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IN SILICO ANALYSIS AND ADMET PREDICTION OF CINNAMON COMPOUNDS ON PPARAα/γ RECEPTOR FOR SEARCHING ANTI-DIABETIC DRUG CANDIDATES

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Abstract

Diabetes is one of the leading causes of death, and while there are currently anti-diabetic drugs available, they do not always work optimally due to reported side effects and resistance. The objective is to identify compounds from Cinnamomum loureiroi with the highest anti-diabetic activity and the lowest toxicity than Metformin Hydrochloride. The study conducted an in silico analysis of six compounds extracted from Cinnamomum loureiroi. The essential oil from cinnamon was extracted through steam distillation and analyzed using GC-MS. The compounds were then subjected to drug-likeness prediction using DruLiTo and ADMET prediction to evaluate absorption, distribution, metabolism, excretion, and toxicity using pkCSM online. Molecular docking was performed using the AutoDock 4.2.6 program to target PPARα/γ, and the results were visualized using Discovery Studio Visualizer softwar. The obtained data revealed that DL-Limonene exhibited a strong binding affinity to PPARα/γ with a free energy of binding (ΔG) of -7 kcal/mol and an inhibition constant (Ki) of 7182 nm. The study's findings suggest that compounds derived from Cinnamomum loureiroi hold promise as potential candidates for anti-diabetic drugs, with DL-Limonene identified as the most promising candidate.

Keywords: Cinnamomum loureiroi, Drug-likeness, GC-MS, Molecular Docking, PPARα/γ, Pharmacokinetics.

1. INTRODUCTION

Diabetes mellitus encompasses a range of disorders characterized by episodes of hyperglycemia and glucose intolerance, resulting from insufficient insulin, impaired insulin function, or both [1]. These complications arise due to disruptions in the regulatory systems responsible for storing and releasing metabolic fuels, including the breakdown and synthesis of carbohydrates, lipids, and proteins resulting from faulty insulin secretion, insulin function, or a combination of both [2].

Type 2 diabetes mellitus (T2DM) accounts for more than 90% of all cases of diabetes. The management of T2DM typically involves the use of modern antidiabetic medications. However, these drugs can be expensive, have significant adverse effects, and may not effectively control glycemia [3]. Various types of antidiabetic medications are commonly used, such as sulfonylureas, biguanides, meglitinide analogues, thiazolidinediones TZD,

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α-amylase inhibitors, and α-glucosidase inhibitors. However, these medications may cause side effects and have unfavorable impacts on health [4].

PPARs belong to the nuclear hormone receptor superfamily and function as transcription factors dependent on ligands to regulate lipid and glucose metabolism. There are three known isoforms of PPARs, namely PPARα, PPARγ, and PPARδ [5]. Activation of PPARα results in decreased levels of triglycerides, while activation of PPARγ enhances glucose metabolism by increasing insulin sensitivity. The hypolipidemic drug class, fibrate, activates PPARα, and the antidiabetic agent class, thiazolidinedione, activates PPARγ [6].

According to research, cinnamon oil has been found to regulate blood glucose levels and improve the function of pancreatic islets, making it a potentially useful treatment for type 2 diabetes mellitus [7]. Cinnamon bark has also been found to be effective in alleviating diabetes due to its antioxidant and insulin-potentiating properties, among other activities. These benefits are attributed to the water-soluble polyphenolic oligomers present in cinnamon [8], [9].

Molecular docking plays a crucial role in structural molecular biology and computer-assisted drug design. Its primary purpose is to predict the most probable binding mode(s) between a ligand and a protein with a known three-dimensional structure. Effective docking methods employ high-dimensional searches and employ a scoring function that accurately ranks potential dockings. Successful ligand-protein docking is essential for identifying potential drug candidates and understanding the molecular mechanisms underlying the interactions between drugs and their target proteins. [10], [11].

