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Evaluation of Antidiabetic Effects of Watermelon Rind Extract: Integrative Computational Simulations and *In Vitro* Studies

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ABSTRACT

Diabetes mellitus is a common metabolic disorder characterized by chronic hyperglycemia, requiring the development of alternative therapies to improve glycemic control. Watermelon rind (*Citrullus lanatus* (Thunb.) Matsum), usually discarded as waste, contains various bioactive compounds potentially having anti-diabetic benefits. This study aimed to evaluate the antidiabetic potential of watermelon rind extract through a comprehensive approach, including secondary metabolite content profiling, Fourier transform infrared spectroscopy (FTIR) analysis, total flavonoid content determination, molecular docking assay, and *in vitro* enzyme inhibition assay targeting *α*-glucosidase. Preliminary phytochemical screening showed the presence of major secondary metabolites such as flavonoids, saponins, and phenolic acids. FTIR analysis confirmed the presence of functional groups typical of these bioactive compounds, including hydroxyl, carbonyl, and aromatic groups. The total flavonoid content was relatively high, indicating significant antioxidant and therapeutic potential. Molecular docking studies were conducted to explore the interaction of bioactive compounds from watermelon rind with *α*-glucosidase enzyme. The docking results identified a strong binding affinity of specific flavonoids, particularly quercetin derivatives, within the active site of *α*-glucosidase, indicating a potential inhibitory mechanism. The *in vitro* assay further validated these findings by showing significant inhibitory activity of watermelon rind extract against *α*-glucosidase, which plays an important role in carbohydrate digestion and glucose absorption. The results of the *in vitro* assay aligned with the predictions from molecular docking. These findings suggest that watermelon rind extract has promising antidiabetic potential through *α*-glucosidase inhibition, supported by the content of flavonoids and other bioactive compounds.

Keywords: Diabetes Mellitus, Watermelon rind extract, *In vitro*, Computational simulations.

Introduction

Diabetes mellitus is one of the most common metabolic diseases in the world, characterized by chronic hyperglycemia due to impaired insulin secretion, insulin resistance, or both. The prevalence of diabetes continues to increase globally, posing a significant health burden, both to individuals and health systems.[1] Therefore, the search for new effective, safe, and affordable antidiabetic agents continues to be a significant focus in pharmacological and biomedical research.[2]

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α-Glucosidase is a hydrolase enzyme that plays an important role in the process of carbohydrate digestion, especially in the final stages of digestion in the small intestine. This enzyme breaks down disaccharides, oligosaccharides, and polysaccharides into monosaccharides, such as glucose, which are absorbed into the bloodstream. High α-glucosidase activity can rapidly increase glucose absorption, contributing to the postprandial (after-meal) spike in blood glucose levels.[3] Therefore, α-glucosidase inhibition is an effective therapeutic strategy in managing type 2 diabetes mellitus to lower blood glucose levels and reduce the risk of hyperglycemia-related complications.[4] The *α*-Glucosidase inhibitor drugs, such as acarbose, miglitol, and voglibose, have been widely used in treating type 2 diabetes.[5] The mechanism of action of these drugs is by inhibiting α-glucosidase activity in the gut, slowing the breakdown of carbohydrates and reducing the increase in blood glucose after a meal.[3] Although effective, the use of these drugs is often associated with side effects such as gastrointestinal distress, including bloating, diarrhoea, and abdominal discomfort. This has sparked interest in the search for safer and more natural α-glucosidase-inhibiting agents from plant materials or other natural sources.[5] Recent research has identified various

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bioactive compounds from natural sources, including flavonoids, phenolics, and alkaloids, that exhibit α-glucosidase inhibitory activity.[6–8] These compounds offer potential as safer alternative therapies with minimal side effects. Plant extracts such as watermelon (*Citrullus lanatus* (Thunb.) Matsum) rind, rich in bioactive compounds, show promising prospects in inhibiting α-glucosidase.[9]

Citrullus lanatus (Thunb.) Matsum is widely known as a refreshing fruit rich in nutrients, such as vitamins, minerals, and antioxidants.[10] While the flesh of watermelon is often consumed, the rind is overlooked and considered a waste. However, several studies have shown that watermelon rind contains various bioactive compounds, including flavonoids, saponins, alkaloids, and polyphenols, which have potential therapeutic properties, including antidiabetic activity.[10] *In vitro* and *in silico* studies provide an efficient approach to exploring the biological activity of plant extracts.[11] *In vitro* studies allow direct testing of molecular mechanisms and biological effects on cellular models, whereas *in silico* studies utilise computational techniques to predict the interaction of molecules with specific targets, such as diabetes-related enzymes, including *α*-glucosidase.[12] This study aims to evaluate the antidiabetic potential of watermelon rind aqueous extract through *in vitro* and *in silico* approaches. This approach is expected to identify bioactive compounds in watermelon rind that contribute to its antidiabetic activity while validating the molecular mechanism behind the effect.

