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ASSESSMENT OF GLUCOSE UPTAKE POTENTIAL OF *TRIBULUS TERRESTRIS* AND *GYMNEMA SYLVESTRE* IN YEAST CELL MODEL

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ABSTRACT

Natural plant extracts are extensively investigated for their antihyperglycemic potential. In this study, the glucose-uptake activity of *Tribulus terrestris* and *Gymnema sylvestre* extracts was assessed using a yeast cell glucose-uptake model, with metformin serving as the standard. Yeast cells were incubated with sample concentrations of 100, 200, 300, and 400 µg/mL, and absorbance was measured at 540 nm. The percentage of glucose uptake (expressed as the percentage reduction of residual glucose relative to the control) was calculated accordingly. *Gymnema sylvestre* consistently exhibited higher glucose-uptake activity than *Tribulus terrestris* and showed a response pattern comparable to metformin. At 400 µg/mL, the percentage uptake values were 80.533% for *Gymnema sylvestre*, 77.057% for *Tribulus terrestris*, and 74.855% for metformin, indicating dose-dependent enhancement in both extracts. Overall, both plant extracts demonstrated significant glucose-uptake potential in the yeast model, with *Gymnema sylvestre* showing the most pronounced activity. Further mechanistic studies and *in-vitro* evaluation using mammalian cell lines are recommended to validate and extend these findings.

KEYWORDS: *Tribulus terrestris*, *Gymnema sylvestre*, glucose uptake, yeast model, metformin, antihyperglycemic.

INTRODUCTION

Diabetes mellitus, especially type 2 diabetes mellitus (T2DM), constitutes one of the major global health challenges of the 21st century. Impaired glucose homeostasis, characterized by reduced insulin sensitivity and defective peripheral glucose uptake, leads to chronic hyperglycaemia and associated complications such as cardiovascular disease, nephropathy and neuropathy. In this scenario, promoting glucose uptake into peripheral tissues is a key therapeutic target. While conventional drugs (e.g., metformin, insulin sensitizers) remain central to diabetes management, there is growing interest in identifying affordable, safe phytomedicinal agents that can enhance glucose handling and mitigate the burden of disease in low- and middle-income countries.[1]

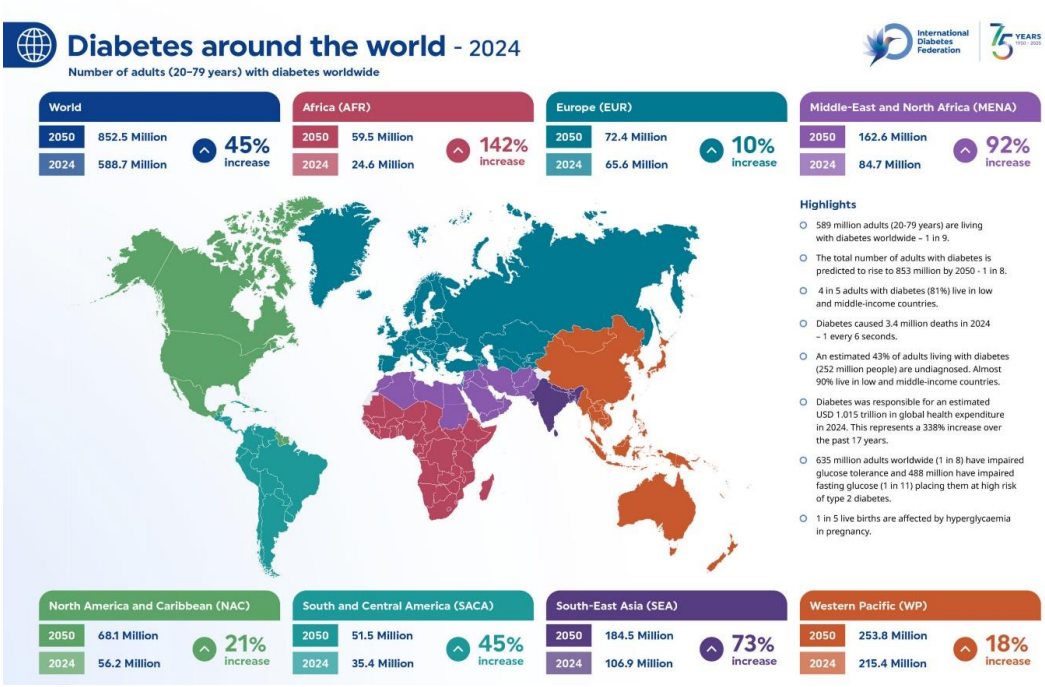

Figure 1: Global Estimates and Distribution of Diabetes.

