



Exploring The Antidiabetic Efficacy Of Almonds, Walnuts, Flaxseeds And Tamarind Seeds:

A SCIENTIFIC CONVERGENCE OF EXPERIMENTAL ANALYSES AND MOLECULAR DOCKING INVESTIGATIONS

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Abstract: Diabetes mellitus is one of the most serious health challenges confronting the world today, with its prevalence and associated mortality increasing. Poor blood sugar regulation is hazardous to one's health. Traditional anti-diabetic drugs are effective, but they have unavoidable side effects. Groundnuts and seeds, on the other hand, can provide an alternative supply of anti-diabetic drugs. Groundnuts and seeds can be highly useful to folks. They have a low glycemic index, which means they cause blood sugar levels to rise more slowly than high-glycemic foods. This can aid with blood sugar control. They're high in Fiber and healthy fats, both of which can help with blood sugar regulation. The antioxidant and alpha-amylase inhibitory activities of groundnut and seed extracts were determined using the 3,5-dinitrosalicylic acid method and the diphenyl-2-picrylhydrazyl assay model, respectively. Tamarind seeds and almonds had the highest percentage inhibition (76.40% and 71.3%, respectively). Flaxseed had a moderate amount of enzyme inhibition of 54.0%, whereas Walnut had the lowest level of enzyme inhibition of 46.8%. The Glucose Yeast Uptake Model was used to compute the percentage increase in glucose uptake, which was highest in flaxseeds (76.0%) and tamarind (72.3%), 56.4% in almond, and lowest in walnut (40.4%). To learn more about the nature of the samples, the Phytochemicals test was done on all of them. Alkaloid, Tannins, Steroid, Protein, and Carbohydrate were all positive, meaning they were present in all four samples. Flavonoids were found in flaxseeds and tamarind seeds. Saponins and Phlobatannin were only found in Almond. Keller Killani Test was positive for Almond and Tamarind seeds. The phenolics test was used to determine the unknown value for samples, and the results were as follows: Almond 75.88mg/ml, Walnut 85.57 mg/ml, Flaxseeds 85.57 mg/ml, and Tamarind Seed 77.55 mg/ml. Tamarind and flaxseeds were found to be significantly more active than other samples in vitro, however each sample has its own variance and effectiveness. The in-silico molecular docking method was used to determine the efficiency of the samples, and the samples were docked with Human Receptor Protein GLP 1 and the ligand of each sample.

Index Terms - antidiabetic, diabetes mellitus, antioxidant, a-amylase, almond, walnut, flaxseeds, tamarind seeds, molecular docking

METHODOLOGY

1.1 Preparation of Crude extract

Groundnuts like almond, walnut and seeds like flaxseeds and tamarind seeds were collected. The seeds and groundnut samples were thoroughly washed with distilled water to remove dirt and then dried under shade at room temperature (25 to 27 °C). The dried materials were ground into coarse powder by the electrical mill. Then, the coarse powdered groundnut and seed materials were macerated separately in 80% methanol for 48 hours and then the extracts were filtered

by using muslin cloth.(Ayoola et al., 2008)

1.2 Determination of α -Amylase Inhibition Activity

The 3,5-dinitrosalicylic acid (DNSA) technique was used to carry out the α -amylase inhibition experiment. The buffer (Na₂HPO₄/NaH₂PO₄ (0.02 M), NaCl (0.006 M) at pH 6.9) was used to dissolve the crude and solvent portions of the samples to produce concentrations ranging from 50 to 1000 μ g/mL. Following the addition of 200 μ L of the extract and 2 units/mL of α -amylase solution (Molychem), the mixture was incubated at 30°C for 10 minutes. Subsequently, 200 μ L of the 1% starch in water w/v solution was added to each tube, and it was incubated for three minutes. 200 μ L of DNSA reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM 3,5-DNSA solution) was added to end the reaction, and it was then heated for 10 minutes at 85 °C in a water bath. After bringing the mixture down to room temperature, it was diluted with five milliliters of distilled water, and a UV-visible spectrophotometer (Agilent Technologies) was used to detect the absorbance at 540 nm. 200 μ L of the buffer was used in place of the plant extract to create the blank, which had 100% enzyme activity. In the same way, a blank reaction was made without the enzyme solution, utilizing the plant extract at every concentration. The reaction was carried out in a manner akin to the reaction with plant extract previously described, utilizing acarbose (Bayer) to create a positive control sample.(Sharma et al., 2016)

The following equation was used to calculate the percentage of inhibition of α amylase, which was stated as follows:

$$\text{Percentage of } \alpha\text{-amylase Inhibition (\%)} = \frac{(\text{Abs. of control} - \text{Abs. of sample})}{\text{Abs. of control}} \times 100$$

where Abs is Absorbance and where control is the solution having all reagents except the test sample.

