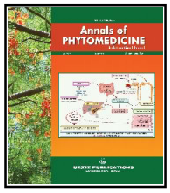
Annals of Phytomedicine 13(1): 577-588, 2024

DOI: http://dx.doi.org/10.54085/ap.2024.13.1.60

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

In silico molecular docking analysis of *Cichorium intybus* L. phytochemical compounds against two related targets of type 2 diabetes mellitus

Kurma Devakrishna, Gohar Taj◆ and Sandhya Upadhyay

Department of Molecular Biology and Genetic Engineering, G.B Pant University of Agriculture and Technology, Pantnagar-263145, Uttarakhand, India

Article Info

Article history
Received 13 February 2024
Revised 29 March 2024
Accepted 30 March 2024
Published Online 30 June 2024

Keywords
Cichorium intybus L.
Antidiabetic
Adenosine monophosphate dehydrogenase enzyme
Protein kinase A and
Molecular docking.

Abstract

To investigate the antidiabetic properties of the selected *Cichorium intybus* L. phytochemical compounds against two target proteins of type 2 diabetes mellitus (T2DM), *i.e.*, adenosine monophosphate deaminase 1 isoform 1 (AMPD1) and protein kinase A (PKA).We examined the active phytochemical compounds present in *C. intybus* to unveil their potential. This involved conducting molecular docking analysis to assess their efficacy against type 2 diabetes mellitus (T2DM) targets, a process performed using Schrodinger Maestro v12.1 docking software.

During the molecular docking process, Schrodinger Maestro 12.1v software generated a broad spectrum of docking scores. Among the chosen phytochemical compounds, chlorogenic acid exhibited the highest docking scores against AMPD1 and PKA, measuring –8.41 kcal/mole and –12.56 kcal/mole, respectively. Chlorogenic acid from *C. intybus* was observed with a significant docking score though concluded to be a more potent antidiabetic compound.

1. Introduction

The frequency of type 2 diabetes mellitus (T2DM), in the human population is increasing at a rapid rate. T2DM and its associated disorders, known as metabolic syndrome, affect over 380 million people worldwide (Chowdhury, 2010). A report from the International Diabetes Federation (2011), revealed that 366 million people are anguish from diabetes mellitus presently while by 2023 it will rise to 552 million (Whiting *et al*., 2011). T2DM has become a serious medical issue and each country is facing plenty of financial burden regarding this disorder. Hence, it is important to develop effective management strategies in preventing T2DM and also with its associated complications. T2DM accounts for over 90-95 % of all people with diabetes and in this disorder, the pancreas produces ample insulin but some misleads either with the insulin binding receptor or insulin signalling inside the target cells (WHO, 1994). Insulin resistance is the basic abnormality in the metabolism of glucose that underlies these disorders (Zhou *et al*., 2019). T2DM is an insulin independent disorder. Insulin resistance occurs due to a variety of factors which include genetic factors, more consumption of processed food, obesity, inactive lifestyle, and adult onset (>30 years) (Isganaitis and Lustig, 2005). Among the main factors, *i.e.*, obesity promotes a metabolic inflammatory response, enduring inflammation in adipose tissue and thereby causing insulin resistance, the fundamental cause of T2DM (Zietek and Rath, 2016). Various studies have evident that skeletal muscle is a crucial site of insulin action, adds to the state of T2DM observed in humans and animals due to consumption of the food that has high fat content. Children less than 15 years of age can also be affected by T2DM; however, the symptoms tend to be mild (Zimmet *et al*., 2014). If not detected at an early stage, children may develop multiple diseases, including kidney disorders and organ damage involving the kidneys, eyes, nerves, heart, blood vessels, tuberculosis, and even mucormycosis over an extended period. Additionally, most of the time in T2DM disorder the blood glucose level appear normal while abnormal at some periods. If random blood sugar (RBS) examination at normal periods is performed, it might not be detected. However, the haemoglobin A1c (HbA1c) test detects average glucose levels in blood over 3 months while T2DM is diagnosed when the HbA1c level in the blood is 6.5 per cent or higher though it is highly essential that we have to manage this disorder after its detection.

Skeletal muscle is the main location of insulin-mediated glucose absorption in humans during the postprandial state (DeFronzo and Tripathy, 2009). Insulin increases the translocation of the glucose transporter GLUT4 from intracellular vesicles to the plasma membrane and transverse tubules, stimulating glucose uptake (Suzuki and Kono, 1980). By turning on glycogen synthase, insulin increases the production of muscle glycogen. The two main target proteins of insulin resistance are adenosine monophosphate deaminase 1 isoform 1 (AMPD1) and protein kinase A (PKA). T2DM has been linked to insulin's inability to effectively inhibit AMPD1 and PKA activity in skeletal muscle, which is necessary for such effects of insulin to function at least in part. So, it has been disclosed that the skeletal muscle is among the significant organs involved in insulin resistance

Corresponding author: Dr. Gohar Taj
Professor, Department of Molecular Biology and Genetic Engineering, College of Basic Sciences and Humanities, G.B Pant University of Agriculture and Technology, Pantnagar-263145, Uttarakhand, India
E-mail: gohartajkhan@rediffmail.com
Tel.: +91-7906553007

(Bouzakri *et al*., 2005), so the therapies that direct targets expressed in such tissue may offer novel approaches for treating T2DM disorder (Subramoniam, 2014). Moreover, a recent study revealed that metformin, the most well-known medications to treat of insulin resistance, inhibits AMPD1 and the PKA which are obtained from skeletal muscle (Ouyang *et al.,* 2011).

Cichorium intybus L. (Chicory) is a medicinal herb belonging to the family Asteraceae that grows up to 1.5 meters ht. In India, chicory is widely cultivated within the states of Gujarat and region of Uttar Pradesh specifically in the districts of Etah and Aligarh (Srivastava *et al.,* 2003). Chicory leaves have abundant medicinal properties and the leaves consist of high dietary fibre sources favourable for poultry and livestock nutrition (Mahmoud, 2021). Chicory is mostly grown for its roots which are employed in the food processing industry and also accepted as a coffee substitute internationally. Chicory plant consists of many secondary metabolites including tannins, coumarins, and flavonoids and they are reported to have biological activities including antioxidant, anti-inflammatory, anticancer, antiparasitic, antihepatotoxic properties which have a supportive health effect on livestock as well as in humans (Das *et al.,* 2016; Arya *et al*., 2022). Furthermore, the ethanol extract of chicory is extensively utilized for treating diabetes mellitus, as it effectively reduces the activity of hepatic glucose-6-phosphatase (Pushparaj *et al*., 2007; Yadav and Srivastava, 2014).

Molecular docking is a computational simulation of a candidate that predicts the favored orientation of ligand binding to an active site of a receptor and forms a stable complex with minimum energy (Mukesh and Rakesh, 2011). As there are several stages and workflows involved in the finding of new drugs, *in silico* tools, particularly molecular docking, are used to streamline the process overall. With molecular docking, the therapeutic potential of any compound can be evaluated in advance, saving time and money on the drug development process. For instance, the present analytical study utilizes a computational approach to evaluate the pharmacological properties of chicory phytoconstituent against T2DM disorder.

