

EXTRACTION, IDENTIFICATION, INSILICO MOLECULAR DOCKING STUDY FOR PROTEIN KINASE INHIBITION OF PHLORETIN FROM APPLE PEELS (*MALUS DOMESTICA*)

¹*Dr. Syed Azhar Nizami, ²Harshitha A. G., ³Aishwarya V. R., ⁴Sinchana K. T.,
⁵Sinchana U. K.

¹*Professor, Dr. HLT College of Pharmacy, Kengal, Channapatana., Ramnagram, Dist.,
Karnataka.

²8th Semester B. Pharma., Dr. HLT College of pharmacy, Kengal, Channapatana.,
Ramnagram, Dist., Karnataka.

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***Corresponding Author**

Dr. Syed Azhar Nizami

Professor, Dr. HLT College
of Pharmacy, Kengal,
Channapatana., Ramnagram,
Dist., Karnataka.

ABSTRACT

Phloretin is 3-(4-hydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)propane-1-one found in the peels and roots of fruits like apples, pears, and strawberries. It is a type of flavonoids with various antioxidant, anti-inflammatory and potential anticancer properties. In this study we have reported a simple method of extraction and identification of phloretin from apple peels. For extraction simple maceration technique was employed using ethanol and water (30:70) as a solvent. The yield was 63.7%. Identification of phloretin was carried by performing TLC method using n-hexane and ethyl acetate (40:60) as mobile phase, the R_f value of phloretin was found to be 0.7. Insilico molecular docking and ADMET properties of phloretin was also done to identify key interactions such as hydrogen bonds and hydrophobic forces, and determining binding affinities to guide the rational design of Kinase

enzymes inhibitors for therapeutic purposes. To forecast ADMET properties and the Lipinski rule of five parameters the Molinspiration and Ptoctox3 version software were used. The ADMET predictions revealed that the compounds had good and safe pharmacokinetic features. Swiss Dock with AutoDock Vina was utilized for the docking process. The results revealed that phloretin was found to be a potent inhibitor against (CDK4) 2W96, with binding affinities of -6.8321 kcal/mol to -6.4548 kcal/mol and displayed strong conventional hydrogen bonding hydrophobic and π interactions with interacting amino acids residues.

This study concluded that phloretin have the potential to modulate the activity of cyclin D1-cyclin-dependent kinase 4 (CDK4 and Aurora B) complexes, making them potential lead molecules would pave the potential lead medication for anticancer therapeutic strategies.

KEYWORDS: Nutraceuticals, Phloretin, Molecular docking, CDK4/CycD1.

1. INTRODUCTION

Molecular Docking has become an essential aspect of in-silico drug development in recent years. This technique involves predicting the interaction between a small molecule and a protein at the atomic level. This enables researchers to study the behaviour of small molecules, such as nutraceuticals, before in vitro investigations, molecular docking studies are used in the field of nutraceutical research to provide crucial information. Within the binding site of a target protein and understand the fundamental biochemical process underlying this interaction. There are several computational tools and algorithms available for molecular docking techniques, these programs and tools have been developed and are currently being used in drug research and academic fields. According to Sahoo et al.^[1] some of the most commonly used docking programs include Swiss Dock, AutoDock Vina, Discovery Studio, Surflex, AutoDock GOLD, Glide, MCDock, MOE-Dock, FlexX, DOCK, LeDock, rDock, ICM, Cdcker, LigandFit, FRED, and UCSF Dock. Among these programs, AutoDock Vina, Glide, and AutoDock GOLD have been identified as top-ranking choices with the best scores.^[2-6] However, flexible receptor docking, specifically receptor backbone flexibility, remains a challenge for contemporary docking programs. Sahoo et al, stated that the ligand-receptor complexes can be assessed, screened, and predicted through the docking study. This study typically follows two distinct steps, according to Mohapatra et al.^[7] First, ligand conformations are sampled according to the protein's active site. Second, the conformations are ranked according to a scoring function. Sampling algorithms should theoretically reproduce experimental binding modes, and the confirmations obtained should be ranked according to a scoring function, Dash et al.^[8] Nutraceuticals are natural substances with therapeutic benefits on human health that are often sourced from dietary sources. Because of their potential to prevent and control chronic diseases including cancer, diabetes, cardiovascular, and neurodegenerative disorders, these have grown in popularity over the past several years. Before in vitro investigations, molecular docking studies are used in the field of nutraceutical research to provide crucial information.^[9-10] The goal of this study is to examine

