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Pharmacokinetic Virtual Screening and Molecular Docking Simulation for Evaluating Cytochrome P450 CYP3A4 Interactions Between Glibenclamide and Eurycoma longifolia Jack Extract

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ABSTRACT

Type 2 diabetes mellitus is a chronic disease with a globally increasing prevalence. The treatment regimen for this condition often includes oral blood glucose lowering agents such as glibenclamide. While glibenclamide is effective, its use can lead to severe hypoglycemia, prompting individuals to combine it with herbal remedies like Eurycoma longifolia root extract (ELRE), commonly known as Tongkat Ali in Malaysia and Indonesia. ELRE has been reported to exert anti-diabetic effects through mechanisms such as enhanced insulin sensitivity and antioxidant activity. However, the pharmacokinetic interaction between ELRE and glibenclamide, particularly in relation to CYP3A4 which is responsible for the metabolism of glibenclamide, has not been extensively studied. In this study, an in silico approach utilizing a pharmacokinetic virtual screening server followed by molecular docking analysis was employed to evaluate the potential interaction between these compounds. The results revealed that most of the chemical constituents of ELRE exhibited high predicted affinity for CYP3A4, with two compounds, 14,15β dihydroxyklaineanone and niloticin, showing a higher affinity for CYP3A4 than glibenclamide. These compounds are predicted to act as competitive substrates, suggesting a potential modulation of glibenclamide metabolism through CYP3A4 inhibition or competition.
Keywords: *Eurycoma longifolia*; Glibenclamide; CYP3A4; *In Silico*

INTRODUCTION

Type 2 diabetes mellitus is one of the chronic diseases with an increasing global prevalence (Khan *et al*., 2020). The treatment of type 2 diabetes involves a multifactorial approach, including the use of oral hypoglycemic agents. Glibenclamide, a frequently used oral sulfonylurea antidiabetic derivative, is a therapeutic option for enhancing insulin secretion from pancreatic beta cells and is considered safe for pregnant and breastfeeding women (Costello *et al*., 2023). However, long-term use of glibenclamide is often associated with the risk of side effects such as severe hypoglycemia, prompting its combination with traditional medicines like the root extract of Eurycoma longifolia, commonly known as "Tongkat Ali" (Wizneh and Asmawi, 2014).

Eurycoma longifolia Jack. (Tongkat Ali) is a herbal plant that has long been used in traditional medicine in Southeast Asia. Its extract has been reported to possess various pharmacological activities, including anti-diabetic effects through mechanisms such as increased insulin sensitivity and antioxidant activity (Rehman *et al*., 2016). The potential combination of *Eurycoma longifolia Jack.* extract with glibenclamide as a diabetes therapy is worth further exploration, particularly in the context of possible pharmacokinetic interactions between the two.

Eurycoma longifolia Root Extract (ELRE) contains a diverse range of bioactive compounds that can be categorized based on their chemical structures, including quassinoids, alkaloids, saponins, and other secondary metabolites. Quassinoids represent the major bioactive constituents of ELRE, comprising eurycomanone (pasakbumin A), pasakbumin B, pasakbumin C, eurycomalactone,

eurycomalide A, and their derivatives such as 13,21-dihydroeurycomanone, 13,21-dehydroeurycomanone, and 6α-hydroxyeurycomalactone. These compounds are known for their potent biological activities, including antiparasitic and cytotoxic properties. Alkaloids, another important class of compounds in ELRE, include various canthin-6-one derivatives such as canthin-6-one, 9-methoxycanthin-6-one, 9-methoxycanthin-6-one-N-oxide, 9-hydroxycanthin-6-one, and canthin-6-one 9-O-β-glucopyranoside, which have been reported for their antimicrobial and anticancer potential. Additionally, β-carboline alkaloids, including 7-methoxy-β-carboline-1-propionic acid and β-carboline-1-propionic acid, contribute to the extract's pharmacological effects, particularly in modulating neurotransmission and exhibiting neuroprotective properties. Saponins, such as laurycolactones A and B (C18), have been identified as key bioactive constituents with potential immunomodulatory and adaptogenic effects. Other secondary metabolites present in ELRE include eurylactone A, eurylactone E, eurycolactone D, niloticin, 5-iso-eurycomadilactone, 14,15β-dihydroxyklaineanone, and 2,3-dehydro-4a-hydroxylongilactone, which contribute to the extract's broad pharmacological spectrum. The structural diversity of these compounds underlies the multifaceted biological activities of ELRE, highlighting its significance in traditional medicine and modern pharmacological research (Abubakar *et al*., 2017).

