SUPPLEMENTARY MATERIAL

In-Silico comparison of Cytochrome P450 inhibitory and Dopaminergic activity of Piperine, Curcumin and Capsaicin

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ABSTRACT

Psychiatric disorders are a heterogeneous group of mental disorders that manifest as abnormal mental or behavioral habits that cause the individual discomfort or disability. Dopamine imbalance plays a major role in many psychiatric disorders. Piperine, Curcumin and Capsaicin are CYP P450 3A4 and 2D6 inhibitors. The objective of this study is to determine the dopaminergic activity of Piperine, Curcumin and Capsaicin and also to compare cytochrome P450 3A4 and 2D6 inhibition activity by *in-silico* methods. In this *in-silico* study we utilized compounds such as Piperine, Curcumin and Capsaicin were subjected to Lipinski's rule of five, and ligands were also evaluated for toxicity profile and ADMET properties. Furthermore, the ligands were performed in docking studies. All three compounds were docked with three different targeted proteins (PDB IDs: 4D7D, 4WNW, and 6LUQ). According to the docking result, Piperine has higher binding energy (-8.55 kcal/mol) (-8.1 kcal/mol) (-8.57 kcal/mol) when compared with Curcumin (-7.39 kcal/mol) (-5.61 kcal/mol) (-6.57 kcal/mol) and Capsaicin (-6.86 kcal/mol) (-6.57 kcal/mol) (-5.42 kcal/mol) and also with standard drug (-8.61 kcal/mol) (-7.65 kcal/mol) (-6.16 kcal/mol). The present study concluded that the bioactive compound Piperine has a better inhibitory activity of CYP 3A4, 2D6 enzymes and dopamine D2 receptor among the three compounds and also with the standard drug thioridazine.

KEYWORDS

Piperine, Curcumin, Capsaicin, ADMET, Molecular docking

Experimental S1

1. Materials & Methods

1.1 Selection of ligands

The ligand molecules, Piperine, Curcumin and Capsaicin were collected from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and identified as CYP3A4 and CYP2D6 inhibitors from different literature reviews (Shamsi S et al. 2016) Their structures were recreated using the Chemsketch software (ACD/Labs, v12.01) (Figure S1). The ligands were optimized using Avogadro software. After optimization, the ligands were saved in PDB format for further process.

1.2 Selection of protein

The three target proteins were retrieved from the Protein Data Bank (http://www.rcsb.org). (PDB IDs: 4D7D, 4WNW, and 6LUQ) (Figure S2-S4). Only the 'a' chain from all three proteins was extracted and it was downloaded from PDB format. It was then supplied into the Auto Dock tool, where ligands and water molecules were eliminated before docking, and hydrogen atoms were supplied to the protein to correct the ionization and tautomeric states of the amino acid residues. Kollaman charges and compute gasteiger were also included, and the protein was saved in .pdbqt format (Don et al.2020).

1.3 Drug-likeness Analysis

Selected constituents were processed for additional selection based on Lipinski's rule of five (RO5). Lipinski's rules were implemented using the molinspiration server (https://www.molinspiration.com/). The rule states that compounds with strong membrane potential have log P $\leq$ 5, molecular weight $\leq$ 500, $\leq$ 10 hydrogen bond acceptors and $\leq$ 5 hydrogen bond donors. The log P assessment is utilized to know the solubility behavior of a substance and result, its oral absorption and bioavailability. The number of rotatable bonds, molecule volume, and topological polar surface area (TPSA) are other important rules in the computational prediction of drug-likeness. The number of rotatable bonds in a compound determines its conformational flexibility and ultimately, its ability to bind to receptors or ion channels. The molecular volume can predict the compound transport characteristics, including intestinal absorption or Blood Brain Barrier (BBB) penetration. TPSA has been shown as an excellent predictor of medication absorption in the gut and BBB penetration (Lipinski CA et al.2001).

