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Potential of *Combretum Indicum* var. B flowers as Hepatoprotector Through Molecular Dynamic Methods

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ABSTRACT

The challenge of liver disease is predominantly found in developing countries due to the use of drugs, parasites, alcohol, bacteria, and viruses. To overcome this challenge, *Combretum indicum* var. B plant has been identified as potential hepatoprotector. Therefore, this study aimed to screen compounds in *C. indicum* var. B flowers with the potential to be CYP2C9 enzyme inhibitors using pharmacophore and molecular dynamic methods. The compounds contained in the *C. indicum* var. B flowers were extracted and screened based on pharmacophore, drug-likeness, absorption probability, docking, and molecular dynamics. The screening results showed that there were five compounds with potential as hepatoprotector and docking obtained the ranking based on their lowest binding energies. These compounds included 6-quanolinecarboxylic acid, ellagic acid, 2-(2-thienyl)-4H-chromen-4-one, N-[4-(diethylamino)phenyl]-N'-phenylurea, and AMPA, with binding energies of -5.52, -8.93, -7.04, -6.16, and -5.54 kcal/mol, respectively. Based on the results, the most potential compound as hepatoprotector was N-[4-(diethylamino)phenyl]-N'-phenylurea.

Keywords: Molecular dynamic, Flowers, Hepatoprotectors, *C. indicum* var. B

Introduction

The liver is the most important metabolic organ in the process of synthesis, storage, and metabolism. It is a wedge-shaped organ with mean weight of 1.5 kg, or 2.5% of the normal adult body weight. One of the liver functions is detoxification, causing high susceptibility to becoming the main target of toxicity.[1] Liver disease is classified as a major problem in developing countries,[2] which is often caused by the use of drugs, parasites, alcohol, bacteria, and viruses,[3] An example of drugs that can cause liver disease is paracetamol, which has antipyretic and analgesic properties.[4] Paracetamol is hepatotoxic when used long-term and given in a single dose of 10-15 grams or 200-250 mg/kgBW. This is because of the ability to metabolize and produce NAPQI (N-acetyl-p-benzoquinone) compound, which cannot bind to receptors, leading to the formation of free radicals and toxicity.[5] Free radicals are molecules or atoms that do not have an electron pair in the outer orbital, thereby remaining unstable and highly reactive to cells in the body.[6] When the body administers paracetamol, the liver endogenous antioxidant, namely Glutathione (GSH), is unable to control NAPQI. This leads to the formation of free radicals that bind to the unsaturated fatty acids in the cell membrane, causing lipid peroxidation and the formation of malondialdehyde (MDA).[7] High levels of MDA in plasma can be used as an indicator of the presence of free radicals and oxidative damage to cell membranes. This is because free radicals attack lipid membranes containing polyunsaturated fatty acids to form MDA, which is the end product of lipid peroxidation.[8]

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Antioxidant compounds inhibit and prevent free radicals as well as oxidative damage by stabilizing unpaired electrons with hydrogen atom groups.[9] The cytochrome P450 enzyme system plays a significant role in the metabolism of various xenobiotics, including drugs and other chemical compounds. This enzymatic system also contributes to oxidative stress through the formation of reactive oxygen species, which can cause cell damage when not properly regulated by antioxidants.[10] Therefore, there is a need to maintain a balance between CYP2C9 activity and antioxidant effect for cellular homeostasis and mitigating harmful oxidative effects. Understanding the balance is essential as antioxidant can modulate CYP2C9 activity, affecting metabolic efficiency and therapeutic pharmacokinetics.[11] Consequently, plants with antioxidant capabilities such as *Combretum indicum* var. B requires further exploration to determine the potential as hepatoprotector. Previous studies on the genus Combretum had explored the potential as hepatoprotector. These included water extract of *C. sericeum* roots, which showed protective effects on liver by affecting parameters of urea levels, CRT, ALP, AST, ALT, and antioxidant enzymes such as catalase and SOD.[12] The protective ability was also identified in *C. albidum*,[13] water extract of *C. dolichopentalum* leaves,[14] and ethanol extract of *C. Hypopilinum.*[15] The extract showed antioxidant and anti-inflammatory properties and *C. micranthum* induced by paracetamol.[16] Moreover, compounds from the genus Combretum were found to inhibit hepatic cell death induced by D-GalN/TNF-alpha. These compounds included 1-O-Galloil-6-O-(4-hydroxy-3,5-dimethoxy) benzoyl-beta-D-glucose, methyl gallate, and epicatechin with inhibitory ability of 7.2 microM, 19.9 microM, and 71.2 microM, respectively.[17] Although several experiments conducted on ethanol extract of *C. indicum* as potential hepatoprotector had focused on parameters ALP, AST, and ALT,[18] there is no information on the screening of hepatoprotective compounds. Therefore, this study aimed to screen compounds in *C. indicum* var. B flowers with the potential to be CYP2C9 enzyme inhibitors using pharmacophore and molecular dynamic methods.