One of the key factors influencing the pharmacokinetics of a drug is its interaction with metabolic enzymes, particularly from the cytochrome P450 (CYP) family. CYP3A4 is the enzyme that plays a significant role in the metabolism of many drugs, including glibenclamide (Marbun *et al*., 2023). The interaction between glibenclamide and the active compounds from *Eurycoma longifolia Jack.* root extract (ELRE) can affect the metabolism of this drug, making it essential to study the interactions between them.

Research on drug interactions with herbal ingredients has rapidly advanced in recent decades. *In silico* approaches, such as the use of ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) and molecular docking, have become efficient methods for screening potential interactions between active herbal compounds and drug metabolic enzymes (Asha *et al*., 2021). ADMETLab is one platform that provides preliminary predictions about the pharmacokinetic profile of a compound, including toxicity assessment and interactions with CYP enzymes (Fu *et al.*, 2024). Meanwhile, molecular docking is used to model molecular interactions more specifically, such as between active compounds from *Eurycoma longifolia Jack.* and the CYP3A4 enzyme to predict potential inhibition or activation of the enzyme (Agu *et al*., 2023; Torres *et al*., 2019). Although research on the potential interactions of herbal ingredients with antidiabetic medications has progressed, particularly using *in silico* approaches, studies specifically examining the pharmacokinetic interactions between glibenclamide and *Eurycoma longifolia Jack.* extract is still limited. This study aims to provide new contributions to the field of pharmacokinetic interactions between drugs and herbal ingredients, especially in the context of diabetes treatment. Through the *in-silico* approaches based on ADMETLab and molecular docking, this research provides initial data on the potential interactions between glibenclamide and *Eurycoma longifolia* extract, which have not been extensively studied. The findings of this study are expected to offer important insights for developing safer and more effective combination therapies and to expand knowledge about the pharmacokinetic interactions between modern medicines and herbal ingredients.

METHODS

Equipment and Software

The hardware used for this study was a Windows 10 Pro computer with specifications of Intel® Core™ i7-3770 CPU @ 3.40 GHz, 8.00 GB RAM, and a 64-bit operating system based on x64 architecture. The software utilized included MarvinSketch 2020, the online pharmacokinetic screening platform ADMETLab 3.0 (https://admetlab3.scbdd.com/) (Fu *et al.,* 2024), and the Molecular Docking and visualization application MOE (Molecular Operating Environment) Version 2015 under the license of the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gadjah Mada University. The two-dimensional structure of glibenclamide and 28 active constituent compounds from *Eurycoma longifolia Jack.* root was drawn using MarvinSketch and converted into three-dimensional forms using the builder feature of the MOE application. The CYP3A4 protein

Table I. Active Constituents of *Eurycoma longifolia* Root Extract (ELRE) (Abubakar *et al*., 2017)

Pasakbumin C	7-methoxy-beta-carboline- 1-propionic acid
Eurycomanone (Pasakbumin A)	Eurylactone A
14,15β-dihydroxyklaineanone	Pasakbumin B
3b,18-dihydroeurycomanol	Canthin-6-one 9-O- beta-glucopyranoside
9-methoxycanthin-6-one	6alpha-Hydroxyeurycomalactone
9-methoxycanthin-6-one-N-oxide	Eurylactone E
Laurycolactones A and B (C18)	Eurycomalide A
Laurycolactone B	14,15β-dihydroxyklaineanone
5-iso-eurycomadilactone	13,21-dihydroeurycomanone
Eurycomalactone	13,21-dehydroeurycomanone
Eurycolactone D	Niloticin
7-methoxy-beta-carboline-1-propionic acid	beta-carboline-1-propionic acid
9-methoxycanthin-6-one	9-hydroxycanthin-6-one
Canthin-6-one	2,3-dehydro-4a-hydroxylongilactone

structure was obtained in PDB (Protein Data Bank) format from the RCSB website (http://www.rcsb.org) with PDB ID 3UA1.