The aim of this research is to study the preliminary phytoconstituents of Cinnamomum loureiroi and analyze them using gas chromatography-mass spectrometry (GC-MS). Additionally, the research aims to use in silico activity tests to evaluate the hypoglycemic potential of the identified compounds.

2. MATERIALS AND METHODS

2.1 Plant collection and extraction

The cinnamon barks (Cinnamomum loureiroi) were collected in Vietnamese.

Steam distillation is the simplest method to extract the essential oil from cinnamon. Steam distillation is mostly used to extract various types of essential oils. The process is cheaper than other extraction methods. It does not require any solvent and is safer than other methods [12].

2.2 Gas chromatography-mass spectrum (GC-MS) analysis

The essential oil of cinnamon bark was carried out using the Shimadzu GC-MS system (GCMS-TQ8030) consisting of a mass selective detector (EIMS, electron energy, 70 eV) and an (Agilent ChemStation) data system. The GC column was an HP-5ms fused silica capillary with a 5% phenyl methylpolysiloxane stationary phase, a film thickness of 0.25 μm, a length of 25 m, and an internal diameter of 0.25 mm. The GC settings were as follows: the initial oven temperature was held at 50 °C for 3 min and then heated at 180

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°C at a rate of 2 °C/min, held for 1min, and then heated to 260 °C at 10 °C/min and held for 5 min. The injector temperature was maintained at 250 °C. The sample (1μl, diluted 100: 1 in Hexane) was injected with a split. The carrier gas was helium at a flow rate of 1.0 ml/min. The mass spectral detection was carried out in electronic ionization mode by scanning at 40 to 600 (m / z). Finally, the total time required to analyze a single sample was 35 min.

Most constituents were identified by gas chromatography by comparison of their retention indices with those of the literature or with those of authentic compounds available in our laboratories. Further identification was made by comparison of their mass spectra with those stored in NIST 08 and Wiley 275 libraries or with mass spectra from the literature [13].

2.3 Hardware

The specification of the computer that is used: Intel® Core™ i3 3217U@ 1.80 GHz processor (CPU), HD graphics processing unit (GPU), and 8 GB Random Access Memory (RAM) with Windows 10.

2.4 Compound test preparation

The six cinnamon volatile compounds (CVCs) having activity against diabetes mellitus from *Cinnamomum loureiroi* were the results obtained using the GCMS-TQ8030 instrument. The six compounds from Cinnamomum loureiroi are as shown in figure 1 and table 1.

The test compounds were made in 2D and 3D models, then optimized using the PubChem (https://pubchem.ncbi.nlm.nih.gov/). Then, the structure is translated into SMILES format using the Online SMILES Translator (https://cactus.nci. nih.gov/translate/).

2.5 Drug-likeness prediction

Three filters of the DruLiTo program (Lipinski's rule, Veber rule, and Ghose filter) were used to predict the drug-likeness of the test compound by entering *sdf file format. [14]

2.6 ADMET prediction

Prediction of pharmacokinetics (ADME) and toxicity of the seven compounds from *Cinnamomum loureiroi* was done by the pkCSM website (http://biosig.unimelb.edu.au/pkcsm/prediction) with the SMILES format. [14]

2.7 Molecular docking

The structure of gamma PPARα/γ target receptor (PDB ID: 3G9E) obtained from the Protein Data Bank (https://www.rcsb.org/) that is shown in figure 2. As for a comparison ligand, Metformin Hydrochloride is also used. Molecular docking is done using AutoDockTools 4.2.6 program. The validation results are indicated by the Root Mean Square Deviation (RMSD) value. Center the grid box using a grid box (40 × 40 × 40). The binding site coordinates are x = 3.321; y = 26.529; z = 28.131 with spacing per unit 0.375 angstrom. Visualization analysis of protein-ligand interactions was performed with Discovery Studio Visualizer v.21.1.0.20298 from BIOVIA.

