



Isolation, characterization, and *in vitro* inhibitory activity of a new alpha-glucosidase inhibitor from *Schleichera oleosa* (Lour.) Oken leaves

[Aislamiento, caracterización y actividad inhibitoria *in vitro* de un nuevo inhibidor de glucosidasa a partir de hojas de *Schleichera oleosa* L.]

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Abstract

Context: The development of novel and potent alpha-glucosidase inhibitors from natural sources holds immense promise for treating diabetes.

Aims: To identify the isolated compound, determine its inhibitory potency, explore its molecular interactions with the alpha-glucosidase receptor, and understand the role of specific structural features in its inhibitory activity.

Methods: Research began with the isolation and identification of the new gallic acid derivative of the *Schleichera oleosa* leaf extract. The isolated compound (**1**) was characterized as 3,5-dihydroxy-4-methoxybenzoic acid using spectroscopic techniques. *In vitro* studies were carried out to compare the IC₅₀ values of the ethanolic extract, isolate **1**, and acarbose.

Results: Isolate **1** exhibited significantly higher inhibitory potency against the α -glucosidase enzyme compared to acarbose. Subsequently, molecular docking studies were performed to gain insight into the molecular interactions between the gallic acid derivatives and the active site of the α -glucosidase receptor. The docking simulations revealed that the protection of the methyl group on the hydroxy group of the gallic acid derivatives played a crucial role in enhancing its binding affinity to the alpha-glucosidase receptor. This molecular interaction insight provides valuable information for the design of more potent and selective α -glucosidase inhibitors based on this structural feature.

Conclusions: These results collectively suggest that the newly isolated gallic acid derivative has promising potential as a natural α -glucosidase inhibitor for diabetes management. Further research and optimization of this compound could lead to the development of novel and effective therapeutics for glycemic control in individuals with diabetes.

Keywords: gallic acid; α -glucosidase inhibitor; molecular docking; *Schleichera oleosa*.

Resumen

Contexto: El desarrollo de nuevos y potentes inhibidores de la alfa-glucosidasa a partir de fuentes naturales es una gran promesa para el tratamiento de la diabetes.

Objetivos: Identificar el compuesto aislado, determinar su potencia inhibidora, explorar sus interacciones moleculares con el receptor de la alfa-glucosidasa y comprender el papel de características estructurales específicas en su actividad inhibidora.

Métodos: La investigación comenzó con el aislamiento e identificación del nuevo derivado del ácido gálico del extracto de hoja de *Schleichera oleosa*. El compuesto aislado (**1**) se caracterizó como ácido 3,5-dihidroxi-4-metoxibenzoico mediante técnicas espectroscópicas. Se llevaron a cabo estudios *in vitro* para comparar los valores de CI₅₀ del extracto etanólico, el aislado **1** y la acarbosa.

Resultados: El aislado **1** exhibió una potencia inhibidora significativamente mayor contra la enzima α -glucosidasa en comparación con la acarbosa. Posteriormente, se realizaron estudios de acoplamiento molecular para obtener información sobre las interacciones moleculares entre los derivados del ácido gálico y el sitio activo del receptor de la alfa-glucosidasa. Las simulaciones de acoplamiento revelaron que la protección del grupo metilo en el grupo hidroxilo de los derivados del ácido gálico desempeñó un papel crucial en la mejora de su afinidad de unión al receptor de la alfa-glucosidasa. Este conocimiento de la interacción molecular proporciona información valiosa para el diseño de inhibidores de la alfa-glucosidasa más potentes y selectivos basados en esta característica estructural.

Conclusiones: Estos resultados sugieren colectivamente que el derivado del ácido gálico recientemente aislado tiene un potencial prometedor como inhibidor natural de la alfa-glucosidasa para el tratamiento de la diabetes. Una mayor investigación y optimización de este compuesto podría conducir al desarrollo de terapias novedosas y efectivas para el control de la glucemia en personas con diabetes.

Palabras Clave: ácido gálico; acoplamiento molecular; inhibidor de la α -glucosidasa; *Schleichera oleosa*.

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INTRODUCTION

Type 2 diabetes (T2D) is an impairment in the way the body regulates and uses sugar (glucose) as fuel. This long-term (chronic) condition results in too much sugar circulating in the bloodstream. Eventually, high blood sugar levels can lead to circulatory, nervous, and immune disorders. The number of deaths and the consequences for T2D worldwide reached 463 million in 2019. Predictions from the IDF also show that this figure will continue to increase to 700 million in 2045 (Ogurtsova et al., 2022). Treatment development of T2D must significantly improve the quality of life of sufferers and reduce the risk of complications (Taylor et al., 2021). Pharmacological drugs, such as oral hypoglycemics and insulin, and non-pharmacological therapies, such as diet and physical exercise, have been shown to be effective in controlling blood sugar and preventing diabetes complications (Khan and Baig, 2022).

Controlling postprandial hyperglycemia is very important for treating T2D patients. One therapy method that can be performed is to reduce glucose absorption by inhibiting carbohydrate hydrolyzing enzymes, such as α -glucosidase. This enzyme plays an important role in carbohydrate digestion and glucose metabolism. This therapy method is proven to inhibit the development of T2D by 36%, and T2D sufferers can have a longer life expectancy (Usman et al., 2019). Acarbose and miglitol are two α -glucosidase enzyme inhibitors that are recognized and recommended by the WHO and IDF (Joshi et al., 2021). Although these two compounds have high inhibitory activity, the side effects they cause are also not small. Indigestion and flatulence are the main side effects that may result from the use of acarbose therapy (Dong et al., 2022).

Alpha-glucosidase inhibitors are known for their ability to regulate postprandial hyperglycemia by inhibiting the breakdown of complex carbohydrates into glucose. These inhibitors play a crucial role in diabetes management by slowing carbohydrate absorption, thereby controlling blood glucose levels (Kashtoh and Baek, 2022). As the number of people with diabetes rises around the world, it is important to look for natural sources of alpha-glucosidase inhibitors. *Schleichera oleosa* (Lour.) Oken (family *Sapindaceae*) leaves are a good candidate because they have been used traditionally as medicine. Even though *Schleichera oleosa* leaves (known in Indonesia as kesambi) have been used traditionally in herbal medicine to treat diabetes, not much research has been done on how potent *S. oleosa* leaf extracts might work as alpha-glucosidase inhibitors (Muthukrishnan,

2017). Researchers have investigated various parts of the *S. oleosa* plant for their medicinal properties, but limited research specifically focuses on the leaves as a potential source of alpha-glucosidase inhibitors.

