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ORIGINAL ARTICLE **OPEN ACCESS**

Alpha Glucosidase inhibitory activity on Ethanolic extract of *Pavonia odorata*

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ABSTRACT
A chronic endocrine condition called diabetes mellitus alters how carbohydrates, proteins, fats, electrolytes, and water are metabolized. It comprises a class of metabolic illnesses known as hyperglycemia, in which blood sugar levels are raised due to either insufficient insulin production by the pancreas or ineffective insulin action on the cells. Decreasing postprandial hyperglycemia as a result is a therapeutic strategy for the management of diabetes. This can be accomplished by inhibiting enzymes that hydrolyze carbohydrates, such as alpha amylase and alpha glucosidase. Long chain carbohydrates are broken down by alpha amylase, while starch and disaccharides are converted to glucose by alpha glucosidase. Post-prandial hyperglycemia can be effectively treated with natural alpha amylase and glucosidase inhibitors found in dietary plants with little side effects, therefore in this study we have used the plant Pavonia odorata and aimed to assess its alpha glucosidase inhibitory effect and anti- oxidant activity on diabetes Mellitus. Qualitative tests for phytochemicals, alpha glucosidase inhibitory activity, DPPH assay and molecular docking were evaluated to culminate that Pavonia odorata exhibits high alpha glucosidase activity and can possibly cure diabetes Mellitus. This study provides necessary intel for subsequent studies and shall provide help to researchers in future.
Keywords*- Pavonia odorata, diabetes Mellitus, alpha Glucosidase, anti-oxidant, photochemical, Health, Well-being, Diseases*

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INTRODUCTION

Chronic hyperglycemia is a complication of diabetes mellitus (DM), a metabolic illness with a pathophysiology that may involve abnormalities in insulin secretion and/or activity. One in three Americans will reportedly have diabetes at some point in their lives. kind 2 diabetic mellitus (T2DM), which makes up around 90% of all instances of DM, is the most prevalent kind. T2DM is mostly caused by the body's tissues not responding to insulin or not producing enough insulin. According to a number of academic studies, diabetes reduces people's quality of life by increasing the risk of serious consequences like stroke, amputation, renal failure, and blindness, which results in severe morbidity and early mortality (1). Over 1 in 10 persons between the ages of 20 and 79 had diabetes in 2019, making it the seventh most common cause of death worldwide (2). By 2045, a 45% increase in prevalence is predicted, with low- and middle-income nations expected to experience the largest increases. With one in six of all patients, India has the second-highest population of diabetes worldwide. By 2045, it is expected that the prevalence of diabetes in India will have increased by 74%, driven by a high genetic propensity and deteriorating lifestyle variables (4). Both microvascular and macrovascular damage can result from undiagnosed or untreated cases of T2DM. Amputation, nephropathy, neuropathy, and visual impairment are just a few of the consequences that can arise from the development of microvascular illness. The major cause of death in diabetics is cardiovascular disease, which typically results from coronary heart disease, stroke, and peripheral arterial disease (3,4)

Oral anti-diabetic medications such sulfonylureas, thiazolidinediones, metformin, -glucosidase inhibitors, and glycosuria are being used to treat type 2 diabetes. Peptides including glucagon-like peptide-1 (GLP-1) agonists (exenatide, liraglutide) and dipeptidyl peptidase-IV (DPP-IV) inhibitors (vildagliptin, sitagliptin) are being used in new treatments. New treatments include bile acid sequestrants and cannabinoid receptor type 1 antagonists (5). Controlling postprandial hyperglycemia in type 2 diabetes therapeutic implications include delaying or slowing the digestion and absorption of carbs. Inhibiting digestive -glucosidase is one therapeutic method that slows down the digestion of carbohydrates and the absorption of glucose,

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regulating blood sugar levels and preventing hyperglycemia in diabetic patients (6). To release glucose from the non-reducing end of their oligosaccharide substrates, glycosidases, a broad class of glycoside hydrolase enzymes, cleave the glycosidic bond. A class of enzymes known as glucosidases, which includes -glucosidases (-D-glucoside glucohydrolase; EC 3.2.1.20), is important for digesting glycoproteins and for the metabolism of carbohydrates. In order to liberate glucose, -glucosidases specifically hydrolyze the -glucopyranoside bond (1-4). Glycogen destruction, N-linked oligosaccharide processing for glycoprotein folding and maturation, and intestinal carbohydrate digestion are all tasks performed by -glucosidases in mammals.

