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GC-MS Analysis of phytochemical compounds from Javan olive leaves (Olea javanica) extract and bioactivity screening of antidiabetes through in silico approach

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Abstract: Diabetes is one of the most common metabolic diseases, ranking ninth in mortality worldwide. Although many effective hypoglycemic drugs are available for the treatment of diabetes, researchers are continually seeking more effective drugs with fewer side effects by focusing on various metabolic components such as enzymes, transporters, and receptors. The glucokinase (GK) enzyme, which is primarily found in the liver and pancreatic beta cells, is involved in maintaining blood glucose homeostasis. Therefore, this study was designed to determine the interaction between glucokinase and the compounds (ligands) from Javan olive leaf extract (*Olea javanica*) using GC-MS and in silico analysis. The method used in this study involved maceration with 96% ethanol to produce Javan olive leaf extract, followed by analysis via GC-MS using the Agilent 6980N Network GC System. Subsequently, in silico analysis was conducted using PyRx 0.9 software, the pkCSM website, BIOVIA Discovery Studio 2019, the RCSB PDB database, AutoDock Vina, the CABS-flex 2.0 web server, and Lipinski's rules. The results of the GC-MS analysis identified compounds such as 3 methylpentane, hexane, methylcyclopentan e, alpha-muurolene, (-)-calamenene, methyl 14-methylpentadecanoic acid, methyl ester linoleic acid, trans-squalene, and alpha-tocopherol. In silico analysis revealed that the molecules matching the target protein for diabetes treatment are native ligands exhibiting antidiabetic activity, as determined by molecular docking in this study.

Keywords: Diabetes, GC-MS, Glucokinase, In silico, *Olea javanica*

INTRODUCTION

The genus *Olea* comprises 12 species[1]. The family Oleaceae is commonly known as the olive family, with its members referred to as "olive" in English and "zaitoon" in Arabic. The health benefits of olive fruits and leaves have been widely researched concerning the treatment of respiratory diseases, urinary tract infections, and gastrointestinal disorders, while the oil is applied to the scalp to prevent bone loss and fractures[2]. In addition to traditional uses, the fruit and leaves have recently been reported to have antioxidant effects that could potentially benefit the skin[3].

Research suggests that leaf extracts from olive plants such as *Olea europaea* and *Olea ferruginea* have potential as antidiabetic agents[4,5]. One of the close relatives of the olive that grows in Indonesia is the Javan olive (*Olea javanica*). This plant is an endemic species of Indonesia that has not been extensively studied for its bioprospective potential. Oleuropein is a phenolic compound believed to have hypoglycemic, antihypertensive, antioxidant, anti-

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inflammatory, and cardioprotective properties, as well as supportive effects in obesity therapy.

In general, olive leaves have a rich content of phenols that are beneficial for human health[6]. Oleuropein is part of phenol compounds where oleuropein is thought to play a role as hypoglycemic, antihypertensive, antioxidant, anti-inflammatory, cardioprotective, and supportive effects in obesity therapy. Oleuropein has potential as a hypoglycemic effect with the ability to increase glucose uptake into cells, thus it can be used as an alternative therapy for diabetes to control blood sugar levels[7].

One of the research on diabetes control therapy is by using glucokinase control. Glucokinase (GK or hexokinase IV) as a glucose sensor plays an important role in glucose homeostasis[8]. In pancreatic β-cells, it regulates insulin secretion in response to circulating blood glucose levels. In the liver, it facilitates glycogen storage and post-meal glucose clearance from the bloodstream. Additionally, in pancreatic α-cells, it participates in glucose-dependent regulation of glucagon secretion.

One area of research in diabetes control therapy involves the regulation of glucokinase. Several studies have shown decreased levels of GK expression and activity in pancreatic cells in several animal models and humans with obesity and diabetes. Lu's research revealed that a high-fat diet can damage beta cells and induce diabetes[9]. This enzyme plays a crucial role in maintaining blood sugar balance in liver and pancreatic cells[11]. The use of separation techniques, such as liquid chromatography (LC) or gas chromatography (GC), prior to mass spectrometry (MS) detection, is common when analyzing complex plant-derived samples[12].