1.4 Bioactivity Score of Selected Compounds

A molinspiration screening, which is performed to determine the bioactivity score of Piperine, Curcumin and Capsaicin is a type of *in-silico* screening that involves computational techniques to assess the chemical database to reveal potential drug candidates. This computational technique is used to determine kinase inhibitors, protease inhibitors and enzyme inhibitors as well as ligands modulating G-Protein coupled receptor (GPCR), ion channels and nuclear receptors (Kuchana M et al.2020).

1.5 Toxicity

Thomas Sander of Acetelion Pharmaceuticals Ltd, Gewebestrassem16, and 4123 Allschwil, Switzerland designed the web software OSIRIS property explorer. The OSIRIS property

explorer is an online cheminformatics tool for determining compound toxicity (http://www.cheminfo.org).

In-silico toxicity characteristics are tumorigenicity, mutagenicity, irritants and reproductive effects. The toxicity outcomes are color-coded in green or red. The toxicity parameters of selected compounds are shown in green, indicating that the compounds are non-toxic and harmless. Whether they are highlighted in red, implies a high probability of unfavorable outcomes (Paramashivam et al.2015).

1.6 ADMET Prediction (Absorption, Distribution, Metabolism, Elimination and Toxicity)

Today, plenty of online and offline software is available to assist in the prediction of drug candidate behavior. We used the admetSAR prediction tool in this study (http://lmmd.ecust.edu.cn/admetsar2). The ligand structures were decided to be submitted to the admetSAR online server for analysis of their pharmacokinetic and pharmacodynamics parameters such as blood-brain barrier penetration, human oral bioavailability, P-glycoprotein inhibitor, a CYP3A4 inhibitor, CYP2D6 inhibitor, CYP inhibitory promiscuity, carcinogenicity, AMES, and acute oral toxicity (Malik, Arif, et al.2017).

1.7 Docking

Molecular docking is utilized to detect the scoring function and assess protein-ligand interactions to determine the binding affinity and activity of ligand (Verdonk ML et al.2003). The Auto dock tool was utilized and targeted proteins were docked with Piperine, Curcumin, and Capsaicin. The goal of docking analysis is to bind the ligand into the target protein's determined binding sites and generate the best-docked conformations with the minimum binding energy as the output. A rigid docking was performed. So, the standard protocol of the Lamarckian genetic algorithm was used to calculate the scoring function. For grid generation, the grid map was centered on a target protein. Pymol and Discovery studio visualizers are used to visualize the docking results (Tunga Kuhana A et al.2020).

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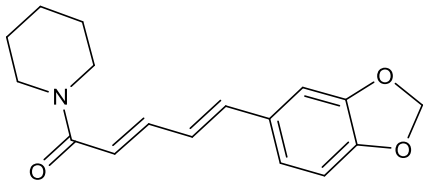
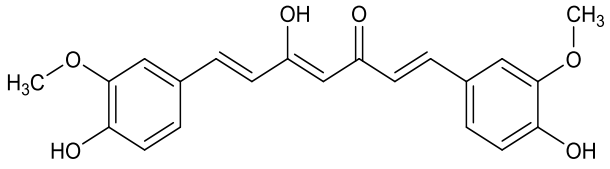
Figures/Tables



Piperine



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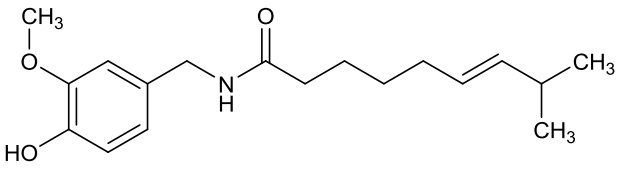


