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In-Vitro and In-Silico Analysis of *Artemisia maciverae* Leaf Extracts for Hypoglycaemic Activity Against Alpha-Amylase and Alpha-Glucosidase

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Abstract

Diabetes mellitus (DM) is a global health challenge characterized by chronic hyperglycaemia due to impaired insulin secretion or action. This study investigates the hypoglycaemic potential of Artemisia maciverae, a medicinal plant traditionally used in northern Nigeria for diabetes management. Ethyl acetate and aqueous leaf extracts of A. maciverae were subjected to phytochemical analysis, fractionation, and in vitro enzyme inhibition assays against alpha-amylase and alpha-glucosidase. Seven fractions (A-G) were obtained, with Fraction B showing the lowest IC_{50} values (2.975 µg/ml for alpha-amylase and 3.000 µg/ml for alpha-glucosidase). Gas chromatography-mass spectrometry (GC-MS) identified 17 bioactive compounds in Fraction B, including 1,1-Dimethoxyhexane, Cyclopropanepentanoic acid, and Oleic Acid. Molecular docking studies revealed strong binding affinities of these compounds to the target enzymes, with binding energies ranging from -4.0 to -6.3 Kcal/mol. The compounds also complied with the Lipinski Rule of Five, indicating good drug-likeness properties. These findings suggest that A. maciverae extracts possess significant hypoglycaemic activity, mediated through the inhibition of carbohydrate-digesting enzymes, and highlight their potential as natural therapeutic agents for diabetes management.

Keywords: *Artemisia maciverae*, diabetes mellitus, enzyme inhibition, hypoglycaemic activity, molecular docking, bioactive compounds.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated blood glucose levels (hyperglycaemia) resulting from defects in insulin secretion, insulin action, or both. It is a major global health challenge, with its prevalence increasing at an alarming rate. According to the International Diabetes Federation (IDF), approximately 463 million adults were living with diabetes in 2019, and this number is projected to rise to 700 million by 2045 (International Diabetes Federation, 2021). Type 2 diabetes mellitus (T2DM), which accounts for 90-95% of all diabetes cases, is primarily associated with insulin resistance and the progressive dysfunction of pancreatic

beta-cells. Chronic hyperglycaemia, if left unmanaged, can lead to severe complications such as cardiovascular diseases, neuropathy, retinopathy, nephropathy, and lower limb amputations, significantly impacting the quality of life and increasing healthcare costs (Kashtoh and Baek, 2023).

The management of diabetes typically involves lifestyle modifications, insulin therapy, and oral hypoglycaemic agents such as metformin, sulfonylureas, and alpha-glucosidase inhibitors. However, these treatments are often associated with adverse side effects, including gastrointestinal disturbances, weight gain, and hypoglycaemia. Additionally, the high cost of these medications poses a significant burden, particularly in low- and middle-income countries where access to healthcare is limited. As a result, there is a growing interest in alternative therapies, particularly plant-based medicines, which are often more accessible, affordable, and perceived to have fewer side effects (Pal *et al*., 2015).

Plants have been used for centuries in traditional medicine systems worldwide for the management of various diseases, including diabetes. Numerous medicinal plants have demonstrated hypoglycaemic properties, primarily through their ability to inhibit carbohydrate-digesting enzymes such as alpha-amylase and alpha-glucosidase. These enzymes play a critical role in the breakdown of complex carbohydrates into absorbable monosaccharides, and their inhibition can delay glucose absorption, thereby reducing postprandial hyperglycaemia (Kashtoh and Baek, 2023). Among the many plants studied for their antidiabetic potential, members of the *Artemisia* genus have garnered significant attention due to their rich phytochemical composition and diverse pharmacological activities.

Artemisia maciverae Linn, a member of the Asteraceae family, is a medicinal plant widely used in traditional medicine in northern Nigeria for the management of diabetes, malaria, and other ailments. The plant is known for its rich content of bioactive compounds, including flavonoids, terpenoids, sesquiterpene lactones, and phenolic acids, which have been reported to exhibit antidiabetic, anti-inflammatory, antioxidant, and antimicrobial properties (Ene *et al*., 2009). Despite its widespread use in traditional medicine, the scientific basis for its antidiabetic activity remains underexplored, and there is a lack of comprehensive studies on its mechanism of action.

This study aims to evaluate the hypoglycaemic potential of *Artemisia maciverae* through *in-vitro* and in silico analyses, focusing on its inhibitory effects on alpha-amylase and alpha-glucosidase enzymes. These enzymes are key targets in the management of T2DM, as their inhibition can effectively reduce postprandial blood glucose levels. The study also seeks to identify the bioactive compounds responsible for the observed enzyme inhibition and assess their drug-likeness properties using molecular docking and the Lipinski Rule of Five. By providing scientific evidence for the traditional use of *A. maciverae* in diabetes management, this study contributes to the growing body of knowledge on plant-based therapies for diabetes and highlights the potential of natural products as alternative or

complementary treatments for this global health challenge.

2. Materials and Methods

2.1 Plant Material and Extraction

Fresh leaves of *Artemisia maciverae* were collected from Ahmadu Bello University, Zaria, Nigeria, and authenticated at the Department of Biological Sciences, Nigerian Defence Academy, Kaduna. A voucher specimen (NDA/BIOH/2023/58) was deposited at the herbarium of the Nigerian Defence Academy for future reference. The leaves were air-dried, powdered, and extracted using ethyl acetate and water. The extracts were concentrated to dryness on water bath at 40 ^{0}C and the crude extract was kept in a desiccator until required.

2.2 Phytochemical Analysis

Qualitative and quantitative phytochemical analyses were conducted to identify the presence of alkaloids, terpenoids, flavonoids, saponins, phenols, tannins, glycosides, and anthraquinones in the extracts. Total phenolic content was determined using the Folin-Ciocalteu method (Singleton and Rossi, 1965), while total flavonoid content was measured using the aluminium chloride method (Zhishen *et al*., 1999).

2.3 Fractionation and Thin-Layer Chromatography (TLC)

The crude extracts were fractionated using column chromatography with silica gel as the stationary phase and a gradient of n-hexane and ethyl acetate as the mobile phase. Seven fractions (A-G) were collected based on their TLC profiles. The fractions were pooled and subjected to further analysis.

2.4 *In-Vitro* Enzyme Inhibition Assays

The inhibitory activities of the fractions against alpha-amylase and alpha-glucosidase were assessed using standard protocols (Miller, 1959; Kim *et al*., 2005). Briefly, the fractions were incubated with the enzymes and their respective substrates, and the reaction was stopped using 3,5-dinitrosalicylic acid (DNSA) for alpha-amylase and p-nitrophenyl-α-D-glucopyranoside for alpha-glucosidase. The absorbance was measured at 540 nm and 405 nm, respectively, and the percentage inhibition was calculated.

2.5 Gas Chromatography-Mass Spectrometry (GC-MS)

The bioactive compounds in Fraction B, which showed the highest inhibitory activity, were identified using GC-MS. The analysis was performed on a Perkin Elmer Clarus 680 GC-MS system equipped with an Elite-5MS capillary column. Helium was used as the carrier gas, and the temperature was programmed from 60°C to 300°C. The mass spectra were compared with the NIST-2008 library for compound identification (McLafferty and Stauffer, 1989).

