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α-amylase inhibition activity of phytoconstituents present in the roots of *Cyclea peltata*-an *in-silico* and *in-vitro* investigation

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ABSTRACT

The primary goal of this research is to examine the α-amylase inhibitory effect of phytoconstituents found in the roots of the *Cyclea peltata* plant. The extract will also be used to investigate the effect of root extract on α-amylase inhibition assay. The roots were gathered, processed, and extracted in petroleum ether before being kept at 4ºC. The extract was exposed to a preliminary phytochemical examination. The extract was employed in the α-amylase inhibition experiment at concentrations of 50, 100, 200, 400, 600, 800, and 1000 µg/ml with acarbose as a control. Molecular docking analysis was performed on the phytoconstituents cycleapeltine, cycleadrine, cycleacurine, cycleanorine, cycleahomine chloride, and acarbose on human pancreatic alpha-amylase 1B2Y. A preliminary phytochemical study revealed the presence of alkaloids, saponins, and terpenoids. The tests came back negative for flavonoids, steroids, and tannin. The root extract inhibited α-amylase at 69.42 ± 0.74 % at 1000 µg/ml and acarbose at 94.63 ± 0.57 %. The IC_{50} value was calculated and found to be 484.08 µg/ml for the extract and 42.47 µg/ml. The docking studies, revealed that cycleacurine (-4.751 Kcal/mol) has a comparable anti-diabetic effect to Acarbose (-6.713 Kcal/mol). Furthermore, the function groups –OH and –NH found in phytoconstituents interacted with the active site of 1B2Y similarly to acarbose. This provides evidence that the function groups –OH and –NH present in the phytoconstituents might inhibit alpha amylase.

Keywords: *Cyclea peltata*, Diabetes Mellitus, Docking, Schrodinger, Alpha amylase, Inhibition

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1. INTRODUCTION

The management of diabetes mellitus affects approximately 9% of the global population, and its management without side effects remains a serious challenge for the healthcare system [1]. Type 2 diabetes is a complicated illness characterized by an improper dynamic connection between insulin production and insulin activity [2]. A glucose deficit elevates blood glucose levels, which impairs many of the body's processes, particularly the blood vessels. Among the ailments were retinal degeneration, nephropathy, neuropathy, and angiopathy. The disease's natural history is marked by increasing cell loss [3]. Amylin antagonists, insulin secretagogues, insulin sensitizing agents, α-glucosidase inhibitors, biguanides, incretinmimetics, and SGLT2 inhibitors are the most often used medications to treat type II diabetes. In patients who are not capable to meet treatment goals with first-line oral hypoglycemic medicines alone, dual pharmacological regimens are commonly prescribed. Despite the apparent therapeutic advantages, traditional dosage forms exhibit varied bioavailability and a short half-life, necessitating repeated administration and creating increased side effects, resulting in therapy ineffectiveness and patient noncompliance [4]. Reduced postprandial hyperglycemia is one type of therapy for diabetes in its early stages. This is performed by suppressing the carbohydrate-hydrolyzing enzymes -amylase and -glucosidase in the digestive system, which prevents glucose absorption. As a result, inhibitors of these enzymes reduce the incidence of glucose absorption, decreasing the post-prandial plasma glucose increase.

Amylase, a main enzyme produced by exocrine pancreatic cells, has been found to be a good predictor of organ function in both healthy and diseased settings. Endoamylase and exoamylase are the two forms of amylases. Endoamylases hydrolyze carbohydrates into a range of linear and branching chain lengths, whereas exoamylases convert carbs into short molecules. Alpha amylase, one of the utmost essential enzymes in the body, is responsible for the breakdown of starch into tiny sugar molecules, making it a potential target for pharmacological treatment. All of the alpha amylase enzyme molecules work on the α 1-4 and 1-6 links of glucose residues [5].

At higher doses, these amylase inhibitory medications might cause hypoglycemia, as well as liver problems, lactic acidosis, and diarrhea. In addition to the currently existing therapeutic treatments, various herbal therapies have been pushed for the management of

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diabetes [6]. Traditional plant medicines are used to cure a range of diabetic symptoms all around the world.

