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Screening of Flavonoid-Rich Fraction from a Polyherbal Formulation for *In-Vitro* Antidiabetic Activity

Ittagi Shanmukha[1*], C M Manu[1],Vinuth C M[1], H M Nandeesha[1], B C Meghana[1], Samreen B[1], Shweta Mugandamath[1]

Department of Pharmacology, S.C.S College of Pharmacy Hrapanahalli-583131 Karnataka, India

[1*]***Corresponding author:*** *Ittagi Shanmukha, Professor and Head Department of Pharmacology, S.C.S College of pharmacy Hrapanahalli-583131 Karnataka, India*

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ABSTRACT

OBJECTIVE: The present study aimed to scientifically evaluate the in-vitro antidiabetic potential of the flavonoid-rich fraction isolated from the traditional polyherbal formulation Madhumardhan**.** A flavonoid-enriched fraction was isolated and characterized using standard phytochemical and chromatographic techniques. The presence of flavonoids was confirmed through qualitative and quantitative analyses, including HPLC profiling of key bioactive markers. The in-vitro antidiabetic efficacy of the isolated fraction was assessed based on its α-amylase inhibitory activity, and the results were compared with the standard drug acarbose**.** This investigation seeks to establish a scientific basis for the traditional therapeutic claims of Madhumardhan in the management of diabetes mellitus.

METHODS: The flavonoid fraction was extracted from the commercially available polyherbal formulation Madhumardhan using standard liquid–liquid extraction with dichloromethane. The presence of flavonoids was confirmed through Shinoda and Lead acetate tests. Quantitative phytochemical analysis and HPLC profiling identified and quantified key bioactive compounds such as tannic acid, catechol, and quercetin. The in-vitro antidiabetic activity was assessed using α-amylase inhibition assay**,** with acarbose serving as the standard reference drug. The IC_{50} value of the test fraction was determined to evaluate potency.

RESULTS: The flavonoid fraction represented approximately 11.4% (w/w) of the total extract. HPLC analysis revealed concentrations of tannic acid (8.98 µg/mL)**,** catechol (3.14 µg/mL)**,** and quercetin as dominant constituents, each showing excellent linearity **(**R^2 = 0.999**).** The flavonoid fraction exhibited a strong, dose-dependent α-amylase inhibitory effect, achieving 83% inhibition at 2.5 mg/mL**,** comparable to the activity of acarbose. The calculated IC_{50} value was approximately 1.8 mg/mL**,** indicating significant enzyme inhibition capacity. These findings suggest effective modulation of carbohydrate metabolism and postprandial glucose control.

CONCLUSION: The study provides scientific evidence that the flavonoid-rich fraction of Madhumardhan exerts potent in-vitro antidiabetic activity, primarily through α-amylase inhibition. These results support its traditional therapeutic use in diabetes management and warrants for further in-vivo and clinical evaluations to confirm its efficacy, safety, and mechanism of action.

KEYWORDS: HPLC analysis of Polyherbal formulation, Madhumardhan, Separation of Flavonoid fraction, α-amylase inhibition, In-vitro antidiabetic activity, IC_{50} value, Quercetin.

I. INTRODUCTION:

In vitro pharmacology, which involves evaluating biological effects of drugs outside living systems, is a cornerstone of modern drug discovery. It enables rapid, cost-effective, and precise screening of candidate compounds before advancing to more complex in vivo studies.

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin resistance, or both. It is associated with oxidative stress, dyslipidemia, and progressive complications such as neuropathy, nephropathy, retinopathy, and cardiovascular disease. According to the International Diabetes Federation, an estimated 451 million people worldwide were living with diabetes in 2017, a number projected to reach 693 million by 2045. Nearly half of these cases remain undiagnosed. In the same year, approximately five million deaths were attributed to diabetes. Despite the availability of synthetic anti-diabetic drugs, their long-term use is limited by high costs, adverse effects, and drug resistance, underscoring the need for safer, affordable alternatives[1,2]. These challenges have spurred renewed interest in polyherbal formulations (PHFs), which have been an integral part of traditional medicine systems such as Ayurveda for centuries.

