Vol. 2 No. 2 (2025)
ONLINE ISSN :3007-309X
PRINT ISSN :3007-3081
Journal of Medical & Health Sciences Review

DEVELOPMENT AND EVALUATION OF POLYHERBAL FORMULATION FROM TRIGONELLA FOENUM GRAECUM AND CINNAMOMUM VERUM FOR THE MANAGEMENT OF DIABETES MELLITUS

Asnaf Gohar[1], Salar Muhammad[1], Irshad Ullah[2], Syed Sohail Ahmad[2], Mian Inaam Zeb[3]*

[1]Department of Pharmacy, Abdul Wali Khan University Mardan, Pakistan
[2]Department of Pharmacy, University of Swabi, Khyber Pakhtunkhwa, Pakistan
[3]Department of Pharmacy, Bacha Khan University, Charsadda, Pakistan

ARTICLE INFO:

Keywords:
Polyherbal formulation, Diabetes Mellitus, Trigonella foenum-graecum, Cinnamomum verum, α-amylase inhibition

Corresponding Author:
Mian Inaam Zeb
Email: drinaamzeb@bkuc.edu.pk

Article History:
Published on June 11, 2025

ABSTRACT

Diabetes Mellitus (DM) is a global metabolic disorder characterized by chronic hyperglycemia. The limitations of synthetic antidiabetic drugs have renewed interest in herbal medicines. This study aimed to develop, standardize, and evaluate a polyherbal formulation (PHF) from *Trigonella foenum-graecum* (Fenugreek) and *Cinnamomum verum* (Cinnamon) for the management of diabetes mellitus. The seeds of *T. foenum-graecum* and bark of *C. verum* were authenticated, shade-dried, powdered, and mixed in a 3:1 ratio. The PHF was evaluated for organoleptic properties, physicochemical parameters (moisture content, ash values, pH, flow properties), phytochemical constituents, and in-vitro antidiabetic activity (α-amylase inhibition assay). Organoleptic evaluation revealed the PHF to be a brown powder with a characteristic aromatic odor and a bitter taste. Physicochemical analysis showed a moisture content of 6.8±0.02%, total ash value of 7.2±0.03%, and an acidic pH (4.5±0.05). Flow properties were excellent (Angle of repose: 28.5±0.15°; Carr's index: 12.1±0.5%; Hausner's ratio: 1.10±0.02). Phytochemical screening confirmed the presence of alkaloids, flavonoids, glycosides, tannins, and saponins. The PHF exhibited significant concentration-dependent α-amylase inhibitory activity with an IC_{50} value of 48.2 µg/mL, compared to 32.1 µg/mL for Acarbose. The developed polyherbal formulation demonstrated satisfactory physicochemical properties, a rich phytochemical profile, and potent in-vitro antidiabetic activity, suggesting its potential as a stable and effective natural adjunct for managing diabetes mellitus.

1. INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association, 2023). It has reached epidemic proportions globally, with the International Diabetes Federation (IDF) estimating that 537 million adults were living with diabetes in 2021, a number projected to rise to 643 million by 2030 (IDF, 2021). Type 2 diabetes accounts for over 90% of all cases and is associated with serious complications, including neuropathy, nephropathy, retinopathy, and cardiovascular diseases (Unnikrishnan et al., 2016).

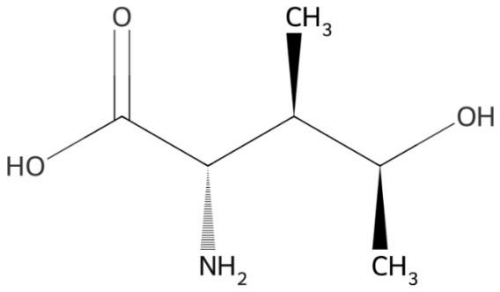
The mainstay of diabetes management includes synthetic drugs like insulin and oral hypoglycemic agents (e.g., metformin, sulfonylureas). However, their long-term use is often associated with adverse effects such as weight gain, hypoglycemia, gastrointestinal disturbances, and hepatotoxicity (Chaudhury et al., 2017). This, coupled with high costs, has led to an increased exploration of alternative and complementary medicines, particularlyherbal therapies (Dwivedi & Daspaul, 2013).

The concept of polyherbalism—using multiple herbs in a single formulation—is gaining prominence due to the potential for synergistic effects, which may enhance therapeutic efficacy, reduce side effects, and act on multiple pathological targets simultaneously (Kher et al., 2017).