Capsaicin

Figure S1: Structure of Piperine, Curcumin, Capsaicin

Figure S2: Crystal structure of Cytochrome P450 3A4

Figure S3: Crystal structure of Human Cytochrome P450 2D6

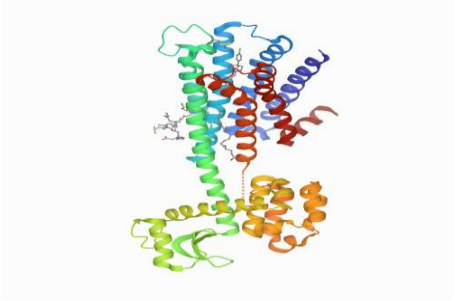


Figure S4: Crystal Structure of D2 dopamine receptor

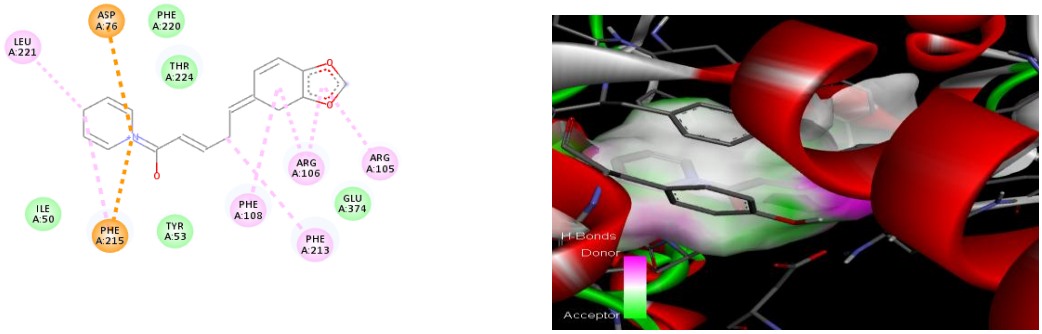


Figure S5: 2D and 3D structure of the Binding activity of Piperine with 4D7D protein

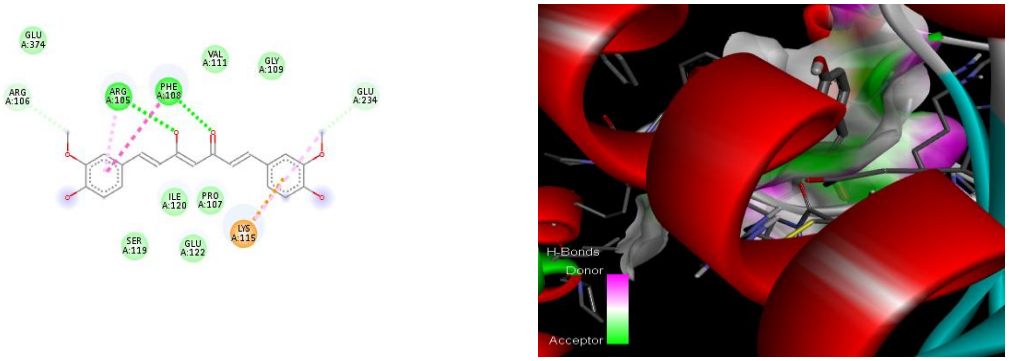


Figure S6: 2D and 3D structure of the Binding activity of Curcumin with 4D7D protein

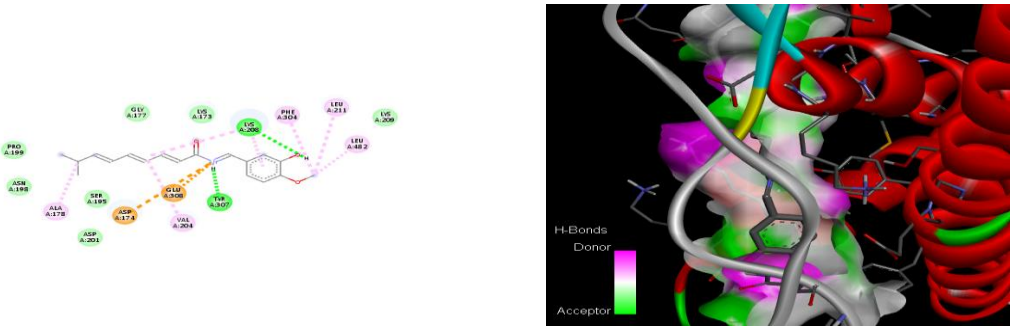


Figure S7: 2D and 3D structure of the Binding activity of Capsaicin with 4D7D protein