Materials and Methods

Materials

These include glassware (Pyrex®), food hydrator (Ira Star®), analytical scales, pH meter (Thermo Fisher Scientific®), micropipette (Soccorex®), centrifuge (Thermo Fisher Scientific®), freeze dryer, UV-VIS spectrophotometry (Agilent®), GC-MS (Shimadzu®). Other include watermelon rind, Aquadest (Onelab®), H_2SO_4(Merck®), H_3PO_4(Merck®), p-nitropheni-*α*-D-glucopyranoside (pNPG) (Merck®), Na_2CO_3 (Merck®).

Plant Collection, Preparation, and Analysis

The plant sample was collected in March 2024. The plant sample (Watermelon) *Citrullus lanatus* (Thunb.) Matsum was identified at the Botany Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, Makassar State University, Indonesia, where a voucher specimen was deposited with an identification number LAB/2024/V/15.

Sample Preparation

Ripe watermelon fruits with no defects in the fruit skin and dark green skin grooves were selected for this experiment. The fruits were washed, the red flesh and skin were separated, then cut into small pieces.

Watermelon Rind Extraction Using Kinetic Maceration Method

The sample was dissolved with water (1:2 ratio) and extracted 2x at a temperature of 50°C while stirring for approximately 1 hour. The extract was filtered using a sieve, and the filtrate was stored in a Freezer at -300°C for 50 hours and then freeze-dried for 24 hours. The extract yield was then calculated by comparing the watermelon rind's initial weight with the extract's final weight.

$$\%\ Extract\ Rendamen = \frac{(Weight\ of\ extract\ produced)}{(Initial\ weight\ of\ simplisia)}\ x\ 100 \quad (1)$$

Phytochemical Screening of Watermelon Fruit Rind Extract

Phytochemical screening includes the examination of alkaloids, flavonoids, tannins, triterpenoids/steroids, and saponin compounds.

Alkaloid screening

Four grams of sample was added to enough chloroform, followed by 10 mL of 10% ammonia was added. The solution was stirred and filtered using filter paper. The filtrate was transferred into an Erlenmeyer bottle, and 10 drops of 2M H_2SO_4 were added. The mixture was homogenized regularly and left for a few minutes until two layers were formed. 2.5 mL of the top layer was transferred to three test tubes. The three solutions were tested with Meyer, Dragendorf, and Wagner reagents. The tubes were observed for the formation of precipitates.

Saponins Screening

About 50 mg of the extract was added to 10 mL of water, followed by 1 mL of concentrated HCl, and shaken vigorously. The formation of foam that persists for 15 minutes indicates the presence of saponins.

Steroids/Triterpenoids Screening

Drops of glacial CH_3COOH and 2 drops of concentrated H_2SO_4 were added to 50 mg of extract solution. The solution was shaken gently and left for a few minutes. The formation of a blue or green precipitate indicates the presence of steroids, while a red or purple precipitate indicates triterpenoids.

Tannins Screening

The extract (50 mg) was added to 1 mL of 10% $FeCl_3$ solution. The formation of a dark blue or greenish-black colour indicated the presence of tannins.

Flavanoids Screening

The extract (50 mg) was added to 100 mL of hot water, boiled for 5 minutes, and filtered using filter paper. 50 mg of Magnesium powder and 1 mL of concentrated HCl were added to 2 mL of the filtrate and shaken vigorously. The formation of red, yellow, or orange colour indicates a positive test.

GC-MS Analysis

The analysis was performed with a Shimadzu GC-MS fitted with a column of length 30 m and an internal diameter of 0.22 mm. Helium gas was used as the carrier gas. The GC-MS equipment conditions used were injector temperature 320°C, pressure 13.7 kPa, total flow 40 mL/min, column flow 0.50 mL/min, linear velocity 25.90 cm/s, purge flow 3 mL/min, split ratio 73:0, programmed column temperature from 70°C (maintained for 5 minutes) to 300°C (maintained for 52 minutes) with a temperature increase rate of 10 °C/min, ion source temperature 250°C and interface temperature 320°C.[13]

FTIR Analysis

A total of 10 mg of dry extract powder was encapsulated in 100 mg of KBr pellets and inserted into the chamber of the FT-IR instrument. The transmittance wavelength was set between 4000 cm^{-1} to 500 cm^{-1}.[14,15]

Molecular Docking Evaluation

The ligands (16) were downloaded from the PubChem website (https://pubchem.ncbi.nlm.nih.gov) in 3D SDF and optimized using the Autodock Tools program by setting their rotatable bonds.[17] Furthermore, the Receptor with PDB code 3WY1 was downloaded from the RSCB.PDB website (https://www.rcsb.org),[18] which results from X-ray crystallography and crystallization of Protein *α*-Glucosidase. Water molecules were then removed from the structure. Kollman charges were added to the protein.[19] Ligands and receptors that have been prepared and charged were opened using the application of the autodock tools connected to the autodock vina.[20] The first step was determining each receptor's grid box size, and the next step was the molecular docking process using auto dock vina. The visualization results were done using Discovery Studio Visualizer.[21]

Total Flavonoids Analysis

The sample (50 mg) was weighed, dissolved in 10 mL of ethanol, and filtered. Then, the quercetin standard was made by weighing 100 mg, in 100 mL of ethanol to obtain a 1000 ppm solution and further diluted to 100 ppm with ethanol. The stock solution was serially diluted to obtain different concentrations as follows: 2, 4, 8, 16, and 32 ppm.