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Materials and Methods

Materials

The materials used *C. Indicum* var. B flowers were collected from cordinate number -3^{0}72'01.30"S 115^{0}61'72.57"E (Tanah Bumbu, Kalimantan Selatan) with Herbarium specimen with number 032/LB.LABDASAR/II/2022 and collection number XII-21-018-S, ethanol 96 %, acetonitrile (Tedia, USA), formic-acid (Pro analytical, Merck), methanol (merk).

Tools

LC-MS/MS (WatersSYNAPT G2-Si HDMS), The database included Chemspider (5.0; 2023) (https://www.chemspider.com/), SwissADME (CC BY 4.0.; 2023) (http://www.swissadme.ch/index.php), AdmetSAR (2.0; 2022) (http://lmmd.ecust.edu.cn/admetsar2/), chemaxon (16.8.8.0; 2016) (https://chemaxon.com/marvin), RCSB (PDB101; 2023) (https://www.rcsb.org/), YASARA (21.6.2; 2021) (https://www.yasara.org/), Autodock (4.2.6; 2014) (https://autodock.scripps.edu/download-autodock4/), and discovery studio (2.0; 2008) (https://www.3ds.com/products/biovia/discovery-studio)

Methods

The screening stages of active compounds from *C. indicum* as a hepatoprotector are attached in **Figure 1.**

