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Studies on Molecular Docking of *Moringa Oleifera Leaf* Phytochemical Constituents on Alpha Glucosidase, Alpha Amylase and Dipeptidyl Peptidase

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AN, EIO, HUN, DCN, INN and RE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GUO, CCNV, COA, CJO, VE, UC and MEA managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Moringa oleifera leaf have been used for treatment of diabetes, in this work we studied pancreatic gene expression, trace elements, enzymatic antioxidants, kidney injury biomarkers in streptozocin induced diabetic rats treated with *M. oleifera* leaf powder and molecular ducking of *M. oleifera* leaf

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ethanolic, ethyl acetate, hexane and aqueous extracts phytochemicals into protein bank, focusing on the ligands that possesses inhibitory affinity closer to the co-crystalline ligands of alpha amylase, alpha glucosidase and dipeptidyl peptidase-4, for detection of active polyphenols that aid glucose reduction. Molecular docking methods used for predicting binding modes to proteins and energies of ligands [1]. Using the Autodock vina program compiled under Ubuntu 14.04 LTS, the compounds were docked into the target protein to get the respective binding affinity. The proteins were viewed on pymol to show the amino acid sequence and the co-crystallized ligandsStevioside Stigmaste, γ-Sitosterol and Campesterol has affinity energy (-6.893,-5.500, -5.294, -5.260) respectively, close to the co-ligand of α-amylase (-7.811). 2-Butyloxycarbonyloxy-1 has affinity energy (-5.583) closer to the co-ligand of DPP-4 (-6.102). Butanoic acid has affinity energy (-4.239) close to the co-ligand of α-glucosidase (-6.488). Ethanolic and ethyl acetate extract contains 24 compounds; hexane extract contains 22 compounds while aqueous extract contains only 6 compounds.

Keywords: Molecular docking; moringa oleifera leaf; phytochemical constituents; alpha glucosidase; alpha amylase; dipeptidyl peptidase.

1. INTRODUCTION

Moringa oleifera belongs to the family *Moringaceae,* the order *Brassicales,* and the genus *Moringa,* there are 13 species of the plant ranging in height from 5 to 10 m. It has an open crown of drooping, feathery foliage, flowers with distinctive green patches at the tips of the petals and sepals, tripinnate leaves and trunk. It's flowers, pods, and leaves have medicinal benefits owing to various phytochemical constituents. The flower is used to treat inflammation for its stimulant content, the spots and seeds have liver-protective and antihypertensive properties, while the leaves are used to treat microbial infections and to control blood glucose levels.*Moringa oleifera* contains soluble fibers that enhance reduction of glucose levels, proliferation of lymphocytes and induced nitric oxide from macrophages. The leaves contains polyphenols and has been found to be useful in diabetes conditions because of their possible capacity to decrease blood glucose concentrations [2-3].

The study was done on ducking of the plants component into protein bank, focusing on the inhibitory ligands to Dipeptidyl Peptidase 4 DPP-4, Alpha amylase(α-Amy), and Alpha glucosidase (α-Gluc).

2. MATERIALS AND METHODS

2.1 Plant Materials and Preparation

The plant was harvested from garden within Madonna University and was identified in the department of pharmacognosy of the University by Dr P. Osuagwu. The leaves were air dried at room temperature for two weeks, after which it was pulverized using electronic blender, the pulverized sample was subjected for extraction using four different solvents namely; ethanol, ethyl acetate, hexane and water. Each of the extracts was analyzed for bioactive components using Gas Chromatography – Mass Spectrometry (GC-MS).

2.2 Animals Handling

Male wistar albino rats (n=40) six weeks old weighing 150-250g were purchased from the animal farm of Madonna University Elele. Each of the animals was housed in animal cage with wire mesh and saw dust lining, and they were kept in a room inside the animal house, with 12 hours light/dark circle. The animals were allowed to acclimatize for 2 weeks, and were given food and water.

2.3 Experimental Design

After two weeks, they were numbered and separated into four groups of 10 rats each, group one were fed with animal feed throughout the experimental period, while other groups were fed with high fat diet (HFD) for seven weeks to increase the body mass index. At the end of the 9th week, 0.5ml of treptozocin 37mg/kg body weight in citrate buffer was administered intraperitoneally to the rats in groups 2, 3 and 4. The rats in groups 3 and 4 in addition to streptozocin were fed with pulverized *Moringa oleifera* leaf daily with the aid of rats cannular, according to the experimental deisgn below. Fasting blood sugar was measured weekly by

cutting the tip of the animals tail, using Easy Touch HealthPro glucose monitoring system.

Group 1 (Negative control): The animals in this group were fed with only animal mesh and water throughout the experiment.

