Original Research Article

INTEGRATING *IN SILICO* WITH *IN VITRO* STUDIES OF RUTIN AS AN ANTI HYPERTENSIVE

ABSTRACT

Rutin was evaluated for its antihypertensive effect by *In vitro* ACE inhibitory activity using Captopril as standard. For molecular docking the proteins namely PDB ID: 1UZE, 1O86, 2XY9 and 3L3N are selected and modelled. The abilities of the 3D model were assessed *via* the PROCHECK database and considered with the Ramachandran plot. The G-score of rutin was found to be more than the glide score of standard drug captopril affirming that the compound had similar affinity to impasse to the proteins. Proteins with PDB ID 1UZE, 1O86, 2XY9, and 3L3N showed more than 90% favoured area which evidently specifies that the designated protein in the current research is of noble eminence. ADME (Absorption, Distribution, Metabolism and Excretion outcome shown the three violations of rutin out of the five and the Captopril has got zero violations which noticeably shown the possibility for its greater oral bioavailability. The results of bioactive score revealed that rutin and captopril showed moderately active against GPCR ligand, ion channel modulator, kinase inhibitor and nuclear receptor but both rutin and the standard captopril were found to be highly dynamic counter to protease inhibitor and enzyme inhibitor. The PASS results clearly stated that rutin and captopril as cardio protectant, vasoprotector, vasodilator, antihypertensive. Rutin was predicted with hepatoxicity and nephrotoxicity while captopril was predicted with myocardial infarction, hepatotoxicity and nephrotoxicity. The direct targets of rutin have interventions with Cytochrome P4503A, Carbonic anhydrase II, Sodium /glucose cotransporter/2 and TNF-alpha for direct targets. The possible targets were with vascular endothelial growth factor receptor 1, and Sodium /glucose cotransporter/2. *In vitro* ACE inhibition assay demonstrated that rutin has shown antihypertensive activity by inhibiting angiotensin converting enzyme. From the above results the mechanism of action of rutin was validated through *in silico* interactions with the suitable PDB's from MCULE software.

Keywords: Rutin, Molecular docking, Swiss ADME, PASS (Predicted activity spectra for substances), bioactive score.

1. INTRODUCTION

The phytoconstituents like flavonoids located in flora and are used up withinside the shape of fruitlets, nuts, vegetables, and spinoff meals including wine and brunette. The meals utilized by the western international locations usually consist of of quercetin [1]. Quercetin is a pattern of a flavonoid magnificence located in nutriments having sugars, basically as β-glycosides. Rutin as properly termed rutoside is the glycoside linking the flavonol quercetin and the disaccharide rutinose. This citrus flavonoid is located in a enormous variety of plants. Rutin, a dietary flavonoid which has set up prodigious consideration, because of their pharmacological properties, which includes antimicrobial, anti-inflammatory, anticancer, antidiabetic and inter alia [2].

Though flavonoids are missing usual nutritive value, they're steadily extra appeared as precious nutritional parts that act as probably defenders opposite to human sicknesses including coronary coronary heart disease, cancers and inflammatory bowel disease. Rutin seems to be a quercetin releaser to the gut; moreover, quercetin is extensively damaged down withinside the gut, launched from rutin and/or its colonic metabolites would possibly play a crucial role [3].

To apprehend the ligand binding houses of rutin with the angiotensin changing enzyme, the take a look at compound rutin become subjected to molecular docking research. This research act as a computational device to anticipate the probably interactions among rutin and protein. An *in silico* look at of rutin become carried out via way of means of Swiss ADME to calculate its pharmacokinetics, drug-likeness and medicinal chemistry friendliness of trivial molecules to guide drug discovery [4]. PASS (Prediction of Activity Spectra for Substances) on line software program become carried out to assess the organic activity, unfavourable drug reactions, direct and feasible goals to set up molecular mechanism of rutin and fashionable drug captopril. In the prevailing studies a try is made to look at the *in silico* and *in vitro* strategies of rutin as an anti-hypertensive.

2. MATERIALS AND METHODS

In silico studies

2.1 Docking studies

Molecular docking is an appealing scaffold to apprehend drug biomolecular interactions for the rational drug layout and discovery, in addition to withinside the mechanistic look at with the aid of using setting a molecule (ligand) into the desired binding webweb page of the goal precise place of the DNA/protein (receptor) particularly in a non-covalent style to shape a strong complicated of capability efficacy and greater specificity [5, 6]. The records received from the docking method may be used to signify the binding electricity, loose electricity and balance of complexes. At present, docking method is applied to be expecting the tentative binding parameters of ligand-receptor complicated beforehand. In this look at the crystallographic shape of the enzymatic goal ACE (Angiotensin changing enzyme) became received from the Protein Data Bank (PDB) database (PDB: 1UZE, 1O86, 2XY9, 3L3N). The molecular docking look at became accomplished the usage of Mcule. The software program permits us to surely display a database of compounds and is expecting the most powerful binders primarily based totally on diverse scoring functions. The series of enzyme substrate complexes became diagnosed thru docking, and their relative stabilities have been evaluated the usage of their binding affinities. Ligand suit became used for as it should be docking ligands into protein lively webweb sites using a hollow space detection algorithm. A high-throughput screening look at implemented to the ACE receptor is likewise supplied wherein ligand suit whilst blended with LigScore, an internally advanced scoring function, yields excellent hit prices for a ligand pool seeded with acknowledged actives [4].

