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ANTI DIABETIC POTENTIAL OF CITRULLUS COLOCYNTHIS BY INVITRO AND IN SILICO APPROACH

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Abstract
Introduction: In traditional medicine, citrullus colocynthis is a popular fruit that is used as a treatment against a variety of ailments, most notably diabetes. A problem in the metabolism of proteins, fats, and carbohydrates is commonly associated with diabetes mellitus (DM). It arises as a result of insulin resistance, loss, or insufficiency. The need for new anti-diabetic medications is driven by the prevalence of diabetes as a metabolic condition.
Objective : The objective of the present study was to assess the alpha-glucosidase inhibition activity of *Citrullus colocynthis* extract and determine its impact on cell viability.
Methods: Using a colorimetric technique with acarbose as the reference standard, the ethanolic extract of *Citrullus colocynthis* is examined for its ability to inhibit alpha-glucosidase. Various extract concentrations are used to gauge the vitality of *C. colocynthis* cells. The binding affinity of the chemicals in the extract to the target protein is examined using molecular docking experiments.
Results: The *Citrullus colocynthis* ethanolic extract exhibits considerable alpha-glucosidase inhibitory activity, with a concentration-dependent pattern similar to acarbose, though with significantly lower activity. *C. colocynthis* exhibits exceptional cell vitality at low concentrations; even at high concentrations (1000 ug/ml), the cell viability is still greater than 70%, showing little cytotoxicity. A significant affinity for the target alpha-glucosidase protein has been found by molecular docking experiments between the chemicals in the extract.
Discussion: In vitro and in silico studies on *C. colocynthis* have revealed its potential as an anti-diabetic agent. In vitro experiments showed enhanced glucose uptake, insulin secretion, and antioxidant activity, while in silico simulations demonstrated binding affinity with relevant proteins.
Conclusion: The potential for *C. colocynthis* as a natural anti-diabetic compound seems promising. Despite being slightly less effective than acarbose, *C. colocynthis* alpha-glucosidase inhibitory efficacy may be improved through purification. The high cell viability of *C. colocynthis* further supports its safety. The outcomes of the molecular docking offer information on the potential mechanism of action. These results support the need for safe and effective diabetes management alternatives and call for additional study to confirm the efficacy and safety of these plant extracts as possible anti-diabetic treatments.

Keywords: Citrullus colocynthis,alpha-glucosidase inhibition, cell viability, molecular docking, Health, Well-being, Dental, Diseases, Universal health

Introduction

Among the most frequent metabolic diseases is diabetes mellitus that affects more than a billion people worldwide and leads to a significant number of fatalities out of all the endocrine diseases (1). There are many known risk factors for this condition, such as nutrition and age. Approximately 422 million individuals worldwide suffer from diabetes, and the disease is directly linked to 1.5 million fatalities annually. According to the report, India, the world's most populated country, has 101 million diabetics. Diabetes has increased in both the number of cases and the prevalence during the previous few decades. Reactive oxygen species are produced at higher levels when hyperglycemia promotes non-enzymatic glycation of proteins. In diabetic patients, IOS also results in the degeneration of red blood cells.This hematological issue interferes with erythrocyte function (2). A lot of work is being done to discover safe and efficient hypoglycemia drugs that might protect diabetic people from developing additional diabetes-related issues (3). In this situation, WHO has recommended alternative medicine that aids in the treatment of metabolic disorders more efficiently. Herbal remedies have been used for anti-diabetic therapy for a very long time. In the last ten years, controlled studies in healthy and ill animals as well as humans have shown that numerous plant medicines have valid antidiabetic potential. By restoring the integrity and function of the cells, these plant medicines' mode of action for treating diabetes involves modulating carbohydrate metabolism (4)(5). Even though many drugs are used to treat type 2 diabetes, researchers are still looking for new anti-diabetic substances because long-term results have not been satisfactory and because some treatments, like those associated with the insulin-sensitizing thiazo-lidinediones (TZDs), have significant adverse reactions (6) (7).