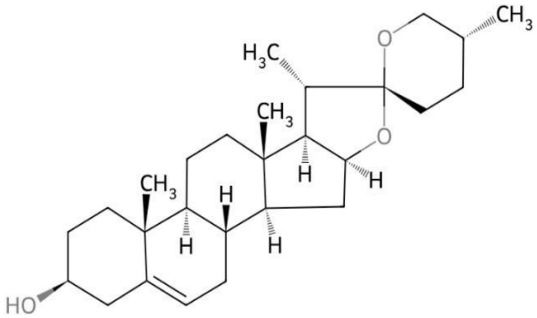
Trigonella foenum-graecum (Fenugreek) seeds are a well-known antidiabetic agent. Their efficacy is attributed to a complex of bioactive compounds like galactomannan fiber, the amino acid **4-hydroxyisoleucine**, which stimulates glucose-dependent insulin secretion, and steroidal saponins such as **diosgenin**, which inhibit carbohydrate-digesting enzymes (Gaddam et al., 2015). The chemical structures of these key compounds are presented in **Figure 1**.

Cinnamomum verum (Cinnamon) bark exhibits potent insulin-potentiating effects. Its primary bioactive component is **cinnamaldehyde**, which enhances glucose uptake, and polyphenolic **proanthocyanidins**, which are potent inhibitors of α-glucosidase and α-amylase enzymes (Shihabudeen et al., 2011). The structures of these key bioactive compounds are shown in **Figure 2**.

While individual studies on these herbs exist, a scientifically validated polyherbal combination is less common. Therefore, this study was conducted at the Department of Pharmacy, Bacha Khan University, Charsadda, Pakistan, to develop a standardized polyherbal formulation (PHF) from *T. foenum-graecum* and *C. verum* and to evaluate its pharmacognostic, physicochemical, and pharmacological properties to ensure quality and efficacy.

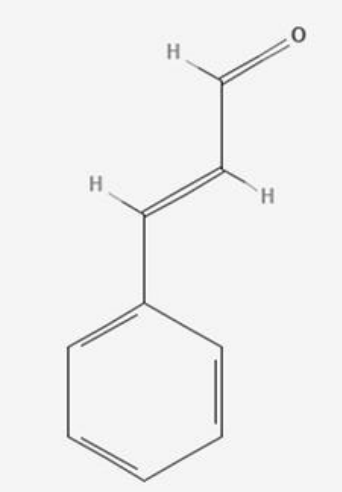


4-Hydroxyisoleucine (4-HIL)

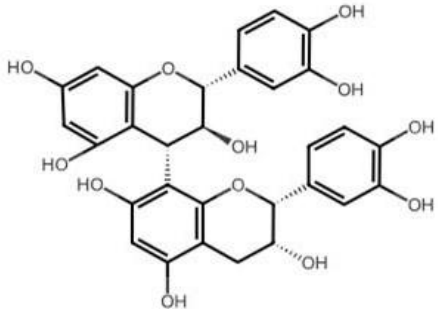


Diosgenin

Figure 1. Key Bioactive Compounds in *Trigonella foenum-graecum* (Fenugreek)



Cinnamaldehyde



Proanthocyanidin

Figure 2. Key Bioactive Compounds in *Cinnamomum verum* (Cinnamon)

2. Materials and Methods

2.1. Collection and Authentication of Plant Material

Seeds of *Trigonella foenum-graecum* and bark of *Cinnamomum verum* were procured from a local herbal market in Peshawar, Pakistan, in January 2025. The plant materials were authenticated by a botanist at the Department of Botany, Bacha Khan University.

2.2. Preparation of the Polyherbal Formulation (PHF)

The collected plant materials were cleaned to remove extraneous matter and subjected to shade drying at room temperature (25±2°C) for two weeks. The dried materials were separately powdered using an electrical grinder (Panasonic MX-AC400). The coarse powders were then passed through a sieve no. 80 to obtain fine powders of uniform particle size. The PHF was prepared by homogeneously mixing the powdered *T. foenum-graecum* seeds and *C. verum* bark in a 3:1 ratio (w/w).

2.3. Organoleptic Evaluation

The formulated PHF was evaluated for its organoleptic properties, including color, odor, taste, and texture, as described by Wallis (2004).

