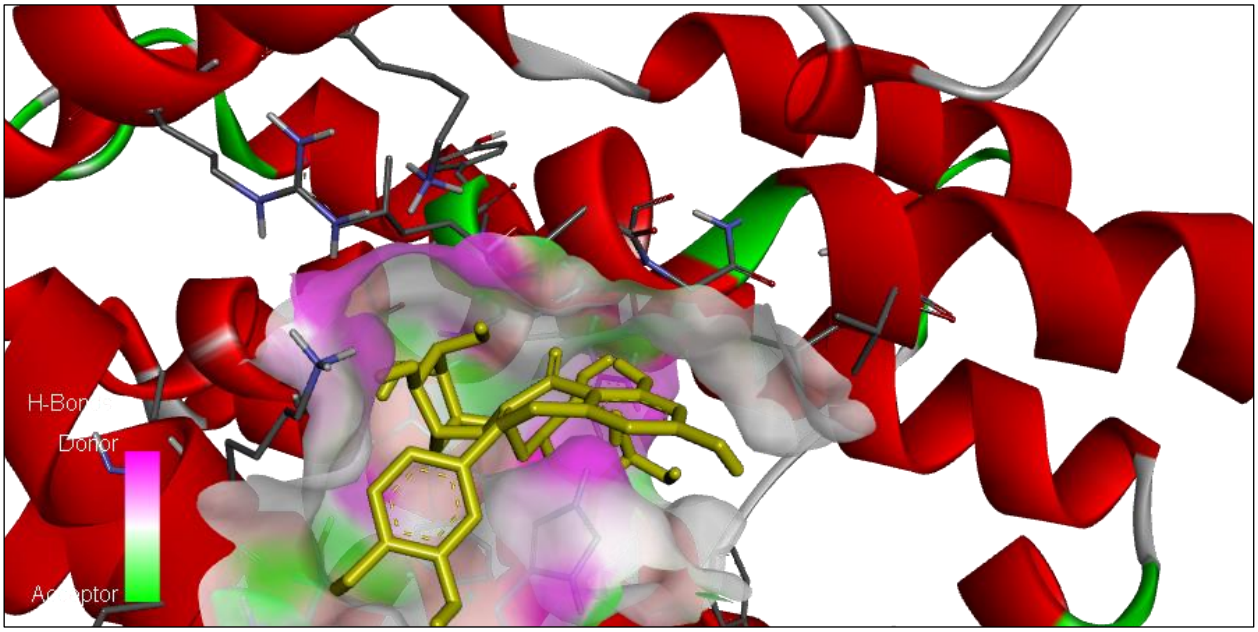


Figure 13: Three-dimensional binding mode of rutin within the active site of human HO-1 receptor

CONCLUSION

Computer based predicative validation of current investigation was designed by molecular docking of rutin and quercetin with *HO-1* protein. The cellular stress protein heme oxygenase-1 (HO-1) is essential for the oxidative degradation of heme, which results in the synthesis of biliverdin (BV), free iron, and carbon monoxide (CO). As a result, HO-1 has the capability to control oxidative and inflammatory processes, which helps to effectively manage metabolic illnesses and makes it a target of several studies aimed at validating its antioxidant capabilities. The finding of the *in-silico* molecular docking showed that both lead compound are effective binds & inhibitory action on target protein. The molecular docking of ligands like quercetin and rutin with human *HO-1* receptor revealed that it has exhibited the chemical interaction with the amino acids in the active pockets. Theoretically, all the ligand molecules have shown encouraging docking score. The docking result of quercetin revealed that their docking scores was -6.18 kcal mol^{-1}, and it can be predicted as good inhibitor of human *HO-1* receptor.

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