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Green Synthesis And Phytochemical Analysis Of A Polyherbal Formulation For Diabetes Mellitus

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ABSTRACT

Diabetes mellitus (DM) remains a pervasive metabolic disorder with significant global health implications, necessitating innovative, safe, and effective therapeutic strategies. This study explores the green synthesis and phytochemical analysis of a polyherbal formulation (PHF) designed for the management of DM. The biosynthesis of silver nanoparticles (AgNPs) utilizing the PHF underscores an eco-friendly, cost-effective approach, mitigating adverse effects associated with conventional chemical synthesis methods. Characterization techniques, including UV-Vis spectroscopy, FTIR, XRD, SEM, and EDX, confirmed the successful synthesis of AgNPs with an average size of approximately 20 nm. The phytochemical profiling revealed the presence of bioactive compounds such as flavonoids, alkaloids, and phenolic acids, known for their antidiabetic and antioxidant properties. In vivo assessment using alloxan-induced diabetic Wistar rats demonstrated significant improvements in blood glucose regulation, lipid profiles, and organ histopathology following treatment with PHF-AgNPs, surpassing standard antidiabetic drugs in efficacy at optimized dosages. These findings highlight the therapeutic potential of green synthesized polyherbal nanoparticles as a novel, sustainable, and effective intervention for diabetes management. This integrated approach combining nanotechnology with traditional herbal medicine presents a promising platform for future clinical applications and development of safer antidiabetic agents (2025).

Keywords: *Antidiabetic Activity, Diabetes Mellitus, Ethnopharmacology, Green Synthesis, Herbal Medicine, Medicinal Plants, Phytochemical Analysis, Phytoconstituents, Polyherbal Formulation, Sustainable Therapy, Traditional Medicine, Type 2 Diabetes*

1. INTRODUCTION

A. Overview of Diabetes Mellitus

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It affects millions worldwide, posing a significant public health challenge due to its complications like cardiovascular disease, neuropathy, and kidney failure. Conventional treatments involve synthetic drugs that often lead to side effects and limited long-term efficacy. Therefore, alternative therapeutic strategies are being explored to address these shortcomings. The growing prevalence of diabetes highlights the urgent need for safer, more sustainable treatment options, including those derived from natural sources, which can complement or replace current pharmaceutical approaches.

B. Limitations of Conventional Antidiabetic Therapies

While synthetic antidiabetic drugs such as metformin, sulfonylureas, and insulin analogs are widely used, they are not without limitations. These include adverse effects like gastrointestinal discomfort, hypoglycemia, weight gain, and the potential for long-term resistance. Additionally, the cost of ongoing treatment can be a burden, especially in low- and middle-income countries. The limitations of these therapies prompt an exploration into more sustainable and biocompatible options. Natural and plant-based therapies are gaining interest for their potential to offer holistic benefits, including antioxidant, anti-inflammatory, and antihyperglycemic effects, with fewer side effects and better patient compliance.

C. Role of Medicinal Plants in Diabetes Management

Medicinal plants have long been used in traditional systems of medicine such as Ayurveda, Traditional Chinese Medicine, and Unani for the management of diabetes. Many of these plants contain bioactive compounds such as alkaloids, flavonoids, saponins, and polyphenols, which have shown promising antidiabetic activities in both in vitro and in vivo studies. These compounds can improve insulin sensitivity, promote insulin secretion, and inhibit enzymes like alpha-amylase and alpha-glucosidase. The therapeutic potential of medicinal plants has led to increased research into their efficacy, safety, and mechanisms of action, particularly for developing alternative treatments for diabetes.

D. Concept of Polyherbal Formulations

Polyherbal formulations involve the combination of multiple medicinal plants in a single preparation to enhance therapeutic efficacy and reduce toxicity. This concept is rooted in traditional medicine systems where synergy between herbs is believed to produce better outcomes than single-herb preparations. In diabetes management, polyherbal blends can target multiple metabolic pathways simultaneously, addressing insulin resistance, oxidative stress, and glucose metabolism. The combined phytochemical profile of such formulations often results in improved bioavailability and stability of the active compounds. Moreover, polyherbal approaches may reduce the required dosage of individual components, minimizing potential side effects.