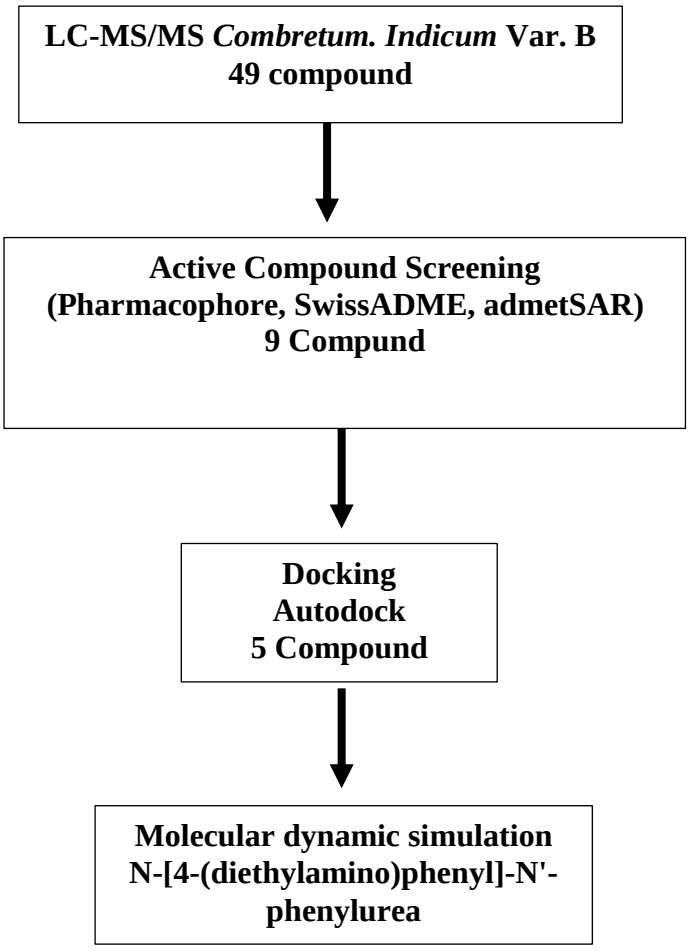

Figure 1: Research scheme

Compounds screening

Dry powder of *C. indicum* var. B flowers as much as 10 grams was extracted with 96% ethanol using the maceration method to produce 700 mg of thick extract. The thick extract weighing 10 grams was dissolved with 10 methanol p.a and filtered with a 0.22 µm filter. The volume injected into the LC-MS/MS was 5 µL. Compounds contained in flower of *C. indicum* var. B was obtained from LC-MS/MS analysis of *C. indicum* flowers with a database from ChemSpider. LC-MS/MS conditions Stationary phase C18 column (2.1 × 100 mm, 1.8 µm), Mobile Phase; mobile phase A: acetonitrile + 0.% formic acid; flow rate 0.3 mL/min; Electrospray ionisation (ESI), ESI+(positive), capillary cone 35 V, collision energy 18 V and dessolvation temperature 600ºC. Mass detector was performed with multiple reaction (MRM) at transition 138 m/z → 121 m/z. Initial screening was carried out through pharmacophore. Compounds were selected based on pharmacophore criteria Based on the native ligand, namely (2R)-N-{4-[(3-bromophenyl)sulfonyl]-2-chlorophenyl}-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide from RCSB with the code 4NZ2, which included the presence of aromatic with coordinates X: -61.999; Y: -49.126; Z: -24.144, Hydrogen acceptor (X; -58.947; Y: -46.426; Z: -24.641), First hydrophobic (X: -61.699; Y: -49.126; Z: -24.144) second hydrophobic (X: -62.032; Y: -51.781; Z-22.193). Followed by SwissADME to assess the similarity of the drugs when used orally. Subsequently, AdmetSAR was used to observe the drugs absorption in the body and predict the compounds bioavailability when used orally.

Docking

Study on compounds from *C. indicum* var. B with potential as hepaprotector was carried out targeting protein enzyme CYP2C9 with the code 4NZ2. This protein had a native ligand which was used coordinates[19]. The docking process used autodock found in YASARA with coordinates of 3Å from the native ligand and gridbook in the form of a cube X: 18.88 Å, Y: 18.88 Å, Z: 18.88 Å with angles α: 90; β: 90; λ: 90.

Molecular dynamics

Molecular dynamics simulations were performed in YASARA Structure version 21.6.2 under license 237458916, using the Amber14 force field. The Coulomb distance interactions were calculated through the particle Ewald algorithm, and the Van der Waals forces were limited to 8. The simulation box was shaped similarly to a cube and placed around the simulated molecules at a distance of 5 nm. The size was 50 × 50 × 50 Å with n = 6, and the boundaries were conditioned periodically. The system conditions were 0.9% NaCl (physiological solution), pH 7.4, temperature 310^0 K, and water density set at 0.997. The simulation lasted for 100 ns with images taken every 100 ps.

Statistical analysis

This study analyzed docking data based on binding energy, while molecular dynamics data were evaluated through several parameters. These included stability of the interaction through root mean square deviation (RMSD) data, solvent-accessible surface area (SASA), radius of gyration (Rg), hydrogen bond, root mean square fluctuation (RMSF), and binding energy.

Results and Discussion

Compound screening

Based on the analysis using LC-MS/MS, a total of 49 compounds were obtained from *C. indicum* var. B flowers[20] with ChemSpider database (**Table 1 and Figure 2**). A total of 12 compounds were selected based on pharmacophore criteria. After being analyzed using swissADME, 11 compounds were obtained. Subsequently, further analysis was conducted using admetSAR to obtain nine compounds that were docked, as shown in **Table 2.**

Docking

YASARA performed docking on nine compounds using the AutoDock software. This process used AutoDockLS mode (local search) which allowed the compounds to move freely with 100 conformations while keeping the CYP2C9. To prevent interference with the docking simulation and ensure that only ligand interact with proteins, the CYP2C9 protein was prepared with the code 4NZ2, removing water molecules and other residues. Subsequently, validation was performed by re-docking the native ligand of a separated target protein. The result showed RMSD value of 0.9833, which met the docking validation requirements.[21]

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Table 1. Prediction of compounds from *C. indicum* var. B flowers using the LC-MS/MS method

