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# Molecular Docking of *Rhus Longipes* Leaves Phytocompounds Against Dipeptidyl Peptidase IV (DPP4) and Alpha-Amylase Protein Associated with Diabetes Mellitus

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## *Abstract*

*In southwestern Nigeria, Rhus longipes is famous for its traditional medicinal uses. In our latest study, we presented GC-MS analysis, antioxidant activity, and the determination of components of the methanol extract of this plant. Using in silico methods, the present work aimed to perform molecular docking and ADME/drug screening on the newly discovered potentially active candidate compounds obtained from the methanol extract of Rhus longipes leaves. Using PyRx software, the drugs were molecularly attached to the target proteins amylase and dipeptidyl peptidase-IV. The online tool SwissADME was used to calculate physicochemical, ADME, and drug-like properties. With significant binding interactions between the compounds ferulic acid, 4-hydroxycoumarin, estriol, miglitol (a standard amylase inhibitor), sitagliptin (a standard dipeptidyl peptidase-IV inhibitor), and ferulic acid, the result has confirmed the antidiabetic properties of the plant leave extract. 2,2-dibromocholestanone, deoxymorellin, cholest-2-eno(2,3-B)-quinoxaline, and on the active sites of two target proteins. Six compounds (ferulic acid, 4-hydroxycoumarin, estriol, cholest2eno(2,3B)quinoxaline, deoxymorellin, and 2,2-dibromocholestanone) produced the best binding sites compared to the target proteins, and four of them performed well, showing that the drug is relatively optimal chemical and medicinal properties. Accordingly, the present study concluded that these chemicals may have contributed to the reported antidiabetic properties of these plants. They can now be studied further as potential pharmaceuticals or drug-like molecules.*

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## Introduction

A metabolic disease with multiple etiologies, diabetes mellitus (DM) is typified by aberrant metabolism of fat, carbohydrates, and proteins due to defects in insulin action, synthesis, or both (Latti, Kalburge, Birajdar, & Latti, 2018). Type 1 and type 2 diabetes are two of the most complex heterogeneous disorders, according to Tuomi, Santoro, Caprio, Cai, & Weng (2014) and Yi, Huang, & Zhou (2016). In the past, clinical characteristics like age of onset, presence of ketosis, and dependence on insulin secretion were the main indicators of type 1 and type 2 diabetes. The death of T cells in the B cells of the pancreatic islets, which stops the pancreas from making and secreting insulin, is the defining feature of type 1 diabetes, which primarily affects children and young adults (Tseng, 2012). Type 2 diabetes primarily affects adults and is primarily caused by insulin resistance and relative insulin insufficiency. People with type 2 diabetes are more likely to develop cancers of the breast,

endometrial, stomach, colon, liver, pancreas, bladder, and lymphatics (Arcidiacono, Iiritano, Nocera, Possidente, and Nevolo, 2012; Tseng, 2015). Joydip et al. (2019) suggest that elevated insulin resistance, hyperinsulinemia, inflammation, and oxidative stress could be the cause of diabetes-related cancer risk. One of the most prevalent chronic illnesses in the world, diabetes is becoming more and more prevalent in the population. The World Health Organization (WHO) estimates that 200 million people worldwide suffer from diabetes, a number that is predicted to triple by 2030. It has been reported by Pawan, Sukhbir, Rana, and Dhirender (2014) that in middle-income nations, diabetes kills around 80% of the population annually.

Diabetes is a complex disease that can be brought on by abnormalities in a wide range of proteins, enzymes, and organs. No single therapy approach can adequately treat this complex disease, and no single experimental model can be relied upon due to its intricacy. Two protein receptors that are utilized as experimental models in whole-body glucose regulation are alpha-amylase and dipeptidyl peptidase IV (dpp4) (Rawaba et al., 2021). Adenosine deaminase complex protein, or dipeptidyl peptidase-IV (DPP-IV), is a human protein that is encoded by the DPP4 gene. As stated by B. and Rosenstock. Zinman (2007) states that type 2 diabetes can be effectively treated by inhibiting DPP-IV. DPP-IV selectively eliminates N-terminal dipeptides from substrates that also contain proline or alanine as a second residue in order to produce inactive or even hostile species. Incretins, which increase insulin production, are the most significant substrates of DPP-IV. Examples of these include glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (Barnett, 2006). Human pancreatic amylase (HPA, 1,4-glucan-4-glucanohydrolase, E.C.3.2.1.1) plays a crucial role in diabetes. It starts the process of breaking down starch into maltose, which glucosidase then breaks down into glucose. Thus, by postponing starch digestion through HPA inhibition, postprandial hyperglycemia in type II diabetes can be effectively controlled (Tarling, Woods, & Zhang, 2008). Because HPA is blocked in the small intestine, the rate of starch hydrolysis is slowed down, which slows down the digestive process. Prolonging the digestive process can reduce the amount of glucose produced and released into the circulation, making it one of the most effective ways to lower blood sugar levels after meals (Ponnusamy, Haldar, Mulani, Zinjarde, & Thulasiram, 2015).

