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SYNERGISTIC COMBINATION OF QUERCETIN AND 4-HYDROXYISOLEUCINE: A NATURAL STRATEGY FOR MANAGING DIABETES

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ABSRTACT

Fenugreek, a medicinal plant, has demonstrated notable anti-diabetic properties, including the ability to lower fasting blood sugar and enhance glucose tolerance. A systematic review evaluating its hypoglycaemic effects reported significant reductions in fasting blood glucose, postprandial glucose levels, and HbA1c following fenugreek intake. Nonetheless, variability in study quality and consistency remains a limitation. Additionally, the study explored the antidiabetic efficacy of polyphenol extracts derived from guava pulp, seeds, and leaves through in vivo experiments on albino rats. Supplementation with these polyphenols led to increased feed intake and body weight, along with decreased levels of cholesterol, HDL, blood glucose, and triglycerides. The extracts also inhibited α-amylase activity and glucose absorption. These results support the potential use of guava pulp polyphenol extracts at a dosage of 200–250 mg/kg body weight as a natural alternative to conventional antidiabetic medications.

2) KEYWORDS: Trigonella foenum-graecum L. and Psidium guajava L., Combined Therapeutic Effects, Polyherbal Interactions, Diabetes Control, Regulation of Blood Sugar Levels.

3) INTRODUCTION

Diabetes mellitus (DM) is a widespread metabolic disease that currently impacts more than 460 million people worldwide. It primarily arises due to insulin resistance in vital tissues

such as adipose tissue, skeletal muscles, and the liver.[1] *Psidium guajava* (guava), a member of the Myrtaceae family, has long been utilized in traditional medicine for its antimicrobial, hepatoprotective, anti-inflammatory, and antidiabetic properties.[2,3] Guava leaves, which are abundant in triterpenoids and other bioactive compounds, are believed to exhibit blood sugar-lowering effects, though the exact pharmacological pathways are not yet fully understood.[4] This study focuses on evaluating the antidiabetic activity of methanolic and aqueous guava leaf extracts in diabetic rat models.

Type 2 diabetes mellitus (T2DM), accounting for the majority of DM cases, is closely linked to insulin resistance and often results in serious health complications, including heart disease and stroke.[5] Effective control of systemic inflammation and oxidative stress is essential in preventing such outcomes.[6] *Trigonella foenum-graecum* (fenugreek), a well-known medicinal herb in traditional healthcare systems, has shown promising results in managing T2DM. Its seeds contain bioactive constituents such as diosgenin, galactomannan, and 4-hydroxyisoleucine, which have been reported to reduce blood glucose levels and combat oxidative stress and inflammation.[7,8] Although various studies support these effects, further research is needed to better understand fenugreek's anti-inflammatory actions in human subjects.[9] With projections indicating that the global diabetic population will exceed 537 million by 2021, there is an urgent need for affordable and effective treatment strategies.[10] Fenugreek, known for its antidiabetic, antioxidant, and anti-inflammatory effects, has been traditionally used for glycemic regulation in many regions, particularly across Asia and Africa.[11] This review compiles recent research highlighting fenugreek's therapeutic potential in diabetes management through its role in reducing hyperglycemia and mitigating oxidative and inflammatory stress.[12]

4) MATERIALS AND METHODS

4.1 Reagents

Aqueous guava leaf extracts were prepared according to the method outlined by Bai et al.[13] Primary antibodies—ATP1A1, phospho-AKT (Ser473), total AKT, phospho-GSK3β (Ser9), total GSK3β, and mouse β-actin—were purchased from Cell Signaling Technology Inc. (Beverly, MA, USA). The GLUT2 antibody was sourced from Proteintech (Chicago, IL, USA).

