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Original Research Article

Virtual Screening Using a Ligand-based Pharmacophore Model from Ashitaba (*Angelica keiskei* K.) Isolates and Molecular Docking to Obtained New Candidates as α-Glucosidase Inhibitors

Anne Yuliantini[1], Syitha Ocktavyanie[1], Ellin Febrina[2], Aiyi Asnawi[1]*

[1]*Faculty of Pharmacy, Universitas Bhakti Kencana, Jl. Soekarno Hatta No.754, Bandung, West Java, 40617, Indonesia.*
[2]*Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang Km. 21, Jatinangor, Sumedang, West Java, 45363, Indonesia.*

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ABSTRACT

Diabetes mellitus (DM) is a serious, long-term disease when the pancreas doesn't make enough insulin or when the body can't use the insulin it makes well. Type 2 Diabetes Mellitus (T2DM) is a metabolic disorder characterized by elevated blood glucose levels due to insulin resistance and impaired secretion, primarily due to inefficient intestine glucose absorption through the α-glucosidase enzyme. Long-term synthetic drug use can cause issues in the digestive system, kidneys, and liver. Alternative treatments that use herbal products include the ashitaba (*Angelica keiskei* Koidzumi) plant, which has been evaluated as an α-glucosidase inhibitor. The purpose of this study was to use molecular docking and virtual screening to identify potential α-glucosidase inhibitors from Ashitaba (*Angelica keiskei* Koidzumi) isolates using a ligand-based pharmacophore model. The screening methods used were ligand-based virtual screening, docking-based virtual screening, and molecular docking. By using 8 training sets of ashitaba isolates, the best model was obtained with 18 features, including two aromatic ring bonds, nine hydrophobic bonds, three hydrogen bond donors, and four hydrogen bond acceptors. The pharmacophore model and docking-based virtual screening simulations of 270,547 molecules in the ZINC Natural Product database and further investigation using molecular docking yielded (*R*)-*N*-(diaminomethylene)-3-hydroxy-3-((*S*)-6-(4-(hydroxyamino)benzyl)-2-(2-hydroxypropan-2-yl)-4-methoxy-5-oxo-2,3-dihydro-5H-furo[3,2-g]chromen-7-yl)propan-1-aminium (ZINC000085594472), (*R*)-3-((*S*)-4-(cyclopentyloxy)-6-(4-(hydroxyamino)benzyl)-2-(2-hydroxypropan-2-yl)-5-oxo-2,3-dihydro-5H-furo[3,2-g]chromen-7-yl)-*N*-(diaminomethylene)-3-hydroxypropan-1-aminium (ZINC000085594416), and (*S*,*E*)-1-(1-(2-hydroxy-5-(7-(4-hydroxy-3-methoxyphenyl)-2-isopropyl-5-oxohept-3-en-1-yl)-3-((iminio(methylamino)methyl)amino)phenoxy)cyclopentyl)-*N*-methylmethanaminium (ZINC000085597046) as the three top hits with binding energies of -16.09, -15.83, and -15.76 kcal/mol, respectively. In conclusion, the (*R*)-*N*-(diaminomethylene)-3-hydroxy-3-((*S*)-6-(4-(hydroxyamino)benzyl)-2-(2-hydroxypropan-2-yl)-4-methoxy-5-oxo-2,3-dihydro-5H-furo[3,2-g]chromen-7-yl)propan-1-aminium (ZINC000085594472) was a more potential candidate for α-glucosidase inhibitor.

***Keywords*:** α-Glucosidase, Ashitaba, Hits, Inhibitor, Molecular Docking, Virtual Screening.

Introduction

Diabetes Mellitus (DM) is a serious chronic disease that occurs when the pancreas does not produce enough insulin or when the body cannot use the insulin it produces effectively. High blood sugar caused by DM increases the risk of cardiovascular disease and other illnesses. In the United States, 37.3 million people have diabetes, which is 11.3 % of the population. Of these, 28.7 million people have been diagnosed with diabetes, while 8.5 million people are undiagnosed.[1] In 2019, 96 million American adults, which is more than 1 in 3, had prediabetes.[2]

*Corresponding author. E mail: aiyi.asnawi@bku.ac.id
Tel: +62 22 783076

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Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Globally, about 422 million people have diabetes, with the majority living in low- and middle-income countries. Diabetes caused 1.5 million deaths in 2019.[3] Diabetes was the direct cause of 1.5 million deaths in 2019, and 48 % of all deaths due to diabetes occurred before the age of 70 years. Another 460,000 kidney disease deaths were caused by diabetes, and raised blood glucose causes around 20 % of cardiovascular deaths.[4] These statistics highlight the significant impact of DM on individuals and society. The high prevalence of diabetes and prediabetes underscores the need for effective prevention and management strategies. The total number of people living with diabetes is projected to rise to 643 million by 2030 and 783 million by 2045.[5] Over 90 % of people with diabetes have Type 2 Diabetes Mellitus (T2DM), which is driven by socioeconomic, demographic, environmental, and genetic factors. These statistics highlight the significant impact of T2DM on individuals and society. The high prevalence of T2DM underscores the need for effective prevention and management strategies.

Acarbose is a pharmacological therapy for T2DM that works by inhibiting the α-glucosidase enzyme. Acarbose is approved for treating adults with T2DM as an adjunct to diet only or diet and exercise, depending on the patient's health status.[6] Acarbose is not a highly effective agent when used as monotherapy, but it is commonly used in

combination with other medications.[7] The most common adverse effects of acarbose are gastrointestinal upset and bloating, as the drug works in the gastrointestinal system.[8] Acarbose is used to treat T2DM by slowing the action of certain chemicals that break down food to release glucose into the blood, thereby keeping blood glucose from rising very high after meals. Acarbose is available in tablet form and is usually taken three times a day, with each dose taken with the first bite of each main meal.[9]

Ashitaba (*Angelica keiskei* K.) is a medicinal plant that has many benefits. Its root, leaf, and stem are used to make medicine. Ashitaba is a medicinal plant that has been found to have potential benefits for people with diabetes. Some studies have suggested that ashitaba may help people with diabetes by improving blood sugar control, but more research is needed to confirm these findings.[10] The chalcone substances xanthoangelol and 4-hydroxyderricin in ashitaba extracts have powerful insulin-like effects. Ashitaba has been found to have antibacterial, anti-inflammatory, anti-cancer, anti-ulcer, and arterial-relaxing effects, which may offer health benefits for people with diabetes.[11] Ashitaba boosts metabolism and assists in blood sugar regulation, contains chlorophyll which aids in wound healing, helps with weight management, enhances vision, lowers blood pressure, controls cholesterol levels, improves kidney and liver function, and acts as a diuretic that helps the body eliminate excess fluids.[11] Research has shown that ashitaba has anti-diabetic properties and can be used in diabetic diets to help regulate blood sugar and promote wound healing.[10] Some diabetes patients in Japan have reported improvements in blood sugar levels after taking ashitaba for several months.[12] While ashitaba shows promise as a potential treatment for diabetes, more research is needed to fully understand its effects on blood sugar control and other aspects of diabetes management.