About 70 species of underbrush and plants make up the genus Pavonia, which is mostly native to North America. There are six species of the genus that are found all over the Indian subcontinent and have lovely, occasionally fragrant flowers. As a result, their fragrant blossoms are a common reason why they are grown in gardens. India is home to the Malvacee family plant known as *Pavonia odorata*. The herb is a perennial that is upright and hairy and is commonly used in Ayurvedic medicine for cooling, diaphoresis, diuretics, and demulcent properties. In addition to other related medications, this plant has been given as an astringent and tonic for fever, inflammation, and hemorrhage (7). According to the results of a study, *Pavonia odorata* root extracts (CHI, EtOAc, and MeH) had a blood glucose-lowering impact in diabetic rats produced by alloxan (8). Reactive oxygen species, or oxidants, are a relative overload that leads to oxidative stress. This affects cellular processes and has a role in the pathophysiology of numerous disorders. There is growing evidence that diabetic people experience oxidative stress, and that oxidative stress may play a role in some of the consequences of diabetes (9). In addition, medication derived from herbal plants hardly poses any threat or hazardous side effects to humans (10). Thus in this study we will be assessing the alpha glucosidase inhibitory effect of ethanolic extract of *Pavonia odorata* along with the assessment of its antioxidant property for a more fruitful management of Diabetes Mellitus.

MATERIAL AND METHODS

Preparation of ethanolic extract

Each of the powdered plant components was steeped in a liter of analytical-grade methanol in a conical flask with a capacity of 2 liters for about 400 g. Each plant material was placed in a flask, which was then corked and permitted to stand for 48 hours at room temperature. Each time, the menstrual fluid was separated using Whatman No. 1 filter paper. The filtrates were then concentrated in a rotary evaporator operating at 50°C before being dried fully in a hot-air oven. In preparation for use in in vitro bioassays, the concentrates were placed in airtight containers and kept at 4°C.

Qualitative phytochemical screening

Using common phytochemical screening techniques, qualitative testing for different phytochemicals found in the methanolic leaf extracts of were conducted. An indicator for the presence or absence of a specific phytochemical group was visual evaluation of color or foaming.

Test for saponins

Each of the investigated plant extracts weighed out to about 2 g was dissolved in 5 cc of distilled water. Then, 2 ml aliquots from each plant extract solution were obtained, mixed for 30 seconds, and vigorously agitated. The settings were then given 15 minutes to settle. When foaming appears and lasts for more than 15 minutes, saponins are present in the sample being analyzed.

Test for alkaloids

A mixture of 10 ml of 0.1 M hydrochloric acid and around 2 g of each of the investigated plant extracts was added, warmed in a water bath for five minutes at 50°C, and filtered using Whatman filter paper No. 1. Three drops of Dragendorff's reagent were added and combined after cooling. A reddish-brown color is a sign that there are alkaloids in the sample, thus this is a good sign.

Test for terpenoids

About 2 ml of alcoholic extracts and 5 drops of acetic anhydride were combined and placed into clean test tubes. Then, carefully pouring 5 drops of strong sulfuric acid into the test tube through the side. When a blue ring forms at the contact, terpenoids are present in the sample under test.

Test for flavonoids

Five drops of strong hydrochloric acid were added to two milliliters of the alcoholic extracts of the plants under study. Flavonoids are indicated by the development of a red color. A further fraction of the alcoholic extracts (two milliliters) were combined with one milliliter of diluted ammonia. The presence of flavonoids is indicated by a greenish-yellow color.