Polyherbal formulations (PHFs), a fundamental concept in traditional medicine systems such as Ayurveda, combine two or more medicinal plants to achieve synergistic therapeutic effects, where the overall activity is greater than the sum of individual components. This synergism can be pharmacodynamic (multiple compounds acting on different targets with a common therapeutic goal) or pharmacokinetic (one herb enhancing the absorption, metabolism, or bioavailability of another)[3,4,5]. Compared with single-herb remedies, PHFs offer several advantages, including enhanced efficacy, reduced toxicity, broader therapeutic activity, and improved patient compliance.

In diabetes management, PHFs are of particular interest due to their multifaceted mechanisms of action. Individual herbs within these formulations have been reported to regulate glucose metabolism, enhance insulin sensitivity, protect pancreatic β-cells, improve lipid profiles, and reduce oxidative stress[1,2]. Collectively, these properties suggest that PHFs may provide a holistic and more sustainable approach to diabetes therapy.

Characterization of marketed PHFs is therefore essential for establishing their quality, safety, and therapeutic reliability. Standardization through phytochemical profiling, chromatographic techniques, and pharmacological assays ensures scientific validation of traditional knowledge and facilitates integration into evidence-based medicine[3,4]. In this context, flavonoids, a prominent group of plant-derived polyphenols are especially important due to their well-documented antioxidant, anti-inflammatory, and anti-diabetic properties. However, limited studies have isolated and characterized the flavonoid-rich fraction of Madhumardhan using chromatographic techniques and correlated it with α-amylase inhibition. Hence, this study aims to bridge that gap.

The present study was undertaken to evaluate the in vitro anti-diabetic potential of the flavonoid-rich fraction isolated from a commercially available polyherbal formulation Madhumardhan, with specific focus on its α-amylase inhibitory activity.

II. MATERIALS AND METHODS:

Procurement of Polyherbal Formulation: The commercially available Ayurvedic formulation Madhumardhan was procured from Ayush Hospital, Harapanahalli, Karnataka, India. The formulation is traditionally prescribed for the management of diabetes mellitus (Madhumeha) Its therapeutic potential is attributed to the synergistic interaction of multiple medicinal plants with reported antidiabetic, antioxidants and metabolic-modulating activities[6-8].

Composition and Phytochemical Background: Madhumardhan comprises multiple medicinal plants with established antidiabetic, antioxidant, and metabolic-modulating properties, including *Syzygium cumini* (Jamun)[6], *Momordica charantia* (Karela)[7], *Azadirachta indica* (Neem)[8], *Ocimum sanctum* (Tulsi)[9], *Emblica officinalis* (Amla)[10], *Curcuma longa* (Haridra)[11], *Trigonella foenum-graecum* (Methi)[14], *Gymnema sylvestre* (Gurmar)[12] and *Withania somnifera (*Ashwagandha)[13].

The synergistic action of these constituents attributes to glycemic control, β-cell protection, and improved insulin sensitivity[14].

Chemicals and Reagents: Analytical grade solvents and reagents—ethyl acetate, hexane, acetone, ethanol, and distilled water—were used throughout the study. Additional reagents included sodium hydroxide (Noah), sodium chloride (Nacl), calcium chloride, Arabic gum, sodium alginate, and HPMC. The enzyme α-amylase and the standard drug acarbose were obtained for enzymatic inhibition studies.

Instruments: Analyses were performed using a Shimadzu LC-2010A-HT HPLC system equipped with a C18 column (150 × 4.6 mm, 5 µm) and photodiode array (PDA) detector. Spectrophotometric measurements were recorded on a UV–Visible spectrophotometer at appropriate wavelengths.

Extraction and Fractionation of Flavonoids: Approximately 30 g of Madhumardhan powder was suspended in 250 mL distilled water and transferred to a separating funnel. Liquid–liquid extraction was performed with 250 mL dichloromethane (CH_2Cl_2), and the aqueous phase was re-extracted twice with the same volume. The combined organic layers were dried over anhydrous magnesium sulfate ($MgSO_4$), filtered, and concentrated under reduced pressure at 40 °C using a rotary evaporator, yielding a flavonoid-rich fraction (≈ 11.4% w/w) of dark brown color[15].

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Fig.1- Fractionation of flavonoid-rich extract using separating funnel.