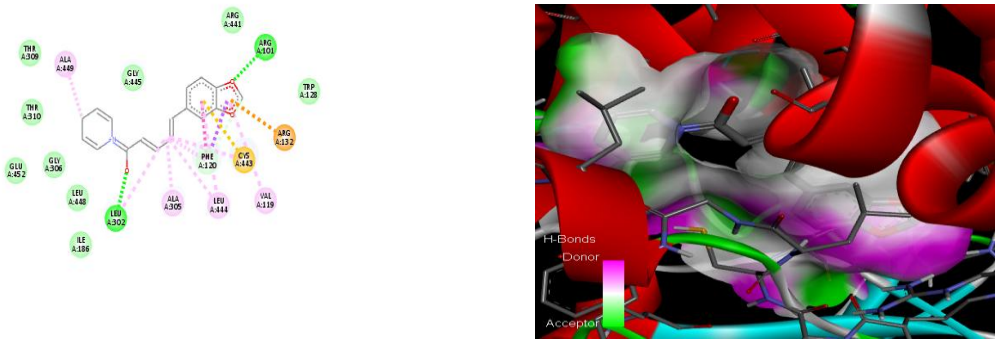


Figure S8: 2D and 3D structure of the Binding activity of Piperine with 4WNW protein

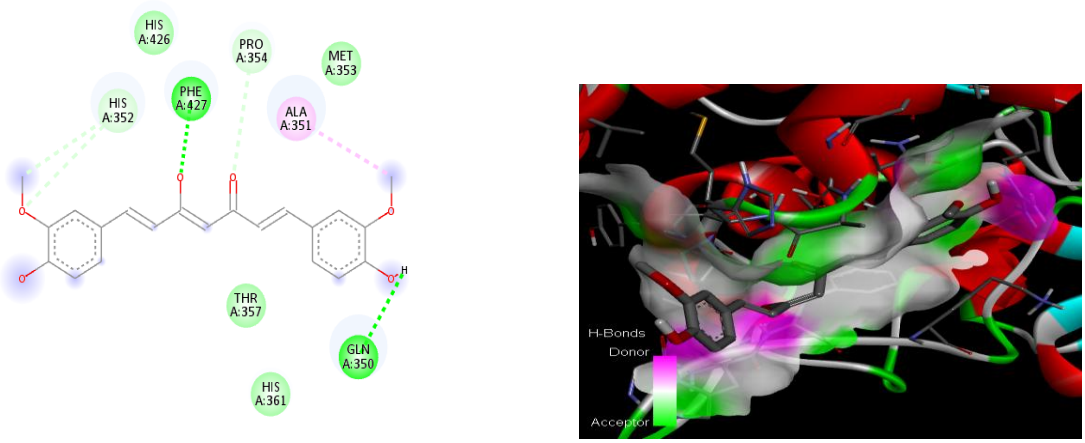


Figure S9: 2D and 3D structure of the Binding Activity of Curcumin with 4WNW protein