4.2 Plant Material

Fresh leaves of *Psidium guajava* L. (family Myrtaceae) and seeds of *Trigonella foenum-graecum* L. (family Fabaceae) were collected and authenticated with reference to the "Flora of Kolhapur District".[14]

4.3 Sample Preparation

Guava leaves were thoroughly washed with double-distilled water, shade-dried at ambient temperature, ground into powder, and sieved through a 1 mm mesh. The powder was then stored in airtight containers. Fenugreek seeds were dried in an oven at 50 °C for 24 hours, ground, passed through a 0.5 mm mesh, and stored similarly.

To obtain the aqueous extract, 20 g of guava leaf powder was boiled in 100 mL of double-distilled water at 90 °C for 30 minutes. The mixture was centrifuged at 4000 rpm for 10 minutes, and the resulting supernatant was stored at 4 °C for subsequent use.[15]

For fenugreek seed extraction, 100 g of powdered seeds underwent Soxhlet extraction using hexane for 3 hours. The extract was filtered through Whatman No. 1 paper, and the solvent was evaporated at 40 °C using a rotary evaporator. The concentrated extract was collected and stored in an airtight container.[16]

4.4 Proximate Composition

The nutritional composition of guava leaves and fenugreek seeds—including moisture, fat, ash, crude fiber, and protein—was determined using standard AOAC methods (2000).[17]

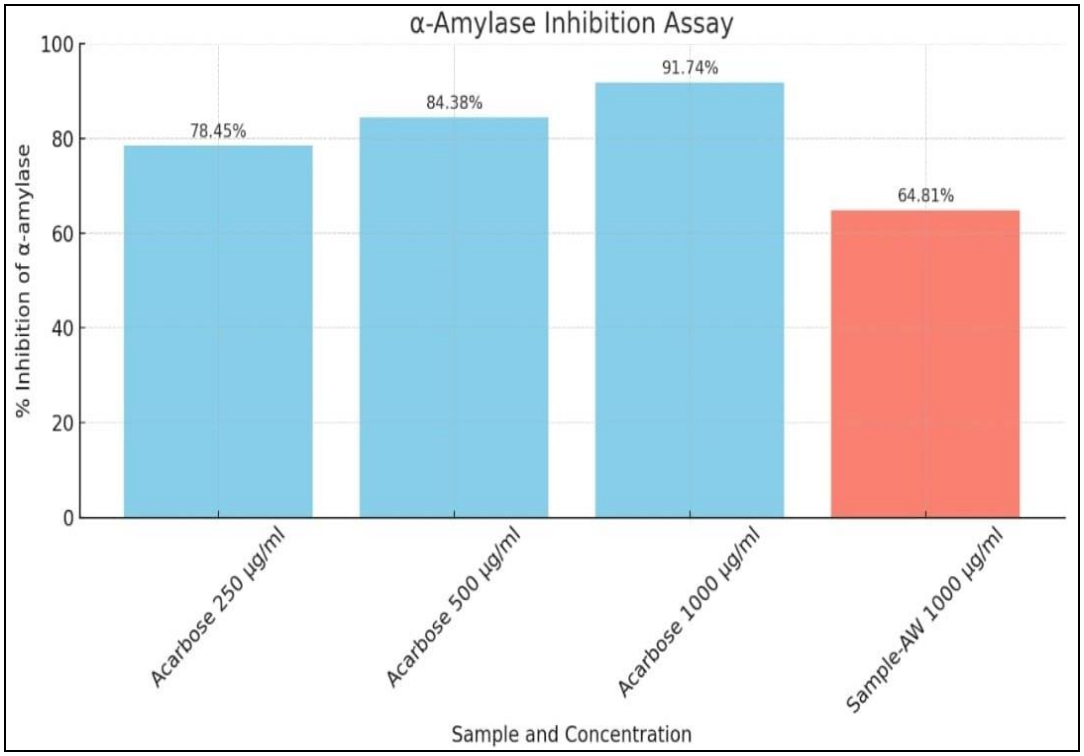
Moisture content was measured by oven drying, fat by Soxhlet extraction, ash using a muffle furnace, fiber via acid-base digestion, and protein content was calculated using the Kjeldahl method, with a nitrogen-to-protein conversion factor of 5.64.