However, the chemical composition of Javan olive (*Olea javanica*) leaves remains largely unexplored. This study aims to identify phytochemicals through GC-MS analysis, quantify their abundance in Javan olive leaf extract, and assess their potential involvement in antidiabetic signaling pathways using an in silico approach.

MATERIAL AND METHOD

The materials used in this study included leaves from endemic Javan olive trees (*Olea javanica*) collected from wild populations in Mojokerto Regency, East Java. Additional materials used included 96% ethanol, filter paper, and aluminum foil. The laboratory equipment used in this study included an oven, analytical scales, beakers, dropper pipettes, a rotary evaporator, a vortex mixer, and a water bath.

Leaf Collection

Javan olive (*Olea javanica*) leaves were collected on July 1, 2023, from Mojokerto District, East Java, Indonesia (coordinates: -7.558369, +112.547966; altitude: 108.7 meters). The collected leaves were cleaned to remove dust and dirt, then dried in an oven at 60°C for 5 hours[14]. The dried leaves were then pulverized into a fine powder using a blender.

Olive Leaf Extraction

Olive leaf powder (250 grams) was homogenized using the maceration method by adding 96% ethanol (w/v) for 72 hours. The resulting macerate was filtered using filter paper and a rotary evaporator. The filtrate was then concentrated using a rotary vacuum evaporator and further processed with a water bath to produce pure olive leaf extract[15].

GC-MS Analysis

Javan olive leaf extract was dissolved in 6 mL of ethanol, vortexed for 2 minutes, and then sonicated for 15 minutes. The resulting solution was filtered through a 0.45 µm membrane filter, and a 1 µL aliquot was injected into the GC-MS instrument. GC-MS analysis was performed using an Agilent 6980N Network GC System equipped with an Agilent 5973 inert MSD detector and a J&W

Scientific HP-5MS column (0.25 mm × 30 m × 0.25 µm). The oven temperature was programmed to increase from 150°C to 230°C at a constant flow rate of 1 mL/min.

Mass spectra were obtained and compared with the Wiley spectral library (version 8.0), which contains more than 62,000 reference spectra[16]. Compounds were identified based on the similarity of their mass spectra, retention times, and molecular formulas. The relative abundance of each identified compound was determined by calculating its peak area as a percentage of the total peak area of all detected compounds[17].

In Silico Analysis

In silico docking simulations were performed to evaluate the binding interactions between secondary metabolites identified in Javan olive extract and target proteins.

Ligand Preparation

The 3D structures of the identified compounds (shown in Figure 2) were retrieved from public databases such as PubChem (https://pubchem.ncbi.nlm.nih.gov/) and ChemSpider (https://www.chemspider.com). For PubChem-derived ligands, conformational optimization was performed using the Open Babel 2.3.1 plug-in integrated within PyRx 0.9 software[18]. Ligands obtained from ChemSpider/1were downloaded in molar file format and converted to Canonical SMILES format using the CACTUS (Chemical Abstracting Computer Toolkit Service) website (https://cactus.nci.nih.gov/translate)[19]. Ligands obtained from ChemSpider were downloaded in mol file format and converted to Canonical SMILES format using the CACTUS (Chemical Abstracting Computer Toolkit Service) website (https://cactus.nci.nih.gov/translate)[19]. This optimization process enhances ligand flexibility and facilitates docking simulations[20].

Control Ligand Preparation

Control ligands are known modulators or activators of the target protein. In this study, glucokinase activators were used as control ligands.

Protein Preparation

The 3D structure of the target protein, human glucokinase (GCK), was retrieved from the Protein Data Bank (PDB) database (http://www.rcsb.org/pdb/home/home.do) using its specific identifier (ID). The protein structure was then processed using Biovia Discovery Studio 2019 software to remove any contaminant molecules[21].

