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Research Article Open Acess

In Silco Screening of *Parkia biglobosa* Fatty Acids as Inhibitors of A-Glucosidase, Aldehyde Reductase (ALR1) and Aldose Reductase (ALR2) Enzymes

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Abstract

Diabetes mellitus is a world health problem with high mortality and morbidity due to the complications; as a result of increased level of glucose concentration. The search for new antidiabetic drugs from natural products has been on increase. Though discovery of drug is time consuming with numerous challenges, therefore, *in silico* screening is now being used for the preclinical search and development of drugs within limited time. In this study, fatty acids determined from *P. biglobosa* seeds were screened *in silico* via molecular docking against α-glucosidase, ALR1 and ALR2 enzymes linked to type 2 diabetes mellitus complications using AutoDock Vina. These enzymes play different roles in glucose metabolism and associated to diabetes complications development. The results obtained from the docking studies revealed that docked ligands (fatty acid) bind firmly to the enzymes with the binding energy in the range of -4.12 Kcal mol^{-1} to -13.61 Kcal mol^{-1}. Inhibition constant obtained for α-glucosidase was in micromolar and nanomolar for both ALR1 and ALR2 enzymes. Docking analysis showed different orientations of the ligands inside the active pocket of the enzymes, of all the ligands, linoleic acid forms perfect orientation with different amino acid residues of all the enzymes via hydrogen bonding formation when compared to the rest of fatty acids.

Keywords: Diabetes; Plant seeds; Fatty acid; *In silico* screening; Enzyme inhibitor

Introduction

Diabetes mellitus is a metabolic endocrine disorder that causes morbidity and mortality. It is characterised by hyperglycemia with the association of carbohydrate, protein and lipid metabolism in balance [1]. It also results from partial, complete or relative lack of insulin secretion by the beta cells of the pancreas and insulin impairment [2]. The World Health Organization (WHO) estimates that the number of people suffering from diabetes will be doubled by the year 2025. New forms of insulin and its delivery [3] and therapeutic modalities have been achieved for the control of hyperglycemia [4]. But it is still impossible to completely prevent the hyperglyceamic occurrence. Thus, diabetic therapy problems and challenges are increasingly shifted. Diabetes' long-term complications are manifested in both microvascular and macrovascular. The new approach for the treatment of diabetes is the improvement on the control of glycaemic and the long-term complications.

In this study, *in silico* molecular docking of fatty acids from *P. biglobosa* seeds, an antidiabetic agent was carried on α-glucosidae, aldehyde reductase (ALR1) and aldose reductase (ALR2) enzymes. This is considered as an approach for the search of new inhibitors of these enzymes from the natural source for the control of postprandial hyperglyceamia. Glucosidase is an intestinal enzyme that produces glucose via the breakdown of carbohydrates; inhibition of this enzyme limits the amount of glucose absorption in the blood, thus, helpful in treatment of type 2 diabetes. Inhibitors of α-glucosidase and aldose reductase (ALR2) have been considered to be the target enzymes for the treatment of diabetes and diabetes complications. Voglibose (voglitab), glucobay (acarbose) and glyset (miglitol) and are known inhibitors of α-glucosidase that have already been marketed as medicines.

Also, aldose reductase (ALR2) inhibition, a key rate-limiting enzyme in the polyol pathway that catalyses sorbitol conversion to glucose in the presence of NADPH as a cofactor. Under normoglycemia ALR2 has low affinity for glucose and small protein of glucose to enter polyol pathway. On the contrary, the influx of glucose into polyol pathway increases to one third of the total glucose turn over with increase in sorbitol accumulation in the cells during hyperglycemia follow by tissue damage by the increase intra cellular osmolarity as the main cause of long term complications. Therefore, inhibition of ALR2 delays the rate of sorbitol conversion. Consequently, prevents diabetes complication progression or on set.

Materials and Methods

Seeds collection

The seeds of the plant were bought in the month of July, 2014 from Bode market in Ibadan, Oyo State, Nigeria. The seeds sample of *P. biglobosa* was taken to Herbarium Unit at Botany Department, University of Ibadan, and Ibadan, Nigeria for authentification.