Preliminary Phytochemical Screening: Presence of flavonoids was confirmed through standard qualitative tests[15]:

- **Shinoda Test:** Pink coloration after treatment with ethanol, concentrated HCl, and magnesium turnings indicated flavonoids.
- **Lead Acetate Test:** Formation of a white precipitate confirmed the presence of flavonoids.

Quantitative Analysis[16,17]: The concentrations of major phytoconstituents—tannic acid, catechol, and quercetin—were quantified by HPLC using specific regression equations derived from calibration curves. Linearity for each compound was confirmed within the range of 1–100 µg/mL with correlation coefficients (R^2) = 0.999.

In-Vitro α-Amylase Inhibition Assay[18]: The antidiabetic potential of the flavonoid fraction was assessed using the α-amylase inhibition assay. Test samples and standard (acarbose) solutions were prepared in sodium phosphate buffer (pH 6.9).

1. **Incubation:** A mixture of α-amylase enzyme and test sample was incubated at 37 °C for 10 minutes.
2. **Reaction Initiation:** The reaction was initiated by adding 1% starch solution, followed by further incubation for 10 minutes.
3. **Reaction Termination:** The reaction was stopped by adding dinitrosalicylic acid (DNSA) reagent and heating the mixture in a boiling water bath for 5 minutes to develop color.
4. **Measurement:** Absorbance was measured at 540 nm, and percentage inhibition was calculated using:

% Inhibition = [(Absorbance of control – Absorbance of sample) / Absorbance of control] × 100

Statistical Analysis: All experiments were conducted in triplicate, and results were expressed as mean ± SEM. The IC_{50} value (concentration required to inhibit 50% of α-amylase activity) was determined by linear regression analysis of dose–response data.

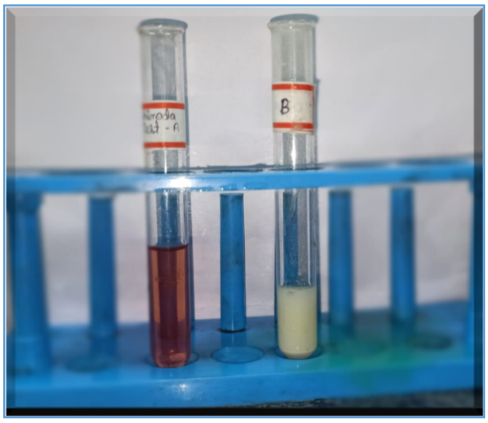
III. RESULTS:

Extraction and Yield of Flavonoid Fraction: Liquid–liquid extraction of the polyherbal formulation Madhumardhan yielded a flavonoid-rich fraction equivalent to 11.4% (w/w) of the totalcrude extract. The fraction appeared as a dark brown, semi-solid mass, indicating a high concentration of polyphenolic compounds.

Qualitative Confirmation of Flavonoids: Preliminary phytochemical screening confirmed the presence of flavonoids in the isolated fraction. The Shinoda test produced a pink coloration, while the Lead acetate test resulted in the formation of a white precipitate, both characteristic of flavonoid compounds.

TEST	OBSERVATION	INFERENCE
a)Shinoda Test To dry powder add 5ml 95% ethanol few drops of conc.HCL and 0.5g Magnesium turning	Pink color is formed	Flavonoids are present
b)Lead Acetate Test To small quantity of powder add lead acetate solution	White precipitate is formed	Flavonoids are present and confirmed

Table-1: Chemical tests for flavonoids



HPLC Characterization

Quantitative chromatographic analysis using Shimadzu LC-2010A-HT with a C18 column (150 × 4.6 mm, 5 µm) revealed distinct peaks corresponding to tannic acid (2.0 min), catechol (2.8 min), and quercetin (3.6 min). Linearity for each standard was established between 1–100 µg/mL with excellent correlation ($R^2 = 0.999$). The concentrations of the identified compounds in the Madhumardhan sample were determined as follows:

- Tannic acid: 8.98 µg/mL
- Catechol**:** 3.14 µg/mL
- Quercetin: Detected, confirming flavonoid presence

The total flavonoid concentration indicated that Madhumardhan is a rich source of flavonoid and phenolic constituents.