Target Protein Modeling Analysis

Protein preparation primarily involved the removal of water molecules and any bound ligands to facilitate visualization, docking simulations, and potentially molecular dynamics simulations.

Specific Docking

Molecular docking simulations were conducted using AutoDock Vina, integrated within the PyRx 0.9 software platform. It is important to note that docking primarily focuses on predicting ligand interactions within the protein's active site. Binding affinity, a measure of the strength of protein-ligand interactions, is inversely correlated with stability; lower binding affinity values indicate more stable complexes[22].

Chemical Interactions

The resulting docking poses were visualized using Biovia Discovery Studio 2019 software to identify key interactions, including hydrogen bonds, hydrophobic interactions, electrostatic interactions, and unfavorable contacts.

The formation of multiple hydrogen bonds is often indicative of a stable protein-ligand complex, as these interactions contribute significantly to binding affinity[23].

Structural Visualizations

Both 3D and 2D visualizations were generated using Biovia Discovery Studio 2019 software. Initial 3D representations of the ligands and the target protein were created to provide a global perspective. Subsequently, the focus shifted to a 2D view of the protein-ligand binding interface for a detailed analysis of intermolecular interactions.

RESULTS AND DISCUSSION

GC-MS Analysis Result

Phytochemical analysis was conducted using gas chromatography-mass spectrometry (GC-MS). This technique enables the separation and identification of volatile compounds, including plant secondary metabolites. The resulting chromatogram visually represents the sample components, with each peak corresponding to a distinct compound[24]. The chromatogram of the ethanol extract from Javan olive leaves is presented in Figure 1, while the identified compounds and their relative abundances are summarized in Table 1.

Table 1. Chemical Components of GC-MS Test Results of Endemic Javan Olive Leaf Extract

Chemical Compound	RT (Retention Time)	Normalization%	Qual
3-methylpentane	1,81	2,71	91
Hexane	1,88	50,46	93
Methylcyclopentane	2,04	14,95	91
Alpha-muurolene	17,51	0,12	99
-(-calamenene)	17,81	0,12	91
Methyl 14-methylpentan decanoic acid	22,09	0,47	98
Methyl ester linoleic acid	23,80	0,89	99
Trans squalene	29,95	1,68	91
Alpha tocopherol	34,26	1,13	95

The chromatogram presented in Figure 1 illustrates the relative abundance of each identified chemical class as a percentage of the total chromatographic peak area, determined using five analytical methods. A total of 107 distinct compounds were detected in the chromatogram, with each peak corresponding to a unique chemical entity. However, the identification of certain compounds was hindered by low-quality scores (<90), indicating a lack of confidence in the spectral match. This discrepancy suggests that the sample's mass spectrum deviated significantly from the reference library spectrum, potentially due to factors such as background noise, ion source decomposition, weak signal intensity, or variations in collision energy. Consequently, this study provides an estimate of the overall percentage of unidentified compounds across the different analytical platforms. A substantial limitation of GC-MS phytochemical analysis is the necessity of authentic reference standards to precisely quantify and identify target analytes within intricate herbal matrices[25]. While GC-MS libraries offer a valuable resource for tentative compound identification based on spectral similarity, establishing definitive compound identity remains challenging due to potential spectral variations[26]. Known compounds identified in the GC-MS analysis included 3-methylpentane, hexane, methylcyclopentane, α-muurolene, (-

)-calamenene, methyl 14-methylpentanoate, methyl linoleate, trans-squalene, and α-tocopherol.