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The samples (0.5 mL) were each added to 3 mL of methanol, followed by 0.2 mL of $AlCl_3$ and 0.2 mL of CH_3COOH 1 M, and then the volume was adjusted to 10 mL by adding 6.2 mL of distilled water. After that, the absorption was measured at a maximum wavelength of 400 to 500 nm using a UV-vis spectrophotometer. Quercetin was used as a standard, while the mixture of ethanol and methanol was used as a blank[22].

Test for α-Glucosidase Enzyme Inhibitory Activity

The alpha-glucosidase enzyme used in this study comes from *Saccharomyces cerevisiae* and p-nitrophenyl-α-D-glucopyranoside (pNPG), which serves as a substrate. Watermelon rind extract was prepared with 10, 20, 30, 40, and 50 ppm concentrations using Dimethylsulfoxide (DMSO). 100 µL of *α*-glucosidase enzyme (1.0 unit/mL) was pre-incubated with 50 µL of extract concentrations for 10 minutes. Next, 50 µL of pNPG (3.0 mM) dissolved in 20 mM phosphate buffer solution with pH 7 was added to the test solution to initiate the reaction and then incubated at 37℃ for 20 minutes. The reaction was stopped by adding 2000 µL of Na_2CO_3 (0.1 M) and then measured on a UV-VIS spectrophotometer at a wavelength of 400 nm. Acarbose was used as a positive control, and a blank test was performed. Replication of measurement was done 3 times. Enzyme activity was measured based on the absorbance of p-Nitrophenol, measured at a wavelength of 400 nm on a UV-Vis spectrophotometer.[9]

The percentage of *α*-glucosidase enzyme inhibition activity was computed from the following equation:

$$\%\ Inhibition = \frac{Control\ absorbance - Sample\ absorbance}{Control\ absorbance}\ x\ 100\ \% \quad (2)$$

The IC_{50} value was calculated using a linear regression equation (y = a + bx) with extract concentration as the x-axis and percentage inhibition as the y-axis.

Results and Discussion

This study uses watermelon rind (*Citrullus lanatus* (Thunb.) Matsum), family Cucurbitaceae, obtained from Cakura hamlet, Cakura village, south Polongbangkeng Takalar district. The pieces of the rind were dried using a food dehydrator at a temperature of 50° C. The drying shrinkage of the watermelon rind was obtained from the drying process at 91.98% (Table 1). Also, the percentage extract yield was 11.53% (Table 2). The results of phytochemical screening are presented in Tables 3-6 and Figures 1-2.

Table 1: Drying shrinkage of watermelon rind

Wet weight (g)	Simplisia weight (g)	Drying shrinkage (%)
17.083	1.370	91.98

Table 2: Yield of watermelon rind extract

Wet weight (g)	Simplisia weight (g)	Drying shrinkage (%)
1370	158	11.53

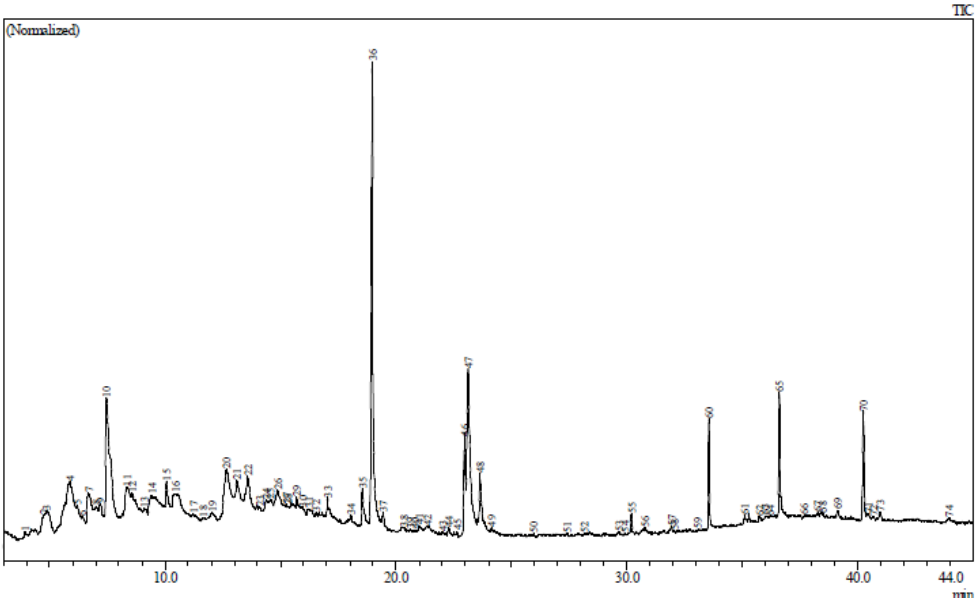

Figure 1: GC-MS chromatogram of watermelon rind extracts

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Table 3: Phytochemical screening results

Group Compound	Reaction Results
Alkaloids	+
Saponins	-
Flavonoids	+
Steroids	-
Tannins	+