Cyclea peltata (Lam) Hook. F. Thoms is a traditional plant from India's west coast that has been used for about 600 years. In the Indian medical system, the roots of Cyclea peltataare known as Rajapatha and are used for a number of medicinal applications. [7] Cyclea peltataleaves have long been used as a sedative, anti-dandruff, anti-fever, and diuretic [8]. Cyclea peltataleaves included cycleanine, bebeerins, hayatinin, hayatidine, and hayatin. The five bisbenzylisoquinoline alkaloids identified from Cyclea peltataroots are cycleapeltine, cycleadrine, cycleacurine, cycleanorine, and cycleahomine chloride. [9] Cyclea peltataleaves have been found to protect against nephrotoxicity produced by cisplatin and oxidative damage. The findings imply that Cyclea peltata leaf extracts may lower the oxidative stress factors reported in cisplatin-induced kidney damage and could be employed as a natural anti-stress antioxidant [10].

Cylcleapeltata was used as an ancient traditional medicine for a number of ailments [11]. The anti-diabetic property, in particular, needs to be investigated further, as the danger of diabetes is increasing at an alarming rate. As a result, the goal of this study was to investigate the influence of Cyclea peltata root extract on the α-amylase inhibitory test, as well as to evaluate the -amylase inhibitory property of the phytoconstituents found in the roots of Cyclea peltata through in silico studies.

2. METHODS AND MATERIALS

2.1. Processing of Root Extract:

The root plant was collected from Kozhikode, Kerala. The root was cleaned, shade dried, powdered, and extracted with petroleum ether. This Cyclea peltata extract was used for preliminary phytochemical screening as well as the α-glucosidase assay. In a 1 litre flask, 100 g of powdered plant material was combined with 500 ml of petroleum ether and shaken for 16 hours. The solution was then extracted using a separatory funnel and concentrated by the solvent evaporation technique using a rotary evaporator, and the sample material was preserved in an airtight container at 4^{o}C [12].

2.2.Preliminary Phytochemical Analysis:

The plant extracts were sent to preliminary qualitative phytochemical screening according to established methods to determine the occurrence of several phytoconstituents in

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the extracts such as tannins, alkaloids, saponins, flavonoids, phytosterols, and terpenoids [13, 14, 15].

2.2.1. Test for flavonoids:

A fraction of the plant extract was taken in a test tube and few drops of sodium hydroxide was added to it. A yellow color appears which upon addition of few drops of acid (dilute) disappears. This indicates the presence of flavonoids.

2.2.2. Test for alkaloids:

On a water bath, 5 ml of 1% aqueous HCl acid was mixed with 0.5 g of the extract. A few drops of Dragendorff's reagent were added to 1 ml of the filtrate. Turbidity or precipitation with this reagent was used to test the presence of alkaloids.

2.2.3. Test for saponins:

In a steam bath, 1 g of plant extract was warmed in 10 mL of distilled water and filtered. Then 10 mL of the filtrate was mixed with 5 mL of distilled water and aggressively agitated for 5 mins and allowed to stand for 10 mins to create a stable persistent froth.

2.2.4. Test for steroids:

To 0.5 g of plant extract was dissolved in anhydrous CHCl3 and filtered, 2 mL of acetic anhydride was added. Then 2 mL of concentrated H2SO4 were added slowly into the sides of the test tube. The presence of steroids is indicated by an appearance of green color.

2.2.5. Test for terpenoids:

0.5 g of plant extract was combined with 2 mL chloroform and acetic anhydride and 3 mL H2SO4 was carefully added to form a layer. Terpenoids were identified by the reddish violet coloration of the contact.

2.2.6. Test for tannins:

The extract (0.5 g) was diluted and filtered in 5 to 10 ml of distilled water. The filtrate was treated with a few drops of 5% ferric chloride solution. The presence of tannins was established by the formation of a greenish black precipitate.