The many side effects of current treatments make them less effective in controlling diabetes. The shortcomings of existing treatments surpass financial constraints in addition to raising the prevalence of diabetes. Treatments for each of these side effects should be safer, more efficient, simpler to use, and more affordable (Gout-Zwart, de-Jong, Saptenno, & Postma, 2020). Due to the lower side effects of natural medicines compared to synthetic ones, traditional treatments utilizing antidiabetic compounds from different plant species are becoming more and more popular (Marella and Tollamadugu, 2018). To determine the right lead compound for the intended activity, tests were also conducted on the corresponding bioactive plant species (Lankatillake, Huynh, & Dias, 2019). According to Maroyi (2011), the roots are used as a laxative and when combined with the leaf juice, can result in miscarriage. Rhus longipes leaves were traditionally used to treat toothaches and were thought to have antidiabetic, antibacterial, and antioxidant properties; however, other Rhus genera have demonstrated significant pharmacological activities (Olasunkanmi Project Students, 2018 and 2022). Molecular dynamics and in silico molecular docking computer simulations of drug-target interactions are widely used for logical drug design and screening (Jorgensen, 2004). Kellenberger, Rodrigo, Muller, and Rognan (2004) state that molecular docking is one of the key computational methods for predicting receptor-ligand interactions. Arun, Saranraj, Balachandran, and Perumal (2014) stated that a proficient docking strategy must possess the ability to comprehensively consider the local ligand functioning as the receptor's binding site (i.e., determining the conformation of the ligand test element within the restricted parameters of a specific protein), resistance, and molecular bonding of physically related compounds). Investigating the molecular processes

underlying the antidiabetic activity of substances isolated from *Rhus longipes* was the study's main goal.

## Materials and Methods

### Collection of plant sample

A new Rhus longipes specimen was successfully identified and verified by a taxonomist from the Department of Pure and Applied Botany, Federal University of Agriculture, Abeokuta, Ogun State, University of Natural Sciences. The specimen came from a traditional house in Oja Odan, Ogun State, Nigeria. After being cleaned, dried, and allowed to air dry at room temperature (37°C), the leaves were allowed to reach a stable weight.

### Preparation of plant extract

The leaves of R. Longipes were crushed and then extracted with methanol using the cold maceration method (plant weight ratio: 1:10 per extraction solvent), according to Dieudonné et al. (2010). As stated by Azadi et al. (2019). 180 g of powdered material were soaked in 1. 8 L of 95% ethanol for 72 hours in different ways, stirring constantly to help with extraction. The water mixture was kept at 4°C throughout the soaking process to prevent microbial growth. The mixture was filtered through Whatman No. 1 filter paper after 72 hours. The filtrate was concentrated using a rotary evaporator (RE 300) running at 40°C. Refrigerated at 4°C until needed, the concentrated extract was kept.

### Gas chromatography-mass spectroscopic (GC-MS) analysis of *R. longipes*

For the GC-MS investigation, an Agilent Technologies 789OA mass spectrometer with a three-axis detector (VL5675C) and an automatic injector (10 L syringe) was utilized. Helium was used as the carrier gas in a chromatographic separation on a capillary column (30 m2; 250 lm; 0. 25 lm) with a constant flow rate of 1.5 ml/min. In split mode, the sample injection volume was 1 LL with a split ratio of 1:3. After raising the column temperature from 35°C to 150°C for five minutes, it was raised to 2500°C at a rate of 200°C per minute for five minutes. By comparing the spectra of individual components with reference mass spectra from the National Library of Standards and Technology (NIST), located in Maryland, the United States, compounds were identified.

### Molecular Docking

### Protein Preparation

The amylase and dipeptidyl peptidase-IV crystal structures were obtained from PDB files, which can be found on the Protein Data Bank (http://www.rcsb.org/). PDB is a global repository that provides access to the three-dimensional structures of biological macromolecules (Burley et al., 2021). In this work, we used amylase and a series of dipeptidyl peptidases IV for docking analysis. Chimera was used to prepare the proteins. In macromolecules, polar hydrogen takes the place of co-crystallized ligands and water molecules.

### Ligand Preparation

Initially, the SDF format of the chosen ligands' 3D structures was downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) (Rawaba et al., 2021). PubChem is a database of chemical compounds and the microbiological reactions associated with them. Next, the optimized structures were converted into PDB format using Open Babel.