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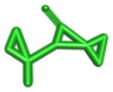
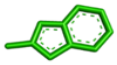
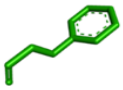
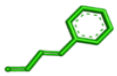
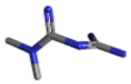
3. RESULTS

The compounds from ***Cinnamomum loureiroi*** (Figure 1).

Table 1: The seven compounds from *Cinnamomum loureiroi* using the GCMS-TQ8030 instrument

	No	RT (min)	Mass M/Z	Formula	IUPAC	3D Models
C1	α-Pinene	7.385	136.23	$C_{10}H_{16}$	Bicyclo [3.1.1]hept-2-ene, 2,6,6-trimethyl-	
C2	Camphene	7.767	136.23	$C_{10}H_{16}$	2,2-dimethyl-3-methylidenebicyclo[2.2.1]heptane	
C3	DL-Limonene	9.805	136.25	$C_{10}H_{16}$	1-methyl-4-prop-1-en-2-ylcyclohexene	
C4	2-methylbenzofuran	13.703	132.16	C_9H_8O	2-methyl-1-benzofuran	
C5	Benzenepropanal	13.320	134.17	$C_9H_{10}O$	3-phenylpropana	
C6	Cinnamaldehyde	16.279	132.16	C_9H_8O	(E)-3-phenylprop-2-enal	
S	Metformin Hydrochloride (standard drug)	/	165.62	$C_4H_{12}ClN_5$	1,1-dimethylbiguanide hydrochloride	

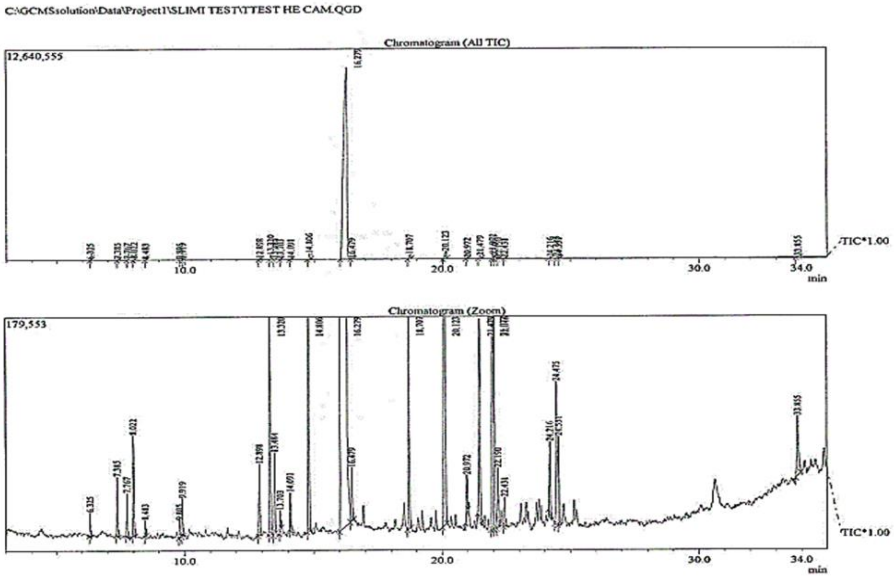


Figure 1: GCMS-TQ8030 chromatogram

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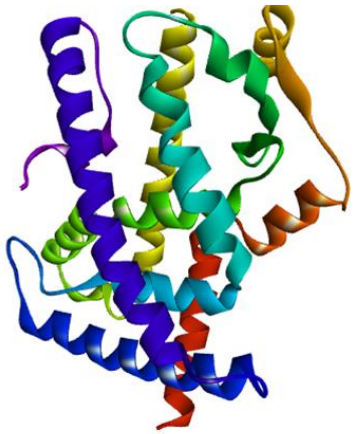

Figure 2: PDBQT format of PPARα/γ (ID: 3G9E)

3.1 Drug-likeness prediction

Prediction results of drug-likeness of the compounds from ***Cinnamomum loureiroi*** are shown in table 2.