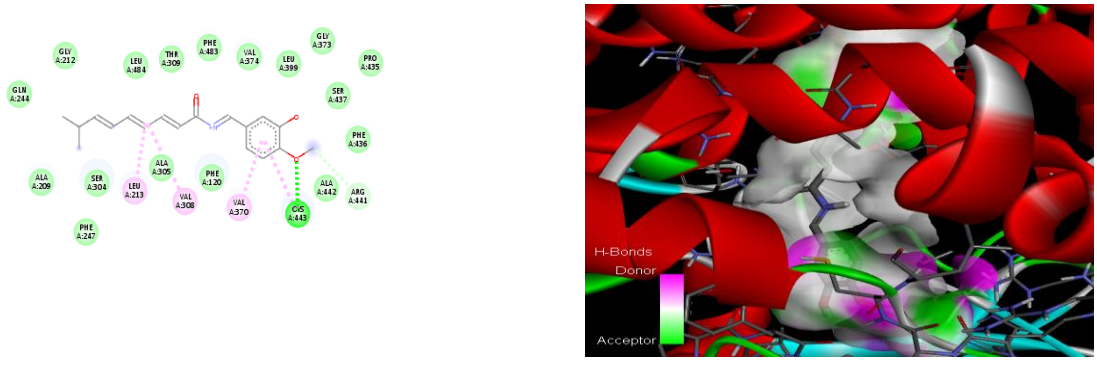
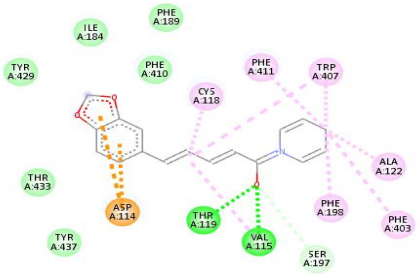


Figure S10: 2D and 3D structure of the Binding Activity of Capsaicin with 4WNW protein



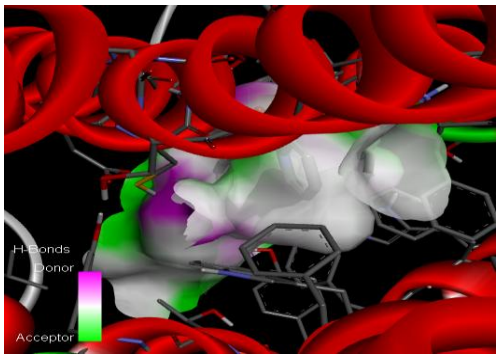
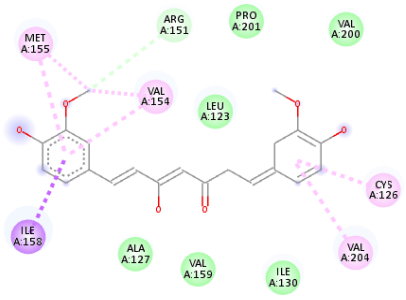


Figure S11: 2D and 3D structure of the Binding activity of Piperine with 6LUQ protein



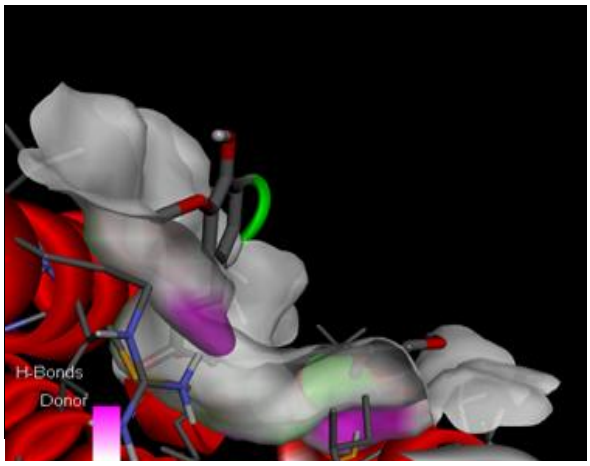
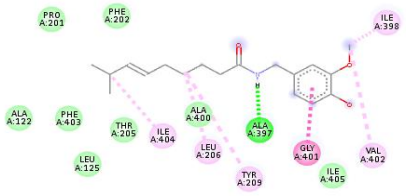


Figure S12: 2D and 3D structure of the Binding Activity of Curcumin with 6LUQ protein