No	Name	Calc. MW	RT [min]	ChemSpider Results
1	Trigonelline	137.0476	1.066	15
2	Choline	103.1001	1.033	1
3	Palmitic Acid	273.2664	15.294	1
4	trimethadione	143.0582	2.462	5
5	Betaine	117.0791	1.057	14
6	Muramic acid	251.1005	1.048	3
7	2-Amino-1,3,4-octadecanetriol	317.2925	15.465	1
8	Erucamide	337.3338	26.723	1
9	L-alpha-Glycerylphosphorylcholine	257.1029	1.056	2
10	6-(alpha-D-glucosaminyl)-1D-myo-inositol	341.1317	1.002	4
11	Glu-Gly	204.0748	1.092	6
12	6-Quinolinecarboxylic acid	173.0476	1.623	5
13	1-[(3-Carboxypropyl)amino]-1-deoxy-beta-D-fructofuranose	265.1162	1.057	3
14	5-Hydroxymethyl-2-furaldehyde	126.0317	2.799	11
15	5-Methoxybenzimidazole	148.0637	1.089	2
16	8-Hydroxyquinoline	145.0527	5.346	8
17	sphinganine	301.2974	17.715	2
18	3-Acetyl-2,7-naphthyridine	172.0636	5.593	2
19	D-(+)-Pyroglutamic Acid	129.0426	1.116	11
20	L-Proline	115.0636	1.069	8
21	Stigmatellin Y	484.2813	23.72	1
22	D-(+)-Pipecolinic acid	129.0791	1.103	19
23	.alpha.-Aminoadipic acid	161.0688	1.006	16
24	Volkenin	287.1005	2.308	1
25	Desethylatrazine	187.0632	11.783	1
26	Dihydrothymine	128.0588	1.056	6
27	4-Aminophenol	109.0531	1.086	11
28	N-Acetylglucosaminitol	223.1057	1.019	1
29	benzoquinone	108.0214	2.79	2
30	1-O-Phosphonopentitol	232.0351	1.085	5
31	L-Phenylalanine	165.079	2.564	17
32	Ellagic acid	302.0058	8.597	1
33	(+/-)-Camphor	152.1202	9.115	108
34	Deferiprone	139.0633	1.107	5
35	Pyridoxal	167.0582	6.474	11
36	D-Glucosamine	179.0794	0.998	11
37	2-(2-thienyl)-4H-chromen-4-one	228.0245	2.083	1
38	MFCD00036904	495.3326	19.922	2
39	N-[4-(diethylamino)phenyl]-N'-phenylurea	321.121	1.913	1
40	4-Methoxyaniline	123.0687	1.086	11
41	L-Isoleucine	131.0946	1.559	18
42	ACETYL PROLINE	157.074	4.406	6
43	carglumic acid	190.0591	1.121	6
44	Deferiprone	139.0633	1.615	5
45	AMPA	186.0643	1.097	3
46	Orientin	448.1005	8.01	22
47	Adenosine	267.0965	1.469	12
48	MFCD00037215	218.0905	1.108	8
49	N-Acetylneuraminic acid	309.106	1.089	6

The coordinates obtained were considered suitable for nine test compounds from *C. indicum* var. B. After the docking, five compounds with the lowest binding energy were obtained, namely 6-Quinolinecarboxylic acid, Ellagic acid, 2-(2-thienyl)-4H-chromen-4-one, N-[4-(diethylamino)phenyl]-N'-phenylurea, and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), as shown in **Table 3**. Meanwhile, the interaction of compounds and their visualization targets are presented in **Figure 3.** 6-Quinolinecarboxylic acid forms hydrogen bonds with target proteins as hydrogen donors to LEU208:O and ASN217:OD1. Additionally, it forms hydrogen bonds with target proteins through hydrogen acceptors to ASN217:HD21 and GLY475:HA2. The compound also has residue similarities compared to native ligands,[22] LEU208, SER209, and PHE476, with binding energy of -5.52 kcal/mol. Ellagic acid has hydrogen acceptor with two amino acids, namely ALA103: H and PHE476: H, ILE99: HA. The compound is a hydrogen donor to LEU208: O, ASN217: OD1, and PHE100: O. It also has a more negative binding energy than the native ligand d, namely -8.93 kcal/mol, with residues similar to the native ligand PHE114, LEU208; SER209, PHE476, and ALA477. N-[4-(diethylamino)phenyl]-N'-phenylurea is a hydrogen donor to LEU208:O. It has more negative binding energy than the native ligand, which is -6.16 kcal/mol, with residue similarities to the native ligand, namely ILE205, LEU208, SER209, GLU300, THR301, PHE476, and ALA477.

Table 2. Physicochemical properties of selected compounds from *C. Indicum*

Name	MW	H acceptor	H donor	TPSA	MLogP	HIA	HO
Trigonelline	137.0476	2	0	44.01	0.33	0.7939	0.7429
6-Quinolinecarboxylic acid	173.0476	3	1	50.19	1.34	0.9969	0.7571
5-Methoxybenzimidazole	148.0637	2	1	37.91	0.7	1	0.7429
3-Acetyl-2,7-naphthyridine	172.0636	3	0	42.85	-0.01	0.9971	0.7
Ellagic acid	302.0058	8	4	141.34	0.14	0.8113	0.6714
Pyridoxal	167.0582	4	2	70.42	-1	0.9813	0.5714
2-(2-thienyl)-4H-chromen-4-one	228.0245	2	0	58.45	1.79	1	0.7286
N-[4-(diethylamino)phenyl]-N'-phenylurea	321.121	1	2	44.37	3.37	0.9672	0.5286
AMPA	186.0643	5	3	109.32	-3.07	0.7752	0.7143

