

Anti-diabetic Activity of Ethanolic Stem Extract of *Hylocereus undatus*: Phytochemical Screening and In-vitro Evaluation

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Abstract

Background

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels resulting from impaired insulin secretion, insulin action, or both. The increasing prevalence of diabetes worldwide has become a major public health concern. Although several synthetic antidiabetic drugs are available, their long-term use is often associated with adverse effects and high treatment costs. Therefore, there is a growing interest in exploring medicinal plants as safer and cost-effective alternatives.

Objective

The present study was designed to evaluate the antidiabetic activity of the ethanolic stem extract of *Hylocereus undatus* and to identify the phytochemical constituents responsible for its biological activity.

Materials and Methods

Fresh stems of *Hylocereus undatus* were collected from Sangli district, Maharashtra, India. The plant material was washed, shade-dried, powdered, and extracted using ethanol by Soxhlet extraction. Preliminary phytochemical screening was carried out using standard qualitative methods. The in-vitro antidiabetic activity was assessed by the α -amylase inhibition assay at different concentrations of the extract. Acarbose was used as the standard drug.

Results

The phytochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, and saponins in the ethanolic stem extract. The extract exhibited concentration-dependent inhibition of α -amylase activity, demonstrating its ability to reduce carbohydrate breakdown and glucose release. Although the inhibitory activity was lower than that of acarbose, the extract showed significant antidiabetic potential.

Conclusion

The findings of the present investigation indicate that the stem extract of *Hylocereus undatus* possesses promising antidiabetic activity due to the presence of various bioactive phytoconstituents. The study supports the traditional use of the plant in diabetes management and suggests that further in-vivo and clinical studies are required to establish its efficacy and mechanism of action.

Keywords

Hylocereus undatus; Diabetes mellitus; α -Amylase inhibition; Ethanolic extract; Phytochemical screening; Herbal antidiabetic agents

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Introduction

1.1 Diabetes Mellitus

Diabetes is a group of metabolic disorders caused by defects in insulin secretion, insulin action, or both. Hyperglycemia causes long-term damage, dysfunction, and failure of various organs. Diabetes arises from various pathogenic processes, including the autoimmune destruction of pancreatic β -cells or

insulin deficiency. Diabetes is a condition characterized by blood sugar levels that exceed the normal range. Symptoms of diabetes include polyuria, polydipsia, weight loss, and blurred vision. [1]

1.2 Types of Diabetes Mellitus

If any characteristic can define the new intentions for DM classification, it is the intention to consolidate

etiological views concerning DM. The old and confusing terms of insulin-dependent (IDDM) or non-insulin-dependent (NIDDM) which were proposed by WHO in 1980 and 1985 have disappeared and the terms of new classification system identifies four types of diabetes mellitus: type 1, type 2, other specific types and gestational diabetes. [10]

Type 1 Diabetes Mellitus

It is not very common and makes up only 5 to 10% of all diabetes cases. The rate at which the insulin-making cells in the pancreas get damaged can vary. It can happen quickly in babies and young children, but more slowly in adults. Some people with Type I diabetes may first show symptoms like severe acidosis in the blood. In some cases, the exact cause of the disease is unknown. [1]

Type 2 Diabetes Mellitus

The development of diabetes is based on a complex set of biochemical reactions. For type 2 diabetes, there is resistance to insulin in the body. As a result, less glucose enters muscle tissue, while glucose production by the liver increases. Over time, β -cells of the pancreas lose their ability to produce sufficient amounts of insulin. Chronic hyperglycemia leads to the formation of advanced glycation end products, oxidative stress, and inflammation, creating favorable conditions for the development of such complications as diabetic neuropathy, nephropathy, retinopathy, and cardiovascular diseases. The management of diabetes usually consists of both changing one's lifestyle and medication therapy. The latter may include the use of insulin injections and oral anti-diabetic drugs. [2]

Gestational diabetes mellitus

It is an operational classification (rather than a pathophysiologic condition) identifying women who develop diabetes mellitus during gestation. Women who develop Type 1 diabetes mellitus during pregnancy and women with undiagnosed asymptomatic Type 2 diabetes mellitus that is discovered during pregnancy are classified with Gestational Diabetes Mellitus (GDM). In most women who develop GDM; the disorder has its onset in the third trimester of pregnancy. [10]