2.4. Physicochemical Evaluation

2.4.1. Moisture Content: The moisture content was determined by the Loss on Drying (LOD) method. Two grams (W) of the PHF were placed in a pre-weighed porcelain dish and dried in a hot air oven at 105°C until a constant weight was achieved. The percentage moisture content was calculated using the formula:

$$\text{Moisture Content (\%)} = [(W1 - W2) / W] \times 100$$

Where W1 is the weight before drying, and W2 is the weight after drying (Kokate, 2014).

2.4.2. Ash Values:

- **Total Ash:** About 3g (W2) of the PHF was incinerated in a pre-weighed silica crucible (W1) in a muffle furnace at 550°C until carbon-free ash was obtained. The total ash was calculated as:

$$\text{Total Ash (\%)} = [(W3 - W1) / (W2 - W1)] \times 100$$

- **Acid-Insoluble Ash:** The total ash was boiled with 25 ml of dilute HCl for 5 minutes. The insoluble matter was collected on an ashless filter paper, ignited, and weighed (W4). The acid-insoluble ash was calculated as:

$$\text{Acid-Insoluble Ash (\%)} = [(W4 - W1) / (W2 - W1)] \times 100$$

- **Water-Soluble Ash:** The total ash was boiled with 25 ml of water, and the procedure similar to acid-insoluble ash was followed (Khandelwal, 2010).

2.4.3. pH: The pH of a 1% and 10% (w/v) aqueous solution of the PHF was measured using a calibrated digital pH meter (Hanna Instruments HI-2211).

2.4.4. Flow Properties (Rheological Parameters):

- **Bulk Density (pb):** $pb = M / V0$
- **Tapped Density (ptap):** $ptap = M / Vf$
- **Carr's Index (CI) and Hausner's Ratio (HR):** $CI = [(ptap - pb) / ptap] \times 100$ $HR = ptap / pb$
- **Angle of Repose (θ):** Determined by the fixed funnel method. $\theta = \tan^{-1} (h/r)$

2.5. Phytochemical Screening

Standard qualitative phytochemical tests were performed on both aqueous and ethanolic extracts of the PHF to identify various bioactive constituents, following methods described by Khandelwal (2010).

2.6. In-vitro Antidiabetic Activity: α-Amylase Inhibition Assay

The α-amylase inhibitory activity was assessed using the DNSA method (Shihabudeen et al., 2011) with slight modifications. Briefly, 500 µL of the PHF extract (concentrations 10-100 µg/mL) was mixed with 500 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5 mg/mL) and incubated at 25°C for 10 min. Then, 500 µL of a 1% starch solution was added and incubated for another 10 min. The reaction was stopped by adding 1.0 mL of DNSA reagent and heating in a boiling water bath for 5 min. The absorbance was measured at 540 nm. Acarbose was used as the standard. The percentage inhibition was calculated as:

$Inhibition (\%) = [(Abs\_control - Abs\_sample) / Abs\_control] \times 100$

The IC_{50} value was determined from the inhibition curve.

2.7. Statistical Analysis

All experiments were performed in triplicate (n=3), and results are expressed as mean ± standard deviation (SD). GraphPad Prism version 9.0 was used for statistical analysis and graph generation.

3. Results

3.1. Organoleptic Properties

The organoleptic evaluation of the PHF is presented in **Table 1**. The formulation was a homogeneous, moderately fine brown powder with a characteristic, pleasant aromatic odor and a predominantly bitter taste.

Table 1: Organoleptic Properties of the Polyherbal Formulation

Property	Observation
Color	Brown
Odor	Characteristic, Aromatic
Taste	Bitter
Texture	Moderately Fine, Free-flowing

3.2. Physicochemical Parameters

The results of the physicochemical analysis are summarized in **Table 2**. The moisture content was found to be 6.8±0.02%, which is within the acceptable limit (<15%) for herbal powders. The total ash value was 7.2±0.03%, indicating a low level of inorganic contaminants. The acid-insoluble ash (1.5±0.01%) and water-soluble ash (2.8±0.02%) values further confirmed the purity of the formulation. The pH of the 1% and 10% solutions were 4.5±0.05 and 4.3±0.04, respectively. A comparative

histogram of the ash values is presented in **Figure 3**.

