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Molecular Docking of Lipase Inhibitory Activities, Pharmacokinetics and Toxicity Prediction of Chemical Constituents from Curcuma aeruginosa Roxb Rhizome

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ABSTRACT:
The prevalence of obesity continues to increase, and this become risk factor for various diseases. Currently, the use of appetite-reducing drugs for obese sufferers still has uncomfortable side effects. Curcuma aeruginosa Roxb rhizomes are thought to have secondary metabolites having potential as alternatives for anti-obesity. This research aimed to determine the phytochemical components of the Curcuma aeruginosa Roxb rhizome fractions analyzed by GC-MS. Analysis on molecular docking of lipase inhibitory activities, pharmacokinetics, and toxicity prediction of those chemical constituents were also studied. The ethanol extract of Curcuma aeruginosa Roxb rhizomes was separated to produce n-hexane (HF), ethyl acetate (EAF), ethanol (EF), and the insoluble fractions (IF). The GC-MS results showed that there were 34 compounds from the three fractions. Based on molecular docking results, compound Labd-14-ene, 8,13-epoxy- **(1)**; (5R,8R,9S,10R)-2-Formyl- 3-hydroxy- 5-isopropenyl- 8-8-methyl (3a10) octahydronaphtho [1,2-b] furan-9-ol **(2)**; and 9α-D1-5α-Androstan-11-one **(3)**, the test ligand, have the lowest binding affinities (-9.13 kcal/mol; -9.30 kcal/mol; and -9.30 kcal/mol). Respectively, better than orlistat (-6.55 kcal/mol). Thus, the three compounds have the potential to be developed as anti-obesity by inhibiting the pancreatic lipase enzyme protein. All the three compounds were identified from EAF. The pkCMS results showed that the three compounds were predicted to have good pharmacokinetic profiles without any significant toxicity effects, except for compound **(2)** which showed positive in AMES toxicity and compound **(3)** which showed positive in skin sensitisation test.

KEYWORDS: Curcuma aeruginosa Roxb, molecular docking, lipase inhibitor, pharmacocinetics, toxicity

I. INTRODUCTION

According to the World Health Organization's World Obesity Atlas 2022, Indonesia is ranked 131st out of 183 countries for readiness to face obesity, reflecting a poor "global preparedness ranking." The situation is further compounded by a very high annual increase in obesity of 3.9%. Worryingly, by 2035, more than half of Indonesia's population is predicted to be obese, highlighting the ongoing rise in obesity prevalence [1]. From these facts, it can be concluded that obesity is a significant global health problem and needs serious attention from society and governments throughout the world. Prevention and treatment of obesity needs to be carried out holistically and integrated through an approach that involves various sectors, such as health, education, environment and public policy. The use of natural ingredients as anti-obesity can be an attractive alternative because they are safer and have fewer side effects compared to synthetic drugs. Natural ingredients have the potential as a source of interesting anti-obesity compounds and need to continue to be researched for the development of safer and more effective anti-obesity drugs [2].

The in silico lipase inhibitor test is to identify compounds that can inhibit the activity of the pancreatic lipase enzyme. The pancreatic lipase enzyme plays a role in fat digestion and excess fat in the body can cause obesity. The in silico test was carried out using a structure-based virtual screening method to identify compounds that have potential as pancreatic lipase inhibitors. Several studies have been carried out using this method and succeeded in identifying compounds that have good pancreatic lipase inhibitory activity [2–6]. This in silico test

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can be an effective and efficient alternative in identifying new compounds that can be used as anti-obesity drugs. GC-MS is a highly sensitive and reliable method for separating and reliable method for identifying fraction compounds in a sample mixture, especially for volatile and semi-volatile compounds. It is widely used in many industries, including food and beverage analysis, environmental analysis, and forensic analysis, to identify and quantify the compounds present in a sample mixture. The compounds that can be analyzed using GC-MS must be sufficiently volatile and thermally stable [7]. Curcuma aeruginosa Roxb rhizome is one of example of natural sources used for the treatment of various diseases such as asthma and cough, abdominal pain, scurvy and mental disorders, postpartum or menstruation problems, obesity gout, and rheumatism [8]. There are many benefits of Curcuma aeruginosa Roxb rhizome based on several studies reported. These include anticancer [9], anti-inflammatory [10], hair growth promoter [11,12], antimicrobial [13,14], antifungal [15], antiandrogenic [16], antinociceptive [17], anticancer [18], repellant [19], antiasthmatic [20], uterine relaxant [21], and anthelmintic [22]. There have been many studies to reveal the chemical content of Curcuma aeruginosa Roxb rhizome. In China, curcumenol and isocurcumenol is also found in the rhizome of Curcuma aeruginosa Roxb plus other compounds such as germacrone and curzerenone [23]. Curcuma aeruginosa Roxb rhizome is also distilled to obtain essential oils such as 1,8-cineole, camphor, curcumenol, isocurcumenol, curzerenone, zedoarol, and filranogermenone [24].