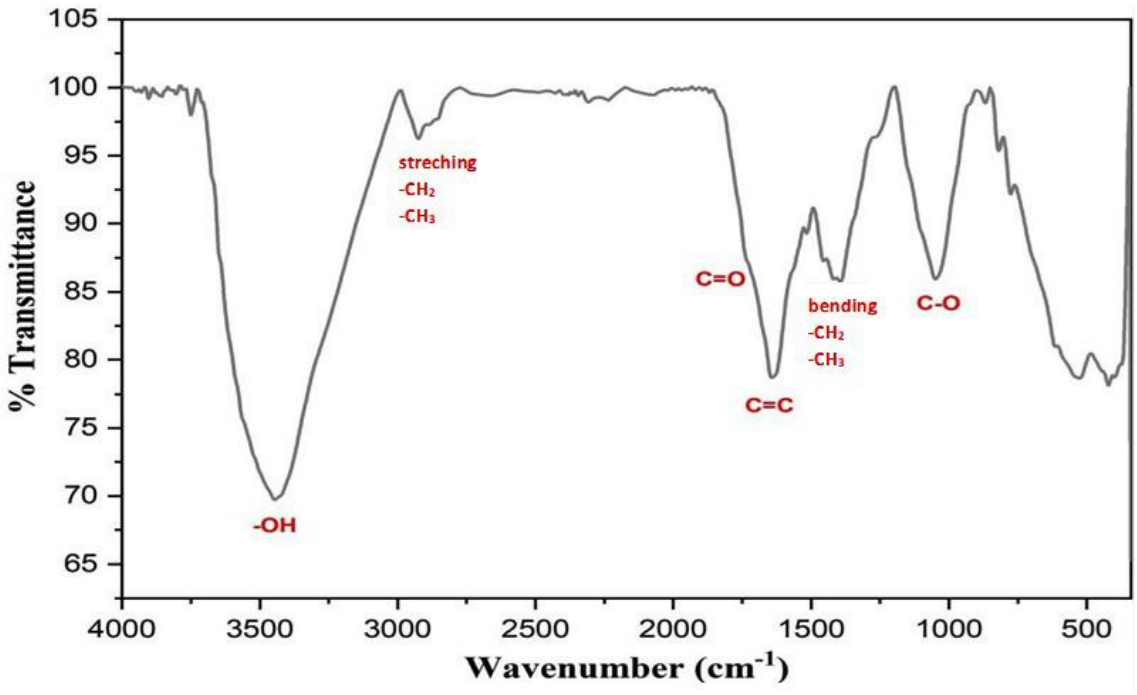

Figure 2: FTIR spectrum of watermelon rind water extracts

Table 4: GC-MS analysis of watermelon rind water extracts

Peak	Retention Time (min)	% Area	Compound Name
1	3.931	3.00	2h-Thiopyran, Tetrahydro-
2	4.742	8.72	1,3-Benzenediol, O-acetoxyacetyl
3	4.865	15.54	Benzenemethanol, Ar-Ethenyl-
4	5.885	28.13	Butane-1,2,3,4-Tetraol
5	6.208	10.13	2,5-Dimethylfuran-3,4(2H,5H)-dione
6	6.425	9.74	Furaneol
7	6.691	14.49	Cyclohexanamine, N-3-butenyl-N-methyl-
8	7.008	11.76	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]eth
9	7.189	10.31	Silane, dimethyl(dimethyl(3methylpentyloxy)silyloxy) propoxy-
10	7.465	15.86	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
11	8.352	18.66	1-(1-Methoxypropan-2-yloxy)propan-2-yl acetate
12	8.575	20.32	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-
13	9.096	13.46	1-Piperidinecarboxylic acid, ethyl ester
14	9.441	33.24	2-FURANCARBOXALDEHYDE, 5(HYDROXYMETHYL)-
15	10.067	11.47	2-Methoxy-4-vinylphenol

