Renoprotective effects of Cermai Leaves (*Phyllanthus acidus* (L.) Skeels) as a candidate for antidiabetic in silico

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Abstract. Renal complications are a major concern in individuals with diabetes, necessitating the search for effective antidiabetic agents with renoprotective properties. *Phyllanthus acidus* (L.) Skeels, commonly known as Cermai leaves, have been recognized for their medicinal properties, including potential antidiabetic effects. This in silico study aimed to investigate the renoprotective effects of Phyllanthus acidus as an antidiabetic agent by targeting the insulin-like growth factor-1 (IGF-1) (PDB code 1GZR) and angiotensin-converting enzyme 2 (ACE2) (PDB code 6M0J) receptors. The prediction of phytochemical properties was made based on the five laws of Lipinski as seen from the values of absorption, distribution, and metabolism with Swiss ADME. The in-silico method was conducted with the assistance of computer devices, databases like Protein Data Bank (PDB) and PubChem, and software like PyRx and Discovery Studio. The results showed the compounds kaempferol and cafestol meet the five laws of Lipinski. The compound kaempferol has the lowest binding affinity, with a value of -5.8 kcal/mol on IGF-1 and -6.1 kcal/mol on ACE2 and cafestol, -5.6 kcal/mol on IGF-1 and -5.8 kcal/mol on ACE2. It's compared to the native ligand value -3.9 kcal/mol on IGF-1 and -5.2 kcal/mol on ACE2. Based on the results, it is known that this compound has a high potential to be a drug candidate for renoprotective.

1 Introduction

Type 2 diabetes mellitus (DMT2) is a metabolic condition characterized by elevated blood glucose levels and a reduced responsiveness to insulin. DMT2 is one of the most significant public health issues since it could affect millions of people worldwide. According to predictions, the number of persons in Indonesia with diabetes mellitus (DM) will double to quadruple by 2030 compared to 2000 [1][2]. About 85% to 90% of all instances of diabetes mellitus are type 2 diabetes. Better lifestyle changes, treatment measures, and other alternative therapies, such as using and formulating medicinal plants are all part of type 2

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diabetes therapy. The use of medicinal plants to manage various health conditions has been a practice that has lasted for centuries, largely associated with the existence of biologically active compounds with significant therapeutic properties [3].

Type 2 diabetes mellitus (DMT2) patients frequently have kidney issues, with diabetic nephropathy emerging as a key factor in the emergence of late-stage kidney disease. Renoprotective actions of sodium-glucose inhibitor cotransporter 2 (SGLT2) have been found in studies on people with DMT2 [4]. However, due to their possible renoprotective characteristics, using medicinal plants as therapeutic agents for diabetes mellitus needs further research and expansion. In previous studies, it was discovered that plant extracts from medicinal plants were evaluated for their potential to have renoprotective qualities in diabetic nephropathy animal models, such as ginger extract, which functions as a suppressant of cell apoptosis potential as a renoprotective [5].

Phyllanthus acidus (L.) Skeels have historically been used in traditional medicine for various medical ailments, including type 2 diabetes mellitus. The effectiveness of medicinal herbs in controlling glycemic levels in people with type 2 diabetes mellitus (DMT2) has been extensively studied. Strong research that lasts at least three months and involves a comprehensive follow-up method is required to determine the impact of possibly beneficial plant preparations on diabetes mellitus [6]. More efficient ways of examination must be conducted because they take less time. The molecular docking approach is one of the many analytical approaches. The use of molecular docking as an in-silico drug development technique has grown in favor. By predicting the interaction between ligands and targets at the molecular level, molecular docking aids in discovering possible benefits of substances. This method also enables the investigation of the produced structural-activity relationships (SARs) [7]. One of the most significant advantages of molecular docking is the capacity to optimize and save time and money by minimizing the number of molecules that must be synthesized for in vitro or in vivo testing [8]. This research aims to discover the possible renoprotective benefits of cermai plants as anti-diabetic medicines. The development of new therapeutic strategies to treat type 2 diabetes mellitus may, therefore benefit greatly from this investigation.