In an *in vitro* study, it was found that eight Ashitaba isolates could stop the α-glucosidase enzyme from working.[10] The ashitaba extract and xanthoangelol indicated excellent activity in inhibiting α-glucosidase, with IC_{50} values of $\leq$20 µM for substrate 4-nitrophenyl-α-D-glucopyranoside.[13] The plant Angelica keiskei contains two main physiologically active flavonoid chalcones, 4-hydroxyderricin, and xanthoangelol, which have been found to have α-glucosidase inhibitory activities.[13] These findings suggest that Ashitaba may have potential as a natural therapy for T2DM by inhibiting the α-glucosidase enzyme and improving blood sugar control. However, further research is needed to develop these findings for diabetes management.

Ligand-based virtual screening (LBVS) is a method for developing discoveries for diabetes drugs. LBVS has been used to find new possible sodium-glucose cotransporter type 2 inhibitors, which are a target for treating diabetes.[14] Virtual screening has also been used to find compounds that could be used to treat peroxisome proliferator-activated receptor-gamma (PPAR-γ) for the treatment of diabetic nephropathy.[15] Bioinformatics techniques, such as virtual screening, were used to understand the complexity of medicinal natural product mixtures used to treat diabetes.[16] Molecular docking-based virtual screening was used to predict what compounds could be made from Songga (*Strychnos lucida* R.Br.), an Indonesian native plant, that has potential as antidiabetic agents.[17] In a study by Singh et al. (2013), virtual screening was used to identify new anti-amyloid compounds for the treatment of diabetes.[18] These studies demonstrate the potential of ligand-based virtual screening in identifying potential drug compounds for diabetes management. By using computational methods to screen large databases of compounds, researchers can identify potential drug candidates that can be further tested *in vitro* and *in vivo*.

LBVS is a computational approach that can be used to identify compounds that inhibit the α-glucosidase enzyme and have potential for diabetes management. One effective therapeutic approach for controlling hyperglycemia associated with T2DM is to target α-amylase and α-glucosidase enzymes. α-glucosidase inhibitors stand out as a non-invasive treatment associated with mild, short-lived, and dose-dependent gastrointestinal side effects, including diarrhea, abdominal pain, and flatulence.[19] α-Glucosidase inhibitors, such as acarbose, miglitol, and voglibose, have been found as an alternative treatment for T2DM.[20] These findings suggest that natural compounds, including those found in ashitaba, have potential as a natural therapy for T2DM by inhibiting the α-glucosidase enzyme and improving blood sugar control. By using LBVS, researchers can identify potential drug candidates that can be further tested *in vitro* and *in vivo*.

This research aimed to obtain candidate α-glucosidase inhibitors from a ligand-based pharmacophore model from Ashitaba (*Angelica keiskei* Koidzumi) isolates using virtual screening and molecular docking. Screening α-glucosidase inhibitors from ZINC15 natural product ligand-based virtual screening and molecular docking. By using these methods, it can identify compounds that have the potential to inhibit α-glucosidase.

Materials and Methods

Hardware

The hardware used was an HP Z820 WorkStation Server with the following specifications: 32 GB of random-access memory (RAM), an Intel Xeon E5-2667 Double Processor, a Nvidia® RTX 3060 graphics processing unit (GPU), and a dual system running Ubuntu 22.04 LTS for Pharmagist and Windows 10 Pro-64-bit for molecular docking.

Software

Several software tools, including PharmaGist[21], PyRx 9.0[22], DecoyFinder[23], AutoDock 4.2[24], Discovery Studio Visualizer 2021[25], OpenBabel GUI[23], and Avogadro 1.2.0 [26], were used in the investigation into the potential of eight ashitaba isolates through LBVS to obtain compounds that were potent in inhibiting the α-glucosidase enzyme.

Training set

Eight Ashitaba isolates that have been shown *in vitro* to have α-glucosidase inhibitory activity were utilized to create the training set (Table 1). SMILES data for each compound was used, and it was found on the website pubchem.ncbi.nlm.nih.gov. One notepad file containing the SMILES data for each compound was organized using the writing rule "SMILESspace>compound name" and then saved in *.smi format. The files were then transformed using the OpenBabel GUI program into the *.sdf and *.mol2 formats.

Active dataset

The active dataset was created using data downloaded from the ChEMBL website (https://www.ebi.ac.uk/chembl). CHEMBL3833502 was α-glucosidase. The data in the form of a *.csv file containing the 605 compounds in the code was then downloaded. It then examined 37 compounds using the standard relation "=" and an IC_{50} value ranging from 1 to 100 nM. The SMILES data for these compounds was structured in Notepad using the formatting rule "SMILESspace>ID ChEMBL" before being saved in *.smi format. The files were then converted into *.sdf and *.mol2 formats using the OpenBabel GUI application.

Decoys

The decoy was made with the help of 37 active datasets and drug-like ZINC compounds. This allowed them to prepare the decoys. On the left, the active dataset in *.sdf format was entered, and on the right, the ZINC drug-like file was inserted. The initial search was 1,332 decoys in *.sdf format. The OpenBabel GUI software was used to complete the file's transformation into the *.mol2 format.

ZINC natural product database

The ZINC Natural Product database can be obtained at https://zinc15.docking.org/. The database in *.sdf format was downloaded and converted to *.mol2 format. There are a total of 270,547 chemicals in the database.