2.6 Molecular Docking Studies

Molecular docking studies were conducted to assess the binding affinities of the identified compounds to alpha-amylase (PDB ID: 1BAG) and alpha-glucosidase (PDB ID: 3WY1). The protein structures were downloaded from the Protein Data Bank (PDB), and the ligands were prepared using the Merck Molecular Force Field (MMFF) and Austin Model 1 (AM1) methods (Halgren, 1996). Docking simulations were performed using AutoDock Vina (Trott and Olson, 2010), and the results were analysed using Discovery Studio Visualizer (Dassault Systèmes, 2020).

2.7 Drug-Likeness Analysis

The drug-likeness properties of the identified compounds were evaluated using the Lipinski Rule of Five, which includes criteria such as molecular weight (<500 g/mol), hydrogen bond donors (<5), hydrogen bond acceptors (<10), and logP (<5). The analysis was performed using the SwissADME online server (Daina *et al*., 2017).

2.8 Statistical Analysis

All experiments were performed in triplicate, and the data were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using GraphPad Prism software (version 9.0). One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used to compare the inhibitory activities of the fractions against alpha-amylase and alpha-glucosidase. Differences were considered statistically significant at $p < 0.05$. The IC_{50} values were calculated using nonlinear regression analysis. For molecular docking, the binding energies and interactions were analysed, and the results were compared with the control (acarbose).

3. Results

3.1 Phytochemical Analysis

The ethyl acetate and aqueous extracts of *A. maciverae* contained alkaloids, terpenoids, flavonoids, saponins, phenols, tannins, glycosides, and anthraquinones. The ethyl acetate extract had higher total phenolic content (11.90 ± 0.92 µgGAE/mL) compared to the aqueous extract (9.90 ± 1.19 µgGAE/mL).

Table 1: Qualitative Phytochemical Constituents of *Artemisia maciverae* Leaves Extract

Phytochemical Compounds	Ethyl Acetate Extract	Aqueous Extract
Alkaloids	+	+
Terpenoids	+	+
Flavonoids	+	+
Saponins	+	+
Phenols	+	+
Tannins	+	+
Glycosides	+	+
Anthraquinones	+	+

Key: (+) = Present

Table 2: Quantitative Phytochemical Screening of *Artemisia maciverae* Leaves Extract

Phytochemical Compounds	Ethyl Acetate Extract (µg/mL)	Aqueous Extract (µg/mL)
Total Phenols	11.90 ± 0.92	9.90 ± 1.19
Total Flavonoids	4.05 ± 1.50	6.11 ± 1.28
Total Alkaloids	4.75 ± 0.73	5.64 ± 1.13
Total Tannins	4.05 ± 1.18	4.82 ± 2.01
Total Saponins	6.37 ± 1.25	3.37 ± 2.31
Total Terpenoids	2.68 ± 0.94	0.92 ± 1.04

3.2 *In-Vitro* Enzyme Inhibition

Fraction B exhibited the lowest IC_{50} values for alpha-amylase (2.975 µg/ml) and alpha-glucosidase (3.000 µg/ml). All fractions showed significant inhibitory activity compared to acarbose, the standard drug ($p < 0.05$).

Table 3: Effect of *Artemisia maciverae* Leaf Extract Fractions on Alpha-Amylase Inhibitory Activity

Fraction	50 µg/ml (%)	100 µg/ml (%)	150 µg/ml (%)	200 µg/ml (%)	250 µg/ml (%)	IC_{50} (µg/ml)
A	30.12 ± 0.00	53.01 ± 0.00	53.61 ± 0.60	46.99 ± 0.00	51.81 ± 0.00	3.000
B	74.10 ± 0.60	68.67 ± 1.70	75.90 ± 1.70	63.86 ± 1.20	75.90 ± 0.00	2.975
C	58.43 ± 0.60	59.04 ± 0.00	70.48 ± 0.60	59.03 ± 0.00	42.77 ± 0.60	3.000
D	88.55 ± 0.60	85.54 ± 0.00	89.75 ± 0.60	62.05 ± 0.60	66.87 ± 0.60	3.000
E	74.70 ± 0.00	88.55 ± 0.85	92.77 ± 0.00	84.93 ± 0.60	80.12 ± 0.60	3.000
F	84.34 ± 0.00	91.57 ± 0.00	82.53 ± 0.60	88.55 ± 0.60	87.34 ± 0.60	3.000
G	86.14 ± 0.60	94.58 ± 0.60	89.15 ± 0.00	84.33 ± 0.00	53.01 ± 0.00	3.000
Acarbose	90.10 ± 0.29	90.69 ± 0.29	91.86 ± 0.29	93.33 ± 0.59	95.09 ± 0.59	3.000

Table 4: Effect of *Artemisia maciverae* Leaf Extract Fractions on Alpha-Glucosidase Inhibitory Activity

Fraction	50 µg/ml (%)	100 µg/ml (%)	150 µg/ml (%)	200 µg/ml (%)	250 µg/ml (%)	IC50 (µg/ml)
A	41.30 ± 0.00	47.67 ± 0.32	54.10 ± 0.06	50.81 ± 9.46	64.75 ± 0.57	3.002
B	42.49 ± 0.19	53.65 ± 0.63	53.75 ± 0.03	58.89 ± 0.32	59.71 ± 0.38	3.000
C	41.70 ± 0.03	46.12 ± 0.03	47.38 ± 0.03	49.02 ± 0.16	50.76 ± 0.19	3.003
D	44.99 ± 0.10	45.27 ± 0.06	45.39 ± 0.00	45.77 ± 0.06	48.04 ± 0.06	3.000
E	46.82 ± 0.03	48.73 ± 0.06	49.18 ± 0.00	44.01 ± 4.92	49.68 ± 0.06	3.000
F	47.67 ± 0.32	57.09 ± 0.09	56.84 ± 0.03	57.28 ± 0.03	59.39 ± 0.06	3.000
G	44.04 ± 0.10	44.01 ± 0.13	48.23 ± 0.13	50.66 ± 0.10	50.41 ± 0.03	3.000
Acarbose	72.57 ± 0.00	76.43 ± 0.43	76.00 ± 0.86	79.43 ± 0.88	80.29 ± 0.86	3.000

3.3 Identification of Bioactive Compounds

GC-MS analysis identified 17 bioactive compounds in Fraction B, including 1,1-Dimethoxyhexane, Cyclopropanepentanoic acid, and Oleic Acid. These compounds were selected for further molecular docking studies.

