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Research Article

Antidiabetic activity of aqueous root extract of *Chlorophytum borivilianum* on streptozotocin induced diabetic rats

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Abstract

To identify and characterize the synthetic components from the roots that are responsible for the activity, as well as to assess the plant *Chlorophytum borivilianum's* pharmacological effectiveness in diabetes both in vitro and in vivo. The goal of the current study was to examine the ethnomedical application of aqueous extract from the roots of *Chlorophytum borivilianum* as a possible anti-diabetic drug in rats with STZ-induced diabetes. The biological activities of the extract were examined both in vitro and in vivo. Water was utilized as a solvent for cold maceration, while TLC and column chromatography were employed for fractionation. The structures of the isolated compounds were validated by mass spectroscopy and liquid chromatography. The aqueous extract of *Chlorophytum borivilianum Sant. & Fern. (Liliaceae)* roots revealed the presence of phytoconstituents with therapeutic effects, including dihydrocapsaicin, deserpidine, reserpine, biliverdin-IX-α, and vitamin C. After giving a dose orally in comparison to the oral hypoglycaemic medication metformin, dihydrocapsaicin was detected, and the *Chlorophytum borivilianum* root aqueous extract fraction significantly decreased elevated blood glucose levels. Every outcome is dose-dependent. The presence of the anti-diabetic chemical dihydrocapsaicin was demonstrated from the aqueous extract. The parameters associated with diabetes can be inhibited by the aqueous extract of *Chlorophytum borivilianum* root.

Keywords: *Alpha-amylase, Alpha-glucosidase, Diabetes, Chlorophytum borivilianum*

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Introduction

Diabetes mellitus is a metabolic disease with several etiologies that is typified by persistently high blood sugar levels and abnormalities in the metabolism of proteins, lipids, and carbohydrates. It is caused by abnormalities in the secretion of insulin. (1)

A chronic metabolic disease called diabetes mellitus (DM) is typified by hyperglycemia. (2). Human health is negatively impacted by elevated blood sugar levels. Diabetes affects the metabolism, which is how our body uses the food we eat to make energy and grow. Numerous health advocacy organizations, including the World Health Organization (WHO), have acknowledged diabetes mellitus as a chronic global epidemic. According to WHO estimates, during the next 25 years, diabetes will rank among the world's top causes of mortality and disability. (3). *Chlorophytum borivilianum Sant. & Fern. (Liliaceae)*, widely recognized as Safed Musli, belongs to a genus comprising approximately 200 to 220 species of perennial flowering plants within the Asparagaceae family. This genus is indigenous to the humid and subtropical regions of Asia and Africa. (4). With long, lanceolate leaves that are 20–70 cm long and 0.5-2 cm wide, they emerge from a thick, meaty root and reach a height of 30 cm. The tiny white blossoms measure 120 centimeters in length. Plantlets with roots spreading out in the ground are occasionally found in the panicle species. Numerous chemical components, including vitamins, proteins (5–10%), alkaloids (30%), steroids, saponins (10–20%), potassium, calcium, magnesium, polysaccharide (40–45%), phenols, and mucilage, are present in *Chlorophytum borivilianum*.(5). The chemical components exhibit antihypertensive, aphrodisiac, immune system modulator, anthelmintic, antioxidant, antibiotic, antimicrobial, antiviral, antifungal, and stomach ulcer prevention properties..(6). Infectious diseases include gastrointestinal, respiratory, and urinary tract infections are briefly explained in this article. (7)

To evaluate how *Chlorophytum borivilianum Sant. & Fern. (Liliaceae)* root extract affects diabetic rats' fasting blood glucose levels. The purpose of this study is to assess the antidiabetic effects of *Chlorophytum borivilianum Sant. & Fern. (Liliaceae)* aqueous root extract in rats with diabetes induced by streptozotocin (STZ).

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Materials and methods

Collection and Authentication of Plant

Chlorophytum borivilianum roots have been collected at Mahatma Phule Krishi Vidyapeeth Rahuri in Ahmednagar. Verified by Dr. A.S. Wable, a botanist in the Department of Botany, Maharashtra. PVP College Pravaranagar, Loni Ahmednagar's Department of Botany received a specimen sample (Ref.no./PVPC/Bot/2023-24/91).