This study aims to identify innovative ligand compounds targeting adenosine monophosphate deaminase 1 isoform 1 and protein kinase A and assess their ADMET pharmacokinetic properties (Indumathy *et al*., 2023). These analyses are pivotal for gauging the safety profile of identified compounds, and determining their potential as drug molecules for further development.

2. Materials and Methods

2.1 *In silico* analysis (Software and database)

Schrodinger-Maestro 12.1v software, PubChem Database https://pubchem.ncbi.nlm.nih.gov/, PubMed Database https://pubmed.ncbi.nlm.nih.gov/, RCSB-PDB https://www.rcsb.org/ , Swiss-model (https://swissmodel.expasy.org/) – homology modelling server of protein, SwissADME (http://www.swissadme.ch/) a free web tool to predict ADME parameters and ProTox-II Server (https://tox-new.charite.de/protox_II/). Endocrine disruptome (http://endocrinedisruptome.ki.si/).

2.1.1 Swiss-model

Swiss-model, a fully automated server that can cast protein structure through homology modelling (Biasini *et al*., 2014) including the retrieval of complete amino acid sequences in fasta format using the protein database of the National Center for Biotechnology Information (NCBI). The second step is to submit the fasta sequence of amino acids into the swiss-model server to discover a known template structure that resembles to the target sequence. Thereafter, the basic local alignment search tool (BLAST) was employed to choose a set of proteins with greater homology which was present in the protein data bank (PDB). Third, using the template structure as a basis, a 3 dimensional (3D) model of the target protein structure was modelled. Fourth, the modelled structure's molecular dynamics and thermodynamics were optimized to remove unnecessary components. Lastly, until the model's quality was optimized, the previous three phases were repeated.

2.1.2 Phylogenetic analysis

To construct and analyze phylogenetic trees to study ancestral relationships, a popular software known as MEGA11.0 (Molecular Evolutionary Genetic Algorithm) is used on AMPD1 and PKA. When building the phylogenetic tree, the distance-based method was used with neighbour joining and the bootstrap value of 1000 replications was considered (Tamura *et al.,* 2021).

2.1.3 Molecular docking studies

Docking studies performed through Schrodinger-Maestro v 12.1 software. The pattern of molecule binding was explored using the XP visualizer tool which was present in this software. The ligand compounds possessing highly efficient affinity binding were picked for further studies of absorption, distribution, metabolism, and excretion profiling through SwissADME analysis and toxicity prediction done through ProTox-II server (Dheeraj *et al*., 2023).

2.1.4 Preparation of *Homo sapiens* AMPD1 and PKA protein structures

The polyphenol compounds reported in this paper have shown a desirable antidiabetic activity (Sun *et al*., 2020). To study interactions between these polyphenol compounds and the diabetes causing target proteins like AMPD1 and PKA (Plaideau *et al*., 2012; Hafizur *et al*., 2018), computational work has to be done by performing molecular docking. From NCBI, we retrieved the protein sequence of AMPD1 (Accession: NP_000027.3) and PKA (Accession: AAL40923.1) enzymes. The 3D protein structure was built using a Swiss-model server. Then the protein was prepared, refined, optimized, and minimized before docking by using the protein preparation wizard tool of Schrodinger-Maestro (Release, 2016). In protein preparation, all water molecules were eliminated, polar hydrogens were attached to the heavy atoms so that charges were stabilized, bond order and charges were allocated and all selenomethionines were converted into methionines. By using optimized potentials for liquid simulations (OPLS3e) force field the protein was minimized by converging heavy atoms to RMSD 0.30 Å. The tautomeric states and the protonation were kept at pH 7.0 ± 2.0. The Ramachandran plot was used to assess the protein's overall stability and its stereochemical quality in the three dimensional structure (Yadav and Khandelwal, 2019).

2.1.5 Ligand preparation

Compounds that are used as input for docking studies were retrieved from PubChem. The polyphenol compounds belonging to chicory plants are taken as ligands and the compounds that are retrieved are caffeic acid, chlorogenic acid, cichoric acid, coumarin, kaempferol, and ferulic acid. Some prescribed drugs including metformin,

sitagliptin, nateglinide, farxiga and dasatinib are taken as ligands to compare with natural compounds. After incorporation of compounds into the working station, parameters like Epik 2.2 are used for the neutralization of compounds at pH 7.0 ± 2.0, and OPLS3e force field is used in energy minimization of ligands which is included in the Ligprep module of Schrodinger-Maestro software (Release, 2019). All possible ring conformations, stereochemistry, and tautomers were generated. Stereoisomers were generated by retaining specified chiralities considering a maximum of 32 isomers per ligand. The energy minimization of compounds helps in assigning bond order, assigning hydrogens to the ligands, and also in the conversion of ligand structures from 2D to 3D which is required for docking studies. Later on, the generated output file was applied in docking studies.

2.1.6 Binding site prediction and receptor grid generation

On the protein surface, the hydrophobic cavities are responsible for its specificity (del Sol *et al.*, 2006). The active site of protein can be theoretically determined by using tools, based on algorithmic results of computational geometry namely the sitemap of Schrodinger-Maestro suite (Dundas *et al.*, 2006; Halgren, 2007). This Sitemap prediction tool scientifically furnishes the volume and area of these hydrophobic cavities. Five standard sitemaps are provided and select first sitemap conformation is to pick an atom in the ligand. Then, the receptor grid is generated around the predicted active site by using the glide module. The grid was in the shape of a cube which was located in the centre of the centroid of the residues of the active site of the receptor and all the parameters were kept in default condition (Kawatkar *et al*., 2009). Additionally, sitemap can provide an altered version of the scoring that correctly categorizes the drug ability of proteins. Sitemap can identify the binding sites in a large scale validation with the best outcomes for ligand binding sites with the sub-nanomolar association (Halgren, 2009).

2.1.7 Glide extra precision (XP) ligand docking

Extra precision (XP) ligand docking was performed using Schrodinger glide to obtain high-accuracy docking results (Halperin *et al.*, 2002). To soften the potential for ligand nonpolar parts, the scaling of the van der waals factor and partial charge cut off were kept at 0.80 and 0.15, respectively. Flexible ligands docking with sample inversions of nitrogen and sample ring conformations were selected. For all predefined functional groups bias sampling of torsions was applied and also Epik state penalties were applied to the docking score. The final scoring function was carried out using energy-minimized conformations and then it shows the result as docking score and glide score. The conformation that had docked with the lowest docking score was obtained for each ligand.

2.2 Visualization of results

2.2.1 Docking and glide score

The XP visualizer tool was utilized to view the docking and glide score after performing ligand docking (Halperin *et al.,* 2002). The outcome was obtained in an excel sheet in CSV format.