4.5 In Vitro α-Amylase Inhibition Assay

The α-amylase inhibitory potential of the extracts was assessed using the protocol established by Patil et al.[5] A 500 µL sample of the combined guava leaf and fenugreek seed extract (LBF) was mixed with 500 µL of 0.1 M phosphate buffer (pH 6.9) containing 0.5% fungal α-amylase and incubated at 25 °C for 10 minutes. After pre-incubation, 500 µL of 1% soluble starch solution (prepared in 0.1 M phosphate buffer, pH 6.8) was added, followed by a second 10-minute incubation at the same temperature.

For the control, phosphate buffer replaced the enzyme solution. After incubation, 1000 µL of 3,5-dinitrosalicylic acid (DNS) reagent was added to both test and control tubes. The mixtures were boiled for 10 minutes in a water bath, then cooled to room temperature, and absorbance was recorded at 540 nm using a UV–Vis spectrophotometer. Acarbose served as the positive control.

The percentage inhibition of α-amylase activity was calculated using the formula: Inhibition (%) = [(Abs_{540} control – Abs_{540} extract) / Abs_{540} control] × 100



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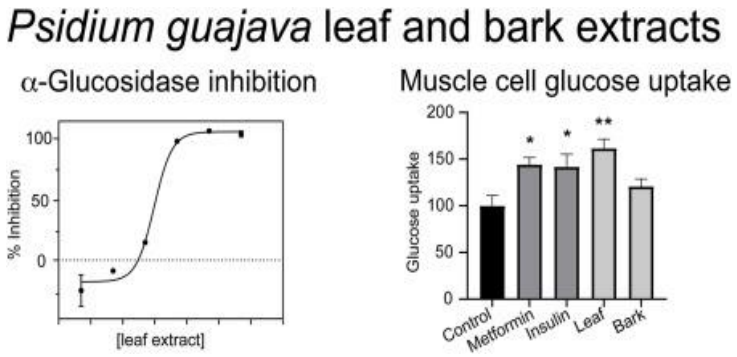
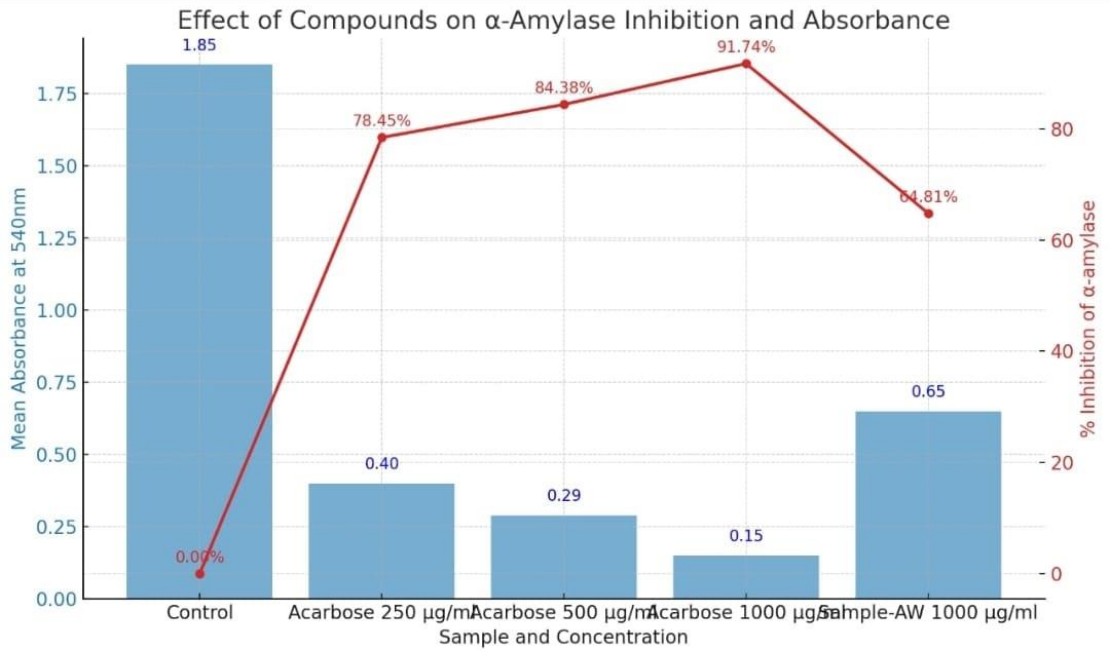