Test for cardiac glycosides

The extract (0.5 g) was diluted in 2 ml of glacial acetic acid that also contained 2 drops of a 10% ferric chloride solution in order to test for the presence of cardiac glycosides. The underlying mixture was then

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slowly infused with one milliliter of concentrated H_2SO_4. A positive test for the deoxy sugars (cardenolides) would be the appearance of either a violet band at the boundary.

Test for steroids

This study identified the presence of steroids in the plant extracts under investigation.

In 2 ml of chloroform, 0.5 g of each extract were dissolved. The Liebermann-Burchard reagent was then added in three drops, and the mixture was gently stirred after that. Reddish-purple coloring is a sign of the presence of steroids.

Test for phenols

In a waterbath for five minutes, five grams of each of the investigated plant extracts were heated in five milliliters of 70% ethanol before being filtered using Whatman filter paper No. 1. Five drops of 5% ferric chloride were then stirred in after cooling. The presence of phenols in the sample is indicated by the development of a green precipitate.

Determination of 1,1, dipheny-2-picrylhydrazyl (DPPH) Radical Scavenging Activities

Following 1,1 diphenyl-2-picrylhydrazyl (DPPH), the DPPH radical scavenging assay was carried out following the method reported by Brand-Williams et al. [18] with a few changes. In methanol (analytical grade), five different concentrations of the investigated plant extracts (0.0625, 0.125, 0.25, 0.5, and 1 mg/ml) were produced. L-ascorbic acid, a common antioxidant, was made at the exact same amounts. 0.5 ml of 0.3 mM DPPH in methanol was added to a clean test tube after 1 ml of each extract under study had been put there. After shaking, the mixture was allowed to stand at room temperature in the dark for 15 minutes. As a starting point, blank solutions containing 2.5 ml of the extract solutions under study and 1 ml of methanol were utilized. The positive control was L-ascorbic acid at the same concentrations as the extracts under study, while the negative control was 2.5 ml of DPPH solution and 1 ml of methanol. A spectrophotometer was used to measure the absorbance values at 517 m after incubation in the dark. The tests were carried out three times. The Brand-Williams et al. equation, where As is the absorbance of the sample and Ac is the absorbance of the control, was used to determine the DPPH radical scavenging activity.

% Radical scavenging activity = [(Abs of control - Abs of sample) / Abs of control] × 100

Methodology of a-glucosidase inhibitory activity

Extract and fraction a-glucosidase inhibitory activity was measured using the standard procedure with a few minor modifications. A 96-well plate was preincubated at 37°C for 15 minutes with the reaction mixture, which contained 50 ml of phosphate buffer (100 mM, pH = 6. 8), 10 ul of alpha-glucosidase (1 U/ml), and 20 ul of different concentrations of extract and fractions (2, 4, 6, 8, and 10 mg/ml). 20 MI P-NPG (5 mM) was then added as a substrate, and the mixture was incubated once again for 20 min at 37°C. The addition of 50 Ml of Na 2 CO3 (0.1M) stopped the process. Using a Multiplate Reader, the emitted p-nitrophenol's absorbance was calculated at a distance of 405 m. Acarbose was used as a standard in a range of concentrations (0.1-0.5 mg/ml). Without test substance was set up in parallel as a control. The results were expressed as percentage inhibition, which was calculated using the formula, Inhibitory activity (%) = (1 - As/Ac) ×100