Many fundamental human requirements, such as different types of medicinal drugs, have been met by plants (7). *C. colocynthis*, also known as "bitter apple", "colocynth", "vine-of-sodom", and "Tumba", is an annual tropical plant found in Europe, Asia, and Africa (8). With its herbaceous stalks, triangular, hairy leaves, yellow flowers, and globular bitter fruit, *C. colocynthis* has characteristics similar to those of a watermelon. The fruit of this plant has an inside white spongy pulp and an outside hard rind. Its pulp contains a significant quantity of seeds (9).The arid plant Numerous bioactive compounds, including as lipid, glycosides, flavonoids, alkaloids, and essential oils, may be found in C. colocynthis. Its pulp has been found to contain the primary phytochemicals pectin, colocynthin, colocynthetin, and gum; its seeds have yielded albuminoids and fixed oil. The pulp of the fruit and seeds are an important part of this plant's medicinal composition because of these compounds that have therapeutic value. Additionally therapeutic are the root and leaves of C. colocynthis (10).The immune system is enhanced by medicinal herbs. Intestinal parasites, gastroenteritis, and indigestion have all been treated with the dried fruit pulp of C. colocynthis. As a laxative and purgative, C. colocynthis has superior pharmacological properties as well. Moreover, it has anthelmintic, anti-inflammatory, anti-diabetic, and anticancer properties. The fruit's antibacterial, antioxidant, and anti-inflammatory properties have been well investigated (11)
The purpose of this research is to look at the anti-diabetic potential of *C. colocynthis* utilizing both in vitro and in silico methods.

Materials and Methods

MTT assay for cell viability

Utilizing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and neutral red uptake (NRU) assays, assessments of the cytotoxicity and biosafety of plant ethanol extracts were conducted. The concentration of Citrullus colocynthis extracts that is considered biologically safe or non-cytotoxic was also established, as was the cytotoxicity of ethanol.
For 24 hours, *C. colocynthis* extracts in varying doses (from 10 to 1000 mg/ml) were applied to HepG2 cells. HepG2 cells were treated to ethanol at a range of doses (50–1000 mM) for 24 hours to test for cytotoxicity.
Using the MTT assay as specified, the percentage of cell viability was evaluated. In summary, $1x10^4$ cells were plated in 96-well culture dishes and cultured for a full day at 37 °C in a CO2 incubator. After the proper exposure, 10 ml/well in 100 ml of cell suspension was added to each plate, along with 5 mg/ml of MTT stock in phosphate-buffered saline (PBS), and the plates were allowed to incubate for 4 hours. The supernatants were disposed of, and 200 ml of DMSO was put in and gently mixed into each well. The generated colour was measured at 550 nm using a multiwell spectrophotometer (Thermo Scientific, USA). Sets that were not processed were also run in the same conditions as a control.

In vitro test for inhibiting alpha amylase

Alpha amylase enzyme (Sigma, India) and phosphate buffer (2 mM, pH = 6.9) were added to 100 ML of the test extract and allowed to react with 100 ML of the test extract. A 100ml solution of 1% starch was added following a 20-minute incubation period. The identical procedure was followed for the controls, except that 200ul of the enzyme was swapped out for a buffer. About 500 ml of the dinitrosalicylic acid reagent was added to both the control and the test after 5 minutes of incubation. The tubes were boiled in water for five minutes. With the aid of a spectrophotometer, the absorbance was measured at 540 nm.
The formula % inhibition = [(Control - Test)/Control *100 was used to compute the percentage inhibition of the a-amylase enzyme. Acarbose was used as a reference standard drug.

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. Acarbose's chemical composition serves as a guide.
Protein Data Bank provided the three-dimensional structure of alpha amylase (PDB ID: 2QV4). The protein had its water molecules from the receptor crystallographic structure removed. Using Hex 8.0.0, each of the recovered phytocompounds was subjected to docking with the Alpha Amylase 2QV4 Protein Receptor. 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

The results of the alpha-glucosidase inhibitory activity for the ethanolic plant extract of Citrullus Colocynthis show remarkable potential as an anti-diabetic medication. Although the activity was significantly lower than that of normal acarbose, it showed a similar trend of increased inhibition with concentration. Table 1 shows that the plant extract includes bioactive substances that can efficiently target and inhibit alpha-glucosidase, which is essential for controlling postprandial glucose levels. The promise of *C. colocynthis* as a natural alternative for treating diabetes is suggested by the fact that the action is comparable to acarbose. The results may be improved by separating and purifying the active component(s) from the plant extract. Purification concentrates the beneficial molecules and enables the elimination of undesirable components, potentially increasing efficacy. Understanding the specific substances responsible for the anti-diabetic activity through further research in this area may be useful for the eventual creation of more potent and precise medications to manage diabetes.