Preparation of enzyme structure

The docking target enzyme was PDB ID 2QMJ, which was retrieved from the website https://www.rcsb.org/. In *.pdb format, the proper target protein was downloaded. It was then produced using Discovery Studio Visualizer 2021 by first eliminating water molecules, atoms, and other ions. The macromolecular structures were then separated with native ligands and recorded in *.pdb format.

Generating pharmacophore models
The pharmacophore model was created using the previously prepared *.mol2 training set (Table 1). PharmaGist software was used to generate the pharmacophore model using Ubuntu 22.04. The best model was automatically selected from the PharmaGist program with output in the form 1.pha. The pharmacophore model that was chosen was then utilized to screen the active dataset, decoys, and the ZINC Natural Product database.

Pharmacophore model validation
The website http://stats.drugdesign.fr was used to validate the pharmacophore model. The ROC curve and enrichment curve were what came out of the process. ROC curves and enrichment curves are two types of validation methods that can be used to figure out how well the pharmacophore model works and how often it gives false positives. These methods can help make the virtual screening method more accurate and reliable.

Pharmacophore-based virtual screening
The PharmaGist software was used to screen 36 database files containing a total of 270,547 ZINC Natural Products for a pharmacophore-based virtual screening simulation. The software searched the database for compounds with the same pharmacophore characteristics as the native ligand pharmacophore features, yielding findings in the form of the number of hits (PharmaGist hits).

Docking-based Virtual Screening
Autodock Vina and AutoDock under PyRx software were utilized to accomplish virtual screening. AutoDock Vina is generally faster than AutoDock. AutoDock uses a Lamarckian genetic algorithm, while AutoDock Vina employs stochastic global optimization with a local search strategy. The PharmaGist hits in *.mol2 file format were converted to *.pdbqt format after being energy reduced. The enzyme α-glucosidase (PDB ID 2QMJ) and its native ligand (acarbose) structures were input and grouped as macromolecules and ligands, respectively. The grid box and grid center were then configured at the center of the native ligand of the enzyme α-glucosidase (PDB ID 2QMJ). The results were acquired in the form of binding affinity and analyzed by comparing the binding energy values of hits with the native ligand.

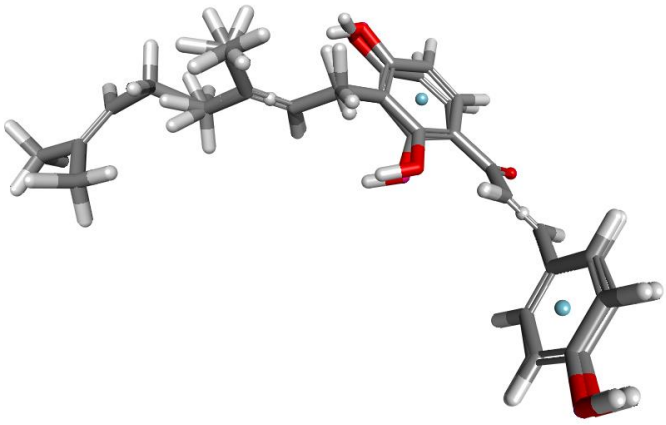
Molecular docking using AutoDock 4.2
The AutoDock hits were docked again using AutoDock 4.2. The docking parameters were determined using the dimensions and coordinates received during the docking procedure's validation stage. Autogrid run was used for simulating docking. Following the completion of the Autogrid process, the Lamarckian Genetic Algorithm (GA) docking parameter was chosen, with the docking parameter number of GA runs being 20 and the number of evals being medium, with a maximum evaluation of 2,500,000. The results were saved as a *.dlg file, and the 30 compounds with the lowest binding energy value relative to the native ligand were chosen for further investigation for interaction and binding energy.

2D and 3D visualization of best hits interaction
A total of 30 best hits obtained from docking using AutoDock 4.2 were visualized in 2D and 3D using Discovery Studio Visualizer 2021 software.

Result and Discussion

Pharmacophore-based virtual screening
Pharmacophore model
Pharmacophore modeling is a widely used method in drug discovery for identifying the critical chemical features of active compounds. The method involves the construction of a pharmacophore model that describes the spatial arrangement of groups for the chemical features of the active site. The pharmacophore model can be assembled to select promising compounds from chemical databases. The pharmacophore features can be hydrogen bond donors, hydrogen bond acceptors, aromatic planes, aliphatic, hydrophobic, positive and negative ionizable groups.[27]
The training sets used in pharmacophore modeling can be composed of compounds with different numbers of atoms and pharmacophore features (Table 1). For example, the first training set used in this research was the isobavachalcone compound, which has 44 atoms with a total of 18 features, consisting of 2 aromatic ring bonds, 9 hydrophobic bonds, 3 hydrogen bond donors, and 4 hydrogen bond acceptors. The second training set used was the xanthoangelol compound, which has 57 atoms and 24 pharmacophore features consisting of 2 aromatic structures, 15 hydrophobic bonds, 3 hydrogen bond donors, and 4 hydrogen bond acceptors. The third training set was the compound 4-hydroxyderricin, which has 47 atoms with 18 features, including 2 aromatic ring bonds, 10 hydrophobic bonds, 2 hydrogen bond donors, and 4 hydrogen bond acceptors. The fourth training set was the demethylsuberosin compound with a total of 31 atoms accompanied by 13 pharmacophore features consisting of 2 aromatic ring bonds, 7 hydrophobic bonds, 1 hydrogen bond donor, and 3 hydrogen bond acceptors. The fifth training set was the compound 3'-senecioyl chelactone, which has the chemical formula $C_{19}H_{20}O_6$.