Research Procedure

ADME-CYP3A4 Interaction Screening All test compounds, including glibenclamide and the 28 active constituents from Eurycoma longifolia Jack., were input in SMILES code format into the ADMETLab 3.0 screening server. The interaction data obtained was represented by a "+" symbol in the phase I CYP3A4 metabolism parameter, indicating a probability of interaction greater than 0.5.

Protein Structure Download

The CYP3A4 protein structure was downloaded from the Protein Data Bank (PDB) via the website http://www.rcsb.org/ using the PDB ID 3UA1.

2D and 3D Ligand Structure Building

Glibenclamide and the 28 chemical constituent compounds from Eurycoma longifolia Jack. were derived from the study by Abubakar et al. (2017). All test ligands were converted from 2D structure to SMILES code using MarvinSketch 2020, then constructed in 3D using the builder feature of MOE version 2015 (MOE,2024).

Protein Preparation

The downloaded protein was prepared using the MOE 2015 application. Preparation was conducted in three stages: the first stage involved sequence editing to remove mutated subunits, water molecules, and residual solvents; the second stage added hydrogen using the Protonate 3D feature, setting conditions to normal physiological conditions: temperature of 310 K (37°C), pH 7.4, and a salt concentration of 0.1; the third stage involved energy minimization using the energy minimize feature.

Ligand Preparation

The 3D structure of the ligands built with MOE was stored in a single ligand database in MDB format. Preparation was conducted in two stages: residual ion cleaning using the wash feature and semi-empirical charge assignment using the PM3 partial charge feature.

Molecular Docking Validation

The docking method validation was performed in three stages: determining the binding site by creating a pocket using the surface and maps feature, validating redocking by assessing the RMSD value, which should not exceed 2 Å, and scoring function conversion of docking scores into predictive pIC50 using the docking scoring function equation developed and validated in the study by Marbun *et al.* (2023).

Visualization

The docking results were visualized in both 2D and 3D formats using the MOE software. The visualization depicted the interactions between the ligands and binding site residues, serving as qualitative markers for protein-ligand interactions.

RESULT AND DISCUSSION

Pharmacokinetics (ADME) Virtual Screening

The pharmacokinetic screening results obtained from the ADMETLab platform indicated that glibenclamide and four active compounds from *Eurycoma longifolia Jack.* root extract (ELRE) have the potential to interact with CYP3A4 as both substrates and competitive inhibitors.

Based on virtual screening with the ADMETLab server, it was found that 3 out of 28 compounds from *Eurycoma longifolia Jack.* root extract (ELRE) are predicted to be metabolized by CYP3A4, with probabilities above 0,5. These include Niloticin with a probability of 0,9 and two other compounds: 9-methoxycanthin-6-one and Canthin-6-one 9-O-beta-glucopyranoside with a probability value of 0,7. Based on these findings, there is a predicted potential interaction between ELRE and glibenclamide due to the similarity in metabolic targets, P450 isoform CYP3A4.

The pharmacokinetic screening results indicated that the three ELRE constituent compounds with high affinity for CYP3A4 comply with Lipinski's Rule of 5. According to Lipinski's Rule of 5, a compound is more likely to exhibit poor absorption when it exceeds the following criteria: (i) molecular weight (MW) greater than 500 g/mol (5 x 100), (ii) more than 5 hydrogen bond donors (nHD), (iii) more than 10 hydrogen bond acceptors (nHA) (2 x 5), (iv) molar refractivity less than 40 and greater than 130, and (v) a calculated degree of polarity (cLogP) greater than 5 (Lipinski, 2004). Although these criteria slightly differ from ADMETLab physicochemical rules (MW 100-600; nHD ≤ 7; nHA ≤ 12; logP ≤ 3), the three ELRE constituent compounds still meet the physicochemical requirements, with deviations of no more than 1, thus they are predicted to be absorbable and able to enter phase I metabolism in hepatic circulation.