Each researcher performs the isolation of the chemical composition of Curcuma aeruginosa Roxb in various ways. Methods used to obtain essential oils from Curcuma aeruginosa Roxb can be hydro and steam distillation, but also other methods such as microwave-assisted hydrodistillation or fractional vacuum liquid chromatography. Based on previous studies, the components in the rhizome extract of Curcuma aeruginosa Roxb using gas chromatography-mass spectrometry (GC-MS) have been identified as belonging to the Sesquiterpenoids: α-curcumene, curzerene, curcumenol, curzerenone, epicurzerenone, caryophyllene oxide [25], monoterpenes; Phenolics; Diterpenes; Phenanthrene; Tetrapeptides [26,27]. These compounds could have anti-obesity potential so an initial in silico screening was carried out to support this potential. Analyze the compound content using GC-MS. GC-MS examination of fractination results using solvents with increased polarity has never been carried out. The polarity of the solvent used in the fractination process can affect the solubility of the compounds present in the sample mixture. There also has never been any research on compounds in Curcuma aeruginosa as anti-obesity in silico.

II. MATERIALS AND METHODS

a. Chemicals

All the chemicals and reagents used for the research were of analytical grade. Ethanol, methanol, n-hexane, and ethyl acetate as solvents from Smart Lab, Indonesia.

b. Plant Collection

Curcuma aeruginosa Roxb rhizome was purchased and identified from the Center for Research and Development of Traditional Medicinal Plants and Medicines Tawangmangu, Central Java, Indonesia with collection number CA-01-03-20 and harvested in February 2000.

c. Instrumentation

1. Instrument Analysis

GCMS analysis was carried out in GCMS (Shimadzu QP 2010 SE) and mass spectrophotometer. The columns used are Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane) and Carbowax (Polyethylene glycol), thickness 0.25um, length: 30.0m, inside diameter: 0.25mm.

2. Software and Hardware

PDB ID: 1LPB was downloaded from the Protein Databank (PDB, www.rcsb.org) [2]. The 3D structures' files of the natural compounds were downloaded from PubChem (www.pubchem.ncbi.nlm.nih.gov). Ligands prepared for molecular docking using chemdraw 3D 15.0. In-silico screening used molecular docking protocol in AutoDock Tool 1.5.6 Sep_17_14 and the output was visualized using Biovia Discovery Studio V21.1.0.2.20298. Pharmacokinetics and toxicity prediction using Lenovo laptop with Core i3 processor on Windows 10 operating system with 4 GB RAM and 64-bit operating system, x-64 based processor. The compound was translated in SMILES format by using the online SMILES Translator (https://cactus.nci.nih.gov). The SMILES formatted compound was processed using pkCMS online tool (https://biosig.lab.uq.edu.au/pkcsm) to predict pharmacokinetics and compound toxicity [28].

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d. Extraction and Fractination

Dried Curcuma aeruginosa Roxb rhizomes was powdered and then 1 kg of powdered material was macerated using 70% ethanol in a ratio of 1:5 overnight for 3 days. Upon filtration, the filtrates were combined and evaporated to dryness using a rotating vacuum evaporator at 60 ℃ and 100 rpm to give ethanol extract (EE). The ethanol extract (EE) was then separated by solid (SiO2)-liquid employing solvents ranging from non-polar (n-hexane) to semi-polar (ethyl acetate) to polar (ethanol) to give n-hexane (FH), ethyl acetate (EAF), ethanol (EF), insoluble fractions (IF). Fractions were concentrated by rotating the vacuum evaporator at 60 ℃ and 100 rpm, respectively.

e. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GCMS analysis was carried out in GCMS (Shimadzu QP 2010 SE) and mass spectrophotometer. The columns used are Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane) and Carbowax (Polyethylene glycol), thickness 0.25um, length: 30.0m, inside diameter: 0.25mm. The mobile phase used is helium and was adjusted to a column velocity flow of 0.74 mL/min. Other GC-MS conditions are ion-source temperature, 250 °C; interface temperature, 300 °C; pressure, 42,3 kPa; and 1 µl injector in split mode with a split ratio of 153.0 with injection temperature of 300 °C. The temperature was raised to 320 °C at the rate of 10 °C/min and held for 5 min. The total elution was 24 min. The relative percent amount of each component was calculated by comparing its average peak area to total areas. MS solution software provided by the supplier was used to control the system and to acquire the data.