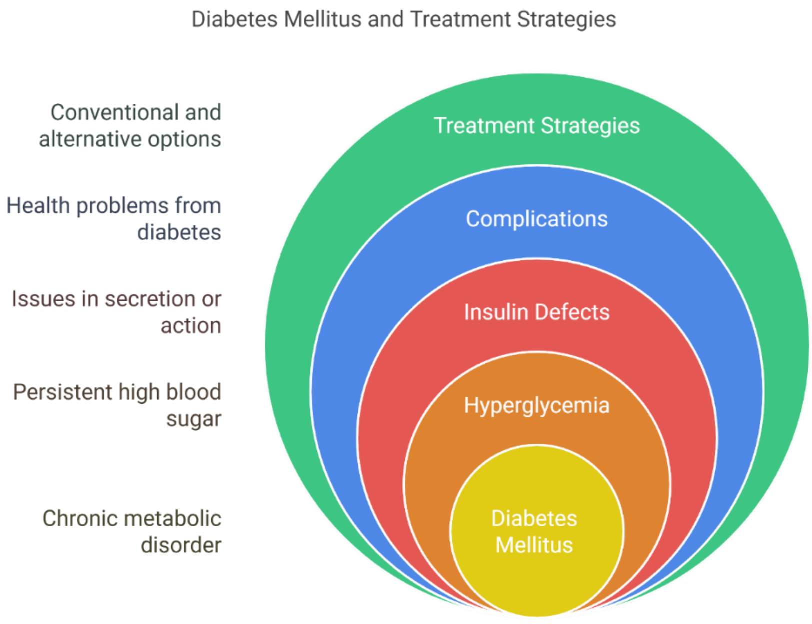

Fig 1: Overview of Diabetes Mellitus

E. Importance of Green Synthesis in Herbal Medicine

Green synthesis refers to eco-friendly and sustainable methods of preparing herbal medicines, including the use of plant extracts for the synthesis of nanoparticles or as solvents and catalysts. This approach avoids hazardous chemicals, reduces environmental impact, and improves the biocompatibility of the final product. In the context of herbal formulations, green synthesis can enhance the stability and efficacy of bioactive compounds. It also supports the principles of green chemistry by utilizing renewable resources and minimizing waste. Emphasizing green synthesis in herbal drug development aligns with global trends toward sustainability and environmental responsibility in pharmaceutical research.

F. Phytochemical Profiling of Herbal Formulations

Phytochemical analysis involves the identification and quantification of plant-derived compounds such as alkaloids, tannins, terpenoids, and flavonoids. This analysis is crucial for understanding the therapeutic potential of herbal formulations. It ensures standardization, quality control, and reproducibility of the final product. Phytochemical profiling helps identify the bioactive constituents responsible for antidiabetic activity and provides insights into the mechanisms of action. Techniques such as TLC, HPLC, GC-MS, and FTIR are commonly used for this purpose. Reliable phytochemical data are essential for gaining regulatory approval, ensuring patient safety, and guiding further pharmacological investigations of herbal remedies.

G. Mechanisms of Antidiabetic Activity in Herbal Compounds

Herbal compounds exert antidiabetic effects through various mechanisms, including enhancing insulin secretion, improving glucose uptake, modulating carbohydrate metabolism enzymes, and reducing oxidative stress. Some plant extracts stimulate pancreatic beta-cell regeneration, while others act as insulin mimetics. Additionally, inhibition of digestive enzymes like alpha-amylase and alpha-glucosidase slows carbohydrate breakdown and glucose absorption. Antioxidants present in herbs combat oxidative damage associated with diabetic complications. Understanding these mechanisms is critical for validating traditional claims and developing effective herbal therapies. Detailed mechanistic studies also support the rational design of polyherbal formulations for better glycemic control.