Table 1: Training set and visualization of the best pharmacophore model

No.	Training set	IC_{50} (µM)	Visualisasi Model Farmakofor Terpilih (*Best Pairwaise*)
1.	Isobavachalcone	20.32	
2.	Xanthoangelol	11.54	
3.	4-hydroxyderricin	33.76	
4.	Demethylsuberosin	9.51	
5.	3’-senecioyl khellactone	90.36	
6.	Munduleaflavanone A	39.90	
7.	(10*S*,15*R*,*Z*)-10,15-Dihydroxyheptadeca-8,16-dien-11,13-diynylacetate	53.26	
8.	Falcarindiol	19.13	

This compound has 45 atoms with 18 pharmacophore features consisting of 11 hydrophobic bonds, 1 hydrogen bond donor, and 6 hydrogen bond acceptors. The sixth training set was the compound munduleaflavanone A, which has 47 atoms. This compound is composed of 15 pharmacophore features, including 2 aromatic ring bonds, 8 hydrophobic bonds, 1 hydrogen donor bond, and 4 hydrogen bond acceptors. The seventh training set was the compound (10*S*,15*R*,*Z*)-10,15-dihydroxyheptadeca-8,16-dien-11,13-diynylacetate with a total atomic arrangement of 49, which has 18 pharmacophore features including 12 hydrophobic bonds, 2 bond donors hydrogen, and 4 hydrogen bond acceptors. The eighth training set was the falcarindiol compound with a total of 43 atoms which is composed of 17 pharmacophore features including 13 hydrophobic bonds, 2 hydrogen bond donors, and 2 hydrogen bond acceptors.

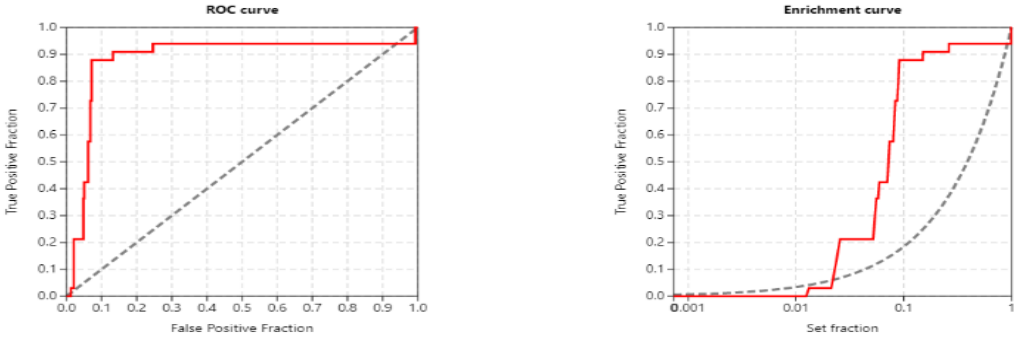
PharmaGist is a web server for ligand-based pharmacophore detection that can be used to identify the critical chemical features of active compounds.[21] In this research, various combinations of pharmacophore features from 8 training sets were carried out by PharmaGist to produce optimal features. PharmaGist searches for models with the most similar features, starting from base eight combinations to base three yielding as many as 10 combinations each. However, this combination did not produce suitable pharmacophore features, so the best pharmacophore model was produced, which was built from a combination of two compounds. The selected pharmacophore model was the *.pha model, indicating that it is the best model produced by PharmaGist for screening compounds in the database. The model score obtained was 19.3613, which shows a general similarity between the database and the training set. Of the eight training sets, the selected model was composed of 2 training sets, namely training set 1 and training set 2, respectively, the isobavachalcone and xanthoangelol (Table 1). The model was composed of 18 features, including 2 aromatic ring bonds, 9 hydrophobic bonds, 3 hydrogen bond donors, and 4 hydrogen bond acceptors. This feature was used to select the best compound to proceed to the validation stage or the next stage. If we compare the IC_{50} of isobavachalcone and xanthoangelol have values of 20.32 and 11.54 µM, respectively, and were classified as having very strong activity.

Pharmacophore model validation

Pharmacophore model validation is necessary to obtain authentic pharmacophore analysis and evaluate the quality of the molecular model. To validate the pharmacophore model generated, the active and decoy datasets were used. The pharmacophore model generated in this study was validated before database screening to evaluate whether the obtained model was able to differentiate between active compounds and decoys.[28] The validation of the pharmacophore model was carried out on the website http://stats.drugdesign.fr/ using a total of 37 active dataset compounds. However, the validation results showed that only 33 compounds could be validated. Apart from that, 1,332 decoy compounds were used. The pharmacophore model validation results were obtained in Figure 2.

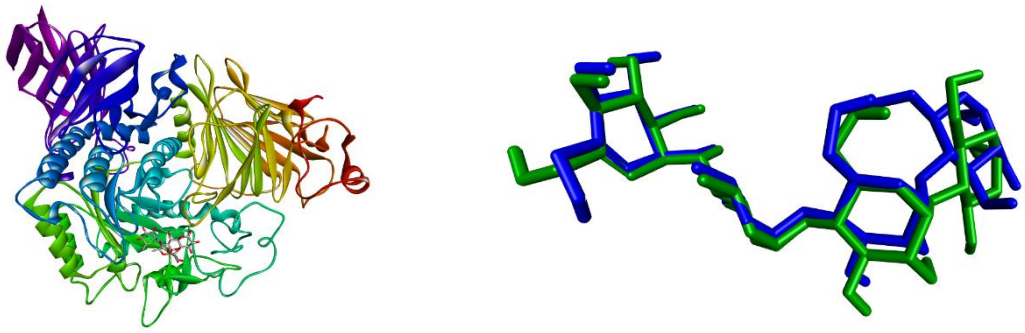
The Receiver Operating Characteristic (ROC) curve is a widely used parameter in virtual screening validation. The ROC curve shows the model's ability to differentiate between true positive compounds and false positive compounds.[29] The ROC curve applied to virtual screening analysis is a plot of the fraction of true positive compounds (y-axis) and false positive compounds (x-axis) for all compounds in the data set. Qualitatively, the closer the curve is to the upper left corner, the higher the overall test accuracy. Based on the validation results, an AUC value of 0.883 was obtained from the ROC curve (Figure 1a). The value obtained was greater than the minimum requirement, namely greater than 0.5, and was included in the good category for distinguishing compounds.[30]

The Enrichment Factor (EF) is a parameter that is often used in virtual screening validation to measure the model's ability to measure genuine positive compounds in proportion to the ratio of all active compounds in the entire database. The maximum EF that can be produced was 41.36 with the condition that it is more than 1 (Figure 1b). The values obtained indicate that the model can be used well. Both parameters have met the requirements for pharmacophore model validation parameters. Therefore, the obtained pharmacophore model can be used for virtual screening simulations.