Sample preparation

The collected seeds of the plant were cleaned and dried at a temperature of 35°C in oven to prevent growing of fungi, weighed and blended into powder by using blending machine. The pulverized seeds of the selected plants for this study were re-weighed and then stored for extraction.

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Preparation of *P. biglobosa* extract

Extract from the pulverized *P. biglobosa* powder was obtained with methanol (b. pt 67°C) through cold maceration as described by Ajayi et al. [5]. The powder of the seeds (900 g each) was placed in 5.0 L of aspirator bottle and 3.0 L of methanol was put in them, the mixture was kept at temperature of 25°C for seven days with constant stirring daily. On the 7th day, the mixture was filtered under reduced pressure using Buchner funnel connected to the vacuum pump. The extract used for this study was concentrated from the solvent mixture phase by using rotary evaporator at 40°C and pressure of 626 mmHg; yields were determined and stored at -4°C until required for further uses.

GC-FID

Fatty acids determination by the conversion of *P. biglobosa* extract to fatty acid methyl esters was made by the addition of 1 mL of n-hexane to 40 mg of extract and 200 µL of 2 M sodium methoxide in a one neck round bottom [6]. The mixture was heated at a temperature of 50°C in water bath for 30 sec after which 200 µL of 2.0 M HCl was added. The top layer from the reaction mixture was collected and 1.0 µL from the top layer was injected into gas chromatograph (HP7820A Agilent) equipped with flame ionization detector and HPINNOWAX 15 m × 0.25 mm × 0.20 µm (HP) at the gradient temperature of 120°C.

Molecular docking

Molecular docking of the fatty acids from *P. biglobosa* seeds was performed using Auto Dock v4.2 and MGL Tools v1.5.64. The fatty acids structures were drawn using ACD/ChemSketch, (2015) and 3D optimized. Crystal structure of human α-Glucosidase (PDB ID 3WY1) with co-crystallized ligand (3R, 5R, 7R)-octane-1, 3, 5, 7-tetracarboxylic acid, human aldose reductase (PDB ID 1US0) and porcine aldehyde reductase (PDB ID 3FX4) crystal structures were downloaded from RCSB protein data bank [7]. The co-crystallized reference ligands of ([(5Z)-5-{[3-(carboxymethoxy)-4-methoxyphenyl] methylidene}-2, 4-dioxo-1, 3-thiazolidin-3-yl] acetic acid) and (2-(2-{[(4-bromo-2-fuorophenyl) methyl] carbamothioyl}-5-fuorophenoxy) acetic acid) were also downloaded from PDB for ALR1 and ALR2 enzymes respectively. Nicotinamide adenine dinucleotide phosphate (NADP) was selected as ALR1 co-factor; while in dihydro nicotinamide adenine dinucleotide phosphate (NADPH) was the cofactor used for the molecular docking of ALR2. Active site of the receptor was defined as the amino acid residues in 7.5°A radii of the co-crystallized reference ligand. The docking parameters were first optimized by re-docking of the reference ligands with the determination of their RMSD values with the co-crystallized structures and further by the removal of water molecules and co-crystallized ligands and addition of hydrogen atoms and charges. The re-docking of the reference ligand and molecular docking of all the fatty acids were carried out with Lamarckian Genetic Algorithm of AutoDock v4.2. The active site dimensions for each enzyme were recorded by using their co-crystallized ligands.100 different poses were generated and then clustered according to their RMSD values. Putative binding modes of best and lowest energy poses were selected for further visualization using discovery studio visualizer for the investigation of the binding interactions. The inhibition constants for each of the enzymes were determined using the equation:

$$G=RTInKi$$

Where G is the binding energy (Kcal/mol), R is the gas constant (1.987 cal/mol/k) and T is temperature, assumed to be absolute (298 K) [8].

