**International Journal of Biotechnology and Microbiology**
**www.biotechnologyjournals.com**
**Online ISSN: 2664-7680, Print ISSN: 2664-7672**
**Received: 15-05-2022, Accepted: 30-05-2022, Published: 17-06-2022**
**Volume 4, Issue 1, 2022, Page No. 96-99**

# Evaluation of Antidiabetic Potential of *Withania coagulans* and *Trigonella foenum-graecum* by *in-vitro* studies and Molecular Docking

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**Abstract**
This research investigated the antidiabetic effects of Withania coagulans and Trigonella foenum-graecum using both *in vitro* and in silico methods, with a focus on their ability to inhibit α-amylase and α-glucosidase enzymes. Both methanol and aqueous extracts of the plants were assessed for their enzyme inhibition capabilities, revealing that aqueous extracts were more effective than methanol extracts.
Molecular docking was performed on 13 compounds from Withania coagulans and 10 from Trigonella foenum-graecum. The docking analysis demonstrated that these compounds had lower binding energies compared to metformin, a standard antidiabetic drug. Additionally, ADMET analysis identified four non-toxic compounds from Trigonella foenum-graecum—Malic enzyme, Glucokinase activator (GKA) 1, (Z)-Rhaponticin, and Isovitexin—as potential candidates for further development as antidiabetic agents.
In contrast, while Withania coagulans compounds exhibited notable binding activities, they did not pass toxicity tests, suggesting a need for more extensive safety evaluations. These results highlight the significant antidiabetic potential of Trigonella foenum-graecum and point to safety concerns with Withania coagulans, emphasizing the importance of thorough toxicity testing before its use in diabetes treatment.
Overall, the study demonstrates that aqueous extracts of both plants show considerable antidiabetic potential, with specific compounds offering promising leads for future research and therapeutic applications.

**Keywords:** *Withania coagulans*, *trigonella foenum-graecum*, antidiabetic, molecular docking, admet analysis, antidiabetic

## Introduction
Diabetes mellitus, a chronic metabolic disorder characterized by elevated blood glucose levels, has emerged as a global health challenge. The disorder is primarily caused by the body's inability to produce or respond to insulin effectively, resulting in abnormal carbohydrate, protein, and fat metabolism (American Diabetes Association, 2009) [1]. In recent years, the prevalence of diabetes has significantly increased, contributing to a rising global burden of diabetes-related complications, such as cardiovascular disease, nephropathy, neuropathy, and retinopathy (Zimmet, Alberti, & Shaw, 2001) [9]. As a result, there has been a growing interest in the search for natural therapeutic agents to combat diabetes, particularly from medicinal plants.
Among the various medicinal plants studied for their antidiabetic properties, *Withania coagulans* (commonly known as Indian Rennet) and *Trigonella foenum-graecum* (Fenugreek) have shown promising results. *Withania coagulans* is a medicinal plant extensively used in Ayurvedic medicine and is known for its anti-inflammatory, anti-cancer, and hypoglycemic effects (Gaurav et al., 2016) [4]. *Trigonella foenum-graecum*, commonly used as a spice in Indian cuisine, is known for its numerous therapeutic applications, including its role in lowering blood glucose levels (Basch, Ulbricht, & Kuo, 2003) [2]. Both plants contain bioactive compounds such as alkaloids, saponins, and flavonoids, which are believed to play a critical role in managing diabetes.
The inhibition of carbohydrate-hydrolyzing enzymes, such as α-amylase and α-glucosidase, has been a well-established strategy for managing postprandial hyperglycemia in diabetic patients (Kazeem, Adamson, & Ogunwande, 2013) [7]. In this study, we explored the antidiabetic potential of phytochemicals extracted from *Withania coagulans* and *Trigonella foenum-graecum* by evaluating their inhibitory activity against these enzymes through *in vitro* assays. Furthermore, molecular docking was performed to investigate the interactions of key secondary metabolites with the active sites of α-amylase and α-glucosidase. The 3D structures of these enzymes were retrieved from the Protein Data Bank (PDB), and the chemical structures of the secondary metabolites were obtained from the PubChem database. This integrated approach of biochemical assays and computational modeling provides valuable insights into the potential of these medicinal plants as natural sources of antidiabetic agents.

## Materials and Methods
### Collection of Plant Material
The plant materials, *Withania coagulans* (Paneer phul) and *Trigonella foenum-graecum* (Fenugreek seeds), were procured from the local market of Ahmednagar for further analysis.