1.3 Glucose Yeast Uptake Model

Determination of Glucose Uptake Capacity by Yeast Cells The assay was carried out in compliance with Cirillo's clearly described protocol.[3] [4] To create a 1% suspension, commercial baker's yeast was dissolved in distilled water. The suspension was maintained at room temperature (25°C) for the entire night. The suspension of yeast cells was centrifuged for five minutes at 4200 rpm (Microfuge® 16 Centrifuge, FX241.5P Rotor, 50/60 Hz and 220–240 V) on the following day. Until a clear supernatant was obtained, the procedure was repeated by adding distilled water to the pellet. To create a 10% v/v suspension of the yeast cells, precisely 10 parts of the clear supernatant fluids were combined with 90 parts of distilled water. Samples weighing 1-2 mg w/v were combined with DMSO until they dissolved. The 1 ml mixture was incubated at 37°C for 10 minutes with 1 mL of glucose solution. After adding 100 μ L of yeast suspension to the glucose and extract mixture, vortexing it, and letting it sit at 37°C for an additional 60 minutes, the reaction was started. Following incubation, the tubes were centrifuged for five minutes at 3800 rpm, and the amount of glucose was measured at 520 nm using a UV 5100B spectrophotometer. At the same wavelength, the control's absorbance was also measured.(Cirillo', n.d.)

The following formula was used to determine the percentage increase in uptake:

$$\text{Percentage \% increase in glucose uptake} = \frac{(\text{Abs. of control} - \text{Abs. of sample})}{\text{Abs. of control}} \times 100$$

where Abs is Absorbance and where control is the solution having all reagents except the test sample.

1.4 Phytochemical analysis

Phytochemical screening was performed using standard procedures. (Ayoola et al., 2008)

1.4.1 Test for Tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black coloration

1.4.2 Test for alkaloids

About 0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Dragendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish-brown precipitate (with Dragendorff's reagent) was regarded as positive for the presence of alkaloids.

1.4.3 Test for Flavonoids

Three methods were used to test for flavonoids. First, dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow colouration that disappears on standing indicates the presence of flavonoids. Second, a few drops of 1% aluminium solution were added to a portion of the filtrate. A yellow colouration indicates the presence of flavonoids. Third, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration indicates the presence of flavonoid

1.4.4 Test for Saponins

About 0.5 g of extract was added with 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

1.4.5 Test for Phlobatannin

About 0.5 g of extract was boiled for five minutes in 10 ml of distilled water. The mixture was filtered and, in the filtrate, a few drops of 1% aqueous HCl was added. The mixture was kept in boiling water bath and a red precipitate was observed which indicates the presence of Phlobatannin.

1.4.6 Test for Steroid

About 0.5 g of extract was added with 1 ml of chloroform to 1 ml of sample and 0.5 ml of sulfuric acid. The lower layer becomes red when this reaction occurs this indicates the presence of Steroid.

1.4.7 Test for Protein

About 0.5 g of extract was added with 2ml of distilled water and A few drops of 1% copper sulphate solution should be added to the mixture. A few drops of a 10% sodium hydroxide solution were added to the mixture. The development of a violet hue, which denotes the presence of proteins.

1.4.8 Test for Keller Killani

About 0.5 g of extract was added to 1 ml of strong sulfuric acid, and then a few drops of a 5% ferric chloride solution are added. Then 2 ml of acetic acid and 0.5ml of ferric chloride, a reddish-brown ring that is indicative of cardenolides forms at the interface. The results are confirmed by a greenish ring in the acetic acid layer and a violet ring beneath the brown ring.