### Docking Protocol

To perform molecular docking studies using AutoDock Vina, the ligand and protein PDB data were converted to an enhanced PDB format called PDBQT. Like many previous studies (2019), a docking procedure was used. Alpha-amylase and dipeptidyl peptidase-IV were fitted with grid sizes of 65-70-81 and 81-77-52 xyz, respectively, and a grid spacing of 1. The center of the grid was set according to the dimensions (x, y, and z): 4.444, 17.423, 61. 676 and 14. 551 and 13. 139, 16. 154 and 27. 245 for amylase and glucosidase, respectively. In the configuration file, the generated PDBQT files were stored. In terms of the bioactive chemicals, the shape of the ligand with the lowest binding energy was thought to be the most stable one. Using the free Biova Discovery Studio 2020 client, the data were examined.

**Analysis of Docking Results**

The accuracy of the docking technique was assessed by comparing reference ligands that were docked into the binding site of the target protein with co-crystallized ligands of the target protein. Target protein binding sites and the immobilized state of the ligand at these sites are important aspects of the study of protein-ligand interactions. Using PyMOL and Biova Discovery Studio 2020, the ligand-binding interactions, such as H-bonding and hydrophobic interactions, were further examined.

**ADME/T prediction**

The absorption, distribution, metabolism, excretion, and toxicity of ligands are all part of the pharmacokinetic properties, or ADMET, that must be assessed in order to ascertain their activity in the body. The ligands' ADMET characteristics were investigated using Swissadme and AdmetLab, an online tool for ADMET prediction (Cheng et al., 2012). The following are some examples of physicochemical properties: mutagenicity, hepatotoxicity, biodegradation, ocular corrosion, eye irritation, acute oral toxicity, carcinogenicity, and CYP450 inhibitors. toxicity parameters and simplicity (Anza et al., 2021).

## Results

**Table 1: GC-MS profile of compounds identified in the ethanol leaf extract of *Rhus longipes***

| Retention Time | Compound | Percentage (%) |
|---|---|---|
| **3.441** | Oleic acid | 0.12 |
| **5.791** | 1-(3-cyclohexylaminopropyl)guanidine | 0.11 |
| **5.893** | Cholest-2-eno[2,3-b]quinoxaline | 0.40 |
| **6.428** | Aziridine | 0.35 |
| **7.135** | Picyclopentadienyl | 0.22 |
| **7.868** | Deoxymorelin | 0.25 |
| **7.895** | N-Heptafluorobutyrylmorphine | 0.16 |
| **8.771** | Nicotinic acid | 0.22 |
| **9.800** | Ethyl formate | 0.27 |
| **11.185** | 2,2 dibromocholestanone | 0.09 |
| **11.735** | Oleanan-29-oic acid | 0.09 |
| **11.839** | Methylester | 0.08 |
| **14.224** | Diacetate | 0.14 |
| **14.486** | 4a,7a-Epoxy-5H-cyclopropa[F] | 0.47 |
| **16.666** | Cholestan-6-en-3-ol | 1.48 |
| **16.992** | Acetic acid | 0.72 |
| **17.206** | Alphalongipinene | 0.45 |
| **18.118** | 6R,7R-bisabolone | 0.18 |
| **18.617** | Neophytadiene | 0.46 |
| **19.608** | Silane | 0.22 |
| **19.661** | Bis(Trimethylsilyl)Ether | 0.16 |
| **20.508** | 3-diethoxyphosphonyldimethylthiocolchicine | 0.44 |
| **25.508** | Elaidic acid | 0.11 |
| **26.253** | Z-28-Heptatriaconten-2-one | 0.13 |

| | | |
|---|---|---|
| **26.347** | Trans-4-Trimethylsilyloxy | 0.12 |
| **19.608** | 3-Diethoxyphosphonyl-demethylthiocolchicine | 0.22 |
| **19.661** | 9-Octadecenoic acid, (E)-, TMS derivative | 0.15 |
| **20.508** | Z-28-Heptatriaconten-2-one | 0.4 |
| **25.508** | *cis*-4-Trimethylsilyloxy-cyclohexyl (trimethylsilyl)carboxylate | 0.11 |
| **7.868** | N-Heptafluorobutyrylmorpholine | 0.33 |
| **7.895** | 3-Pyridinecarboxylic acid, 1,4,5,6-tetrahydro-1,2-dimethyl-6-oxo-, ethyl ester | 0.46 |

**Table 2: Molecular docking result of APHAL AMYLASE protein**

| **Compound Name** | **Compound Id** | **Binding Affinity** | **Residual Interactions** | | |
|---|---|---|---|---|---|
| | | | H-Bond | Hydrophobic/ Pi-Cation | Hydrophobic/ Pi-Anion |
| **Miglitol (STD)** | 441314 | -5.7 | Gly403, Arg421, Asp402,Gly334, Arg398, Phe335 | - | - |
| **Ferulic Acid** | 445858 | -5.8 | Ser4,Arg398,Asp 402,Pro332, Gly334 | - | - |
| **4-Hydroxy-Coumarin** | 54682930 | -5.8 | Arg421 | Arg398 | Asp402 |
| **Estriol** | 5756 | -6.9 | Asp290, Gly334, Rrg10 | Phe335 | |