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16	10.453	33.26	Glutaric acid, 2-ethylhexyl 1-naphthyl ester
17	11.229	15.93	Phenol, 4-(ethoxymethyl)-
18	11.642	15.79	1,3-OCTADIENE, 1,1,3-TRIDEUTERO-2,4-DIMETHYL-
19	12.045	16.08	Permethylated And Reduced Product of Degradation Product
20	12.663	21.38	2-Amino-9-(3,4-Dihydroxy-5-Hydroxymethyl-Tetrahydro-Furan-2
21	13.114	18.26	D-Allose
22	13.576	16.80	Phosphonofluoridic acid, (1-methylethyl)-, cyclohexyl ester
23	14.092	15.97	Bicyclo[4.1.0]heptane,-3-cyclopropyl,-7-carbethoxy, trans-
24	14.375	13.68	3,4-Altrosan
25	14.562	10.81	Acetic acid, 2-ethylbutyl ester
26	14.870	16.47	beta.-D-Glucopyranoside, methyl (CAS)
27	15.258	12.38	(4'S,4'Ar,5'R,8'aS)-spiro-[1,3-dithiolan-2,1'-(4'-isopropyl-4'a,5'-dimethyl-(1'H)-octahydro
28	15.308	1.94	Pentane, 1,1,1,5-tetrachloro-
29	15.701	22.21	Tetradecanoic acid
30	15.958	10.12	9,10-SECOERGOSTA-5,7,10(19),22-TETRAENE-1,3,25-TRIO, (3.BETA.,5Z,7E,22E
31	16.210	12.99	(-)-Loliolide
32	16.542	3.61	Neophytadiene
33	17.042	5.88	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
34	18.046	10.23	Hexadecanoic acid, methyl ester
35	18.553	7.26	Palmitoleic acid
36	18.974	6.12	n-Hexadecanoic acid
37	19.443	3.07	Hexadecanoic acid, ethyl ester
38	20.330	14.92	D-Arabinose
39	20.608	8.14	1h-Indole-3-Acetic Acid, Bis(Trimethylsilyl)Hydrazide, Mono(Tr
40	20.808	3.43	n-Octanoic acid isopropyl ester
41	21.008	9.52	cis-10-Heptadecenoic acid
42	21.360	12.63	Heptadecanoic Acid
43	22.025	8.09	6-Octadecenoic acid, methyl ester, (Z)-
44	22.279	5.81	2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL-, [R-[R*,R*-(E)]]-
45	22.642	6.43	Octadecanoic acid, methyl ester (CAS)
46	22.964	5.00	9,12-Octadecadienoic acid (Z,Z)-
47	23.125	10.21	7-Tetradecenal, (Z)-
48	23.637	9.79	Octadecanoic Acid
49	24.142	4.68	Octadecanoic acid, ethyl ester
50	25.958	6.50	10-Bromo-7-Hydroxy-11-Iodolaurene
51	27.394	6.17	4,8,12,16-Tetramethylheptadecan-4-olide
52	28.158	8.16	Succinic acid, ethyl 2-ethylhexyl ester
53	29.667	10.20	Glutaric acid, 2,2,3,3-tetrafluoropropyl 2-chloro-6-fluorophenyl ester
54	29.908	9.10	4'-Chloro-.alpha.-carbomethoxy-cis-3-stilbenzole
55	30.197	4.13	Hexatriacontane
56	30.787	9.87	Bis(2-ethylhexyl) phthalate
57	31.922	6.16	Hexatriacontane
58	32.058	4.70	4-Chloro-8-fluoroquinoline
59	33.058	13.10	Methyl 10-methoxycarbonyl-17-oxooctadecanoate
60	33.552	3.42	Hexatriacontane

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61	35.095	5.64	Hexatriacontane
62	35.735	7.82	Alpha-Tocospiro B
63	35.975	13.13	Hexanedioic Acid, Diethyl Ester
64	36.192	19.09	1-(beta-d-Arabinofuranosyl)-4-difluoromethyl-5-bromouracil
65	36.594	4.67	Hexatriacontane
66	37.675	28.64	17 alpha -Ethynyl-5(10)-estrene-3 alpha,17 beta-diol-di-TMS
67	38.257	6.03	Docosane (CAS)
68	38.458	10.35	1-Eicosanol
69	39.112	5.80	Henicosanal
70	40.232	5.01	Hexatriacontane
71	40.458	8.79	1-Heptacosanol
72	40.658	8.92	11,14-Eicosadienoic acid, methyl ester
73	40.959	6.51	DL-alpha-Tocopherol
74	43.952	7.85	Nonacosanal

Table 5: FTIR spectrum interpretation of watermelon rind water extracts

No.	Wavenumber (cm^{-1})	Functional Groups
1.	717.54	C-H Bend rocking
2.	1068.60	C-O stretching
3.	1151.54	C-O stretching
4.	1465.95	C-H bending
5.	1516.10	C-N stretching
6.	1612.54	C=C stretching
7.	1680.05	C=O stretching
8.	1737.92	C=O stretching
9.	2928.04	C-H stretching
10.	3068.85	=C-H stretching
11.	3389.04	O-H stretching

Table 6: Results of total flavonoid assay of watermelon rind extracts

Sample Code	Abs	Measured Flavonoids (ppm)	G Sample	Sample Volume (L)	Flavonoid Total (mg QE/g)
Water Extract	0.106	0.5846003	0.05021	0.01	0.582155242

Molecular docking is used to understand biomolecular drug interactions in rational drug design and discovery. Molecules (ligands) are placed into the preferred binding sites of specific receptor regions to form stable complexes with potential efficacy and specificity based on molecular interactions and binding affinity.[23] This study used the alpha-glucosidase protein obtained from Protein Data Bank with PDB ID: 3WY1. The ligand molecule used was obtained from watermelon rind extract. The initial validation was done by redocking between the target protein and its native ligand. The validation results are in RMSD (Root Mean Square Deviation) value, which shows the similarity value between the native ligand of redocking results and the native ligand of the protein itself. The validation process on molecular docking is said to be an acceptable validity value if the RMSD value is 2-3 Å.[24] The redocking process of native ligands to its protein can also identify its binding site, which is determined using Gridbox.[25] The results of redocking protein 3WY1 with native ligand PRU at grid box coordinates X = 6.63, Y = 16.267, and Z = 19.72 obtained RMSD value = 2.2497A (Figure 3). Based on these results, the docking process can be considered acceptable and valid. The docking results of the ligands and protein complexes' binding free energy (ΔG) scores and the RMSD values. The binding free energy indicates the affinity between the ligand and the receptor.[26] A low affinity indicates that the ligand and receptor require little binding energy. Thus, the smaller the free energy of the bond, the stronger and more stable the bond between the ligand and the receptor.[27] Bond Energy is obtained from the docking result between the protein and ligand that was previously prepared. The docking results produced 20 poses with different binding free energy. From the 20 docked poses of docking results on each protein, the conformation closest to the native ligand ΔG score is selected. The data in Table 7 shows the ΔG score of the best conformation of each ligand on each protein. The results obtained total free energy of different bonds in each protein. The smaller the (negative) value of the free energy of the bond,