Methodology of molecular docking

According to maximum binding energy, docked molecules were screened. The binding energy of the interaction between the target enzyme and substrate was calculated by docking, and the analysis of the docking results was done to find possible inhibitors. From a thorough literature search, phytochemicals from plants were chosen to function as ligands against MMP8. Their corresponding structured data format (SD) two-dimensional chemical structures were obtained from the PubChem-NCBI database, and the SDF format was then transformed to Protein data bank (PDB) format using OpenBabel 2.3.1. Control is provided by Glucosidase's chemical composition. Protein Data Bank provided the three-dimensional structure of glucosidase (PDB ID:3L4Y). The protein had its water molecules from the receptor crystallographic structure removed. Using Hex 8.0.0, each of the recovered phytocompounds was examined separately. Hex server, a first Fourier Transform (FFT)-based analytics, is a protein docking tool (http://hex.loria.fr). This approach uses 6D analysis to rigidly dock while taking into account various orientations. By rotating and translating the expansion coefficients, the HEX software performs an exhaustive search over all six rigid-body degrees of freedom. This was done by keeping appropriate parameters such the FFT mode set to 3D fast lite, the grid size set to 0.6, the receptor and ligand ranges set to 180 and 360, respectively, and the distance range set to 40. In Pymol, the docked complex of protein and ligand interaction was seen. More negative E-total values in Hex Docking server 8.0 versions indicated that there was a strong contact between the ligand and receptor, which resulted in the activation of receptor activity.

RESULTS

Phytochemicals in plant roots have various mechanisms of action to control diabetes, including inhibition of -amylase and -glucosidase enzymes, reduction of oxidative stress, insulin secretion, improvement of diabetic retinopathy/nephropathy, slowing starch digestion, and contribution against hyperglycemia (Table 1).

Table 1- Qualitative analysis of plant extract

Phytochemicals	Qualitative analysis of plant extract
Flavonoids	+
Phenols	-
Steroids	+
Saponins	+
Alkaloids	+
Cardiac glycosides	+
Terpenoids	+

Antioxidants play a crucial role in diabetes, as low plasma antioxidant levels have been linked to the disease's onset and its progression, and circulating levels of radical scavengers are compromised. (Table 2)

Table 2- DPPH scavenging activity (%inhibition)

Concentration in mg/ml	L ascorbic acid	Plant extract
0.250	55	50
0.125	43	38
0.25	40	32
0.5	35	29
0.1	29	25

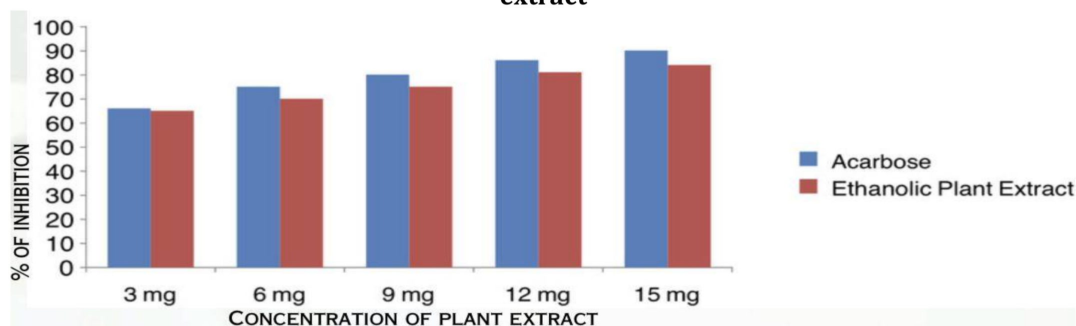
Reduction of postprandial glycemia and reduction of the extremes between maximal and minimal postprandial glucose levels are the main benefits of alpha-glucosidase inhibitor therapy in diabetic patients. (Table 3)

Table 3- Alpha glucosidase inhibitory effect

Concentration mg/ml	Acarbose (% of inhibition)	Ethanolic plant extract (% of inhibition)
3mg	66	65
6mg	75	70
9mg	80	75
12mg	86	81
15mg	90	84