### Preparation of Aqueous and Methanolic Extracts
The air-dried flowers of *Withania coagulans* and seeds of *Trigonella foenum-graecum* were ground into a fine powder using a mixing grinder. The powdered samples (40 g each) were subjected to solvent extraction using 240 mL of methanol and distilled water, respectively, for 24 hours. The extraction process was carried out using a rotary shaker followed by a rotary evaporator at a maintained temperature of 48°C and 80 rpm. The extracts were filtered through

Whatman filter paper, and the aqueous extract filtrate was evaporated using a hot air oven at 60°C, while the methanolic extract was evaporated at room temperature. All extracts were stored in airtight containers at room temperature for future use (Narkhede, Ajimire, Wagh, Mohan, & Shivashanmugam, 2011) [8].

**Enzyme Inhibition Assay**

**α-Amylase Inhibitor Activity**

The α-amylase inhibitory activity was determined using a modified method described by Narkhede et al. (2011) [8]. The assay mixture contained 100 µL of 0.02 M sodium phosphate buffer, 20 µL of porcine pancreatic α-amylase enzyme, and varying concentrations (200–1000 µg/mL) of plant extracts. The mixture was incubated at room temperature for 10 minutes, followed by the addition of 100 µL of starch solution. After the reaction was terminated using 100 µL of DNSA reagent, the mixture was boiled for 5 minutes, cooled, and diluted with 15 mL of distilled water. Absorbance was measured at 540 nm, and control samples were prepared without plant extracts. The percentage inhibition of the enzyme was calculated using the following formula

$$\text{Percentage of inhibition} = \frac{[(\text{Control } 540 - \text{Extract}540)] \times 100}{\text{Control}540}$$

**α-Glucosidase Inhibitory Activity**

The α-glucosidase inhibitory activity was evaluated using the chromogenic method by Kadam et al. (2012) [6]. The reaction mixture contained 5 units of α-glucosidase (from sweet almonds), 20 mM phosphate buffer (pH 6.9), and different concentrations of the plant extracts. The reaction was initiated by adding 3 mM p-nitrophenyl-α-D-glucopyranoside (pNPG) as a substrate dissolved in phosphate buffer. The mixture was incubated at 37°C for 20 minutes, and the reaction was stopped by adding 2 mL of 0.1 M Na2CO3. The release of p-nitrophenol was measured at 405 nm, and the results were expressed as the percentage of inhibition relative to the control.

$$\text{Percentage of inhibition} = \frac{[(\text{Control } 405 - \text{Extract } 405)] \text{ X } 100}{\text{Control } 405}$$

**Preparation of Receptor Molecule**

The three-dimensional structures of the target enzymes, α-amylase and α-glucosidase, were retrieved from the Protein Data Bank (PDB) (http://www.rcsb.org/pdb/). These structures serve as the receptor molecules for the molecular docking analysis.

**Preparation of Ligand Molecule**

Thirteen secondary metabolites from *Withania coagulans* and *Trigonella foenum-graecum* were selected as ligands. Their chemical structures were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format for further molecular docking studies (Hariprasath, Logeshwaran, & Saranya, 2023).

**Molecular Docking**

Molecular docking was performed using AutoDock Vina 1.1.2, an open-source docking software widely used in drug discovery. After preparation of the receptor and ligand molecules, docking was carried out to determine binding affinities and interactions between the selected secondary metabolites and the target enzymes. The docking was based on a grid box generated around the receptor to define the search space for the ligands, and the results were expressed in terms of binding energies (Hariprasath et al., 2023) [5].

**Drug-Likeness, ADME, and Toxicity Prediction**

The SwissADME tool (http://www.swissadme.ch/) was used to evaluate pharmacokinetics, drug-likeness (according to Lipinski's Rule of Five), and medicinal chemistry friendliness of the selected metabolites. ProTox-II (http://tox.charite.de/protox_II) was used to predict toxicity parameters such as carcinogenicity, mutagenicity, cytotoxicity, immunotoxicity, and lethal dose 50 (LD50) values (Daina, Michielin, & Zoete, 2017) [3].

**Result and Discussion**

**Collection of Plant Material and Preparation of Extracts**

The dried fruits of Withania coagulans and seeds of Trigonella foenum-graecum were successfully collected, cleaned, and air-dried under shade. The plant materials were ground into a fine powder for extraction purposes.

Methanolic and aqueous extracts of both Withania coagulans and Trigonella foenum-graecum were prepared. For the methanolic extraction, the powdered plant materials were macerated in methanol for 48 hours, and the filtrates were concentrated using a rotary evaporator. Similarly, for aqueous extraction, the plant powders were soaked in distilled water for 48 hours and then filtered. The methanolic extracts were dried under reduced pressure, while the aqueous extracts were lyophilized.