Glibenclamide is extensively metabolized in the liver via the cytochrome P450 system. The two main metabolites of glibenclamide, 4-trans-hydroxyglibenclamide (M1) and 3-cis-hydroxyglibenclamide (M2b), are also pharmacologically active as the parent drug. Several studies have shown that CYP3A4 is the major CYP enzyme involved in the in vitro metabolism of glibenclamide (Naritomi *et al.*, 2004; Zharikova *et al.*, 2009; Zhou *et al.*, 2010). However, little information is known about similar interactions with ELRE, as noted in the study by Purwantiningsih *et al.* (2013), which found weak inhibitory interactions between standardized ELRE extract and CYP3A4. Therefore, preliminary molecular docking studies are needed following ADME screening to predict the interaction between the two based on affinity strength in the form of docking scores correlated with predictive pIC50 values.

Docking Method Validation

The validation of the docking method was carried out through protein preparation in PDB format, followed by RMSD calculations. The PDB file used was downloaded from the RCSB website with ID code 3UA1. As a result, a 3D PDB structure was obtained with the native ligand bromoergocryptine (Sevrioukova and Poulos, 2012).

The RMSD calculation results showed a value of 0,9811. This RMSD value meets the criteria of RMSD ≤ 2Å, confirming the validity of the docking protocol (Mena-Ulecia *et al*., 2015) According to Sevrioukova and Poulos (2012) in Marbun *et al*. (2023), key interactions that indicate the presence of interaction activity between the native ligand and the CYP3A4 receptor in PDB ID 3UA1 involve binding with two essential amino acid residues: Arg 212 and Thr 224. Thus, in this study, the

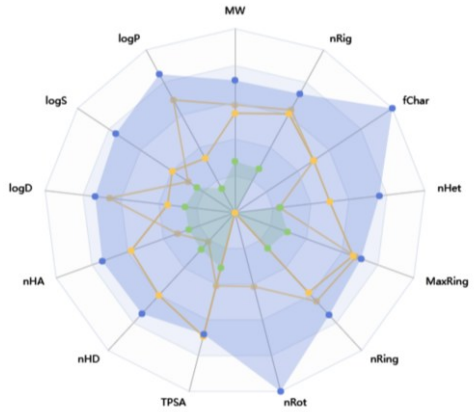

Figure 2. Physicochemical Radar Diagram of 14,15β-Dihydroxyklaineanone and Niloticin Based on ADMETLab 2.0. The two compounds (yellow line) generally exhibit physicochemical parameter values within the upper (blue line) and lower (green line) threshold limits, suggesting their potential for absorption, distribution, and metabolism.

Table II. Screening Results of Interaction Probability Between Glibenclamide and Active Constituents of *Eurycoma longifolia* Root with CYP3A4 Receptor via ADMETLab 3.0 Pharmacokinetic Screening Server

Testing Ligands	Probability of Interaction with CYP3A4 Based on ADMETLab
Glibenclamide	1
9-methoxycanthin-6-one	0,7
Canthin-6-one 9-O- beta-glucopyranoside	0,7
Niloticin	0,9

interaction activity is evaluated not only based on the docking score output but also on the interaction with one or both of these residues. The determination of the binding site for the test compound was conducted by marking the pocket formed in the area of the native ligand's binding.

Molecular Docking Result

After the docking test protocol was validated, docking experiments were conducted on glibenclamide and 28 ELRE active chemical constituents. Docking was performed using the London dG scoring algorithm, Pocket Atoms site, Triangle Matcher placement, and Induced Fit refinement. The scoring function for converting the docking score to pIC50 was obtained from the study by Marbun *et al*. (2023) using the following equation:

Predictive pIC50 = (-0.869446 x LondonDG Docking Score)-0.744353

Through the results of molecular docking tests, it was found that almost all active compounds in *Eurycoma longifolia* extract (ELRE) exhibit high predicted affinity towards CYP3A4, similar to glibenclamide (IC50 < 1 nM). However, two compounds, 14,15β-dihydroxyklaineanone and niloticin, showed docking scores correlated with IC50 values that were higher than that of glibenclamide, indicating a greater affinity. Therefore, these two compounds are predicted to be responsible for the interactions with glibenclamide in the CYP3A4 system, acting as competitive substrates.

Both compounds exhibited affinity-inhibition activity, where 14,15β-dihydroxyklaineanone showed a docking score of -12.382 kJ/mol with a predicted pIC50 of 10.02 (IC50 0.095 pM), and niloticin had a docking score of -12.873 kJ/mol with a predicted pIC50 of 10.45 (IC50 0.036 pM).