f. Molecular Docking Studies, Pharmacokinetics, and Toxicity Prediction of Chemical Constituents

First step in molecular docking is the preparation of proteins and ligands. Interaction, docking, and binding analyses in 3D were performed using AutoDock Tools-1.5.6. Target proteins were downloaded from the Protein Data Bank (PDB ID: 1LPB) [2]. Protein files (.pdb) are prepared by removing water molecules and native ligands from 3D structure using Biovia Discovery Studio visualizer software. Test ligand file (.sdf) that has been downloaded from PubChem was prepared for molecular docking using chemdraw 3D 15.0. Before molecular docking is carried out, The receptor adds charge then the ligand adds charge and torsion. The size and coordinates of the grid box were determined. The grid box size is 40 × 40 × 40Å while the grid box coordinates are X: 8.980, Y: 25.120, Z: 50.590, spacing 0.375. Parameters used in Molecular docking is binding affinity (kcal/mol) and interacting amino acid residues. Interaction 2D and 3D between ligand and 1LPB protein were visualized using Biovia Discover Studio visualizer.

The prediction of chemical properties explained were molecular weight (BM), logarithm of coefficient octanol/water partition (Log P), the number of bonds between atoms that can rotate (Torson), Hydrogen Bond Acceptors (HBA), Hydrogen Bond Donors (HBD), and polar surface activity (PSA). These were carried out using the pkCMS online tool application, known as Lipinski's rule of five, which was a set of rules that helps differentiate between drug-like molecules and non-drug-like molecules [29]. This method could predict the greater likelihood of success or failure due to drug absorption and permeation. After the compound structure was drawn in 3D format using chemdraw 3D 15.0 and it was saved in a specific format (.pdb), and translated in SMILES format by using the online SMILES Translator (https://cactus.nci.nih.gov). The SMILES formatted compound was processed using the pkCMS online tool (https://biosig.lab.uq.edu.au/pkcsm) to predict pharmacokinetics and compound toxicity [28].

III. RESULTS AND DISCUSSION

a. GC-MS Identification

The ethanol extract of Curcuma aeruginosa Roxb rhizome was separated using solid-liquid fractination in stages with 3 solvents of different polarity (n-hexane, ethyl acetate, ethanol) to produce n-hexane (HF), ethyl acetate (EAF), ethanol (EF) and the insoluble fraction (IF). A total of 34 compounds were identified from the GC-MS analysis fractions of Curcuma aeruginosa Roxb rhizoma. The chemical constituents with their retention time (RT), molecular formula, molecular weight (MW), and concentration (%) in the HF, EAF, EF, and IF are presented in **table 1**.

The GC-MS results showed that HF contained mostly of sesquiterpenes (63.12%), and diterpenes (5.26%), 31.58% of it are unidentified compounds. The EAF consisted of sesquiterpenes (42.86%), diterpenes (21.43%), steroids (7.14%), and others (28.57%), whilst the EF was composed of sesquiterpenes (68.42%) and others (31.58%). The identification of IF showed the presence of saturated fatty acids. The HF and EF main compounds included curcumenol and

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epicurzerenone, and the main compounds of EAF were curcumenol and 2,4-Di-spironorbornylcyclobuta-1,3-dione (ketene dimers). In IF, only one compound was detected by GC-MS, namely dodecanoic acid because in this fraction. Polar compounds were not detected by GC-MS. Other instruments are needed to detect the presence of polar compounds, for example using LC-MS. It is reported that the most dominant metabolite in the ethanol extract of Curcuma aeruginosa Roxb rhizome is isocurcumenol, with the highest production came from Kulonprogo, Yogyakarta accession. The isocurcumenol was detected in nine accessions except for Bogor, West Java accession [26].

Table 1. Identified Chemical Compounds by GC-MS in Curcuma aeruginosa Roxb Rhizomes Sequential Fraction