1.5 Antioxidant studies DPPH radical scavenging assay

(DPPH) radical assay. (Ayoola et al., 2008) 2.64 grams of DPPH should be precisely weighed and added to an 11 ml volumetric flask. At that point, the solution's DPPH content is 0.004%. This solution's absorbance was measured at 517 nm using methanol as a blank, and the results were recorded as the standard for the control solution. A fresh DPPH 1 mM solution was prepared in methanol, and 3 ml of this solution was mixed with 100 µl of test samples at various concentrations (12.5, 25, 50, 100, 250, and 500 µg/ml). The ascorbic acid reference was used. The samples were incubated for 30 minutes at 37 °C before their absorbance at 517 nm was measured using a spectrophotometer. The radical scavenging activity was calculated using this formula:

$$\text{Percentage (\%)} \text{ of scavenging of the DPPH free radical} = \frac{[Ac-As]}{Ac} \times 100$$

where: Ac is the absorption of the blank sample; As is the absorption of the extract

1.6 Phenolics

Using the Folin-Ciocalteu method described by (Chumbhale et al., 2017) the total phenol content of the samples was ascertained. After 15 minutes of room temperature incubation, samples containing the reaction mixture were analysed using a spectrophotometer for absorbance at 765 nm. The values of total phenol were represented as Gallic acid equivalents. After giving the combination a good shake, distilled water was added to make up to 10 ml. For two hours, the mixture was let to stand. The absorbance at 765 nm was then calculated. Next, using an equation derived from a standard gallic acid graph, the concentration of total phenol content in SA extract was calculated as mg of gallic acid equivalent.

2.1 Molecular Docking Study

The PDB structure of 7DUQ 'Cryo-EM structure of the compound 2 and GLP-1-bound humanGLP-1 receptor-Gs complex' was taken from protein database (<http://www.rcsb.org>) and molecular docking was done using [Auto Dock Vina](#) program. Docking calculations were carried out using Discovery Studio 2017 [BIOVIA Discovery](#) for designing. The water molecules and ligands were removed from the protein structure before performing docking. The 3D structures of the most active compounds which is present in almond that is epicatechin, for walnut Ellagic acid, for flaxseeds SDG Seloisolariciresinoldiglucocyaninde and for tamarind seeds Gallic acid were taken from [NCBI PubChem](#) and were converted to a PDB file using [PyMol Molecular Graphics System \(San Carlos, CA, USA\)](#) and finally to pdbqt file using [AutoDock 4.2.6](#). The cubic grid dimensions were set at 88 × 104 × 104 and was placed in coordinates x = 35.098, y = 31.028, z = 15.155 for a while for 7DUQ grid dimension were set at 50 × 50 × 50 and was placed in coordinates x = 22.6225, y = - 8.069, z = 24.158 as previously described with a spacing of 0.375 Å. The docking of the active compound was done with instead. The molecular docking was done to understand the efficiency of the samples and their effectiveness and binding capability

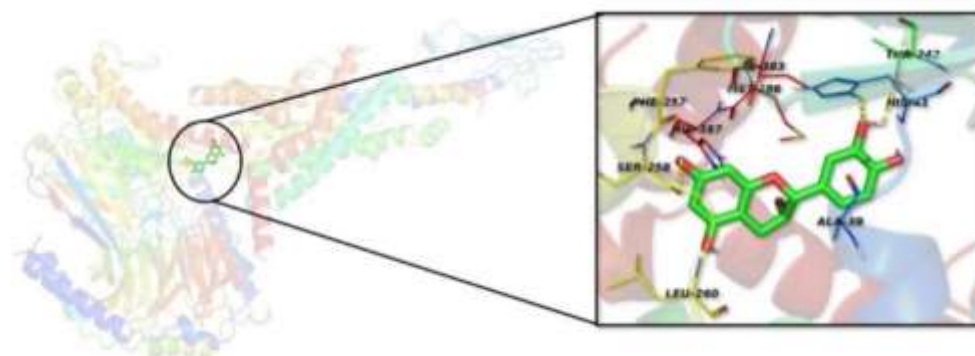


Figure 1: Molecular docking of Almond polyphenol epicatechin with 7DUQ Human insulin protein. The boxes indicate the binding region.