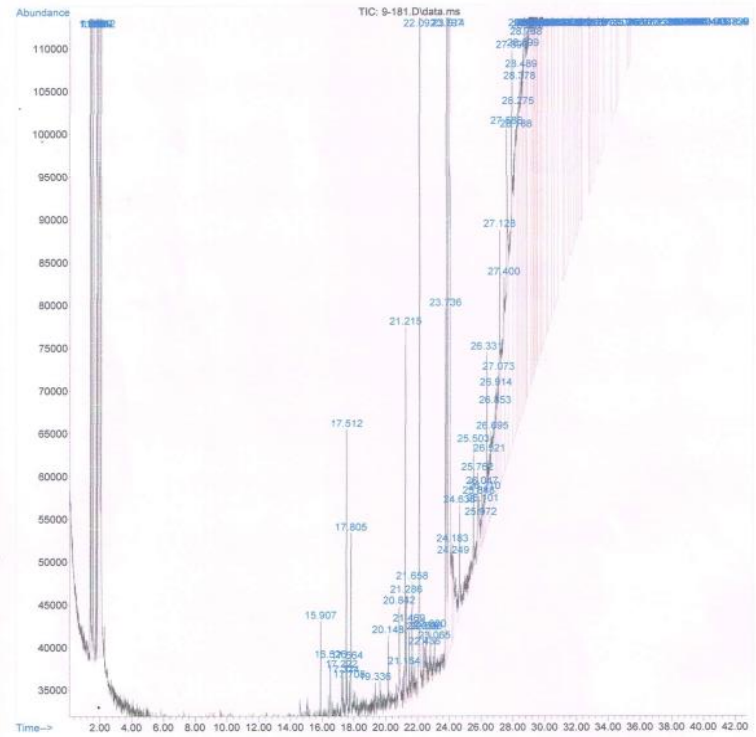


Figure 1. This is a figure GC-MS chromatogram of endemic javan olive leaf extract (*O. javanica*)

The chromatogram revealed nine distinct peaks, each subjected to mass spectrometric analysis. GC-MS analysis confirmed the presence of nine volatile compounds belonging to various chemical classes. In descending order of relative abundance, these classes were identified as esters (21.61-60.49%), alcohols (20.73-49.2%), hydrocarbons (3-38.88%), ketones (0.16-3.87%), acids (0.07-2.62%), and aldehydes (0.12-1.47%).

In Silico Results

GC-MS analysis identified several compounds[27] that adhered to Lipinski's rule of five, suggesting potential drug-like properties. These compounds were subjected to computational docking with the GCK protein, with the lowest binding energy pose selected for each. Binding energy is a predictor of protein-ligand complex stability, wherein lower values indicate stronger interactions[28]. Table 2 presents the docking results for the identified compounds: 3-methylpentane, hexane, methylcyclopentane, α-muurolene, (-)-calamenene, methyl 14-methylpentadecanoic acid, methyl linoleate, trans-squalene, and α-tocopherol, demonstrating their potential to form stable protein-ligand complexes.

Table 2. Results of molecular docking to determine binding affinity

Chemical Compound	Protein	Binding Affinity	RMSD
3-methylpentane	1,81	-4,1	0, 0
Hexane	1,88	-4,0	0, 0
Methylcyclopentane	2,04	-4,5	0, 0
Alpha-muurolene	17,51	**-7,9**	0, 0
-(-calamenene)	17,81	**-8,3**	0, 0
Methyl 14-methylpentan	22,09	-6,6	0, 0

decanoic acid			
Methyl ester linoleic acid	23,80	-6,4	0, 0
Trans squalene	29,95	**-7,9**	0, 0
Alpha tocopherol	34,26	-7,7	0, 0

Results of the Drug-likeness Property Assessment Based on Lipinski's Rule of Five

The drug-likeness properties of the identified compounds were evaluated using Lipinski's rule of five, which stipulates that drug-like molecules typically possess a molecular weight below 500 Da, a Log P value less than 5, fewer than 5 hydrogen bond donors, and fewer than 10 hydrogen bond acceptors[29]. As summarized in Table 3, α-muurolene and α-tocopherol from Javan olive leaf extract exceeded the Lipinski Log P threshold of 5. In contrast, 3-methylpentane, hexane, methylcyclopentane, methyl 14-methylpentadecanoic acid, methyl linoleate, and trans-squalene adhered to this criterion. Molecules with excessive hydrogen bond donors or acceptors tend to exhibit reduced chemical stability, potentially impacting their drug-like properties[30]. Non-compliance with any of these parameters can adversely affect a compound's absorption and bioavailabilit y[31].