No	Compounds	Group Compounds	MF	MW	Rt (min)	% Total in Fraction			
						HF	EAF	EF	IF
1	Curzerene	Monoterpenes	$C_{15}H_{20}O$	216	9.566	0.44		0.44	
2	Caryophyllene oxide	Monoterpenes	$C_{15}H_{24}O$	220	10.742	0.40		0.38	
3	Epicurzerenone	Monoterpenes	$C_{15}H_{18}O_2$	230	11.028	28.71	14.81	29.69	
4	2,4-Di-spironorbornylcyclobuta-1,3-dione	Others	$C_{16}H_{20}O_2$	244	11.111	0.63		0.62	
5	alpha.-Guaiene	Monoterpenes	$C_{15}H_{24}$	204	11.269		2.60	0.24	
6	Isocurcumenol	Monoterpenes	$C_{15}H_{22}O_2$	234	11.305	3.95		3.98	
7	Veridiflorol	Monoterpenes	$C_{15}H_{26}O$	222	11.981	1.28			
8	Germacrone	Monoterpenes	$C_{15}H_{22}O_2$	218	12.109	0.95		0.76	
9	Curdione	Monoterpenes	$C_{15}H_{24}O_2$	236	12.341	1.15		1.03	
10	9.alpha.-D1-5.alpha.-Androstan-11-one	Steroids	$C_{19}H_{29}$	274	12.658		1.72		
11	Curcumenol	Monoterpenes	$C_{15}H_{22}O_2$	234	12.641	34.47	24.31	32.69	
12	Ledane	Monoterpenes	C15H26	206	12.725		1.62		
13	Diepicedren-1-oxid	Monoterpenes	$C_{15}H_{24}O$	220	12.772	1.90		1.47	
14	13.149 1H-Naphtho[2,1-b]pyran-8(4aH)-one, 3-ethenyldecahydro-3,4a,7,7,10a-pentamethyl-	Diterpenes	$C_{20}H_{32}O_2$	304	13.149		9.78		

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	(terpenoid)							
15	Methyl stearolate	Others	$C_{19}H_{34}O_2$	294	13.196			9.30
16	9-Octadecynoic acid, methyl ester	Others	$C_{19}H_{34}O_2$	294	13.213	8.11		
17	iso-velleral	Monoterpenes	$C_{15}H_{20}O_2$	232	13.269	1.30		1.34
18	4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one	Others	$C_{10}H_{14}O$	150	13.384	0.24		0.26
19	Elemene	Monoterpenes	$C_{15}H_{24}$	204	13.707	6.04	4.02	6.13
20	1,5,9-Trimethyl cyclododecatriene	Others	$C_{15}H_{24}$	204	13.847	3.92		3.99
21	Germacrene B	Monoterpenes	$C_{15}H_{24}$	204	13.979			1.07
22	Thunbergen	Diterpenes	$C_{20}H_{32}$	272	13.998	0.95		
23	beta.-elemene	Monoterpenes	$C_{15}H_{24}$	204	14.333		3.32	
24	Octahydroanthracene	Others	$C_{14}H_{18}$	186	15.243	0.82		1.42
25	5R,8R,9S,10R)-2-Formyl-3-hydroxy-5-isopropenyl-8-8-methyl-(3a10)-octahydronaphthO	Others	$C_{15}H_{22}O_2$	234	15.245		12.81	
26	6-(1-hydroxymethyl-vinyl)-4,8A-dimethyl-3,5,6,7,8,8A-Hexahydro-1H-Naphthalen-2-O	Others	$C_{15}H_{22}O_2$	234	15.292	0.71		
27	15.422 1-Cyclohexyl-2-propen-1-one	Others	$C_{15}H_{22}O$	218	15.422		6.74	
28	Isolongifolene	Monoterpenes	$C_{15}H_{24}$	204	15.481	5.04		5.00
29	Labd-14-en-3-one, 8,13-	Diterpenes	$C_{20}H_{32}O_2$	304	15.618		11.10	

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	epoxy-(diterpen)							
30	2-methyl-4-(2,6,6-trimethyl-Cyclohex-1-enyl)-but-2-enal	Others	$C_{14}H_{22}O$	206	15.908	2.74		
31	Linolelaidic acid, methyl ester	Others	C19 H34 O2	294	16.020		0.19	
32	Gaillardin	Sesquiterpenes	$C_{17}H_{22}O_5$	306	18.461	1.80		
33	Cyclooctenone, dimer	Others	$C_{16}H_{24}O_2$	248	19.338	2.63		
34	Dodecanoic acid, ethenyl ester	Others	$C_{14}H_{26}O_2$	226	22.867			100

Note :GC-MS identification results come from Hexan **(HF)**, Ethyl acetata **(EAF)**, Ethanol **(EF)**, and Insoluble **(IF)** Fractions