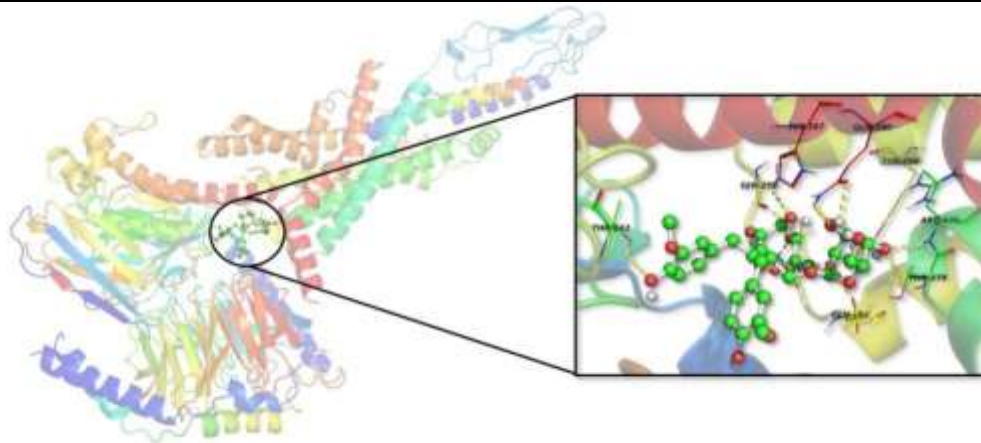


Figure 2: Molecular docking of Walnut polyphenol Ellagic acid with 7DUQ Human insulin protein. The boxes indicate the binding region.

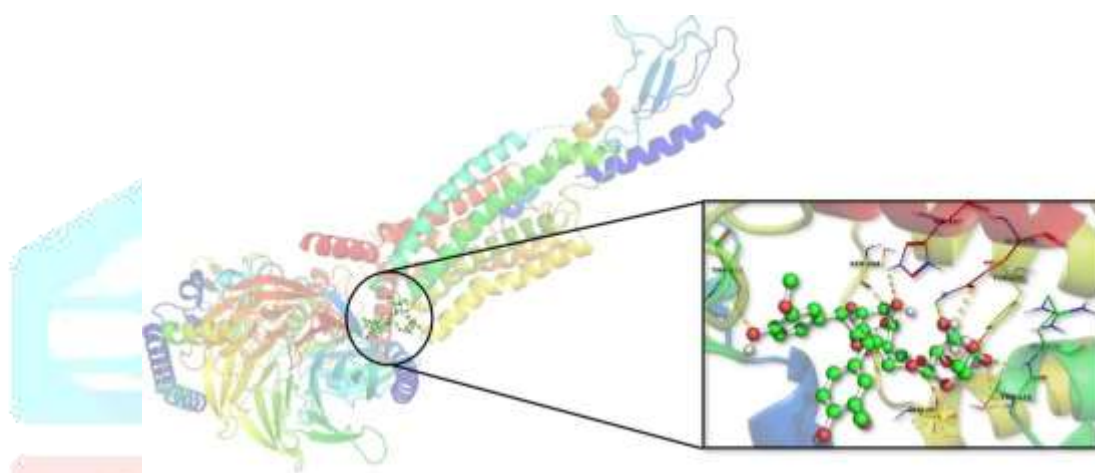


Figure 3: Molecular docking of Flaxseeds with polyphenol Seloissolariciresinddiglucocyanidin with 7DUQ Human insulin protein. The boxes indicate the binding region.

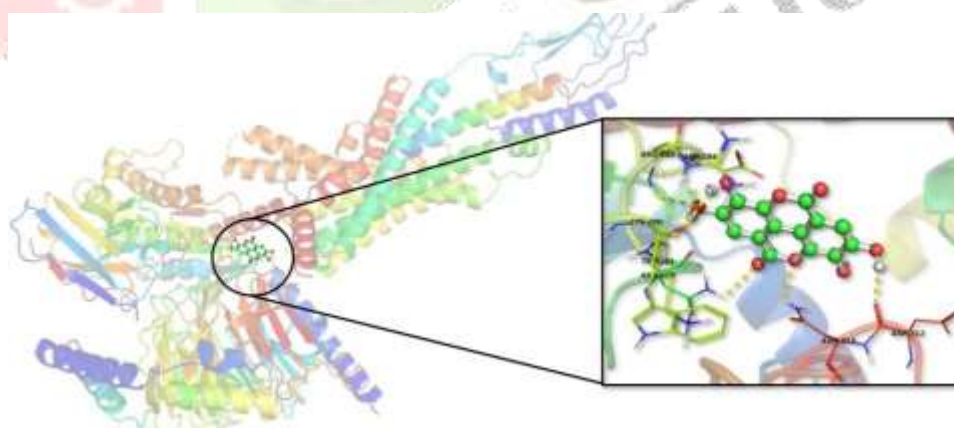


Figure 4: Molecular docking of Tamarind Seeds with polyphenol Gallic acid with 7DUQ Human insulin protein. The boxes indicate the binding region.