Tribulus terrestris is a well-known medicinal herb traditionally valued for its cardiotonic, appetite-enhancing, antidiabetic, aphrodisiac, anti-urolithiasis, diuretic, emollient, anthelmintic, digestive, and antibacterial properties. It has also exhibited antihypertensive, antihyperlipidemic, and antioxidant effects. Across various traditional medical systems, this plant has been used to manage numerous health conditions. Extensive scientific investigations have explored its phytochemical composition and therapeutic activities, highlighting its diuretic, anti-urolithiasis, antihypertensive, analgesic, lipid-lowering, immunomodulatory, antidiabetic, anticancer, anthelmintic, aphrodisiac, antibacterial, hepatoprotective, and anti-inflammatory actions. Owing to its potassium-sparing, cardioprotective, and lipid-modulating effects, *Tribulus terrestris* is considered a promising herbal option for managing hypertension. Its applications are deeply rooted in traditional Chinese and Indian medicine, with numerous studies supporting its diverse pharmacological benefits.[2]

Gymnema sylvestre, a member of the Apocynaceae family, has been traditionally utilized for managing numerous health conditions. This wild herb grows widely across India, Africa, Australia, and China. Commonly known as "Gurmar" due to its notable sugar-suppressing activity, it has been a prominent component of Ayurvedic medicine for centuries. Recognized in the Indian Pharmacopoeia as an antidiabetic plant, *G. sylvestre* has historically been used to address ailments such as diabetes, malaria, and even snakebites. Owing to its therapeutic potential, various formulations including tea bags, health supplements, and tablets are commercially available. Its broad medicinal value extends to conditions like cardiovascular disorders, asthma, cancer, diabetes, and obesity, making it a widely utilized natural remedy.[3]

The medicinal plants *Gymnema sylvestre* (GS) and *Tribulus terrestris* (TT) have long been used in traditional systems—particularly Ayurvedic medicine—for the treatment of "madhumeha" (diabetes) and related metabolic disorders. GS, a climbing shrub native to the tropical forests of India and other regions, is commonly referred to as the "sugar destroyer" owing to its reputed ability to reduce sugar cravings and modulate glucose metabolism. Numerous in vitro, in vivo and clinical studies have investigated its glycaemic benefits. For example, a systematic review and meta-analysis of ten studies involving 419 participants found that GS supplementation significantly reduced fasting blood glucose, post-prandial blood glucose and HbA_1c in T2DM patients.[4] In another randomized controlled trial in patients with impaired glucose tolerance (IGT), GS administration (300 mg twice daily) decreased 2-h oral glucose tolerance test (OGTT) values and HbA_1c and improved the Matsuda index of insulin sensitivity.[5] At the mechanistic level, GS leaf extract has been shown in L6 myotubes and 3T3-L1 adipocytes to up-regulate GLUT-4 and PPAR-γ gene expression, increase adiponectin and leptin levels, and thereby enhance glucose uptake in a dose-dependent manner.[6] Together these data point to GS as a promising botanical for augmenting peripheral glucose disposal.

Conversely, TT is a prostrate herb from the Zygophyllaceae family, widely distributed as a weed but also valued as a medicinal plant across many cultures. Phytochemical investigations show that TT contains steroidal saponins (such as protodioscin), flavonoids, alkaloids and sterols.[7] Although TT is more commonly

studied for its effects on fertility and libido, increasing evidence supports its anti-hyperglycaemic and anti-dyslipidaemic potential. In animal models, alcohol extracts of TT lowered fasting glucose, HbA_1c, triglycerides and LDL-cholesterol in streptozotocin-induced diabetic rats.[8] In humans, a 3-month randomized trial in women with non-insulin-dependent diabetes mellitus who consumed 1000 mg/day TT hydro-alcoholic extract showed significant reductions in fasting glucose and LDL compared to placebo.[9] These findings highlight TT's utility as a complementary anti-diabetic phytomedicine.