Group 2 (Positive control): The animals in this group were given 0.5ml of 37mg/kg of Streptozotocin intraperitoneally in addition to feed and water.

Group 3: The animals in this group were given 0.5ml of 37mg/kg of streptozotocin and 150mg/kg of *Moringa oleifera* leave powder daily, in addition to food and water throughout the experiment period.

Group 4: The animals in this group were given 0.5ml of 37mg/kg of streptozotocin and 300mg/kg of *Moringa oleifera* leave powder daily, in addition to food and water throughout the experiment period.

2.4 Determination of Lethal Dose

This involves two steps; in the first step, nine animals were used grouped into three animals, each group were given different doses of the *Moringa oleifera* leaf powder (50, 100, 150mg/kg). The animals were monitored for 24 hours. Second step three groups of one animal each were given different higher doses of *Moringa oleifera* leaf powder (200, 300, and 400mg/kg). The animals were monitored for 24 hours.

LD50 was determined using the formular;

$$LD50 = \sqrt{(D_O \times D_{100})}$$

Where Do = the highest dose that gave no mortality
D_{100} the lowest dose that produced mortality

2.5 Sample Collection

At the end of the experimental period, the animals were euthanized by exposure to chloroform, blood sample was collected via cardiac puncture. Blood was collected into test tubes labeled accordingly, Serum samples were separated and used for determination of different biochemical parameters. Liver and kidney were surgically removed. Liver and kidney were washed with ice cold (4^0C) phosphate buffer saline (immediately after removal) to remove blood, tissue homogenate was prepared by homogenization of 1g of liver/ kidney using BeadBug 6 position tissue homogenizer, the remaining part of the tissue was preserved using formalin for histological studies.

2.6 Protein Preparation for Docking (www.rcsb.org)

The crystallized 3D structure of the human Dipeptidyl Peptidase 4 (DPP4), Glucagon-like peptide-1 receptor (GLP-1R), Alpha-amylase, and Alpha-glucosidase, receptors was downloaded from the protein data bank. The proteins were viewed on pymol to show the amino acid sequence and the co-crystallized ligands (the ligands crystallized together with the protein, so it is downloaded in complex with the protein). The crystallized ligands; 6B1E, 1B2Y, and 5NN3, Dipeptidyl peptidase 4 (DPP4), Alpha-amylase, and Alpha-glucosidase respectively, were extracted to show the active site or the grid around the binding site of the protein. This grid is called the 'configuration text'. This defines the region around the active site of the target. The receptor was also generated in the pdbqt format on the pymol software. The grid center was placed in the active site pocket center. The grid boxes included the entire binding site of the enzymes and provided enough space for the ligand translational and rotational movement.

2.7 Ligand Preparation for Docking

Following the GC-MS analysis, a library of compounds from the GC-MS result was generated by downloading the various plant constituents from the NCBI pubchem database in the 2D sdf format. The ligands in sdf were converted to pdb using the babel command. The ligands were further converted to the pdbqt format using the Autodock MGLTool for ligand preparation.

2.8 Molecular Docking (www.ebi.ac.uk/ chembl/)

Molecular docking methods used for predicting binding modes to proteins and energies of ligands [1]. Using the Autodock vina program compiled under Ubuntu 14.04 LTS, the compounds were docked into the target protein to get the respective binding affinity. The binding affinity predicts the strength of the molecular interaction of the ligand-protein complex. The binding results were validated using the chembl

Database. The faster sequences of the proteins were gotten from Pubmed and blast and the search result was downloaded in the text format, using the IC_{50} activity type. The smile format of the compounds were converted to sdf using Data warrior software and saved as 2D. These 2D structures were converted to pdb and pdbqt using Babel and ligand preparation command lines respectively to generate the 3D structure of the compounds. The results were analyzed using binding energy.

For each ligand, a docking experiment consisting of 100 stimulations was performed and the analysis was based on binding free energies and root mean square deviation (RMSD) values, and the ligand molecules were then ranked in the order of increasing docking energies. The binding energy of each cluster is the mean binding energy of all the conformations present within the cluster, the cluster with the lowest binding energy and higher number of conformations within it was selected as the docked pose of that particular ligand. The clusters were ranked by the lowest-energy representative of each binding mode. At the end of a docking experiment with multiple runs, a cluster analysis was performed.

3. RESULTS

Table 1. Shows that *Moringa oleifera* leaf extract contained 41 compounds, it could be seen that ethanolic extract contains 24 compounds, ethylacetate extract contains 24 compounds, hexane extract contains 22 compounds and aqueous extract contains 6 compounds.