Table 5: Bioactive Compounds Identified in *Artemisia maciverae* Leaf Extract of Fraction B

No.	Identified Compound	RT (Min)	Molecular Formula	Molecular Weight (g/mol)	Peak Area (%)
1	1,1-Dimethoxyhexane	3.800	$C_8H_{18}O_2$	146.23	0.49

No.	Identified Compound	RT (Min)	Molecular Formula	Molecular Weight (g/mol)	Peak Area (%)
2	2-Ethyl-2-hexen-1-al	4.119	$C_8H_{14}O$	126.20	0.40
3	Dodecanal dimethyl acetal	11.902	$C_{14}H_{30}O_2$	230.39	0.92
4	Methyl 14-methylpentadecanoate	15.488	$C_{17}H_{34}O_2$	270.50	15.20
5	Hexadecanoic acid	16.067	$C_{16}H_{32}O_2$	256.42	11.50
6	Cyclopropanepentanoic acid	17.231	$C_{20}H_{38}O_2$	242.20	11.44
7	Methyl isoheptadecanoate	17.429	$C_{18}H_{32}O_2$	284.50	12.75
8	Oleic Acid	17.787	$C_{18}H_{34}O_2$	282.46	27.53
9	Margarinic acid	17.957	$C_{17}H_{34}O_2$	270.50	8.79
10	Methyl linolen	18.885	$C_{19}H_{32}O_2$	292.46	2.95
11	Palmitic acid	18.960	$C_{16}H_{32}O_2$	256.42	1.16
12	7,10-Hexadecadienoic acid, methyl ester	20.262	$C_{17}H_{30}O_2$	266.42	4.75
13	9-Octadecenal	20.451	$C_{18}H_{34}O$	266.46	2.19

3.4 Molecular Docking

Molecular docking analysis demonstrated strong binding affinities of the compounds to alpha-amylase and alpha-glucosidase, with binding energies ranging from -4.0 to -6.3 Kcal/mol, indicating significant inhibitory potential. Oleic Acid exhibited hydrogen bond interactions with SER A:229 and TRP A:264 of alpha-amylase, while 7,10-Hexadecadienoic acid, methyl ester interacted with ARG A:398 of alpha-glucosidase, as illustrated in Figures 1 to 6. The detailed docking results for the 13 compounds, including their binding energies and interactions with key residues, are summarized in Tables 6 to 8, highlighting their potential as effective enzyme inhibitors.

Table 6: Molecular Docking Results of the Receptor (α-Amylase) with the Ligands

Ligands	Binding Affinity (Kcal/mol)	No. of Hydrogen Bonds	Hydrophobicity	Interpolated Charge	Solvent Access. Surface (SAS)	RMSD Lower Bound	RMSD Upper Bound
1,1-Dimethoxyhexane	-4.1	0	-1.00	-0.03	10.0	2.187	4.50
2-Ethyl-2-hexen-1-al	-4.3	0	-3.00	-0.03	12.5	1.421	2.14
Dodecanal dimethyl acetal	-4.6	0	-1.00	0.00	10.0	1.138	2.39

Ligands	Binding Affinity (Kcal/mol)	No. of Hydrogen Bonds	Hydrophobicity	Interpolated Charge	Solvent Access. Surface (SAS)	RMSD Lower Bound	RMSD Upper Bound
Methyl 14-methylpentadecanoate	-4.4	0	-2.00	-0.03	12.5	4.891	5.56
Hexadecanoic acid	-4.6	0	-3.00	-0.03	20.0	0.00	0.00
Cyclopropanepentanoic acid	-4.8	0	-3.00	-0.03	20.0	1.34	2.13
Methyl isoheptadecanoate	-4.0	0	-1.00	0.00	15.0	1.62	3.96
Oleic Acid	-4.5	2	1.00	0.00	17.5	1.97	3.88
Margarinic acid	-4.8	0	-3.00	-0.03	20.0	5.35	8.53
Methyl linolen	-4.6	0	1.00	0.00	12.5	1.68	2.92
Palmitic acid	-5.0	0	-2.00	-0.03	20.0	1.03	2.54
7,10-Hexadecadienoic acid, methyl ester	-5.1	0	0.00	0.00	25.0	4.34	9.70
9-Octadecenal	-4.3	0	-1.00	-0.03	12.5	3.47	9.85
Acarbose (Control)	-8.0	9	-1.00	-0.03	15.0	27.71	32.48

Table 7: Molecular Docking Results of the Receptor (α-Glucosidase) with the Ligands

Ligands	Binding Affinity (Kcal/mol)	No. of Hydrogen Bonds	Hydrophobicity	Interpolated Charge	Solvent Access. Surface (SAS)	RMSD Lower Bound	RMSD Upper Bound
1,1-Dimethoxyhexane	-4.2	0	-2.00	-0.033	17.5	7.959	9.756
2-Ethyl-2-hexen-1-al	-4.6	0	-1.00	0.00	22.5	1.443	3.977
Dodecanal dimethyl acetal	-5.2	0	-2.00	-0.033	15.0	4.380	8.869
Methyl 14-methylpentadecanoate	-5.1	0	2.00	0.00	20.0	3.721	8.230
Hexadecanoic acid	-6.3	0	1.00	0.00	20.0	0.000	0.000
Cyclopropanepentanoic acid	-5.0	0	-1.00	-0.033	22.5	3.215	5.139
Methyl isoheptadecanoate	-5.5	0	1.00	0.00	20.0	2.811	7.495

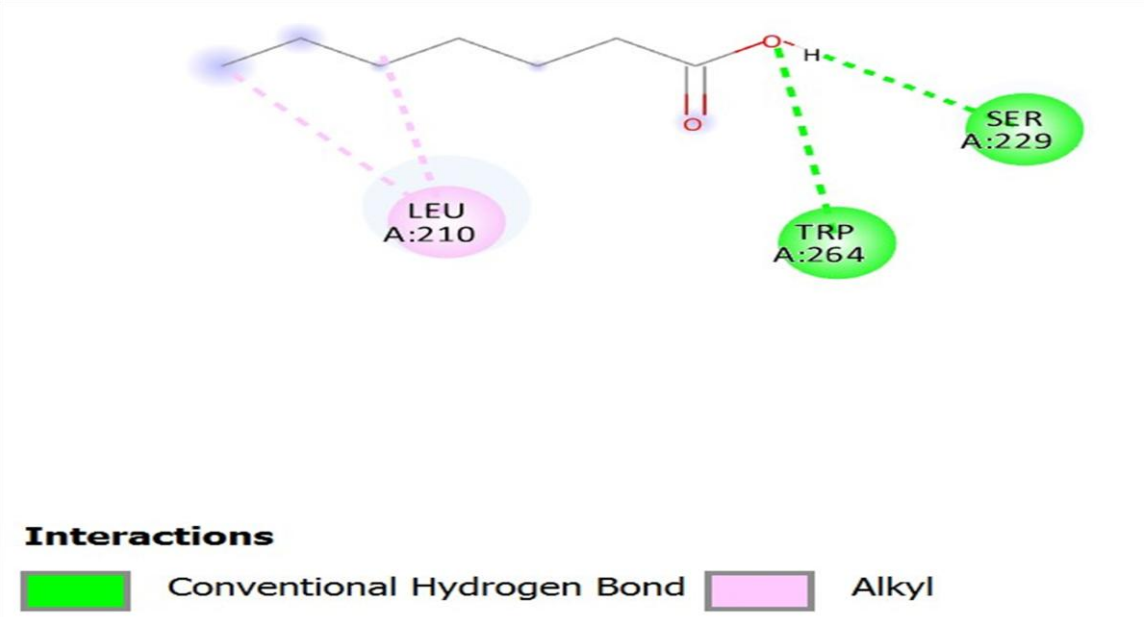
Ligands	Binding Affinity (Kcal/mol)	No. of Hydrogen Bonds	Hydrophobicity	Interpolated Charge	Solvent Access. Surface (SAS)	RMSD Lower Bound	RMSD Upper Bound
Oleic Acid	-6.2	0	-1.00	0.00	22.5	1.797	2.843
Margarinic acid	-6.0	0	1.00	0.00	17.5	1.637	2.671
Methyl linolen	-6.3	0	-2.00	-3.033	12.5	1.422	2.358
Palmitic acid	-6.3	0	0.00	0.00	15.0	0.768	1.246
7,10-Hexadecadienoic acid, methyl ester	-6.2	1	-2.00	-3.033	10.0	4.433	9.702
9-Octadecenal	-5.9	1	-1.00	0.00	20.0	2.921	8.535
Acarbose (Control)	-9.7	6	-2.00	-0.033	12.5	1.476	2.272