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the smaller the energy required for ligand-protein interaction so that the ligand and protein bonds become more stable.[23] So, it is expected to form a good amino acid interaction. The results of molecular docking on the 3WY1 receptor showed different affinity values. The data obtained, PRU used as a comparison, has an ΔG value of -6.1. This value is a standard value used to predict that compounds that have a score of ± 5% of this value have the same affinity value as PRU against the 3WY1 receptor. The compounds from the (*Citrullus lanatus* (Thunb.) Matsum) plant content that have ΔG values close to PRU are 1,3-Benzenediol, O-acetoxyacetyl; Benzenemethanol; Cyclohexanamine, N-3-butenyl-N-methyl-; 2-Methoxy-4-vinylphenol; Glutaric acid, 2-ethylhexyl 1-naphthyl ester; 4-(Ethoxymethyl)phenol; Cyclohexyl isopropylphosphonofluoridate; Ethyl 3-cyclopropylbicyclo[4. 1. 0]heptane-7-carboxylate; Etoposide; Myristic Acid; Neophytadiene; Diisobutyl phthalate; Methyl palmitate; Palmitic Acid; Ethyl palmitate; cis-10-Heptadecenoic acid; Heptadecanoic acid; Phytol; Methyl 12-hydroxyoctadecanoate; Linoleic Acid; (Z)-Tetradec-7-enal; Stearic Acid; 4,8,12,16-Tetramethylheptadecan-4-olide; Bis(2-ethylhexyl) phthalate; 4-Chloro-8-fluoroquinoline; predicted to have similar activity to PRU. In molecular docking, ligand interaction is characterized by forming bonds between the ligand and its target protein. Hydrogen, van der Waals, and hydrophobic bonds are parameters that help determine the relationship between structure and activity. Hydrogen bonding is stronger than van der Waals bonding (Table 8). Hydrogen bonds can be formed despite the distance between the ligand and the receptor. In addition, hydrophobic interactions also play a role in determining the stability of the ligand towards the receptor. The formation of hydrophobic bonds minimizes the interaction of nonpolar residues with water.[23]

The *α*-glucosidase enzyme inhibition test results showed that watermelon rind extract had an inhibitory activity close to the positive control (Table 9). The positive control showed an inhibition (IC_{50}) value of 40.077 ppm, which indicates strong inhibitory activity against the *α*-glucosidase enzyme. Watermelon rind extract also showed significant inhibitory activity, with an IC_{50} of 47.094 ppm. Although slightly higher than the positive control, the IC_{50} value of the watermelon rind extract showed that this extract had an inhibitory potency close to the effectiveness of the positive control. This indicates that the watermelon rind extract has competitive inhibition ability, although it requires a slightly higher concentration to achieve the same level of inhibition.[9] The small difference in effectiveness between the watermelon rind extract and the positive control may be influenced by several factors, such as the concentration and potency of the active compounds in the extract that is close to, but not equivalent to the positive control. Watermelon rind is known to contain bioactive compounds such as flavonoids and polyphenols that have been shown to have the potential to inhibit the *α*-glucosidase enzyme, suggesting that the concentration and effectiveness of these compounds in the extract are significant and relatively compared to the positive control. Previous molecular docking studies have also shown a good affinity of the compounds in watermelon rind towards *α*-glucosidase. This supports the *in vitro* results, which show inhibitory activity close to the positive control.

Table 7: Binding energy of metabolite compounds

No.	Ligand	ΔG	Receptor I.b	u.b	Rotatable Bond
1	PRU	-6.1	1.534	2.914	10
2	Thiane	-3.5	0.977	2.273	0
3	1,3-Benzenediol, O-acetoxyacetyl	-6.2	1.542	1.843	6
4	Benzenemethanol	-6.2	2.182	3.250	6
5	Butane-1,2,3,4-tetrol	-4.5	1.634	3.158	7
6	2,5-Dimethylfuran-3,4(2H,5H)-dione	-4.5	3.478	4.644	0
7	Furaneol	-4.7	1.567	1.601	1
8	Cyclohexanamine, N-3-butenyl-N-methyl-	-6.2	1.225	1.526	4
9	4H-Pyran-4-one	-4.8	2.457	3.280	2
10	Dipropyleneglycol methyl ether acetate	-4.9	1.100	5.257	7
11	5-Hydroxymaltol	-4.9	2.406	3.218	2
12	Ethyl 1-piperidinecarboxylate	-5.1	1.264	2.079	2
13	5-Hydroxymethylfurfural	-4.5	1.834	2.209	3
14	2-Methoxy-4-vinylphenol	-5.6	1.963	3.966	3
15	Glutaric acid, 2-ethylhexyl 1-naphthyl ester	-5.5	2.922	5.510	13
16	4-(Ethoxymethyl)phenol	-5.8	0.070	1.462	4
17	D-Allose	-5.3	1.460	4.295	6
18	Cyclohexyl isopropylphosphonofluoridate	-6.2	1.488	1.846	3
19	Ethyl 3-cyclopropylbicyclo[4.1.0]heptane-7-carboxylate	-6.5	1.060	1.418	4
20	3,4-Altrosan	-4.9	1.389	1.822	4
21	2-Ethylbutyl acetate	-5.0	2.185	4.658	5
22	Etoposide	7.5	0.000	0.000	8
23	1,1,1,5-Tetrachloropentane	-4.8	0.603	1.773	4