Table 1. The gas chromatography mass spectroscopy analysis of *m. Oleifera* ethanolic, ethyl acetate, hexane and aqueous extracts

Compound	m/w	Ethanol	ethylacetate	hexane	aqueous
2-+-butyl-5-propyl-1,3-dioxolan-4-one	186	32.0	_	_	_
α- d-glucopyranoside,o- α-d-	504	16.6	_	_	_
glucopyranosyl	164	9.37	14.5	_	_
b-d-glucopyranose,4-o-b-d-gluctopyranosyl(b-actose)	290	22.7	_	_	_
12,15-octadecadiyniocadiynoic acid,	279	17.8	13.8	_	_
methyl esther	200	15.8	31.1	10.4	_
	228	56.9	69.3	72.8	_
desulphosinigrim	296	36.4	56.9	58.6	_
dodecanoic acid (β hydroxylodecanoic	256	63.9	79.9	29.1	_
acid)	284	64.2	49.4	56.4	_
tetradecanoic acid	296	61.4	54.1	44.9	_
3,7,11,15-tetramethyl-2-hexadecan-1-ol					
n-hexadecanoic acid	278	61.4	54.9	55.6	_
n-hexadecanoic acid ethyl esther	284	63.4	_	_	_
phytol	312	57.9	49.8	-	__
	380	20.8	21.9	30.8	_
9,12,15-octadecatrienoic acid (linolenic	340	39.1	_	_	_
acid) 13-heptadecyn-1-ol	436	44.1	26.8	24.7	_
octadecanoic acid	430	47.1	49.2	58.2	_
eicosanoic acid	400	50.5	55.2	_	_
heptacosane	412	63.4	7.06	_	_
decosanoic acid	414	53.0	53.4	44.8	_
ethy iso-allocholate	412	42.9	37.8	_	_
vitamin e (α tocopherol)	424	20.4	_	_	_
campesterol	418	30.0	_	_	
stigmasterol	420	_	24.5	29.8	_
γ-sitosterol	310	_	6.26	_	_
stigmasta-5-24(28) dien 3-ol	394	_	12.1	12.4	_

Compound	m/w	Ethanol	ethylacetate	hexane	aqueous
4,4,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14	228	_	17.5	20.4	
b-octade	804	_	15.1	_	_
butanoic acid					
α-amyrin	326	-	16.3	_	_
cis-11-eicosenoic acid					_
octacosane	174		14.2	22.2	_
1-dodecanol	426		-	39.8	_
stevioside	490		-	_	_
	454		_	15.9	_
2-butyloxycarbonyloxy-1,1,10-trimethyl-6,9	310		_	17.4	__
epidioxydecalin	592		_	5.89	15.3
	296		_	11.6	11.1
naphthalene.1,2,3,4-tetrahydro-1,1,6	292		_	12.1	4.53
trimethyltetralin(α-lonene)	374		_	12.9	12.6
choles-5-en-3-ol,24-propylidene	256		_	_	5.49
17-pentatriacontene	310		_	_	6.41
1,30-triacontanediol					
2-14-octadecen-1-ol acetate					
oleic acid					
heneicosane					
9-octadecen-12-ynoic acid, methyl esther					
cyclopropanebutanoic acid					
1-hexadecanol					
3-trifluroacetoxytetradecane					

Table 2. The inhibitory ligands to dipeptidyl peptidase-4(dpp4) from ethanolic, ethylacetate, haxane and aqueous extracts

Compounds DPP4	Ethanol -6.102	Ethylacetate	Hexane	Aqueous
Eicosanoic acid	-3.852	5.8	5.1	-
Butanoic acid	2.711	3.0	-	-
Cis-11-eicosenoic acid	-3.000	6.3	-	-
2-Butyloxycarbonyloxy-1	-5.583	-	1.6	-

From the table above, we noticed that only 2-Butyloxycarbonyloxy-1 has inhibitory energy (-5.583) closer to that of the co-ligand (-6.102). Also, eicosanoid and cis-11-ecosanoid has energy (-3.852 and -3.000) which is up to half of the co-ligand. And they are contained in the ethanolic extract. But 2-Butyloxycarbonyloxy-1 having the highest energy is also present in the ethylacetate extract in little amount.

From the table above, stevioside (poorly contained in hexane extract), campesterol and stigmasterol (contained in ethanolicand ethylacetate extracts) has inhibitory energy (-6.893, -5.260 and -5.500) respectively, closer to the co-ligand of α-Amy (-7.811).

From the table above, only butanoic acid and stigmasterol which are contained in the ethanolic and ethylacetate extracts has inhibitory energy to α-Gluc (-4.239 and -3.948) respectively, more than half of that of the energy of the co-ligand.