I. RESULTS & CONCLUSIONS

2.1 Results of Determination of α -Amylase Inhibition Activity

The alpha amylase assay showed that tamarind seed had the highest enzyme inhibition at 78.4%. This means that the compounds present in tamarind seed were able to effectively inhibit the activity of the alpha amylase enzyme, which plays a role in carbohydrate digestion. Almond also demonstrated significant enzyme inhibition at 71.3%. This suggests that almond contains bioactive compounds that can interfere with the enzyme's function. Flaxseeds exhibited a moderate level of enzyme inhibition at 54.0%. While not as high as tamarind seed and almond, this still indicates that flaxseeds have the potential to inhibit alpha amylase activity to some extent. Interestingly, walnut showed the lowest enzyme inhibition at 40.4%. This could be attributed to the different chemical composition of walnut compared to the other seeds.

Table 2.1 Descriptive percentage of α -Amylase Inhibition Activity

Samples	Absorbance at 540nm	Percentage of enzyme inhibition
Control	1.404	-
Sample 1 Almond	0.403	71.3%
Sample 2 Walnut	0.747	46.8%
Sample 3 Flaxseeds	0.645	54.0%
Sample 4 Tamarind seeds	0.303	78.4%

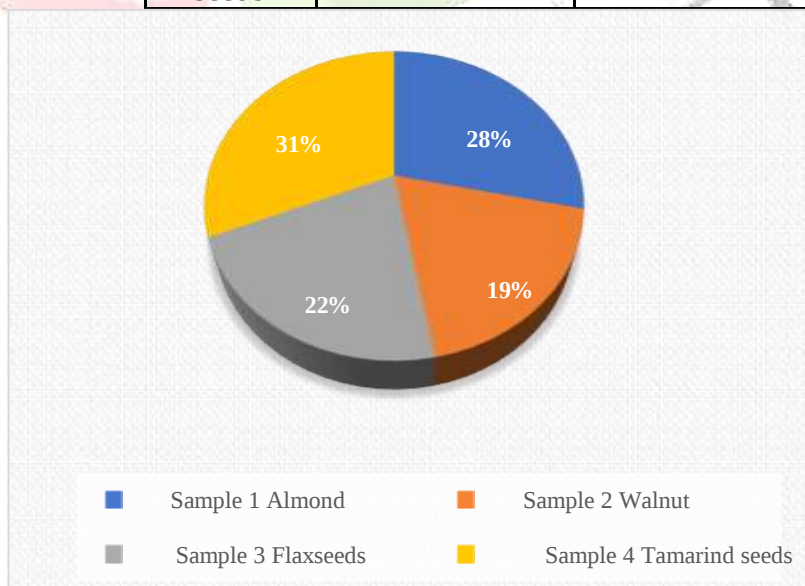


Figure 2.1: Percentage of α -Amylase Inhibition Activity

2.2 Results of Determination of Glucose Yeast Uptake Model

The glucose yeast uptake model, they tested different seeds to see how well they can take up glucose. The results showed that flax seeds and tamarind had the highest glucose uptake percentages at 76.2% and 72.3% respectively. Almond had a medium uptake at 56.4%, while walnut had the lowest uptake at 40.4%. The reason for these variations could be because of the different bioactive compounds present in the samples. Flax seeds and tamarind might. This means is that if you want to have better glucose uptake, it could be a good idea to include flax seeds and tamarind in your diet. They might help you maintain healthy blood sugar levels.

Table 2.2 Descriptive percentage increase in Glucose Yeast Uptake Model

Samples	Absorbance at 540nm	Percentage increase in glucose uptake
Control	0.728	-
Sample 1 Almond	0.317	56.4%
Sample 2 Walnut	0.434	40.4%
Sample 3 Flaxseeds	1.283	76.0%
Sample 4 Tamarind seeds	0.196	72.3%