2.3. α –Amylase inhibition assay:

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The 3,5-dinitrosalicylic acid (DNSA) approach was employed to carry out the -amylase inhibition experiment. The extract fractions were diluted in buffer Na2HPO4-0.02 M, NaCl-0.006 M at pH 6.9 to get concentrations of 50, 100, 200, 400, 800 and 1000 g/mL.200 µL of anti-amylase solution was combined with 200 µL of extract and incubated for 10 minutes at 30°C.The starch solution (1 percent w/v) was then added in 200 µL increments to each tube and incubated for 3 minutes. The reaction was halted by adding 200 µL DNSA reagent and boiling for 10 minutes in an 85°C water bath. The mixture was diluted with distilled water after cooling to 25°Cand the absorbance at 540 nm was measured with a UV-visible spectrophotometer. A blank with enzyme activity was created by exchanging the plant extract with 200 µL of buffer. In the nonexistence of the enzyme solution, a blank reaction was prepared in the same manner using the plant extract at each dosage. Acarbose was utilized as a control sample and the reaction as performed as same. [16].

%inhibition= (absorbance of control – absorbance of control blank)– (absorbance of extract - absorbance of extract blank) / (absorbance of control – absorbance of control blank) X 100

2.4. Molecular Docking

Molecular docking is the computational study of protein-ligand interactions or their geometries. Besides these uses, docking is also useful for hit identification, lead optimization, and bioremediation [17]. There is a plethora of software available to perform docking studies, with the GLIDE standing out as the best due to its accuracy and user-friendly options. Docking involves various steps such as ligand preparation, protein preparation, site map and receptor grid generation, docking and scoring.

2.4.1. Ligand preparation:

The ligand preparation is done using the Ligprep tool in the software. The concept of ligand preparation involves taking the 2D or 3D structure and producing a corresponding low-energy 3D structure, with the option to expand the input structure by expanding the variations in ionisation state, stereochemistry, and ring conformations. The ligands cycleapeltine, cycleadrine, cycleacurine, cycleanorine, cycleahomine chlorine, and acarbose were imported in.mol format and the forward field OPLS3 was chosen. Furthermore, tautomeric forms were chosen to carry out the ketoenol tautomerisation. The Epik option was chosen so that the best ligand is obtained from the state penalty score (Kcal/mol), which represents highly favorable energy for docking. Finally, the ligands were processed with the Ligprep tool.

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2.4.2. Protein preparation:

The target protein used in this investigation is 1B2Y, obtained from the PDB website [18]. Some structures are multimeric and need to be reduced to a single unit. The PDB compounds may omit certain atom and continuation information, which must be updated with bond order and charges. This may be done through the protein preparation wizard. The errors in the proteins were rectified using the color based error reporting scheme. The bond order, hydrogens, zero-order bonds for metals, disulfide bonds, filling of side chains (using prime), and deletion of water beyond the proteins were performed and preprocessed using the wizard. The errors were reviewed, modified, minimized, and prepared for docking [19].

2.4.3. Receptor grid generation [20]:

The grids represent the physical volume of the receptor specifically the active site where an attempt to dock the ligand is performed. The grid volume was adjusted to the volume of the binding site obtained from the site map generation and processed for docking.

2.4.4. Docking and scoring:

The docking wizard was used to start the process where the ligands, processed proteins, and generated receptor grid were selected. The Glide XP method was adopted in this study, and the process was run. The docking and glide scores were calculated by the software and displayed once the process was completed. These scores were tabulated, and the binding sites were analysed in the workspace. Once all the information was retrieved, the project was saved in the maestro format.

3. RESULTS AND DISCUSSION

3.1. Preliminary phytochemical analysis:

Table 1: Phytochemical analysis of root extract of *Cyclea peltata*

S.NO	Phytoconstituents	Petroleum ether extract
1.	Alkaloids	+
2.	Flavonoids	-
3.	Saponin	+
4.	Terpenoids	+
5.	Steroids	-

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6.	Tannins	-

The phytochemical analysis resulted in the occurrence of alkaloids, saponins, and terpenoids and the absence of flavonoids, steroids, and tannins in the root extract. According to Pillai R et al. (2010) the roots of Cyclea peltata contain bisbenzylisoquinoline alkaloids, which support the alkaloid test for the root extract [21]. Also shine vj et al. (2020) extracted the terpenoids present in the Cyclea peltata and also the terpenoids test suggests that there may be terpenoids present which can be identified further by elucidation methods [22]. Though the tests for saponins are positive, there is no strong evidence for the presence of saponins in Cyclea peltata roots.