Table III. Physicochemical Properties Screening Results of ELRE Compounds Predicted to Interact with CYP3A4 Using Lipinski-Veber Rules Based on ADMETLab

Physicochemical Properties	Reference Values		9-methoxycanthin-6-one	Canthin-6-one 9-O-beta-glucopyranoside	Niloticin
	Lower Limit	Upper Limit			
Molecular Weight (MW) (g/mol)	100	600	250,07	394,14	456,36
LogP	0	3	2,21	1,69	4,70
Logs	-4	0,5	-3,40	-4,02	-5,75
LogD	1	3	2,47	2,07	-4,10
Number of Hydrogen Bond Acceptors (nHA)	0	12	4	6	3
Number of Hydrogen Bond Donors (nHD)	0	7	0	4	1
Topology of Polar Surface Area (TPSA)	0	140 A2	43,6	107,22	49,83
Number of Rotated Bonds (nRot)	0	11	1	3	4
Number of Rings (nRing)	0	6	4	5	5
Number of Atom in The Biggest Ring (MaxRing)	0	18	15	15	17
Number of Heteroatoms nHet	1	15	4	6	3
Formal Charge (fChar)	-4	4	0	0	0
Number of Rigid Bonds (nRig)	0	30	20	26	24
Lipinski-Veber Rules Deviation	0	1	0	0	0

Table IV. Crystallographic Identity of CYP3A4 Protein with PDB Code 3UA1

PDB ID	3UA1
PDB DOI	https://doi.org/10.2210/pdb3UA1/pdb
Organism	Homo sapiens
Expression System	Eshericia coli BL21 (DE3)
Mutatio	No
Native Ligand	Bromoergocryptine

These values are higher compared to glibenclamide, which had a docking score of -12.165 kJ/mol and a predicted pIC50 of 9.832 (IC50 0.147 pM).

The atomic interactions between the ligand and receptor specifically demonstrate that glibenclamide binds to four amino acid residues at the binding site. The oxygen atom of glibenclamide forms hydrogen bonds with residues Arg 212, Ser 119, Ser 120, and Phe 108. The compound 14,15β-dihydroxyklaineanone forms hydrogen bonds with the oxygen atoms of its structure interacting with residues Ser 119, Arg 212, Arg 105, and Ser 119. Meanwhile, niloticin shows only one interaction, forming a hydrogen bond between its oxygen atom and Arg 212. Key residues indicating significant inhibitory affinity are Arg 212 and Thr 224 (Marbun *et al*., 2023, in Sevrioukova and Poulos, 2021). In this study, two compounds, 14,15β-dihydroxyklaineanone and niloticin, predicted to be potential

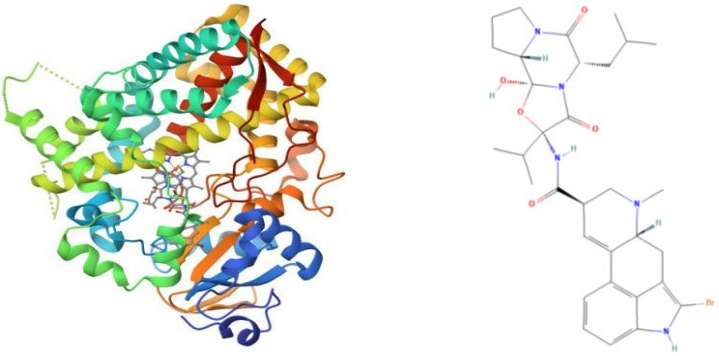

Figure 3. Crystallographic Structure of CYP3A4 PDB ID 3UA1 (Sevrioukova and Poulos, 2012). The binding site area containing the native ligand is located near the HEME cofactor, which plays a crucial role in oxidation (primarily hydroxylation) processes (Yan *et al*., 2022).