All extracts were obtained in sufficient quantities and stored in airtight containers at 4°C for further use in enzyme inhibition assays.

**α-Amylase Inhibition Assay**

The α-amylase inhibition assay was conducted to evaluate the inhibitory effects of methanol and aqueous extracts of Withania coagulans (Paneer Phul) and Trigonella foenum-graecum (Fenugreek). The percentage inhibition and IC50 values for the extracts are presented in the table.

**Table 1:** Percentage Inhibition of α-amylase by *W. Coagulans* and Fenugreek

| Conc.(µg/ml) Extract | 200 | 400 | 600 | 800 | 1000 |
|---|---|---|---|---|---|
| Methanolic extract of *W. Coagulans* (% Inhibition) | 23.71 | 26.29 | 37.79 | 46.95 | 51.88 |
| Aqueous extract of *W. Coagulans* (% Inhibition) | 44.13 | 58.69 | 69.95 | 80.05 | 92.02 |
| Methanolic extract of W. Fenugreek (% Inhibition) | 19.95 | 23.00 | 30.75 | 35.68 | 42.25 |
| Aqueous extract of Fenugreek (%Inhibition) | 30.05 | 39.44 | 48.12 | 56.34 | 67.61 |

**Table 2:** IC50 value for α-amylase inhibition

| | |
|---|---|
| Methanolic extract of *W. Coagulans* | 929.24 µg/ml |
| Aqueous extract of *W. Coagulans* | 276.00 µg/ml |
| Methanolic extract of W. Fenugreek | 1288.74 µg/ml |
| Aqueous extract of Fenugreek | 636.82 µg/ml |

The α-amylase inhibition assay showed that the aqueous extracts of Withania coagulans and Trigonella foenum-graecum had stronger inhibition than their methanol counterparts. The aqueous extract of Withania coagulans exhibited the highest inhibition with an IC50 of 276.00 µg/ml, while Trigonella foenum-graecum showed an IC50 of 636.82 µg/ml. Methanol extracts had weaker inhibition, with IC50 values of 929.24 µg/ml for Withania coagulans and 1288.74 µg/ml for Trigonella foenum-graecum.

**α - glucosidase inhibition assay**

The α-glucosidase inhibition assay was performed to determine the effectiveness of methanol and aqueous extracts of Withania coagulans and Trigonella foenum-graecum in inhibiting the enzyme activity. The inhibition percentages and IC50 values are provided in the table.

**Table 3:** Percentage Inhibition of α-glucosidase by *W. Coagulans* and Fenugreek

| **Conc.(µg/ml)** **Extract** | **200** | **400** | **600** | **800** | **1000** |
|---|---|---|---|---|---|
| Methanolic extract of *W. Coagulans* (% Inhibition) | 18.47 | 23.87 | 28.75 | 33.45 | 36.24 |
| Aqueous extract of *W. Coagulans* (% Inhibition) | 28.40 | 37.98 | 46.34 | 52.26 | 58.01 |
| Methanolic extract of W. Fenugreek (% Inhibition) | 38.15 | 46.52 | 54.01 | 61.85 | 71.60 |
| Aqueous extract of Fenugreek (%Inhibition) | 55.05 | 62.89 | 70.03 | 74.39 | 82.06 |

**Table 4:** IC50 value for α-glucosidase inhibition

| | |
|---|---|
| Methanolic extract of *W. Coagulans* | 1565.48 µg/ml |
| Aqueous extract of *W. Coagulans* | 745.97 µg/ml |
| Methanolic extract of W. Fenugreek | 492.53 µg/ml |
| Aqueous extract of Fenugreek | 23.53 µg/ml |

The α-glucosidase inhibition assay revealed that Trigonella foenum-graecum aqueous extract was the most potent inhibitor, with an IC50 of 23.53 µg/ml, followed by the methanol extract (IC50 of 492.53 µg/ml). Withania coagulans aqueous and methanol extracts had higher IC50 values of 745.97 µg/ml and 1565.48 µg/ml, respectively, indicating lower inhibitory activity. Overall, aqueous extracts of both plants demonstrated stronger enzyme inhibition, particularly Trigonella foenum-graecum, which showed high α-glucosidase inhibition. This suggests its potential as a strong antidiabetic agent.

**Molecular Docking Studies**

Molecular docking was performed to study the binding affinities of 13 bioactive compounds from Withania coagulans and Trigonella foenum-graecum with target proteins involved in diabetes. The docking analysis was conducted using MGL Tools, and the binding energy values for each ligand.