Other Specific Types

Types of diabetes mellitus of various known etiologies are grouped together to form the classification called "Other Specific Types". This group includes persons with genetic defects of beta-cell function (this type of diabetes was formerly called MODY or maturity-onset diabetes in youth) or with defects of insulin action; persons with diseases of the exocrine pancreas, such as pancreatitis or cystic fibrosis; persons with dysfunction associated

with other endocrinopathies (e.g. acromegaly); and persons with pancreatic dysfunction caused by drugs, chemicals or infections and they comprise less than 10% of DM cases. [10]

1.3 Epidemiology of Diabetes mellitus

The application of epidemiology to the study of DM has provided valuable information on several aspects of this disease such as its natural history, prevalence, incidence, morbidity and mortality in diverse populations around the world. Identification of the cause of the disease and the possible preventive measures that could be instituted to arrest or delay the onset of this disease which has reached epidemic proportions in both the developed and the developing nations. [10]

The discovery of sugar in the urine and its detection using laboratory Tests made it possible for this information to be transferred to the 18th century. The estimated number of diabetics in India stood at 22 millions in 1990, 28 millions in 1995 and 33 millions in 2000. Diabetes is one of the most common metabolic disorders found all over the world. NIDDM is the most common form of diabetes affecting almost 90 percent of diabetics worldwide. [2]

1.4 The Indian Scenario

A national survey of diabetes conducted in six major cities in India in the year 2000 has shown that the prevalence of diabetes in urban Indian adults was 12.1%. The onset of diabetes among Indians is about a decade earlier than their western counterparts and this has been noted in Asian Indians in several studies. In the national survey 54.1% of diabetes developed it in the most productive years of their lives i.e. before the age of 50 years and they also had a higher risk of developing chronic complications of diabetes. The prevalence of Type 2 diabetes is 4-6 times higher in the urban areas as compared to rural areas. The prevalence of impaired glucose tolerance (IGT) in the rural population is also high at 7-8%, which indicates presence of a genetic basis for Type 2 diabetes in ethnic Indian population. [19]

1.5 Symptoms

- Frequent urination
- Excessive thirst
- Extreme hunger
- Weightloss
- Fatigue
- Blurred vision
- Slow wound healing

1.6 Pathophysiology of Diabetes Mellitus

- Diabetes mellitus develops when the body cannot properly regulate blood glucose

levels due to problems with insulin. This may happen either because the pancreas does not produce enough insulin or because the body's cells do not respond effectively to it (insulin resistance). As a result, glucose builds up in the bloodstream instead of being used by the cells for energy.

- In type 1 diabetes, the immune system mistakenly destroys insulin-producing beta cells in the pancreas, leading to little or no insulin production. In type 2 diabetes, the body becomes resistant to insulin, and over time the pancreas cannot produce enough insulin to overcome this resistance. Persistent high blood sugar affects multiple organs and systems. It alters fat and protein metabolism, increases fatty acids in the blood, and reduces glucose uptake in tissues like muscles. Over time, these changes can damage blood vessels and organs, leading to complications such as heart disease, kidney damage, and nerve problems. Overall, diabetes is a complex disorder involving both impaired insulin function and disrupted glucose balance in the body. [10]

1.7 Diagnostic Criteria

For the diagnosis of diabetes, at least one criterion must apply-

- Symptoms of diabetes (polyurea, polydipsia, unexplained weight loss, etc) as well as casual plasma glucose concentration = 11.1 mmol/L (200 mg/dL).
- Fasting plasma glucose = Its normal range is 70-110 mg/dl with no caloric intake for at least 8 h.

The World Health Organization (WHO) classification includes both clinical stages (normoglycaemia, impaired glucose tolerance/impaired fasting glucose (IGT/IFG), diabetes) and etiological types of diabetes mellitus, identical to the ADA except that WHO group includes classification formerly known as gestational impaired glucose tolerance (GIGT) and GDM: fasting glucose = 7.0 mmol/L (126 mg/dL) and/or 2-h glucose = 7.8 mmol/L (140 mg/dL) after a 75-g OGTT 4.[12]

2.1 Hylocereus undatus

Medicinal plants have many interesting species, including dragon fruit, known scientifically as *Hylocereus undatus*, which has garnered considerable interest due to its nutritious and curative properties. *Hylocereus undatus* belongs to the Cactaceae family and can be found in tropical and subtropical zones

across the globe. This plant has thick green stems, large white flowers, and brightly colored fruit. These fruits are a rich source of vitamins, minerals, fibers, and antioxidants that confer their benefits. [4]

2.2 Geographical source

Dragon fruit, which was originally from Central America, is extensively grown in various countries, including Vietnam, Thailand, Malaysia, and India. It grows in India in various states, such as Maharashtra, Gujarat, Karnataka and Andhra Pradesh among others. [4]