**Table 3: Molecular Docking Result of DPP IV Protein**

| **Compound Name** | **Compound Id** | **Binding affinity** | **Residual Interactions** | | |
|---|---|---|---|---|---|
| | | | H-Bond | Hydrophobic | Vander wall interaction |
| Sitagliptin (STD) | 4369359 | -8.7 | Trp629, lys554, ser630 | Arg125, | Try547 |
| Cholest-2-eno(2,3-B) Quinoxaline | 538059 | -9.3 | Arg669, | Trp629, tyr547 | Phe357 |
| Deoxymorellin | 635828 | -9.1 | Val546, gly632, tyr752 | Trp629 | Try547, his740 |

| | | | | | |
|---|---|---|---|---|---|
| 2,2-Dibromocholestanone | 22212696 | -8.8 | - | Tyr666 | Tyr631, tyr547, trp629, try662 |

**Table 4: Basic ADMET Properties And Computational Descriptors of the Screened Compounds**

| Compounds name | MW | GI absorption | BBB | Pgp | Lipinsk | TPSA | CYTP450 Inhibitor | Carcinogenicity |
|---|---|---|---|---|---|---|---|---|
| Miglitol (STD) | 207.22 | Low | No | Yes | 0 | 104.39 | No | No |
| Ferulic Acid | 194.18 | High | Yes | No | 0 | 66.76 | No | No |
| 4-Hydroxy Coumarin | 162.14 | High | Yes | No | 0 | 50.44 | No | Yes |
| Estriol | 288.38 | High | Yes | Yes | 0 | 60.69 | No | No |
| Sitagliptin (STD) | 407.31 | High | Yes | Yes | 0 | 77.04 | No | Yes |
| Cholest-2-eno(2,3-B) Quinoxaline | 517.75 | Low | No | No | 2 | 71.6 | Yes | No |
| Deoxymorellin | 530.65 | High | No | Yes | 1 | 82.06 | No | Yes |
| 2,2-Dibromocholestanone | 544.45 | Low | No | No | 2 | 17.07 | Yes | No |

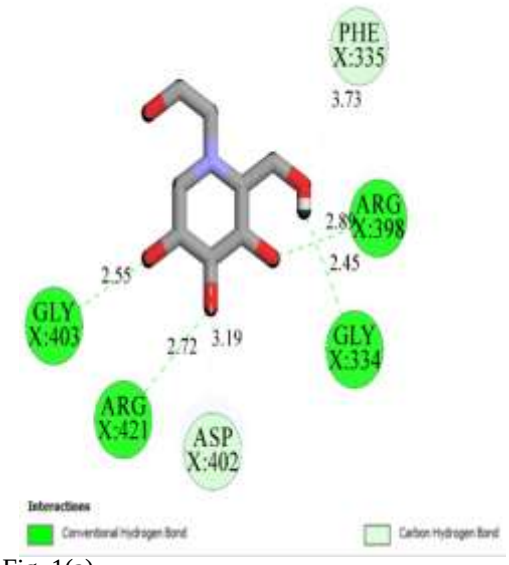



Fig. 1(a)

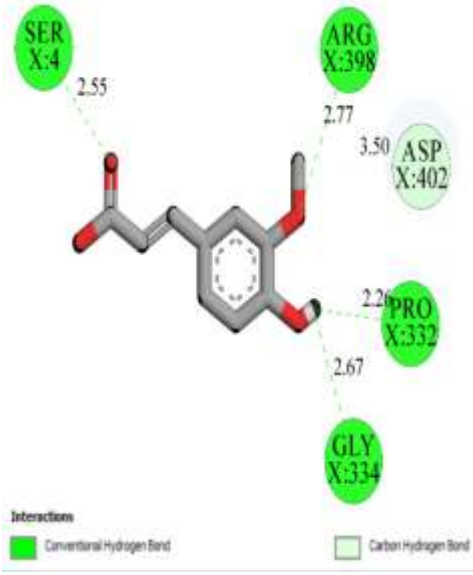



Fig. 1(b)

Fig.1: The optimal poses of (a) MIGLITOL and (b) FERILLIC ACID interact with alpha-amylase (PDB ID: 3BLP) schematically.


Fig. 2(a)


Fig. 2(b)

Fig. 2: The optimal poses of (a) 4-HYDROXY COUMARIN and (b) ESTRIOL interact with alpha-amylase (PDB ID: 3BLP) schematically.