The isolation of phytochemicals from *S. oleosa* has also been carried out for the effective active content material in the treatment of T2D. Some common phytochemicals in *S. oleosa* include tannins, flavonoids, alkaloids, saponins, and phenolic acids. These phytochemicals are known to have various medicinal properties, including antioxidant and anti-inflammatory effects (Khandekar et al., 2015). Furthermore, studies have also shown that *S. oleosa* contains fatty acids, including oleic acid, linoleic acid, and linolenic acid (Bhatia et al., 2013). These fatty acids are believed to have a potential role in controlling blood sugar levels and improving insulin sensitivity, making them important for the management of T2D. Further research is needed to determine the other phytochemical compositions of *S. oleosa* and fully understand its potential health benefits.

We can use *in vitro* to understand how active compounds from *S. oleosa* control blood sugar levels in T2D. However, *in vitro drug discovery* has weaknesses compared to computer-aided drug discovery, such as a lack of validity, a limited system, and the potential for inaccurate results. The computer drug discovery technique, for example, the docking technique, is extensively used to predict interactions between active compounds and protein targets in cells. Consideration of the interaction between active compounds and protein targets, along with the demonstration of efficacy and safety in humans or animals, enhances the accuracy and validity of computer drug discovery. Molecular docking is a computer-simulated method for predicting interactions between drug molecules and their protein targets in cells. We have used the molecular docking technique to understand the mechanisms by which compounds control blood sugar levels in T2D (Nursamsiar et al., 2022).

Traditional medicine uses *S. oleosa* leaves, and our research provides scientific validation and understanding of these traditional uses, potentially leading to the development of new medicinal agents. The new isolated glucosidase inhibitor could provide a new approach to the treatment of diabetes, a condition that affects millions worldwide. This compound helps to control blood sugar levels by inhibiting glucose, which is critical for diabetes treatment. The isolation and characterization of new compounds enriches the pharmaceutical industry's arsenal of drugs, particularly natural product-based therapies that often have unique modes of action and reduced side effects. The demonstration of the medicinal value of *S. oleosa*

leaves could stimulate their agricultural cultivation, benefiting local economies and promoting the sustainable use of plant resources.

Ethnomedical records indicate that *S. oleosa* leaves have traditionally been used to treat symptoms associated with high blood sugar levels, such as excessive thirst, frequent urination, and general fatigue (Meshram et al., 2015). These traditional applications suggest that leaves may contain compounds that regulate glucose metabolism. It is known that glucose inhibitors play a crucial role in diabetes management by slowing down the breakdown of carbohydrates into glucose, thus helping to control postprandial blood sugar levels. The traditional use of *S. oleosa* leaves for blood sugar management coincides with the potential presence of these inhibitors. By scientifically validating the inhibitory activity of glucosidase in isolated compounds, our study bridged the gap between ethnomedicine and modern pharmacology. This not only supports the empirical knowledge of traditional healers but also contributes to the development of new natural therapies for the management of diabetes. Our research underscored the importance of integrating traditional medical practices with contemporary scientific approaches to discover new treatments and validate ethnobotanical knowledge.

The research problem addressed in this study was the need for new, effective, and natural glucose inhibitors to treat diabetes, which is currently treated with synthetic drugs that may cause significant side effects. The aim of this study was to investigate the potential of *S. oleosa* leaves, traditionally used in ethnomedicine to treat symptoms associated with high blood sugar, as a source of natural glucosidase inhibitors. To solve this problem, we proposed the following approach: 1. Using solvent extraction methods, we extracted compounds from *S. oleosa* leaves. Extracts were fractionated using chromatography techniques to isolate individual bioactive compounds. 2. The isolated compounds were characterized by spectroscopic methods such as NMR, mass spectrometry, and IR to determine their structures. 3. Isolated compounds were tested for their glucosidase inhibitory activity using standard *in vitro* tests. Their efficacy would be compared to known glucosidase inhibitors such as acarbose. 4. Docking studies were conducted to determine the mode of inhibition of isolated compounds. By identifying and writing about the glucosidase inhibitors found in *S. oleosa* leaves, we hope to give traditional uses of these plants a scientific basis and help the search for natural alternatives to artificial diabetes treatments.

The purpose of this study was to isolate bioactive compounds from *S. oleosa* leaves, identify their chemi-

cal composition, and evaluate their inhibitory activity against alpha-glucosidase. Additionally, molecular docking studies were performed to identify specific structural features of the isolated compounds that contribute to their inhibitory activity. This would aid in the rational design of more potent and selective alpha-glucosidase inhibitors derived from *S. oleosa* leaves.

MATERIAL AND METHODS

Chemicals

The chemicals used were *S. oleosa* leaves, acetic anhydride, HCl, HNO₃, H₂SO₄, distilled water, 70% ethanol, ethyl acetate, chloroform, methanol, *n*-hexane, DPPH solution, Dragendorff reagent, Mayer's reagent, Wagner's reagent, sitoborate reagent, AlCl₃ reagent, silica gel G-60 (0.063-0.200). Sigma-Aldrich (MilliporeSigma, Germany) provided the α -glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20) and PNPG. All other commercially available reagents were used without further purification.

General experimental procedures

The IRspirit FTIR spectrophotometer (Shimadzu Corporation, Japan) was used to obtain IR spectra (KBr). NMR analysis was used to determine the structural conformation, number of protons, and number of carbons in a chemical compound's structure. NMR spectra of ¹H and ¹³C were acquired by nuclear magnetic resonance spectroscopy (Jeol JNM ECA-500, Japan) with chemical shift (δ) provided in parts per million (ppm) relative to TMS as an internal standard. In the context of FTIR, the acronyms s = singlet and br = broad were used. The molecular weight of the reaction product was determined by mass spectrometry. A Thermo Fisher LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, United States) was used to measure the spectra. 60-column silica gel chromatography was used for purification. Thin-layer chromatography was used to monitor both the formation of reaction products and the completion of the reaction (TLC). TLC was performed on Merck 25 TLC aluminum sheets using silica gel 60 F₂₅₄ (Merck KGaA, Germany).