Table 2: Prediction of Druglikeness with DruLiTo program.

	MW	logP	HBA	HBD	TPSA	AMR	nAtom	Lipinski's Rule	nRB	Ghose Filter	Veber Rule
C1	136.13	4.177	0	0	0	44.54	26	1	0	0	1
C2	136.13	4.024	0	0	0	41.93	26	1	0	0	1
C3	136.13	3.729	0	0	0	46.02	26	1	0	0	1
C4	132.02	1.549	1	0	9.23	46.06	18	1	11	0	1
C5	134.07	2.047	1	0	17.07	45.44	20	1	7	0	1
C6	132.06	1.968	1	0	17.07	46.27	18	1	8	0	1

3.2 ADMET prediction

The prediction results of the ADMET of the compounds from ***Cinnamomum loureiroi*** are shown in table 3.

Table 3: Prediction of ADMET

code	Intestinal absorption (human)	Skin Permeability	VDss human	BBB permeability	CYP2D6 substrate	CYP2D6 inhibitior	Total Clearance	Renal OCT2 substrate	Ames toxicity	Hepatotoxicity
C1	96.041	-1.827	0.667	0.791	non	non	0.043	non	non	non
C2	94.148	-1.435	-1.43	0.787	non	non	0.049	non	non	non
C3	95.898	-1.721	0.396	0.732	non	non	0.213	non	non	non
C4	94.965	-1.485	0.246	0.288	non	non	0.354	non	non	non
C5	95.203	-1.422	0.299	0.483	non	non	0.341	non	non	non
C6	95.015	-2.355	0.266	0.436	non	non	0.203	non	non	non

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Table 4: Molecular docking binding affinity of compounds from Cinnamomum loureiroi, ranked by the lowest free energy of binding (ΔG) and inhibition constant (Ki)

Code	Compound name	ΔG (kcal/mol)	Ki (nM)
C1	α-Pinene	-6.7	11400
C2	Camphene	-6.8	10130
C3	DL-Limonene	-7.0	7182
C4	2-methylbenzofuran	-5.9	46179
C5	Benzenepropanal	-5.2	150944
C6	Cinnamaldehyde	-5.9	46179
S	Metformin Hydrochloride (standard drug)	-4.4	581442

Table 5: Interaction details of the target enzyme PPARα/γ

compounds	Ligand type	Receptor pocket	Interactions Category	Distance(Å)
	C = O	ILE341	Hydrophobic	5,34314
	C = O	PHE247	Hydrophobic	5,4488
	C = O	ILE249	Hydrophobic	5,11084
	C = O	PHE247	Hydrophobic	5,23112
DL-Limonene	C = O	ARG288	Hydrophobic	4,62993
	C = O	ALA292	Hydrophobic	3,88971
	C = O	LEU330	Hydrophobic	4,14183
	C = O	ILE326	Hydrophobic	4,35993
	C = O	MET329	Hydrophobic	4,58437
2-methylbenzofuran	CZ - NH1	ARG288	Electrostatic	3,23545
	CZ - NH2	ARG288	Hydrogen Bond	2,55872
	C = O	MET329	Hydrophobic	4,54852
	C = O	MET329	Hydrophobic	3,50969
	C = O	LEU330	Hydrophobic	4,42734
	C = O	ALA292	Hydrophobic	4,0916
	C = O	LEU333	Hydrophobic	5,28651
	CA - C	ILE296	Hydrophobic	4,93345
Benzenepropanal	HG - OG	SER342	Hydrogen Bond	2,16352
	CD – OE1	GLU259	Electrostatic	3,8908
	CG1 - CD1	ILE341	Hydrophobic	3,91769
Cinnamaldehyde	CB - OG	SER332	Hydrogen Bond	3,55072
	C = O	LEU228	Hydrogen Bond	3,29913
	HE - NE	ARG288	Hydrogen Bond	3,8236
	C = O	MET329	Hydrophobic	4,51275
	C = O	ALA292	Hydrophobic	5,30025
	C = O	ILE326	Hydrophobic	5,48393
	C = O	LEU330	Hydrophobic	4,46984