Table 1: Inhibition of alpha amylase at various concentrations of the plant extracts

Concentration	Acarbose (%)	Plant extract (ethanolic) %
2 mg	72	35
4 mg	77	40
6 mg	79	53
8 mg	81	60
10 mg	87	68

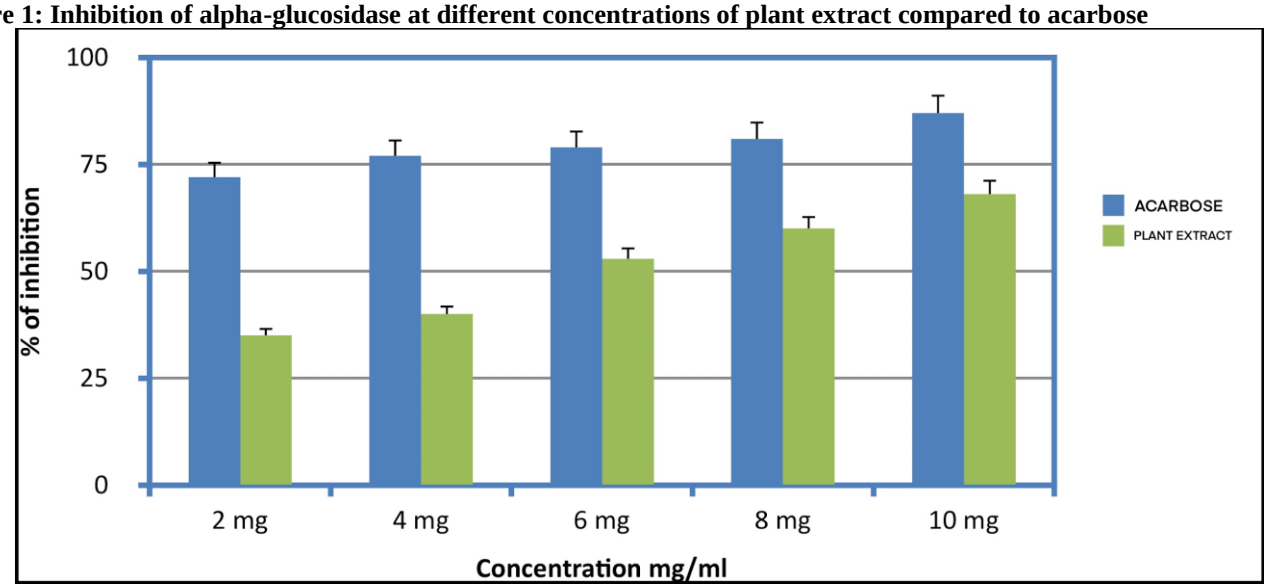
Concentration-dependent alpha-glucosidase inhibitory activity of *C. colocynthis* extract, demonstrating comparable effectiveness to acarbose.
The results of the *C. colocynthis* cell viability experiment are quite encouraging from a safety perspective. The plant extract showed remarkable cell viability at extremely low concentrations, which suggests the cells were barely harmful (Table 2). Cell viability remained very high at more than 70% even at high concentrations (1000 ug/ml), indicating that the extract is not harmful. This important discovery shows the potential medical safety of Citrullus Colocynthis as a natural product.High cell survival at various concentrations suggests that the extract may inhibit alpha-glucosidase with only a minor impact on other cellular processes. This selectivity is a desired property for possible anti-diabetic drugs since it signals a more focused and targeted activity while minimizing negative effects on regular cellular functions.
Additionally, the molecular docking study (figure 2) adds to our understanding of the potential mechanism of action. A persistent interaction between the active substance(s) and the target enzyme is suggested by the drug's potent affinity for binding to the protein, which is demonstrated by the outstanding E-score of minus 374 (figure 3).
This interaction could stop the enzyme from converting complex carbs into glucose, lowering postprandial glucose levels and assisting in the control of diabetes.