Plant material

Samples of *S. oleosa* leaves were obtained from Merdekayya Village, Takalar Regency, South Sulawesi, Indonesia (5°17'00.6"S 119°27'01.5" E). Specimens of *S. oleosa* plants were determined at the Biology Research Center, Herbarium Bogoriense, National Research and Innovation Agency (BRIN), with specimen number B-132-DI-05.07.

Extraction and isolation

The collected leaves of *S. oleosa* were sorted by wet sorting, then washed with water, and drained. The sample was then chopped and dried using a drying cabinet. The dried simplicial was then powdered using a 40-mesh sieve. The powdered simplicial (1 kg) was macerated using 70% ethanol with a ratio of 1:10 (v/v). Maceration was carried out at room temperature for three days. The filtrate was filtered, and the residue was re-macerated under the same conditions as in the previous step. The filtrate was collected and evaporated using a rotary evaporator to obtain a thick extract (114.88 g). The thick ethanolic extract (15 g) was fractionated by column chromatography with silica gel and successively eluted with *n*-hexane: ethyl acetate (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9, v/v), chloroform and methanol (7:3, 1:1, and 3:7, v/v), and methanol to give four fractions A-D, respectively. The chemical contents of the A-D fraction were monitored using TLC silica gel. Fraction B was further purified by recrystallization to amorphous isolate **1** (63 mg). In this work, we report the isolation of fraction B because fraction B shows two spots on the surface of the TLC silica gel, making it easier to purify using the recrystallization method.

Isolate **1** (3,5-dihydroxy-4-methoxybenzoic acid): brownish white amorphous powder; UV (MeOH) λ_{max} : 217, 261, 285 (sh) nm; IR (KBr) ν_{max} : 462.92, 1082.07, 1460.11, 1743.65, 2854.65, and 2922.16 cm^{-1} ; $^1\text{H-NMR}$ (400 MHz, CD_3OD) δ_{H} 7.038 (2H, s, H-2, H-6), 4.872 (br, OH), and 3.813 (3H, s, OCH_3); $^{13}\text{C-NMR}$ (100 MHz, CD_3OD) δ_{C} 121.45 (C-1), 110.260 (C-2), 139.722 (C-3), 146.453 (C-4), 139.722 (C-5), 110.260 (C-6), 52.261 (OCH_3), and 169.002 (C8); ESIMS (pos.): m/z 207.02553 [$\text{M} + \text{Na}^+$] and m/z 185.04372 [$\text{M} + \text{H}^+$] (calcd. for $\text{C}_8\text{H}_8\text{O}_5$, 184.04).

Biological evaluation

Acarbose and isolate **1** solution with concentrations of 100, 50, 25, 12.5, and 6.25 ppm (30 μL each) were added to 50 μL of phosphate buffer (pH 6.8) and 10 μL of 5 mM *p*-nitrophenyl- α -D-glucopyranoside (pNPG) substrate in a 96-well plate and incubated for 5 minutes at 37°C. Then, 10 μL of 0.5 U/mL α -glucosidase enzyme solution was added, and the mixture was incubated for another 15 minutes at 37°C. After incubation, 100 μL of sodium carbonate was added. Absorbance was measured at a wavelength of 405 nm. The test was replicated three times.

Molecular modeling simulation

Molecular modeling was used to investigate possible targets and their candidate drugs for the treatment of diabetes. The simulation was carried out on a

personal computer equipped with an Intel(R) Core(TM) i7-4600U CPU running at 2.10 GHz and 2.70 GHz, 8.00 GB of installed RAM, 512 GB of SSD, and Windows 10 Pro 64-bit operating system, x64-based processor. AutoDock Tools software was used to perform all docking calculations. The RCSB Protein Data Bank (<https://www.rcsb.org>, 3 January 2023) provided the crystal structures of the α -glucosidase protein with PDB ID 2QMJ. The α -glucosidase protein was separated from all water molecules and natural ligands using Discovery Studio Visualizer 4.5. Using AutoDock Tools, the α -glucosidase protein was further prepared by assigning hydrogen polarities and computing Gasteiger charges on protein structures. Avogadro created the 3D structures of gallic acid derivatives (Snyder and Kucukkal, 2021). Molecular docking simulations were performed using AutoDock4 and AutoDockTools 1.5.6rc3 (Morris et al., 2009). The molecular docking was studied using the published approach (Asnawi et al., 2022; Nursamsiar et al., 2020). The Discovery Studio 4.5 Client was used to visualize the docking findings.

Statistical analysis

All values were presented as mean $\pm$ SEM. The data were analyzed using the fitting of a curve using nonlinear regression and the significance level stated in r^2 .

In silico data analysis

Data obtained at each step were analyzed using a combination of computational and visualization techniques:

1. Validation of the docking method: The docking procedure was validated by redocking known inhibitors into the active site of the target protein. The predicted positions of the inhibitors were then compared to the actual structures seen in the experiments. The RMSD values were computed, and any values below 2 Å were considered indicative of satisfactory validation. The findings were condensed into a table that displayed the root mean square deviation (RMSD) values for each established inhibitor.

2. Chemical interaction of the ligand and the target protein: The ligands were positioned within the active site of the protein using AutoDock Vina. The docking scores, which indicate the strength of binding, were documented. The analysis of interactions was conducted using LigPlot+ to build 2D interaction diagrams and Discovery Studio Visualizer for 3D visualizations. The table provided a comprehensive list of important interactions, including hydrogen bonds and hydrophobic contacts. Additionally, figures were

included to visually depict the 2D and 3D binding postures.

3. Free energy calculation: The MM/GBSA method in the AMBER software suite was employed to compute the binding free energy. The values were calculated and recorded in conjunction with the docking scores for the sake of comparison. A graph illustrating the binding free energy of each ligand was developed to provide a visual comparison of their binding affinities.