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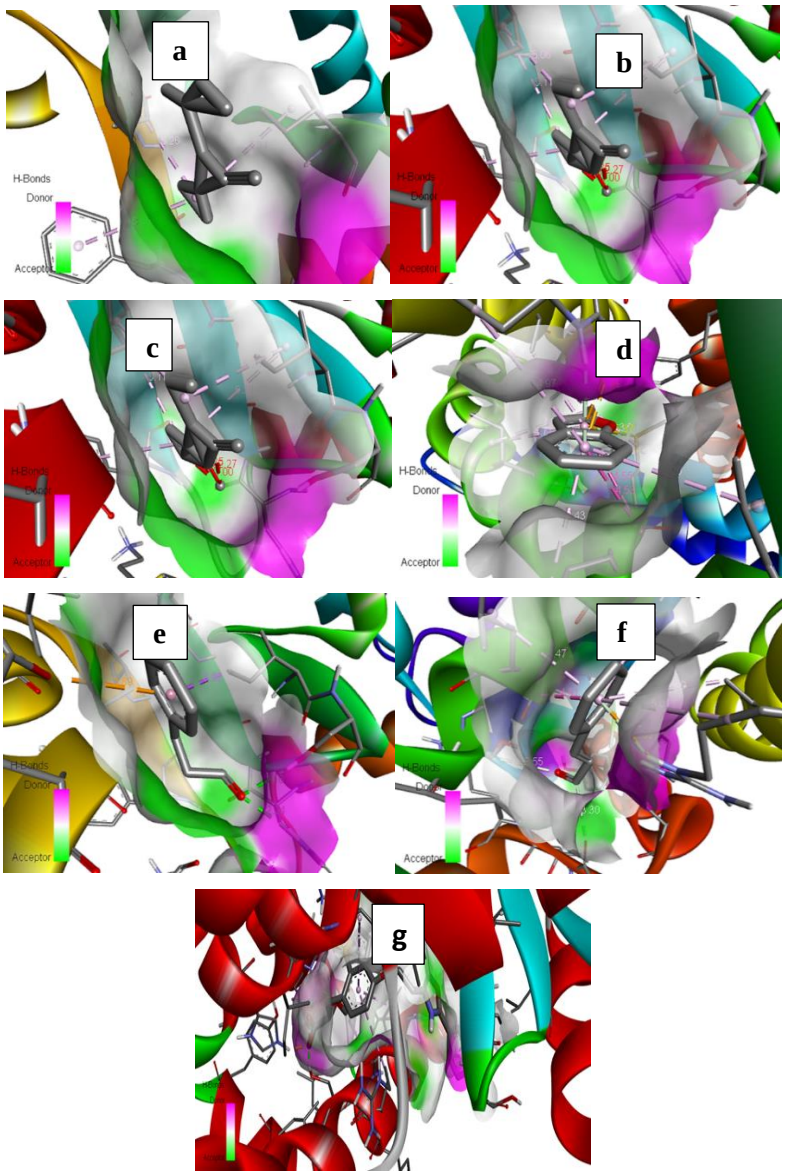

Figure 3: Visualization of (a) alpha-pinene, (b) Camphene, (c) DL-Limonene, (d) 2-methylbenzofuran, (e) Benzenepropanal, (f) Cinnamaldehyde, (g) Metformin Hydrochloride

4. DISCUSSION

The RMSD value, or root-mean-square deviation, is a measure of the similarity between the predicted and actual conformations of the ligand-receptor complex. A low RMSD value indicates a good fit between the ligand and receptor, and suggests that the docking protocol is valid. A commonly accepted threshold for RMSD in docking studies is 2 Å. [15]

In the case mentioned, the RMSD value of 1.16 indicates that the docking protocol used in the study is valid, and the predicted conformations of the ligand-receptor complexes are accurate.