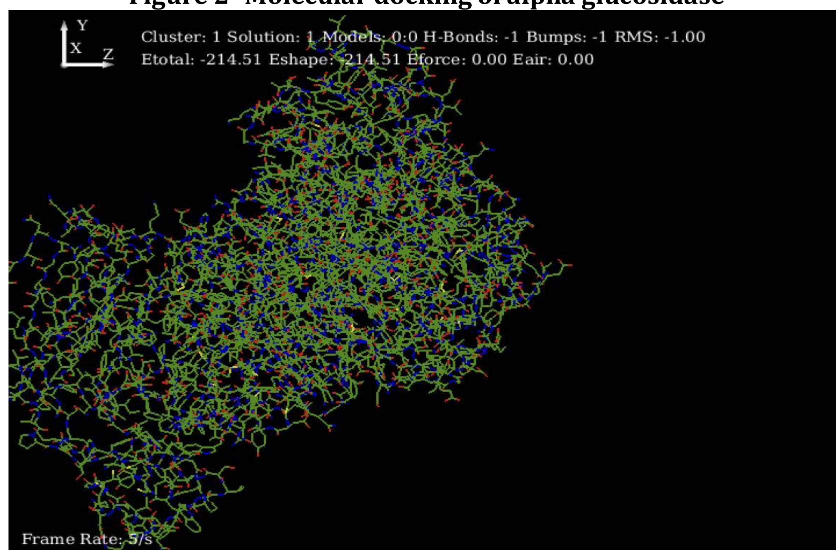
The graph demonstrates that with increasing concentration of the plant extract the percentage inhibition of alpha glucosidase activity too increased considerably.
(Figure 1).

Figure 1- Percentage of inhibition of alpha glucosidase in contrast with concentration of plant extract



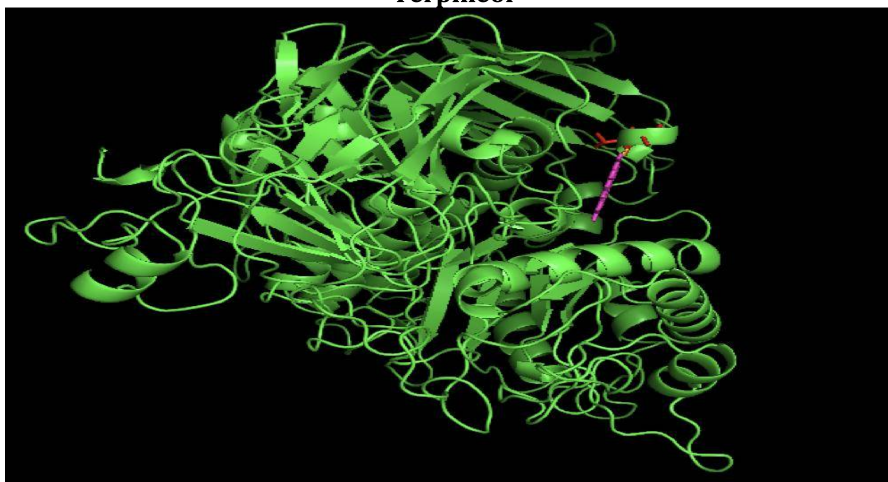
The binding energy of the interaction was ascertained by docking the target enzyme with the substrate.(Figure 2)

Figure 2- Molecular docking of alpha glucosidase



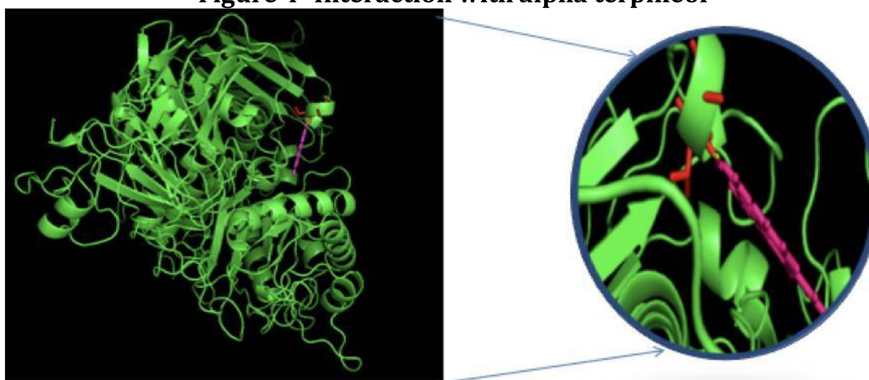
In silico- Alpha Glucosidase inhibitory activity on Alpha Glucosidase enzyme (PDB ID:3L4Y with Alpha-Terpineol (pubchem ID: 17100) (Figure 3)