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ISSN 2616-0692 (Electronic)

24	Myristic Acid	-5.8	1.130	1.799	13
25	Loliolide	-5.3	1.878	3.764	1
26	Neophytadiene	-6.9	0.892	8.415	13
27	Diisobutyl phthalate	-7.1	0.956	1.217	8
28	Methyl palmitate	-5.7	3.374	7.799	15
29	Palmitoleic Acid	-6.1	3.157	6.969	14
30	Palmitic Acid	-5.7	3.322	4.868	15
31	Ethyl palmitate	-5.5	3.537	5.285	16
32	DL-Arabinose	-4.7	1.230	3.802	8
33	Isopropyl octanoate	-5.4	2.266	3.525	8
34	cis-10-Heptadecenoic acid	-5.9	3.333	4.920	15
35	Heptadecanoic acid	-5.7	3.603	5.183	16
36	Methyl petroselinate	-5.2	3.677	7.473	16
37	Phytol	-6.2	3.791	7.824	14
38	Methyl 12-hydroxyoctadecanoate	-5.8	-3.389	4.842	18
39	Linoleic Acid	-6.4	1.854	3.000	15
40	(Z)-Tetradec-7-enal	-5.8	2.020	2.650	11
41	Stearic Acid	-5.9	1.050	2.035	17
42	Ethyl stearate	-4.9	4.001	7.826	18
43	4,8,12,16-Tetramethylheptadecan-4-olide	-6.5	1.101	1.640	12
44	Succinic acid, ethyl 2-ethylhexyl ester	-5.4	1.980	3.444	12
45	Glutaric acid	-5.4	2.014	3.674	12
46	4'-Chloro-alpha-carbomethoxy-cis-3-stilbenzole	-5.1	2.847	4.140	4
47	Hexatriacontane	-1.4	1.703	6.889	33
48	Bis(2-ethylhexyl) phthalate	-6.1	1.670	7.820	16
49	4-Chloro-8-fluoroquinoline	-5.6	2.915	3.623	0
50	Methyl 10-methoxycarbonyl-17-oxooctadecanoate	-5.3	1.843	5.686	19
51	alpha-Tocospiro-B	-1.9	2.235	5.530	14
52	Diethyl adipate	-5.0	2.265	6.512	9
53	Limonin	9.0	0.000	0.000	1
54	1-Eicosanol	-5.4	3.683	7.672	19
55	Heneicosanal	-4.9	1.421	2.357	19
56	1-Heptacosanol	-4.0	1.373	5.852	26
57	11,14-Eicosadienoic acid, methyl ester	-4.9	1.445	2.918	17
58	DL-alpha-Tocopherol	-0.6	2.530	7.485	13
59	Nonacosanal	-4.5	1.332	2.908	27

Table 8: Amino Acid Interaction of the ligand with the lowest docking binding energy

Ligand	Receptor ΔG	Ligand Structure	Amino Acid Residue	Bond Interaction
PRU	-6.1		PRO:277, GLY:273, GLY:270, VAL:204, ASP:274, TYR:284, GLU:271, THR:296, MET:281, ASN:205, PHE:297	Van der Waals
			TYR:295	Conventional Hydrogen Bond
			ARG:280, ALA:294	Alkyl
			GLU:271,GLY:273	Covalent bond