Table 2: Cell viability assay performed to assess the cytotoxicity profile of *C. colocynthis*

Concentration ug/ml	Cell viability in (%)
Control ug/ml	100
10 ug/ml	98
25 ug/ml	95
50 ug/ml	93
100 ug/ml	94
250 ug/ml	90

500 ug/ml	85
1000 ug/ml	82

Figure 1: Inhibition of alpha-glucosidase at different concentrations of plant extract compared to acarbose

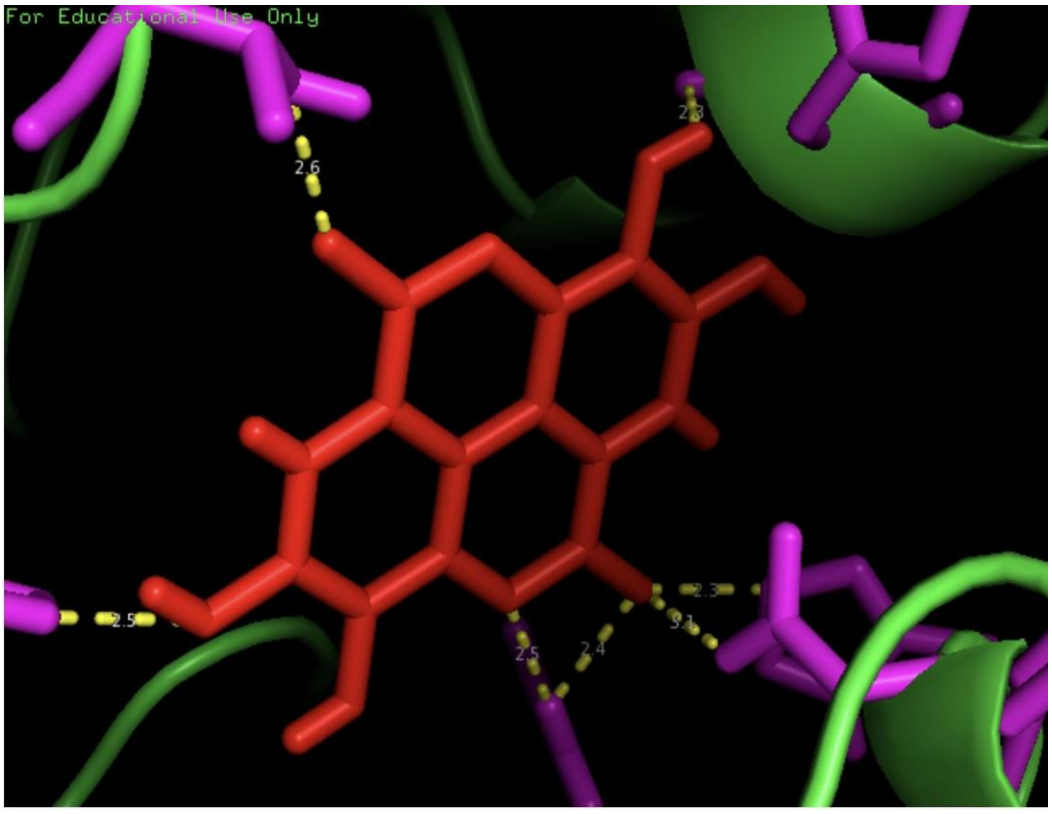


Concentration-dependent alpha-glucosidase inhibitory activity of *C. colocynthis*, demonstrating comparable effectiveness to acarbose.

Molecular Docking

The results of the interaction of the ligand (gallic acid) with the protein using molecular docking approach showed strong binding with the lowest E score of -178Kcal/mol. The best scoring docking model did not show either Pi stacking or hydrogen bonding but showed strong hydrophobic interactions. The three-dimensional structure of the binding of the ligand with the alpha-glucosidase protein is shown in Figure 2.

Figure 2: Molecular docking of gallic acid with alpha-glucosidase enzyme



The gallic acid compound is indicated in red; the protein in magenta and the binding interactions in yellow dotted lines.

Figure 3: The three-dimensional protein-ligand interaction between gallic acid and alpha-glucosidase enzyme

Discussion

C. colocynthis is a viable possibility for the creation of anti-diabetic medications, according to the study's overall findings. Despite being marginally less effective than acarbose, Citrullus Colocynthis has tremendous potential as a natural substitute. *C. colocynthis* is a viable option for further research because the cell viability experiment also shows that it is safe, even at high concentrations. Increased anti-diabetic action may result from purification of active ingredients of *C. colocynthis*. A thorough knowledge of the probable processes underlying the anti-diabetic effect of these plants can be obtained by combining the findings of these assays and the molecular docking studies.