Figure 3- Alpha Glucosidase inhibitory activity on Alpha Glucosidase enzyme with Alpha-Terpineol



The interacting or the binding energy obtained through the molecular docking was 214.51 kcal/mol. As it is, less the binding energy more the interaction. Our result demonstrated good interaction with alpha terpineol. (Figure 4)

Figure 4- Interaction with alpha terpineol



The TPC, (total phenolic content; mgGAE/g, milligrams gallic acid equivalent per gram of sample) of the plant extract was found to be 55. While the TFC, (total flavonoid content; mg QE/g, milligrams of quercetin equivalent per gram of sample) of the plant extract was 49.

DISCUSSION

Reducing postprandial hyperglycemia is a treatment strategy for diabetes. The suppression of enzymes that hydrolyze carbohydrates, such as alpha glucosidase and alpha amylase, can accomplish this. Alpha glucosidase converts starch and disaccharides to glucose, while alpha amylase breaks down long-chain carbohydrates. Type 2 diabetes mellitus is treated with alpha glucosidase inhibitors, which are oral anti-diabetic medications. They work by stopping the breakdown of starches and other carbs. However, the issue at hand is that.The synthetic enzyme inhibitors that are now in use have gastrointestinal adverse effects that include bloating in the abdomen, diarrhea, and flatulence. Thus, postprandial hyperglycemia can be effectively treated with natural alpha amylase and glucosidase inhibitors derived from dietary plants, with little to no adverse effects.

The onset of diabetes mellitus is significantly influenced by free radicals. According to Ceriello and Motz 2004 (11), oxidative stress is the common pathogenic cause that leads to insulin resistance, B-cell dysfunction, reduced glucose tolerance, and type 2 diabetes. In turn, diabetes will make a diabetic patient's body more susceptible to oxidative stress. Compromised antioxidant defenses, glucose autoxidation, the production of advanced glycation end products, and a shift in the glutathione redox status are among the potential pathways that could cause increased oxidative stress in diabetes patients (12). Diabetes's complication with cardiovascular disease is caused by increased oxidative stress (13). Antioxidant consumption is therefore helpful in the treatment of diabetes, especially type 2 diabetes.

Plant roots contain phytochemicals that have various mechanisms of action to control diabetes. These mechanisms include the inhibition of α-amylase and α-glucosidase enzymes, reduction of oxidative stress, secretion of insulin, amelioration of diabetic retinopathy/nephropathy, slowing down the digestion of starch, and protection against hyperglycemia. (14-18)

Our results demonstrate the qualitative analysis of the phytochemicals present in the plant and further exhibit their potential anti-oxidant and alpha glucosidase inhibitory effect. We can confirm that *Pavonia odorata* possesses excellent potential to be used as a medicine occurring from natural sources to cure diabetes Mellitus.

CONCLUSION

After a careful analysis of our results we can conclude that *Pavonia odorata* possesses great potential for its usage as a treatment for Diabetes Mellitus. It can effectively inhibit alpha glucosidase activity alongside its commendable anti-oxidant potential. Since it is a plant based cure it tends to demonstrate the least or null health hazardous effects to humans. It can also be easily accessible along with its excellent potency. Although we require further studies on the clinical use of this plant as medicinal treatment for its consumption.

CONFLICT OF INTEREST: none

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