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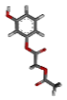
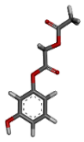
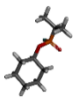
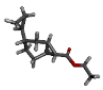
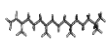
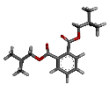
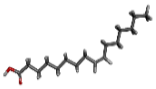
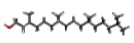
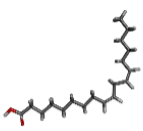
1,3-Benzenediol, O-acetoxyacetyl	-6.2		ARG:404, ARG:200, ILE:146, PHE:206, TYR:235, GLY:273, PHE:297, , PHE:166, GLU:271, THR:203, HIS:105, PHE:147, GLN:170, ASP:62,TYR:65 ARG:400, HIS:332 GLY:228 ASP:333 ASP:202	Van der waals Conventional Hydrogen Bond Carbon Hydrogen Bond Unfavorable Acceptor-Acceptor Pi-Anion
Benzenemethanol	-6.2		PHE:397, TYR:389,PHE:166, GLU:271, ARG:200, ASP:202, ASP:62, PHE:147,ILE:146,PHE:297,GLY:228, VAL:334 ASP:333, HIS:332 ARG:400	Van der waals Conventional Hydrogen Bond Carbon Hydrogen Bond
Cyclohexanamine, N-3-butenyl-N-methyl-	-6.2		GLY:228, TYR:389, ARG:400,THR203, GLN:170,PHE:147, ASP:62, ASP:333, ARG:200 GLU:271, ASP:202	Van der waals Carbon Hydrogen Bond
Cyclohexyl isopropylphosphonofluoridate	-6.2		GLY:228, TYR:389, PHE:147, ASP:62, ASP:202, HIS:332, ARG:200, PHE:297, ILE:146 ARG:400 TYR:65, PHE:166	Van der waals Conventional Hydrogen Bond Pi-Alkyl
Ethyl 3-cyclopropylbicyclo[4.1.0]heptane-7-carboxylate	-6.5		ASP:333, ARG:400, PHE:297, THR:226, ALA:229, GLU:271, THR203, PHE,147, ASP:202 GLY:273, TYR:235	Van der waals Carbon Hydrogen Bond
Neophytadiene	-6.9		THR:203, GLU:271, ASP:202, HIS:105, GLN:170, ASP:62, VAL:335, GLY:228, PHE:297, ASP:333, PHE:397, ALA:229, LEU:227, ASN:301	Van der waals
Diisobutyl phthalate	-7.1		PHE:166, TYR:65 PHE:206, GLY:273, GLU:271, THR:203, GLN:170, HIS:105, TYR:65, ASP:62, ARG:200, ARG:400, PHE:147, ASP:333, PHE:297, GLY:228, PHE:297, GLY:228, PRO:230, PHE:397, VAL:334, ALA:229, ASP:202 ILE:146, PHE:166 PHE:166 TYR:389	Pi-Sigma Van der waals Carbon Hydrogen Bond Pi-Sigma Pi-Pi T-Shaped Pi-Alkyl
Palmitoleic Acid	-6.1		ASN:301, LEU:227, PRO:230, ALA:229, GLY:228, PHE:397, PHE:147, ARG:400, GLU:271, THR:203, ASP:202, ARG:200, TYR:65 ASP:333, HIS:332	Van der waals Conventional Hydrogen Bond
Phytol	-6.2		GLU:271, THR:203, GLY:273, ARG:340, VAL:334, LEU:227, ASP:333, ARG:400, ALA:229, GLY:228, THR:226 LEU:300, ASN:301	Van der waals Conventional Hydrogen Bond
Linoleic Acid	-6.4		ASN:301, LEU:227, PRO:230, ARG:400, ALA:229, PHE:397, ASP:333, THR:203, GLU:271, PHE:206, GLY:273, LEU:244, ASP:274, THR:226, TYR:235 GLY:228	Van der waals Conventional Hydrogen Bond Carbon Hydrogen Bond

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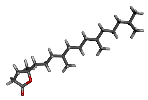
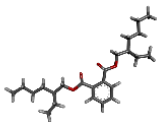
4,8,12,16-Tetramethylheptadecan-4-olide	-6.5		HIS:105, ASP:202, GLN:170, ASP:62, THR:203, GLY:273, GLU:271, ARG:400, GLY:228, ASP:333, VAL:334, LEU:227, ASN:301 ALA:229 PHE:166, TYR:65	Van der waals Conventional Hydrogen Bond Pi-Sigma
Bis(2-ethylhexyl) phthalate	-6.1		VAL:334, PHE:397, TYR:389, PHE:147, GLN:170, ASP:62, ASP:202, GLU:271, GLY:273, THR:226, ALA:229, THR:203, ILE:146, ARG:200, ASP:274, LEU:244, PHE:297 ARG:400 ASP:333 PHE:166, TYR:65	Van der waals Conventional Hydrogen Bond Pi-Anion Pi-Sigma

Table 9: Results of the α-Glucosidase Enzyme Inhibition and IC_{50} (ppm) value of Watermelon Rind Extract

No	Sample	Absorbance	% Inhibition	IC_{50} (ppm)
1	Positive control (Acarbose)	1.565	15.860	40.077
		1.322	28.925	
	Positive control (Duplo)	1.031	44.570	
		1.551	16.613	
		1.309	29.624	
		1.027	44.785	
2	Watermelon rind extract	1.801	3.172	47.094
		1.778	4.409	
	Watermelon rind extract (Duplo)	1.717	7.688	
		1.791	3.709	
		1.788	3.871	
		1.716	7.742	

Figure 3: Overlay of native ligand before and after docking

Conclusion

This study evaluated the antidiabetic potential of watermelon rind extract (*Citrullus lanatus* (Thunb.) Matsum) through *in vitro* and *in silico* approaches, focusing on inhibiting the α-glucosidase enzyme. The results showed that watermelon rind extract had an inhibitory activity close to the positive control, with a competitive IC_{50} value. The study also identified flavonoids and polyphenols as the major secondary metabolites in watermelon rind that may have contributed to α-glucosidase inhibitory activity. FTIR spectra analysis confirmed the presence of bioactive compounds with functional groups that support inhibitory activity. Molecular docking studies supported these results by showing good binding affinity of the compounds in the extract towards the α-glucosidase enzyme. In conclusion, watermelon rind extract has potential as a natural antidiabetic agent by inhibiting α-glucosidase.

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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