3.2. α -amylase inhibition assay:

In the in vitro -amylase inhibitory study, the percentage of α-amylase inhibition as a function of extract concentrations was evaluated, and IC50 values were calculated. The IC50 values of the root extract were 484.08 g/ml and that of Acarbose were 42.47 g/ml, indicating concentration dependent inhibition.

Table 2: α -amylase inhibition assay of root extract and acarbose

Concentration (µg/ml)	**% inhibition of α-amylase**	
	Petroleum ether extract	**Acarbose**
50	10.53 ± 0.43	54.14 ± 0.61
100	15.31 ± 0.29	62.75 ± 0.42
200	22.92 ± 0.49	73.48 ± 0.39
400	44.77 ± 0.37	85.16 ± 0.71
600	53.37 ± 0.34	90.42 ± 0.48
800	61.18 ± 0.92	92.31 ± 0.62
1000	69.42 ± 0.74	94.63 ± 0.57
IC_{50}	484.08 (µg/ml)	42.47 (µg/ml)

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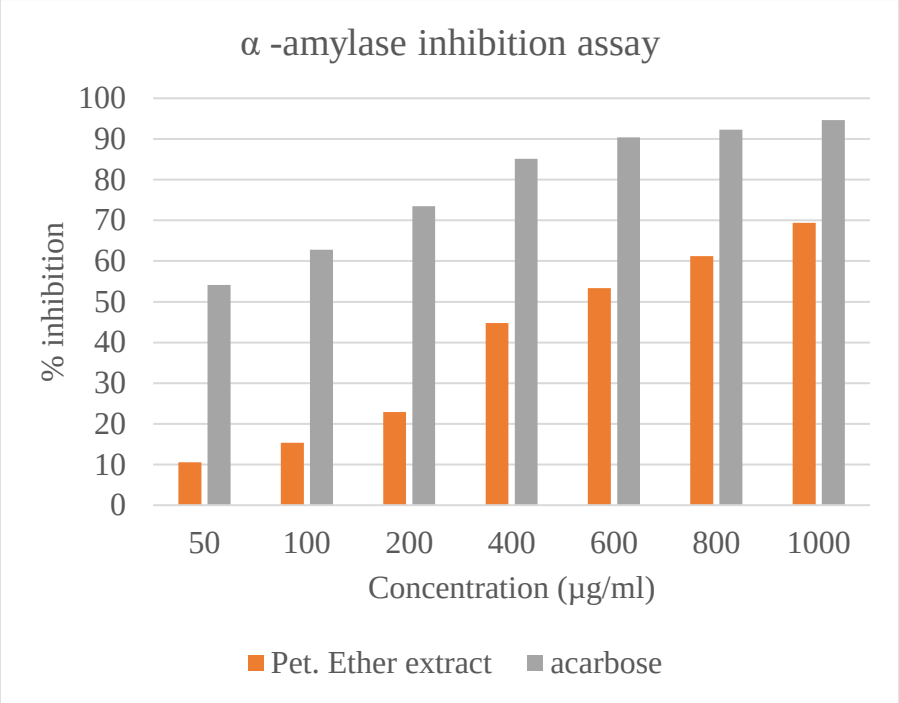

Figure 1-Alpha-amylase inhibition assay

3.3. Docking Results:

The ligands cycleapeltine, cycleadrine, cycleacurine, cycleanorine, and cycleahomine chloride were docked against human pancreatic alpha amylase 1B2Y protein with acarbose as a standard. The poses and scores were extracted and analysed for the best fit. It was found that acarbose (Figure 2) the standard, was found to bind with ARG195 (h-bond), ASP197 (h-bond), ASP300 (salt-bridge) and a few water molecules in the protein. Cycleacurine (Figure 3) was found to interact with THR163 (h-bond), HIE305 (h-bond), TRP59 (pi-cation) and a few water molecules in the protein. Cycleadrine (Figure 4) showed interaction with TRP59, HIE305 (pi-pi-stacking) and cycleanorine (Figure 5) was found to interact with GLY308 (h-bond). The phytoconstituent cycleapeltine (Figure 6) was found to be in interaction with TRP59 (h-bond), whereas the cycleahomine chloride (Figure 7) showed no type of interaction with the alpha amylase protein. The docking score of acarbose was -6.713 Kcal/mol while the best of the phytoconstituents was cycleacurine with -4.751 Kcal/mol. The completed docking score of the compounds docked is expressed in Table 3.