(a) (b)

Figure 1: Characteristics of the pharmacophore model. (a) ROC curve and (b) enrichment curve



a b

Figure 2: Visualization of re-docking of the native ligand (acarbose) into the binding site of the enzyme structure of α-glucosidase (PDB ID 2QMJ). The structure of α-glucosidase (PDB ID 2QMJ) (a). Overlaying of the native ligand X-ray crystallography (Green) and native ligand resulting from re-docking (Blue) (b).

Pharmacophore-based virtual screening simulation

Virtual screening of the database with pharmacophore models was carried out using PharmaGist software. The virtual database screening was carried out on 36 database files with a total of 270,547 ZINC Natural Product compounds. The screening results obtained a total of 260,301 compounds with varying scores. The score obtained from the screening results indicates the similarity of the compound to the pharmacophore model. A higher score indicates that the compound has better activity against the target macromolecule when adapted to the desired environment. From each of the 36 databases, compounds with a fit score of more than 44.4802 were selected so that a total of 1,000 hits were obtained. These hits were further filtered using Vina Wizard in the PyRx Screening Tool 0.8 software to obtain better compounds.

Virtual screening using AutoDock Vina

Virtual screening was further carried out using the AutoDock Vina under the PyRx Screening Tool 0.8 software. Virtual screening was carried out by setting the grid maps with grid box sizes x = 25, y = 25, and z = 25, while the grid centers used were x = -21.184 Å, y = -6.491 Å, and z = -5.049 Å.

After virtual screening using PharmaGist, a total of 1000 best hits were filtered again using Vina Wizard in the PyRx Screening Tool 0.8 software to obtain better compounds. The main results of the virtual screening process were the best-predicted results and the corresponding binding affinity values. Binding affinity is a measure of the ability of a drug to bind to a receptor, or vice versa. Negative values for binding affinity or binding free energy indicate that the ligand is expected to bind to the target macromolecule. The more negative the numerical value for the binding affinity, the better the prediction of binding between the ligand and the macromolecule. This value was compared with the value of the native ligand, namely acarbose. The final result was called Vina Hits with a total of 460 hits that are better than the native ligand. The hits obtained were continued with virtual screening with AutoDock.

Virtual screening using AutoDock

Binding site locations

The binding site location of the molecular docking for AutoDock 4.2 was determined by redocking the macromolecule with its native ligand. The acceptance parameter is a Root Mean Square Deviation (RMSD) value < 2.0 Å. The RMSD value describes the suitability of the ligand position from the crystallography results compared to the ligand from the re-docking results. In obtaining the expected results, an algorithm method was used during the validation process, namely the Lamarckian Genetic Algorithm (LGA). This method is a combination of local search methods and genetic algorithms. This method was chosen because LGA is more accurate, efficient, and optimal than other methods. Validation was carried out using a number of GA runs of 20, where once the molecular docking process is carried out it will produce 20 interaction conformations between the ligand and the macromolecule. There is also a medium number of evaluations with a maximum evaluation of 250,000. The location of the binding site had a grid box size of 50 Å × 50 Å × 50 Å with coordinates -21.727 Å, -6.323 Å, and -5.281 Å with an RMSD value of less than 2.0 Å (Figure 2). The provided search results include several resources that can be used to learn more about molecular docking and its applications in drug discovery.

Visualization of the re-docking results shows the position of the native ligand before and after re-docking (Figure 3). This aims to see the similarity of the re-docked ligand structure. Based on the results of molecular tethering between native ligands and macromolecules, the best conformation was obtained with an RMSD value of 0.95 Å in the 6th run with a bond energy (ΔG) value of -8.43 kcal/mol and an inhibition constant (Ki) value of 659.88 nM. In addition, the interaction between the native ligand and the receptor before and after re-bonding (Figure 2a). There is a difference in the position of the native ligand after redocking. However, the results of the interaction of native ligands with receptors are not much different.

The study utilized molecular docking simulations to analyze the conformational structure of the ligand before and after docking, and the RMSD values were compared to evaluate the similarity of the re-docked ligand structure.[31]

Changes in the position of the native ligand into the binding site of the enzyme structure of α-glucosidase (PDB ID 2QMJ) (Fig. 3) affect the results of ligand-receptor interactions. There is a difference in the number of hydrophobic bonds and hydrogen bonds produced from the amino acid residues. In obtaining activity as an α-glucosidase inhibitor, ligand-receptor interactions involve amino acid residues on the active side of the receptor that plays a role in this activity. The binding of acarbose on the active side involves multiple hydrogen bonds through Asp327, Asp542, His600, and Arg526. Additional residues lining the sugar-binding site are Asp443, Tyr299, Ile328, Ile364, Trp441, and Met444. One of the differences can be seen in the number and type of hydrogen bonds. There are six hydrogen bonds formed between the native ligand-receptor before tethering, including Arg526, Asp203, Asp327, Asp542, His600, and Met444. In the ligand-receptor interaction after re-redocking, eight hydrogen bonds were obtained with two different hydrogen bonds, namely Asn207 and Thr205. However, both bind to amino acids on the active side, namely Asp327, Asp542, His600, and Arg526. The study utilized molecular docking simulations to evaluate the interaction between curculigoside A and its derivatives with α-glucosidase identified the stability of the binding interaction and the role of Asp542 residue in the stability of the binding interaction.[32]

Virtual screening simulation using AutoDock

A total of 460 VINA hits were further screened using AutoDock 4.2 and used the free energy bonding (ΔG) to filter the hits. The ΔG value of the 30 hits was sorted (Table 2) with the smallest value compared to the native ligand to proceed to the interaction visualization.