Table 3. Results of the analysis of the properties of drug-like chemical compounds based on the Lipinski rule

Chemical Compound	MW (≤500 Da)	Log P (≤5)	HBD (≤5)	HBA (≤10)	MR(75-150)
3-methylpentane	86,18	3,52	0	0	30.96
Hexane	86,18	3,52	0	0	30,96
Methylcyclopentane	84,16	3,12	0	0	28,84
Alpha-muurolene	204,35	4,63	0	0	69,04
-(-calamenene)	202,34	5,45	0	0	68,07
Methyl 14-methylpentan decanoic acid	254,41	4,09	1	2	80,32
Methyl ester linoleic acid	294,47	4,09	0	2	93,78
Trans squalene	410,72	4,09	0	0	143,48
Alpha tocopherol	430,71	6,14	1	2	139,27

Protein Structure and Active Site

The target protein for in silico docking simulations was chosen based on its relevance to the study and the availability of a suitable structure. Ideally, the target protein should possess a known activatory ligand and a high-resolution structure deposited in the Protein Data Bank (PDB). In this study, human glucokinase (PDB ID: 6E0E) was selected as the target protein due to its established role in glucose metabolism and the availability of a complex structure with an activatory ligand (HKM) deposited in the PDB. Control compounds, defined as known activators of the target protein or its original ligand, were also identified based on information from scientific databases. The downloaded PDB file for human glucokinase contained information about the protein's three-dimensional (3D) structure, including its dimensions along the x, y, and z axes (23.6161 Å, 27.7023 Å, and 25.8563 Å, respectively) as reported in the reference source. The protein's active site residues were identified as Arg63, Pro66, Ile211, Val455, Met210, Tyr214, Ile159, Met235, Val62, and Val452. For visualization purposes, the 3D structure of the protein was rendered in a ribbon style, highlighting its secondary structure elements[33]. In this representation, red

indicates α-helices, light blue represents β-sheets, white represents loops, and green represents coils,as shown in Figure 2.

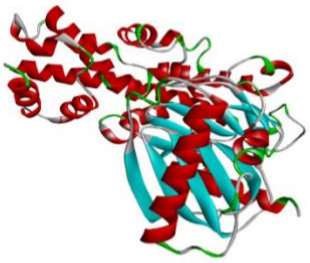
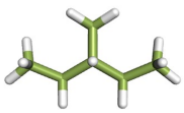
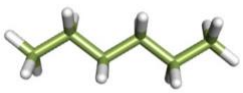
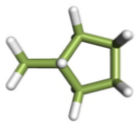
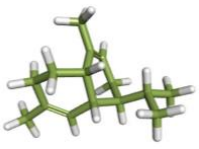
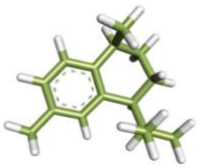
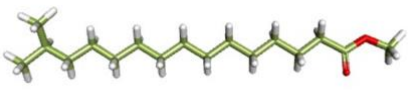
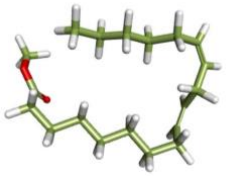
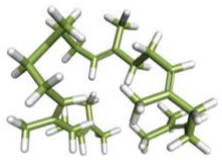
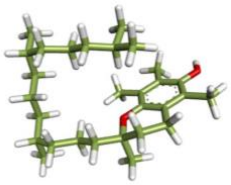


Figure 2. 3D structure of the target protein: Human glucokinase (insulin production activator)

The identified compounds within the *O. javanica* leaf extract are listed in Table 4, along with their corresponding Chemical Identifier (CID) and source database. The 3D structures of these compounds are depicted in stick representation in Figure 2, where carbon atoms are colored green, oxygen atoms red, and hydrogen atoms white. It is important to note that some compounds could not be identified or retrieved from the available databases.