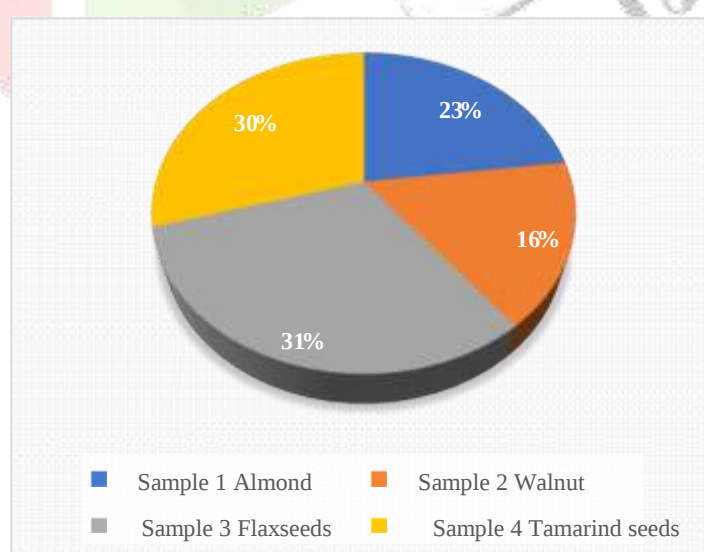


Figure 2.2: Percentage increase in glucose uptake (yeast)

2.3 Results of Phytochemical analysis

The presence of phytochemicals such as tannins, alkaloids, steroids, proteins, and carbohydrates in all four samples. Phlobatannin was present in almond and flax seeds only and was absent in walnut and tamarind seeds. Flavonoid was present in only flax seeds and tamarind seeds and was absent in almond and walnuts. Saponins was present only in almond and absent in the other samples, that is flax seeds and walnut. Keller Killani was positive for almond and tamarind seeds only and was absent in flaxseed and walnut.

Table 2.3 Descriptive Phytochemicals analysis

Tests	Sample 1 Almond	Sample 2 Walnut	Sample 3 Flaxseeds	Sample 4 Tamarind seeds
Alkaloid	+	+	+	+
Tannins	+	+	+	+
Flavonoids	-	-	+	+
Saponins	+	-	-	-
Phlobatannin	+	-	+	-
Steroid	+	+	+	+
Protein	+	+	+	+
Keller Killani	+	-	-	+
Carbohydrate	+	+	+	+

2.4 Results of Phenolics

Table 2.4 Descriptive Phytochemicals analysis

Tube No.	X Axis Concentration mg/ml	Y Axis Optical density at 765nm
1	1	0.039
2	10	0.197
3	20	0.345
4	30	0.598
5	40	0.741
Unknown Sample 1 Almond	75.88	1.418
Unknown Sample 2 Walnut	87.57	1.635

Unknown Sample 3 Flaxseeds	85.57	1.598
Unknown Sample 4 Tamarind seeds	77.55	1.449