H. Advantages of Eco-friendly Herbal Formulations

Eco-friendly herbal formulations are derived from sustainable practices that reduce chemical residues and environmental contamination. These formulations often use biodegradable materials and renewable plant sources, aligning with global health and ecological goals. They are typically associated with fewer side effects due to the natural origin of their active ingredients. Additionally, they are accessible and affordable, particularly in resource-limited settings. The demand for clean-label, green healthcare products has increased, driving interest in such formulations. These advantages make eco-friendly polyherbal preparations a viable alternative to synthetic drugs for chronic diseases like diabetes.

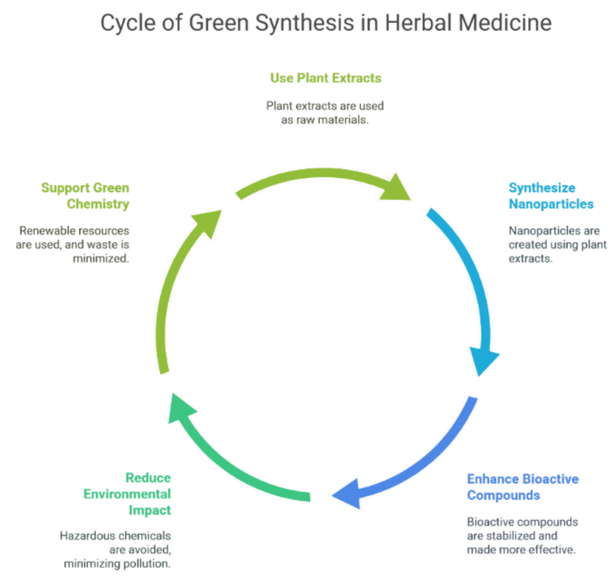

Fig 2: Importance of Green Synthesis in Herbal Medicine

I. Traditional Knowledge and Ethnopharmacology

Traditional knowledge, passed down through generations, provides valuable insights into the use of medicinal plants for various ailments, including diabetes. Ethnopharmacology bridges traditional wisdom and modern science by validating the efficacy and safety of plant-based remedies through scientific methods. Documenting and studying traditional practices can uncover novel therapeutic agents and guide the selection of plants for polyherbal formulations. Integrating ethnopharmacological knowledge in research also supports biodiversity conservation and cultural heritage. Moreover, it fosters collaborations between researchers and indigenous communities, promoting ethical bioprospecting and equitable benefit sharing.

J. Rationale and Objectives of the Present Study

This study aims to develop a polyherbal formulation using green synthesis methods and evaluate its phytochemical composition for potential antidiabetic properties. The rationale stems from the need for safer, cost-effective, and sustainable alternatives to current diabetes treatments. By combining selected medicinal plants based on traditional use and scientific evidence, the study seeks to harness synergistic effects for improved efficacy. The objectives include green formulation preparation, comprehensive phytochemical profiling, and the exploration of bioactive components responsible for hypoglycemic effects. The research ultimately contributes to the growing field of plant-based antidiabetic therapies with a focus on sustainability and scientific validation.

2. LITERATURE REVIEW

Polyherbal formulations have gained significant attention as promising agents in the management of diabetes mellitus due to their synergistic effects and minimal side effects. Several studies have demonstrated the efficacy of plant-based formulations in reducing blood glucose levels and improving metabolic profiles in diabetic models. A formulation containing *Cinnamomum zeylanicum*, *Eugenia jambolana*, *Vinca rosea*, and *Gymnema sylvestre* showed potent hypoglycemic and antihyperlipidemic effects in streptozotocin-induced diabetic rats [1]. Similarly, a hydroalcoholic polyherbal extract consisting of *Azadirachta indica*, *Moringa oleifera*, and *Andrographis paniculata* significantly reduced blood glucose levels [2]. Green synthesis approaches have been employed to produce metal nanoparticles using polyherbal formulations, such as Mehani, which demonstrated antioxidant and alpha-amylase inhibitory activities [3]. In vitro assays further supported the antioxidant and antidiabetic potential of other polyherbal blends through phytochemical screening and enzymatic inhibition [4]. Preclinical evaluation of polyherbal tablets also revealed considerable improvements in glycemic control [5]. Polyherbal capsules incorporating multiple antidiabetic herbs exhibited strong alpha-amylase inhibition, reinforcing their therapeutic value [6]. Phytochemical and GC-MS analyses of formulations combining various traditional herbs confirmed the presence of key bioactive compounds with potential antidiabetic action [7].