While many studies focus on GS or TT in animal or human models, comparatively fewer investigations explore their direct effect on cellular glucose uptake, especially in simpler in vitro systems such as yeast cell models. The yeast (e.g., *Saccharomyces cerevisiae*) glucose-uptake assay provides a simple, cost-effective preliminary screening tool for plant extracts to evaluate their ability to promote glucose removal from the medium. For instance, in a study of *Annona reticulata* leaf extracts, a dose–dependent increase in % glucose uptake by yeast cells was observed, establishing the assay's feasibility in antidiabetic screening.[10] Using a yeast model allows rapid comparison of extracts, concentration–response profiling and serves as an entry point before more complex mammalian cell or *in vivo* studies.

The rationale for the present study is thus founded on three points:

a. GS and TT have documented antihyperglycaemic effects, but direct comparisons in a uniform assay (glucose uptake) are less common.
b. Using a yeast cell glucose uptake model allows efficient comparative screening of the two extracts and a standard (metformin) across defined concentrations.
c. Establishing dose–response curves in such a model enriches the phytopharmacological evidence and supports further mechanistic and mammalian cell investigations.

Therefore, the objective of this study was to evaluate and compare glucose-uptake activity of GS and TT extracts over a concentration range (100–400 µg/mL) in a yeast-cell assay, using metformin as a reference standard. We hypothesized that both extracts would enhance glucose uptake dose-dependently, and that there might be differences in potency or efficacy between them, reflecting their differing phytochemical profiles and mechanisms of action.

By demonstrating the glucose-uptake promoting potential of GS and TT in a simple yeast model, this work contributes to the pharmacological validation of these botanicals, provides comparative efficacy data, and supports the rationale for subsequent mechanistic studies (e.g., GLUT-4 translocation in mammalian cells) and possible development of standardized extracts for antidiabetic supportive therapy.

MATERIALS AND METHODS

Materials required

1. Soxhlet apparatus
2. Bakers yeast (saccharomyces cerevisiae)
3. Glucose
4. Metformin 500 mg
5. Distilled water
6. DNSA reagest (for glucose estimation)
7. Spectrophotometer or colorimeter (505-540 nm)
8. Incubator
9. Centrifuge (3000 rpm)
10. Organic solvents (Ethanol, Pet ether, Chloroform)

METHODS

1. **Collection and Authentication of Plant Materials:** The fresh aerial part of *Tribulus terrestris* plant was collected from Kadur Chikmagalur district local region during January and *Gymnema sylvestre* aeriel part were collected from Nagpur Maharastra. The plant materials ware identified and authenticated by a taxonomist Dr. Haleshi C, Department of Botany, Davanagere University.

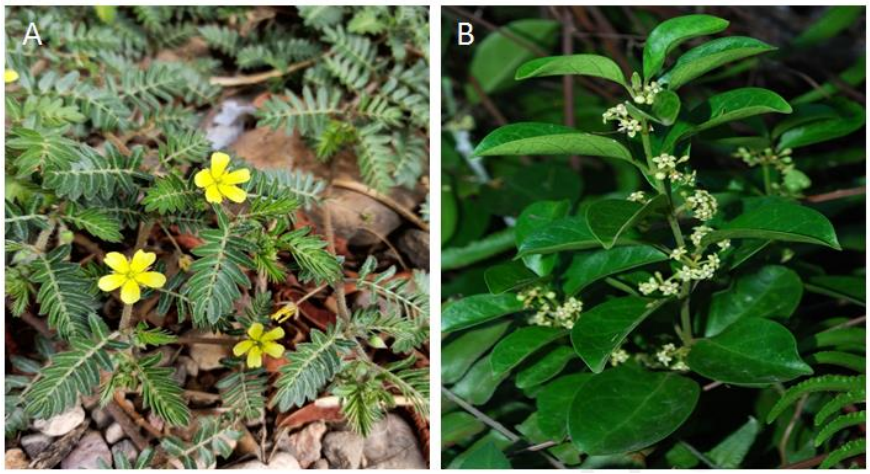

Figure 2: (A) *Tribulus terrestris* plant (B) *Gymnema sylvestre* plant.