Table 4. Phytochemical Compounds in PubChem: Those without HMDB and Those with HMDB

Chemical Compound	CID	Figure of Compound
3-methylpentane	7282	
Hexane	HMDB0029600	
Methylcyclopentane	7296	
Alpha-muurolene	12306047	
-(-calamenene)	HMDB0059910	
Methyl 14-methylpentadecanoic acid	HMDB0041422	

Methyl ester linoleic acid	HMDB0034381	
Trans squalene	HMDB0000256	
Alpha tocopherol	14985	

Molecular docking results of Javan olive compounds activating human glucokinase protein

Molecular docking simulations were performed to evaluate the binding interactions between the test compounds and human glucokinase. Binding affinity, a measure of the strength of the ligand-protein complex, was determined for each compound[34]. A lower binding energy value corresponds to a more stable complex, often indicative of increased activatory potency. The native ligand for glucokinase exhibited the most favorable binding affinity, serving as a benchmark for comparison. Similar to other known glucokinase activators, α-muurolene, trans-squalene, and (-)-calamenene displayed significant hydrophobic interactions with the protein. These compounds demonstrated potential as drug candidates based on their predicted binding modes, as visualized in Figure 3.

Table 6. List of Phytochemical Compounds in PubChem (without HMDB) and HMDB (with HMDB)

Ligand	Hydrogen Bonding	Hydrophobic Interactions	Other Bonds
Alpha-muurolene	-	Pro66, Pro66 (alkyl), Pro66, Tyr214, Tyr214 (pi-orbital)	-
-(-calamenene)	-	Trp99, Trp 99 (pi-pi stacked), Val101, Lys90, Val101 (alkyl), Trp99, Trp99, Trp99, Trp99 (pi-orbital)	-
Trans squalene	-	Pro66, Pro66, Pro66, Met210, Met235(alkyl), Tyr214, Tyr214, Tyr214 (pi-alkyl)	-
Native ligand	Arg63 (konvensional), Pro66 (C-H)	Ile211, Val455 (pi-sigma), Tyr214 (pi-pi T-shaped), Arg63, Ile159, Val455 (alkyl), Met236, Pro66, Val62, Val452 (pi-	Met210 (pi-sulfur)

alkyl)