Chemicals and reagents

The study was conducted using methanol and chloroform. Additionally, throughout the entire research project, reagents such as Mayer's, Fehling's, Benedict's, Dragendorff's, and ruthenium red were used.

Extraction

By cold macerating 100 g of shade-dried root powder in 500 ml of distilled water for five days, the aqueous extract was created. After the extract was filtered, concentrated, and vacuum-dried (resulting in 3.4 g), the residue was kept in a refrigerator between 2 and 8 0C for use in the experiment.

Physico-chemical parameters and preliminary phytochemical screening

The standard approach was used to evaluate the various physicochemical properties of root powder, including extractive values, total ash, acid insoluble ash, water soluble ash, and moisture content. Several qualitative tests were performed on the aqueous root extract of *Chlorophytum borivilianum* to ascertain the presence of distinct phytoconstituents. (Table 1, 2)(8)

Column Chromatography

Adsorbent (activated silica for the column) was mixed with solvent (mobile phase) to create a slurry, which was then poured into a glass tube filled with solvent. Before the slurry was poured into the tube, the cotton provided a level base for the adsorbent column. After allowing the adsorbent to settle, the sample was loaded. The extract and silica were combined until the mixture flowed freely to create the sample.
To prevent sample disruption when a new mobile phase was added to the column, cotton was positioned above the loaded sample. The solvent level was never permitted to drop below the sample level. After that, a gradient was used to elute the column with a mobile phase. The colour of the column determined the fraction that was collected. They gathered three fractions.
60–120 mesh adsorbent silica gel activated for 1 hour at 110°C. The column's length is 40 cm. A column's diameter is one centimeter. The elution rate is 20 drops/min. Mobile Phase: Methanol: Chloroform (50:30) (9)(Fig.1)

Liquid Chromatography- Mass Spectroscopy

Three fractions were gathered. Thin layer chromatography (TLC) was performed on fractions A, B, and C using the mobile phase chloroform: methanol (9.5: 0.5) and the stationary phase silica gel G. Fraction B demonstrated that a better spot was seen in TLC and the spot was seen when exposed to UV light. Following TLC, fraction B was set aside for LC-MS analysis via natural evaporation. IIT Bombay receives the observed spot of fraction B for LCMS analysis. (Table 3).

In-vitro Antidiabetic activity

Alpha amylase inhibition

Assay of Amylase Inhibition

The inhibition of amylase was investigated using the Bernfeld method. Briefly, 500 µL of 0.1M phosphate buffer pH 6.9 containing the α-amylase enzyme (fungal diastase (0.5%)) was allowed to react with 20, 40, 80, and 100 µg/ml of Chlorophytum borivilianum (Safed Musli). 500 µL of 1% starch solution in 0.1M phosphate buffer pH6.8 was added following a 10-minute incubation period at 25°C.
Once more, incubated for 10 minutes at 250°C. The controls underwent the identical procedure, substituting buffer for 500 µL of the enzyme. Following incubation, both the control and test groups received 1000 µL of the dinitro salicylic acid reagent. After ten minutes in a boiling water bath, they were cooled (10).

The absorbance was recorded at 540 nm using a spectrophotometer and the percentage inhibition of α-amylase enzyme was calculated using the formula Inhibition (%) = Absorbance 540 (control) sample – Absorbance 540 (extract) * 100 Absorbance 540 (control) sample Simultaneously, appropriate blank reagent and inhibitor controls were carried out (11)(Table4)

Alpha-glucosidase inhibition assay

A previously reported approach was modified to perform the inhibitory activity of α-Glycosidase. Glutathione (50 µL), α-glucosidase solution (50 µL) in phosphate buffer (pH = 6.8), and pNPG (4-Nitrophenyl β-D-glucopyranoside) (50 µL) were combined with sample solution of Chlorophytum borivilianum (Safed Musli) (20, 40, 80, and 100 µg/ml) in a 96-well microplate. The mixture was then incubated for 15 minutes at 37°C. On the other hand, a blank was made using the sample and reaction ingredients but without an enzyme (α-glucosidase) solution. The addition of sodium carbonate (50 µL, 0.2 M) then stops the process. The absorbance at 400 nm was then determined (12). (Table 5).