2. **Preparation of Plant extract**: The collected plant materials were washed, shade-dried for 10–15 days, and powdered using a mechanical grinder. The dried and coarse powdered plant material of *Tribulus terrestris* and gymnema sylvestre were subjected to successive Soxhlet extraction using organic solvents of increasing polarity, namely petroleum ether, chloroform, and ethanol.

Initially, 250 g of the coarse powdered material of *Tribulus terrestris* was extracted with petroleum ether (60–80 °C) until the solvent in the siphon tube became colorless, indicating completion of extraction(25 cycles). The marc was dried and subsequently extracted with chloroform under the same conditions. Finally, the marc was extracted with ethanol (95%) at a controlled temperature of 45-50^{0}C. The extracts were evaporated to get concentrate and preserved in a desiccator.

The aerial part of *Gymnema sylvestre* dried powdered material (250 gm) was subjected to Successive Soxhlet extraction with Petroleum ether and Ethanol (95%). The temperature (45-50^{0}C) was maintained on an electrical heating mantel with thermostat control. Appearance of colourless solvent in the siphon tube was taken as the end point of extraction (approximately 20-30 cycles for each solvent). The extract was then concentrated to 3/4th of its original volume by distillation. The extracts were evaporated to get concentrate and preserved in a desiccator.

3. Reagent preparation[11]

A. Glucose solution (2% w/v)

Weigh 2 gm of glucose, dissolve it in 100 ml of distilled water and mix thoroughly.

B. Yeast cell suspension.

Weigh 1 g of dry baker's yeast and suspend it in 10 ml of distilled water. Incubate at 37 °C for 1 hour to activate, then centrifuge at 3000 rpm for 5 minutes. Wash the pellets twice with distilled water and resuspend them in 10 ml of distilled water.

C. Plant extract solution

Dissolve the dried *Tribulus terrestris* extract in distilled water or 1% DMSO to prepare a stock solution of 1 mg/ml, and filter or centrifuge if necessary to remove any particulate matter.

a) To prepare 10 ml of 1 mg/ml stock.

Weigh 10 mg of extract Dissolve in 10 ml of DW or 1% DMSO Stir or vortex until fully dissolved.

Clarify the solution

Option 1: Centrifuge at 3000 rpm for 10 mins and collect supernatant.

Option 2: Filter using whatman filter paper or 0.22 µm syringe filter.

4. Experimental setup

Prepare the following groups in labelled test tubes (do it in triplicates)

The experimental setup included four groups: the **blank** containing 1 ml of glucose only; the **control** with 1 ml of glucose and 1 ml of yeast suspension; the **test** group with 1 ml each of glucose, extract, and yeast suspension; and the **positive control** containing 1 ml of glucose, 1 ml of metformin, and 1 ml of yeast suspension.

5. Procedure

1) Mix all components gently by tapping or vortexing.
2) Incubate all tubes at 37°C for 1 hour.
3) After incubation, centrifuge at 3000 rpm for 5 mins.
- This seperates yeast cells which have absorbed glucose.
4) Carefully collect the supernatant from each tube.
5) Take 1 ml of supernatant from each tube and perform the glucose estimation using DNSA method.

DNSA Assay steps

1) Add 1 ml DNSA reagent to 1 ml of supernatant.
2) Heat in boiling water for 5 mins.
3) Cool under tap water.
4) Add 8 ml distilled H_2O to dilute.
5) Measure absorbance at 540 nm using a spectrophotometer or colorimeter.

6. Data analysis

% glucose uptake is calculated as,

$$\textbf{\% Uptake} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

7. Precautions

Use freshly prepared yeast suspension and glucose solution

Handle DNSA with gloves.