2.2 SWISS ADME STUDIES USING MOLINSPIRATION

The ADME properties of Rutin and Captopril are evaluated the use of the device Molinspiration Cheminformatics server (http://www.molinspiration.com). There are numerous pharmacokinetic parameters and physicochemical descriptors which have been evaluated for numerous pills thru utility of the device Molinspiration. These properties are in particular hydrophobicity, digital distribution, hydrogen bonding characteristics, molecule length and versatility and of path presence of diverse pharmacophoric capabilities that impact the behaviour of molecule in a dwelling organism, along with bioavailability, delivery houses, affinity to proteins, reactivity, toxicity, metabolic balance and plenty of others. The Lipinski rule of 5 offers 4 easy

physicochemical parameter ranges (MWT ≤ 500, log P ≤ 5, H-bond donor`s ≤ 5, H-bond acceptors ≤ 10). [7]

2.3 BIOACTIVITY SCORE USING MOLINSPIRATION

The bioactivity rating of rutin and captopril also are evaluated the use of the device Molinspiration Cheminformatics server (http://www.molinspiration.com). In this computational chemistry approach massive chemical databases are analysed as a way to discover viable new drug candidates. Only SMILES or SDfile systems of energetic molecules are enough for the training, no statistics approximately the energetic web website online or binding mode is necessary. This is mainly beneficial in initiatives wherein shape-primarily based totally method cannot be implemented due to the fact statistics approximately 3-d receptor shape isn't always available [8].

2.4 PASS (Prediction of Activity Spectra for Substances)

PASS software program is a prediction of pastime spectra for biologically energetic substances. The idea of the organic pastime spectrum becomes added to explain the residences of biologically energetic substances. The PASS (prediction of pastime spectra for substances) software program product, which predicts greater than three hundred pharmacological consequences and biochemical mechanisms on the idea of the structural formulation of a substance, can be successfully used to locate new targets (mechanisms) for a few ligands and, conversely, to show new ligands for a few organic targets. Prediction of pastime spectra of substances (PASS) is this sort of tool that could are expecting the pharmacological houses ahead and might assist in screening pharmacological attainable leads for a specific condition [9, 10].

2.4.1 Input and Output of PASS

PASS makes use of as enter facts a MOL- or SD-file23 representing the structural facts approximately the molecules below observe. On the premise of those facts, MNA descriptors (Multilevel Neighbourhoods of Atoms) are generated automatically. Based at the information of MNA descriptors for energetic and inactive compounds from the education set, chances are calculated for every pastime: Pa - the chance of the compound being energetic and Pi - the chance of being inactive. Being chances, the Pa and Pi values range from 0.000 to 1.000 (with 3

applicable decimals being calculated), and in widespread Pa + Pi < 1, for the reason that those chances are calculated independently. Pa and Pi may be taken into consideration to be measures of the compound below observe belonging to the lessons of energetic and inactive compounds, respectively, or may be visible as estimates for the primary and 2nd sorts of mistakes within side the prediction. All MNA descriptors impact the estimates within side the pastime prediction. Their impact may be both positive (if the descriptors are determined in compounds with the specific pastime), or negative (if the descriptors are determined in compounds without the specific pastime), or maybe neutral (if the descriptors are determined in each energetic and inactive compounds). In the final case, they lower the relative effect of the "positive" and "negative" descriptors.

2.4.2 Interpretation of Predictions

PASS predictions can be interpreted and used in flexible ways. The most likely activity for a particular compound is characterized by a Pa value close to 1 and a Pi value close to 0. First, consider the case where the Pa value is high and much larger than the Pi. Although a set of statistically significant samples with predictions obtained with the threshold Pa> 0.9 should be expected to lose 90% of the drug if selected and tested from a much larger database. , The false positive rate is lost. It will be very low. With a cut-off of Pa> 0.8, only 80% of the active ingredient is lost, but the rate of false positives is slightly higher. Finally, going down to the criterion Pa> Pi, the probability of the first type of error is equal to the probability of the second type of error i.e., one can miss a true asset, much like finding a false positive.

However, maximizing the Pa value for the desired activity is not the only criterion for selecting the most promising compounds. Another aspect could be the novelty of the compound. If Pa is very high, the compound may be an analogue of a known drug. Therefore, if you are interested in finding new traces, especially new chemical units (NCEs), it is advisable to choose a compound whose specified activity is predicted with a lower probability, for example 0.5< Pa < 0.7 In this case, there is a high probability of false positives, but if the activity is confirmed in the experiment, it is likely that you have received an NCE [11, 12, 13].