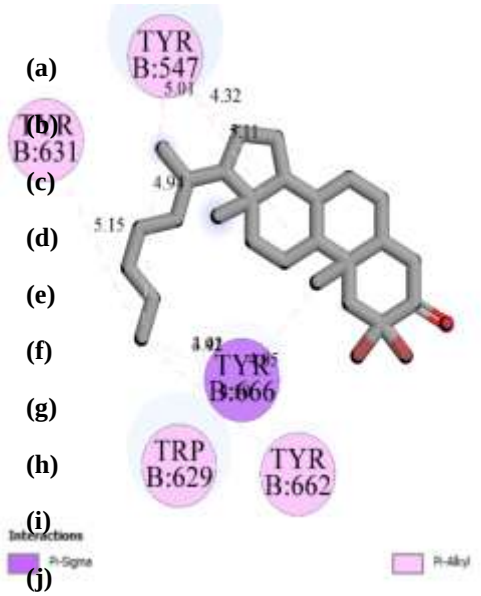



Fig. 3(a)

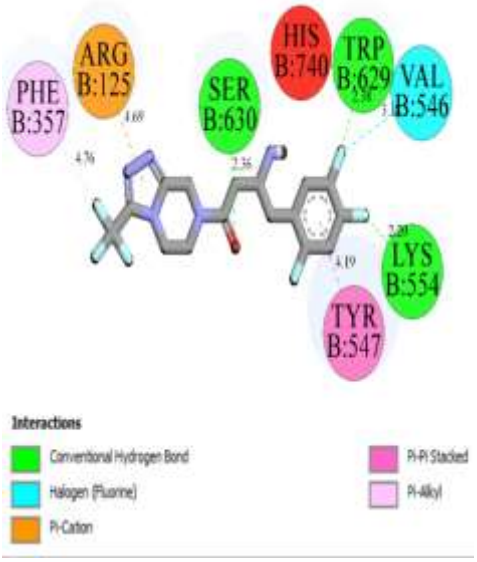
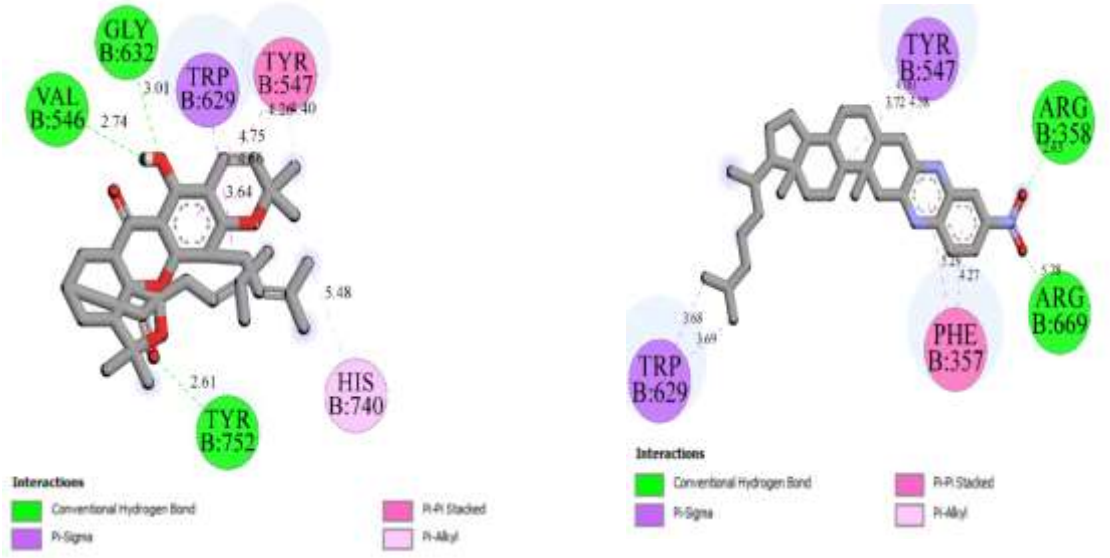



Fig. 3(b)

Fig. 3: The Optimal poses of (a) SITAGLIPTIN and (b) 2,2-Dibromochlostanone interact with dipeptidyl peptidase-IV (PDB ID: FT4F) schematically.




**(a)** **(b)**

Fig. 4: The optimal poses of (a) Deoxymorelin and (b) Cholest2-(2,3-B) Quinoxaline interact with dipeptidyl peptidase-IV (PDB ID: FT4F) schematically.