Results and Discussion

P. biglobosa fatty acids composition

Fats and lipids are common food component and may perform essential roles. The type of fats and lipids are more important with respect to their health benefit and disease than their amount. Foods are considered as functional, if they provided reduction in the risk of disease and promote good health (Roberfroid, 2000). Fatty acid analysis of *P. biglobosa* seeds indicated that linoleic acid (42.72%) is the most abundant followed by oleic acid (30.70%). It also contains 13.46% and 11.34% of palmitic acid and stearic acid respectively. Palmitoleic acid (0.27%) and arachidic acid (0.36%) were the least dominant saturated fatty acids found in *P. biglobosa* seeds. The ratio of PUFA/SFA was 1.70. More so, the percentage composition of unsaturated fatty acid (73.69%) was found higher than saturated fatty acids (25.16%) and the oleic ratio to linoleic was 0.72. Lipids like Omega-3, conjugated linoleic acid, medium chain triglycerides, omega-6 and phytosterol are known as functional lipids, because they offer many beneficial effects to human health such as obesity, diabetes, blood pressure, cardiovascular disease and depression management.

α-glucosidase enzyme docking study

The putative binding modes of *P. biglobosa* fatty acids inside the active site pocket of α-glucosidase are shown in Figure 1. Inside the active pocket of α-glucosidase, linolenic acid was determined to be having highest binding energy value of -10.34 Kcal mol^{-1} with amino acid residue of the enzymes while the binding energy of other fatty acids inside the active pocket were found to be mild to moderate affinity in the range of -7.23 Kcal mol^{-1} to -10.08 Kcal mol^{-1}. Figures 1a-1g illustrated the binding interaction of individual fatty acid within the active site of α-glucosidase enzyme; it was revealed that amino acid residues of Arg400, Pro230, Phe166, Gln170 and Asp62 were the main interacting groups with the fatty acids (ligands) in the active pocket of α-glycosidase.

ALR1 enzyme docking study

Molecular docking studies of the fatty acids revealed their interaction modes within the active site of ALR1 enzyme. The different binding energy affinities calculated for all the fatty acids were shown in Table 1. In the docking studies of the fatty acids with ALR1 enzyme, linoleic acid was also found to be exhibiting highest free binding energy of -14.49 Kcal mol^{-1} while other fatty acids displayed free binding energy in the range of the values obtained for α-glucosidase and ALR2 enzymes. The free binding affinity value obtained for linoleic acid against ALR1 enzyme (-14.49 Kcal mol^{-1}) was found to be almost double when compared to -10.04 Kcal mol^{-1} free binding energy obtained for linoleic acid against ALR2 enzyme (Tables 2 and 3). The key interacting amino acid residues of the enzymes with the docked ligands were Tyr210, Trp22, Arg309, Trp114, Arg312, Trp50 and Ile29 as shown in Figures 2a-2g.

ALR2 docking study

Aldose reductase enzyme (ALR2) molecular docking study with all the fatty acids revealed the different interaction modes of the acids and their binding affinity trends in the active pocket of the enzyme (ALR2); the trend was different from what was observed for the fatty acids molecular docking against α-glucosidase and ALR1 enzymes. The free binding energy for the molecular docking studies of the fatty acids with the ALR2 enzymes was found to be in the range of -4.12 Kcal mol^{-1} to -9.59 Kcal mol^{-1}. The binding energy value was two folds lower than

Fatty acids	Binding free energies (E=Kcal mol^{-1})		
	α-glucosidase	ALR1	ALR2
Palmitoleic	-9.17	-13.61	-9.27
Oleic	-9.64	-10.68	-6.93
Linoleic	-10.08	-13.16	-9.59
Linolenic	-10.34	-14.49	-8.33
Palmitic	-9.08	-11.76	-5.52
Stearic	-7.35	-10.44	-4.14
Arachidic	-7.23	-10.07	-4.12

Table 1: Binding free energy of the fatty acids (ligands) against the receptors.

Fatty acid	Inhibition constant (Ki)		
	α-glucosidase (μM)	ALR1 (nM)	ALR2 (nM)
Palmitoleic	0.19	0.11	0.09
Oleic	0.09	14.85	8.33
Linoleic	0.04	0.23	0.16
Linolenic	0.03	0.02	0.78
Palmitic	0.22	2.40	89.93
Stearic	4.10	22.26	955.2
Arachidic	5.02	41.57	923.5

Table 2: Inhibition constants of the fatty acids (ligands) against the receptors.