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Table 3: Molecular docking score of Phytoconstituents and Acarbose

Compounds	Docking score	Glide score	Glide emodel
Acarbose	-6.713	-7.041	-76.108
Cycleacurine	-4.751	-5.184	-52.381
Cycleadrine	-3.851	-4.129	-40.361
cycleanorine	-3.778	-3.842	-47.865
cycleapeltine	-3.609	-3.735	-29.272
cycleahomine chloride	-3.315	-3.592	-40.464

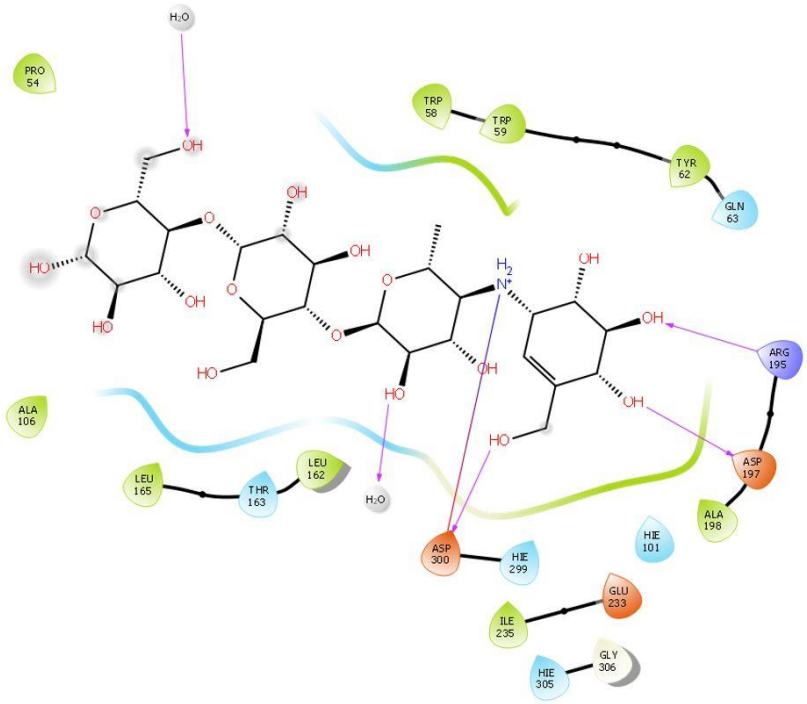

Figure 2 - Interaction of Acarbose with active site of 1B2Y

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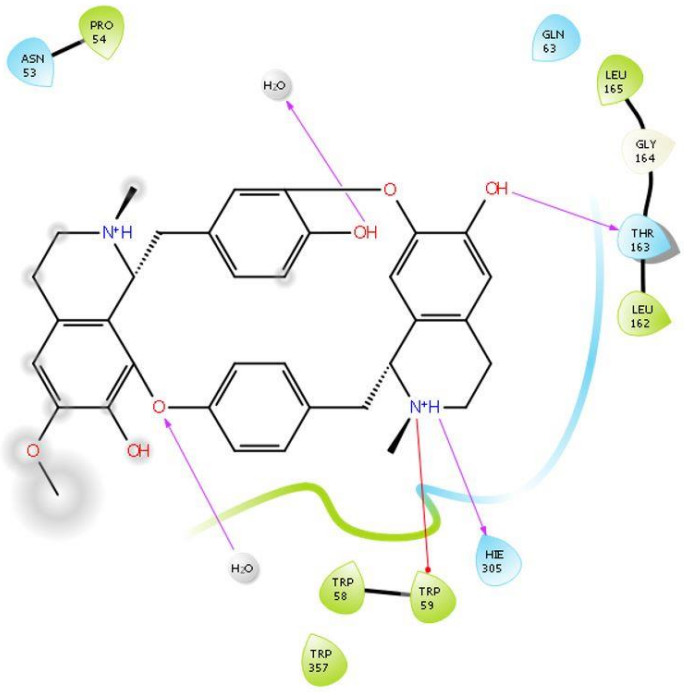