RESULTS AND DISCUSSION

Phytochemical profile of the ethanolic extract

The isolation and phytochemical characterization of *S. oleosa* involve the extraction of bioactive compounds from the leaves of *S. oleosa*, followed by the identification and analysis of these compounds using various analytical techniques. Maceration was employed using ethanol as a solvent. Once extraction was complete, the obtained ethanolic extract was subjected to fractionation to classify the phytochemical content according to its polarity. The ethanolic extract and its fraction were subjected to various phytochemical screening tests. These tests aim to identify the presence of different classes of compounds, including alkaloids, flavonoids, phenolic, and terpenoid compounds (Sasidharan et al., 2011). Phytochemical screening involves specific chemical or colorimetric tests that react with the characteristic functional groups of these compound classes.

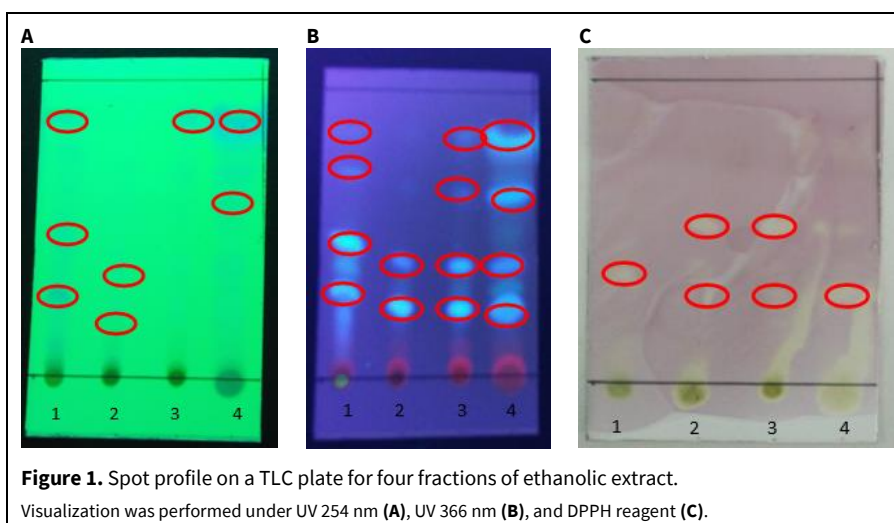
The extraction of 1 kg of dried *S. oleosa* leaf using the maceration method with 70% ethanol yielded 114.8 g (yield = 11.48%) of condensed extract. The initial characterization of the crude ethanolic extract is

a phytochemical screening. The results of the characterization of the main chemical content of this ethanol extract contain secondary metabolites, including flavonoids, tannins, terpenoids, saponins, and phenolics.

TLC profile of fractions

To further characterize the isolated compounds, various analytical techniques were employed. Chromatographic techniques such as thin-layer chromatography (TLC), nuclear magnetic resonance (NMR) spectroscopy, liquid chromatography-mass spectrometry (LC-MS), and infrared (IR) spectroscopy were used to determine the chemical structure of isolated compounds (Pettit et al., 2000). These techniques provide valuable information about the connectivity of atoms, functional groups, and the overall molecular structure.

The ethanolic extract was then fractionated by column chromatography with *n*-hexane: ethyl acetate (3:7) as the eluent and silica gel as the stationary phase (Fig. 1). The results of the fractionation obtained were four fractions, namely fraction 1, fraction 2, fraction 3, and fraction 4 with a crude fraction weight of 1.37, 4.66, 3.69, and 4.50 g, respectively. Visualization of spots on the TLC plate under 254 nm UV light, fraction 1, fraction 2, fraction 3, and fraction 4 have the numbers 3, 2, 1, and 1, respectively. While the visualization of spots on the TLC plate under 254 nm UV light, fractions 1, 2, 3, and 4 have the number of 4, 2, 4, and 4 spots, respectively. The antioxidant activity of the spots in the fractions was evaluated using a DPPH reagent. Visualization of the antioxidant activity of the spots on the TLC plate for fractions 1, 2, 3, and 4 have a number of 1, 2, 1, and 1 spot, respectively.