CN	Fatty acid	RT (min)	C Composition (%)
$C_{14:0}$	Myristic	2.86	____
$C_{16:0}$	Palmitic	4.254	13.46
$C_{16:1}$	Palmitoleic	4.46	0.27
$C_{18:0}$	Stearic	6.322	11.34
$C_{18:1}$	Oleic	6.518	30.70
$C_{18:2}$	Linoleic	7.05	42.72
$C_{18:3}$	α-linoleic	7.639	----
$C_{20:0}$	Arachidic	8.608	0.36
Total SFA	-------	-----	25.16
Total UFA	-------	-----	73.69
Total MUFA	-------	-----	30.97
Total **PUFA**	------	-----	42.72
PUFA/MUFA	-------	-----	1.70

Table 3: Fatty acid composition of the seeds.

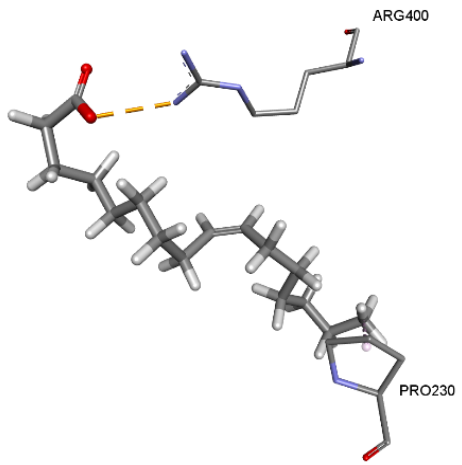

Figure 1a: Putative binding interaction of 3WY1 receptor with palmitoleic acid.

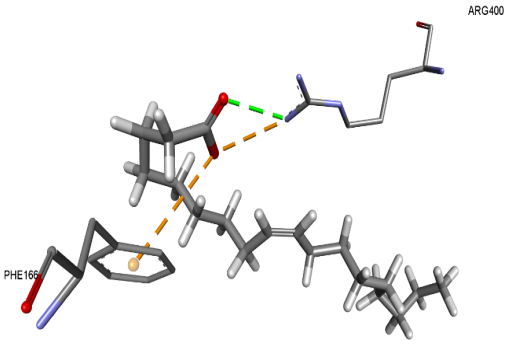

Figure 1b: Putative binding interaction of oleic acid with 3WY1 receptor.

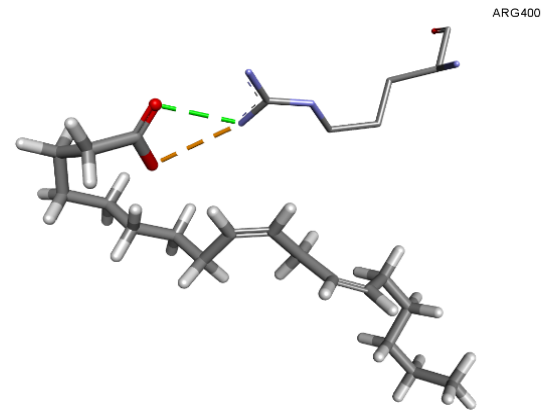

Figure 1c: Putative binding interaction of linoleic acid with 3WY1 receptor.

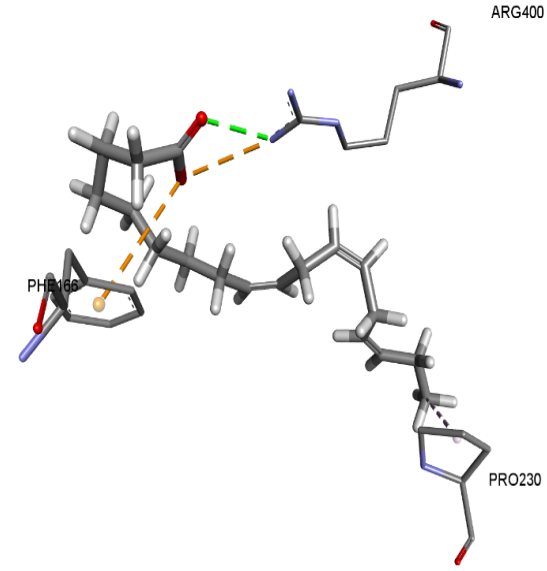

Figure 1d: Putative binding interaction of linolenic acid with 3WY1 receptor.