Linearity study

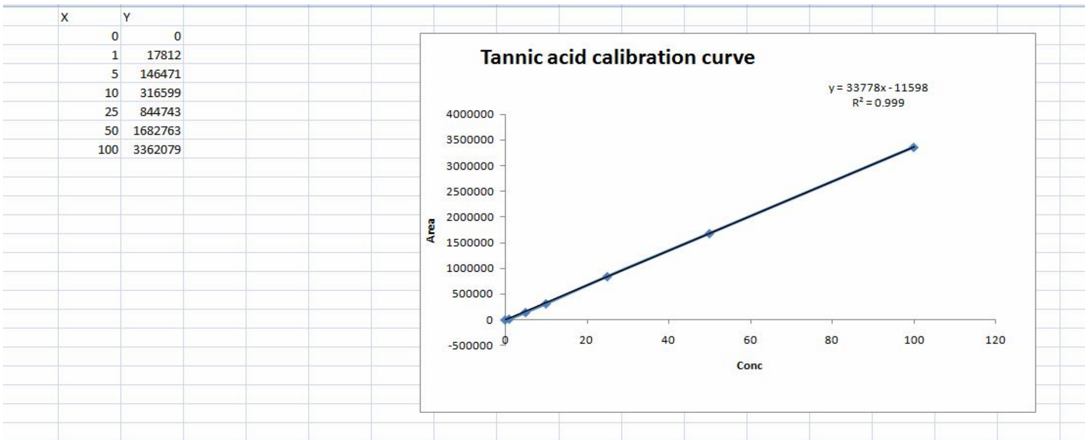


Fig-2: Linearity data for tannic acid

The linearity was established for Tannic acid in the range of 1, 5, 10, 50, 100ug/mL with a correlation coefficient (R^2) of 0.999.

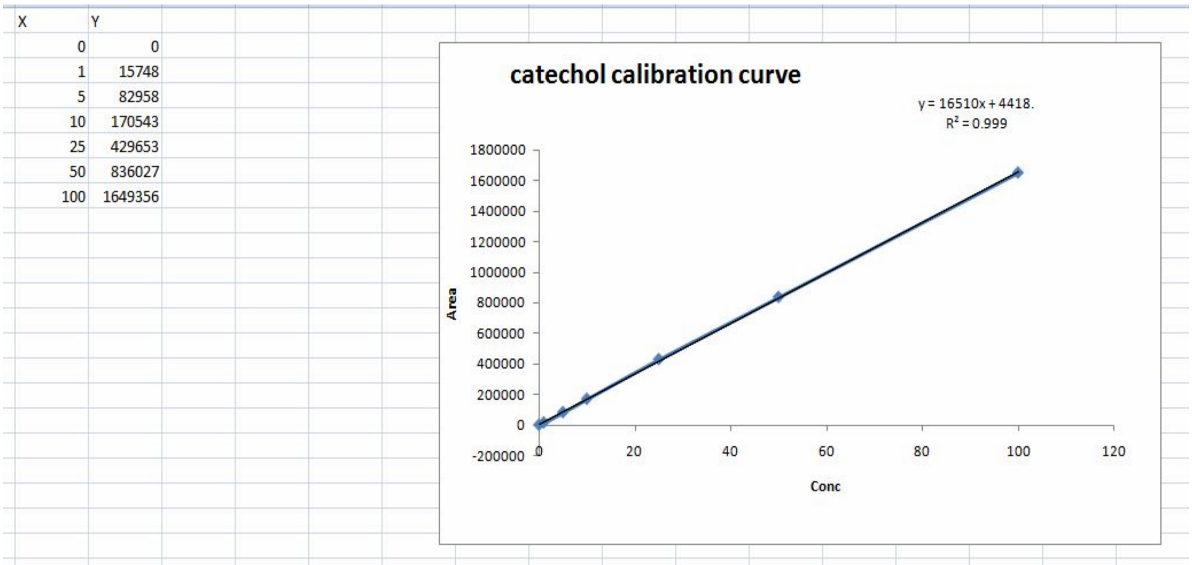


Fig-3: Linearity data for catechol

The linearity was established Catechol in the range of 1, 5, 10, 25, 50,100ug/mL with a correlation coefficient (R^2) of 0.999.