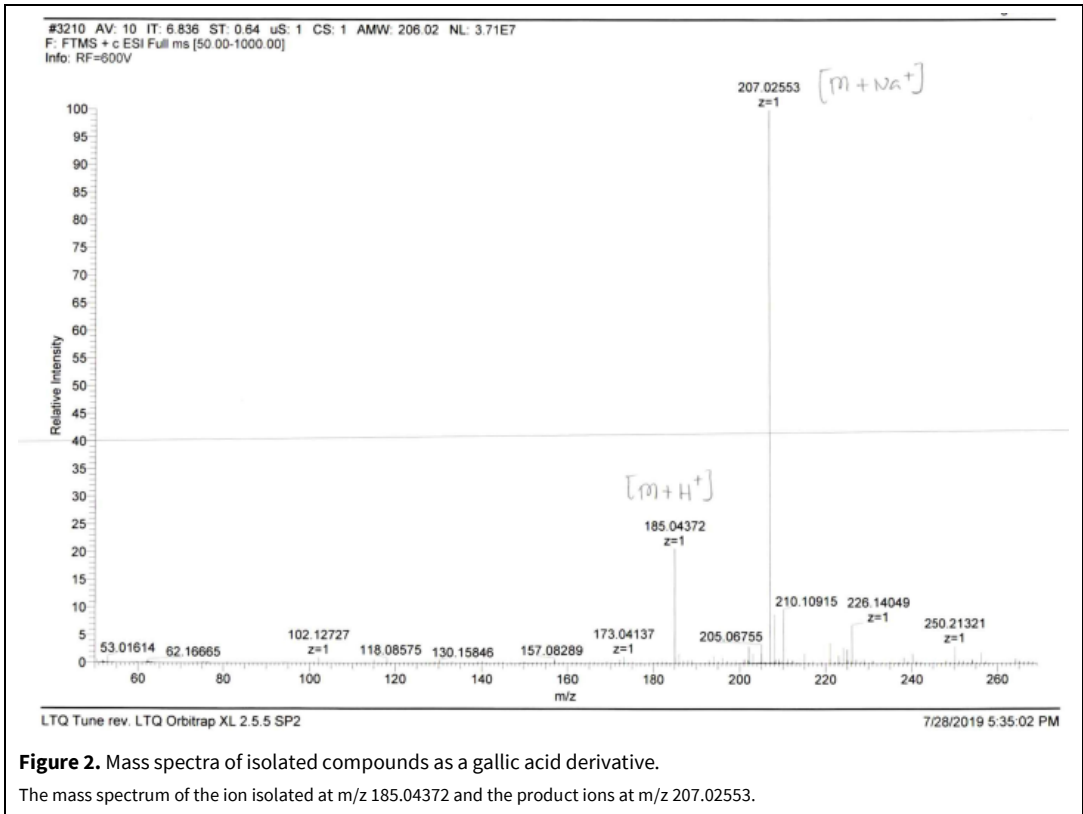


Figure 2. Mass spectra of isolated compounds as a gallic acid derivative. The mass spectrum of the ion isolated at m/z 185.04372 and the product ions at m/z 207.02553.

Characterization of isolate 1 of *S. oleosa* leaves

The TLC profiles of all fractions (Fig. 1) showed that fraction 2 had two spots, both under UV 256 nm and 366 nm, and these two spots had antioxidant activity, as indicated by the appearance of yellow spots on the surface of the TLC plate after being sprayed with the DPPH reagent. Only fraction 2 underwent subsequent isolation, as it contained just two spots, one of which was more dominant than the others. In other words, fraction 2 was isolated with little impurity. This fraction could be purified using a simple re-crystallization method. Other fractions were not reported in this article. Initially, fraction 2 was dissolved in methanol and then evaporated at room temperature, redissolved using *n*-hexane, and continued using chloroform. As a result of the difference in solubility from the addition of solvents of different polarities, the main compound would crystallize first. The recrystallization process was repeated several times to obtain a pure isolate.

ESIMS data from isolate 1 provide information on molecular weight and the presence of specific ions (Fig. 2). The signal m/z of 207.02553 [M + Na⁺] was the ion that represented the molecular weight of the compound with the addition of a sodium ion (Na⁺). The m/z value provided information on the molecular weight, and the presence of the sodium ion suggested that the compound might be capable of forming a complex with sodium. The signal m/z of 185.04372 [M + H⁺] indicated that this ion represents

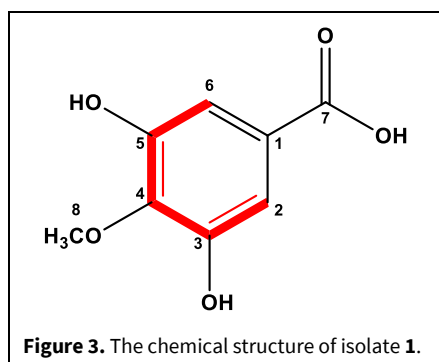
the molecular weight of the compound with the addition of a hydrogen ion (H⁺). The calculated molecular weight for C₈H₈O₅ was 184.04, which closely matched the observed m/z value. This suggested that the compound had a molecular formula of C₈H₈O₅.

Table 1. NMR ¹H and ¹³C spectroscopic data of isolate 1 in CD₃OH₃ (δ_H 7.26. δ_C 77.0 ppm).

Atom positions	Chemical shift	
	δ _H	δ _C
1	-	121.396
2	7.038 (1H. s)	110.260
3	4.872 (1H. br)	139.722
4	-	146.453
5	4.872 (1H. br)	139.722
6	7.038 (1H. s)	110.260
7	4.872 (1H. br)	169.002
8	3.813 (3H. s)	52.261

Characterization of isolate 1 based on proton NMR showed information about the chemical shifts and integrals for various positions of proton and carbon (Table 1 and Fig. 3). Atom position 2, with the proton at 7.038 ppm, was observed as a singlet (s) with an integral value of 1 H. This chemical shift suggested the presence of an aromatic or olefinic proton. Typically, benzene rings or double bonds were found. Atom position 3 with the proton at 4.872 ppm was

labeled as a broad singlet (br) with an integral of 1 H. The broadness indicated the presence of some kind of exchange or dynamic behavior. This signal was commonly observed for protons adjacent to heteroatoms, such as oxygen or nitrogen. Atom position 5 with the proton at 4.872 ppm was also labeled as a broad singlet (br) with an integral of 1 H. This signal was similar to the one observed at position 3, suggesting a similar chemical environment or structural motif. Atom position 6 with another proton at 7.038 ppm was observed as a singlet (s) with an integral of 1 H. Similar to atom position 2. This chemical shift suggested the presence of an aromatic or olefinic proton. Atom position 7 with the proton at 4.872 ppm was again labeled as a broad singlet (br) with an integral of 1 H, indicating a similar chemical environment or structural motif as observed in positions 3 and 5. Atom position 8 with the proton at 3.813 ppm was observed as a singlet (s) with an integral value of 3H. This chemical shift and integral were characteristic of methyl groups (-CH₃) or methoxy groups (-OCH₃).



On the basis of the analysis of the proton NMR data, we proposed a possible structure for the isolate. It appeared to contain two aromatic or olefinic protons (positions 2 and 6) along with three protons in a chemical environment similar to that adjacent to heteroatoms (positions 3, 5, and 7). Furthermore, a methyl group (position 8) was present in the molecule. One possible structure that fit these data was a compound with a benzene ring, two double bonds, and a methyl group. The broad singlets at positions 3, 5, and 7 suggested the presence of oxygen or nitrogen atoms in the molecule. Possible use of the metals as substituents on the benzene ring or as part of a functional group.