Analysis of the form of interaction that occurs between the 2QMJ protein with native ligands and 30 test ligands (Table 2) was carried out using Discovery Studio Visualizer 2021 software. The screening results of the three top hits were further analyzed for their binding energy (ΔG), hydrogen bonding, and other interaction patterns with amino acid residues on the active side of the 2QMJ protein (Fig. 4). Hydrogen bonds are generally considered to be facilitators of protein-ligand binding.[33] The free energy for hydrogen bonding can vary between -1.5 kcal mol^{-1} to -4.7 kcal mol^{-1}, and the contribution of a hydrogen bond to binding can be modest if the new interaction formed does not outweigh the desolvation penalty upon ligand binding.[34] Hydrogen bonds between C-H groups adjacent to an ammonium cation and an oxygen atom (N^+-C-H···O hydrogen bonds) in protein-ligand complexes can contribute significantly to the formation of protein-ligand complexes and the activity of the ligand.[35] Hydrogen bonds regulate molecular interactions via a donor-acceptor pairing mechanism that minimizes the free energy of the system.[33] Electrostatic interactions, such as ionic bonds, play a key role in determining protein-ligand binding affinity and selectivity.[36] Van der Waals interactions, such as π/π interactions, also play a critical role in ligand-receptor interactions.[35]

Acarbose, a known α-glucosidase inhibitor, exhibits a binding energy of -1.25 kcal/mol in its interaction with specific residues within the target protein. This negative binding energy value indicates a favorable interaction, as it represents the energy released when acarbose binds to its target. The interaction involves a total of 12 hydrogen bonds, demonstrating a high level of specificity and affinity between the ligand (acarbose) and the protein. These hydrogen bonds are formed with amino acid residues Arg526, Asn207, Asp203, Asp327, Asp542, His600, Met444, and Thr205. Hydrogen bonds are crucial in molecular recognition and play a significant role in the stabilization of the ligand-protein complex.

In addition to hydrogen bonds, acarbose also forms 2 hydrophobic bonds with amino acid residues Phe575 and Trp406. Hydrophobic interactions are essential for maintaining the structural stability of proteins and can contribute to ligand binding. Furthermore, the interaction between acarbose and its target involves 8 van der Waals interactions. These van der Waals forces are weaker than covalent bonds but are vital for close-range interactions, contributing to the overall stability of the ligand-protein complex. The amino acid residues involved in this van der Waals interaction include Ala576, Arg598, Asp443, Ile328, Ile364, Thr544, Trp441, and Trp539. The combination of these various types of interactions highlights the intricate nature of the binding between acarbose and the target protein, emphasizing the

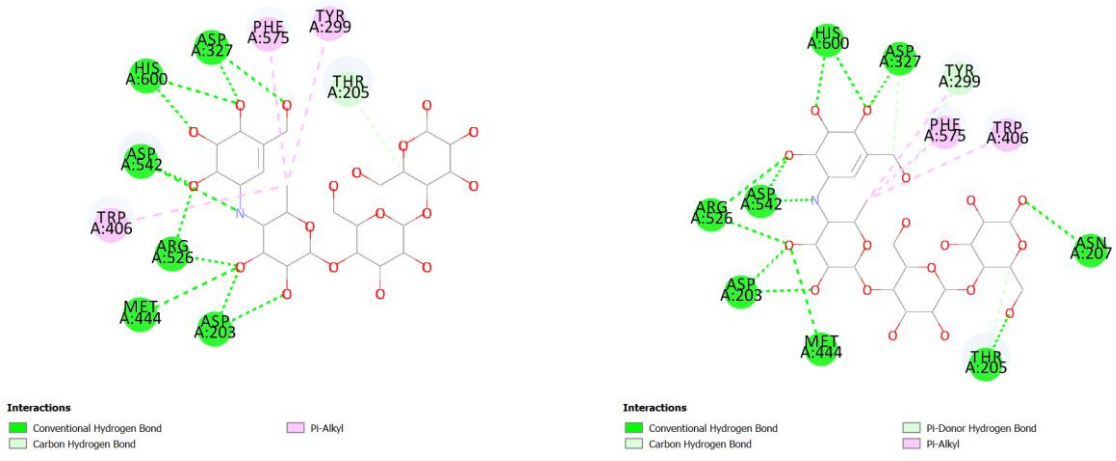
importance of both specific and non-covalent interactions in the ligand's mechanism of action.

The ligand ZINC000085594472 demonstrates a remarkable binding energy of -16.09 kcal/mol when interacting with its target protein, signifying a highly favorable and robust binding affinity. This exceptional binding energy value reflects the strength of the interaction and the energy released upon binding. The interaction involves 9 hydrogen bonds, showcasing a strong and specific engagement between ZINC000085594472 and the protein. These hydrogen bonds are formed with amino acid residues Arg202, Asp203, Asp327, Asp443, Asp542, Gln603, and Tyr605, highlighting the precise molecular recognition and the role of hydrogen bonding in stabilizing the ligand-protein complex. In addition to hydrogen bonds, ZINC000085594472 forms a single hydrophobic bond with Tyr299, which contributes to the structural stability and specificity of the ligand-protein interaction. Furthermore, this interaction comprises 12 van der Waals interactions, which are essential for maintaining close-range contacts and overall complex stability. These van der Waals forces are formed with amino acid residues Arg526, Gly604, His600, Ile328, Ile364, Leu473, Lys480, Met444, Phe575, Thr205, Trp406, and Trp441, emphasizing the intricate nature of the binding and the combined effects of various types of interactions. The impressive binding energy, the number of interactions, and the specific amino acid residues involved in this interaction underscore the strength and specificity of the ZINC000085594472-protein binding, which is crucial for its biological activity or potential therapeutic use.

ZINC000085594416, a ligand interacting with a specific protein, demonstrates a substantial binding energy of -15.83 kcal/mol, reflecting a robust and highly favorable binding affinity. This binding energy value underscores the strength of the interaction and the energy released upon the ligand's binding to its target. The interaction includes the formation of 9 hydrogen bonds, signifying a precise and strong molecular engagement between ZINC000085594416 and the protein. These hydrogen bonds are established with amino acid residues Arg202, Asp203, Asp327, Asp443, Asp542, Gln603, and Tyr605, highlighting the significance of hydrogen bonding in stabilizing the ligand-protein complex and enabling molecular recognition.