2 Method

This descriptive study employs an in-silico technique. The Protein Data Bank website (PDB; https://www.rcsb.org) was used to download the three-dimensional (3D) structure of the insulin-like Growth Factor-1 (IGF-1; PDB code: 1GZR) and Angiotensin-Converting Enzyme 2 (ACE2; PDB code: 6M0J) proteins. The natural ligands employed in this work were isolated from experimental data on the PDB database website [9]. Furthermore, the bioactive chemicals found in cermai leaves serve as test ligands in molecular docking experiments. The bioactive components in the leaf were discovered using Liquid Chromatography-Mass Spectrometry (LC-MS). The researchers used the PubChem database website (https://pubchem.ncbi.nlm.nih.gov/) to determine the chemical structure of a known compound of cermai leaves. [10]. The structure of the chemical was then chosen using the Swiss ADME website (http://www.swissadme.ch/) in accordance with Lipinski's Rule of Five (RO5). PyRx software was used to execute the molecular docking test to determine the bonding affinity value between the target protein and the test ligand [11]. The Biovia Discovery Studio program was used to identify complicated protein-ligand interactions that arose from the docking test [12].

3 Results and Discussion

The implementation of renoprotective strategies holds substantial promise in mitigating the burgeoning concerns surrounding kidney dysfunction within the realm of type 2 diabetes. In this context, exploring natural compounds as potential therapeutic interventions becomes pivotal. Cermai emerges as a compelling candidate for medicinal composition, offering a reservoir of bioactive compounds that may play a pivotal role in formulating renoprotective agents tailored to counteract the renal problems associated with type 2 diabetes. Cermai bioactive chemicals have a lengthy track record in medicinal uses and as a potential substitute composition for the use of synthetic medications, particularly those with negative side effects. Because of the diversity of chemical components and bioactivity in plants, the mechanisms of action of plant chemicals are still being investigated to this day. The therapeutic bioactivity of plant chemicals would make it a very interesting choice for illness therapy [3]. Therefore, it becomes crucial to isolate and characterize the bioactive cermai chemicals and evaluate their biological activity. The Liquid Chromatography-Mass Spectrometry (LC-MS) examination of cermai leaves yielded 37 distinct bioactive chemicals (see Table 1). These compounds were further selected based on Lipinski's Rule of Five (RO5).

Table 1. Liquid Chromatography-Mass Spectrometry (LC-MS) results of cermai leaves

No	Compounds Name	Acceptance of Lipinski's Rules of Five
1	Adenosine	
2	L-Phenylalanine	Yes
3	Trigonelline	Yes
4	L-Histidine	Yes
5	Adenine	Yes
6	DL-Tryptophan	Yes
7	Kaempferol	Yes
8	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	Yes
9	L-Tyrosine	Yes
10	Dibenzylamine	Yes
11	3,5-di-tert-Butyl-4-hydroxybenzoic acid	Yes
12	L(-)-Pipecolinic acid	Yes
13	Choline	Yes
14	Tributyl phosphate	Yes
15	γ-Aminobutyric acid (GABA)	Yes
16	Diisobutylphthalate	Yes
17	9(Z),11(E),13(E)-Octadecatrienoic Acid methyl ester	No
18	D-Glucosamine	Yes
19	Glycitein	Yes
20	Sorbic acid	Yes
21	D-(+)-Proline	Yes
22	Oxolinic acid	Yes
23	Palmitelaidic acid methyl ester	No

No	Compounds Name	Acceptance of Lipinski's Rules of Five
24	(+)-Aphidicolin	Yes
25	N,6-diphenylthieno[2,3-d]pyrimidin-4-amine	Yes
26	6-Methoxyquinoline	Yes
27	4-Methoxycinnamic acid	Yes
28	4-Hydroxybenzaldehyde	Yes
29	α-Eleostearic acid	No
30	2,2,6,6-Tetramethyl-1-piperidinol (TEMPO)	Yes
31	NP-004713	Yes
32	Cafestol	Yes
33	6-Aminocaproic acid	Yes
34	DL-Arginine	Yes
35	12-Oxo phytodienoic acid	Yes
36	MDPBP	Yes
37	α-Linolenic acid	No