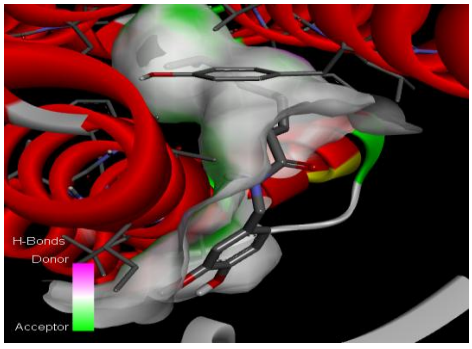


Figure S13: 2D and 3D structure of the Binding Activity of Capsaicin with 6LUQ protein

Molinspiration

Table S1: Comparison of drug likeness properties of Piperine, Curcumin and Capsaicin

Drug likeness properties	Piperine	Curcumin	Capsaicin
Molecular Weight	285.4	368.38	305.42
Log P	3.33	2.30	3.10
TPSA	38.78	93.07	58.56
n atoms	21	27	22
noN	04	06	04
noHNH	0	02	02
n Violation	0	0	0
nrotb	03	08	09
Volume	267.74	332.18	310.37

TPSA- Total Polar Surface Area, noN – Number of hydrogen bond acceptors, noHNH – Number of hydrogen bond donors, nrotb – Number of rotatable bonds

Table S2: Bioactivity score of Piperine, Curcumin and Capsaicin

Bioactivity Score	Piperine	Curcumin	Capsaicin
GPCR ligand	0.15	-0.06	0.03
Ion Channel Modulator	-0.18	-0.20	-0.01
Kinase inhibitor	-0.13	-0.26	-0.28
Nuclear receptor ligand	-0.13	0.12	0.01
Protease inhibitor	-0.10	-0.14	-0.02
Enzyme inhibitor	0.04	0.08	0.07

GPCR – G Protein-coupled receptor

Table S3: Toxicity

Chemical Constituents	Mutagenicity	Tumorigenic	Irritant	Reproductive Effect	Drug likeness	Drug Score
Piperine	Non-Toxic	Non-Toxic	Non-Toxic	Non-Toxic	0.60	0.43
Curcumin	Non Toxic	Non Toxic	Non Toxic	Non Toxic	-3.95	0.44
Capsaicin	Non Toxic	Non Toxic	Non Toxic	Non Toxic	-9.65	0.42

Table S4: ADMET Properties

ADMET Properties	Piperine	Curcumin	Capsaicin
BBB	0.9964	0.6162	0.6219
Human Oral Bioavailability	0.5714	0.6000	0.5000
P-gp inhibitor	Non-inhibitor (0.8218)	inhibitor (0.682)	No (0.7854)
CYP3A4 inhibitor	inhibitor (0.7959)	inhibitor (0.6392)	inhibitor (0.8287)
CYP2D6 inhibitor	inhibitor (0.8307)	inhibitor (0.6715)	inhibitor (0.8932)
CYP inhibitory Promiscuity	inhibitor (0.8136)	inhibitor (0.5716)	No (0.7551)
Carcinogenicity	No (0.9385)	No (0.8689)	No (0.9153)
AMES	No (0.9133)	No (0.9132)	Yes (0.7678)
Acute Oral Toxicity	0.8002	0.6349	0.6676

BBB – Blood Brain Barrier, P-gp – P- glycoprotein

Table S5: Comparison of Binding energies (ΔG) between the ligands.

Target Proteins	Binding Energy for Active Constituents			Standard drug
PDB Code	**Piperine (kcal/mol)**	**Curcumin (kcal/mol)**	**Capsaicin (kcal/mol)**	**Thioridazine (kcal/mol)**
4D7D	-8.55	-7.39	-6.86	-8.61
4WNW	-8.1	-5.61	-6.57	-7.65
6LUQ	-8.57	-6.57	-5.42	-6.16