2.2.2 Residues that stabilize AMPD1 and PKA - ligand complexes

The identification of AMPD1 and PKA amino acid residues that interact with different sets of ligands in the most stable complex conformation was done by using a ligand-protein 2D interaction diagram.

2.2.3 *In silico* study: Determination of pharmacokinetic parameters by SwissADME

After performing docking studies, the physicochemical descriptors as well as ADME parameters of the selected lead compounds were predicted by *in silico* analysis using the SwissADME web tool (Daina *et al.*, 2017). Refractive index, topological polar surface area, molecular weight, hydrogen-bond donors and acceptors, topological polar surface area (TPSA), and number of rotatable bonds (nRB) are some of the descriptors of phytochemical compounds that are obtained by using this tool. It also predicts the pharmacokinetic properties of the obtained hit compound to support its drug discovery (Egan *et al.,* 2000). The canonical smiles notations of ligand compounds were retrieved from the PubChem database and then it was used as an input file for the SwissADME web tool. Based on Lipinski's rule of five, one can determine whether a ligand molecule has drug-like activity or not. It is possible to have inadequate permeability and absorption if more than two of the five parameters are out of range.

2.2.4 *In silico* study: Toxicological properties prediction by ProTox-II server

The process of creating new drug designs includes a significant amount of compound toxicity prediction. In addition to being quicker than figuring out toxic doses in animals, computational toxicity estimations can also reduce the need for animal testing. The toxicity profile of the chicory chemical compounds and also the prescribed drugs were predicted using the ProTox-II online server (Banerjee *et al*., 2018). The toxicity parameters like hepatotoxicity, immunotoxicity, carcinogenicity, mutagenicity, and cytotoxicity were predicted.

The LD_{50} values for toxic doses are frequently expressed in mg/kg body weight. The median lethal dose, or LD_{50}, is the dose in which half of the test subjects pass away after being exposed to a substance.

According to the globally standardized system of classification and labeling of chemicals, toxicity classes are established. Values for LD_{50} are provided in [mg/kg].

Class I: If swallowed, fatal ($LD_{50} \leq 5$)

Class II: If swallowed, fatal ($5 < LD_{50} \leq 50$)

Class III: Toxic if swallowed ($50 < LD_{50} \leq 300$)

Class IV: If swallowed, harmful ($300 < LD_{50} \leq 2000$)

Class V: May be harmful, if swallowed ($2000 < LD_{50} \leq 5000$)

Class VI: Non-toxic ($LD_{50} > 5000$).

2.2.5 *In silico* study: Endocrine disruption potential prediction

It is very critical to anticipate the endocrine disruption potential of the chosen phytoconstituents and the prescribed drugs. The software predicts the potential for endocrine disruption using an online platform available at (http://endocrinedisruptome.ki.si), well-validated web application for evaluating endocrine disruption potential via nuclear receptor binding (Kolšek *et al*., 2014). This software uses docking interface for target systems (DoTS) for the docking simulation and the docking calculation is performed by AutoDock Vina. The evaluation includes 16 different nuclear receptors: Androgen receptor antagonist (AR an.) and Androgen

receptor (AR); Estrogen receptors alpha antagonist (Er α an.), Estrogen receptor alpha (Er β), Estrogen receptor beta antagonist (Er β an.) and Estrogen receptor beta (ER β); Glucocorticoid receptor antagonist (GR an.) and Glucocorticoid receptor (GR); Liver X receptor β (LXRβ) and Liver X receptor β (LXRβ); Peroxisome proliferator-activated receptors α(PPARα), β (PPARβ), and γ(PPARγ); Retinoid X receptor α(RXRα); Thyroid receptor α(TRα) and Thyroid receptor β(TRβ) (Kenda and Sollner Dolenc, 2020).

3. Results

3.1 *In silico* studies

3.1.1 Homology modelling

The AMPD1 and PKA protein sequences of *Homo sapiens* were acquired from NCBI with accession numbers NP_000027.3 and AAL40923.1, respectively. The amino acid sequence length for AMPD1 and PKA are 747 and 2813 amino acids respectively. When the 3D structures for AMPD1 and PKA were examined in the PDB structure database, they were unavailable. So, the protein sequences were employed for template selection by performing homology modelling using the Swiss model. Both the templates (PDB ID: 2a3l.1.A and PDB ID: 4d0n.1.B) for AMPD1 and PKA were selected and homology modelling of the protein was done and their structures are illustrated in Figure 1. AMPD1 template shows 47.29% sequence identity with an X-ray crystal structure atomic resolution having 3.3Å and QMEAND score is 0.73 ± 0.05 whereas for PKA template shows 100% sequence identity with an X-ray crystal structure atomic resolution having 2.1Å and QMEAND score is 0.76 ± 0.05.

3.1.2 Model evaluation and validation

The modelled 3D structure of the protein was stereochemically validated by using the Ramachandran plot. Drug design must consider the targeted protein's reliability and quality in its 3D shape. The allowed and disallowed regions are displayed on the Ramachandran plot that was generated by using the Schrodinger's suite tool (Figure 2). For AMPD1 as well as PKA proteins, more than 99% of the residues were present in the allowed regions (96% of residues were in the favoured region and 4% in the allowed regions and 0% in the disallowed regions and this result infers the reliability and the attribute of the protein structures.

3.1.3 Phylogenetic analysis

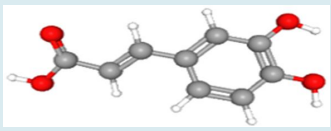
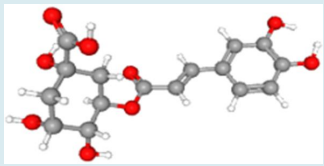
MEGA 11.0 software was used to construct a neighbour joining phylogenetic tree of the selected AMPD1 and PKA proteins to find out their evolutionary history among humans, chimpanzees, and gorillas. The phylogenetic trees of AMPD1 and PKA are presented in Figure 3. As per phylogenetic studies, the *Homo sapiens* AMPD1 isoform 1 is closely related to AMPD1of *Homo sapiens* and then is also related to *Pan paniscus* (pygmy chimpanzee) up to some extent. *Pan troglodytes* (chimpanzees) were ascertained as an outgroup in the primates whereas the PKA protein is closely linked to the PKA isoform 2 protein of *Homo sapiens* and then it is related with gorillas up to some extent. *Pan paniscus* and *Pan troglodytes* are in the same cluster for PKA protein and it infers that they are closely related.

3.1.4 Molecular docking analysis

This study's primary goal is to determine novel AMPD1 and PKA inhibitors based on their binding affinity with AMPD1 and PKA target proteins. Generally, phytochemical compounds have one to many medicinal properties and they are taken as inhibitor compounds. In this study, seven different phytochemical compounds from the *C. intybus* along with the five prescribed drugs available in the market are taken into consideration and their 3D conformers are presented in Table 1. Then we performed virtual screening studies to know their inhibitory effect on AMPD1 and PKA proteins. Chlorogenic acid (phenolic compound) present in roots of *C. intybus* had shown highest significant Glide score of -8.42 and Dock score of -8.41 for AMPD1-chlorogenic acid complex whereas Glide score of -12.56 and Dock score of –12.56 for PKA-chlorogenic acid complex. The results of docking studies are detailed in Table 2 and the Figures of docking are presented in Figure 4 and the best 3D binding poses are presented in Figure 5.