the most pertinent molecular docking applications for assessing the possible health-promoting effects of nutraceuticals in disease management.

2. MATERIALS AND METHODS

Locally available fresh Apple fruit "*Malus domestica*" were obtained from local market of Maddur, Mandya (dist). Solvents such as Acetone, Ethanol, Dimethyl formamide, Silica gel G, Calciumphosphate, n-Hexane, Ethyl acetate, of AR grade were purchased from the SD Fine chemicals, Mumbai, India. For the separation of different phytoconstituents by TLC technique was followed using silica coated glass plates, by using an appropriate solvent mixture. TLC spots of the compounds were visualized in Iodine chambers.

2.1.2. Sample preparation

Fully matured and ripened apple Fruits was washed in order to remove any dust, dirt, or residues of any kind of pesticides etc. The peels were separated from the pulp and cut into small pieces. Then, peels were dried with the assistance of 'hot air oven' at 50°C temperature for 48 h. the dried peels, were grinded into fine powder. Maceration technique was employed in order to extract polyphenols from apple peels powder. The sample-to-solvent ratio was set to 1:15 and temperature for the maceration technique was set to 40°C. Nearly about three grams of dried peels' powder was taken in 250 ml of conical flasks and 45 ml of solvent was added. The conical flasks were hired in the shaking water bath for about 20 h. After 20 h, the entire sample was filtered through Whatman filter paper 41. The filtrate was concentrated to dryness to obtain the crude extracts.

2.1.3. Detection method

To detect the Phloretin contents in the extracted material, thin layer chromatography (TLC) method was performed. The samples were dissolved in methanol and spot was placed on the TLC plates with the help of capillary tube, the sample was applied on the marked points. The plates were then kept in the beaker containing nhexane and ethyl acetate (40:60) as mobile phase and allowed to travel up to three-fourth of the length, it was removed from the beaker and marked the solvent front on the plates and dried. Then the spots were identified on the chromatogram and the Rf value was calculated.

2.1.4. Ligand preparation

Phloretin, 3-dimensional (3D) structure was downloaded from PubChem database as smile and used for assessing their inhibitory potential against protein kinase inhibitors.

2.1.5. Protein preparation

The crystallographic structures of CDK4/CycD1 and Aurora B (Figure 1) were obtained from Protein Data Bank (PDB ID: 2W96 with resolution 2.3 Å with resolution 1.49 Å) Structures of selected targets were downloaded from Chemdraw software and finalized for docking studies using AutoDock Tool “Swiss Dock”.

3. RESULTS AND DISCUSSION

Phloretin was extracted from Apple peels by simple maceration technique. The extraction yield was found to be 63.7 %. (Fig no 1)



Fig. No. 01: Extaction of phloretin from apple peels.

3.1. TLC studies: TLC was done to identify phloretin from the extract. The R_f value for phloretin was found to be 0.70. (Fig no 2)



Fig. No. 02: Identification of phloretin by TLC method.

3.2. ADME and Drug-likeness criteria of Phloretin

ADMET prediction: Table 3 shows the ADMET profiles of phloretine. The compound had a high rate of intestinal absorption in humans, indicating that they are highly absorbed and may be ingested. The drug-likeness criteria of phloretine was assessed using protex3 software the results are presented in fig no3 table no 3 and 4.