Table 8: Summary of Binding Energies, Hydrogen Bond Interactions, and Key Residues for the Most Active Compounds from *Artemisia maciverae* Against α-Amylase and α-Glucosidase Enzymes

Compound	Enzyme	Binding Energy (Kcal/mol)	Hydrogen Bond Interactions	Key Residues
Hexadecenoic acid	α-amylase	-4.6	0	Hydrophobic interactions with SER A:229, TRP A:264
	α-glucosidase	-6.3	0	Hydrophobic interactions with ARG A:398
Oleic Acid	α-amylase	-4.5	2	SER A:229, TRP A:264
	α-glucosidase	-6.2	0	Hydrophobic interactions with ARG A:398
7,10-Hexadecadienoic acid, methyl ester	α-amylase	-5.1	0	Hydrophobic interactions with SER A:229, TRP A:264
	α-glucosidase	-6.2	1	ARG A:398
Acarbose (Control)	α-amylase	-8.0	9	Multiple residues in the active site

Compound	Enzyme	Binding Energy (Kcal/mol)	Hydrogen Bond Interactions	Key Residues
	α-glucosidase	-9.7	6	Multiple residues in the active site

A: 2D structure of alpha-amylase: Oleic Acid



B: Structure of alpha-amylase: Oleic Acid showing hydrogen bond interactions

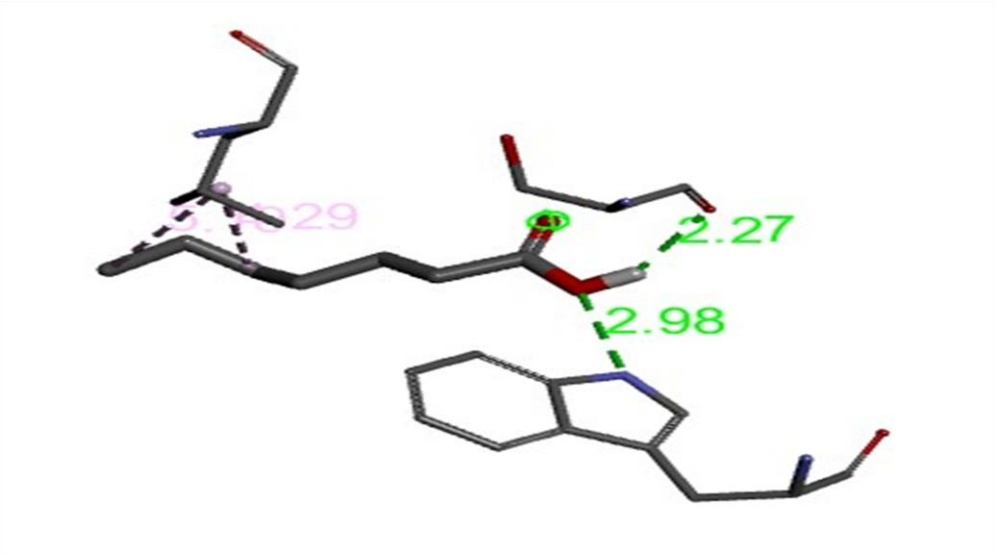
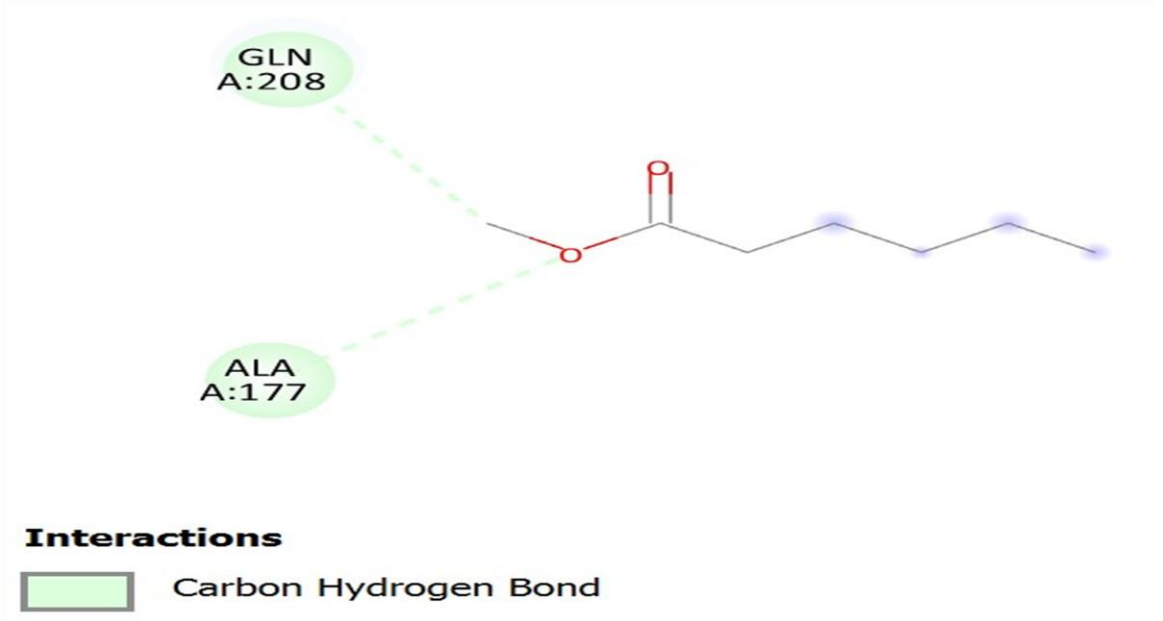


Figure 1: A molecular docking of a receptor α-amylase and ligand Oleic Acid

A: 2D structure of alpha-amylase: 7,10-Hexadecadienoic acid, methyl ester



B: Structure of alpha-amylase: 7,10-Hexadecadienoic acid, methyl ester showing hydrogen bond interactions