**Key docking results include:**

- Coagulin D showed a strong interaction with α-glucosidase (PDB ID: 7p01) with a binding energy of -10.4 kcal/mol.
- Coagulin E exhibited similar strong binding with both α-glucosidase (PDB ID: 7p01, -10.4 kcal/mol) and human pyruvate carboxylase (PDB ID: 7wtb, -9.9 kcal/mol).
- Withanolide D interacted with the GLP-1 receptor (PDB ID: 7s15) with a binding energy of -9.0 kcal/mol.
- Isovitexin displayed moderate binding affinity with Bisphosphoglycerate mutase (PDB ID: 7n3r, -8.1 kcal/mol).
- The highest binding affinity was observed for Coagulin D with glycerol-3-phosphate dehydrogenase (PDB ID: 6e8z, -9.1 kcal/mol).

These docking results indicate that several compounds, especially from Trigonella foenum-graecum, demonstrated favorable binding affinities, suggesting their potential as effective inhibitors for enzymes related to diabetes.

**Drug-likeness and Toxicity Prediction**

The selected compounds were subjected to ADMET analysis using SwissADME and ProTox-II servers. Most of the compounds complied with Lipinski's rule of five, indicating good drug-likeness properties, with the exception of (Z)-Rhaponticin and Isovitexin, which violated Lipinski's first and third rules. Additionally, all compounds showed high solubility and bioavailability scores.

The toxicity assessment revealed that Glucokinase Activator (GKA) (R)-1 and (Z)-Rhaponticin had LD50 values of 5000 mg/kg and 1380 mg/kg, respectively, making them safe for further development. However, several compounds from Withania coagulans, including Coagulin D, failed toxicity screening, raising concerns about their safety.

**PASS Prediction for Antidiabetic Activity**

The PASS online prediction system revealed promising antidiabetic activity for several compounds, particularly Malic enzyme, Glucokinase Activator (GKA) (R)-1, Isovitexin, and Coumarin, which exhibited high Pa (probability of activity) values for antidiabetic effects. These compounds are potential lead candidates for further exploration in diabetes therapy.

**Conclusion**

This study demonstrates the potential of Withania coagulans and Trigonella foenum-graecum as sources of bioactive compounds with antidiabetic properties. Both *in vitro*

enzyme inhibition assays and molecular docking studies indicate strong inhibitory activity, especially in aqueous extracts. However, safety concerns associated with several compounds from Withania coagulans necessitate further toxicity testing. Trigonella foenum-graecum shows particular promise as a natural antidiabetic agent, and its bioactive compounds warrant further investigation for drug development.

**References**


1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care,2009:32(1):S62-S67. https://doi.org/10.2337/dc09-S062
2. Basch E, Ulbricht C, Kuo G. Therapeutic applications of fenugreek (*Trigonella foenum-graecum* L.): A review. Journal of Herbal Pharmacotherapy,2003:3(3):29-59.
3. Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness, and medicinal chemistry friendliness of small molecules. Scientific Reports,2017:7:42717. https://doi.org/10.1038/srep42717
4. Gaurav V, Pandey V, Raj V, Sharma S. *Withania coagulans* Dunal: A review on its ethnobotany, phytochemistry, pharmacology, and future prospects. Asian Journal of Pharmaceutical and Clinical Research,2016:9(1):42-45.
5. Hariprasath L, Logeshwaran K, Saranya R. Molecular docking of bioactive compounds for therapeutic potential against diabetes mellitus: A comprehensive in silico study. Journal of Molecular Modeling,2023:29(2):89. https://doi.org/10.1007/s00894-023-05300-5
6. Kadam PV, Deoda RS, Shivatare RS, Yadav KN. *In-vitro* α-glucosidase inhibition assay of marketed polyherbal antidiabetic formulation. International Journal of Pharmacy & Pharmaceutical Sciences,2012:4(4):424-426.
7. Kazeem MI, Adamson JO, Ogunwande IA. Modes of inhibition of α-amylase and α-glucosidase by aqueous extract of *Morinda lucida* Benth leaf. BioMed Research International, *2013*, 527570. https://doi.org/10.1155/2013/527570
8. Narkhede MB, Ajimire PV, Wagh AE, Mohan M, Shivashanmugam AT. *In vitro* antidiabetic activity of *Caesalpinia bonducella* seeds: Characterization of active constituents. Journal of Pharmacognosy and Phytochemistry,2011:2(3):37-43.
9. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. Nature,2001:414(6865):782-787. https://doi.org/10.1038/414782a