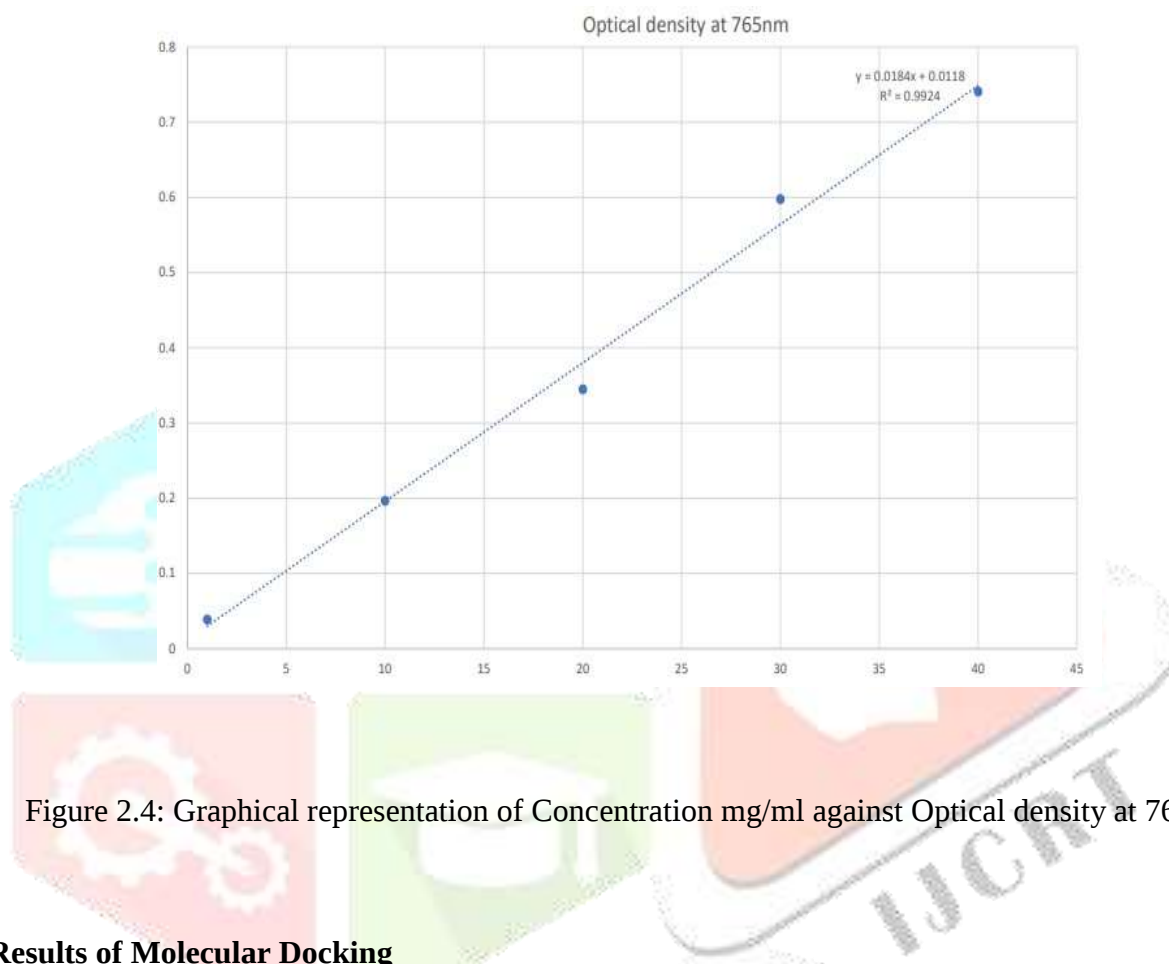


Figure 2.4: Graphical representation of Concentration mg/ml against Optical density at 765nm

2.4 Results of Molecular Docking

The compounds of each extract were used to run the dockings. Molecular docking analysis was carried out with one protein (7DUQ identified from the literature as the glucagon-like peptide-1 (GLP-1) receptor is a well-established therapeutic target for metabolic diseases. Ago-allosteric modulators can operate as agonists on their own as well as effectiveness enhancers for orthosteric ligands). The compounds to be docked with target proteins were Epicatechin, Ellagic acid, Seloissolariciresinddiglucocyanidin, Gallic acid for Almond, Walnut, Flaxseed and Tamarind seed respectively. In total, 4 in-silico docking analyses were performed. Their free energy binding is showed in Table 2.4

Table 2.4: Target protein 7DUQ interaction with compounds of each extract

Sr no.	Ligand	Free energy of binding (kcal/mol) Target protein 7DUQ
1.	Epicatechin	-7.6
2.	Ellagic acid	-7.9
3.	Seloissolaricires inddglucocyani din	-8.6
4.	Gallic acid	-7.9

We conclude that the most important result of this research is that we were able to design and propose our samples as a treatment for diabetes. The results of this compound are satisfactory. In addition, the study of molecular docking helps us with the better binding affinity of samples with target protein. Our in vitro experiments demonstrated significant anti-diabetic effects, with tamarind and flax seeds showing the greatest effectiveness in suppressing key indicators related with diabetes. Following that, molecular docking simulations were used to determine the molecular underpinnings of the reported effects. All of the samples had strong binding affinities to important molecular targets. The combination of experimental and computational studies supports the claim that Almond, Walnut Tamarind, and Flax seeds should be considered as potential antidiabetic therapeutic options. The molecular insights derived from docking simulations give a structural basis for the reported inhibitory effects, broadening our understanding of the mechanistic bases of their anti-diabetic activity. The complexity inherent in the experimental and computational methodologies used must be highlighted. Future study should focus on a more in-depth examination of the bioactive chemicals present in the samples, explaining specific mechanisms and molecular interactions that contribute to their anti-diabetic efficacy. These samples, almond, walnut, flax seeds, and tamarind seeds, have the potential to help with diabetes management. Importantly, they are safe to consume on a daily basis, with no adverse effects even at higher doses. These nutrient-dense seeds are simple to add into our everyday diets. Regularly including them may help to reduce the impact of diabetes. The fact that these advantages are derived from natural sources underscores the need of incorporating them into our daily diets, providing a straightforward and health-conscious approach to diabetes control.

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