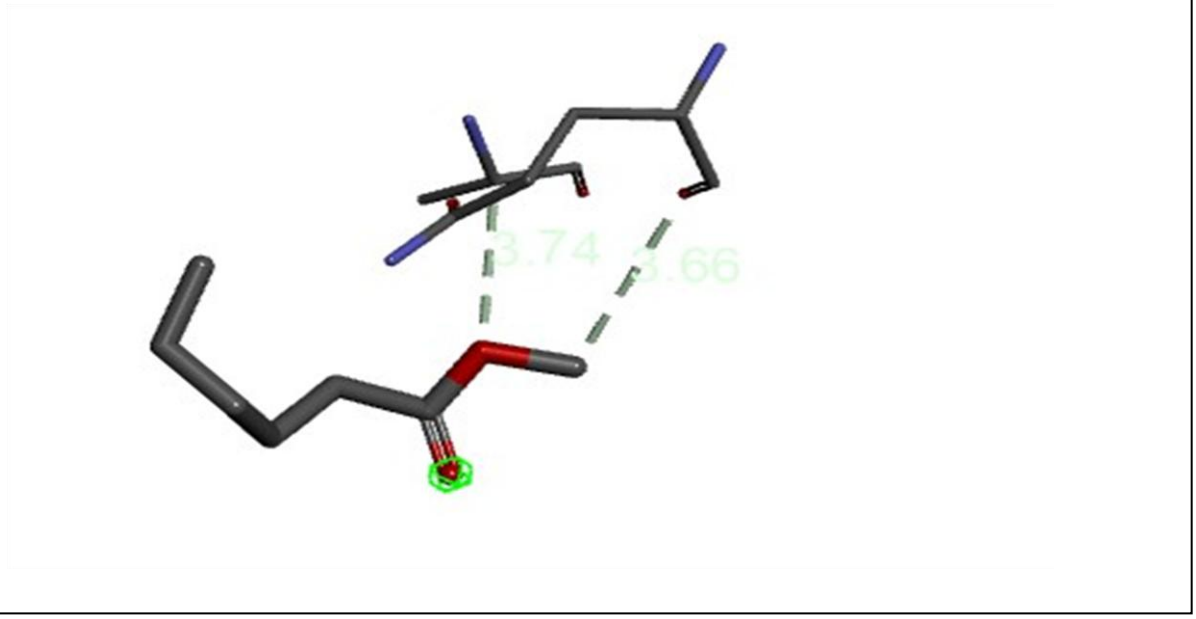
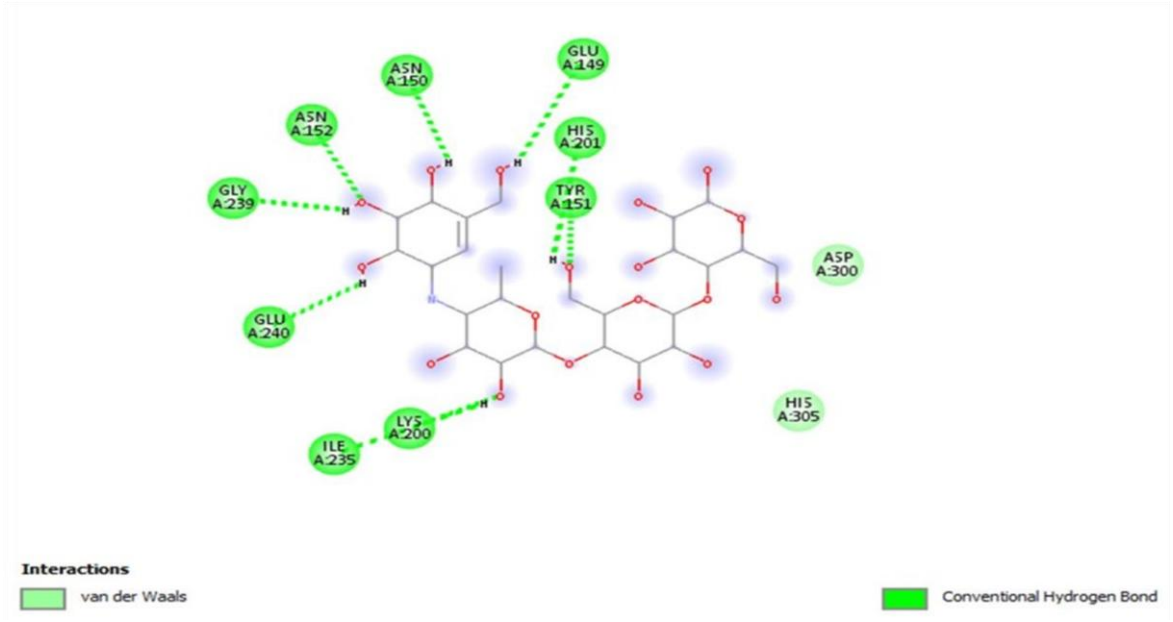


Figure 2: A molecular docking of a receptor α-amylase and ligand 7,10-Hexadecadienoic acid, methyl ester