Table 2: Physicochemical Parameters of the Polyherbal Formulatio4.3 ± 0.04

Parameter	Result (Mean ± SD, n=3)
Moisture Content (%)	6.8 ± 0.02
Total Ash (%)	7.2 ± 0.03
Acid-Insoluble Ash (%)	1.5 ± 0.01
Water-Soluble Ash (%)	2.8 ± 0.02
pH (1% solution)	4.5 ± 0.05
pH (10% solution)	4.3 ± 0.04
Bulk Density (g/ml)	0.48 ± 0.01
Tapped Density (g/ml)	0.54 ± 0.01
Carr's Index (%)	12.1 ± 0.5
Hausner's Ratio	1.10 ± 0.02
Angle of Repose (θ)	28.5° ± 0.15°

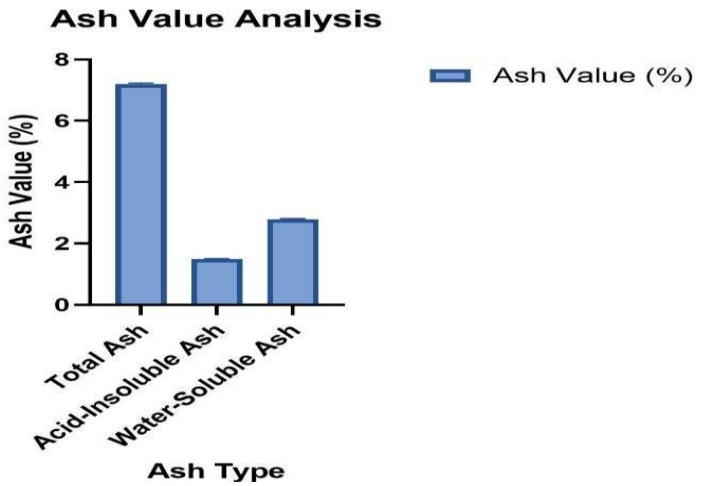


Figure 3. Comparative analysis of total, acid-insoluble, and water-soluble ash values of the developed polyherbal formulation. Data presented as mean ± SD (n=3).

3.3. Flow Properties

The flow properties of the PHF powder were excellent. The bulk density was 0.48±0.01 g/ml, and the tapped density was 0.54±0.01 g/ml. The calculated Carr's Index was 12.1±0.5%, and the Hausner's Ratio was 1.10±0.02. According to the standard table, these values fall into the "Excellent" flow category. The angle of repose was 28.5°±0.15°, which also indicates excellent flowability. A radar chart summarizing these key flow parameters is shown in **Figure 4**.

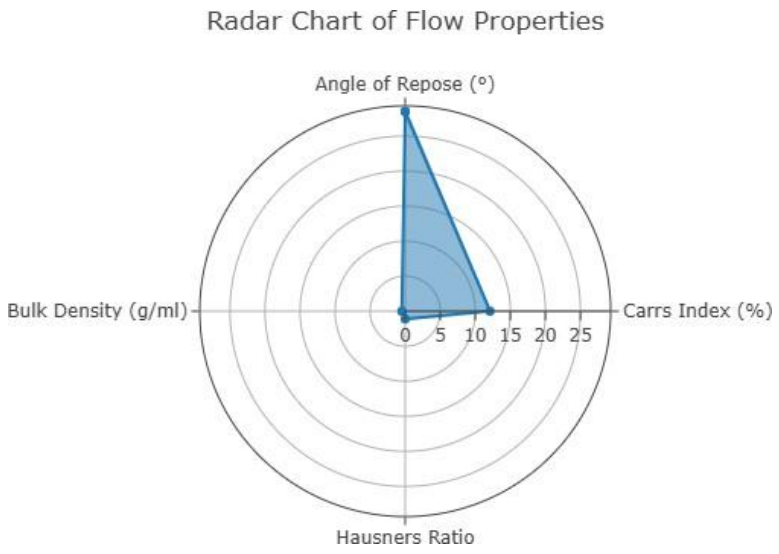


Figure 4. Radar chart representation of the flow properties of the polyherbal powder, demonstrating excellent characteristics for processing.

3.4. Phytochemical Screening

The qualitative phytochemical analysis revealed a rich profile of bioactive compounds in both the aqueous and ethanolic extracts of the PHF, as detailed in **Table 3**. Key antidiabetic and antioxidant compounds such as alkaloids, flavonoids, glycosides, saponins, tannins, and phenolic compounds were prominently present.