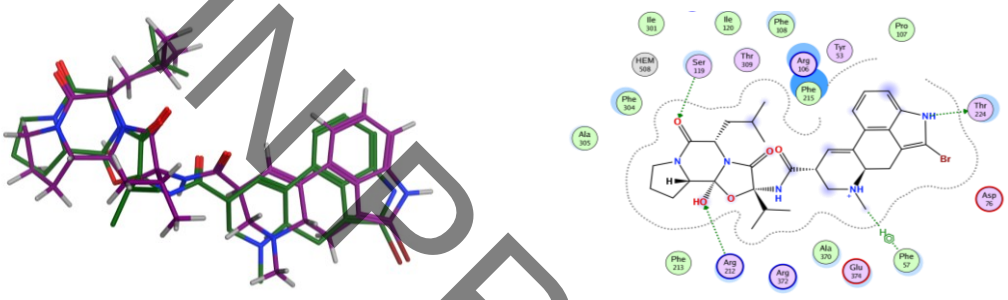

Figure 4. Visualization of the Redocking Pose of the Native Ligand 3UA1 with an RMSD of 0,9811 and its Interaction with CYP3A4 in 2D. An RMSD value of no more than 2 indicates that the observed deviation is still within a tolerable range, making the use of MOE software and PDB ID 3UA1 along with its native ligand valid for molecular docking simulation purposes.

Figure 5. Results Graph of Molecular Docking and Predictive pIC50 for Glibenclamide and the Chemical Constituents of ELRE. Two compounds, 14,15β-dihydroxyklaineanone and niloticin, exhibit higher docking scores and predictive pIC50 values than glibenclamide. These compounds are predicted to be responsible for the potential inhibition of glibenclamide metabolism by CYP3A4.

Table V. Predictive Docking Score and pIC50 of Active Constituents in *Eurycoma longifolia* Jack Root Against CYP3A4. The predictive pIC50 score was derived from the translation of docking scores using a scoring function equation.

Testing Ligands	Docking Score (kJ/mol)	Predictive pIC50
Glibenclamide	-12.164791	9.832275876
Eurycomanone (pasakbumin A)	-11.904709	9.606148621
14,15β-dihydroxyklaineanone	-12.382257	10.02135082
3b,18-dihydroeurycomanol	-12.049284	9.731848777
9-methoxycanthin-6-one	-8.6180048	6.748536801
9-methoxycanthin-6-one-N-oxide	-9.3043137	7.345245329
Laurycolactones A and B (C18)	-9.1190472	7.184166112
Laurycolactone B	-9.78337	7.761758913
5-iso-eurycomadilactone	-11.199231	8.992773596
Eurycomalactone	-10.03198	7.977911883
Eurycolactone D	-9.1972742	7.252180264
7-methoxy-beta-carboline- 1-propionic acid	-9.9611225	7.916305113
Eurylactone A	-10.359201	8.262412873
Pasakbumin B	-12.105647	9.780853362
Canthin-6-one 9-O- beta-glucopyranoside	-9.9849777	7.937045921
6alpha-Hydroxyeurycomalactone	-10.622926	8.491707519
Eurylactone E	-9.6075611	7.608902568
Eurycomalide A	-10.168897	8.096953821
14,15β-dihydroxyklaineanone	-10.549601	8.427955391
13,21-dihydroeurycomanone	-12.018846	9.705384579
13,21-dehydroeurycomanone	-11.210795	9.00282787
Niloticin	-12.87306	10.44807752
5,6-dehydroeurycomalactone	-10.746866	8.599466656
7-methoxy-beta-carboline-1-propionic acid	-10.13803	8.070116631
beta-carboline-1-propionic acid	-9.9690228	7.923173997
9-methoxycanthin-6-one	-8.8183079	6.92268953
Canthin-6-one	-7.3875217	5.678698192
2,3-dehydro-4a-hydroxylongilactone	-10.098651	8.035878717
9-hydroxycanthin-6-one	-7.1871448	5.504481298

Table VI. Amino Acid Residues Interacting with Glibenclamide, 14,15β-dihydroxyklaineanone, and Niloticin

Ligands	Ligand Atoms	Receptor Atoms	Interaction	Distance (Å)	Binding Energy (kJ/mol)
Glibenclamide	O	NH1-Arg 212	H-acceptor	2,73	-1,4
	O	OG-Ser 119	H-acceptor	3,32	-1,0
	O	OG-Ser 120	H-acceptor	3,10	-0,8
	C	6 ring-Phe 108	H-pi	3,77	-0,7
14,15β-dihydroxyklaineanone	O	O-Ser 119	H-donor	2,73	-1,2
	O	NH-Arg 212	H-acceptor	2,75	-2,0
	O	C-Arg 105	H-acceptor	3,24	-0,6
	O	NH-Arg 105	H-acceptor	3,06	-1,7
	O	C-Ser 119	H-acceptor	3,20	-0,6
Niloticin	O	O-Arg 212	H-acceptor	2,80	-1,8