❖ Reagent preparation

A. Metformin 500 mg (standard)

The tablets were crushed into a fine powder and the excipients were removed. The resulting powder was then dissolved in distilled water (DW) to a final volume of 50 ml. This preparation yielded a stock solution with a concentration of 10 mg/ml.

Serial dilution

Dilution formula

$$C_1V_1 = C_2V_2$$

Where,

C_1 = stock concentration (10000 µg/ml)

V_1 = volume of stock needed

C_2 = desired concentration

V_2 = final volume to prepare

Working solutions of 100, 200, 300, and 400 µg/ml were prepared from the stock solution. For this, 0.1, 0.2, 0.3,

and 0.4 ml of the stock solution were measured, respectively, and each was diluted with 9.9, 9.8, 9.7, and 9.6 ml of distilled water to obtain a final volume of 10 ml.

B. Plant extract preparation

A stock solution was prepared by dissolving 10 mg of the sample in 10 ml of distilled water, resulting in a final concentration of 10 mg/ml.

Serial dilution preparation

Working standard solutions of 100, 200, 300, and 400 µg/ml were prepared from the 10 mg/ml stock solution. For each concentration, an appropriate volume of the stock solution was transferred to separate volumetric flasks: 0.1 ml, 0.2 ml, 0.3 ml, and 0.4 ml, respectively. Distilled water was then added to each flask to make up a final volume of 10 ml, requiring 9.9 ml, 9.8 ml, 9.7 ml, and 9.6 ml of distilled water accordingly.