Each phytochemical that has been employed for this study needs to qualify for the drug likeness test for *in silico* drug designing. All the phytochemicals should not violate the Lipinski rule of 5 for various descriptors. The independent descriptors of various phytochemicals were determined using the SwissADME web online server and represented in Table 3. Two minimum violations are allowed for each phytochemical to be considered for further analysis. The Varying descriptors of each phytochemical (ligand) along with 5 commercial drugs were compared manually. According to the Table 3, cichoric acid had two violations whereas chlorogenic acid had one violation while the rest of the phytochemical compounds had zero violations. The prescribed drugs which are in comparison with the phytochemicals have zero violation. In this study, we have decided to choose two minimum violators for each phytochemical and they are taken for molecular docking.

Table 1: Selected natural compounds of chicory plant and prescribed drugs with their molecular formula and 3D conformers used for *in silico* study

S.No.	Natural compounds	PubChem ID	Molecular formula	3D conformer
1.	Caffeic acid	CID 689043	$C_9H_8O_4$	
2.	Chlorogenic acid	CID 1794427	$C_{16}H_{18}O_9$	

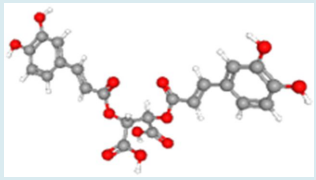
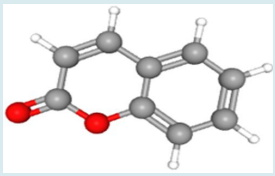
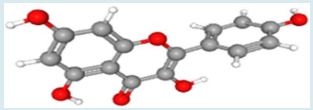
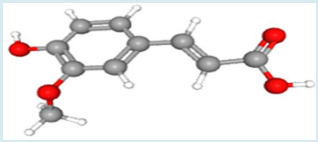
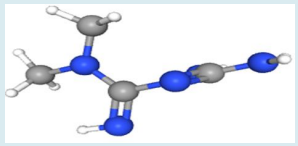
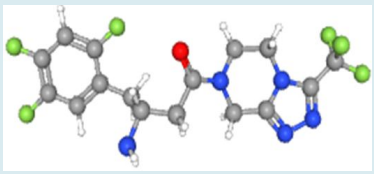
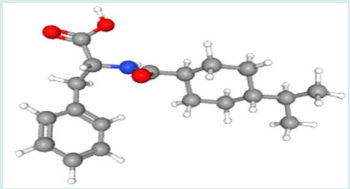
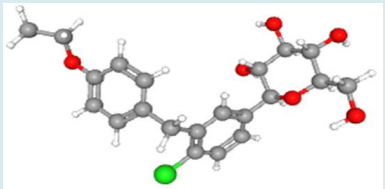
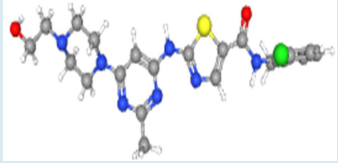
3.	Cichoric acid	CID 5281764	$C_{22}H_{18}O_{12}$	
4.	Coumarin	CID 323	$C_9H_6O_2$	
5.	Kaempferol	CID 5280863	$C_{15}H_{10}O_6$	
6.	Ferulic acid	CID 445858	$C_{10}H_{10}O_4$	
7.	Metformin	CID 4091	$C_4H_{11}N_5$	
8.	Sitagliptin	CID 4369359	$C_{16}H_{15}F_6N_5O$	
9.	Nateglinide	CID 5311309	$C_{19}H_{27}NO_3$	
10.	Farxiga	CID 9887712	$C_{21}H_{25}ClO_6$	
11.	Dasatinib	CID 3062316	$C_{22}H_{26}ClN_7O_2S$	

Table 2: Docking scores of phytochemical compounds of the chicory plant and available prescribed drugs with AMPD1 and PKA enzymes for antidiabetic activity

Compounds	AMPD1			PKA		
	Docking score (kcal/mol)	Glide score (kcal/mol)	Lipophilicity	Docking score (kcal/mol)	Glide score (kcal/mol)	Lipophilicity
Chlorogenic acid	-8.41	-8.42	-3.42	-12.56	-12.56	-3.37
Cichoric acid	-1.37	-1.37	-3.54	-9.28	-9.28	-4.65
Coumarin	3.89	-3.89	-2.82	-6.51	-6.51	-3.25
Kaempferol	-5.57	-5.6	-2.54	-3.78	-3.81	-1.78
Ferulic acid	-2.55	-2.55	-1.53	-4.96	-4.96	-1.03
Caffeic acid	-4.98	-4.98	-1.55	-7.79	-7.79	-2.73
Prescribed drugs available in market						
Metformin	-5.68	-6.04	-0.82	-3.6	-3.6	-0.76
Sitagliptin	-6.38	-6.38	-2.67	-3.08	-3.09	-1.19
Nateglinide	-3.36	-3.36	-2.52	-3.56	-3.56	-1.95
Farxiga	-5.14	-5.14	-2.95	-4.6	-4.6	-1.19
Dasatinib	-6.61	-7.05	-3.74	-2.75	-2.31	-1.98

Table 3: Pharmacological properties of phytochemical compounds of chicory as well as prescribed drugs

S.No.	Natural Compound	Mol.Wt (g/mol)	LogP	nOH	nOHNH	Nb	Bioavailability	Number of violations
1.	Caffeic acid	180.16	0.93	04	03	02	0.56	00
2.	Chlorogenic acid	354.31	-0.38	09	06	05	0.11	01
3.	Cichoric acid	474.37	1.01	12	06	11	0.11	02
4.	Coumarin	146.14	1.82	02	00	00	0.55	00
5.	Kaempferol	286.24	1.58	06	04	01	0.55	00
6.	Ferulic acid	194.18	1.36	04	02	03	0.85	00
Prescribed drugs available in market								
1.	Metformin	129.16	-0.89	02	03	02	0.55	00
2.	Sitagliptin	407.31	2.51	10	01	06	0.55	00
3.	Nateglinide	317.42	3.21	03	02	07	0.85	00
4.	Farxiga	408.87	2.18	06	04	06	0.55	00
5.	Dasatinib	488.01	2.80	06	03	08	0.55	00

Note: Log P (lipophilicity), nOH (no. of H bond acceptors), nOHNH (no. of H bond donors), Mol.Wt (molecular weight), nb (no. of rotatable bonds) and violations based on Lipinski rule.