## Discussion

*Rhus longipes* methanol extract components were identified by GC-MS analysis (see Table 1). The concentration percentages and retention times (RTs) of the active ingredients are listed in Table 1. *Rhus longipes* leaves are a valuable source of medicinal compounds because they contain 34 different types of chemicals. Among the bioactive compounds found, the following significant antioxidant phenolic compounds are frequently found in other plant species: ferulic acid, vanillic acid, gallic acid, catechin, apiginin, caffeic acid, hydroxybenzoic acid, salicyclic acid, 4-hydroxycoumarin, and caffeic acid (Sokamte et al., 2019). *Rhus longipes* contains these compounds in a range of concentrations. The antioxidant capacity of the plant can be greatly impacted by longipes leaf extract. R contains a number of volatile compounds. It has been discovered that *Rhus longipes* leaf extract has advantageous biological characteristics. As an illustration, certain substances have antibacterial effects on various plant species (Gnanaselvan and Sivaraman, 2020; Kaushik et al. 2014; Prabhu et al., 2020).

The interaction between ligands (compounds) and protein targets is studied using the in silico modeling approach known as molecular docking (Iheagwam et al., 2019). The binding energy produced through docking, also known as the docking score, is thought to depend on how well the ligand binds to the protein target. In the current investigation, the target proteins human pancreatic amylase and dipeptidyl peptidase-IV (DPPIV) were docked against in order to identify potentially active chemicals from *Rhus longipes* leaf extract. Alpha-amylase and the DPPIV protein binding interactions of the selected ligands were examined using molecular docking interaction studies. To assess each ligand's binding affinity and interaction with alpha-amylase and the DPPIV protein, one position with a low binding energy was chosen out of the eight poses for each ligand and visualized using PyMOL. More hydrogen bonds and hydrophobic interactions were established with the chosen amino acid residues during the docking procedure, which supports a higher binding affinity between the protein-ligand complex. Anti-diabetic medications that target the alpha-amylase

and DPPIV proteins, respectively, include miglito and sitagliptin. In comparison to miglitol, a common inhibitor of alpha amylase, ferrulic acid, 4-hydroxycoumarin, and estriol exhibit binding affinities of 5.8, 5.8, and 6.9, respectively (table 1). Similar to Sitagliptin, which inhibits the DPP1V protein, Cholest-2-eno(2,3-B)-Quinoxaline, 2,2-Dibromocholestanone, and 2,2-Dibromochlostanone exhibit binding affinities that are -9.3 kcal/mol, -9.1 kcal/mol, and -8.8 kcal/mol, respectively (table 3). The therapeutic potential of the substances can be attributed to these binding affinities. Accordingly, it is thought that the high concentration of hydrogen and hydrophobic interactions accounts for the high binding affinities attributed to ferulic acid, 4-hydroxy coumarin, and estriol for alpha amylase and cholest-2-eno(2,3-b)-quinoxaline, deoxymorellin, and 2,2-dibromocholestanone for DPPIV. This explains the significance of interactions between hydrogen and hydrophobic molecules in medication creation. Target-drug interface binding affinity may be increased via hydrophobic contact. With a different lead compound, this finding was consistent with Ambrose et al. (2018). Figures 1-4 show the interactions of the higher-ranking comp The interactions between ligands (compounds) and protein targets are studied using an in silico modeling approach called molecular docking (Iheagwam et al., 2019). The binding energy generated upon docking, also known as the docking point, is thought to depend on how the ligand binds to the target protein. In the present study, human pancreatic amylase and dipeptidyl peptidase-IV (DPPIV) target proteins were tested to identify potentially active compounds in *Rhus longipes* leaf extract. The alpha-amylase and DPPIV protein binding interactions of selected ligands were examined by molecular docking interaction studies.

To evaluate the binding affinity of each ligand and its interaction with alpha-amylase protein and DPPIV, a position with low binding energy was selected from eight poses for each ligand and visualized in PyMOL. More hydrogen bonds and hydrophobic interactions are established with selected amino acid residues during docking, supporting higher binding affinity between protein-ligand complexes. Antidiabetic drugs that target alpha-amylase and DPPIV proteins include miglito and sitagliptin, respectively. In comparison, miglitol, a common inhibitor of alpha-amylase, ferrulic acid, 4-hydroxycoumarin, and estriol, exhibited binding affinities of -5. 8 kcal/mol, -5. 8 kcal/mol and -6. 9 kcal/mol, respectively (Table 2). Similar to sitagliptin,the DPP1V protein inhibitors cholest-2-eno(2,3-B)-quinoxaline, 2,2-dibromocholestanone, and 2,2-dibromochlostanone have a binding affinity of -9.3 kcal/mol, -9.1 kcal/mol and -8.8 kcal/mol, respectively (Table 3). The therapeutic potential of the agents may be due to these binding affinities. Accordingly, the high concentration of hydrogen and hydrophobic interactions is thought to be responsible for the high binding affinity exerted by ferulic acid, 4-hydroxycoumarin, and estriol for alpha-amylase and cholest-2-eno(2). ,3-b)-quinoxaline. deoxymorellin and 2,2-dibromocholestanone for DPPIV. This explains the importance of interactions between hydrogen and hydrophobic molecules in drug formation. Binding affinity at the drug-target interface may be increased due to hydrophobic contact. With another lead compound, this finding is consistent with that of Ambrose et al. (2018). Figures 1–4 show the interactions of higher-order compounds (in terms of binding energy) and the common drug miglitol with the target protein. According to the expected location and interaction between ferulic acid and amylase, the contacts are supported through convective hydrogen bonds formed by amino acid residues GLY 334, PRO 332, SER 4, and ARG 398, as well as the carbon-hydrogen bond related to ASP402. Convective hydrogen bonds established by amino acid residues ARG42, ARG398, and ASP402 stabilized the 4-hydroxycoumarin contacts. Similarly, the interaction between estriol and the alpha-amylase receptor is stabilized by the formation of a convective hydrogen bond between the amino acid residues GLY 334 and ARG10, while the other bonds involve the Pi-Pi-T form containing PHE 335.