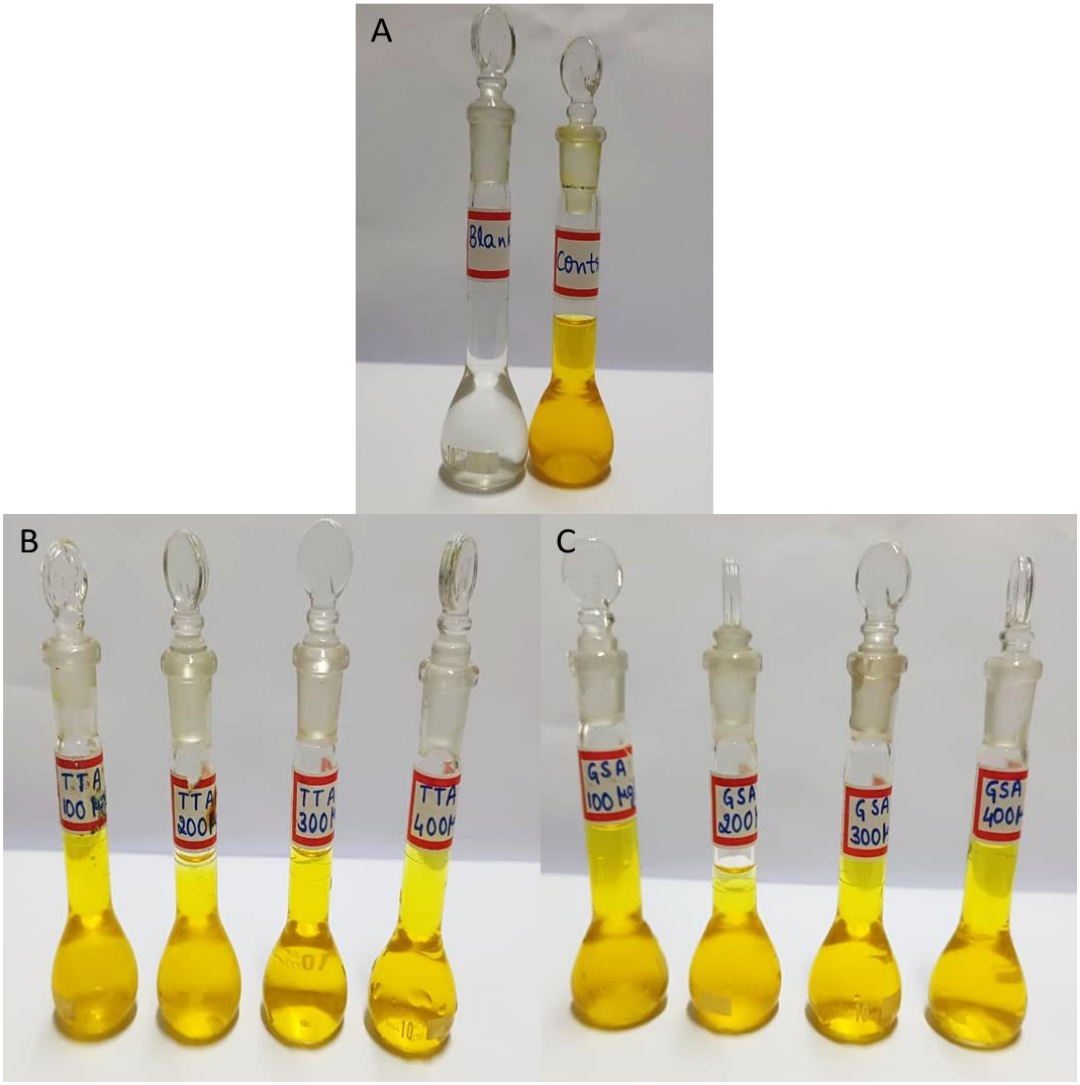

Figure 3: (A) Blank and control tubes. (B) Different concentrations of *Tribulus terrestris* extract (TTA 100–400 µg/mL). (C) Different concentrations of Gymnema sylvestre extract (GSA 100–400 µg/mL).

RESULTS

Effect of *Tribulus terrestris* and *Gymnema sylvestre* Extracts on Glucose Uptake by Yeast Cells.

The glucose uptake activity of the ethanolic extracts of *Tribulus terrestris* and *Gymnema sylvestre* was evaluated using a yeast cell model.

1. Control = **0.863**
2. Standard (Metformin)

The sample showed a concentration-dependent increase in percentage uptake. At 100 µg/ml, the absorbance was 0.352 corresponding to 59.212% uptake. Increasing the concentration to 200 µg/ml resulted in an absorbance of 0.319 and 63.035% uptake. At 300 µg/ml, the absorbance decreased to 0.266 with a corresponding uptake of 69.177%. The highest concentration, 400 µg/ml, exhibited the lowest absorbance value of 0.217 and the highest uptake of 74.855%, indicating an inverse relationship between absorbance and percentage uptake.

3. Test Sample 1 (*Tribulus terrestris*)

A concentration-dependent increase in percentage uptake was observed. At 100 µg/ml, the absorbance was 0.311

with a corresponding uptake of 63.962%. Increasing the concentration to 200 µg/ml resulted in an absorbance of 0.284 and 67.091% uptake. At 300 µg/ml, the absorbance further decreased to 0.229, while the uptake increased to 73.464%. The highest concentration, 400 µg/ml, showed the lowest absorbance value of 0.198 and the highest uptake of 77.056%, indicating an inverse relationship between absorbance and % uptake.

4. Test sample 2 (*Gymnema sylvestre*)

A progressive increase in percentage uptake was observed with increasing concentrations. At 100 µg/ml, the absorbance was 0.259 corresponding to 69.988% uptake. At 200 µg/ml, the absorbance decreased to 0.208 with an uptake of 75.898%. Further reduction in absorbance was seen at 300 µg/ml (0.174), accompanied by an uptake of 79.837%. The highest concentration, 400 µg/ml, showed the lowest absorbance value of 0.168 and the maximum uptake of 80.533%, demonstrating a clear inverse relationship between absorbance and percentage uptake.

DISCUSSION

In the present study, the glucose uptake activity of ethanolic extracts of *Tribulus terrestris* and *Gymnema sylvestre* was evaluated using the yeast cell model. The results demonstrated that both extracts enhanced glucose uptake in a concentration-dependent manner. At 400 µg/mL, *Gymnema sylvestre* showed the highest glucose uptake (60.85%), followed by *Tribulus terrestris* (57.12%), while the standard drug metformin produced the highest effect (63.48%).