Table 4: Toxicity properties of phytochemical compounds

Natural compound	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
Caffeic acid	Inactive	Active	Inactive	Inactive	Inactive
Chlorogenic acid	Inactive	Inactive	Active	Inactive	Inactive
Cichoric acid	Inactive	Inactive	Active	Inactive	Inactive
Coumarin	Inactive	Active	Inactive	Inactive	Active
Kaempferol	Inactive	Inactive	Inactive	Inactive	Inactive
Ferulic acid	Inactive	Inactive	Active	Inactive	Inactive
Caffeoylmalic acid	Inactive	Inactive	Active	Inactive	Inactive
Prescribed drugs available in market					
Metformin	Inactive	Inactive	Inactive	Inactive	Inactive
Sitagliptin	Inactive	Inactive	Inactive	Inactive	Inactive
Nateglinide	Inactive	Inactive	Inactive	Inactive	Inactive
Farxiga	Inactive	Inactive	Inactive	Inactive	Inactive
Dasatinib	Inactive	Active	Active	Inactive	Inactive

3.1.5 Prediction of endocrine disruption potential

This study's primary goal is to determine novel AMPD1 and PKA inhibitors based on their binding affinity with AMPD1 and PKA target proteins. According to predictions made using the computational tool Endocrine Disruptome, a small number of human nuclear receptors can be affected by the selected phytochemicals and prescribed drug action. The possibility that these phytoalexins and prescribed drugs will bind to a few of these receptors, red boxes indicate a high possibility of binding, orange boxes indicate an intermediate possibility of binding, yellow boxes indicate a moderate possibility of binding whereas the green boxes indicate a low possibility of binding.

The selected phytochemicals and the prescribed drugs show moderate to intermediate possibility of binding on the antagonist androgen receptor but the kaempferol compound has shown a high possibility of binding on both the androgen and antagonist androgen receptors. The phytochemicals and the prescribed drug's capitative for endocrine disruption were predicted, as a result of their interactions with several human nuclear receptors are shown in Table 5. The Endocrine Disruptome tool's predictions show that the nuclear receptors that are nearly affected by the studied phytoalexins and the prescribed drugs are: AR an, AR, ER α, GR, TR α, and TR β. This result is in line with the published research showing that antidiabetic drugs can bind with the nuclear receptors, particularly on antagonist androgen receptors, glucocorticoid receptors, and thyroid hormone receptors α and β. (Sakkiah *et al.,* 2018; Niemuth *et al.*, 2015; Lehmann *et al*., 1995).

Table 5: This study aims to identify new inhibitors for AMPD1 and PKA by assessing their binding affinity with target proteins. It also predicts the endocrine disruption potential of selected phytochemicals and prescribed drugs, categorizing their binding probabilities into high, intermediate, moderate, and low likelihoods

Phytochemicals	AR	AR an	ERα	ERα an	ERβ	Erβan	GR	GR an	LXRα	LXRβ	PPARβ	PPARβ	PPARγ	RXRα	TRα	TRβ
Caffeic acid	-6.8	-6.6	-6.4	-6.6	-6.4	-6.3	-7.0	-6.0	-6.6	-7.0	-6.2	-6.1	-6.6	-7.2	-6.9	-6.8
Chlorogenic acid	-6.8	-8.1	-8.5	-7.3	-8.4	-7.0	-8.9	-7.6	-8.8	-8.8	-7.6	-7.8	-7.9	-9.3	-8.7	-8.3
Cichoric acid	-6.0	-6.5	-8.6	-8.7	-4.0	-7.8	-9.3	-8.3	-9.4	-8.2	-7.8	-8.5	-9.0	-9.7	-6.7	-8.9
Coumarin	-6.8	-6.9	-6.3	-6.6	-6.3	-6.4	-6.3	-6.0	-7.0	-7.0	-6.3	-6.9	-6.9	-6.5	-7.0	-6.6
Kaempferol	-8.8	-8.6	-8.4	-8.5	-7.9	-8.4	-8.7	-7.9	-9.0	-9.1	-7.7	-8.6	-9.4	-9.2	-9.2	-9.1
Ferulic acid	-6.6	-6.5	-6.2	-6.3	-6.3	-6.2	-7.2	-6.0	-6.5	-6.9	-6.2	-6.1	-6.5	-7.4	-7.2	-6.6
Caffeoylmalic acid	-7.6	-7.6	-7.6	-7.7	-7.8	-7.1	-7.8	-6.8	-7.8	-7.7	-7.3	-7.3	-7.1	-8.1	-7.9	-8.2
Prescribed drugs available in market																
Metformin	-4.9	-5.3	-5.3	-4.9	-4.9	-4.7	-4.7	-4.9	-5.1	-5.0	-5.1	-5.1	-4.8	-4.8	-5.2	-5.2
Sitagliptin	-7.7	-7.8	-9.2	-8.5	-8.6	-8.0	-9.5	-8.5	-10.1	-10.5	-8.9	-10.0	-9.1	-10.1	-9.7	-9.9
Nateglinide	-7.3	-8.4	-8.5	-8.4	-7.9	-7.7	-8.9	-7.1	-9.3	-8.9	-8.0	-8.5	-7.7	-9.2	-9.2	-9.3
Farxiga																
Dasatinib	7.8	5.1	-6.6	-8.9	3.5	-8.1	-7.0	-9.5	-9.1	-9.1	-7.5	-8.7	-8.0	-5.2	0.2	-0.6