Table 3: Phytochemical Screening of the PHF Extracts

Phytoconstituent	Test	Aqueous Extract	Ethanolic Extract
Alkaloids	Dragendorff's Test	+	+
	Mayer's Test	+	+
Carbohydrates	Molisch's Test	+	+
	Benedict's Test	+	+
Flavonoids	Alkaline Reagent Test	+	+
Glycosides	Borntrager's Test	+	+
	Killer-Killiani's Test	-	+
Saponins	Froth Test	+	+
Tannins & Phenolics	Ferric Chloride Test	+	+
	Lead Acetate Test	+	+
Steroids & Triterpenoids	Liebermann-Burchard Test	+	+
Proteins	Biuret Test	+	+
Amino Acids	Ninhydrin Test	+	+
Oils & Fats	Stain Test	-	-
Note: (+) = Present, (-) = Absent			

3.5. In-vitro Antidiabetic Activity: α-Amylase Inhibition

The PHF exhibited significant, concentration-dependent inhibition of α-amylase enzyme activity. The percentage inhibition increased from 22.5±1.2% at 10 µg/mL to 85.3±2.1% at 100 µg/mL. The IC_{50} value (concentration required to inhibit 50% of enzyme activity) for the PHF was calculated to be 48.2 µg/mL. The standard drug, Acarbose, showed an IC_{50} of 32.1 µg/mL. The dose-response curve is presented in **Figure 5**.

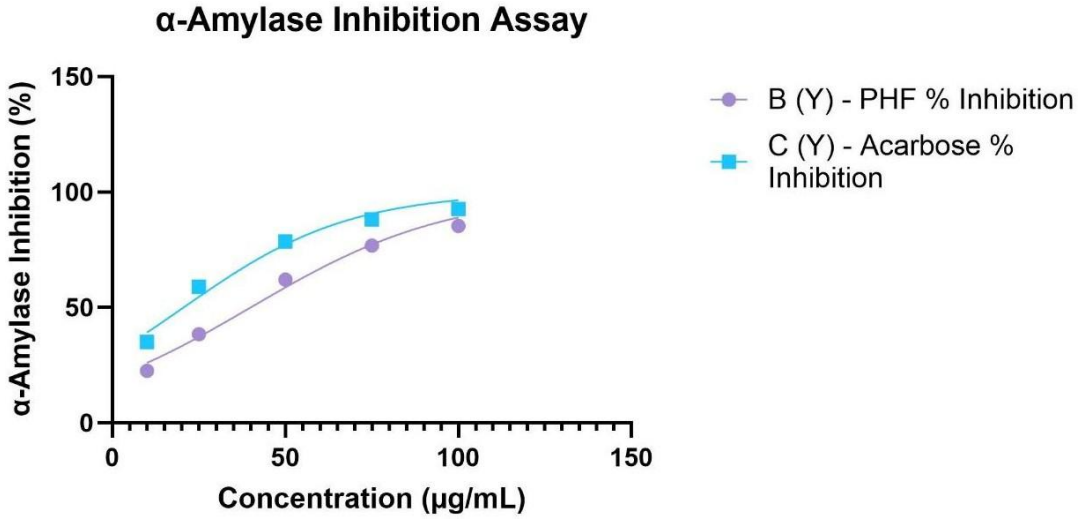


Figure 5. Dose-response curve showing the α-amylase inhibitory activity of the polyherbal formulation (PHF) compared to the standard drug Acarbose. Data points represent mean ± SD (n=3).

4. DISCUSSION

This study successfully developed a standardized polyherbal formulation (PHF) from *T. foenum-graecum* and *C. verum* and established its quality control parameters and in-vitro efficacy.

The organoleptic properties provide the first line of quality assessment and were consistent with the inherent characteristics of its constituent herbs (Wallis, 2004). The low moisture content (6.8%) is critical for ensuring stability by minimizing the risk of microbial growth and chemical degradation during storage (Mukherjee, 2008). The ash values are key indicators of purity. The low total ash (7.2%), acid-insoluble ash (1.5%), and water-soluble ash (2.8%) confirm the purity of the plant material and the cleanliness of the processing method (Kokate, 2014). The acidic pH of the formulation is advantageous, as neutral or alkaline pH levels are known to favor microbial contamination (Abba et al., 2009).

The excellent flow characteristics, as evidenced by the angle of repose (28.5°), Carr's index (12.1%), and Hausner's ratio (1.10), indicate that the PHF powder would handle well in industrial manufacturing processes, ensuring uniform die filling and consistent dosage (Aulton & Taylor, 2013).

The phytochemical screening confirmed the presence of a wide spectrum of bioactive compounds to which the antidiabetic activity can be attributed. The presence of saponins and alkaloids from fenugreek and polyphenols from cinnamon directly correlates with the observed in-vitro antidiabetic activity (Gaddam et al., 2015; Shihabudeen et al., 2011).