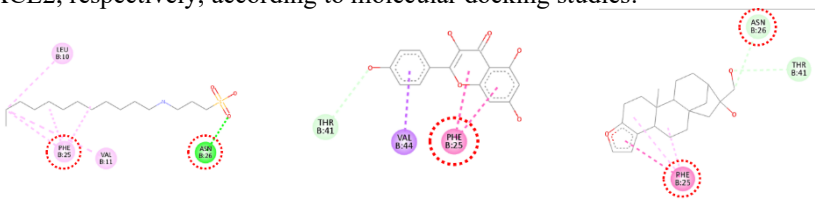
Lipinski's Rule of Five (RO5) is a strategy for predicting the physiochemical properties of pharmaceuticals (drug-likeness) because not all chemicals can be employed as drugs. Several parameters are included in the Lipinski criteria, including a molecular weight of no more than 500 Daltons (Da), a partition coefficient (logP) of no more than 5, a maximum hydrogen binding donor of 5, and a maximum hydrogen bond acceptor of 10 [13]. The Lipinski rule determines whether a ligand is hydrophobic or hydrophilic based on its physicochemical features. These features will determine the compounds or ligand's capacity to diffuse passively through the cell membrane. The log P value, which ranges from -0.4 to 5, represents the solubility coefficient in fat and water. The reason for this scale range is that molecules with molecular weights greater than 500 Da cannot permeate through cell membranes. Molecular hydrophobicity increases as the Log P value increases. Hydrophobic molecules are clearly related to higher toxicity due to prolonged persistence within the lipid double layer (bilayer lipid) and a significantly larger systemic distribution, lowering the selectivity of binding to the target [14].

On the other hand, too-low log P values are also undesirable since they prevent molecules from passing through bilayer lipid membranes. According to the correlation between the number of hydrogen bond donors and acceptors, a higher hydrogen binding capacity correlates with a higher energy need for the absorption process. Lipinski's rule [15] provides a broad picture of various chemicals' ability to passively diffuse across cell membranes based on their solubility. Based on these factors, 33 different types of bioactive chemicals were chosen to fit the Lipinski Five Rule. The molecule from the LC-MS leaf analysis that also satisfied the Lipinski Five Rules was then employed as a test ligand in a molecular docking test involving target insulin-like Growth Factor-1 (IGF-1) and Angiotensin-Converting Enzyme 2 (ACE2). Furthermore, captopril, a medication commonly utilized in clinical use, particularly for kidney function, is used as a comparator agent in molecular docking experiments alongside test ligands in the analysis process. Captopril has been found in studies to enhance kidney blood flow and boost salt excretion rates in patients with congestive heart failure. Renal function protection, or renoprotection, is an important feature of one of the therapeutic targets for type 2 diabetes. Captopril is recommended as a treatment choice for diabetic nephropathy [16].

Table 2. Test results docking between target proteins IGF-1 and ACE2 with test ligands.

Protein Target	Ligands	Binding Affinity (Kcal/mol)
Insulin-like Growth Factor-1 (IGF-1)	*N-Dodecyl-N,N-Dimethyl-3-Ammonio-1-Propanesulfonate* (C15; Native ligand)	-3.9
	Captopril	-3.8
	Kaempferol	-5.8
	Cafestol	-5.6
Angiotensin-Converting Enzyme 2 (ACE2)	*2-acetamido-2-deoxy-beta-D-glucopyranose* (NAG; Native ligand)	-5.2
	Captopril	-4.4
	Kaempferol	-6.1
	Cafestol	-5.8

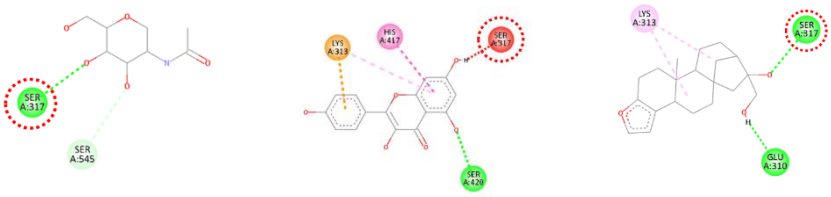
The molecular docking studies yielded two of the best bioactive compounds with lower binding affinity values than natural ligands and comparator pharmaceuticals. Kaempferol and cafestol are two chemicals. According to Table 2, kaempferol exhibits binding affinity for target proteins IGF-1 and ACE2 of -5.8 and -6.1 Kcal/mol, respectively. Cafestol, on the other hand, displays binding affinities of -5.6 and -6.1 Kcal/mol with target proteins IGF-1 and ACE2, respectively, according to molecular docking studies.



(a) (b) (c)

Fig 1. The visualization results of the test docking between target proteins IGF-1 with (a) native ligands, test ligands (b) kaempferol, and (c) cafestol. The red circle of the split line shows the similarity of the binding positions formed between the test ligand and the native ligand. Circle color description: purple-pink indicates a type of hydrophobic interaction; green–cyan indicates a type of Hydrogen interaction.