AMPA has a binding energy close to the native ligand, which is -5.54. This compound has residue similarities to the native ligand, including PHE114, LEU208, and LEU366. Additionally, it forms hydrogen bonds with target proteins by donating hydrogen to GLY98:O and ASN217:OD1. AMPA is also a hydrogen acceptor to PHE:H, ILE99:HA, and PRO367:HD1. 2-(2-thienyl)-4H-chromen-4-one has similarities to the residues that bind to the native ligand, namely ALA297, and THR301. This compound does not form hydrogen bonds but has a smaller binding energy than the native ligand, showing the need for molecular dynamics to validate the interaction.

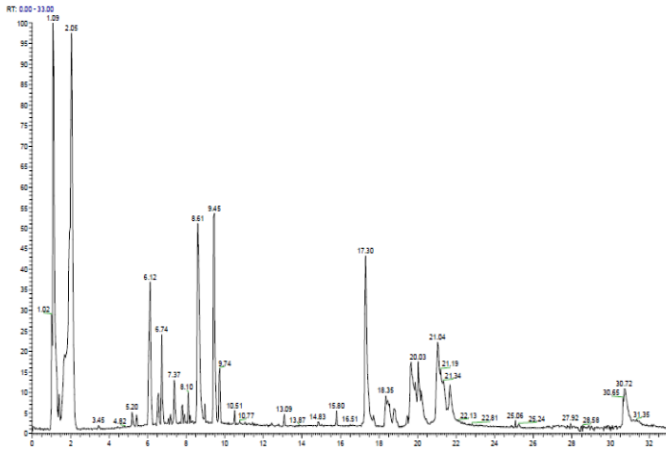

Figure 2. LC-MS/MS profile of flowers of *C. indicum* var. B

Molecular dynamic simulation

Molecular dynamics was conducted to determine the interaction between compounds and CYP2C9 proteins using the YASARA program, with results presented in **Figure 4**. The backbone [RMSDBb] of five compounds screened from *C. Indicum* var. B flowers were as follows. The mean value of 6-quanolinecarboxylic acid was 1.512 Å, with minimum and minimum values of 0.423 and 1.872, respectively. Ellagic acid has 1.716, with minimum and maximum value of 0.427 and 2.125, respectively. 2-(2-thienyl)-4H-chromen-4-one obtained a mean value of 1.536, with minimum and maximum value of 0.414 and 1.835. N-[4-(diethylamino)phenyl]-N'-phenylurea had a mean value of 1.572, with minimum and maximum value of 0.413 and 1.944, respectively. Meanwhile, AMPA obtained a mean value of 1.607, with minimum and maximum value of 0.412 and 2.181, respectively. All compounds when bound to CYP2C9 were still below 3%, showing that bonds formed during the 100 ns period were stable. Based on the molecular dynamics, the interaction of CYP2C9 with the native ligand led to a decrease from 357.328 to 357.236. However, four compounds showed an increase in hydrogen bond from the native ligand, including Ellagic acid, 2-(2-thienyl)-4H-chromen-4-one, N-[4-(diethylamino)phenyl]-N'-phenylurea, and AMPA by 357.799, 360.943, 360.957, and 358.061, respectively. There was a decrease in the hydrogen bond of 6-Quinolinecarboxylic acid by 355.225. Based on the SASA value, the native ligand increased its value by 21142.776 from CYP2C9, and the compounds contained in the *C. indicum* var. B flowers. Among the compounds, only N-[4-(diethylamino)phenyl]-N'-phenylurea had a higher value than the native ligand of 21379.559, while others were lower. Based on the radius of the gyration value, the native ligand formed a more compact structure than CYP2C9, which was 23.095. Compounds from *C. indicum* var. B that have higher compactness of 23.108 and 23118 were Ellagic acid and N-[4-(diethylamino)phenyl]-N'-phenylurea, respectively, while others showed a decrease.

Based on the RMSF value obtained, the fluctuation of amino acid residues above 3 Å was observed when interacting with the CYP2C9 ligand. Native ligands experience fluctuations in LYS27, GLN278, ASN474, and PHE476. 6-Quinolinecarboxylic acid showed fluctuations in LYS48, ARG108, and GLN278. Ellagic acid showed fluctuations in LYS27 and ARG108. 2-(2-thienyl)-4H-chromen-4-one showed fluctuations in LYS48, N-[4-(diethylamino)phenyl]N'-phenylurea was in LYS27, while AMPA did not experience fluctuations. The final analysis was carried out using binding energy in units of kj/mol.