% inhibition = control OD - Test OD / Control OD x100 (12)

In-vivo Antidiabetic activity

Induction of experimental diabetes: Following a 12- to 14-hour fast, male Wistar rats received a single intraperitoneal injection of a freshly made streptozotocin (STZ) solution (55 mg/kg body weight). The rats got diabetes as a result of this. A glucometer was then used to measure weight and fasting blood glucose levels. Each animal's weight was taken into consideration when preparing alloxan. Thirty minutes after receiving streptozotocin, they were fed and given a drink (Srivastava et al., 2020). Blood was taken from the tail of each animal four to five days following the streptozotocin injection in order to measure the plasma blood glucose level of each animal.
Normal fasting blood sugar levels are 99 mg/dL or less, pre-diabetic levels are 100–125 mg/dL, and diabetes is defined as 126 mg/dL or above. (Table 6)

Experimental design for antidiabetic activity

The rats were divided into five groups comprising 6 animals in each group as follows:
Group I: Normal control rats given only buffer.
Group II: Diabetic controls (STZ, 50 mg/kg body weight).
Group III: Diabetic rats treated with glibenclamide (3mg/kg b.w.)
Group IV and V: Diabetic rats treated with *Chlorophytum borivilianum* (250 mg/kg and 500 mg/kg b.w. respectively)

The blood glucose levels of experimental animals were determined at 0, 2, 4 and 6 h after feeding the plant extract by using glu-oxidase peroxidase reactive strips and glucometer (one touch basic plus). Statistical analysis Values are expressed as mean±S.E.M. (n=6). Statistical significance was determined by one way analysis of variance (ANOVA) followed by Dunnet's t test (9).

Results

Physicochemical parameters

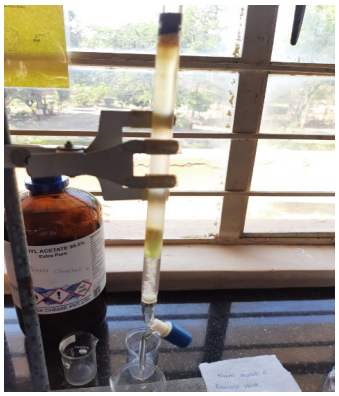
Table 1: Physicochemical Parameters

Sr. No.	Parameter	Value (w/W%)
1	Loss on drying	12
2	Total Ash	0.87
3	Acid insoluble ash	0.15
4	Water Soluble Ash	0.15

Table 2: Preliminary phytochemical screening

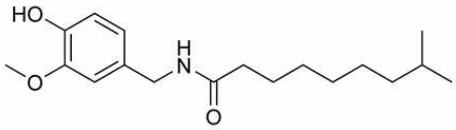
Phytoconstituents	Test	Methanolic Extract
Carbohydrates	Benedicts test	+
Steroids	Liebermann's test	+
Saponins	Foam test	+
Tannins and phenols	Lead acetate test Dilute HNO3 solution	- -
Alkaloids	Dragendorff's test Mayer's test	+ +
Mucilage	Powder + ruthenium red	+

Fig 1: Column Chromatography



LC-MS Analysis

Table 3: LC-MS Analysis

Sr. No.	Compound Label	Name	Mass
1	Cpd 34: Dihydrocapsaicin	Dihydrocapsaicin	308.2194
			

Pharmacological activity

In Vitro Activity

Observation of alpha amylase inhibition

Table 4: Observation of alpha amylase inhibition

Sr. No.	Sample	Concentration	Absorbance at 540 nm	% inhibition
1	Control	-	1.36	-
2	Acarbose	20 40 80 100	0.80 0.73 0.60 0.29	34.60 40.01 49.15 74.90
3	Chlorophytum borivilianum fraction	20 40 80 100	0.73 0.60 0.50 0.46	40.02 49.08 57.01 60.20

Observation of alpha glucosidase inhibition

Table 5: Observation of alpha glucosidase inhibition

Sr. No.	Sample	Concentration	Absorbance at 540 nm	% inhibition
1	Control	-	1.36	-
2	Acarbose	20 40 80 100	0.30 0.23 0.14 0.10	45.60 62.01 75.15 80.70
3	Chlorophytum borivilianum fraction	20 40 80 100	0.43 0.34 0.29 0.25	20.02 39.08 57.10 61.20