Figure 3 - Interaction of Cycleacurine with active site of 1B2Y

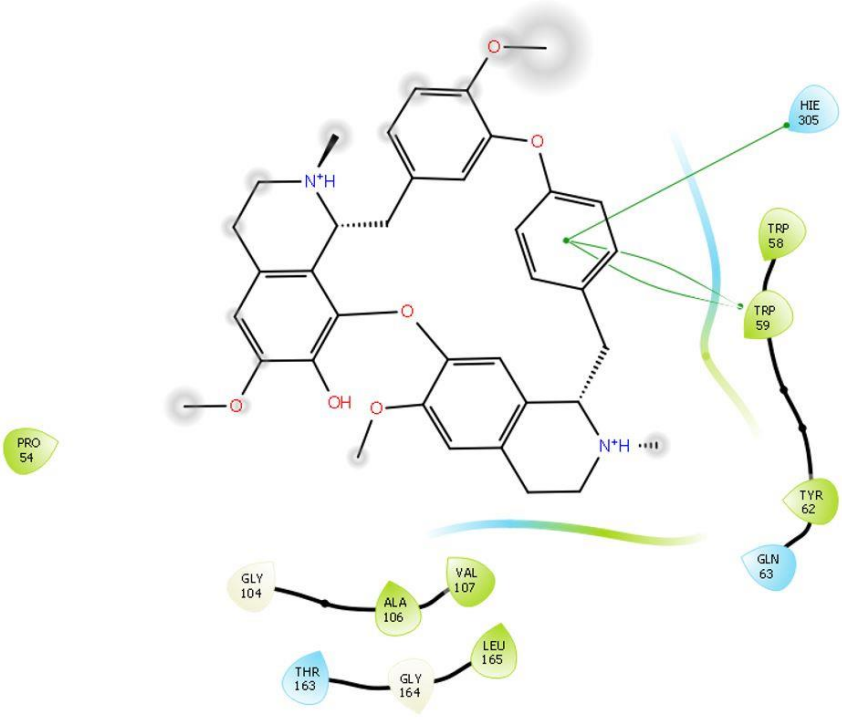

Figure 4 - Interaction of Cycleadrine with active site of 1B2Y

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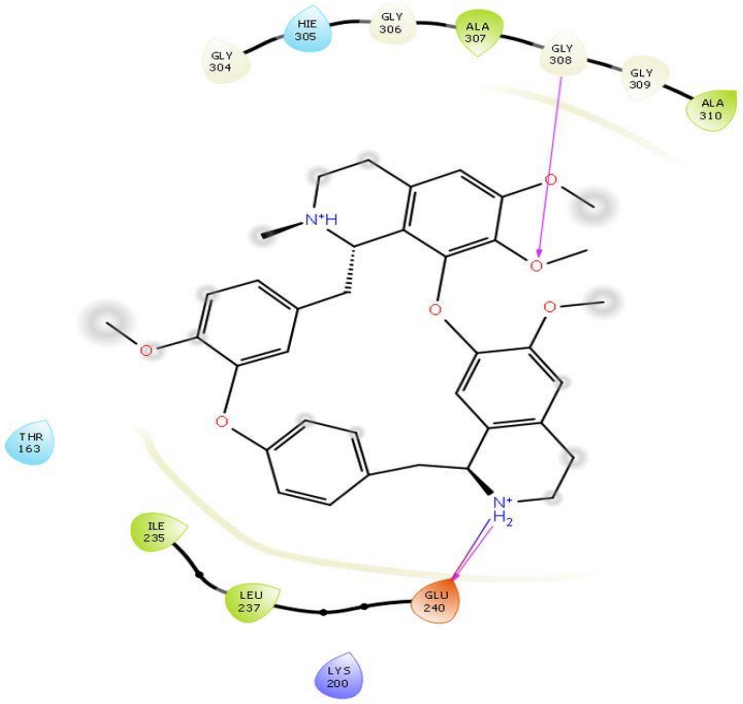