2.5 *In vitro* ACE inhibitor activity

ACE inhibitor activity *in vitro* was measured using hyppuryl histidyl leucine (HHL), ACE (EC3.4.15.1) as a substrate. Different concentrations of rutin (40 µl) were incubated with 100 µl of 0.1 M borate buffer (pH 8.3) containing 5 mM HHL and 0.3 M NaCl and 20 µl ACE (2 mU) for 30 min at 37 °C. Finish the reaction with 150 µl of 1M HCl. The resulting hippuric acid was extracted with ethyl acetate (1000 µl) and centrifuged at 1500 rpm for 10 min to evaporate 750 µl of the organic phase. Residue The substance was converted to 800 µl with distilled water and the absorbance was measured at 228 nm. Three experiments were performed on each sample. Inhibitory activity was expressed as protein concentration (Pierce, Rockford, IL, USA) using bovine serum albumin (half the maximal inhibitory concentration (IC_{50})) as the necessary criterion for 50% inhibition activities [14].

3. RESULTS AND DISCUSSION

3.1 Molecular docking studies

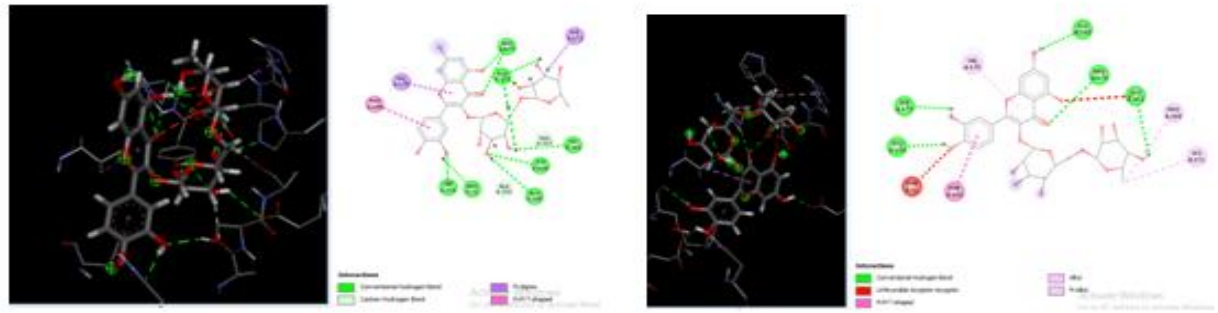
3.1.1 Structure based drug design

Initially the protein downloaded from the PDB was prepared by discovery studio. Water molecules present in the chains are removed. Energy minimization was done. Later, molecules smile image pasted in Mcule online software tool and the structures were docked against protein.

3.1.2 MCULE -docking results

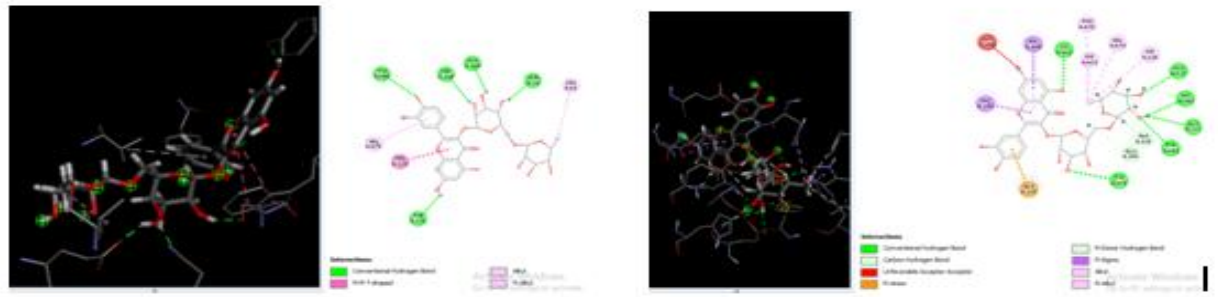
Table 1: Docking Studies of Rutin and Captopril

Sl.no	Compounds	Glide Score			
		1UZE	**1O86**	**2XY9**	**3L3N**
1	Rutin	-9.2	-9.4	-9.8	-11.1
2	Captopril	-5.7	-5.8	-5.4	-5.4



Rutin with PDB ID: 1UZE (-9.2)

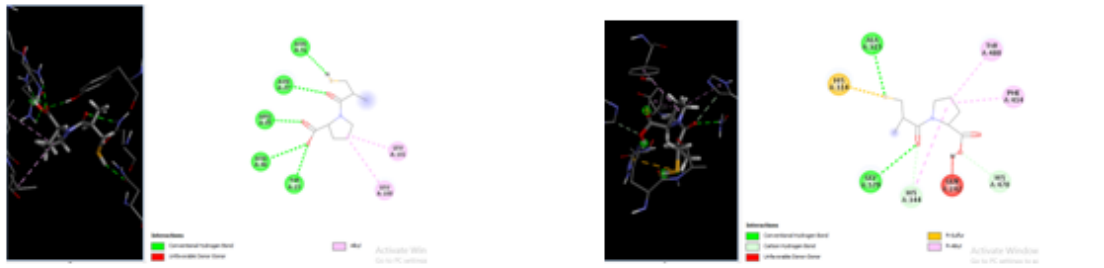
Rutin with PDB ID: 1O86 (-9.4)