2.3 Botanical characteristics

1. **H. polyrhizus** : Flowers are 25–30 cm long. Perianth is red, especially at the tips. Stigma is short, lobed, and yellow in color. Fruit is 10–12 cm long and 130–350 g in weight. It is oblong and covered with scales with different size.
2. **H. venezuelensis** : It is closely related to *H. polyrhizus*, but it has bifid stigma lobes.
3. **H. undatus (Haw.)** : Stems are long and green. Flowers are very long (up to 29 cm). Perianth has outer green (or yellow-green) and inner white segments. Fruit is rosyr-red with 15–22 cm length and 300–800 g weight.
4. **H. megalanthus** : Stems are green, robust, three-ribbed, 1.5 cm thick, with slightly undulating margins, white areoles bearing 1–3 yellowish spines, 2–3 mm long. Flowers are nocturnal, large, white, and funnel-shaped, 32–38 cm long.
5. **H. ocamponis** : It is closely related to *H. purpusii*. They can be distinguished only by the acicular and slender spines of *H. ocamponis*. [6]

2.4 Biological Activities

- **Anti-oxidative**
Dragon fruit contains antioxidants like betalains, flavonoids, and vitamin C, which help protect cells from oxidative damage and improve overall health. Neutralize harmful free radicals due to its rich antioxidant content.
- **Anti- cancer**
Dragon fruit shows potential anticancer activity due to the presence of polyphenols and carotenoids, which help protect cells from damage caused by free radicals.

Regular consumption may support prevention of certain cancers by improving immunity and reducing inflammation.

- **Anti- microbial**

Dragon fruit possesses antimicrobial activity due to the presence of flavonoids, phenolic compounds, and alkaloids. These bioactive compounds may help inhibit the growth of harmful bacteria and fungi, supporting overall health and immunity.

- **Anti- hyper-lipidemic**

Dragon fruit exhibits antihyperlipidemic activity by helping reduce cholesterol and triglyceride levels in the body. Its high fiber, antioxidant, and polyunsaturated fatty acid content may improve lipid metabolism and support heart health.

- **Anti- anemia**

Dragon fruit may help in anemia management due to its iron and vitamin C content, which support hemoglobin formation and iron absorption. Regular consumption can help improve red blood cell production and reduce symptoms of iron deficiency anemia. [6]

2.5 Constituents

Plants used for medicinal purposes are of great importance in traditional management of diabetes. These plants contain many types of phytochemicals such as flavonoids, alkaloids, tannins, and phenolic substances. These phytochemicals act by different means on diabetes such as increased secretion of insulin, increased utilization of insulin, inhibitory action on digestive enzymes, and anti-oxidant effect [3]

- **Bioactive Compounds**

Hylocereus genus comprises a wide range of bioactive compounds that include betalains, flavonoids, phenolic acids, terpenes, sterols, and fatty acids. GC-MS analysis revealed four significant constituents in the *H. polyrhizus* stem MeOH extract, such as terpinolene (3.69%), eucalyptol (6.54%), b-selinene (7.25%), and 5-cedranone (73.05%) which collectively represents 91.15% of the total oil composition.[6]

- **Volatile oils**

121 volatile components were identified in *H. megalanthus* which comprised alcohol, terpene, paraffin, acid, ester, ketone, and other miscellaneous substances. These volatiles were identified by solvent

extraction technique followed by additional concentration.[6]

Though many studies have focused on the fruit and the peels of this cactus, little research has been conducted on its stem which contains phytochemicals that can be pharmacologically useful. Typically considered to be agricultural waste, its application might prove beneficial from both an economic and medical perspective. [7]

Extraction and phytochemical analysis of plants are very crucial processes that can help one identify active constituents of plants. The chemical composition of the plant is identified together with its therapeutic activity through these processes. Anti-diabetic activity assessment can either be carried out in vitro and in vivo. [3]

Materials and Methods

3.1 Collection and Authentication of Plant Material

The stems of *Hylocereus undatus* were collected from Renavi village near Vita, Sangli district, Maharashtra, India. The plant material was authenticated based on its morphological characteristics and transported to the laboratory for further processing.



Fig. 1 Cultivation of Dragon Fruit

3.2 Preparation of Plant Material

The collected stems were washed thoroughly to remove dirt and impurities and then shade-dried at room temperature for several days. The dried material was pulverized into a coarse powder and stored in airtight containers.