4. DISCUSSION

The result obtained from Gas chromatography mass Spectroscopy analysis of *Moringa oleifera leaf* ethanolic, ethyl acetate, hexane, and

Table 3. The inhibitory ligands to alpha amylase (α-amy) from ethanolic, ethylacetate, haxane and aqueous extracts

Compounds	α-Amy(-7.811)	Ethanol	Ethylacetate	Hexane	Aqueous
Campesterol	-5.260	5.1	5.5	-	-
Stigmasterol	-5.500	6.3	7.1	-	-
Stigmasta-5	-3.725	4.3	3.8	-	-
Butanoic acid	-4.792	3.0	-	-	-
Stevioside	-6.893	-	-	1.5	-
2-Butyloxycarbonyloxy-1	-4.690	-	1.6	-	-

Table 4. The inhibitory ligands to alpha glucosidase (α-gluc) from ethanolic, ethylacetate, haxane and aqueous extracts

Compounds	α-Gluc(-6.488)	Ethanol	Ethylacetate	Hexane	Aqueous
Campesterol	-1.741	5.1	5.5	-	-
Stigmasterol	-3.948	6.3	7.1	-	-
Butanoic acid	-4.239	3.0	1.5	-	-
2-Butyloxycarbonyloxy-1	-2.805	-1.6	-	-	-

aqueous extracts shows that ethanolic extract and ethyl acetate extract contain 24 compounds, hexane extract contains 22 compounds while aqueous extract contains only 6 compounds. This indicates that the plant contains more of the organic compounds than inorganic compounds.

In this study also, we evaluated the activities of the compounds contained in *Moringa oleifera* with some proteins that are involved in glucose metabolism. We compared the affinity energy of the compounds with the co-crystalline ligands of the α-amylase, α-glucosidase, and dipeptidylpeptidase-4 (DPP4). From the result, stevioside possesses affinity energy (-6.893) which is closer to the co-crystalline ligand of α-amylase (-7.811). 2-Butyloxycarbonyloxy-1 has affinity energy (-5.583) closer to the co-ligand of DPP-4 (-6.102). Stigmaste, γ-Sitosterol and Campesterol also has affinity energy (-5.500, -5.294, -5.260) respectively, close to the co-ligand of α-amylase (-7.811). Butanoic acid has affinity energy (-4.239) close to the co-ligand of α-glucosidase (-6.488). Many other compounds contained in the plant also possesses a lesser affinity energy to these proteins, as such if all these function to inhibit the proteins, there may be possible total inactivation of the proteins and hence reduced glucose absorption and uninterrupted action of GLP-1R in insulin secretion. Also, DPP-IV inhibitors have significant hypoglycemic effects, prolonging the hypoglycemic effects of GLP-1 and GIP by inhibiting DPP-IV is one of the key mechanisms of type 2 DM treatment [4-5]. Therefore, the discovery of DPP-IV inhibitors with new structures, especially among the secondary metabolites of plants, with higher safety, is a reliable and proven approach for discovering new hypoglycemic drugs [6]. It have been found thatinhibition of pancreatic alpha-amylase is one of the therapeutic targets for delaying oligosaccharide digestion to absorbable monosaccharide in the intestinal brush border, resulting in reduced postprandial hyperglycemia [7]. The inhibition of alpha-amylase is an important therapeutic target in the regulation of postprandial increase of blood glucose in diabetic patients [5,6,8-10].

5. CONCLUSSION

From this study, it could be inferred that *moringa oleifera* leaf ethanolic extract and ethyl acetate extract of *Moringa oleifera* leaf contains more important phytochemicals that function to reduce blood glucose than hexane and aqueous extracts. Ingestion of pulverized *Moringa oleifera* leaf reduced blood glucose level which may be as a result of competitive inhibition of the dipeptidyl peptidase-4, α-amylase and α-glucosidase which are essential for carbohydrate digestion. Dipeptidyl peptidase-4 inhibits the intestinal incretin glucagon-like peptide-1Receptor. Intestinal cells produce incretins which regulate blood glucose level by enhancing insulin production, decreases gastric emptying, regulate appetite, inhibit glucagon, and increases

insulin secretion, hence, decreases blood glucose levels.

Ethanolic and ethylacetate extracts of *Moringa oleifera* leaf may be compounded into tablets to be taken with food containing long chain carbohydrate having been found to reduce intestinal availability of glucose and its corresponding absorption. *Moringa oleifera* leaf should be used with caution in the treatment of diabetes to avoid over accumulation of the many other compounds contained in the plant that does not have any effect on blood glucose maintenance since these may precipitate damage to organs of the body.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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