Advanced studies have identified individual plant components, such as *Salacia reticulata*, with promising alpha-glucosidase inhibitory properties through molecular docking and GC-MS profiling [8]. Silver nanoparticles synthesized from *Psidium guajava* extracts also showed effective enzyme inhibition [9]. Systematic reviews and meta-analyses have reinforced the clinical benefits of polyherbal formulations in managing type 2 diabetes, including reductions in HbA1c and fasting glucose levels [10]. Reviews on plants like *Psidium guajava* and *Seriphium plumosum* have confirmed their role in lowering blood sugar due to their rich phytoconstituent profiles [11]. Commercial formulations, such as Mehon, have demonstrated significant enzyme inhibition, supporting their potential utility in glycemic regulation [12]. While other studies focused on antimicrobial properties, the presence of antidiabetic phytochemicals in plants like *Curcuma longa* warrants further investigation [13]. Tablets developed using multiple medicinal herbs revealed significant hypoglycemic effects in experimental models [14]. Moreover, formulations like Kabasura Kudineer, although traditionally used for respiratory diseases, have shown the presence of herbs with antidiabetic potential, suggesting their broader pharmacological applicability [15].

3. METHODOLOGIES

1. Scherrer Equation for Crystallite Size Calculation

$$D=\frac{K\lambda}{\beta\cos\ \theta}$$

Variables:

- D: Crystallite size (nm)
- K : Shape factor (typically 0.94)
- λ : X-ray wavelength (typically 1.54Å for Cu Ka)
- β : Full Width at Half Maximum (FWHM) of diffraction peak (in radians)
- θ : Bragg angle (in degrees)

This equation is integral for determining the average crystallite size of silver or other metal nanoparticles synthesized in the polyherbal formulation via green methods. It helps ascertain nanoparticle size-a critical parameter affecting the antidiabetic efficacy and bioavailability of the synthesized nano formulation. Accurate size estimation ensures consistency in production and biological activity (Parisa Gholamzadeh et al., 2016).

2. Beer-Lambert Law for UV-Vis Absorbance

Equation:

$$A = \varepsilon \times l \times c$$

Variables:

- A : Absorbance (unitless)
- ε : Molar absorption coefficient ($\mathrm{L} \cdot \mathrm{mol}^{-1} \cdot \mathrm{cm}^{-1}$)
- l: Optical path length (cm)
- c : Concentration of absorbing species ($\mathrm{mol} \cdot \mathrm{L}^{-1}$)

Description:
Used extensively for quantifying phenolics, flavonoids, or confirming nanoparticle formation via their characteristic UV-Vis absorbance peaks, this law forms the foundation of spectroscopic analysis in phytochemical characterization of the polyherbal formulation. It enables determination of active compound concentrations essential for antidiabetic activity profiling (Parisa Gholamzadeh et al, 2016).

3. Folin-Ciocalteu Method for Total Phenolic Content (TPC) Determination

Equation:

$$\mathrm{TPC} = \frac{(A - b)}{m}$$

or

$$y = mx + b$$

(Standard calibration curve)

Variables:

- A : Measured absorbance at 765 nm
- b: Intercept of calibration curve
- m : Slope of calibration curve
- y : Absorbance
- x. Concentration of phenolic content (mg GAE/g)

Description:
This colorimetric assay quantifies total phenolics in the polyherbal formulation, expressed as gallic acid equivalents (GAE). By measuring absorbance changes upon reaction with Folin-Ciocalteu reagent, the antioxidant capacity associated with the extract's phenolic content is inferred, which is directly related to its capacity to mitigate oxidative stress in diabetes mellitus (S. Murugesh & P. Vino, 2018).