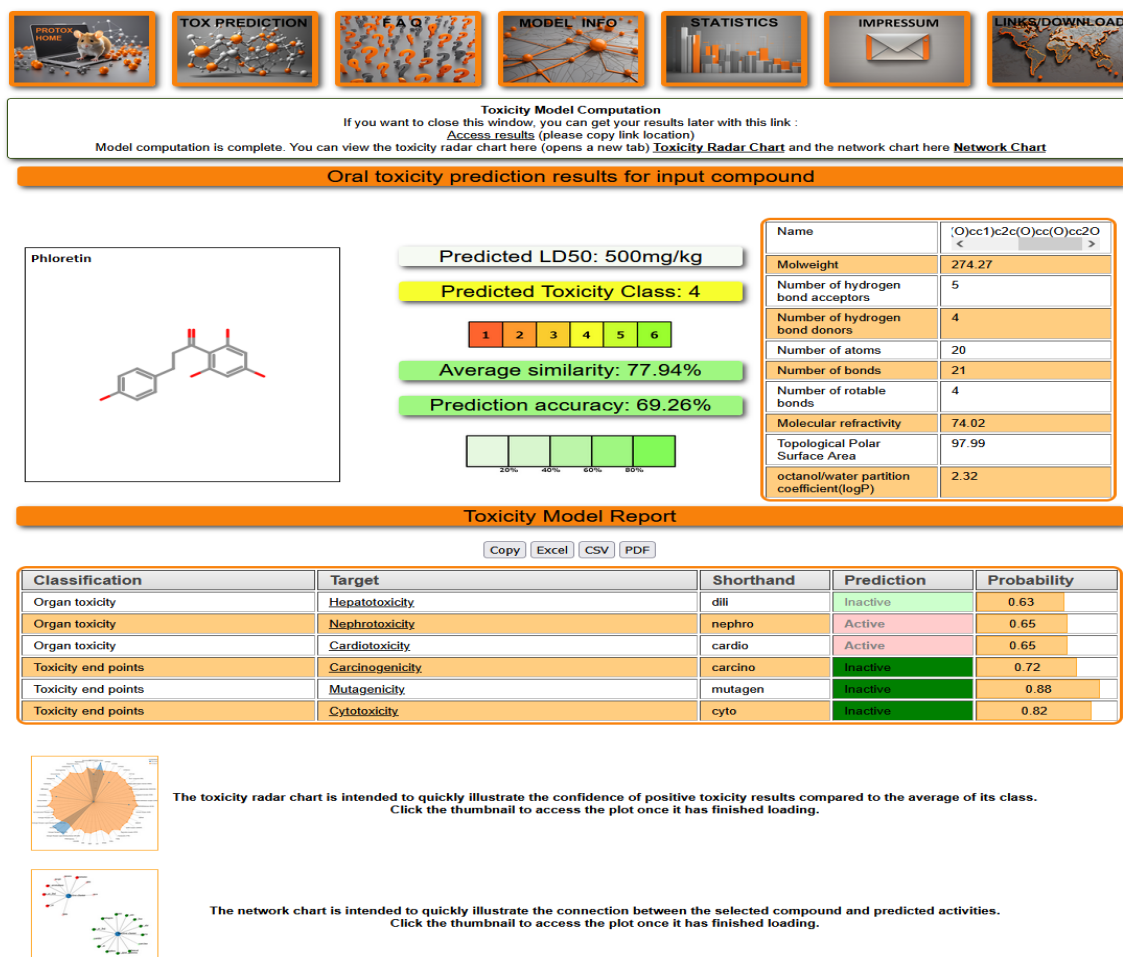


Fig. No. 3: ADMET prediction Drug likeness study of phloretine.

Table No. 3: (ADMET) prediction of Phloretin.