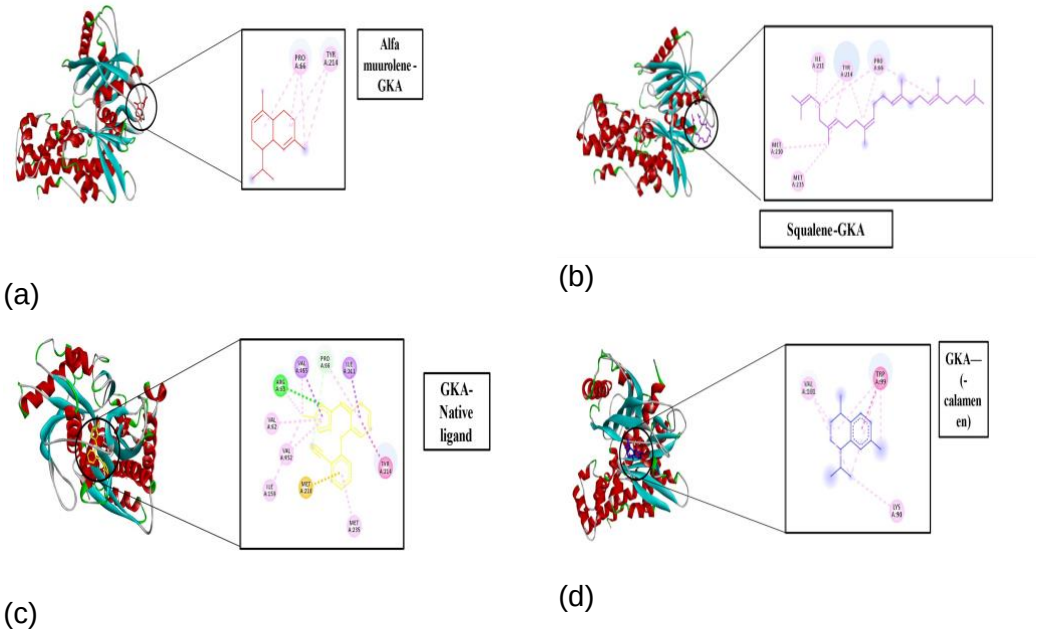


Figure 3. Binding mode visualization of (a) Interaction between human glucokinase protein and the chemical compound α-muurolene; (b) Interaction between human glucokinase protein and the chemical compound trans-squalene; (c) Interaction between human glucokinase protein and the native ligand; (d) Interaction between human glucokinase protein and the chemical compound (-)-calamenene.

Docking simulations between the identified compounds and human glucokinase revealed that α-muurolene, (-)-calamenene, and trans-squalene primarily formed hydrophobic interactions within the protein's active site. Figure 3a illustrates the interaction between α-muurolene and glucokinase, characterized by a binding energy of -7.9 kcal/mol and two π-alkyl interactions involving residues Pro66 and Tyr214. Similarly, trans-squalene exhibited a binding energy of -7.9 kcal/mol with a single π-alkyl interaction (Figure 3b). In contrast, the native ligand displayed a stronger binding affinity of -8.8 kcal/mol, forming four distinct interaction types: π-sigma, π-π T-shaped, alkyl, and hydrogen bonding (Figure 3c). Notably, the native ligand engaged with all active site residues. The (-)-calamenene ligand formed a binding complex with a binding energy of -8.3 kcal/mol, characterized by stacked π-π, alkyl, and π-orbital interactions with specific amino acid residues. Analysis of all docked compounds revealed a common motif of π-alkyl interactions involving Lys169 and Ile225. The presence of π-sigma interactions, including π-alkyl and π-sulfur subtypes, suggests potential charge transfer contributions to ligand binding[35]. These findings corroborate previous observations regarding the diverse chemical interactions of ligands compared to activators, emphasizing the importance of hydrogen bonding for protein stability and ligand affinity[36].

CONCLUSION

GC-MS analysis of Javan olive (*Olea javanica*) leaf extract identified a complex mixture of compounds, including 3-methylpentane, hexane, methylcyclopentane, α-muurolene, (-)-calamenene, methyl 14-methylpentadecanoate, methyl linoleate, trans-squalene, and α-tocopherol. In silico docking studies revealed that the compounds α-tocopherol, trans-squalene, (-)-calamenene, and the native ligand have properties that can activate

glucokinase to stimulate insulin production by binding to the target protein. However, the native ligand exhibits superior binding affinity due to its interaction with several amino acid residues within the active site of the protein. To substantiate these findings and advance the development of native ligand-based antidiabetic therapies, further in vitro, in vivo, and clinical studies are needed. These studies are expected to shed light on the therapeutic potential of *O. javanica* leaf extracts and pave the way for the discovery of new antidiabetic agents with different mechanisms of action.

AUTHORS' CONTRIBUTIONS

Lailatus Fitri: Con-septualization, software, and writing—preparation of the original draft; **Ayu Dewi Wulandari**: Con-septualization and formal analysis; **Tri sumaryono**: investigation and visualization; **Nur Fatimah Azzahra Haibaturrahma**: Methodology and data curation; **Bunga Nanda Agustina**: resources; **Muhammad Badrut Tamam**: validation, writing reviewing, editing, supervision, and project administration; acquisition of funding, Ministry of Education, Culture, Research and Technology.

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DATA AVAILABILITY STATEMENT

There were no studies involving humans and animals as test objects in this research.

DISCLOSURE STATEMENT

The views and opinions expressed in this article are the author's own and do not necessarily reflect the views or policies of the author's institution. The data is original research by the author and has not been published elsewhere.

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