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The ΔG score, as previously mentioned, is a measure of the thermodynamic favorability of the ligand-receptor interaction. A more negative ΔG score indicates a stronger interaction and higher binding affinity between the ligand and receptor. [16]

Table 4 shows the results of the molecular docking study, which reveal that DL-Limonene has the strongest inhibitory activity with a ΔG value of -7.0 kcal/mol, indicating high affinity towards the PPARα/γ target receptor than Metformin Hydrochloride -4.4 Kcal/mol. The Ki values of the compounds range from 7182 to 150944 nM, which suggest high affinity towards the target receptor.

Hydrogen bonds, hydrophobic interactions, and electrostatic interactions are all important types of interactions that can contribute to the stability of a ligand-protein complex. Hydrogen bonds are formed when a hydrogen atom is shared between two electronegative atoms, such as oxygen or nitrogen. These bonds are relatively weak, but they can contribute significantly to the overall stability of a complex when multiple hydrogen bonds are formed.

Hydrogen bonding also contributes to the affinity of the ligand to the protein/receptor due to the electrostatic interaction between the oxygen or nitrogen atom of the ligand and the hydrogen atom of the protein amino acid can be seen in Table 5.

Cinnamaldehyde has 3 hydrogen bonds, with 1 amino acid residue that are the same as 2 methylbenzofuran (Arg 288), Benzenepropanal has 2 hydrogen bonds.

The fact that all compounds from Cinnamomum loureiroi comply with Lipinski's rule and Veber's rule (Table 3) is a positive indication for their potential use as drug candidates. Lipinski's rule and Veber's rule are widely used drug-likeness rules that help predict the oral bioavailability and pharmacokinetic properties of drug molecules. Compliance with these rules suggests that the compounds have good solubility, permeability, and metabolic stability, which are important factors in determining a drug's efficacy. [17], [18]

Table 4 provides information about the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the tested compounds from Cinnamomum loureiroi. The predicted values of intestinal absorption show that the compounds are likely to be well absorbed in the human intestine with values ranging from 94% to 96%.

The steady-state volume of distribution (VDss) values of the test compounds are relatively moderate, ranging from -1.43 to 0.66 (log L/kg), which suggests that the compounds are likely to distribute well throughout the body. The predicted BBB permeability values of the compounds are between 0.2 to 0.8 above -1, indicating moderate ability to penetrate the BBB. [19]

The table also shows that all compounds are predicted to be substrates or inhibitors of CYP2D6, an enzyme involved in drug metabolism. This suggests that the compounds may undergo metabolism by the cytochrome P450 pathway in the liver. [19]

The total clearance values of the test compounds range from 0.203 to 0.37, indicating that the compounds can be eliminated from the body at a moderate rate. OCT2, a transporter protein in the kidney, is also involved in the excretion of some compounds.

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Regarding toxicity, the compounds are predicted to be non-hepatotoxic, which is a positive indication for their potential use as drug candidates. However, it is important to note that all compounds are predicted to be active to cause mutagenic effects, which suggests that further studies are required to evaluate the safety of the compounds.

Overall, the ADMET prediction results suggest that the compounds from Cinnamomum loureiroi have favorable pharmacokinetic properties, which makes them promising drug candidates.

The study suggests that the presence of DL-Limonene enhances the affinity of the compounds from Cinnamomum loureiroi towards the PPARα/γ target receptor, indicating greater activity against the target receptor than Metformin Hydrochloride.

5. CONCLUSION

The results of the study indicate that the compounds from Cinnamomum loureiroi have potential as drug candidates due to their favorable pharmacokinetic properties and strong binding affinity towards the PPARα/γ target receptor. The molecular docking results suggest that DL-Limonene is the most potent inhibitor with high affinity towards the target receptor. Compliance with Lipinski's rule and Veber's rule further support their potential as drug candidates.

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