Fig. 2 Collection and Drying

3.3 Extraction Procedure

The powdered stem material was extracted using ethanol in a Soxhlet apparatus for approximately 48 hours. The solvent was evaporated to obtain a concentrated extract, which was preserved for further analysis.



Fig. 3 Soxhlet Extraction

3.4 Methodology

In this process, the thimble is packed with the solid sample, typically enclosed in a cotton or filter paper casing. A round-bottom flask containing the extraction solvent is then connected below the Soxhlet extractor, while a condenser is fixed at the top.

Upon heating, the solvent reaches its boiling point and vaporizes. The vapors ascend through the distillation arm into the condenser, where they cool and revert to liquid form. This condensed solvent then drips into the extraction chamber containing the sample.

As the solvent accumulates, it dissolves the desired compounds from the solid material. Once the liquid reaches a certain level, it is automatically siphoned back into the flask. This cyclic process continues repeatedly over several hours or even days, allowing for efficient and thorough extraction of the target constituents.

During the Soxhlet extraction process, the desired compound accumulates in the distillation flask after approximately 7 cycles or around 6 hours of

operation. Once extraction is complete, the solvent is removed to obtain the extract, typically by allowing it to evaporate under direct sunlight. The insoluble residue remains in the thimble and is generally discarded.

3.5 Evaporation of Extract

After completing the Soxhlet extraction, the ethanolic extract was carefully transferred into a porcelain dish and kept under direct sunlight for a duration of two days to facilitate solvent evaporation.

3.6 Preliminary Phytochemical Screening

Qualitative phytochemical tests were performed to identify the presence of:

Alkaloids

Flavonoids

Saponins

Tannins

Standard procedures including Dragendorff's, Mayer's, Hager's, Shinoda, Foam, and Gelatin tests were employed.

Table no. 1 Phytochemical Screening

Test	Observation	Inference
To the dry extract (20mg) distilled water (1-2ml) was added, shaken well and filtered.		
With the filtrate the following tests were performed.		
A) Tests for Alkaloids		
Dragendorff's test		
3 ml of test solution was mixed with Dragendorff's reagent (potassium bismuth iodide) was added.	Appearance of reddish brown precipitate.	Indicates the presence of alkaloids
Mayer's test		
To the 3 ml of test solution 3- 4 drops of Mayer's reagent was added.	Creamy white precipitate.	Indicates the presence of alkaloids
Hager's test		
To the 3 ml of test solution 3- 4 drops of Hager's reagent was added.	Yellow precipitate.	Indicates the presence of alkaloids
B)Test for Flavonoids		

Shinoda test		
In extract add 5ml of ethanol+ few drops of HCL+ Magnesium turnings	Red colour	Indicates the presence of flavanoids
Sulphuric acid test		
Extract + 66% to 88% sulphuric acid	Deep yellow colour.	Indicates the presence of flavanoids
Lead acetate test		
In drug extract add lead acetate solution.	Yellow precipitate.	Indicates the presence of flavanoids
Sodium hydroxide test		
In extract add solution of NaoH then add acid.	First gives yellow colour after addition of acid it decolorized.	Indicates the presence of flavanoids
C)Test for Saponin Glycosides		
Foam test		
In drug extract add 5-10 ml water and shake for few minutes.	Formation of foam.	Indicates the presence of Saponin
D)Test for Tannins		
Gelatin test		
In 1% gelatin solution containing 10% NaCl add aqueous extract of drug.	White precipitate.	Indicates the presence of Tannins.



Fig. 4 Phytochemical Tests

3.7 Evaluation of Antidiabetic Activity

The in-vitro antidiabetic activity was assessed using the α -amylase inhibition assay.

Different concentrations of the extract were mixed with α -amylase enzyme solution and incubated. Starch solution was then added as the substrate. The reaction was terminated using DNSA reagent and absorbance was measured at 540 nm using a spectrophotometer.

Percentage inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{[(\text{OD Control} - \text{OD Sample}) / \text{OD Control}] \times 100}{1}$$

Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean values. Statistical analysis was performed using appropriate methods.