Experimental Design for Biological Studies

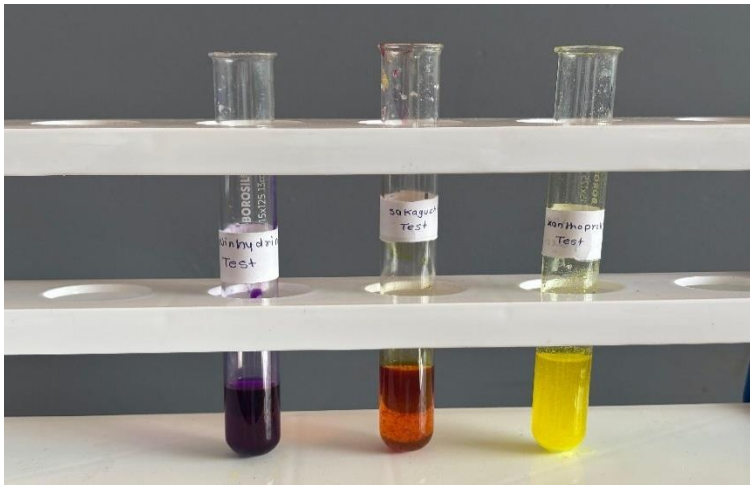
The study was conducted using healthy albino rats housed at the Department of Pharmacy, University of Sargodha, Pakistan. Diabetes was induced by subcutaneous administration of Alloxan monohydrate at a dose of 200 mg/kg body weight. After successful induction, the animals were randomly assigned to eight groups, with each group consisting of five rats.

Two concentrations of guava and fenugreek extracts were incorporated into a basil-enriched diet, which included pulp, leaf, and seed components. This formulation was administered to the respective treatment groups. Throughout the study, no deaths were observed, indicating that the polyphenolic extracts were non-toxic and safe for use.

The diabetic condition, induced by both Alloxan and streptozotocin, caused significant elevations in blood glucose levels. However, administration of fenugreek seed powder led to a substantial decline in blood glucose within 15 days, approaching near-normal levels. Among the tested extracts, the methanolic extract of fenugreek exhibited the most pronounced hypoglycemic activity compared to aqueous and ethereal extracts. The antihyperglycemic effects of fenugreek were attributed to its ability to reduce hepatic glucose production and enhance insulin secretion.[18–20]

Phytochemical Screening

Sr No	Test	Observation	Inference
1	Test for Flavonide	Red colour	Flavonoid present
2	Shinoda Test	Colour change (pink)	Flavonoid present
3	Alkaline Reagent Test	orange colour	Flavonoid present
4	Lead Acetate Test	Yellow colour	Flavonoid present
5	Zinc-HCL Reduction Test	Red colour	Flavonoid present
6	Ninhydrin Test	Violet	Amino acid present
7	Xanthoproteic Test	Yellow colour ppt	Tyrosine or Tryptophan present
8	Sakaguchi Test	Deep orange colour	Arginine present



5) RESULTS

This investigation analyzed the impact of fenugreek supplementation on fasting blood glucose (FBG), postprandial glucose (PPG), and HbA1c across 14 clinical trials conducted in India, Iran, China, France, and Egypt, involving a total of 894 participants. Variations in dosing regimens were observed among the trials, and seven studies did not include a placebo control group.