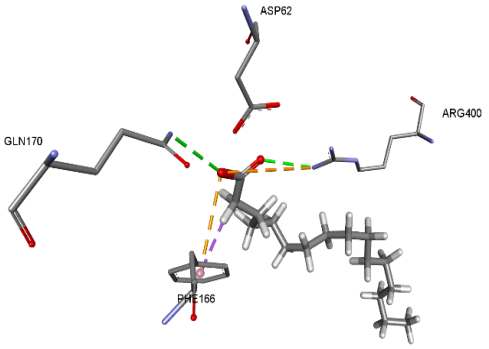

Figure 1e: Putative binding interaction of palmitic acid with 3WY1 receptor.

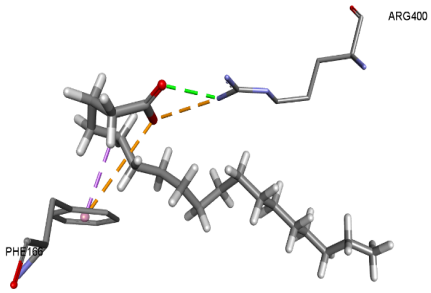

Figure 1f: Putative binding interaction of stearic acid with 3WY1 receptor.

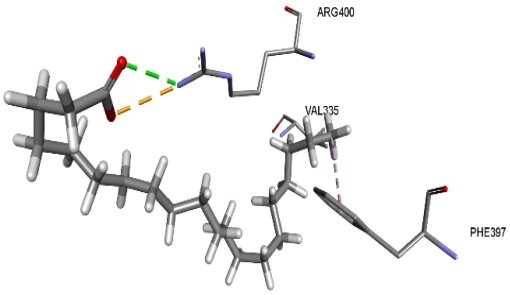

Figure 1g: Putative binding interaction of arachidic acid with 3WY1 receptor.

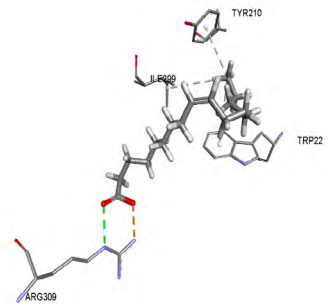

Figure 2a: Putative binding interaction of palmitoleic acid with 3FX4 receptor.

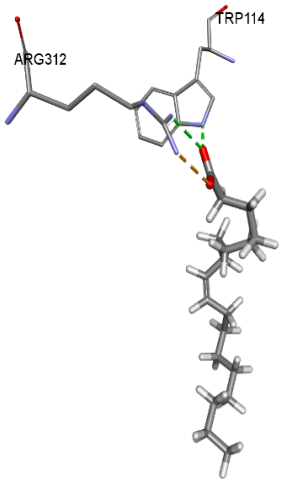

Figure 2b: Putative binding interaction of oleic acid with 3FX4 receptor.

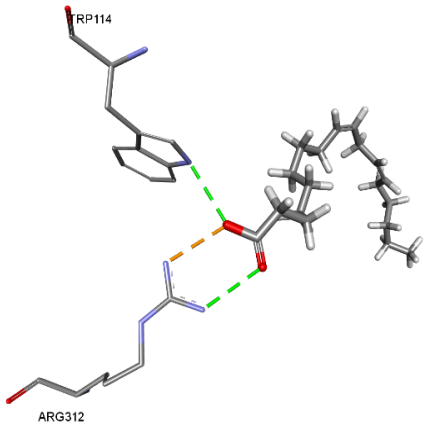

Figure 2c: Putative binding interaction of linoleic acid with 3FX4 receptor.