Table no. 2 Alpha Amylase Inhibition Tests

S r. n o.	Sampl e code	Con c.	OD				me an	Percen t inhibit ion
1	Contr ol	-	0.8 9	0.8 8	0.8 5	0.8 7		
2	Stand ard Acarb ose	200	0.2 9	0.3 1	0.3 2	0.3 1		64.89
		400	0.2 8	0.2 7	0.2 6	0.2 7		69.08
		600	0.2 1	0.2 5	0.2 2	0.2 3		74.05
		800	0.1 5	0.1 6	0.1 7	0.1 6		81.68
		100 0	0.0 4	0.0 5	0.0 4	0.0 4		95.04
3	Sampl e A	200	0.5 6	0.5 6	0.5 6	0.5 6		35.88
		400	0.5 8	0.5 4	0.5 4	0.5 6		35.50
		600	0.5 2	0.5 3	0.5 3	0.5 1		41.22
		800	0.4 4	0.4 5	0.4 5	0.4 4		49.62
		100 0	0.3 8	0.3 5	0.3 5	0.3 5		59.92

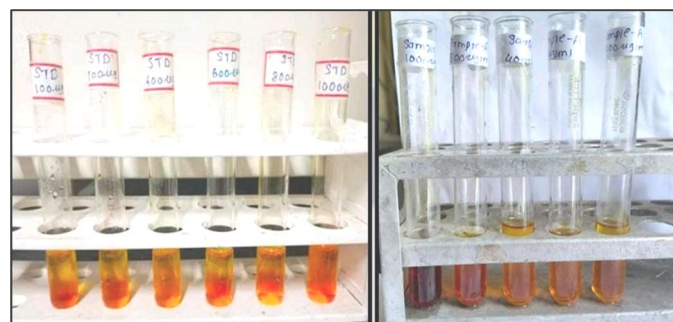


Fig.5 Alpha Amylase Inhibition Tests

Results

Phytochemical Screening

The ethanolic extract of *Hylocereus undatus* stem showed positive results for:

Alkaloids

Flavonoids

Tannins

Saponins

The presence of these compounds suggests that the extract contains biologically active constituents that may contribute to its pharmacological effects.

In-vitro Antidiabetic Activity

The extract demonstrated concentration-dependent inhibition of α -amylase enzyme activity. Increased concentrations of the extract produced higher percentages of inhibition, indicating its ability to delay carbohydrate digestion and reduce glucose absorption.

The standard drug acarbose exhibited greater inhibition than the plant extract. However, the extract showed significant enzyme inhibitory activity and may serve as a potential natural antidiabetic agent.

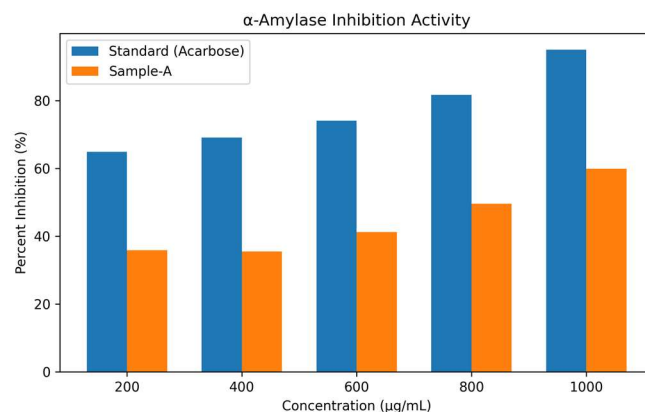


Fig.6 α Amylase Inhibition Activity

Discussion

The present study demonstrated that the ethanolic stem extract of *Hylocereus undatus* possesses considerable antidiabetic activity. The observed activity may be attributed to the presence of flavonoids, alkaloids, tannins, and saponins.

Flavonoids are known to possess antioxidant properties and improve insulin sensitivity. Tannins have been reported to inhibit carbohydrate-digesting enzymes and reduce postprandial hyperglycemia. Saponins contribute to glucose homeostasis by enhancing insulin secretion and improving glucose uptake.

The α -amylase inhibitory activity observed in this study suggests that the plant extract may help control blood glucose levels by slowing the digestion of starch and delaying glucose absorption.

The findings are in agreement with previous studies that have reported antidiabetic properties of different parts of dragon fruit. However, further in-vivo studies and clinical investigations are necessary to isolate the active compounds and establish their mechanisms of action.

Conclusion

The present study confirms that the ethanolic stem extract of *Hylocereus undatus* contains important phytoconstituents and exhibits significant in-vitro antidiabetic activity through α -amylase inhibition. The results support the traditional use of the plant in diabetes management and suggest that the stem of *Hylocereus undatus* could serve as a promising source for the development of novel herbal antidiabetic agents.

Ethical Statement

This study involved only in-vitro experimental procedures and did not involve human participants or laboratory animals. Therefore, approval from an Institutional Ethics Committee was not required.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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