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X	Y
0	0
1	18557
5	90220
10	185778
25	456316
50	884827
100	1722438

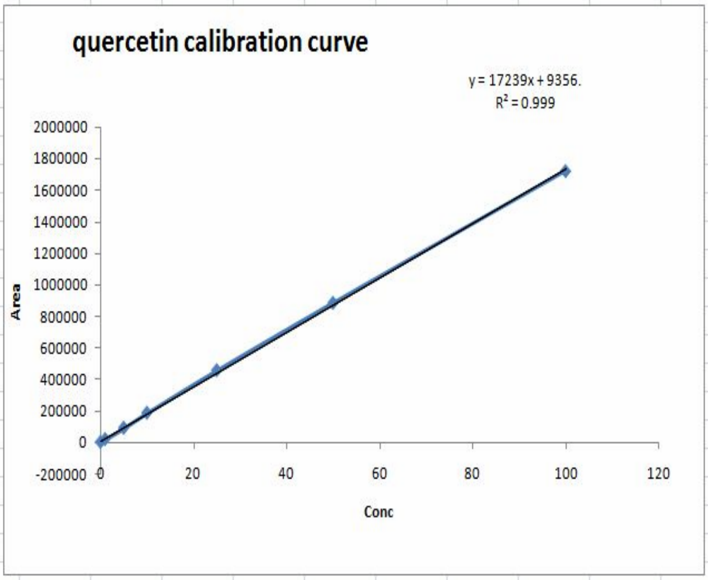

Fig-4 Linearity data for quercetin

The linearity was established for Quercetin in the range of 1, 5, 10, 25, 50,100ug/mL with a correlation coefficient (R^2) of 0.999

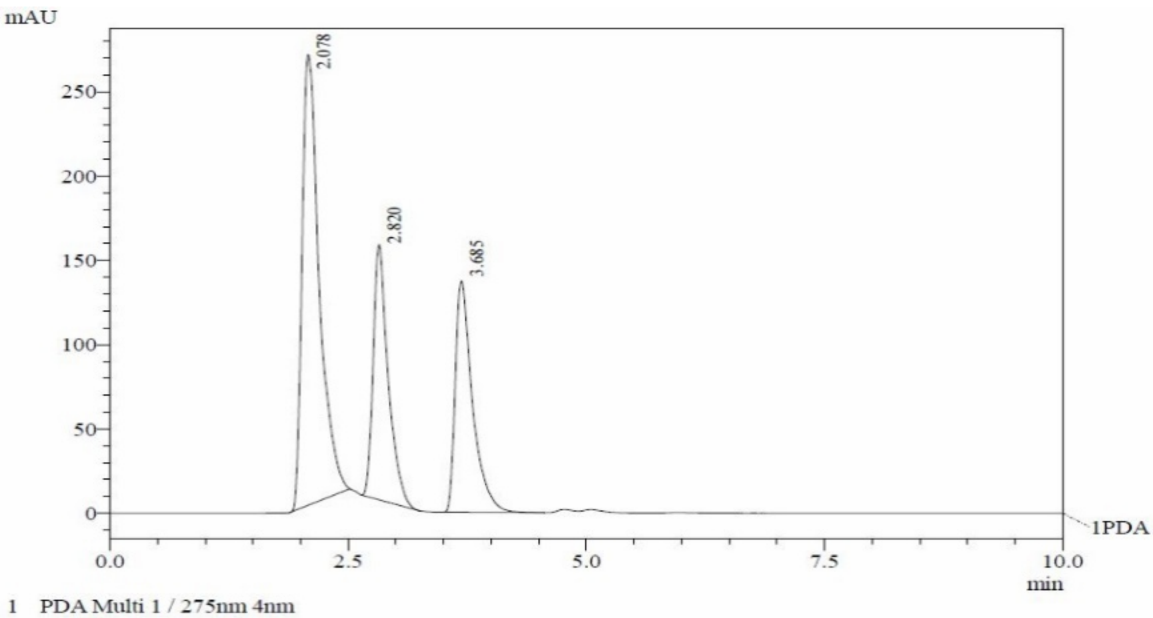

Fig-5: Standard chromatogram of Tannic acid (2.0min), Catechol (2.8min) and Quercetin (3.6min)