Rutin with PDB ID: 2XY9 (-9.8)

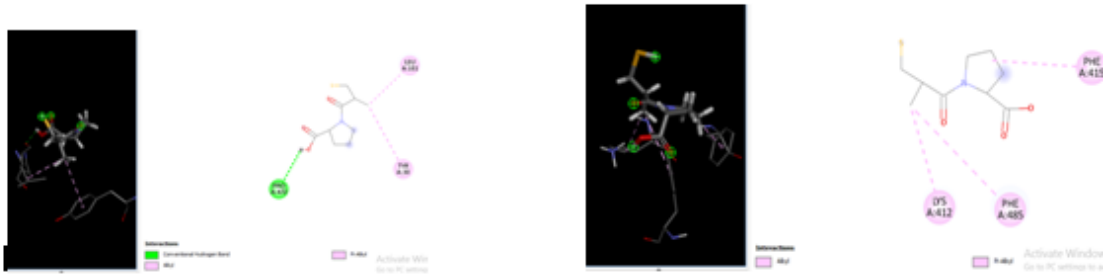
Rutin with PDB ID: 3L3N (-11.1)

Figure 1: Rutin interactions and glide scores with PDB



Captopril with PDB ID: 1UZE (-5.7)

Captopril with PDB ID: 1O86 (-5.8)

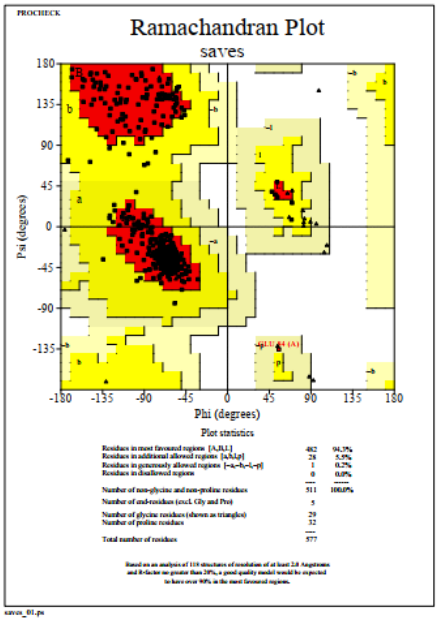


Captopril with PDB ID: 2XY9 (-5.4)

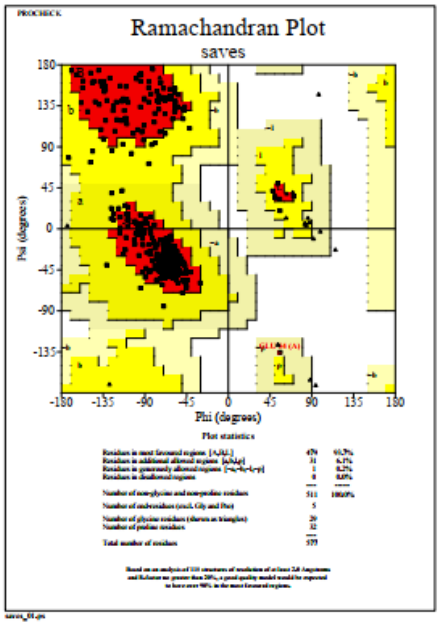
Captopril with PDB ID: 3L3N (-5.4)