Figure 5 - Interaction of cycleanorine with active site of 1B2Y

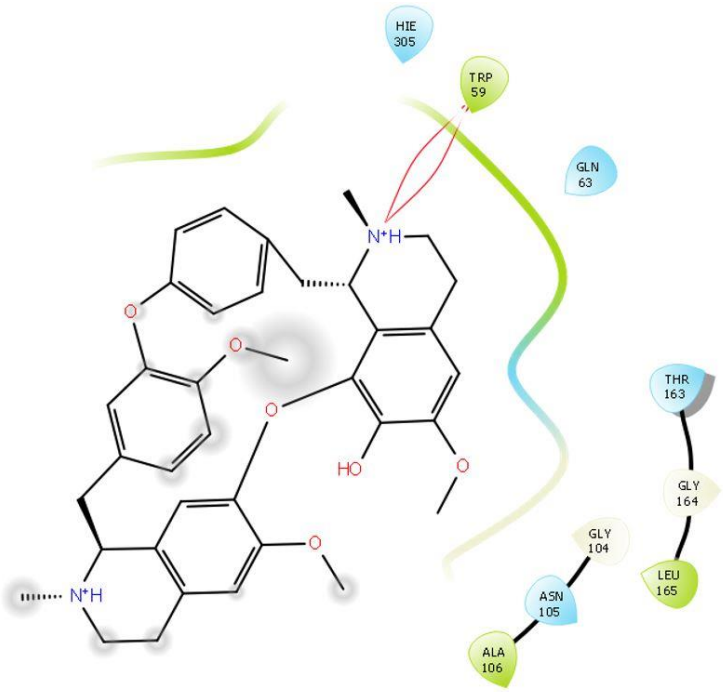

Figure 6 - Interaction of cycleapeltinewith active site of 1B2Y

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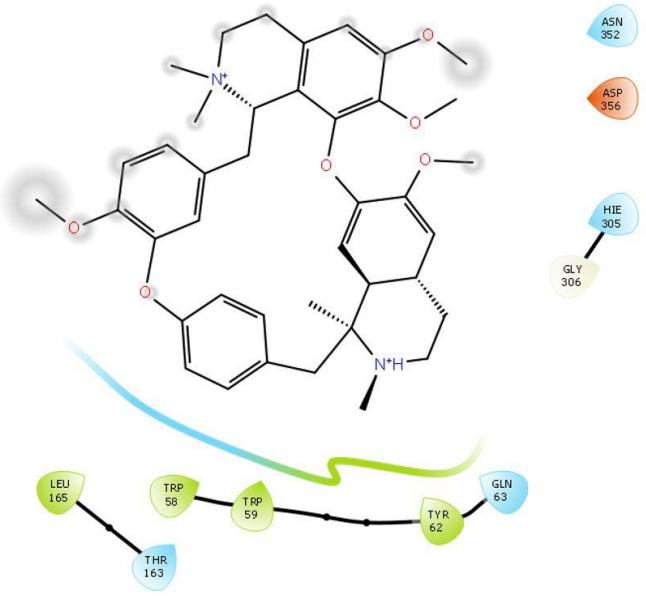

Figure 7 - Interaction of cycleahomine with active site of 1B2Y

A meticulous examination of molecules' interactions with the active area of the protein indicated that the functional group –OH binds effectively. When acarbose was tested with the protein, it was discovered that five –OH groups interacted well with the binding site. The two –OH group of cycleacurine was also discovered to interact with the binding site. Similarly, the –NH group of Acarbose, Cycleapeltine, and Cycleacurine binds to the alpha amylase protein's active region. This might be proof that the functional groups –OH and –NH block the alpha amylase protein.

4. CONCLUSION

Diabetes is among the world's most significant health concerns. Existing drugs are effective with serious side effects for illness prevention or treatment. As a result, the current situation necessitates a continuous quest for new natural products with fewer negative effects. The anti-diabetic effects of a certain plant extract were found to be considerable. We discovered the anti-diabetic activity of phytoconstituents contained in the roots of Cyclea peltata using in-silico molecular docking studies. Furthermore, the molecule Cycleacurine exhibited a similar effect on human pancreatic α-amylase to the commonly used medicine Acarbose. These findings show that plant phytoconstituents might be used to treat diabetes. A deeper knowledge of the molecule via molecular dynamic modelling, isolation, and in-vivo analysis could aid in the synthesis of phytoconstituents as prospective therapeutic options against alpha amylase.

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