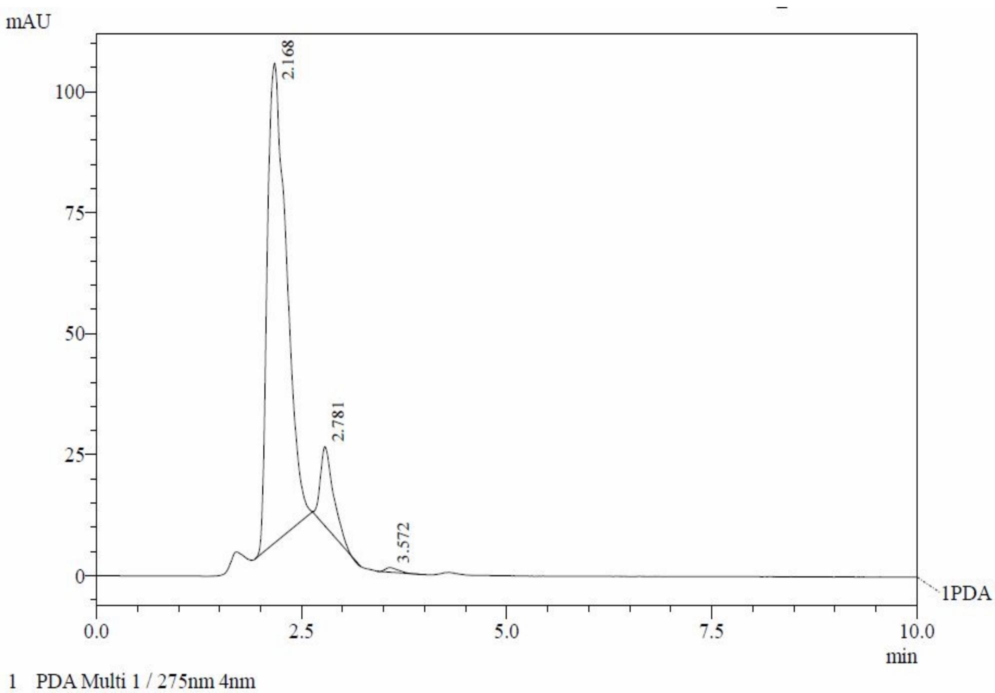

Fig-6 : Chromatogram of polyherbal formulation Madhumardhan which shows elution of Tannic acid and Catechol

In-Vitro α-Amylase Inhibition Assay: The flavonoid fraction demonstrated a dose-dependent inhibition of the α-amylase enzyme, suggesting significant antidiabetic potential, enzyme inhibition increased in a dose-dependent manner, reaching 83% inhibition at 2.5 mg/mL. The standard reference drug acarbose displayed a comparable inhibition profile.

Concentration (mg/mL)	% α-Amylase Inhibition (Mean ± SEM)
0.5	21.4 ± 1.2
1.0	38.7 ± 1.6
1.5	57.8 ± 2.3
2.0	71.2 ± 1.9
2.5	**83.0 ± 2.1**

Table 2:In-vitro α-Amylase Inhibitory Activity of the Flavonoid-Rich Fraction of Madhumardhan.

Values are expressed as mean ± SEM (n = 3). The fraction exhibited a concentration-dependent increase in α-amylase inhibition, comparable to the standard drug acarbose.

The IC_{50} value for the flavonoid fraction was calculated to be **1.8 mg/mL**, confirming potent inhibitory activity comparable to acarbose.