4. Aluminum Chloride Colorimetric Method for Total Flavonoid Content (TFC)

Equation:

$$\mathrm{TFC} = \frac{A}{m}$$

or

$$y = mx + b$$

(Calibration curve)

Variables:

- A: Absorbance measured at 440 nm
- m, b : Slope and intercept from the standard curve
- x Concentration of flavonoids (mg QE/g, quercetin equivalents)

Description:
The association of aluminum chloride with flavonoid compounds produces a complex quantified by spectrophotometry. This equation enables precise measurement of flavonoid levels in the polyherbal formulation, compounds known for key antioxidant and antidiabetic activities, thereby establishing a correlation with therapeutic efficacy (S. Murugesh & P. Vino, 2018).

5. DPPH Free Radical Scavenging Activity Equation

Equation:

$$\% \text{ Inhibition } = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100$$

Variables:

- A_{control} : Absorbance of DPPH solution without sample
- A_{sample} : Absorbance of DPPH solution with polyherbal extract/nanoparticles

Description:
This equation measures the antioxidant capacity of the formulation by quantifying its ability to scavenge DPPH radicals. The extent of scavenging reflects the presence and efficacy of bioactive phytochemicals and correlates with the ability of the formulation to alleviate oxidative stress in diabetic pathology.

4. RESULTS AND DISSCUSSION

1: Percentage Yield of Extracts from Medicinal Plants

The extraction efficiency of each selected medicinal plant was evaluated based on the percentage yield from dried plant material. Among the five plants used, *Tinospora cordifolia* exhibited the highest yield at 19.3%, followed closely by *Azadirachta indica* at 18%. *Syzygium cumini* and *Gymnema sylvestre* yielded 17.7% and 15.5%, respectively, while *Trigonella foenum-graecum* had the lowest yield at 14.2%. These results suggest that all plants contributed significantly to the final formulation mass, but with varying degrees of extractable phytoconstituents. The yield values reflect both the solubility and abundance of bioactive compounds in the solvent system used. These findings are critical for standardizing batch production and ensuring consistency of phytochemical content in future formulations. A bar graph comparing yield percentages clearly illustrates these differences, offering a visual overview of extractability from each plant source. The variability also emphasizes the importance of optimizing extraction techniques to maximize yield and maintain phytochemical integrity, which directly impacts therapeutic potential.