Entry	Mi Log P	%ABS	TPSA	MW	nON accptr	ONHOH Donor	Lipinski violation	nRotb
Rule	1.5	-	-	<500	<10	<5	<1	-
Phloretin	2.32	75.19	97.99	274.27	5	4	0	5
----	✓	--	--	✓	✓	✓	✓	--

mlog-P Octanol/water LOG-P, %-AB – Absorption percentage, M.W-molecular weight in gm/mol, T P S A- Topological polar surface area, nON , no of O-N acceptor, ONHOH, No of ONHOH donor, n rotb- number of rotatable bond, nLV- Lipinski violation,

$$\% \text{-AB} = 109 - (0.345 \times \text{TPSA})$$

Table No. 4: Insilico toxicity prediction of Phloretin.

Comp Code	Hepato toxicity	Nephro toxicity	Cardio toxicity	Carcio genicity	Mutag enicity	Cytoto xicity	Predicted LD50{mg /kg}
phloretin	inactive	Active	Active	Inactive	Inactive	Inactive	500

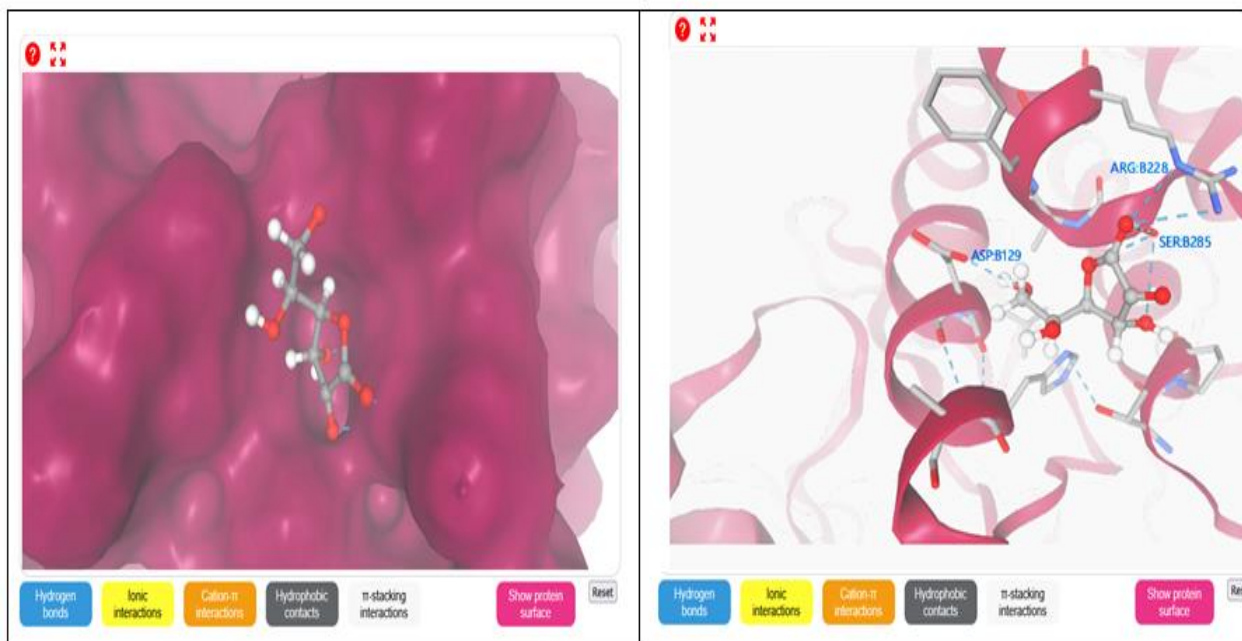


Fig. No. 4: Docking analysis of ascorbic acid with (2w96) targets of protein kinase inhibition.