One of the metabolic diseases that is spreading the fastest is diabetes mellitus. Since there is no cure for diabetes and only symptomatic relief available, the hunt for safer, more cost-efficient, and more effective anti-diabetics continues. Chemical medications must be taken for the rest of one's life and have several adverse effects. Numerous studies on both humans and animals have been conducted on *C. colocynthis*, which has been widely utilized as an anti-diabetic in several nations (12). By taking the aqueous extract orally, one may be able to lessen some of the harmful effects of streptozotocin and blood sugar levels (13). Furthermore, research has demonstrated the insulinotropic effect of fruit extracts (13, 14). Insulin secretion was significantly increased by administering different seed extracts, as demonstrated by in vitro experiments using isolated rat pancreas and islets with 8.3 mM glucose. Glycemia was significantly reduced in normoglycaemic rabbits after the aqueous extract was given orally at a dose of 300 mg/kg. Oral administration of ingredients (tertiary and quaternary alkaloids, glycosides, and saponins) derived from the fruit of Citrullus colocynthis was used to treat normoglycemic rabbits at a dose of 50 mg/kg. We did not observe any hypoglycemic effect from the alkaloids. But the glycosidic component significantly lowered blood sugar levels. In alloxan-induced diabetic rabbits, the saponin component of C. colocynthis demonstrated decreased glycemia at significantly lower dosages (10–20 mg/kg), indicating that the saponin and glycoside components may be responsible for the plant's ability to lower blood sugar. The following parameters were measured: aspartate transaminase, alanine transaminase, alkaline phosphatase, urea, creatinine, low density lipoprotein (LDL), high density lipoprotein (HDL), total cholesterol, glucose, fasting blood sugar, and triglyceride. Every month, patients were seen. HbA1c and fasting blood glucose levels were statistically significantly lower in patients who received Citrullus colocynthis. In both groups, the values of the additional serological variables did not change. There were no notable gastrointestinal side effects observed in either group (11).

In the United Arab Emirates, C. colocynthis is also often used as a diabetic therapy. The effects of the water-based extracts from the seeds on biochemical variables in rats treated with streptozotocin (STZ) and normal rats were found. To induce diabetes mellitus, a single intraperitoneal shot of STZ (60 mg/kg total body weight) was administered. For a duration of two weeks, the plant extract (300 mg/kg) administered orally to diabetic rats significantly reduced plasma concentrations of AST and LDH; however, it was not able to have the same effect on the high blood levels of GGT and ALP (15). To examine the effects of diets supplemented with C. colocynthis, sunflower, or olive oils on the pancreatic M-cell mass, rats with diabetes induced by streptozotocin (STZ) were employed (16). Two months following STZ injection, the pancreatic M-cell mass of rats treated with STZ and given a diet enhanced with colocynth

oil was similar to that of the animals' controls on the same diet. On the other hand, the pancreatic M-cell mass in the STZ-induced diabetic rats given diets high in sunflower and olive oil remained smaller than in the C. colocynthis group. Citrullus colocynthis oil supplementation may benefit the STZ-induced diabetic rat model by partly preserving or regaining pancreatic M-cell mass (17). Researchers looked at how a hydro-ethanolic pulp extract of C. colocynthis affected the hyperlipidemia that diabetic rats developed after being exposed to alloxan. When compared to diabetic untreated rats, the results showed a substantial reduction in the levels of phospholipids, free fatty acids, triglycerides, and total cholesterol in the blood and liver of the C. colocynthis-treated rats (18). These investigations all supported the traditional usage of C. colocynthis in the treatment of diabetes and showed it to be a safe antidiabetic.

It is important to recognize the limitations of this study, though. Because the study occurred in vitro, the findings might not accurately represent the intricate interactions that take place in the human body. In order to validate the results and evaluate the safety and effectiveness of these plant extracts in humans, additional in vivo investigations and clinical trials are required. Future efforts to produce new drugs will also depend on locating and defining the precise bioactive substances that are responsible for the observed activity.

CONCLUSION

The research shows that *C. colocynthis* ethanolic extract showed strong alpha-glucosidase inhibitory action, establishing it as a possible natural anti-diabetic drug. The extract is more active when concentrated, however it is slightly less potent than the regular acarbose. This encourages researchers to look into purified ingredients to increase the extract's effectiveness. Additionally, the Citrullus colocynthis cell viability experiment shows that the plant is safe at both low and high concentrations, proving that it is non-toxic. The probable mode of action is further supported by molecular docking studies, which demonstrate a high affinity for binding to the target enzyme. Collectively, the data point to *C. colocynthis* as a promising candidate for additional research and the creation of brand-new anti-diabetic medications. However, more investigation, such as in vivo investigations and clinical trials, is required to confirm their therapeutic potential and security in the treatment of diabetes.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ETHICAL CLEARANCE NUMBER

Not applicable

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