Table 2: Binding of energy of the first 30 selected hits

Hits	Binding Energy (kcal/mol)	Hits	Binding Energy (kcal/mol)
ZINC000085594472	-16.09	ZINC000085597019	-12.39
ZINC000085594416	-15.83	ZINC000085596043	-12.32
ZINC000085597046	-15.76	ZINC000150349237	-11.93
ZINC000085597063	-15.27	ZINC000085568320	-11.90
ZINC000085597069	-15.10	ZINC000067870596	-11.89
ZINC000085629766	-14.66	ZINC000225465225	-11.84
ZINC000085629826	-14.54	ZINC000103532338	-11.51
ZINC000085631149	-14.16	ZINC000085568304	-11.36
ZINC000085632229	-13.72	ZINC000085568387	-11.36
ZINC000085631180	-13.29	ZINC000103572813	-11.33
ZINC000065074636	-12.72	ZINC000085592803	-11.10
ZINC000085632352	-12.60	ZINC000225473989	-10.97
ZINC000085625506	-12.52	ZINC000225425165	-10.81
ZINC000085631085	-12.52	ZINC000085625945	-10.74
ZINC000150349226	-12.41	ZINC000225476032	-10.56



a b

Figure 3: Visualization of native ligand interaction analysis into the binding site of the enzyme structure of α-glucosidase (PDB ID 2QMJ). (a) Before re-docking and (b) After re-docking.

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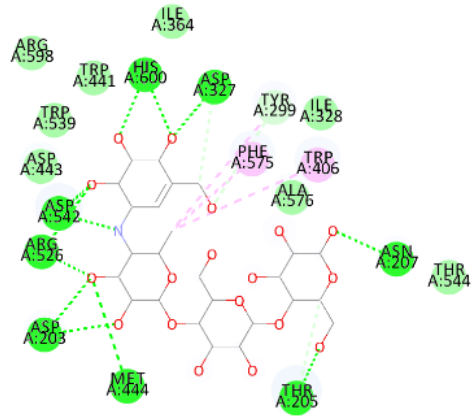
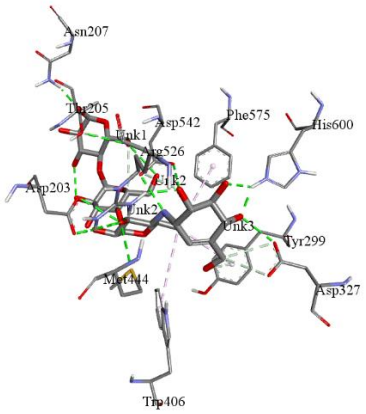
In addition to hydrogen bonds, ZINC000085594416 forms a single hydrophobic bond with Tyr299, contributing to the structural stability and specificity of the ligand-protein interaction. Furthermore, this interaction encompasses 12 van der Waals interactions, which are crucial for maintaining close-range contacts and overall complex stability. These van der Waals forces are formed with amino acid residues Arg526, Gly602, His600, Ile328, Ile364, Leu473, Lys480, Met444, Phe575, Thr205, Trp406, and Trp441, emphasizing the intricate nature of the binding and the combined effects of various types of interactions. The notable binding energy, the multitude of interactions, and the specific amino acid residues involved in this interaction collectively highlight the strength and specificity of the ZINC000085594416-protein binding, which is essential for its potential biological activity or therapeutic application.

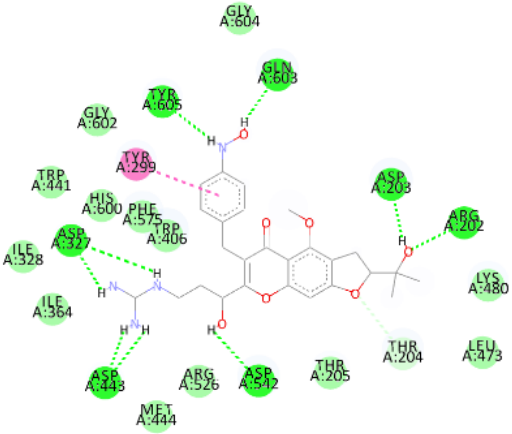
ZINC000085597046, a ligand interacting with a specific protein, demonstrates a substantial binding energy of -15.76 kcal/mol, indicating a strong and favorable binding affinity. This binding energy value reflects the energy released when the ligand binds to its target protein, highlighting the strength of the interaction. In this case, the interaction consists of 4 hydrogen bonds, indicating a selective and robust molecular engagement between ZINC000085597046 and the protein. These hydrogen bonds are formed with amino acid residues Asp203, Asp542, and Tyr299, emphasizing the importance of hydrogen bonding in stabilizing the ligand-protein complex and facilitating molecular recognition.