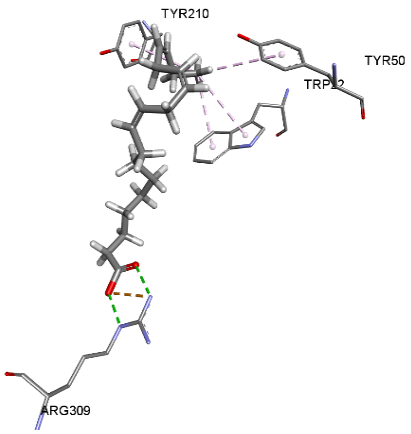

Figure 2d: Putative binding interaction of linolenic acid with 3FX4 receptor.

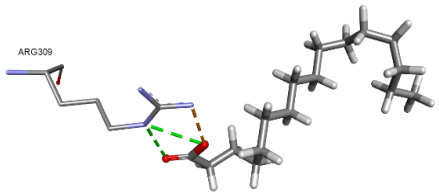

Figure 2e: Putative binding interaction of palmitic acid with 3FX4 receptor.

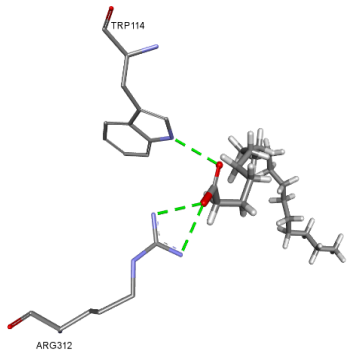

Figure 2f: Putative binding interaction of stearic acid with 3FX4 receptor.

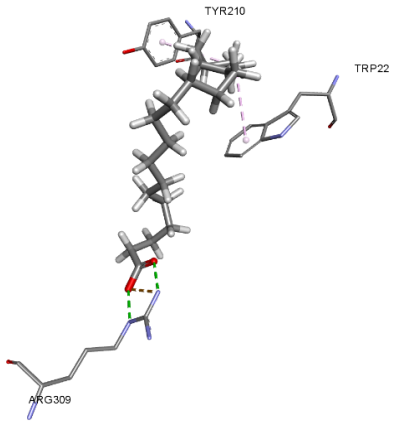

Figure 2g: Putative binding interaction of arachidic acid with 3FX4 receptor.

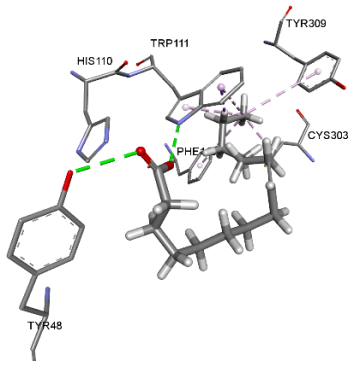

Figure 3a: Putative binding interaction of palmitoleic acid with 1US0 receptor.

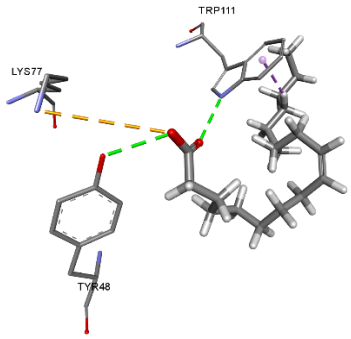

Figure 3b: Putative binding interaction of oleic acid with 1US0 receptor.

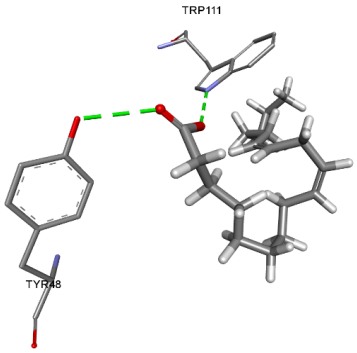

Figure 3c: Putative binding interaction of linoleic acid with 1US0 receptor.

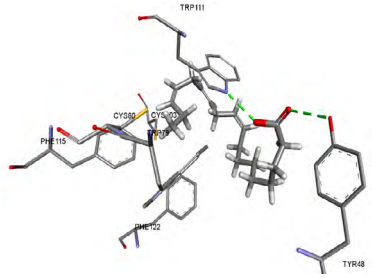

Figure 3d: Putative binding interaction of linolenic acid with 1US0 receptor.