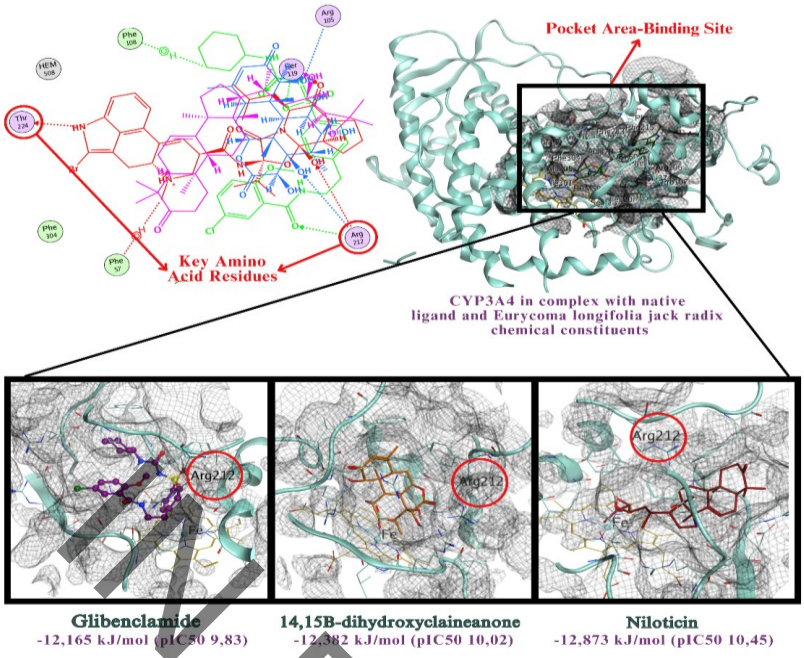

Figure 6. Visualization of Docking Results for Glibenclamide and Active Constituents of ELRE (14,15β-dihydroxyklaineanone and Niloticin) with CYP3A4. The active site (binding site area) is marked by the crystallographic pocket from the PDB structure. Both 2D and 3D visualizations show the binding of these compounds to one of the key residues, Arg 212.

competitive inhibitors of glibenclamide against CYP3A4, demonstrate interaction with the Arg 212 residue.

The compound 14,15βdihydroxyklaineanone is predicted to be a competitive inhibitor of CYP3A4 based on molecular docking tests, while niloticin is predicted to interact with CYP3A4 through both virtual pharmacokinetic screening and molecular docking tests. Although niloticin appears to be the compound more likely to interact with CYP3A4 and cause metabolism issues with glibenclamide, further testing is needed. This further studies includes computational methods, such as molecular dynamics simulations to assess the stability of the interaction (Hollingsworth and Dror, 2018), as well as in vitro or in vivo studies to determine the significance of its impact on glibenclamide metabolism. Several therapeutic management strategies that could be applied if this inhibition potential is proven true include spacing the use of oral diabetes medications from herbal supplements, or discontinuing the consumption of herbal medicines that pose a risk of metabolic disruption if the metabolic phase extends beyond 24 hours.

CONCLUSION

The results of this study indicate the potential for pharmacokinetic interactions between glibenclamide and the active compounds in *Eurycoma longifolia Jack.* root extract, particularly through their effects on the metabolic enzyme CYP3A4. In silico tests revealed that most of the active compounds from the extract show high affinity for CYP3A4, with two compounds, 14,15β-dihydroxyklaineanone and niloticin, demonstrating even higher affinity than glibenclamide. This suggests the possibility of competitive inhibition, which could affect the metabolism of glibenclamide. These findings provide preliminary insights into potential drug-herb interactions, which may influence the efficacy and safety of combining glibenclamide with *Eurycoma longifolia Jact. root* extract for diabetes therapy. Experimental studies are recommended to validate these results.

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AUTHOR CONTRIBUTIONS

Puti Isnaini and Prajona Marbun equally contributed to conducting the virtual screening, docking simulations, the analysis and interpretation of the results, and writing the report. Purwantiningsih and Agung Endro Nugroho were responsible for conceptualizing the study, providing guidance throughout the research process, and evaluating the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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