Figure 2: Captopril interactions and glide scores with PDB

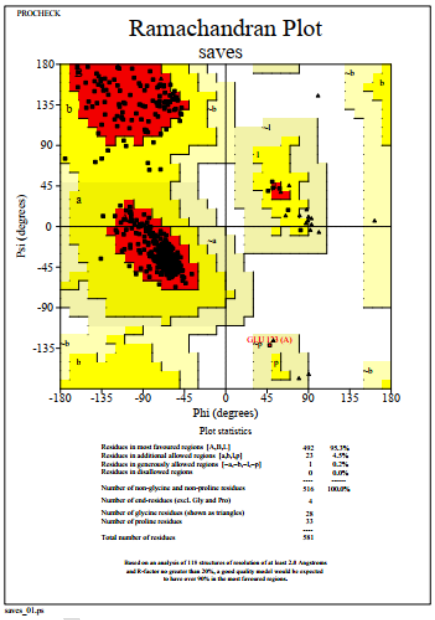
3.2 Ramachandran Plot



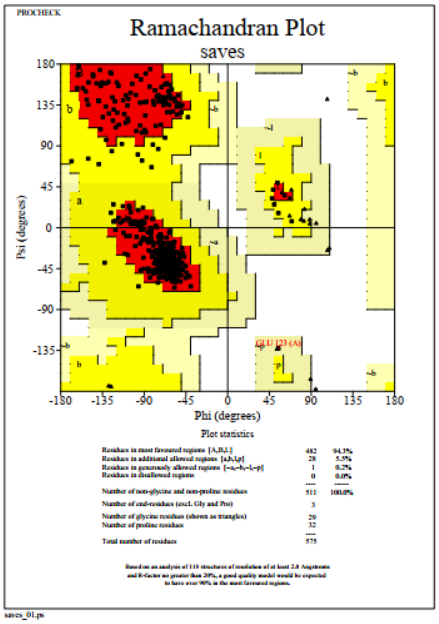
PDB ID: 1UZE



PDB ID: 1O86



PDB ID: 2XY9

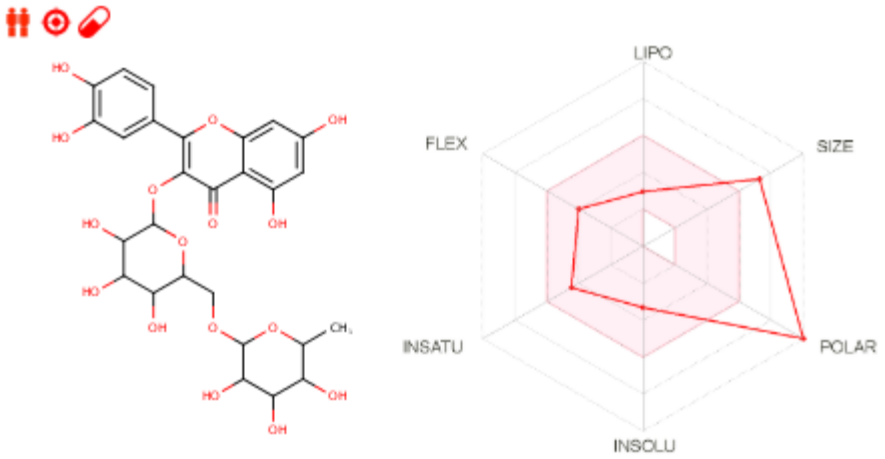


PDB ID: 3L3N

Figure 3: Ramchandran Plot of PDB ID: **1UZE, 1O86, 2XY9, and 3L3N**

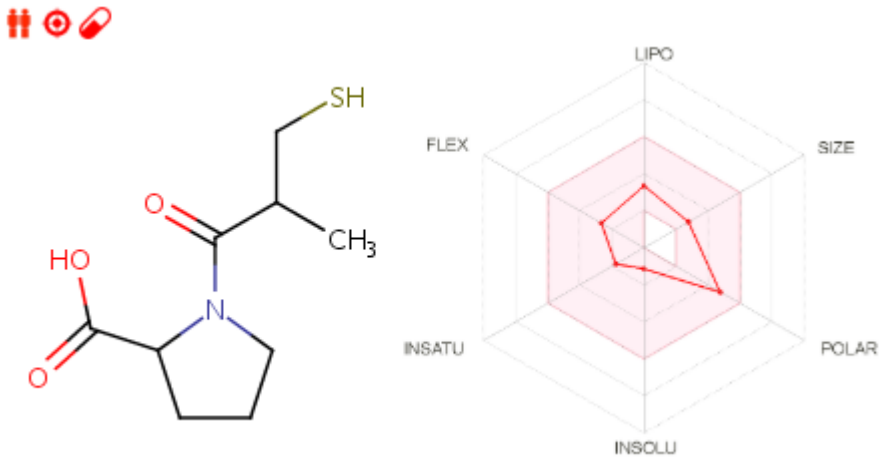
Table 2: Physicochemical properties of Rutin and Captopril using SWISS ADME Software

Compound name	Physiochemical Properties					Lipophilicity WLOGP
	Molwt g/mol	TPSA Å^2	No rotb	No H bond Acceptors	No H bond donors	
Rutin	610.52	269.43	6	16	10	-1.69
Captopril	217.29	96.41	4	3	1	0.25



a. Structure of Rutin **b. Violations from Lipinski depicted in red line**

Fig 4: Physiochemical properties of Rutin



a. Structure of captopril **b. Zero violations from Lipinski depicted in redline**

Fig 5: Physiochemical properties of Captopril

Lipinski`s rule of 5 is to assess drug likeness or decide if a chemical compound with a positive pharmacological or organic interest has chemical and bodily residences that might make it an orally energetic drug in humans. In the prevailing examine rutin has 3 violations (like molecular mass, hydrogen donor and acceptors) of 5. Captopril was given 0 violations which sincerely indicated the chance for its better oral bioavailability. Lipinski violations of rutin and 0 violations of fashionable captopril had been depicted in fig. 4 and 5. Topological polar surface area (TPSA) can predict the place of birth of drug candidates in the intestine and in the blood mind barrier [15]. Rutin has a TPSA score of 269.43, which indicates that this molecule is hydrophilic in nature and cannot be easily transported across the blood mind barrier. Captopril, on the other hand, has a TPSA rating of 96.41, clearly showing the nature of the lipophilic content.