Based on Table 1, it can be seen that there are some similarities of the compounds detected between the fractions.This is caused by several things, including the process of separating compounds that have the same polarity and solubility can be difficult. Sesquiterpenes (C15) and diterpenes (C20) are compounds in the terpenoid group. Both are major compounds identified in HF and EAF. The polarity of solvent-soluble diterpenes can vary depending on the number of polar functional groups in their structure. In general, diterpenes that have polar functional groups such as hydroxyl groups (-OH) or carbonyl groups (C=O) will tend to dissolve in polar solvents more. However, many diterpenes are nonpolar in general because the majority of their structure consists of relatively nonpolar carbon and hydrogen chains [30]. Terpenes that have only carbon and hydrogen structures, such as terpene hydrocarbons, are more likely to dissolve in nonpolar solvents such as nonpolar organic solvents (e.g. hexane or ethyl acetate) [31,32]. As with diterpenes, the solubility of sesquiterpenes can be affected by the presence of polar functional groups in their structure. If sesquiterpenes have polar functional groups such as hydroxyl groups (-OH) or carbonyl groups (C=O), their solubility in polar solvents such as alcohols or water can be increased [33–35]. Therefore, the sesquiterpenes were also identified in EF.

Other classes of compounds detected in HF included ketones, cycloalkenes, aromatic cycloalkanes, and cyclic ketones, whilst in the EAF were ketones, aldehydes, and steroids. Ketones, saturated fatty acids, cycloalkanes, and cycloalkenes were detected in EF, whereas saturated fatty acids were the only detected compounds in IF. This finding is similar to the study reported by Nurcholis [26] who conducted GC-MS analysis of the Curcuma aeruginosa rhizome extracted using methanol/chloroform/water. The study report that 50% of the compounds were dominated by terpenoids and fatty acid groups [36]. Three alkanes (tetracosane, triacontane, and tetratriacontane) and two fatty acid esters were also found in the polar extract [36].

The HF and EF, both contain major sesquiterpene compounds, namely curcumenol, and epicurzerenone. Curcumenol has shown promise as a bioactive compound with various potential therapeutic applications. Curcumenol has been reported to have several activities including antibacterial activity against Gram-negative bacteria such as S. typhi and E. coli [37], a potent CYP3A4 inhibitor with an IC50 of 12.6 μM [38], suppressed LPS-induced NF-κB activity by inhibit the phosphorylation of Akt, inhibit NF-κB activation by suppressed the nuclear translocation of the NF-κB p65 subunit and blocked IκBα phosphorylation and p38 MAPK signaling pathway [39], to mitigate inflammation and ameliorate the catabolism status of the intervertebral discs in vivo and in vitro via inhibit the TNFα/NFκB pathway [40], and Inhibits Osteoclastogenesis Via Blocking IPMK/TRAF6 and Counteracts OVX-Induced Osteoporosis in Mice

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[41]. Epicurzerenone has been reported to have several activities including antioxidant and antimicrobial activities, Inhibit apoptosis of L02 cells induced by H2O2 or TNFα/NFκB pathway [42], also antimicrobial activity against some of Gram-positive and negative pathogenic microorganisms and the components of the extract lead to the apoptosis of human cancer cell line. EAF has the major compounds of curcumenol and 2,4-Di-spironorbornylcyclobuta-1,3-dione (ketene dimers) which are ketone compounds. There is no information specifically about the biological activities of 2,4-Di-spironorbornylcyclobuta-1,3-dione. Only 1 compound was identified in the insoluble fraction /IF, which suggested the need of further analysis using technique such as LC-MS. LC-MS is a suitable method for identifying polar compounds in Curcuma species. Several studies have used LC-MS to analyze polar metabolites in Curcuma species, including Curcuma domestica and Curcuma longa. In addition, LC-MS has been used to separate and detect curcuminoids from turmeric, which are polar compounds [43].

b. Molecular Docking Studies, Pharmacokinetics, and Toxicity Prediction of Chemical Constituents

All 34 compounds identified by GC-MS were used as test ligands and orlistat (drug commercial antiobesity) was used as a control ligand against protein lipase (PDB ID: 1LPB). Molecular docking analysis of orlistat as a control ligand has binding affinity -6.55 kcal/mol. Compound Labd-14-en-3-one, 8,13-epoxy-(diterpene); 9.alpha.-D1-5.alpha.-Androstan-11-one; and (5R,8R,9S,10R)-2-Formyl- 3-hydroxy- 5-isopropenyl- 8-8-methyl (3a10) octahydronaphtho [1,2-b] furan-9-ol are ligands with the lowest binding affinities were -9.13, -9.30, and -9.30 kcal/mol respectively. Detected interacting amino acid residues with ligands can be seen in **table 2**. Based on 3D visualization, the interaction of orlistat against pancreatic lipase protein occurs in the active site, which can bee seen in **figure 1**.