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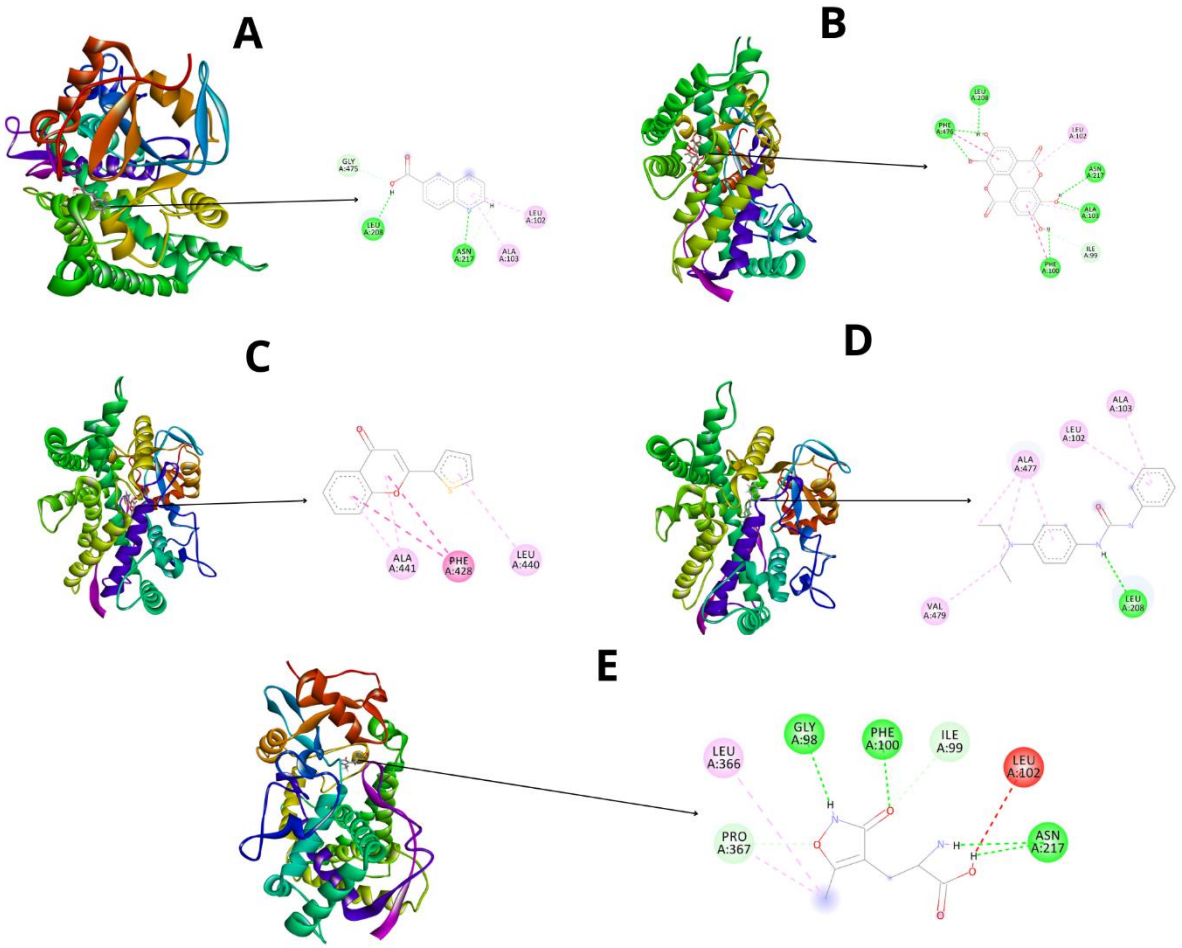

Figure 3. Interaction between protein and ligand.

The native ligand showed a binding of 178.058, while N-[4-(diethylamino)phenyl]-N'-phenylurea had higher value of 191.098. Other compounds including 6-Quinolinecarboxylic acid, ellagic acid, 2-(2-thienyl)-4H-chromen-4-one, and AMPA showed lower values of 65.072, 21.187, 134.596, and 13.691. However, all ligands that were successfully screened from *C. indicum* var. B flowers had positive values in binding energy, which showed good binding to CYP2C9.