Carbon NMR data provided information on the chemical shifts for various carbon positions in the isolate. Atom position 1, with the carbon signal at 121.396 ppm, suggested a carbon atom in a moderately shielded environment. This chemical shift was commonly observed for carbon atoms in aromatic rings, conjugated systems, or carbonyl groups. Atom position 2, with the carbon signal at 110.26 ppm, indi-

cated a carbon atom in a slightly shielded environment. This chemical shift was often associated with carbon atoms in alkene or aromatic systems. Atom position 3, with the carbon signal at 139.722 ppm, suggested a carbon atom in a moderately shielded environment. This chemical shift was consistent with the substitution of carbon atoms in conjugated systems or carbonyl groups. Atom position 4, with the carbon signal at 146.453 ppm, indicated a carbon atom in a relatively deshielded environment. This chemical shift was commonly observed for carbon atoms in carbonyl groups, aromatic rings, or electron-deficient systems. Atom position 5, with the carbon signal at 139.722 ppm, was the same as the signal observed at position 3. This suggested that positions 3 and 5 might be part of the same functional group or structural motif. Atom position 6, with the carbon signal at 110.26 ppm, was the same as the signal observed at position 2. This indicated that positions 2 and 6 might be part of the same functional group or structural motif. Atom position 7, with the carbon signal at 169.002 ppm, suggested a carbon atom in a highly deshielded environment. This chemical shift was often observed for carbon atoms in carbonyl groups, carboxylic acids, or esters. Atom position 8, with the carbon signal at 52.261 ppm, indicated a carbon atom in a shielded environment. This chemical shift was typical for carbon atoms in alkyl groups or aliphatic systems.

On the basis of the analysis of the carbon NMR data, we proposed a possible structure for the isolate. The presence of carbon signals at positions 1, 2, 3, 4, 5, 6, 7, and 8 suggested a molecule with multiple functional groups or structural motifs. A possible structure that fit these data was a compound with an aromatic ring (positions 1, 2, 3, 4, 5, and 6), a carbonyl group (position 7), and an alkyl group (position 8). Carbon signals at positions 1, 2, 3, 4, 5, and 6 indicate the presence of an aromatic system or conjugated pi electrons. The signal at position 7 suggested the presence of a carbonyl group, possibly a ketone or an aldehyde. The signal at position 8 indicated the presence of an alkyl group, possibly a methyl or ethyl group.

Combining information from the ¹H-NMR, ¹³C-NMR, and MS data, we made some initial deductions about the isolates. The ¹H-NMR data suggested the presence of aromatic protons, a hydroxyl group, and a methoxy group. The ¹³C-NMR data supported the presence of aromatic carbon atoms, a carbonyl group, and a methoxy group. MS data suggested a molecular formula for C₈H₈O₅ and provided information on the presence of ions, including the formation of a complex with sodium. These combined findings provided initial indications of the structure of the isolates, sug-

gesting the presence of aromatic compounds with specific functional groups, such as the structure of isolate **1** in Fig. 3.

In vitro alpha-glucosidase

Isolated compounds (**1**) from *S. oleosa* can be further subjected to evaluate for their potential bioactivities as anti-diabetic activities (Anjum et al., 2021). These assays help to understand the therapeutic potential and mechanisms of action of the identified compounds. The isolation and phytochemical characterization of *S. oleosa* was essential for identifying and understanding the chemical constituents responsible for its traditional uses and potential medicinal properties. By isolating and characterizing bioactive compounds, researchers could explore their pharmacological activities, elucidate their mechanism of action, and potentially develop new drugs or therapeutic agents (Hazra et al., 2023).

In pharmacological studies, fitting curves and determination of the IC₅₀ value are important aspects of characterizing the inhibitory potency of compounds against a target enzyme such as alpha glucosidase. The fitting curve analysis allows for a quantitative assessment of the concentration-response relationship, and the IC₅₀ value represents a key parameter in this analysis. Fitting curves are commonly generated by plotting the concentration of the compound on the X-axis against the percentage of enzyme inhibition on the Y-axis. The data points are then fitted to a suitable mathematical model, such as a sigmoidal dose-response curve, using a curve fitting (Tong et al., 2021). The fitted curve provides a visual representation of the inhibitory effect of the compound at a range of concentrations.

The IC₅₀ value is a crucial parameter in pharmacological studies, as it represents the concentration of a compound required to inhibit a specific target enzyme or receptor by 50%. It represents a measure of the potency of the compound, indicating the concentration required for significant inhibition. A lower IC₅₀ value indicates a more potent inhibitor, while a higher IC₅₀ value suggests a weaker inhibitory effect. In the case of gallic acid derivatives as alpha-glucosidase inhibitors, the IC₅₀ value provides valuable information on the inhibitory potency of these compounds and helps to evaluate their potential as antidiabetic agents. Several factors influence the IC₅₀ values of gallic acid derivatives as alpha-glucosidase inhibitors. First, the chemical structure of the derivative plays a crucial role in determining its inhibitory potency. Structural modifications, such as substitutions in the aromatic ring or the addition of functional groups, can significantly impact the interaction with the active site of the enzyme, resulting in variations in IC₅₀ values (Yang et al., 2021).

The ethanolic extract demonstrated an IC₅₀ value at a concentration of 27.35 ppm (Fig. 4). The IC₅₀ value indicated the potency of the extract in inhibiting target activity. A lower IC₅₀ value suggested higher potency, which means that a lower concentration of the extract was required to achieve 50% inhibition. Isolate **1** exhibited an IC₅₀ value at a concentration of 7.55 ppm. The lower concentration of isolate **1** required for 50% inhibition compared to the ethanolic extract suggested that this particular isolate may possess a higher inhibitory potency against the target. Acarbose, which was a reference compound, showed an IC₅₀ value at a concentration of 27.35 ppm. Comparing the IC₅₀ values of the ethanolic extract, isolate **1** and acarbose suggested that isolate **1** had a higher inhibitory potency against the target compared to acarbose.

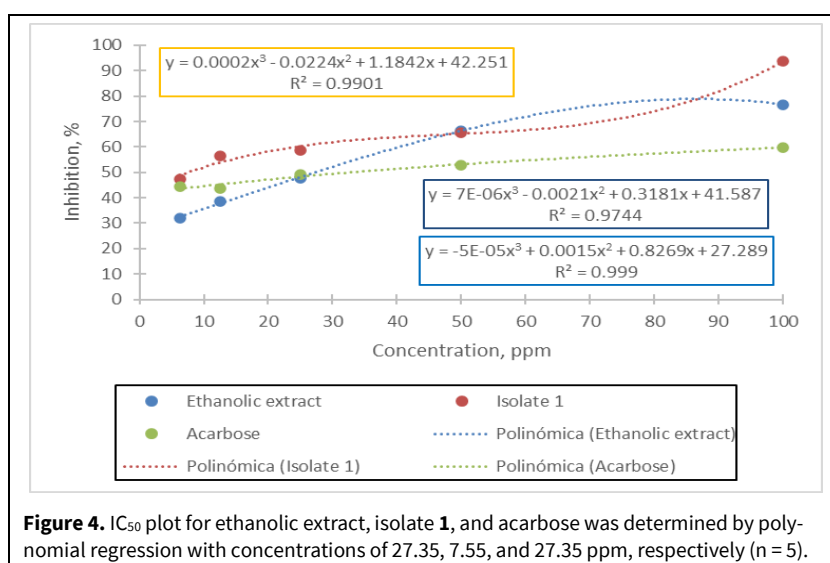


Table 2. The energy of binding and inhibition constant of gallic acid derivatives.