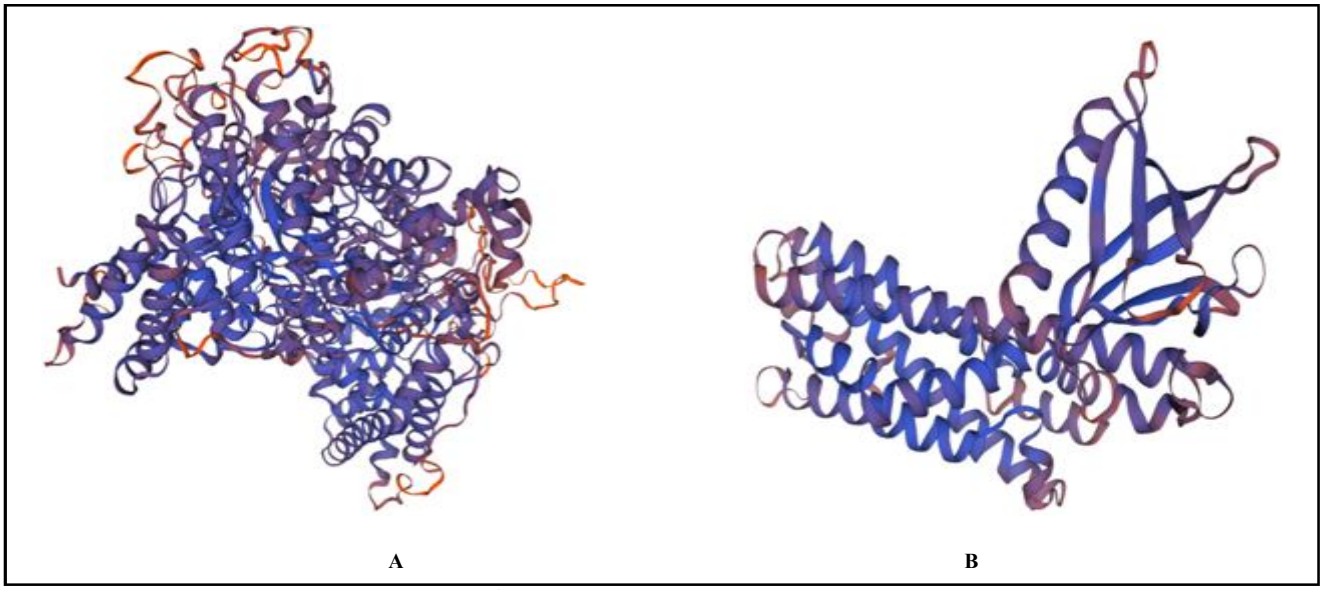
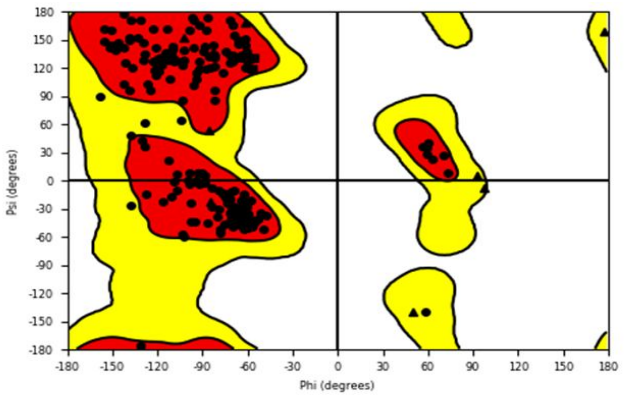
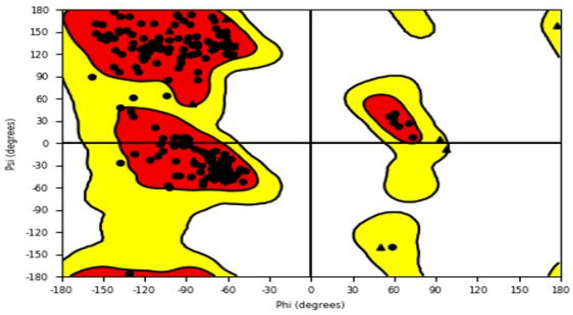


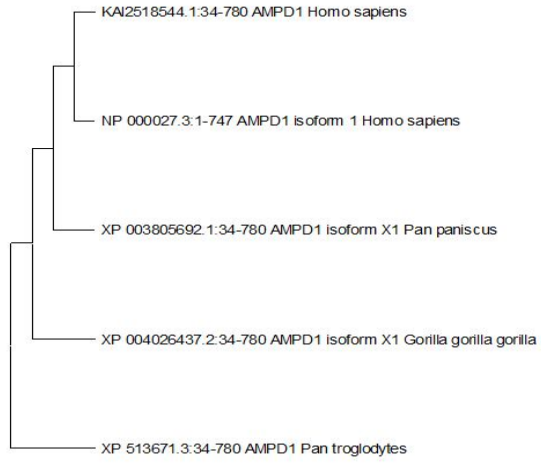
Figure 1: 3D structures of the target proteins (Å) AMPD1 and (B) PKA built using Swiss model tool.





2a3l.1.a 4d0n.1.B

Figure 2: Ramachandran plots for 2a3l.1.A and 4d0n.1.B target proteins generated by PROCHECK.



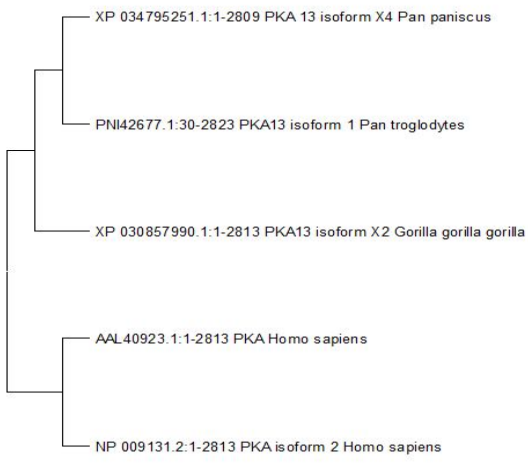
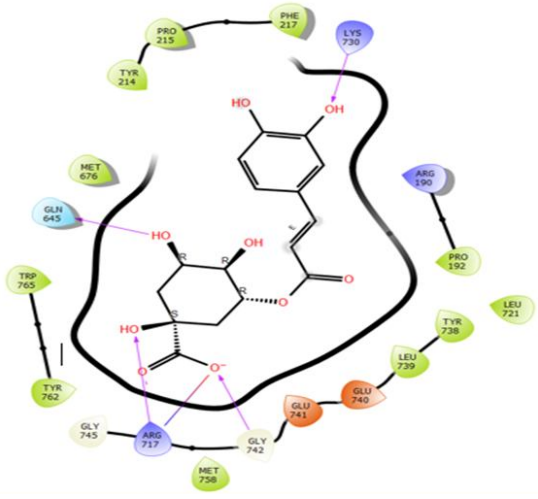
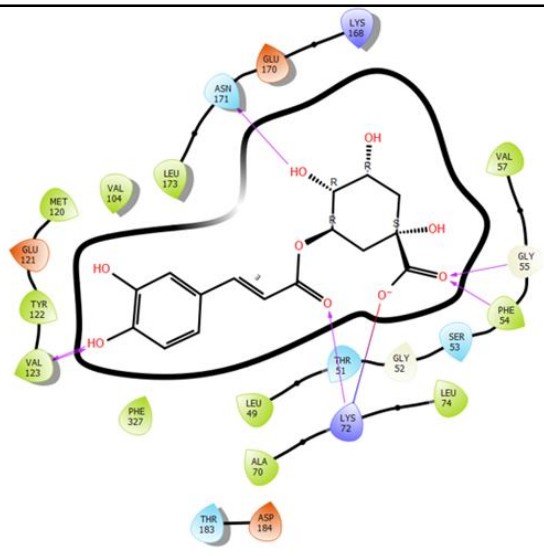
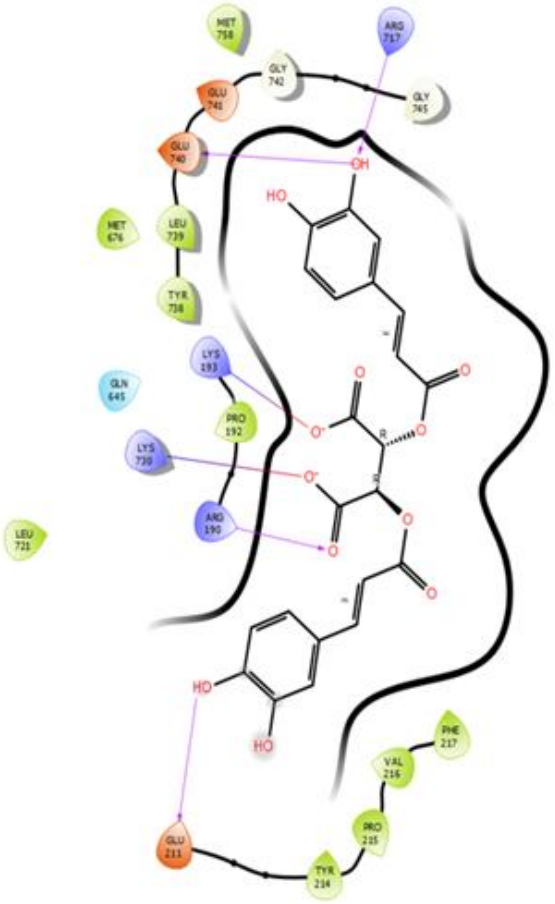


Figure 3: Evolutionary history of AMPD1 and PKA proteins were constructed using MEGA11.0.