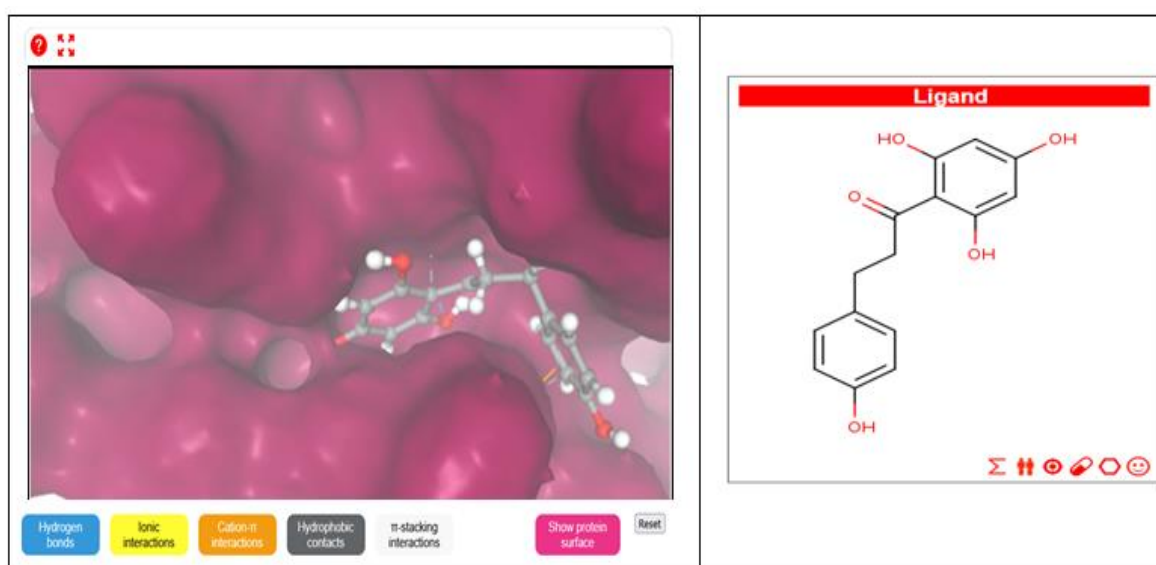


Fig. No. 5: Docking analysis of phloretin with (2w96) targets of protein kinase inhibition.