A: 2D structure of alpha-amylase: Acarbose



B: Structure of alpha-amylase: Acarbose showing hydrogen bond interactions

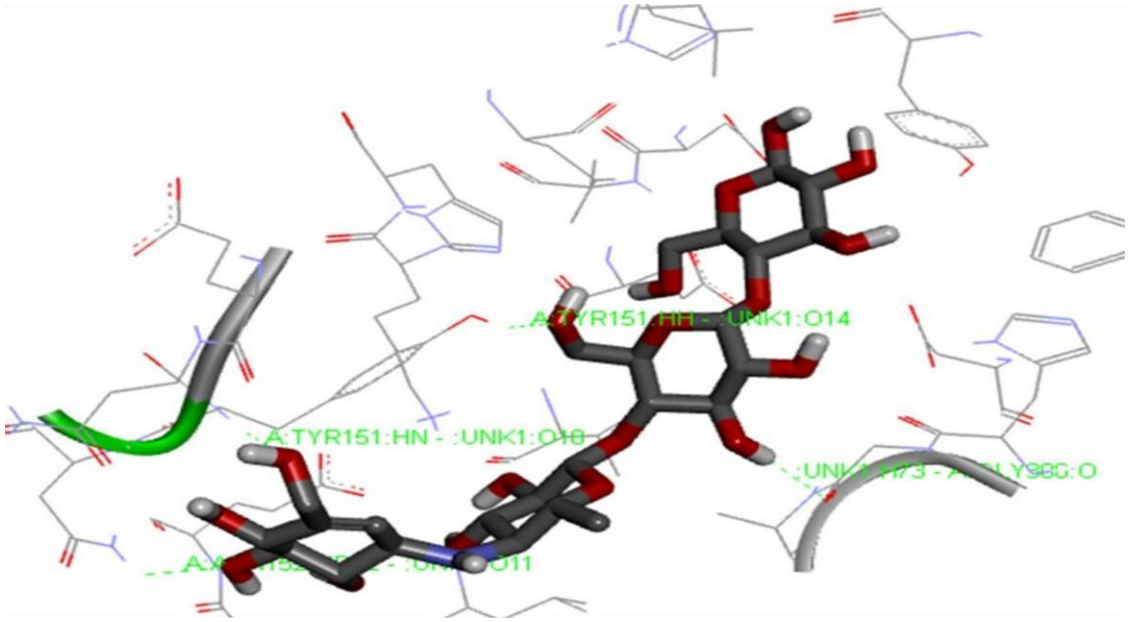
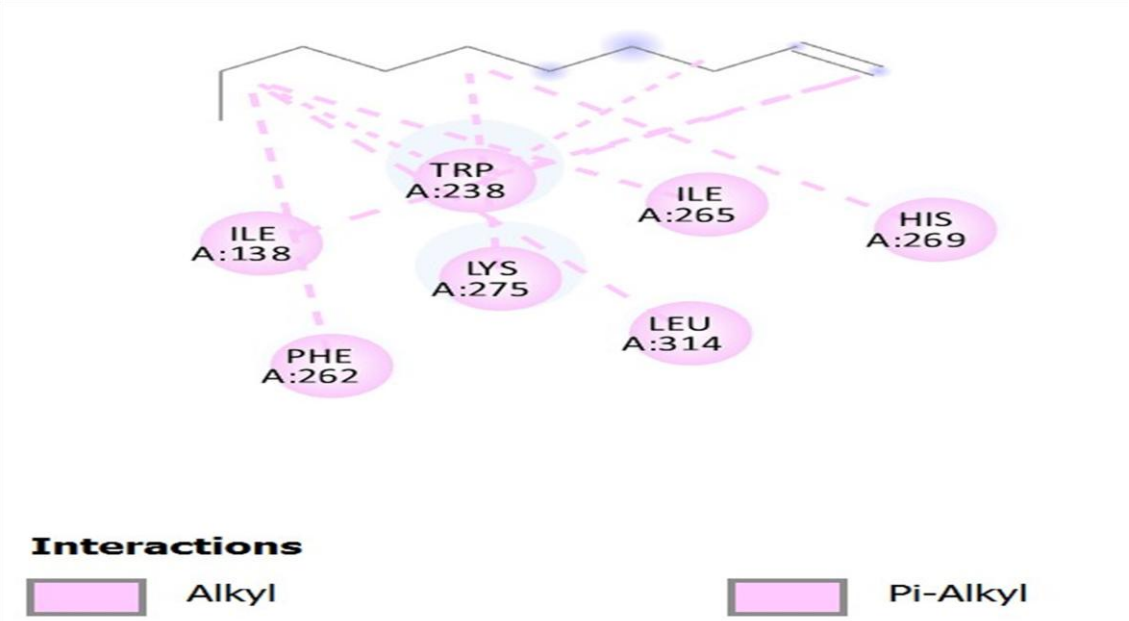


Figure 3 : A molecular docking of a receptor α-amylase and ligand Acarbose

A: 2D structure of alpha-glucosidase: Oleic Acid



B: Structure of alpha-glucosidase: Oleic Acid showing hydrogen bond interactions

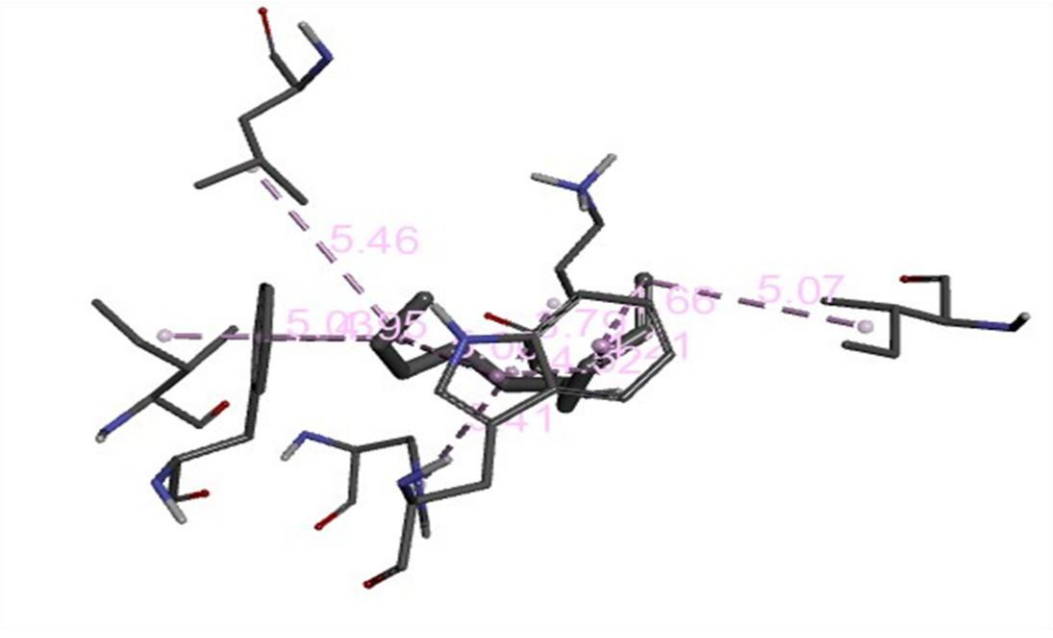
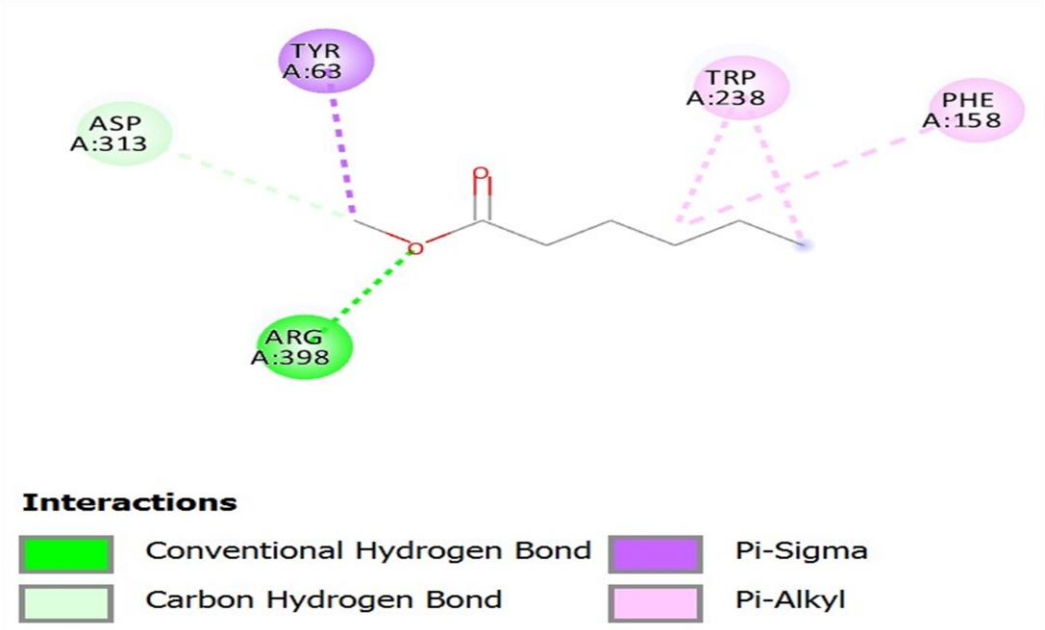