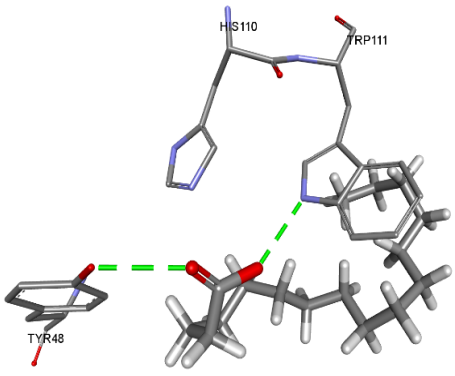

Figure 3e: Putative binding interaction of palmitic acid with 1US0 receptor.

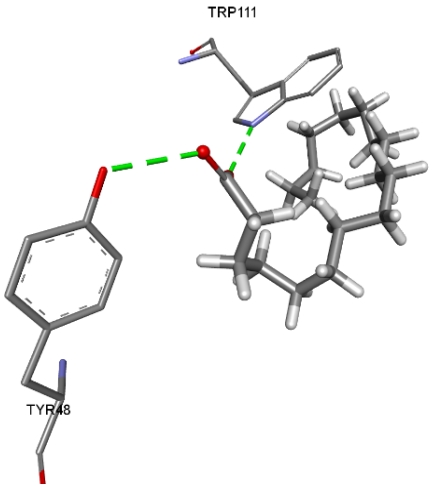

Figure 3f: Putative binding interaction of stearic acid with 1US0 receptor.

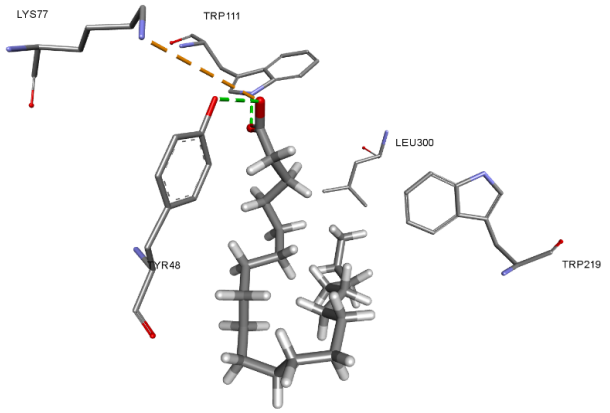

Figure 3g: Putative binding interaction of arachidic acid with 1US0 receptor.

the free binding energy values obtained for α-glucosidase and ALR1 enzymes. Comparison of the docked fatty acids free binding energies against ALR2 enzyme showed that linoleic acid has highest free binding energy value of -9.59 Kcal mol^{-1} and the most binding affinity towards ALR2 enzyme. On the contrary, the lowest binding energy value of -4.12 Kcal mol^{-1} was obtained for the molecular docking of arachidic acid with ALR2 enzyme. The result from the docking analysis also showed that arachidic acid has the least affinity towards α-glucosidase, ALR1 and ALR2 enzymes. Amino acid residues modes of interaction within the active pocket of the enzyme with the ligands were presented in Figures 3a-3g. Lys77, Trp111, Leu300, Tyr48, Phe115, Phe122, His110 and Cys303 were the active sites involved in the interaction.

Conclusion

Fatty acid content of *P. biglobosa* seeds was determined, among the detected fatty acids in the seeds, linolenic acid was found to be the most abundant and they were all screened as novel inhibitors of α-glucosidase, ALR1 and ALR2 enzymes. *In silico* molecular docking investigations of the fatty acids against the selected enzymes revealed the influential affinity and free binding energy of the fatty acids. Linolenic acid was found to be an excellent inhibitor of α-glucosidase, ALR1 and ALR2 inhibitors. Linolenic acid has noticeably high free binding energy and affinity towards all the enzymes. Thus, this study has helped in finding some suitable fatty acids that could lead to synthesis of potential drugs for the management of diabetes and it complications.

Acknowledgements

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