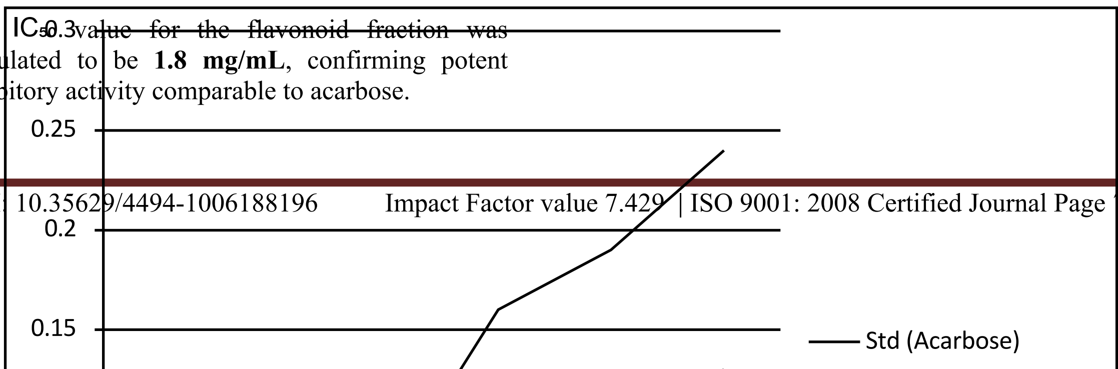

Fig.7: Effect of Std (Acarbose) and Polyherbal formulation Madhumardhan on Alpha amylase enzyme inhibition assay

Summary of findings: The overall results demonstrate that the flavonoid-rich fraction of Madhumardhan contains substantial quantities of biologically active flavonoids and phenolic compounds with strong α-amylase inhibitory activity. This supports its traditional use in diabetes management and indicates its potential as a natural therapeutic agent for controlling postprandial hyperglycemia.

IV. DISCUSSION:

The present study aimed to scientifically validate the in-vitro antidiabetic potential of the flavonoid-rich fraction isolated from the polyherbal formulation Madhumardhan. The findings demonstrated that the isolated fraction exhibited significant α-amylase inhibitory activity, indicating its capacity to modulate carbohydrate metabolism and attenuate postprandial hyperglycemia.

The α-amylase enzyme plays a central role in the breakdown of complex carbohydrates into glucose. Inhibition of this enzyme delays carbohydrate digestion and glucose absorption, thereby lowering postprandial blood glucose levels — a recognized therapeutic mechanism for the management of type 2 diabetes mellitus. In the present study, the flavonoid fraction of Madhumardhan achieved 83% inhibition at 2.5 mg/mL, with an IC_{50} value of approximately 1.8 mg/mL, closely comparable to the standard drug acarbose. These results strongly support the enzyme-inhibitory potential of the flavonoid fraction.

Phytochemical analysis revealed the presence of key flavonoid and phenolic compounds, including tannic acid (8.98 µg/mL), catechol (3.14 µg/mL), and quercetin, which are well-documented for their antioxidant and antidiabetic effects. These bioactive molecules are known to improve insulin sensitivity, protect pancreatic β-cells, and mitigate oxidative stress associated with diabetic complications. The synergistic interaction among these compounds may underlie the overall potency observed in the flavonoid fraction.

Similar observations have been reported in earlier studies where polyphenolic-rich extracts from medicinal plants such as Syzygium cumini, Momordica charantia, and Trigonella foenum-graecum demonstrated enzyme inhibition and glucose-lowering effects through complementary mechanisms. Therefore, the present findings align with existing evidence supporting the role of flavonoids as natural α-amylase inhibitors.

Importantly, the use of a polyherbal formulation such as Madhumardhan enhances therapeutic efficacy through synergism — where multiple phytoconstituents act on diverse metabolic targets, improving glycemic control while minimizing adverse effects associated with single-drug therapy. This reinforces the rationale for the continued exploration of traditional formulations within modern pharmacological frameworks[15-18].

However, while in-vitro results are promising, further in-vivo studies and clinical investigations are essential to confirm pharmacokinetic behavior, bioavailability, and long-term safety profiles of the active constituents.

Therefore, the observed α-amylase inhibitory activity may be attributed to synergistic interactions among flavonoid and phenolic compounds, reinforcing the pharmacological potential of Madhumardhan as a natural antidiabetic agent.

V. CONCLUSION

The study conclusively demonstrates that the flavonoid-rich fraction of the polyherbal formulation Madhumardhan exhibits potent in-vitro antidiabetic activity through α-amylase enzyme inhibition. The presence of bioactive flavonoids and phenolic compounds such as tannic acid, catechol, and quercetin contributes to this effect, highlighting their therapeutic relevance in managing postprandial hyperglycemia.

These findings provide a strong scientific basis for the traditional use of Madhumardhan in diabetes management and emphasize the importance of flavonoid-based polyherbal formulations as potential natural alternatives to synthetic antidiabetic agents. Further in-vivo and clinical evaluations are warrants to establish comprehensive efficacy, safety, and mechanistic profiles for their translational application in integrative diabetes therapy.

VI. ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest

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