The smaller the binding affinity value, the affinity between the receptor and ligand was higher and the vice versa, the greater the binding affinity value, the affinity between receptors and the ligands is getting lower [44,45]. On the structure of the pancreatic lipase enzyme, amino acids His 263, Asp 176 and Ser 152 are lipolytic sites [46]. The enzymatic activity was reduced after amino acid Ser 152 was modified. This showed that Ser 152 has an important role for catalytic activity, which inhibit lipolytic activity [47]. Visualization of 3D complex interactions of ligands and proteins also show that ligands binds to the active site pocket (Figure 1). The active site is an area where the substrate is specific binds to the enzyme and then carries out the catalytic process. The active sites consists of a binding site and catalytic site. Molecular docking analysis with the 1LPB protein revealed all three compounds and orlistat forming bonds with Ser152, suggesting their potential to inhibit the lipase enzyme [46].

Table 2. Results of Molecular Docking Analysis of Compounds Against Pancreatic Lipase Protein

No	Ligands	Bindings affinities (kcal/mol)	Amount bond hydrogen	Interacting amino acid residues
1	Orlistat **(drug control)**	-6.55	5	His 75, Gly 76, Phe 77, Ile 78, Asp 79, Trp 85, Tyr 114, His 151, **Ser 152**, Leu153, Ala 178, Glu 179, Pro 180, Ile 209, Phe 215, Arg 256, Ala 259, Ala 260, His 263, Leu 264
2	Labd-14-en-3-one, 8,13-epoxy- (diterpene) **(compound 1)**	-9.13	-	Gly 76, Phe 77, Ile 78, Asp 79, Tyr 114, His 151, **Ser 152**, Leu 153, Ala 178, Glu 179, Pro 180, Ile 209, Phe 215, Arg 256, Ala 260, His 263, Leu 264, Tyr 267
3	(5R,8R,9S,10R)-2-Formyl- 3-hydroxy- 5-isopropenyl- 8-8-methyl (3a10) octahydronaphtho [1,2-b] furan-9-ol **(compound 2)**	-9.30	2	His 75, Gly 76, Phe 77, Ile 78, Asp 79, Trp 85, Tyr 114, His 151, **Ser 152**, Leu 153, Ala 178, Pro 180, Phe 215, Ala 259, Arg 256, Ala 260, His 263, Leu 264, Tyr 267
4	9.alpha.-D1-5.alpha.-Androstan-11-one **(compound 3)**	-9.30	1	His 75, Gly 76, Phe 77, Ile 78, Asp 79, Trp 85, Tyr 114, His 151, **Ser 152**, Leu 153, Ala 178, Pro 180, Ile 209, Phe 215, His 263, Leu 264, Arg 256, Tyr 267