Computer simulation methods such as molecular dynamics are essential for determining the physical motion of atoms and molecules. By applying the principles of classical mechanics, it simulates the interactions and trajectories of particles over time. In molecular dynamics simulation, the positions, velocities, and forces acting on each atom or molecule are calculated using Newton's equations of motion.[23] The interactions between particles are often described by potential energy functions or force fields, which estimate bonding, angles, Van der Waals interactions, and electrostatics[24]. This information is used to observe the conformation of the protein in its state or when binds to the drug. Molecular dynamic study of *C. indicum* var. B as hepatoprotector started by screening of compounds based on the pharmacophore of the native ligand, namely (2R)-N-{4-[(3-bromophenyl)sulfonyl]-2-chlorophenyl}-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide. [24] This was followed by the selection process through docking to identify five compounds with the lowest binding energies. The final step included using molecular dynamics to assess the stability of the protein conformation when bound, as well as the binding energy between the protein and the compound over a specific period. In the YASARA program, a stronger bond was achieved when the binding energy between the protein and the compound was positive. The results showed that the compound with the potential to be hepatoprotector was N-[4-(diethylamino)phenyl]-N'-phenylurea. This was supported by RMSD during the 100 ns simulation, which was below 3 Å, and the delta RMSD below 2 Å. Therefore, the compound did not change the conformation of the CYP2C9 protein, leading to a stable bond.[25] The stability of this interaction was also supported by a greater binding energy value compared to the native ligand. This showed that more positive binding energy correlated with stronger interaction between the compound and the protein.[26] CYP2C9 is an enzyme found abundantly in the human liver and is the most important among CYP2 subfamily for drug metabolism. It is a member of the CYP2C family highly expressed and metabolizes more than 15% of drugs, including Nonsteroidal anti-inflammatory drug (NSAID).[27] Genetic variations in CYP2C9 are common, resulting in changes in the rate of drug metabolism. [28] Study on CYP2C9 including vatalanib was identified to have inhibitory ability with an IC_{50} value of 0.067 µM. Cloperidone, ticagrelor, and piriqualone had inhibitors with IC_{50} <18 µM, while dasatinib, duvelisib, asapiprant, and sertindole showed moderate IC50 values between 40 - 85 µM. Docking using MOE software showed that all compounds had binding energy <-8.5 kcal/mol.[29] Recent hepatotoxic study of coconut fruit extract induced by doxorubicin showed improvement in liver disease. This was supported by docking using MOE, which showed value of < -7 kcal/mol for compounds L-(+)-Threose, trimethylsilyloxime, Palmitic acid, Stearic acid, 9-Octadecenoic acid, and Fumaric acid[30] with Silymarin as standard (-8.9 kcal/mol). S,S-Palladaheterocycle, and derivatives based on preclinical tests showed potential to protect liver against acute damage caused by CCl_4. This was achieved through activation of catalase, glucuronyltransferase, and inhibition of oxidative stress enzymes. The results were supported by docking against CYP2C9 using LeadIT software package with values ranging from -8.36 to -10.03 kcal/mol.[31] Other studies showed that garcinol was able to inhibit CYP2C9 with an IC50 of 80 µM. After docking using Schrodinger software with the

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Table 3. Docking of the 5 lowest binding energies

Name	Bind. energy [kcal/mol]	Dissoc. constant [pM]	Amino acid recidues
Native	-5.98	41020000	VAL113; PHE114; ILE205; LEU208; SER209; VAL292; ASP293; GLY296; ALA297; GLU300; THR301; LEU362; LEU366; PHE476; ALA477
6-Quinolinecarboxylic acid	-5.52	89790000	PHE100; LEU102; ALA103; LEU208; SER209; SER210; ILE213; GLN214; ASN217; GLY475; PHE476
Ellagic acid	-8.93	283410	ILE99; PHE100; PRO101; LEU102; ALA103; GLU104; PHE114; LEU208; SER209; SER210; ILE213; GLN214; ASN217; LEU366; PRO367; GLY475; PHE476; ALA477
2-(2-thienyl)-4H-chromen-4-one	-7.04	6960000	ILE178; LEU294; ALA297; GLY298; THR301; THR302; THR305; GLN356; ILE359; LEU361; PHE428; CYS435; GLY437; LEU440; ALA441; GLU444; LEU445
N-[4-(diethylamino)phenyl]-N'-phenylurea	-6.16	30680000	PHE100; PRO101; LEU102; ALA103; ILE205; LEU208; SER209; ASN217; GLU300; THR301; THR304; LEU361; ASN474; GLY475; PHE476; ALA477; SER478; VAL479
AMPA	-5.54	87220000	ARG97; GLY98; ILE99; PHE100; PRO101; LEU102; ALA103; GLU104; PHE114; LEU208; ILE213; ASN217; LEU366; PRO367