Rutin's three Rule 5 violations indicate that oral bioavailability may be much lower [15]. The physicochemical locations of rutin and captopril are listed on Desk 2.

3.3 Bioactivity Score of rutin and captopril

Table 3: Bioactivity Score of rutin and captopril using molinspiration

Compounds	GPCR ligand	Ion channel	Kinase inhibitor	Nuclear receptor	Protease inhibitor	Enzyme inhibitor

		modulator		**ligand**		
Rutin	-0.05	-0.52	-0.14	-0.23	-0.07	0.12
Captopril	-0.14	-0.08	-0.98	-0.55	0.97	0.50

3.4 PASS

Table 4: Antihypertensive activity predicted for Rutin and standard Captopril using PASS (prediction of activity spectra for substances)

S. No	Compound	Probable Activity (Pa)	Probable Activity (Pi)	Biological Activity
1.	Rutin	0,988	0,001	Cardioprotectant
		0,980	0,001	Vasoprotector
		0,740	0,006	Vasodilator
2.	Captopril	0,559	0,014	Antihypertensive
		0,661	0,010	Vasodilator, coronary

Table 5: Adverse effects predicted for the Rutin and standard captopril using PASS (Prediction of Activity Spectra for Substances)

S. No	Compound	Pa	Pi	Adverse Effect
1.	Rutin	0.384	0.271	Hepatotoxicity
		0.247	0.228	Nephrotoxicity
2.	Captopril	0.923	0.004	Myocardial infarction
		0.808	0.010	Nephrotoxicity
		0.686	0.106	Hepatotoxicity

Table 6: Direct and possible target Prediction for Rutin and Captopril using PASS (prediction of activity spectra for substances).

S. No	Compounds	Direct target	Confidence	Possible target	Confidence
1.	Rutin	Cytochrome P4503A	0.3519	Vascular endothelial growth factor receptor 1	0.4503
		Carbonic anhydrase II	0.3198		

		Sodium /glucose cotransporter/2	0.1088	Sodium /glucose cotransporter/2	0.0821
		TNF-alpha	0.2162		
2	Captopril	Angiotensin converting enzyme	0.1368	Endothelin converting enzyme-1	0.0222
		Renin	0.0491	Angiotensin II type 2 (AT-2) receptor	0.0081
		Angiotensin II type 2 (AT-2) receptor	0.0486	Vasopressin V2 receptor	0.0107
		Endothelin converting enzyme	0.0218		

3.5 ANTI HYPERTENSIVE ACTIVITY

3.5.1 *In vitro* ACE inhibition assay

Rutin became examined for angiotensin changing enzyme inhibitory hobby the use of ACE inhibition assay method.

The concentrations and percent inhibition of rutin and preferred drug captopril have been recorded. From the percentage inhibition, IC_{50} values have been calculated and mentioned in desk 1. The preferred drug captopril became examined at unique dose degrees and observed to be linear, which substantiates the usefulness of captopril for evaluation of the take a look at doses.

IC_{50} of rutin became 66.01 µg/mL and captopril became 20.31 µg/mL.

Table 7: Effect of Rutin on Angiotensin converting enzyme (ACE) inhibition assay

Rutin			Captopril		
Concentration (µg/mL)	**% Inhibition** (mean ± SEM)	**IC_{50}** (µg/mL)	**Concentration** (µg/mL)	**% Inhibition** (mean ± SEM)	**IC_{50}** (µg/mL)
100	77.25±0.22		-	-	
50	35.50±0.08		50	85.20±0.62	
25	19.50±0.87		25	59.82±0.88	

		66.01			**20.31**
12.5	10.25±0.19		12.5	30.12±0.01	

Assay was performed in triplicate. The above results show that rutin and standard compound captopril has antihypertensive activity.

Rutin has proven antihypertensive interest with the aid of using angiotensin changing enzyme inhibitory action (IC_{50}=66.01 µg/mL). In vitro research have found out the antioxidant interest of rutin and the function of rutin in decreasing oxidative pressure related to hypertension.

4. Discussion

4.1 Molecular docking studies

Molecular docking is a computational approach that predicts the noncovalent interplay among macromolecules or greater again and again in a macro and small molecules. Drug discovery, molecular docking, and digital screening, supplying multi-person capability, improved accuracy. In this study, the hunt of flavonoids withinside the molecular foundation for binding to energetic webweb page of ACE Inhibitors is discovered via way of means of laptop aided docking evaluation. With a developing range of regarded experimental systems of goal molecules, computational strategies were used correctly to complement and accelerate drug discovery [16]. The docking evaluation of rutin and captopril had been finished the use of mcule software program. The rutin and captopril had been subjected to docking towards PDB ID: 1UZE, 1O86, 2XY9, and 3L3N the use of Mcule on-line software program tool. The maximum drift ratings had been determined with rutin and captopril with nearly all the chosen proteins with PDB ID: 1UZE, 1O86, 2XY9, and 3L3N. The drift ratings of rutin become observed to be greater than the drift rating of widespread drug captopril pointing out that the compound would possibly have equal affinity to bind to the proteins. These effects without a doubt suggest that the Rutin would possibly have proven comparable mechanism to that of the same old drug captopril in decreasing hypertension. The proteins recognized in particular 1UZE, 1O86, 2XY9, and 3L3N are demonstrated and the characteristics of the 3D model were assessed utilizing the PROCHECK program and surveyed utilizing the Ramachandran plot. It is clear from the Ramachandran plot that anticipated models have most good areas, moreover permitted locales, by and large permitted districts and refused areas. Such a rate appropriation of the not entirely set in stone by Ramachandran plot shows that the anticipated models are of good quality. As per Ramachandran