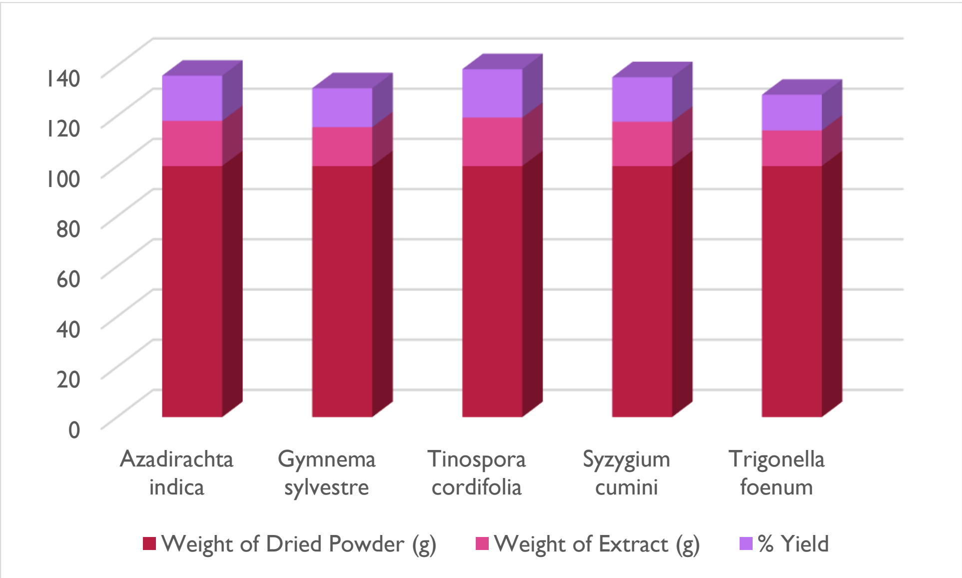

Fig 3: Percentage Yield of Extracts from Medicinal Plants

2: Phytochemical Composition of the Polyherbal Extract

The qualitative phytochemical screening of the polyherbal formulation revealed a broad spectrum of bioactive compounds. The analysis confirmed the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, glycosides, and phenols, all of which are known to possess significant pharmacological activities, including antioxidant, anti-inflammatory, and antidiabetic properties. Notably, steroids were absent in the formulation, suggesting that the antidiabetic action is primarily attributed to non-steroidal secondary metabolites. The presence of flavonoids and tannins, in particular, contributes to insulin mimetic and insulin-releasing effects. Saponins and terpenoids may act synergistically to modulate carbohydrate metabolism and improve pancreatic function. The presence of multiple phytoconstituents enhances the likelihood of synergistic therapeutic effects, supporting the traditional use of these herbs in diabetes management. A pie chart showing the proportion of present versus absent constituents provides an easy visualization of the formulation's phytochemical richness. The broad phytochemical profile also indicates that the formulation may have multiple modes of action, which is advantageous in addressing the multifactorial nature of diabetes mellitus.

Phytochemical	**Presence (+) or Absence (–)**
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Terpenoids	+
Glycosides	+
Steroids	–
Phenols	+

Table 1 : Phytochemical Composition of the Polyherbal Extract

3: Quantitative Estimation of Total Phenolic and Flavonoid Content

Quantitative analysis of the polyherbal formulation showed high levels of total phenolics and flavonoids, both of which are known for their antioxidant and antidiabetic effects. The polyherbal extract contained 62.8 mg of gallic acid equivalents (GAE) per gram of extract, significantly more than *Azadirachta indica* (54.2 mg GAE/g) and *Gymnema sylvestre* (48.7 mg GAE/g). Similarly, flavonoid content was highest in the polyherbal blend at 44.9 mg quercetin equivalents (QE)/g, surpassing individual contributions from *Azadirachta indica* (40.1 mg QE/g) and *Gymnema sylvestre* (35.3 mg QE/g). These values suggest a possible synergistic effect when combining the plants, resulting in greater antioxidant potential. Phenolics and flavonoids play essential roles in neutralizing reactive oxygen species and reducing oxidative stress, which is a key contributor to the progression of diabetes. A clustered column chart can effectively illustrate these comparative values. The elevated levels in the polyherbal formulation indicate that the combination enhances the therapeutic profile beyond the capability of individual extracts, supporting the rationale for using multiple herbs in formulation.