<chem>COc1cc(O)c(O)c(C(=O)O)c1</chem> 1 <chem>COc1cc(OC)c(OC)c(C(=O)O)c1</chem> 2 <chem>Oc1cc(O)c(O)c(C(=O)O)c1</chem> 3				
No.	Ligand	Entry	Estimated free energy of binding (kcal/mol)	Estimated Inhibition Constant, Ki (μM)
1	Acarbose	NL	-5.13	173.15
2	3,5-Dihydroxy-4-methoxybenzoic acid	1	-2.09	29,260
3	3,4,5-Trimethoxybenzoic acid	2	-2.39	17,570
4	Gallic acid	3	-2.53	14,080

Molecular docking

The molecular modeling of gallic acid derivatives as alpha-glucosidase inhibitors have attracted significant attention in the field of drug discovery for the management of diabetes (Nguyen et al., 2023). Alpha-glucosidase is a key enzyme involved in carbohydrate digestion, specifically breaking down complex carbohydrates into absorbable simple sugars. Inhibition of this enzyme can lead to the regulation of postprandial blood glucose levels, making it an attractive target for antidiabetic therapies. Gallic acid derivatives have shown promise as potential alpha-glucosidase inhibitors, and SAR studies have played a crucial role in understanding the relationship between their chemical structures and inhibitory activities (Riyaphan et al., 2021). The gallic acid derivatives, derived from gallic acid through various chemical modifications, exhibit various structural features that influence their interaction with the active site of alpha-glucosidase.

In addition to the SAR studies, computational modeling techniques, such as molecular docking and quantitative structure-activity relationship (QSAR) analyses, have provided valuable insight into molecular interactions between gallic acid derivatives and the active site of alpha-glucosidase (Nag and Majumder, 2023). These computational approaches facilitate the prediction of binding modes, the determination of key structural features responsible for inhibitory activity, and the design of novel derivatives with improved properties.

To compare the strength of the interaction of the gallic acid derivatives, we performed molecular docking by observing the effect of substituents on the aromatic ring for their affinity for the alpha-glucosidase receptor (Table 2).

The results of molecular docking for acarbose as a native ligand (NL), **1**, **2**, and **3** included the estimated free energy of binding and the estimated inhibition

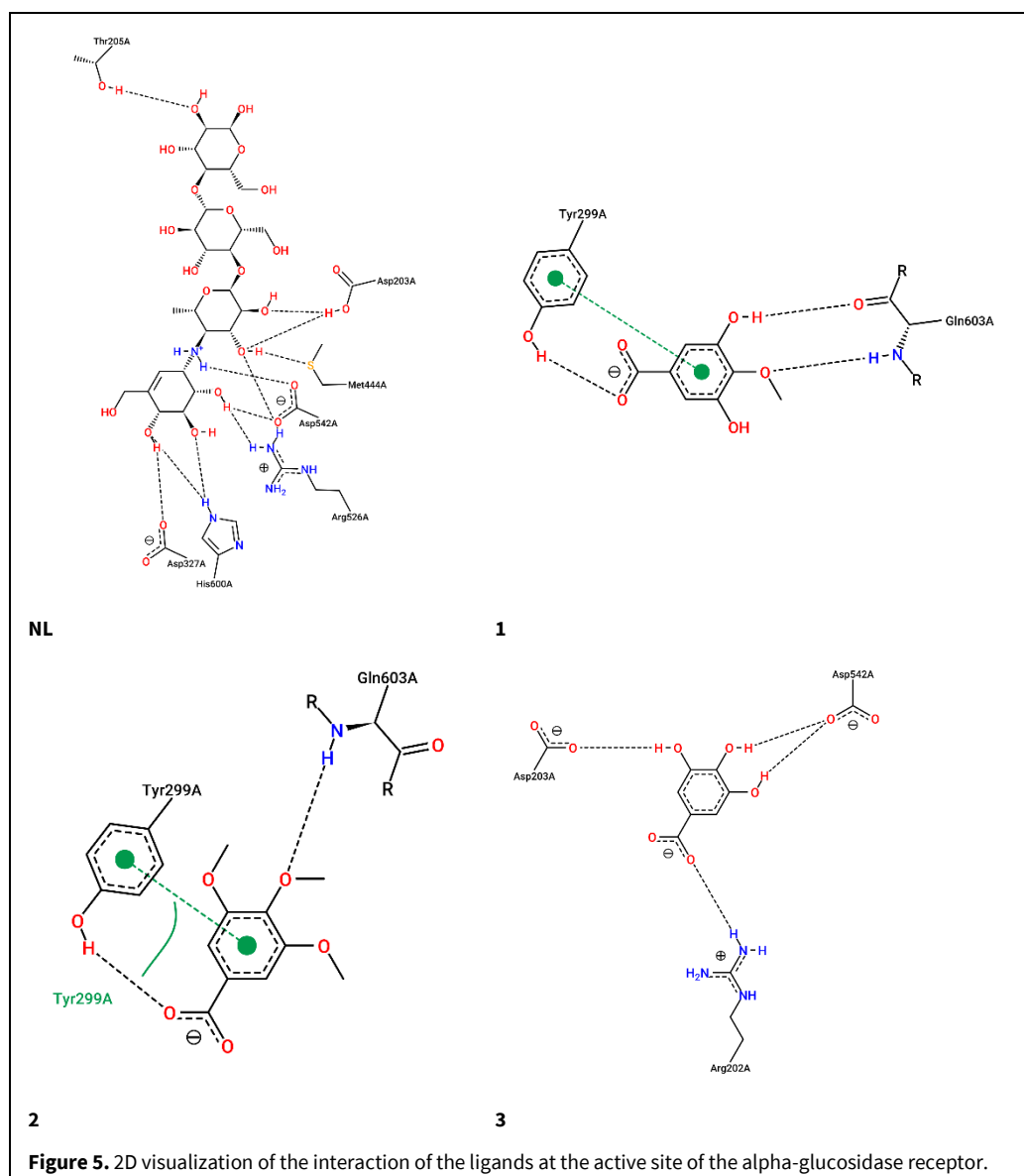
constant (Ki), providing information on the binding affinity and inhibitory potential of each ligand (Table 2). NL exhibited the highest estimated free energy of binding among the three ligands. A higher negative value indicated a stronger binding affinity between the ligand and the target molecule. The estimated Ki of 173.15 μM suggested that NL has a moderate inhibitory potential. Ligand **1** had a less negative estimated free energy of binding compared to NL. This indicated a weaker binding affinity between ligand **1** and the target molecule. The higher estimated Ki of 29.26 mM suggested that ligand **1** had a lower inhibitory potential compared to NL. Ligand **2** also had a less negative estimated free energy of binding than NL, indicating a relatively weaker binding affinity. The estimated Ki of 17.57 mM suggested that ligand **2** had a lower inhibitory potential compared to NL. Ligand **3** had the least negative estimated free energy of binding among the three ligands. This implied a relatively weaker binding affinity compared to NL, **1**, and **2**. However, the estimated Ki of 14.08 mM suggested that ligand **3** has a slightly higher inhibitory potential than ligands **1** and **2**.