plot a decent quality model would be relied upon to have more than 90 % in the most preferred locale. Proteins with PDB ID 1UZE, 1O86, 2XY9, and 3L3N showed practically 90% leaned toward a locale which plainly demonstrates that the chose models in the current review are of good quality.

4.2 SWISS ADME analysis:

Atomic properties were determined based on Lipinski's standard and its parts. Lipinski's standard of five is to assess drug-similarity or decide whether a synthetic compound with a specific pharmacological or organic action has substance properties and actual properties that would make it an orally dynamic medication in people. In the current review rutin has three infringements (like atomic mass, hydrogen giver and acceptors) of five. Captopril got zero infringement which obviously demonstrated the likelihood for its higher oral bioavailability. The molecular weight of captopril is 217.29 g/mol and rutin is 610.52. With less molecular weight the standard captopril might have easily absorbed, diffused and transported and this might be responsible for its high oral bioavailability. Topological polar surface region (TPSA) permits forecast of transport properties of medication up-and-comers in the digestive organs and blood-cerebrum boundary [16]. Rutin has TPSA of 269.43 and this high score of TPSA recommended that this atom specially go about as hydrophilic in nature and can only with significant effort transport through the blood cerebrum obstruction when contrasted with captopril which has TPSA score of 96.41clearly demonstrating lipophilic in nature. Any compound with fewer than 140 displays better penetrability into the tissues.

Molinspiration ADME empowers the calculation of key physicochemical, pharmacokinetic, drug-like and related boundaries for one or different particles. Number of H-bond acceptors should be in a scope of 0-10 and number of H-bond givers should be 0-5. The Number of H-bond acceptors for rutin and captopril are 16 and 3 and the number of H-bond donors for rutin and captopril are 10 and 1. The score of the captopril was viewed as inside the reach. A negative incentive for logP implies the compound has a higher proclivity for the watery stage (it is more hydrophilic); when logP approaches 0 the compound is similarly parcelled between the lipid and fluid stages; a positive incentive for logP means a higher fixation in the lipid stage (i.e., the compound is more lipophilic).

4.3 Bioactivity score

Rutin and Captopril were exposed to bioactivity score utilizing molinspiration. The scores for the

chose mixtures can be deciphered as Active (bioactivity score > 0), tolerably dynamic (bioactivity score: - 5.0-0.0) and inert (bioactivity score < - 5.0). Rutin and captopril were found to modestly dynamic against GPCR ligand, particle channel modulator, kinase inhibitor and atomic receptor yet both rutin and the standard captopril were viewed as profoundly dynamic against protease inhibitor and protein inhibitor.

4.4 PASS software:

Predicition of action spectra of substances (PASS) is an important connection point that ought to be embraced as an original instrument for anticipating the likely particles and to foresee the organic action of Rutin and standard captopril as anti-hypertensive. To understand the molecular mechanisms of rutin and captopril as anti-hypertensive these were subjected to prediction studies by applying PASS online software. Rutin and captopril were anticipated by connecting with the authoritative improved on atomic information line-section framework acquired from PubChem.com followed by utilizing PASS on the web.

Then again, a few new ways were anticipated in which the in vitro and in vivo assessment of a few medications which can be made based on PASS anticipated exercises. It would permit the scientists to smooth out the query all the more effectively. Expectation of action spectra of substances (PASS) is such a gadget which can foresee the pharmacological homes ahead of time and would help in evaluating pharmacological sensible leads for a specific condition [9,10]

The results of rutin and captopril like probable activity (Pa) and probable inactiveness (Pi) and biological activity were given in table 4. The possible interventions of rutin and captopril were found to be Cardio protectant, Vasoprotector, Vasodilator, antihypertensive and Vasodilator, coronary. Rutin and captopril were subjected to pass software for adverse effects. Rutin was predicted with hepatoxicity and nephrotoxicity while captopril was predicted with myocardial infarction, hepatotoxicity and nephrotoxicity.