The visualization of the molecular test docking between the kaempferol and cafestol test ligands and the IGF-1 target protein revealed many amino acid residue implantation locations created similarly to the amino acid native ligand. According to Fig 1, caempferol binds to IGF-1 at amino acid residues PHE 25, THR 41, and VAL 44, whereas cafestol binds to IGF-1 at amino acid residues PHA 25, ASN 26, and THR 41. The natural ligand N-Dodecyl-N, N-Dimethyl-3-Ammonio-1-Propanesulfonate, which affects the physiological functions of IGF-1, was found primarily at amino acid residue PHE 25 [18]. The principal site of this native ligand implantation is the site of the implantation interaction by kaempferol or cafestol, implying that both drugs will play a similar role as the natural ligand against the IGF-1 protein.



(a) (b) (c)

Fig 2. Visualization of the docking test between target protein ACE2 with (a) native ligand, test ligand (b) kaempferol, and (c) cafestol. The red circle of the split line shows the similarity of the binding positions formed between the test ligand and the native ligand. Color description of the circle: purple-pink indicates a type of hydrophobic interaction; green-cyan indicates the type of interaction of hydrogen; red indicates an unfavorable interaction.

Both kaempferol and cafestol indicated the presence of amino acid residues that formed identically to the preservation position of the native amino acid ligand in visualizations with ACE2 proteins. 2-acetamido-2-deoxy-beta-D-glucopyranose, a natural ACE2 ligand, plays a critical role in maintaining amino acid residues while exerting physiological functional influence on ACE2. Figure 2 depicts kaempferol binding to ACE2 at the amino acid residue positions LYS 313, SER 317, HIS 417, and SER 420, whereas cafestol injection occurs at the amino acid residue positions GLU 310, LYS 313, and SER 317. The similarity of kaempferol and cafestol in the primary position of the SER 317 amino acid residues suggests that both drugs are expected to affect ACE2's physiological functions positively. One of the flavonoid components, kaempferol, is found in various plant species, including fruits, vegetables, and medicinal plants. Because of this property, kaempferol is a prominent research focus in pharmacology. Previous research explained kaempferol's pharmacological activities, which included anti-inflammatory, antioxidant, and anti-diabetic qualities [20]. The findings of this study lay the groundwork for future research into the potential use of kaempferol in medical treatment. Further research findings support the importance of kaempferol as an anti-diabetic medicine, as evidenced by comparisons with a control group utilizing the insulin secretagogue glibenclamide [21]. According to the findings, kaempferol has a considerable influence on boosting plasma insulin levels and decreasing blood glucose levels. Because of its ability to modify glucose metabolism in the human body, kaempferol appears to be a viable medication for the treatment of diabetes. The pharmacological investigation considered important aspects of kaempferol's renal protective properties. Another study [5] reported data relating to kaempferol's positive effects on kidney-associated disorders. Oxidative stress, inflammation, and fibrosis are all known to contribute to decreased kidney function, and kaempferol has been shown to mitigate these damaging effects. The findings provide a broader view of the possible usefulness of kaempferol in kidney health preservation.

Cafestol, a coated molecule discovered in coffee seeds, has been the subject of pharmacological investigation, particularly in insulin modulation. Cafestol's pharmacological properties have been demonstrated by increasing blood lipid levels, anti-inflammatory effects, and maybe anti-cancer and anti-diabetic properties. According to Ren's research, cafestol has the ability to promote insulin synthesis in mouse insulinoma cells and boost glucose absorption in muscle cells, a mode of action comparable to rosiglitazone injection. The study suggests that cafestol could be used to modulate glucose metabolism and insulin production in the human body [22]. According to Aminah's research, providing

cermai leaf extract had a favorable effect on reducing blood glucose levels in type II diabetic patients [23]. The affinity binding values and visual visualization of the docking test show that the presence of kaempferol and cafestol in cermai leaves is promising in affecting the physiological functions of IGF-1 and ACE2 proteins, particularly in their capacity as renoprotective agents. The lower bonding affinity values of kaempferol and cafestol, when compared to native ligands and comparator medications, and the similarity of the bonding location of identical amino acid residues to the native ligands of each target protein, provide proof in this molecular research.

4 Conclusion

Kaempferol and cafestol are potentially helpful chemicals that can be employed as renoprotective agents, particularly in the context of type 2 diabetes mellitus. Based on the results of a molecular docking test, which revealed low binding affinities compared to native ligands and comparable drugs, as well as the locations of amino acid residues similar to native ligand positions on each target protein (IGF-1 and ACE2) for both kaempferol and cafestol. To assess the viability of employing these molecules as a potential renoprotective therapeutic alternative, additional steps still need to be taken, such as a thorough investigation of bioavailability, levels of toxicity, and pre-clinical and clinical trials.

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