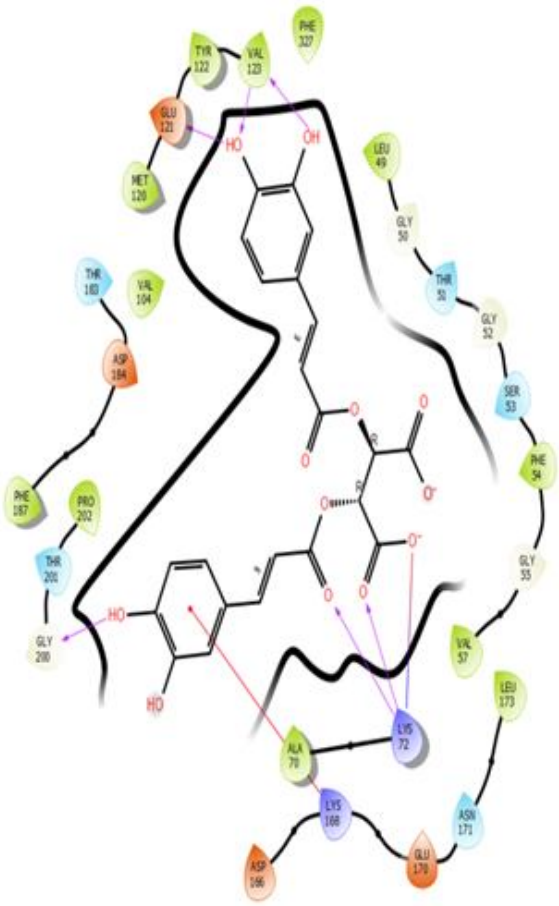




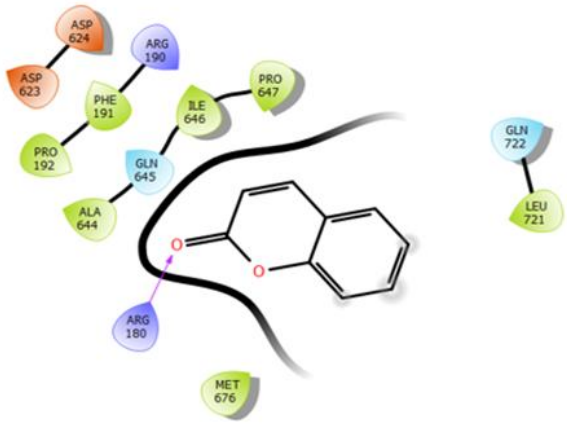
Chlorogenic acid with AMPD1 Chlorogenic acid with PKA