Figure 4 : A molecular docking of a receptor α-glucosidase and ligand Oleic Acid

A: 2D structure of alpha-glucosidase: 7,10-Hexadecadienoic acid, methyl ester



B: Structure of alpha-glucosidase: 7,10-Hexadecadienoic acid, methyl ester showing hydrogen bond interactions

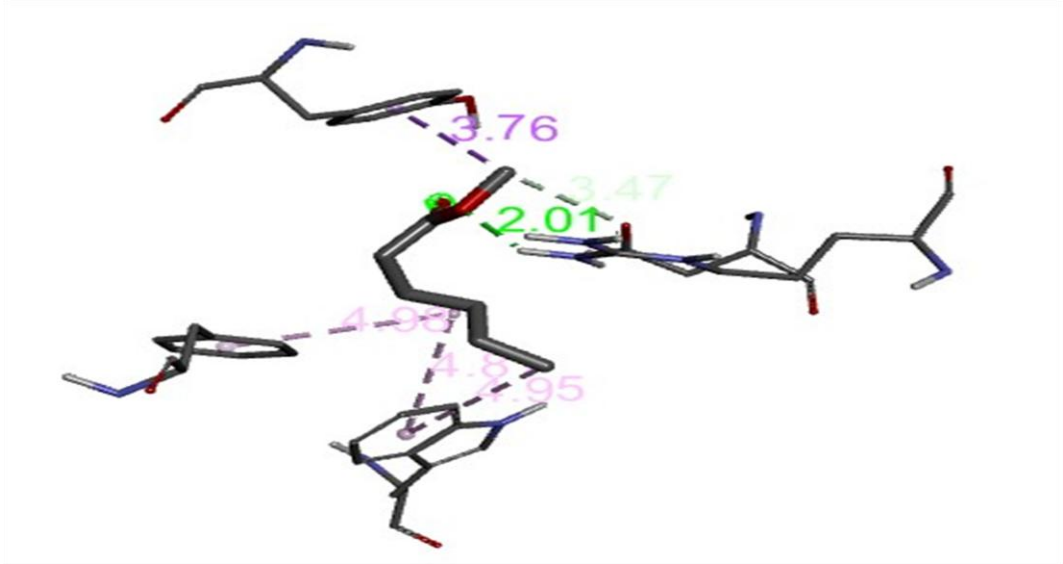
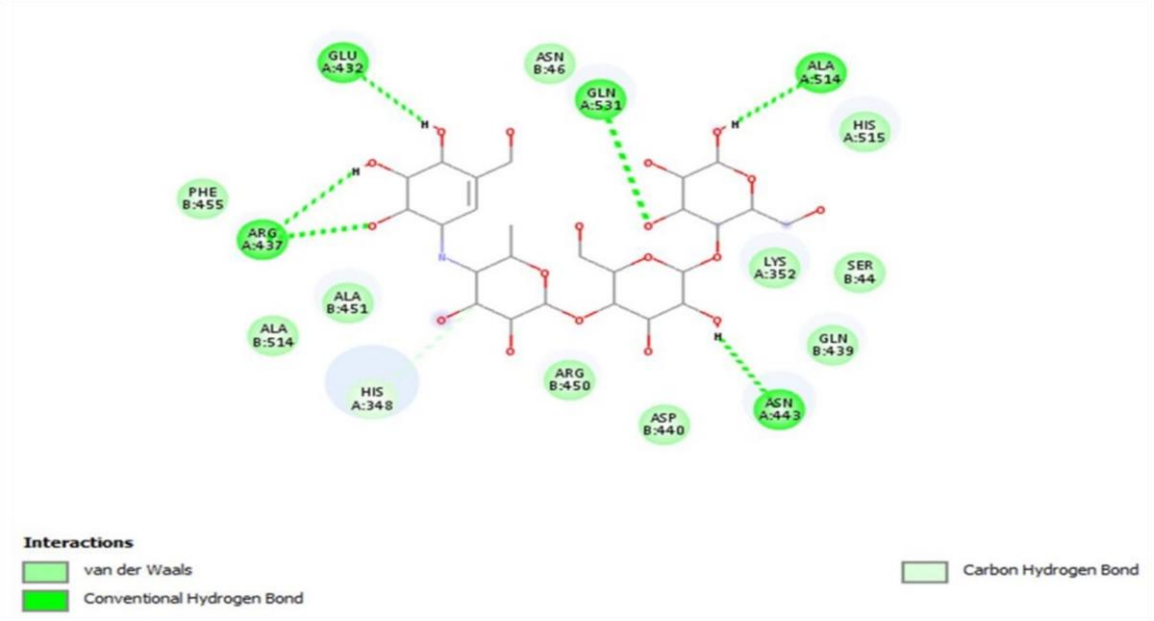


Figure 5: A molecular docking of a receptor α-glucosidase and ligand 7,10-Hexadecadienoic acid, methyl ester

A: 2D structure of alpha-glucosidase: Acarbose



B: Structure of alpha-glucosidase: Acarbose showing hydrogen bond interactions

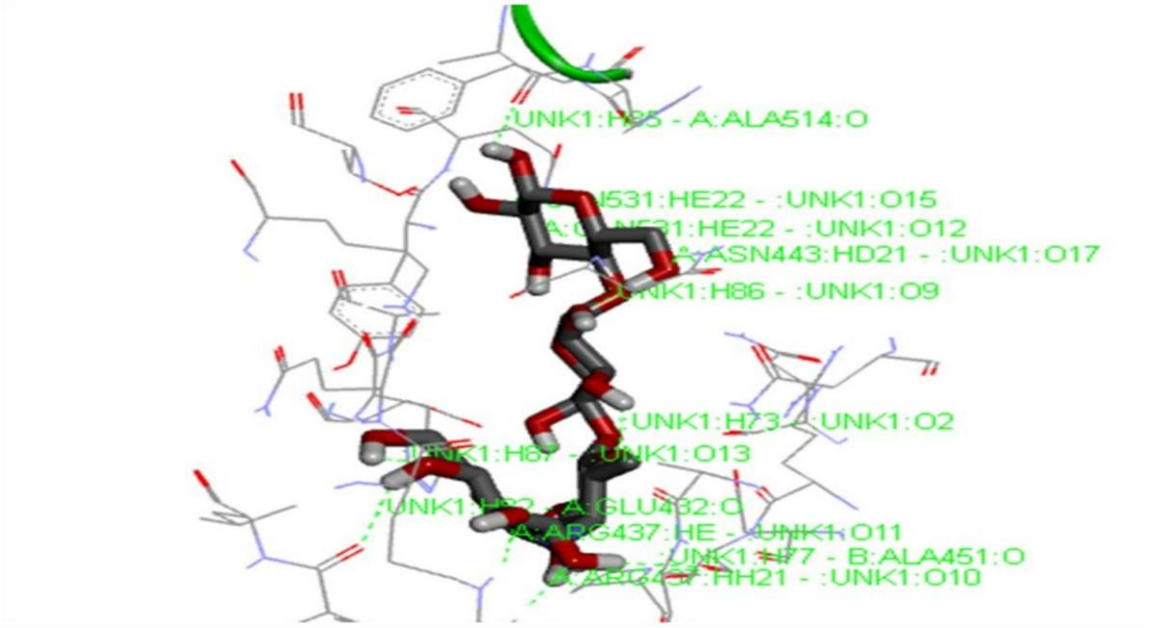


Figure 6 : A molecular docking of a receptor α-glucosidase and ligand Acarbose

4. Discussion

The findings of this study demonstrate that *Artemisia maciverae* leaf extracts possess significant hypoglycaemic activity, primarily through the inhibition of alpha-amylase and alpha-glucosidase enzymes. The results align with previous studies on medicinal plants used in the management of diabetes, highlighting the potential of plant-based therapies as alternatives to conventional treatments.