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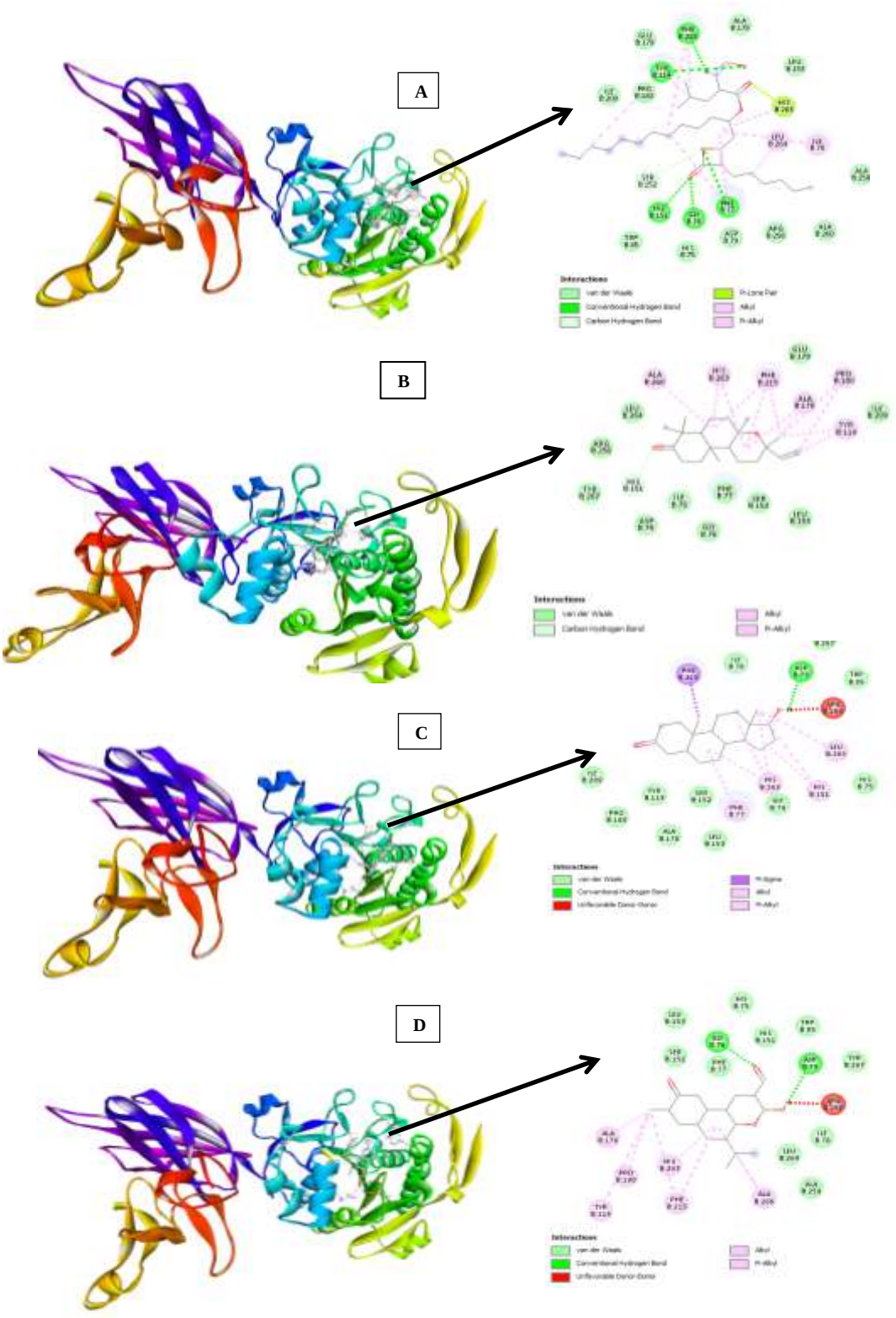

Figure 1. 2D and 3D Visualization of Complex Interactions Between Ligands (A= Orlistat, B= Labd-14-en-3-one, 8,13-epoxy-, C= 9.alpha.-D1-5.alpha.-Androstan-11-one, D= (5R,8R,9S,10R)-2-Formyl- 3-hydroxy- 5-isopropenyl- 8-8-methyl (3a10) octahydronaphtho [1,2-b] furan-9-ol) and Receptors

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The three predicted compounds identified by GC-MS were proven to have the best binding affinity among the other compounds. These compounds were further analyzed for their pharmacokinetic and toxicity profiles (ADMET) using pkCMS online tool. The Lipinski test is carried out to determine the hydrophobic/hydrophilic properties of the compound in cell membranes through passive diffusion. The conditions must be fulfilled by a ligand based on Lipinski's rules, which are a molecular weight < 500 Da, LogP value < 5, donor hydrogen bonds < 5, acceptor hydrogen bonds < 10 and molar refractivity between 40-130. Ligands with a molecular weight < 500 Da penetrate cell membranes more easily than ligands with a molecular weight > 500 Da. The logP value is related to the polarity of the ligand in fat, oil and non-polar solvents. Ligands with a log P value > 5 will interact more easily through the lipid bilayer layer of cell membranes and are widely distributed in the body. This causes the sensitivity of the ligand binding to the target molecule to decrease and the toxicity of the ligand to increase. Excessive hydrophobic molecules tend to have a high level of toxicity because they are retained longer in lipid membranes and are disseminated more widely throughout the body. The smaller the log P value, the ligand tends to dissolve in water and is hydrophobic. The Log P value of the ligand cannot be negative because it cannot pass through the lipid bilayer membrane (the number of hydrogen bonds in the donor and acceptor correlates with the biological activity of a ligand/drug. The greater the hydrogen bonding ability, the greater the absorption energy required [29]. The three compounds subjected to molecular docking tests fulfilled Lipinski's rules and the results can be seen in **table 3**.