Compounds (in terms of binding energy) and the common drug miglitol with the target protein. According to the expected location and interaction between ferulic acid and -amylase, the contacts are

supported through convective hydrogen bonds formed by amino acid residues GLY 334, PRO 332, SER 4 and ARG 398, as well as the carbon-hydrogen bond related to ASP402. Convective hydrogen bonds established by amino acid residues ARG42, ARG 398, and ASP402 stabilized the 4-hydroxycoumarin contacts. Similarly, the interaction between estriol and the alpha amylase receptor is stabilized by the formation of a convective hydrogen bond between the amino acid residues GLY 334 and ARG10, while the other bonds involve the Pi-Pi-T form containing the PHE 335.

Three convective hydrogen bonds involve amino acid residues TRYU752, VAL546, and GLY623, pi-sigma interactions involve residue TRP628, and other bonds, such as the alkyl pi with residue HIS740 and the pi-pi bond stacking introduced by amino acid residue TRY547, stabilize the interactions of DPP1V with deoxymorelin (Fig. 4). The interaction of cholest2-(2,3-B)-quinoxaline with dipeptidyl peptidase-IV (DPPIV) is stabilized by the formation of a convective hydrogen bond between amino acid residues ARG358 and ARG669; other bonds include a pi-sigma bond with residues TRYU547 and TRP629 and a stacked pi-pi bond with residue PHE357 (Figure 4). The combination of dipeptidyl peptidase-IV (DPPIV) with 2,2-dibromochlostanone is stabilized by amino acid residues TRY666 (sigma pi bond) and alkyl pi bonds with TRP629, TRY547, TRY631, and TYR662 (Figure 2). The predicted amylase binding site for the generic drug miglitol shows interactions stabilized by convective hydrogen bonding with ARG421, GLY403, ARG398, and GLY334, as well as carbon-hydrogen bonding with ASP402 and PHE335. Similarly, the generic drug (sitagliptin) also exhibits significant interaction with dipeptidyl peptidase-IV (DPPIV), supported by halogen (fluorine) bonding with VAL546 and convective hydrogen bonding with SER630, TRP629, and LYS554, as well as pi-cation, pi-alkyl, stacked pi-pi, and PHE357.

The high binding scores for these compounds and their activity can largely be attributed to these interactions. As William et al. (2012) discovered in their study using myricetin and ethyl caffeate that such an interaction also has the potential to disrupt the polypeptides that constitute the active site of the target protein, which may affect the activity of the target protein. Therefore, our molecular docking analysis confirms the ability of the plant extract to reduce postprandial glucose levels and confirms the amylase inhibition previously described by Ibrahim et al. (2017). The fact that these substances may have contributed significantly to the reported hypoglycemic activity of the plant extract is a clear indication of this possibility. They can work together to bring about observed activities. This can be explained by structural similarities between chemicals obtained from plant extracts and traditional substrates or ligands of target proteins.

A compound must not only have the necessary biological activity but also exhibit the best safety and pharmacokinetic properties to be a drug of use (Hu et al., 2018). Therefore, we used the SwissADME suite in silico to analyze promising compounds selected from docking simulations to determine optimal pharmacokinetics, drug similarity, and optimal medicinal chemistry properties.