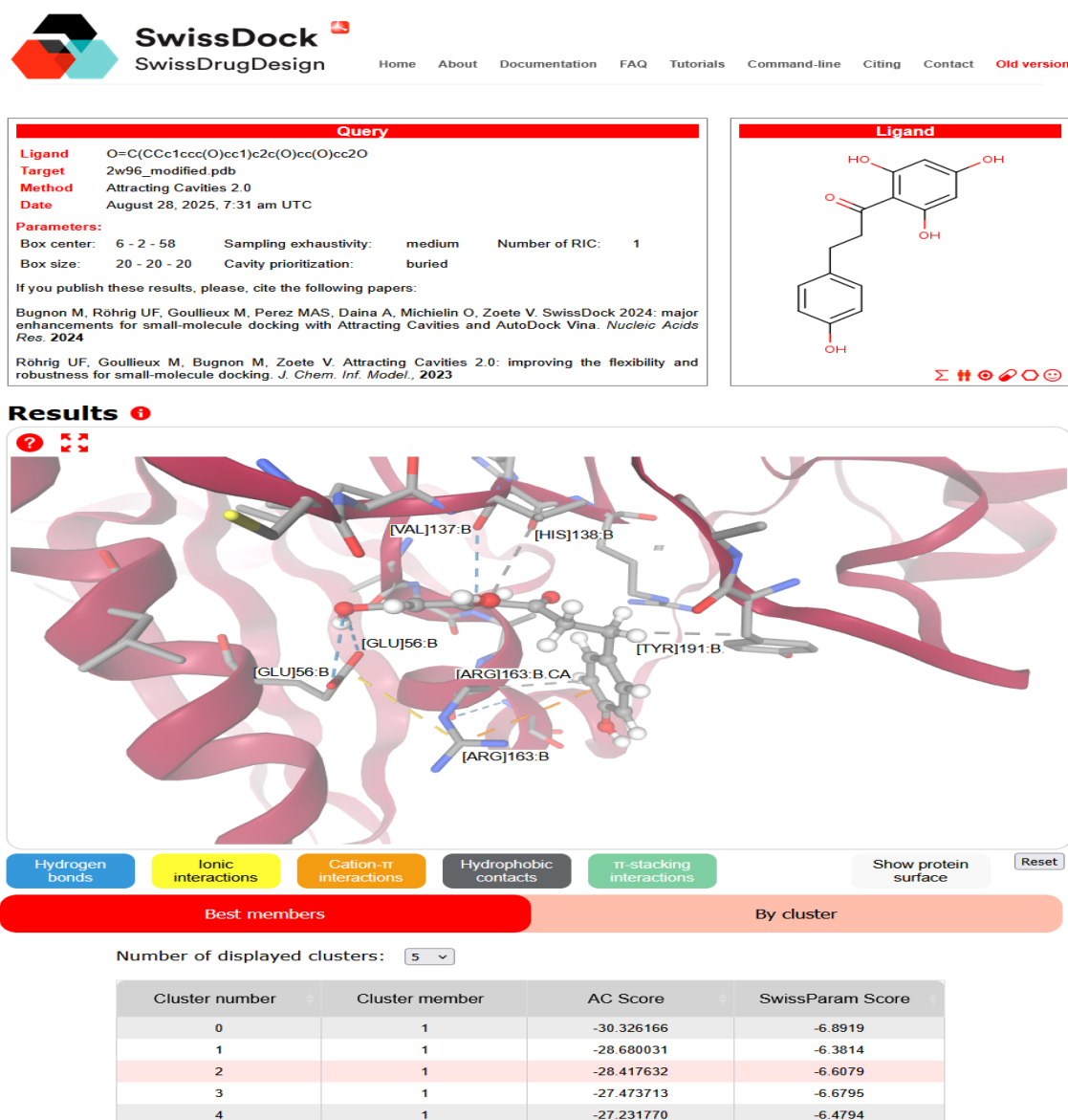


Fig. No. 6: Binding interaction of phloretin with active site of kinase protein (pDB: 2w96).

Table No. 6: Docking interaction of Phloretine with PDB ID-2W96.

Ligand	PDB Id	Binding Interactions		Binding Energies (Kcalories/mol)	Interacting residues
Phloretine	2W96	No of Hydrogen bond	02	-6.8321 -6.6517	GLU-B:56 VAL: B-137
		Hydrophobic Interactions	02	-6.5439 -6.5433	HIS: B-138 TYR: B-191
		Pi-Pi interaction	01	-6.4548	ARG: B-631
Ascorbic acid	2W96	No of Hydrogen bond	03	-7.4328 -6.5710 -6.5439	ASP-B:129 ARG: B-228 SER-B:285

Phloretin shows good binding affinities lesser or higher than the standard drug ascorbic acid. It has exhibited the highest binding affinity with a docking score of -6.54 and -6.83 kcal/mol against target protein PDB ID: 2W96. (Fig no 5 and 6) This is in comparison to the standard drug Ascorbic acid ligand, which showed docking scores of -7.43 and -6.54 kcal/mol respectively. (Fig no 4) Phloretin displayed strong conventional hydrogen bonding interactions with interacting amino acids including GLU-B: 56, TYR-B: 191, respectively. In contrast the standard drug ascorbic acid interacted with ASP-B: 129, ARG-B: 228 and SER-B: 285. (Table no 6)

4. CONCLUSION

In conclusion, Molecular docking is a useful approach for identification of novel therapeutic targets and the creation of secure and efficient dietary supplements for the treatment of diseases. Phloretin is a polyphenol presents in fruits have the unique potential to inhibit the kinase proteins due to their high affinity with all the targeted proteins. When compared with standard drug ascorbic acid, this study discovered that the phloretin have a significantly higher binding free energy to all of the targeted proteins. The ADMET prediction of phloretin indicates that the ligands have favourable pharmacokinetic properties, implying that it has a potential to be developed as drugs. Hence, it can be concluded that, phloretin have the potential to treat protein kinase inhibition because of having immunomodulatory effects and suppressing the cytokine storm, and they have the potential to be developed as drugs because of their good and safe ADMET profile.

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