Table 3. Ligand's Lipinski Rules of Five

Molecule Name	Molecular Weight	Log P	Hydrogen Bond Donor (HBD)	Hydrogen Bond Acceptor (HBA)	polar surface activity (PSA)
Labd-14-en-3-one,8,13-epoxy-(diterpene)	304.474	49.218	0	2	135.241
(5R,8R,9S,10R)-2-Formyl- 3-hydroxy- 5-isopropenyl- 8-8-methyl (3a10) octahydronaphtho [1,2-b] furan-9-ol	308.418	24.323	1	4	132.157
9.alpha.-D1-5.alpha.-Androstan-11-one	290.447	39.591	1	2	128.241

ADMET predictions are critical in assessing the pharmacokinetics of new drug molecules. A compound has high Caco2 permeability if the predicted value is >0.09. Caco-2 cells are human colorectal cancer epithelial cells. Caco-2 cell monolayers are often used as an in vitro model of human intestinal mucosa to estimate oral drug absorption. Steady State Volume of Distribution (VDss) is the volume required for a drug to be distributed uniformly to provide the same concentration as in blood plasma. The higher the VD, the more drug is distributed in tissue rather than plasma. VDss is considered low if <0.71 L/kg (log VDss < -0.15) and high if >2.81 L/kg (log VDss > 0.45). CYP2D6 will assess whether cytochrome P450 is likely to metabolize a particular molecule. Some drugs are metabolized by cytochrome P450. 2D6 and 3A4 are responsible for drug metabolism. Total clearance (CLtot) predicts excretion in log (ml/min/kg). The main components of drug clearance are renal clearance (renal excretion) and hepatic clearance (liver metabolism and biliary clearance). AMES toxicity is widely used to assess the mutagenic potential of compounds using bacteria. Positive results indicate that the compound is cancer-causing and mutagenic [28].

A compound is considered to have high CaCO-2 permeability if has a predictive value > 0.90[48]. The test results obtained more than 0.90 so it has good permeability. The volume of distribution (VDss) is considered low if it is below 0.71 L/kg (log VDss<-0.15) and high if above 2.81 L/kg (log VDss>0.45) [49]. In table 4, all compounds are in the range volume distribution requirements so that it can be predicted that all these compounds can be distributed evenly to provide the same concentration as in blood plasma. In metabolism, all compounds are not affected by metabolism in the presence of

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CYP2D6 and cannot works as an inhibitor for CYP2D6. Total clearance is important for determine the dose size to achieve steady-state concentrations and related to bioavailability. compound with the highest total clearance meaning it is excreted most quickly from the body. The AMES Toxicity test is one of the methods used extensively to assess the mutagenic potential of compounds using bacteria [49]. Based on **table 4**, all ligands have good pharmacokinetic parameter values except (5R,8R,9S,10R)-2-Formyl- 3-hydroxy- 5-isopropenyl- 8-8-methyl (3a10) octahydronaphtho [1,2-b] furan-9-ol which showed toxic effects (AMES toxicity) and 9.alpha.-D1-5.alpha.-Androstan-11-one (Skin Sensitisation); while Labd-14-en-3-one,8,13-epoxy- did not find any toxicity.

Molecule Name	Absorption (CaCo2 permeability) (log Papp in 10^{-6} cm/sec)	Distribution (VDss (human)) (log L/kg)	Metabolism (CYP2D6) (Yes/No)	Excretion (Total Clearance) (log ml/min/kg)	Toxicity (AMES Toxicity) (Yes/No)	Hepato toxicity	Skin Sensitisation
Labd-14-en-3-one,8,13-epoxy-	1.351	0.48	No	0.749	No	No	No
9.alpha.-D1-5.alpha.-Androstan-11-one	1.578	0.433	No	0.698	No	No	Yes
(5R,8R,9S,10R)-2-Formyl- 3-hydroxy- 5-isopropenyl- 8-8-methyl (3a10) octahydronaphtho [1,2-b] furan-9-ol	1.08	0.078	No	0.974	Yes	No	No

Table 4. Pharmacokinetics (ADMET) Parameters of Ligands

IV. CONCLUSION

There were 34 predicted compounds identified by GC-MS from Curcuma aeruginosa Rhizome Fractions. Based on molecular docking analysis there were 3 best compounds, namely Labd-14-ene, 8,13-epoxy-; (5R,8R,9S,10R)-2-Formyl- 3-hydroxy- 5-isopropenyl- 8-8-methyl (3a10) octahydronaphtho [1,2-b] furan-9-ol; and 9α-D1-5α-Androstan-11-one. All three compounds bind to a protein important for the inhibitory activity of the lipase enzyme, Ser 152. The three compounds could be used orally based on the acceptance of Lipinski rule of five. These ligands had good pharmacokinetic (ADMET) parameter values except (5R,8R,9S,10R)-2-Formyl- 3-hydroxy- 5-isopropenyl- 8-8-methyl (3a10) octahydronaphtho [1,2-b] furan-9-ol which have toxic effects (AMES toxicity) and 9.alpha.-D1-5.alpha.-Androstan-11-one based on Skin Sensitisation. There is no toxicity data on Labd-14-en-3-one,8,13-epoxy-.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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