The most significant finding of this study is the potent α-amylase inhibitory activity of the PHF (**Figure 5**). With an IC_{50} of 48.2 µg/mL, the formulation demonstrates a strong ability to retard the breakdown of starch into glucose, thereby potentially lowering postprandial blood glucose levels. While less potent than Acarbose (IC_{50} 32.1 µg/mL), the PHF offers a natural, multi-constituent alternative. This activity is likely due to the synergistic action of saponins from fenugreek and proanthocyanidins from cinnamon, both known α-amylase inhibitors (Shihabudeen et al., 2011; Mukherjee et al., 2016). This multi-targeted approach is a significant advantage of polyherbal formulations over single-drug therapies.

5. Conclusion

The present study concludes that the developed polyherbal formulation from *Trigonella foenum-graecum* and *Cinnamomum verum* possesses satisfactory organoleptic and physicochemical properties, a rich phytochemical profile, and significant in-vitro antidiabetic activity via α-amylase inhibition. The standardization parameters established here can serve as a reference for the quality control of this formulation. This work provides a solid foundation for further research, including in-vivo antidiabetic activity studies, toxicity profiling, and the development of a suitable dosage form for clinical evaluation.

6. References

Abba, D., Inabo, H. I., Yakubu, S. E., & Olonitola, O. S. (2009). Contamination of herbal medicinal products marketed in Kaduna Metropolis with selected pathogenic bacteria. *African Journal of Traditional, Complementary and Alternative Medicines, 6*(1), 70–77.

American Diabetes Association. (2023). Standards of medical care in diabetes—2023. *Diabetes Care, 46*(Supplement 1), S1–S2.

Aulton, M. E., & Taylor, K. M. G. (2013). *Aulton's Pharmaceutics: The Design and Manufacture of Medicines* (4th ed.). Churchill Livingstone.

Chaudhury, A., Duvoor, C., Reddy Dendi, V. S., Kraleti, S., Chada, A., Ravilla, R., ... & Mirza, W. (2017). Clinical review of antidiabetic drugs: Implications for type 2 diabetes mellitus management. *Frontiers in Endocrinology, 8*, 6.

Dwivedi, C., & Daspaul, S. (2013). Antidiabetic herbal drugs and polyherbal formulations used for diabetes: A review. *The Journal of Phytopharmacology, 2*(3), 44-51.

El-Desoky, G. E., Aboul-Soud, M. A. M., & Al-Numair, K. S. (2012). Antidiabetic and hypolipidemic effects of Ceylon cinnamon (Cinnamomum verum) in alloxan-diabetic rats. *Journal of Medicinal Plants Research, 6*(9), 1685-1691.

Gaddam, A., Galla, C., Thummisetti, S., Marikanty, R. K., Palanisamy, U. D., & Rao, P. V. (2015). Role of fenugreek in the prevention of type 2 diabetes mellitus in prediabetes. *Journal of Diabetes & Metabolic Disorders, 14*, 74.

International Diabetes Federation (IDF). (2021). *IDF Diabetes Atlas* (10th ed.). Brussels, Belgium: International Diabetes Federation.

Kher, J. D., Roy, S. P., & Pallavi, K. J. (2017). A review on benefit of polyherbal syrup in diabetes. *World Journal of Pharmacy and Pharmaceutical Sciences, 6*(8), 798-808.

Khandelwal, K. R. (2010). *Practical Pharmacognosy: Techniques and Experiments* (20th ed.). Nirali Prakashan.

Kokate, C. K. (2014). *Practical Pharmacognosy* (5th ed.). Vallabh Prakashan.

Mukherjee, P. K. (2008). *Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals* (2nd ed.). Business Horizons.

Mukherjee, P. K., et al. (2016). Validation of the antidiabetic effects of *Trigonella foenum-graecum* L. (fenugreek) seeds. *Journal of Ethnopharmacology, 185*, 253-262.
Shihabudeen, H. M. S., Priscilla, D. H., & Thirumurugan, K. (2011). Cinnamon extract inhibits α-glucosidase activity and dampens postprandial glucose excursion in diabetic rats. *Nutrition & Metabolism, 8*(1), 46.
Unnikrishnan, R., Anjana, R. M., & Mohan, V. (2016). Diabetes mellitus and its complications in India. *Nature Reviews Endocrinology, 12*(6), 357–370.
Wallis, T. E. (2004). *Textbook of Pharmacognosy* (5th ed.). CBS Publishers & Distributors.