These findings indicate that both plant extracts have the potential to increase glucose uptake, suggesting the presence of bioactive constituents capable of improving cellular glucose utilization. The yeast cell model is a simple and reliable system for studying glucose transport, as glucose enters yeast cells through facilitated diffusion via carrier proteins. An increase in glucose uptake in this model reflects an improvement in glucose transport efficiency, similar to that observed in mammalian cells.

The gradual increase in glucose uptake with rising extract concentrations suggests a dose-dependent effect, implying that higher concentrations of phytochemicals enhance activity. The observed effects may be attributed to the presence of flavonoids, phenolics, and saponins in both extracts, which are known for their roles in modulating carbohydrate metabolism and promoting glucose absorption.

Comparison with Previous Findings

The results obtained in this study are consistent with earlier research showing that *Gymnema sylvestre* and *Tribulus terrestris* possess antidiabetic properties. *Gymnema sylvestre* is rich in gymnemic acids, known for their ability to mimic insulin action and promote glucose uptake in peripheral tissues.[12,13] Studies have demonstrated that extracts of *G. sylvestre* stimulate glucose uptake in skeletal muscle and adipose cells by enhancing glucose transporter expression and activity.[14]

Similarly, *Tribulus terrestris* contains steroidal saponins such as protodioscin, along with flavonoids and alkaloids, which have been reported to modulate glucose metabolism and improve insulin sensitivity.[15-17] Deepak *et al.* (2005) showed that protodioscin enhances glucose uptake in adipocytes and potentiates insulin secretion, supporting the results obtained in the current yeast model.[18]

Although yeast cells do not have insulin receptors, the enhanced glucose uptake observed suggests that the plant extracts may act through mechanisms involving improved membrane transport or increased permeability to glucose molecules.

The observed glucose uptake activity may be attributed to the synergistic effect of several phytochemicals present in the extracts. Flavonoids and phenolic compounds are known to exert insulin-like effects by stimulating glucose utilization and enhancing carbohydrate metabolism.[19] Saponins, abundant in both *G. sylvestre* and *T. terrestris*, may facilitate glucose diffusion through the plasma membrane due to their surfactant properties and interaction with cell membrane lipids.

In *Gymnema sylvestre*, gymnemic acids have also been reported to suppress sweet taste sensation and delay glucose absorption in the intestine, thereby improving glycemic control.[20] On the other hand, *Tribulus terrestris* has shown inhibitory effects on α-glucosidase and α-amylase enzymes, contributing to reduced postprandial glucose levels.[21] These combined mechanisms may explain the enhanced glucose uptake observed in the yeast model.

Overall, the results clearly show that *Tribulus terrestris* and *Gymnema sylvestre* possess glucose uptake–enhancing properties, supporting their traditional use in managing elevated blood glucose levels.

CONCLUSION

The findings of this study clearly demonstrate that ethanolic extracts of *Tribulus terrestris* and *Gymnema sylvestre* exhibit notable glucose uptake activity when evaluated using the yeast cell model. Both extracts enhanced glucose uptake in a concentration-dependent manner, confirming their potential to promote glucose transport across cell membranes. Among the two, *Gymnema sylvestre* showed slightly higher glucose uptake (60.85%) than *Tribulus terrestris* (57.12%), while metformin exhibited the highest activity (63.48%).

These results indicate that both plants possess bioactive constituents capable of improving glucose utilization. The observed activity may be attributed to the presence

of flavonoids, saponins, phenolics, and glycosides, which are known to play a vital role in regulating carbohydrate metabolism and enhancing insulin sensitivity. The positive response obtained through the yeast cell model supports the traditional use of these plants in the management of diabetes and related metabolic disorders.

The study provides preliminary evidence that *Tribulus terrestris* and *Gymnema sylvestre* extracts have promising glucose uptake–enhancing effects. These findings encourage further research involving advanced *in-vitro* and *in-vivo* models to identify and isolate the active compounds responsible for the activity, standardize extract concentrations, and determine their possible therapeutic applications. Collectively, the results contribute to the growing scientific support for the use of medicinal plants as natural alternatives in diabetes management and metabolic regulation.

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