Sulphaphenazole compound, a binding energy of -8,326 kcal/mol was obtained.[32] In silico analysis on anti-inflammatory and apoptosis in colon cancer cases using AutoDock Vina of kaempferitrin on CYP2C9 led to a value of -7.4 kcal/mol.[33]

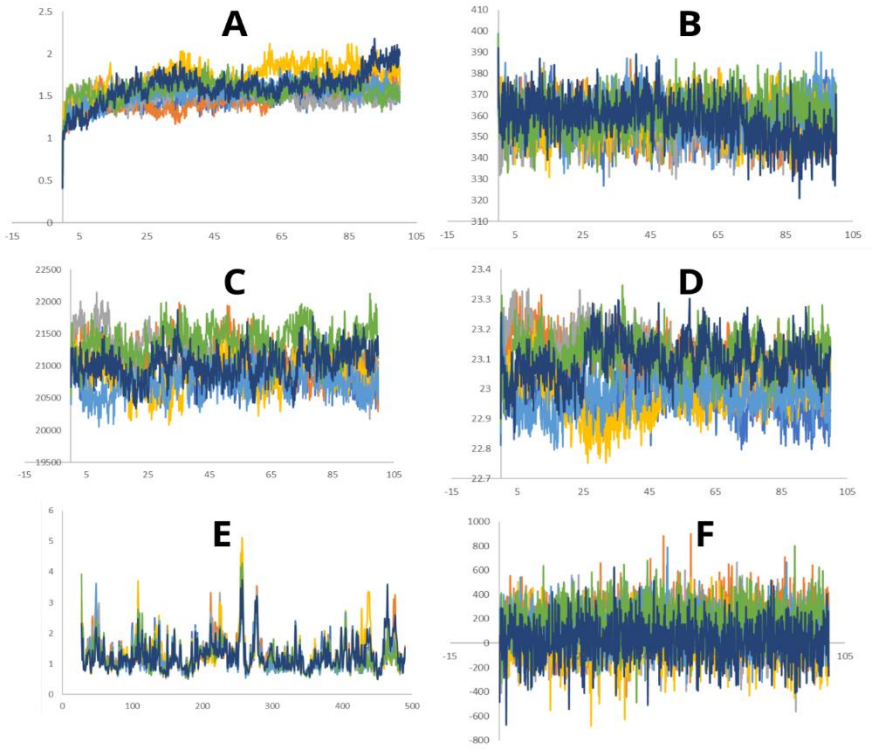

Figure 4. Molecular dynamic simulation analyses

Description: A.RMSD; B. Hydrogen Bond; C. SASA; D. Rg; E. RMSF; F. Binding energy. Blue: protein; Orange: protein-Native; Gray: protein-6-Quinolinecarboxylic acid; yellow: protein- Ellagic acid; Light blue: protein-2-(2-thienyl)-4H-chromen-4-one; Green: protein- N-[4-(diethylamino)phenyl]-N'-phenylurea; Dark blue: protein- AMPA.

Hepatotoxic inducers such as CCl_4, acetaminophen, and ethanol increased the formation of free radicals which could cause lipoperoxidation and damage cell membranes.[34] Natural ingredients that have been recognized as hepatoprotector is Silymarin, consisting of four flavonolignans, namely silychristin, silydianin, isosilybin, and silybin.[35] The mechanism as a hepatoprotector includes increasing glutathione in the liver, which functions as an antioxidant and enhances protein synthesis in hepatocytes by stimulating RNA polymerase II activity.[36] The use of silymarin can improve the survival of patients with cirrhosis.[37]

Conclusion

Conclusion, this study successfully extracted five compounds from *C. indicum* var. B flowers with potential hepatoprotector. These compounds included 6-Quinolinecarboxylic acid, ellagic acid, 2-(2-thienyl)-4H-chromen-4-one, N-[4-(diethylamino)phenyl]-N'-phenylurea, and AMPA. The results of molecular dynamics showed that the most potential compound as hepatoprotector was N-[4-(diethylamino)phenyl]-N'-phenylurea. This study predominantly served as a model, showing the need for validation through in vitro testing.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this study is original and borne responsibility for claims relating to the content.

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