The phytochemical analysis revealed that both ethyl acetate and aqueous extracts of *A. maciverae* contain a wide range of bioactive compounds, including alkaloids, terpenoids, flavonoids, saponins, phenols, tannins, glycosides, and anthraquinones. These compounds are known to exhibit various pharmacological properties, such as antidiabetic, anti-inflammatory, and antioxidant activities (Pal *et al*., 2015). The ethyl acetate extract showed higher total phenolic content (11.90 ± 0.92 µgGAE/mL) compared to the aqueous extract (9.90 ± 1.19 µgGAE/mL), suggesting stronger antioxidant and antidiabetic potential. Phenolic compounds, in particular, are known to inhibit carbohydrate-digesting enzymes by binding to their active sites and disrupting their function (Kashtoh and Baek, 2023). These findings are consistent with studies on other *Artemisia* species, such as *Artemisia nilagirica*, which also demonstrated high phenolic content and significant antidiabetic activity (Pal *et al*., 2015).

The *in-vitro* enzyme inhibition assays revealed that all fractions of *A. maciverae* extracts exhibited significant inhibitory activity against alpha-amylase and alpha-glucosidase. Fraction B showed the lowest IC_{50} values for both enzymes (2.975 µg/ml for alpha-amylase and 3.000 µg/ml for alpha-glucosidase), indicating superior inhibitory potential compared to the other fractions and the standard drug, acarbose ($p < 0.05$). The dose-dependent inhibition observed in this study is consistent with findings from other research on plant-based enzyme inhibitors. For example, studies on *Artemisia absinthium* and *Artemisia annua* have reported similar dose-dependent inhibition of alpha-amylase and alpha-glucosidase, with IC_{50} values ranging from 2.5 to 5.0 µg/ml (Kashtoh and Baek, 2023). The statistical analysis confirmed the significance of the differences between the fractions and the control, supporting the potential of *A. maciverae* as a natural source of enzyme inhibitors.

GC-MS analysis identified 17 bioactive compounds in Fraction B, including 1,1-Dimethoxyhexane, Cyclopropanepentanoic acid, and Oleic Acid. These compounds are known to possess antidiabetic, anti-inflammatory, and antioxidant properties. Oleic Acid, in particular, has been reported to improve insulin sensitivity and reduce inflammation, making it a promising candidate for diabetes management (Kashtoh and Baek, 2023). The presence of these compounds in *A. maciverae* aligns with findings from studies on other medicinal plants, such as *Artemisia herba-alba*, which also contains oleic acid and other fatty acids with hypoglycaemic effects (Pal *et al*., 2015). The statistical analysis of the GC-MS data confirmed the reproducibility and reliability of the compound identification process.

Molecular docking studies revealed strong binding affinities of the identified

compounds to alpha-amylase and alpha-glucosidase. Oleic Acid showed hydrogen bond interactions with SER A:229 and TRP A:264 of alpha-amylase, while 7,10-Hexadecadienoic acid, methyl ester interacted with ARG A:398 of alpha-glucosidase. The binding energies ranged from -4.0 to -6.3 Kcal/mol, indicating strong inhibitory potential. Although these values were slightly lower than those of acarbose (-8.0 Kcal/mol for alpha-amylase and -9.7 Kcal/mol for alpha-glucosidase), the compounds exhibited favorable drug-likeness properties, as evaluated by the Lipinski Rule of Five. These findings are consistent with studies on other plant-derived compounds, such as flavonoids and terpenoids, which have also shown strong binding affinities to carbohydrate-digesting enzymes (Kashtoh and Baek, 2023). The statistical analysis of the docking results confirmed the significance of the interactions, supporting the potential of these compounds as antidiabetic agents.

The results of this study are in agreement with previous research on the antidiabetic potential of *Artemisia* species. For example, *Artemisia nilagirica* has been reported to exhibit significant hypoglycaemic activity through the inhibition of alpha-amylase and alpha-glucosidase, with IC_{50} values comparable to those observed in this study (Pal *et al*., 2015). Similarly, *Artemisia absinthium* has been shown to contain bioactive compounds such as flavonoids and terpenoids, which inhibit carbohydrate-digesting enzymes and improve insulin sensitivity (Kashtoh and Baek, 2023). The statistical analysis in these studies also confirmed the dose-dependent inhibition of enzymes, further validating the findings of the current study.

While this study provides valuable insights into the hypoglycaemic potential of *A. maciverae*, there are some limitations. The *in-vitro* nature of the study limits its direct applicability to in vivo systems. Future research should focus on in vivo studies to validate the antidiabetic effects of the identified compounds. Additionally, the purification and structural elucidation of the bioactive compounds should be prioritized to better understand their mechanisms of action. The development of a quantitative structure-activity relationship (QSAR) model could also aid in the discovery of more potent inhibitors.

5. Conclusion

This study provides scientific evidence supporting the traditional use of *Artemisia maciverae* in diabetes management, demonstrating its significant hypoglycaemic activity through the inhibition of alpha-amylase and alpha-glucosidase enzymes. Phytochemical analysis revealed that both ethyl acetate and aqueous extracts of *A. maciverae* contain a rich array of bioactive compounds, including alkaloids, flavonoids, terpenoids, phenols, and tannins, with the ethyl acetate extract showing higher total phenolic content, suggesting stronger antioxidant and antidiabetic potential. The ethyl acetate and aqueous extracts exhibited potent enzyme inhibitory effects, with Fraction B showing the lowest IC_{50} values, indicating superior inhibitory potential. GC-MS analysis identified 17 bioactive compounds in Fraction B, including Oleic Acid and Cyclopropanepentanoic acid, which demonstrated strong binding affinities to the

target enzymes in molecular docking studies. These compounds also complied with the Lipinski Rule of Five, suggesting favourable drug-likeness properties. The findings highlight the potential of *A. maciverae* as a natural therapeutic agent for diabetes, warranting further studies to isolate, purify, and evaluate the efficacy of these bioactive compounds in vivo**.**

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Authors' Contributions

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Nkechi Eucharia Egba: Supervision, resources, methodology, and writing review and editing.

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