The ability to dissolve a drug in lipids or nonpolar solvents is called lipophilicity. On the total ADMET characteristic of the drug, this characteristic has a significant impact. According to Arnott and Planey (2012), what is important is the transport of drugs across cell membranes. The majority of drug similarity filters (rule of 5) indicate that the lipophilic range of 0 to 5 is generally considered ideal for drug formulation (Waring et al., 2010; Lipinski et al., 2001). The following agents in the present study demonstrated the best lipophilicity: ferulic acid, miglitol (STD), 4-hydroxycoumarin, estriol, sitagliptin, cholest-2-eno(2,3-B)-quinoxaline, deoxymorellin, and 2,2-dibromocholestanone. This suggests that these agents would obtain high bioavailability through efficient membrane absorption into the circulatory system. On the other hand, drug solubility is a physicochemical property that affects formulation, distribution, and absorption (Daina et al., 2017). The drug must be available in an aqueous solution at the absorption site to facilitate absorption (Savjani et al., 2012). All

compounds tested in the present study generally had moderate solubility, suggesting that they can achieve high bioavailability when administered orally and have optimal lipophilicity. However, substances with particularly high solubility and low lipophilicity include sitagliptin, miglitol, and ferulic acid. Gastrointestinal tract (GIT) absorption or poor circulation may occur. However, this effect is unlikely to be severe because sitagliptin and miglitol act at the GIT, where they are expected to intercalate and inhibit the enzymes amylase and DPPIV (Rosak and Mertes, 2014).

The final fate of drugs in the body is determined by their ADME characteristics (Shin et al., 2016). To achieve optimal pharmacokinetics, oral medications must be fully absorbed into the gastrointestinal tract. To prevent drugs from entering the central nervous system ("CNS"), the BBB is important (Abbott, 2002). In the present study, it was found that ferulic acid, 4-hydroxycoumarin, estriol, and sitagliptin penetrated the BBB, and all compounds were well absorbed into the systemic circulation in the GIT (except miglitol). cholest2eno(2,3B) quinoxaline and 2,2dibromocholestanone). Due to potential CNS-related side effects, this may be concerning. The degree of permeation may not be important in cases where it has a marked toxic effect on the central nervous system ("CNS") because the calculation technique does not quantify the degree of permeation.According to Kim (2002; Finch and Pillians, 2014), P-gp usually acts as an efflux transporter that delivers drugs or xenobiotics into the lumen of the GIT, thereby reducing drug concentrations in the blood and tissues. Miglitol, estriol, sitagliptin, and deoxymorellin were all predicted to be non-P-gp substrates in the present experiment, but the other drugs were not. For the generic drug Miglitol, which targets amylase activity in the GIT, this may not be a major concern. Due to the absorption of the drug molecules and perhaps their re-injection into P-pg, agents such as sitagliptin, whose target (DPPIV protein) is located outside the intestinal lumen, may have reduced bioavailability in the target location. Characterizing the pharmacokinetics of drug candidates requires a thorough understanding of the interactions between the substances and the cytochrome P450 (CYP) system, as these interactions are important for their processing and remove pharmaceuticals from the system (Daina et al., 2017). Drug-induced toxicity can occur when drug-inhibited isoforms of this enzyme system are inhibited by the drug due to impaired excretion. Therefore, it is important that a drug candidate have only minimal inhibitory effects on certain enzyme isoforms. The results of the present investigation demonstrated that none of the agents miglitol, ferulic acid, 4-hydroxycoumarin, estriol, sitagliptin, or deoxymorellin were capable of inhibiting any of the five P450 isoforms. This shows that these substances will be effectively digested in the liver and eliminated from the body.

Overall drug-like properties quantitatively evaluate the extent to which the physicochemical and structural properties of compounds comply with or match the majority of well-known drugs. To ensure agreement in predictions, this was predicted using the “rule of five filters” (Lipinski, Pfizer) (Daina et al., 2017). Lipinski's rule was the first "rule of5" for drug similarity to be applied in practice, and the results of this study showed that six substances were evaluated (miglitol, ferulic acid, 4-hydroxycoumarin, estriol, sitagliptin, and deoxymorellin) and were shown to be compliant. According to this study, the substances miglitol, ferulic acid, 4-hydroxycoumarin, estriol, sitagliptin, and deoxymorellin have superior medicinal properties than other substances. In terms of binding interactions with target proteins, ADMET, and drug quality, agents such as ferulic acid, 4-hydroxycoumarin, estriol, and deoxymorellin appear to have the most promising properties. To our knowledge, these substances have not been mentioned in any publications as potential antidiabetic drugs.

## Conclusions

The in silico method was used in this study to investigate potential antidiabetic compounds detected during GCMS analysis of *Rhus longipes* extract. Compared with the reference drugs (miglitol and

sitagliptin), several compounds were found to show significant binding affinity to target proteins, demonstrating the potential of the extract as an antidiabetic drug. Ferulic acid, 4-hydroxycoumarin, estriol, and deoxymorellin were the four candidates found and prioritized during the drug-likeness assessment of these intriguing compounds. Now that these compounds have been studied in more detail, each compound can be evaluated for its antidiabetic effectiveness, clear mechanism of action, and degree of optimization.

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