Rutin and captopril were subjected to pass software for direct and possible targets. Rutin was found to have interventions with Cytochrome P4503A, Carbonic anhydrase II, Sodium /glucose cotransporter/2 and TNF-alpha for direct targets. The possible targets were with vascular endothelial growth factor receptor 1, and Sodium /glucose cotransporter/2. Captopril was found to have interventions with Angiotensin converting enzyme, Renin, Angiotensin II type 2 (AT-2)

receptor and Endothelin converting enzyme for direct targets. The possible targets were with Endothelin converting enzyme-1, Angiotensin II type 2 (AT-2) receptor and Vasopressin V2 receptor. From the above PASS is a significant tool for successfully showing the accumulates of interest for the organic activities of interest. This assists the analysts with defending the exploration.

4.5 *In vitro* ACE inhibition assay

Rutin has shown antihypertensive activity by angiotensin converting enzyme inhibitory action (IC_{50}=66.01 µg/mL). As of late it has been estimated that oxidative pressure is a central member in the pathogenesis of hypertension. A decrease in superoxide dismutase and glutathione peroxidase action has been seen in recently analysed and untreated hypertensive subjects, which are conversely corresponded with circulatory strain. Hydrogen peroxide creation is additionally higher in hypertensive subjects. In vitro investigations have uncovered the cell reinforcement movement of rutin and the job of rutin in lessening oxidative pressure related with hypertension. In the event that oxidative pressure is for sure a reason for hypertension, rutin as a cell reinforcement affects hypertension control and decrease of oxidative harm could have brought about a decrease in pulse.

5. CONCLUSION

Finally it is concluded that the *in silico* and *in vitro* studies clearly demonstrated the anti-hypertensive action of Rutin.

REFERENCES

1. Hertog MG, Hollman PC, Katan MB, Kromhout D. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands. Nutr Cancer 1993; 20: 21–9.
2. Sampson L, Rimm E, Hollman PC, de Vries JH, Katan MB. Flavonol and flavone intakes in US health professionals. J Am Diet Assoc 2002; 102: 1414–20.
3. Benavente-García O, Castilho J. Update on uses and properties of citrus flavonoids: new findings in anticancer, cardiovascular, and anti-inflammatory activity. J Agric Food Chem 2008;56:6185–205.

4. Sjogren E, Thorn H, Tannergren C. *In Silico* modelling of gastrointestinal drug absorption: predictive performance of three physiologically based absorption models. Mol Pharmaceut 2016;13(6):1763–78.
5. Malik A, Manan A and Mirza MU. Molecular docking and *in silico* ADMET studies of silibinin and glycyrrhetic acid anti-inflammatory activity. Trop J Pharm Res 2017; 16 (1): 67-74.
6. Li J, Deng LY, Grove K, Deschepper CF, Schiffrin EL. Comparison of effect of endothelin antagonism and angiotensin-converting enzyme inhibition on blood and vascular structure in spontaneous hypertensive rats treated with N omega-nitro-L-arginine methyl ester. Hypertens 1996; 28: 188-95.
7. Lipinski, C.A., 2004. Lead-and drug-like compounds: the rule-of-five revolution. Drug Discovery Today: Technologies, 1(4), pp.337-341.
8. Sharma, C.S., Mishra, S.S., Singh, H.P. and Kumar N., 2016. In silico ADME and toxicity study of some selected antineoplastic drugs. International Journal of Pharmaceutical Sciences and Drug Research, 8(1), pp.65-67
9. Goel, R.K., Singh, D., Lagunin, A., Poroikov. V., (2011). PASS-assisted exploration of new therapeutic potential of natural products. Medicinal Chemistry Research., 20(9):1509-14.
10. Parasuraman. S., (2011). Prediction of activity spectra for substances. Journal of Pharmacology & pharmacotherapeutics., 2(1):52.
11. Poroikov, V., Filimonov, D., (2001). Computer-aided prediction of biological activity spectra. Application for finding and optimization of new leads. Rational Approaches to Drug Design; Prous Science: Barcelona ., 403-407.
12. Filimonov, D. A.; Poroikov, V. V., (1996). PASS: Computerized prediction of biological activity spectra for chemical substances. In bioactive compound design: possibilities for industrial use; BIOS Scientific Publishers., Oxford: 47-56.
13. Gloriozova, T. A., Filimonov, D. A., Lagunin, A. A., Poroikov, V. V.,(1998). Evaluation of computer system for prediction of biological activity PASS on the set of new chemical compounds.
14. Suvarchala Reddy NVL, Anarthe SJ, Subrahmanyam CVS and Raghavendra NM. Antihypertensive, ACE inhibitory and antioxidant activity of whole plant of *Rhynchosia*

beddomei. Asian J Pharmacol Toxicol 2015;03(10):13-8.

15. Lipinski CA. Lead-and Drug-like compounds: The rule-of-five revolution. Drug Discov Today Technol 2004;1: 337–41.
16. Kumar KA, Jagannath P, and Saleshier F. Computational and molecular designing studies of novel flavonoid analogues as HMG CoA Reductase and cholesterol esterase inhibitors for their Cardioprotective effect using *in Silico* docking studies. Int J Res Pharmacol Pharmacother 2018;7(2):166-177.