In Vivo Antidiabetic activity

Table 6: Anti-diabetic Activity results

Sr. No.	Group	Treatment	Blood Glucose level in mg/dl after			
			0h	2h	4h	6h
1	I	Normal control	70.73±2.46	70.34±2.82	73.21±2.15	71.86±2.42
2	II	Diabetic control	287.53±8.74	283.11±7.62	281.25±7.53	284.75±8.07
3	III	Diabetic control +Standard	290.65±7.42	239.47±6.15*	202.74±6.86*	187.55±5.36*
4	IV	Diabetic control +Extract 250 mg/kg	289.56±7.35	264.57±7.81	250.82±6.42**	211.75±6.15*
5	V	Diabetic control +Extract 500 mg/kg	281.64±8.05	245.61±6.87*	219.24±5.32*	208.82±5.88*

Values are expressed as Mean ± SEM (n=6). P values: **P<0.05 (Highly Significant)
Values are expressed in mean ± SEM (=6). Significant values are * P<0.001 compared to normal control (Group 1) and P<0.05, P<0.01, P<0.001 compared to diabetic control (Group II). NS: Not significant, EM: Standard error of mean.

Discussion

When given aqueous extract of *Chlorophytum borivilianum* roots (250 and 500 mg/kg b.w.) orally to STZ-induced diabetic rats, blood glucose levels decreased dose-dependently, especially 6 hours after treatment (n=6, p<0.01) (-63.77 to -74.80 g/dl, respectively), in comparison to the diabetic control group (Table 1). Six hours after treatment, the high blood glucose level decreased from 285.56 to 221.79 and from 281.62 to 206.82 g/dl, respectively, when administered with an aqueous extract at doses of 250 mg/kg b.w. and 500 mg/kg b.w. Additionally, glibenclamide (3 mg/kg b.w.) significantly decreased blood glucose levels as compared to the control group (289.95 to 187.53 g/dl, P<0.01).

Blood glucose levels rise as a result of insulin insufficiency and diabetes mellitus brought on by STZ. When diabetic rats were given root extract from *Chlorophytum borivilianum Sant. & Fern. (Liliaceae)*, hypoglycemia was noted two hours later, with the greatest impact occurring six hours later.

Based on the findings, it is hypothesized that the root extract may be in charge of the observed restoration of blood glucose levels and activation of insulin release. Moreover, elevated peripheral glucose utilization may potentially be the cause of the extract's observed reduced blood glucose-lowering effect in STZ-induced diabetic rats. ß-cell activation and insulinogenic effects have been documented when medicinal plant extract is used to treat diabetic rats induced by STZ (10).

Glibenclamide, a common hypoglycemic medication, and the aqueous extract of *Chlorophytum borivilianum* roots have similar antihyperglycemic effects.

In streptozotocin-induced diabetic rats, the current study showed that the aqueous root extract of Chlorophytum borivilianum improved the lipid profile, increased antioxidant status, and drastically decreased fasting blood glucose levels. The extract may have both insulinotropic and antioxidant properties, according to histopathological study, which also showed partial regeneration and protection of pancreatic β-cells.

These results are consistent with other studies on C. borivilianum and other potentially antidiabetic medicinal herbs. *Chlorophytum borivilianum Sant. & Fern. (Liliaceae)* has antioxidant and immunomodulatory qualities that could tangentially promote its antidiabetic function. Our findings support these findings and suggest that *Chlorophytum borivilianum's* phytochemicals, especially steroidal saponins, which are known to increase insulin production and improve glucose utilization, may be responsible for its hypoglycemic action.(13)

Our results, however, indicate that the saponin-rich fraction of C. borivilianum may be mostly in charge of the observed benefits, even though some earlier research (14, 15) highlighted the involvement of polyphenols and alkaloids in other plants as important Antidiabetic agents. The significance of precisely determining the bioactive components using sophisticated analytical methods like HPLC, LC-MS, and NMR is highlighted by this variance.

Conclusion

The current study found that the root extract of *Chlorophytum borivilianum* had antidiabetic effects on rats with STZ-induced hyperglycemia.

Alkaloids and saponins were detected in the aqueous root of *Chlorophytum borivilianum* in an initial test. It is highly likely that the comprehensive information provided in this review regarding the various pharmacological and therapeutic actions of the constituents may offer comprehensive proof for the use of various medications in vitro antidiabetic activity in this plant, according to the confirmed pharmacological study. It was determined that the aqueous extract produced a favourable outcome.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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