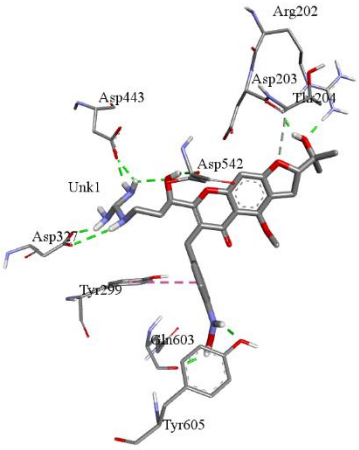
2D **3D**



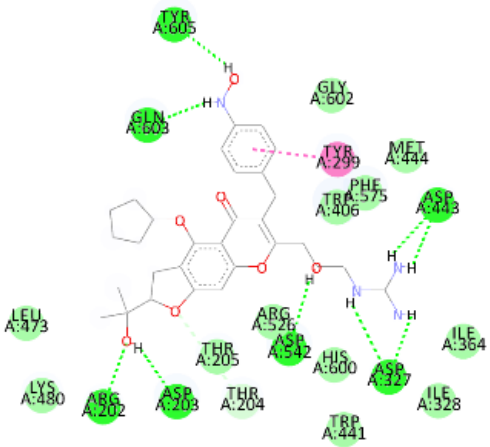


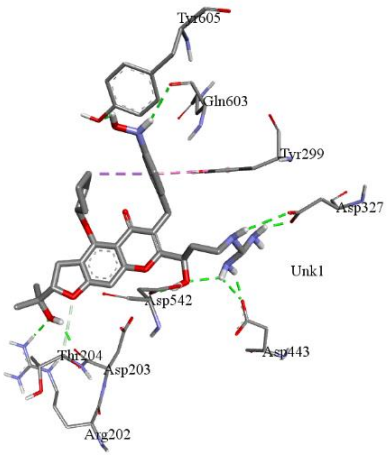
Acarbose





ZINC000085594472





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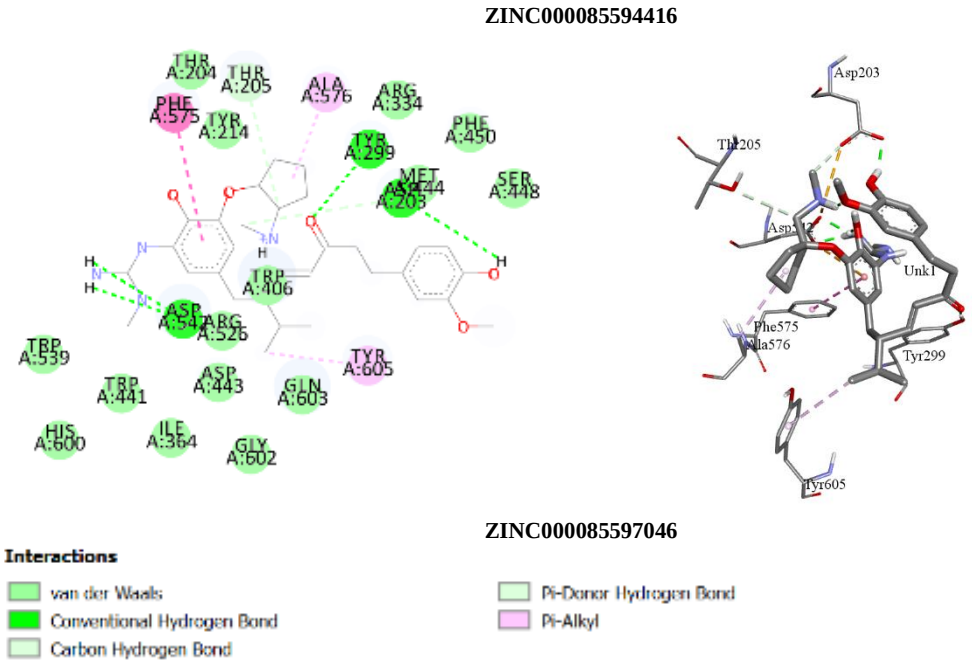

Figure 4: Visualization of 2D and 3D interactions for the top three hits.

In addition to hydrogen bonds, ZINC000085597046 forms 3 hydrophobic bonds with amino acid residues Ala576, Phe575, and Tyr605, contributing to the structural stability and specificity of the ligand-protein interaction. These hydrophobic interactions play a crucial role in the overall binding process. Furthermore, the interaction comprises 14 van der Waals interactions, which are vital for maintaining close-range contacts and stabilizing the complex. These van der Waals forces involve amino acid residues Arg334, Arg526, Asp443, Gln603, Gly602, His600, Met444, Phe450, Ser448, Thr204, Trp406, Trp441, Trp539, and Tyr214. The notable binding energy, the variety of interactions, and the specific amino acid residues involved in this interaction collectively highlight the strength and specificity of the ZINC000085597046-protein binding, which is essential for its potential biological activity or therapeutic application.

The binding energy, number of interactions, and amino acid residues involved in the interactions of acarbose, ZINC000085594472, ZINC000085594416, and ZINC000085597046 with their target proteins offer valuable insights into their respective binding affinities and specificities. Acarbose, while exhibiting a modest binding energy of -1.25 kcal/mol, forms a remarkable 12 hydrogen bonds with amino acid residues Arg526, Asn207, Asp203, Asp327, Asp542, His600, Met444, and Thr205. This extensive hydrogen bonding demonstrates the high specificity of the interaction and the pivotal role of these bonds in stabilizing the ligand-protein complex. Additionally, acarbose engages in 2 hydrophobic bonds with Phe575 and Trp406 and 8 van der Waals interactions with amino acid residues such as Ala576, Arg598, and Thr544. These multiple interaction types contribute to the overall binding strength and specificity.

In contrast, ZINC000085594472, ZINC000085594416, and ZINC000085597046 display significantly higher binding energies, ranging from -15.76 to -16.09 kcal/mol. ZINC000085594472 forms 9 hydrogen bonds with Arg202, Asp203, Asp327, Asp443, Asp542, Gln603, and Tyr605, indicating a strong and specific interaction. In addition to 12 van der Waals interactions, it forms a single hydrophobic bond with Tyr299. ZINC000085594416 also engages in 9 hydrogen bonds with a similar set of amino acid residues and shares the same number of van der Waals interactions as ZINC000085594472, highlighting their comparable specificities. Both ligands form a single hydrophobic bond with Tyr299. ZINC000085597046, on the other hand, forms 4 hydrogen bonds with Asp203, Asp542, and Tyr299, as well as 3 hydrophobic bonds with Ala576, Phe575, and Tyr605. This ligand establishes a substantial 14 van der Waals interactions with the protein, including amino acids like Arg334, Gly602, and Tyr214.

The comparison of these ligands illustrates that while acarbose exhibits a relatively lower binding energy, its multiple interaction types, including hydrogen bonds, hydrophobic bonds, and van der Waals interactions, contribute to its overall binding strength. In contrast, ZINC000085594472, ZINC000085594416, and ZINC000085597046 have substantially higher binding energies, suggesting stronger affinities. They share a commonality in the number of hydrogen bonds and van der Waals interactions, with hydrophobic bonds playing a minor role. The selection of a specific ligand for a given application would depend on the desired binding strength and the specific molecular interactions needed to achieve the intended biological or therapeutic effect.

Conclusion

The *α*-glucosidase inhibitory activity of Ashitaba (*Angelica keiskei* Koidzumi) isolates has been characterized and evaluated *in silico* utilizing a ligand-based pharmacophore model and a molecular docking study. The pharmacophore model and docking-based virtual screening simulations of 270,547 molecules in the ZINC Natural Product database yielded 451 hits. The ZINC000085594472, ZINC000085594416, and ZINC000085597046 were three top hits using molecular docking for 30 best-hits, with binding energies of -16.09, -15.83, and -15.76 kcal/mol, respectively. It denotes the need for additional research and treatment breakthroughs, as well as additional experimental validation.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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