Based on the molecular docking data provided, the ligand NL exhibited the highest estimated free energy of binding, indicating a stronger binding affinity compared to ligands **1**, **2**, and **3**. However, ligands **1**, **2**, and **3** had estimated Ki lower than those of NL, indicating that they might have lower inhibitory potentials. Among ligands **1**, **2**, and **3**, ligand **3** showed a slightly higher inhibitory potential compared to ligands **1** and **2**. Overall, the substitution of hydroxy groups with methoxy on the aromatic ring of gallic acid resulted in a decrease in energy affinity because of a reduced hydrogen bonding ability, an increased steric hindrance, and a reduced ability to stabilize the transition state of the enzyme-catalyzed reaction.

Protein-ligand interaction data from molecular docking for ligands NL, **1**, **2**, and **3** explained the in-

teractions between the ligands and the target protein's active site (Fig. 5). Information on hydrogen bonding and hydrophobic interactions formed between ligands and specific amino acids within the protein. Acarbose (NL) forms 11 hydrogen bonds with the amino acids ASP203, THR205, ASP327, MET444, ARG526, ASP542, and HIS600. Acarbose did not form any hydrophobic interactions. Acarbose showed a significant number of hydrogen bonds with various amino acids within the active site of the target protein. These hydrogen bonds contributed to the strong binding between the acarbose and the protein. However, the absence of hydrophobic interactions suggested that the binding may not involve significant nonpolar interactions. Ligand **1** forms 3 hydrogen bonds with the amino acids TYR299 and GLN603 as

well as forms 1 hydrophobic interaction with the amino acid TYR299. Ligand **1** exhibited fewer hydrogen bonds compared to acarbose, but it still formed interactions with important amino acids at the active site. It also formed a hydrophobic interaction with TYR299, which might contribute to the binding affinity between ligand **1** and the target protein. Ligand **2** forms 2 hydrogen bonds with TYR299 and GLN603, as well as 1 hydrophobic interaction with the amino acid TYR299. Similarly to ligand **1**, ligand **2** formed fewer hydrogen bonds compared to acarbose, but it still engaged in interactions with key amino acids at the active site. It also formed a hydrophobic interaction with TYR299, suggesting a possible stabilization of the protein-ligand complex.



Ligand **3** forms 4 hydrogen bonded with the amino acids ARG202, ASP203, and ASP542; and it did not form any hydrophobic interactions. Ligand **3** formed a moderate number of hydrogen bonds with specific amino acids at the active site. The presence of these hydrogen bonds contributed to the binding affinity of ligand **3** to the target protein, although it lacked hydrophobic interactions.

When the interactions of the ligands with the target protein were compared, it could be observed that acarbose, the reference compound, formed a higher number of hydrogen bonds and did not engage in hydrophobic interactions. Ligands **1**, **2**, and **3** formed fewer hydrogen bonds but exhibited hydrophobic interactions with TYR299. This suggested that ligands **1**, **2**, and **3** had the potential to interact with the active site of the protein but might have different binding affinities and modes of interaction compared to those of acarbose.

CONCLUSION

The isolation of 3,5-dihydroxy-4-methoxybenzoic acid as a new gallic acid derivative found in the *Schleichera oleosa* leaves extract had been presented and its activities had been evaluated for both biological and molecular purposes as an antidiabetic. *In vitro* evaluation, by comparing the IC₅₀ values of the ethanolic extract, isolate **1**, and acarbose, suggested that isolate **1** had a higher inhibitory potency against the target compared to acarbose. Furthermore, the molecular docking study showed that the protection of the methyl group on the hydroxy group of the gallic acid derivative played an important role in the interaction with the active site of the alpha-glucosidase receptor.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Nursamsiar	Marwati	Khairuddin	Sami FJ	Kasmawati H	Aligita W	Febrina E	Asnawi A
Concepts or ideas	x							x
Design	x	x	x	x	x	x	x	x
Definition of intellectual content	x	x	x	x	x	x	x	x
Literature search	x	x	x	x	x	x	x	x
Experimental studies	x	x	x	x	x	x	x	x
Data acquisition	x	x	x	x	x	x	x	x
Data analysis	x	x	x	x	x	x	x	x
Statistical analysis	x	x	x	x	x	x	x	x
Manuscript preparation	x	x	x	x	x	x	x	x
Manuscript editing	x	x	x	x	x	x	x	x
Manuscript review	x	x	x	x	x	x	x	x

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