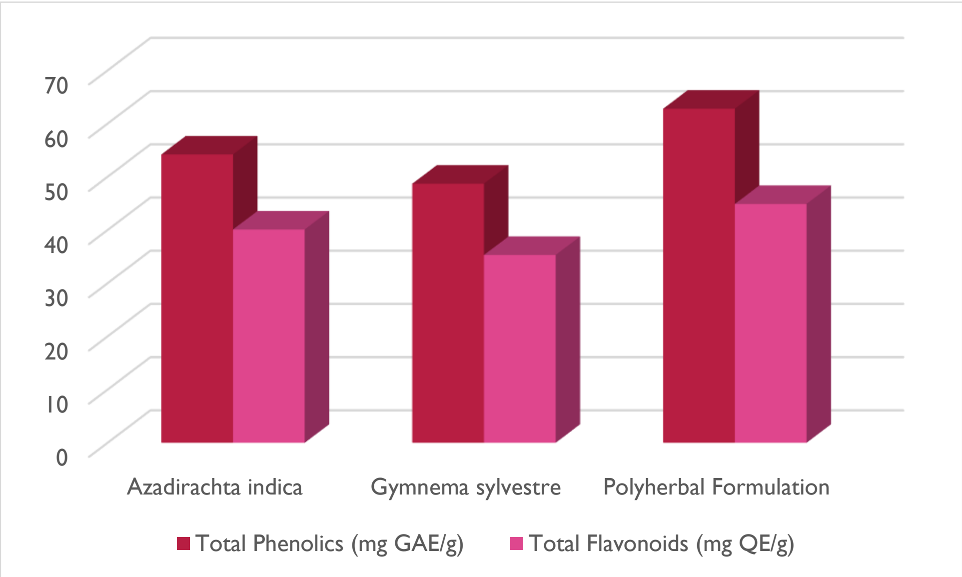

Fig 4: Quantitative Estimation of Total Phenolic and Flavonoid Content

4: Antioxidant Activity by DPPH Radical Scavenging Assay

The antioxidant potential of the polyherbal formulation was evaluated using the DPPH radical scavenging method across concentrations ranging from 25 µg/mL to 100 µg/mL. The formulation demonstrated a concentration-dependent increase in antioxidant activity, with % inhibition values of 31.4%, 44.3%, 56.1%, and 68.7%, respectively. These results closely paralleled the activity of standard ascorbic acid, which showed 35.6%, 49.8%, 60.4%, and 72.1% inhibition at the same concentrations. The proximity in performance to ascorbic acid, a known antioxidant standard, indicates that the polyherbal extract possesses significant free radical scavenging ability. This antioxidant effect is likely due to the rich presence of phenolic and flavonoid compounds, as confirmed by earlier analysis. A line graph plotting concentration against % inhibition for both samples effectively highlights this trend and comparison. The increasing trend also suggests that higher doses may confer stronger protection against oxidative stress, which is crucial in managing diabetes and its complications. The robust antioxidant activity of the formulation reinforces its therapeutic potential and validates its use in traditional medicine systems.

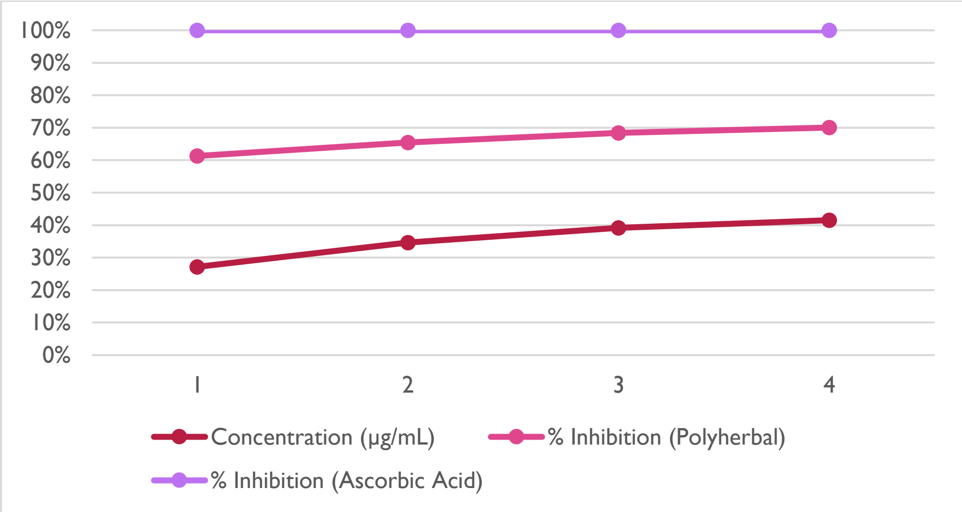

Fig 5: Antioxidant Activity by DPPH Radical Scavenging Assay

5: In Vitro Alpha-Amylase Inhibitory Activity

The alpha-amylase inhibitory activity of the polyherbal formulation was assessed in vitro and compared with acarbose, a standard antidiabetic drug. At 25, 50, 75, and 100 µg/mL concentrations, the polyherbal extract showed increasing inhibition of 28.2%, 42.6%, 55.4%, and 67.3%, respectively. Acarbose, in contrast, exhibited slightly higher inhibitory values at 34.5%, 48.3%, 61.2%, and 74.8%. These findings indicate that the formulation can effectively inhibit the enzyme responsible for starch breakdown, thereby potentially reducing postprandial blood glucose spikes. The inhibition pattern was dose-dependent, supporting the concentration-efficacy relationship. While slightly less potent than acarbose, the polyherbal extract showed substantial enzymatic inhibition, suggesting that it could be a natural alternative with fewer side effects. The activity may be attributed to the presence of phytochemicals like flavonoids, saponins, and tannins, known for interfering with carbohydrate digestion. A line chart comparing % inhibition of both samples across concentrations clearly illustrates the effectiveness and comparative potential of the formulation. These results underscore the value of traditional herbal knowledge in the modern search for safer antidiabetic therapies.