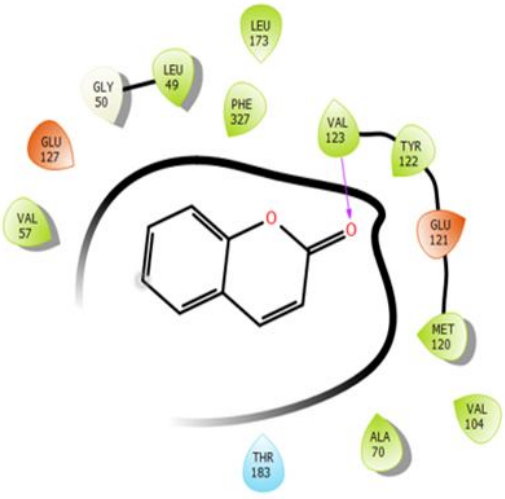
Cichoric acid with AMPD1



Cichoric acid with PKA



Coumarin with AMPD1



Coumarin with PKA

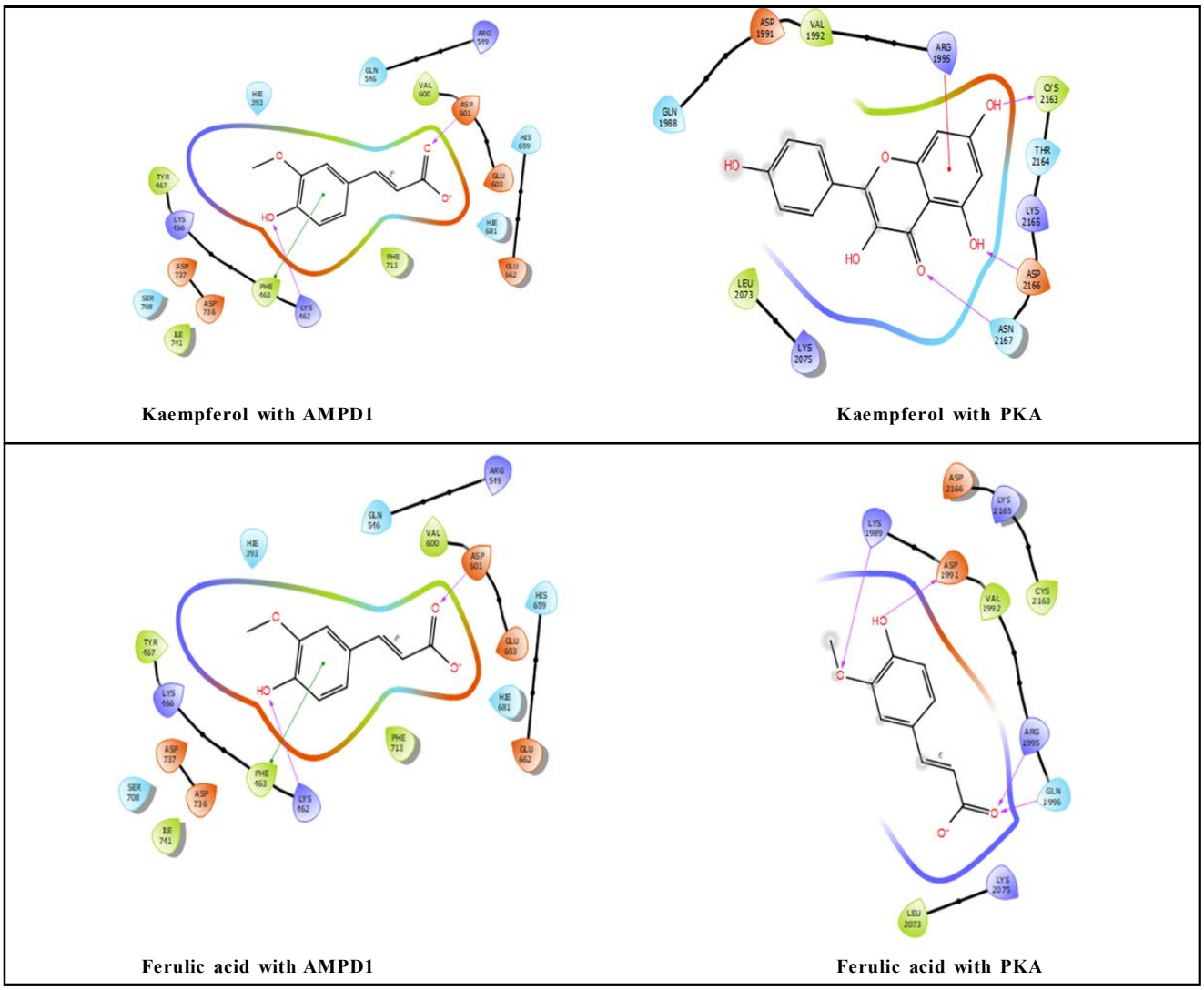


Figure 4: Visualization of 2D AMPD1 AND PKA proteins and their phytochemical ligand interaction, Lid legend tool, Schrodinger software.

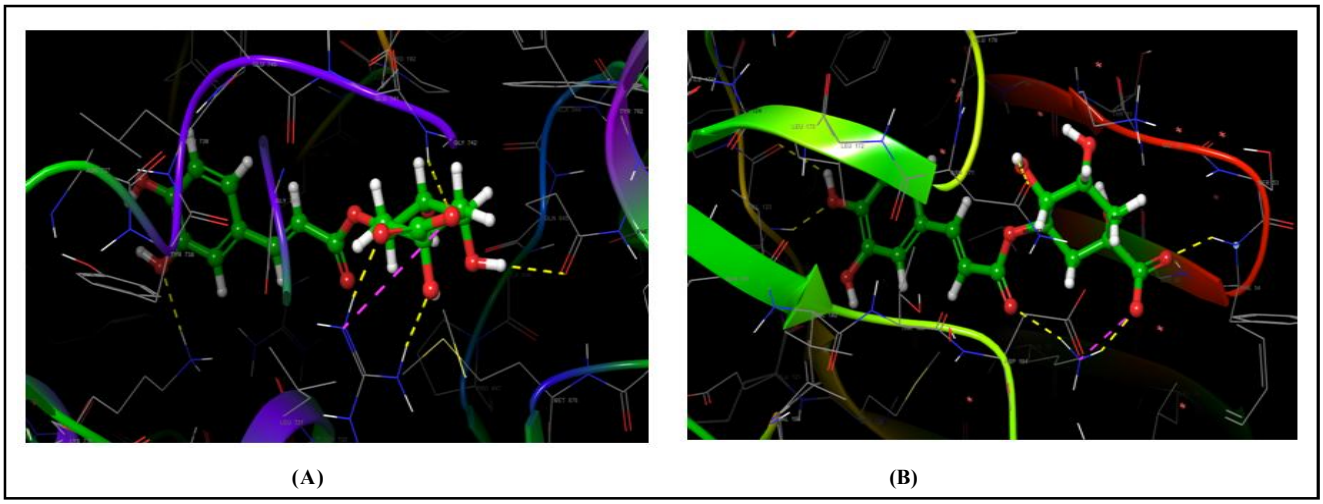


Figure 5: Best 3D binding pose of chlorogenic acid on AMPD1 (A) and PKA 3D (B) protein structure taken from Schrodinger software v 12.1.

3.1.6 Oral toxicity prediction

The toxicity was predicted through ProTox-II web server using chemical similarity, fragment propensity, and machine-learning software and the analysed results are illustrated in Table 4. All the natural compounds have predicted toxicity class either 04 or 05 except coumarin having toxicity class 03. Chlorogenic acid which has the highest docking score for both the target proteins falls under toxicity class 05, slightly immunotoxin in nature, and the predicted LD_{50} value is: 5000 mg/kg, and less than this dose may be made and prescribed as a drug compound.

4. Discussion

The dual targets of diabetes mellitus that are the subject of the present study include essential structural target proteins. The disease can be effectively reduced by inhibiting these targets. The bioactive compounds used in this work were chosen from the *C. intybus* plant, and our *in silico* docking tests revealed that the bioactive compounds interacted with the target receptor molecules. The bioactive herbal compounds have demonstrated higher inhibitory efficacy due to their lower binding energies when compared to the standard medications. Schrodinger Maestro v 12.1 docking analysis revealed that *C. intybus*'s chlorogenic acid had the highest docking score, which was -8.41 kcal/mole for AMPD1 and -12.56 kcal/mole for PKA target proteins. Chlorogenic acid formed hydrogen bonds with the residues Gly742, Arg717, Gln645, and Lys730 to interact with the different amino acids of the AMPD1 receptor. In addition, chlorogenic acid formed hydrogen bonds with the residues Phe54, Gly55, Lys 72, Val123, and Asn171 of the PKA receptor to interact with its amino acids. With one exception, the ADME parameter of chlorogenic acid obeys with the Lipinski rule of five. Our findings revealed that chlorogenic acid had less toxicity with respect to hepatotoxicity, carcinogenicity, mutagenicity and cytotoxicity but it has some immunotoxic properties and there is a need for further studies to be done. Chlorogenic acid has low to moderate binding affinity to the hormone receptors (such as ERs and TRs) whereas kaempferol has highest binding affinity to androgen receptors and it is inactive in toxicity for all the toxic nature properties. Given that a compound with a greater negative docking score will have a higher efficacy, chlorogenic acid from *C. intybus* which was observed with a considerable docking score, may develop into a potent antidiabetic medication.

5. Conclusion

Among all the phytochemical compounds, chlorogenic acid showed the best docking score against AMPD1 and PKA target proteins. So, chlorogenic acid can be the best phytochemical compound as it inhibits both the target proteins and possesses the best molecular docking value when compared to other phytochemical compounds and prescribed drugs. However, in order to confirm the use of biological active molecules from *C. intybus* for drug development, disease therapy, or management, *in vivo* tests needs to be done using the potential diabetic target proteins.

Acknowledgment of sources

Acknowledge the support of the Department of Molecular Biology and Genetic Engineering, CBSH, GBPUA&T, Pantnagar for providing research support throughout the study.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Citation **Kurma Devakrishna, Gohar Taj and Sandhya Upadhyay(2024). *In silico* molecular docking analysis of *Cichorium intybus* L. phytochemical compounds against two related targets of type 2 diabetes mellitus. Ann. Phytomed., 13(1):577-588. http://dx.doi.org/10.54085/ap.2024.13.1.60.**