Meta-analysis revealed a statistically significant reduction in FBG levels in fenugreek-treated groups compared to controls, with a mean difference of −0.88%. However, substantial heterogeneity was present among the included studies. Funnel plots indicated publication bias, though small-study effects were not detected. Meta-regression was conducted to evaluate the effect of dosage, treatment duration, study design, and sample size on FBG outcomes. Results from multivariate analysis indicated that study design significantly influenced FBG reduction. A bubble plot comparing fenugreek dose to effect size did not show a consistent dose-response relationship for FBG, PPG, or HbA1c outcomes.[21–23]

Additionally, the proximate composition of guava (Psidium guajava) leaves, pulp, and seeds was assessed. Leaf powder exhibited the highest moisture and ash content, followed by pulp and seeds. Fat content was most prominent in the seeds, whereas leaves and pulp contained lower levels. Crude fiber content was greatest in the leaves, followed by the pulp and seeds. Vitamin C concentration was highest in the pulp and least in the seeds.[24]

Analysis of the polyphenol content and antioxidant capacity of guava components revealed that total phenolic compounds were abundant in all parts, with antioxidant activity strongest in the pulp, followed by leaves and seeds. DPPH radical scavenging assays further confirmed that pulp extracts had superior antioxidant potential, which may contribute to their antidiabetic effects.[25,26]

In animal studies, phenolic-rich extracts from guava leaves, pulp, and seeds were administered to diabetic mice alongside control diets over a three-week period. Significant changes in feed intake and body weight were observed; mice on extract-supplemented diets generally gained less weight, particularly by the second and third weeks of treatment.

Serum lipid profiles of rats were also examined. Triglyceride levels were highest in Group 2 and lowest in Group 6, with statistically significant differences between groups. Among the guava extracts, pulp extract resulted in the greatest reduction in glucose levels, followed by leaf and seed extracts. Serum insulin levels mirrored glucose trends: treated groups had lower insulin levels than the diabetic control, but higher than the non-diabetic control group.

Haematological parameters such as packed cell volume (PCV), mean corpuscular volume (MCV), and hemoglobin concentration were also analyzed. RBC and WBC counts fluctuated across dietary groups. Group 2 had the lowest RBC count (4.12%), while Group 5 recorded

the highest (4.22%). Platelet counts varied significantly, peaking in Group 8 (358.92%) and lowest in the diabetic control group (332.67%). Although diabetic mice showed reductions in RBC and WBC levels, these changes were mildly reversed in the treated groups.[27–29]

Inhibition (%) = Abs 540 (control) – Abs 540 (extract) * 100 Abs

Table no. 1 Effect of compounds by using alpha amylase inhibition assay

α-amylase enzyme inhibition assay						
Sample code	**Concentration µg/ml**	**Absorbance at540nm**			**Mean**	**% inhibition**
Control		1.82	1.87	1.88	1.85	
Standard Acarbose	**250**	0.42	0.39	0.39	0.40	78.45
	500	0.35	0.28	0.24	0.29	84.38
	1000	0.15	0.12	0.19	0.15	**91.74**
Sample -AW	**1000**	0.69	0.63	0.64	0.65	**64.81**

6) DISCUSSION

This meta-analysis, encompassing 14 clinical trials, demonstrated that fenugreek seed supplementation was associated with reductions in fasting plasma glucose (FPG) by 3.7 mg/dL, postprandial glucose (PPG) by 10.61 mg/dL, and HbA1c by 0.88%. Among these, the reduction in HbA1c reached statistical significance, suggesting a potential long-term benefit on glycemic control. A sensitivity analysis, which excluded the low-quality study by Bordia et al., revealed an even more pronounced decrease in PPG (−37.45 mg/dL), emphasizing the importance of study quality in determining outcomes.[30,31]