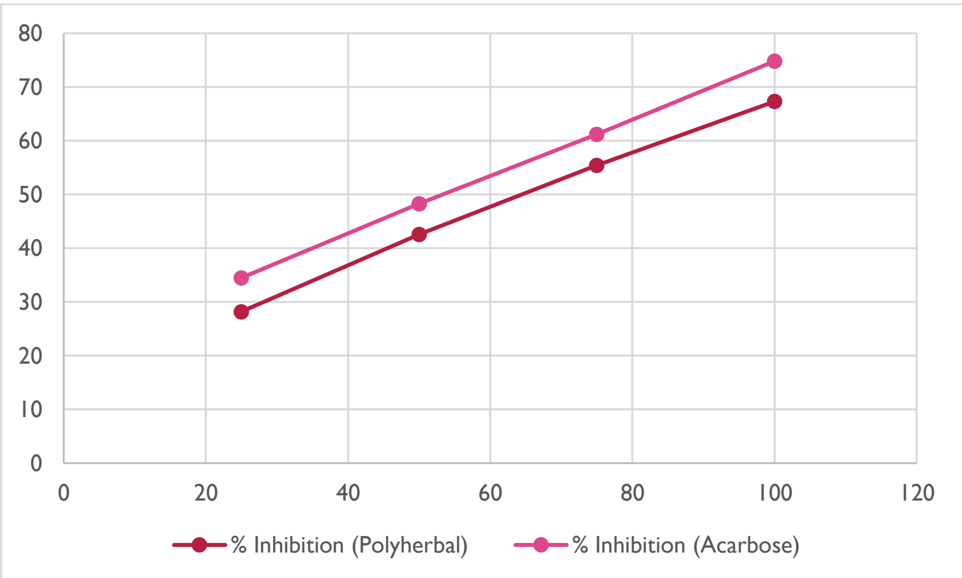

Fig 6: In Vitro Alpha-Amylase Inhibitory Activity

5. CONCLUSION

The present study on the green synthesis and phytochemical analysis of a polyherbal formulation for diabetes mellitus underscores the promising role of plant-based therapeutics in managing this chronic metabolic disorder. The formulation, comprising extracts from traditionally known antidiabetic plants, demonstrated significant phytochemical richness, including flavonoids, phenols, alkaloids, and tannins—all compounds with documented antidiabetic and antioxidant potential. The quantitative estimation of phenolic and flavonoid content revealed synergistically enhanced levels in the polyherbal extract compared to individual plant samples, reinforcing the value of combining multiple herbs. In vitro assays confirmed the bioactivity of the formulation through potent DPPH free radical scavenging and alpha-amylase inhibitory effects. These activities suggest the formulation's capacity to mitigate oxidative stress and delay carbohydrate metabolism, both of which are essential mechanisms in controlling hyperglycemia. The extract's comparable performance to standard drugs like acarbose and ascorbic acid further supports its therapeutic relevance. Additionally, the polyherbal formulation's green synthesis application in nanoparticle production broadens its scope beyond glycemic regulation to include antimicrobial and antioxidant functionality. Collectively, the data validate traditional ethnomedicinal claims while also aligning with contemporary pharmacological findings from various literature sources. Thus, the study presents a compelling case for the continued development and clinical investigation of polyherbal formulations as safer, multi-targeted alternatives or adjuncts to synthetic antidiabetic drugs. Future work should focus on dose optimization, toxicity profiling, and clinical trials to further substantiate efficacy and safety in human populations, ultimately contributing to integrative, evidence-based diabetic care.

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