Subgroup analysis indicated that randomized controlled trials (RCTs) and studies with intervention periods exceeding eight weeks had a more substantial effect on FPG reduction. Meta-regression further confirmed that study design significantly influenced the outcomes. However, inconsistencies in reporting on dietary habits, medication use, and duration of diabetes across trials contributed to the observed heterogeneity.[30,33]

Variability in fenugreek dosage, formulation, and product quality may have also impacted results. Additionally, factors such as physical activity and baseline metabolic status were not consistently controlled, potentially confounding the findings. Despite these limitations, the results are consistent with prior literature supporting fenugreek's glucose-lowering and insulin-sensitizing properties.[32,34]

Biochemical studies suggest that fenugreek may exert its antidiabetic effects by delaying gastric emptying, slowing carbohydrate absorption, and enhancing insulin sensitivity in peripheral tissues, primarily due to its high fiber and saponin content. These mechanisms are likely responsible for improvements in HbA1c and other metabolic parameters. Moreover, fenugreek may positively affect lipid profiles, further highlighting its utility in managing metabolic disorders.[35,36]

Fenugreek is considered cost-effective, widely accessible, and generally safe for individuals with diabetes and dyslipidemia. However, future trials should adhere to standardized protocols regarding intervention duration, dosage, and outcome measures to ensure consistency and reliability.[37]

Parallel to the clinical analysis, this study also investigated the antidiabetic potential of polyphenol-rich extracts from guava (Psidium guajava L.) leaves, pulp, and seeds in diabetic

mice. Among these plant parts, guava leaves exhibited the highest polyphenolic content and antioxidant capacity, while pulp extracts contained the most vitamin C, surpassing even commonly cited sources like orange juice.[38]

Diabetic mice were administered guava extracts at 200 and 250 mg/kg body weight alongside a basal diet. Key biochemical and hematological parameters were monitored on days 1, 7, 14, and 21. Increased feed intake and body weight gain were observed, especially in groups supplemented for longer durations.

Statistical analysis using one-way ANOVA and Duncan's multiple range test ($p < 0.05$) revealed significant improvements in the lipid profile of treated groups. Supplementation resulted in reduced total cholesterol and LDL levels and increased HDL, suggesting cardioprotective properties of guava-derived polyphenols.[39]

Hematological outcomes also improved significantly. Diabetic mice typically exhibited reduced RBC and WBC counts, but groups treated with pulp and leaf extracts showed increases in these parameters. This implies a potential role for guava extracts in stimulating erythropoiesis, possibly through the activation of erythropoietin via renal pathways.[40]

Furthermore, polyphenol supplementation appeared to enhance immune response in diabetic mice. These results are consistent with previous findings involving polyphenols from grape seeds, green tea, and olive pomace, all of which have shown immunomodulatory and antioxidant benefits.[41] The most significant improvements in mean corpuscular volume (MCV), packed cell volume (PCV), and hemoglobin levels were observed in mice administered 250 mg/kg guava pulp extract for 21 days. In contrast, the diabetic control group exhibited decreased values across these indicators, reaffirming the therapeutic effect of guava extracts.

7) CONCLUSION

The study found that P. guajava leaf and bark extracts can naturally produce α-glucosidase inhibitors, α-amylase inhibitors, and increase muscle glucose absorption. These extracts may help prediabetes patients postpone diabetes development. Fenugreek powder reduces blood glucose levels in prediabetes, demonstrating hypocholesterolemic effects without altering serum TG or HDLc levels. The alkaloids of fenugreek may contribute to its hypoglycaemic effects.

The digestive enzyme (alpha-amylase) is responsible for hydrolysing dietary starch (maltose), which breaks down into glucose prior to absorption. Inhibition of alpha amylase can lead to reduction in post prandial hyperglycaemia in diabetic condition. The given Sample AW - 64.81%was showed moderated inhibition of amylase enzyme when compared to acarbose standard- 91.74%.

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