Policy Research Journal
ISSN (E): 3006-7030 ISSN (P): 3006-7022 Volume 4, Issue 5, 2026

FORMULATION AND FUNCTIONAL EVALUATION OF PROTEIN ENRICHED HERBAL ANTIDIABETIC BAR FOR GLYCEMIC CONTROL

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DOI: https://doi.org/10.5281/zenodo.20036016

Keywords
Antidiabetic protein bars; Functional foods; Nutritional composition; Antioxidant activity; Glycemic control; HbA1c

Article History
Received: 11 March 2026
Accepted: 21 April 2026
Published: 05 May 2026

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Abstract

This manuscript presents original research on the formulation and evaluation of plant-based antidiabetic protein bars enriched with chickpea, red and white kidney beans, and functional herbal ingredients such as cinnamon, fenugreek, and Gymnema sylvestre. Nutritional composition, phytochemical content (total phenolic and flavonoid), antioxidant activity (DPPH assay), sensory evaluation and in vivo efficacy in diabetic patients were assessed. Results showed that the fortified bars possessed nutritional profiles, with 20.16-22.26% protein, 14.5-16.03% fiber, and 1.96-2.70% ash. Phytochemical analysis revealed phenolic content of 325-447 mg GAE/100 g and flavonoids of 161-276 mg CE/100 g, with DPPH scavenging activity between 14.4-23.5%. Sensory assessment indicated acceptable consumer preference, although higher herbal concentrations slightly reduced color and taste scores. Efficacy trials (n = 20) demonstrated significant, dose-dependent reductions in HbA1c levels, with greatest improvement observed in the group receiving the highest fortification. Urinary glucose levels decreased gradually (++ to Nil), indicating enhanced metabolic function, while random blood glucose showed some variability. Overall, the outcomes indicate that herbal-enriched protein bars possess notable nutritional, antioxidant, and antidiabetic effects, supporting their potential as an affordable nutraceutical intervention for glycemic control in diabetes mellitus.

1- INTRODUCTION

Diabetes mellitus (DM) is a major global public health concern and a chronic metabolic disorder characterized by persistent hyperglycemia due to impaired insulin secretion and insulin action (Hillier et al., 2021). Its development is associated with multiple risk factors, including unhealthy dietary habits, sedentary lifestyle, obesity, and genetic predisposition (Galicia-Garcia et al., 2020; Shabbir et al., 2026). Current estimates indicate that over 422 million people are affected worldwide, with projections suggesting a substantial increase to nearly 552 million by 2030, particularly in low- and middle-income countries (GBD, 2023; Akbari-Alavijeh et al., 2020). It involves disturbances in carbohydrate, lipid, and protein metabolism and is associated with obesity and increased BMI (Lau et al., 2019). If untreated, it leads to complications such as neuropathy, nephropathy, and cardiovascular diseases, requiring long-term management through lifestyle modification, diet control, physical activity, and pharmacological therapy (Artasensi et al., 2020).

Phytonutrient-based dietary strategies have gained increasing interest due to their ability to reduce healthcare burden (Barrera et al., 2020; Urooj et al., 2026a). Growing demand for nutraceutical and functional foods is largely attributed to their immunomodulatory properties and ability to reduce oxidative stress (Wang et al., 2019; Khan, 2019). Obesity contributes to disease progression by promoting harmful metabolic by-products through excessive nutrient intake (Eizirik et al., 2020; Thomas, 2019). Medicinal plants have shown anti-obesity and metabolic regulatory effects, supporting their role in disease management (Dhara & Nayak, 2022; Bhat et al., 2018). Plant-based strategies are increasingly considered as complementary due to their cost-effectiveness and lower side effect compared to conventional treatments (Ahmad et al., 2020; Padhi et al., 2020).

Composite flour technology (CFT) involves blending of cereals and legumes to produce nutritionally enriched food products using locally available raw materials at reduced cost. Composite flours typically consist of wheat flour, and incorporation of legumes such as red and white kidney beans and chickpeas which explored to enhance functional quality (Urooj et al., 2026b). Blending wheat with alternative flours serves as a cost-effective and nutritionally strategic approach for improving staple food quality, particularly in regions where malnutrition and protein deficiency are prevalent (Shang et al., 2021; Neto et al., 2020).

Chickpea (*Cicer arietinum*) is a widely cultivated legume grown predominantly, characterized by a high protein, lipid, vitamin, and dietary fiber content. Incorporation of chickpea-based ingredients into bars, snacks, and chips has also been associated with improved nutritional quality and a reduction in acrylamide formation (Iqbal et al., 2026). Red kidney beans (*Phaseolus vulgaris*) are rich in lectins, carbohydrate-binding glycoproteins with diverse biological activities (Singhi & Ozturk, 2026). Phytohemagglutinin (PHA) from kidney beans as a plant lectin with antinutritional and insulin or glucose-interaction effects. In diabetes more of glucose metabolism modulation and dietary legume effects, not as a direct therapeutic agent (Wang et al., 2021).

White kidney beans (*Phaseolus vulgaris*) are a rich source of lectins, carbohydrate-binding glycoproteins that interact with structures in a specific and reversible manner (El-Hack et al., 2020). Lectins have gained attention in diabetes due to their potential influence on glucose metabolism and nutrient absorption. Its widely distributed in plant, with more than 70 distinct seed identified across various leguminous species, investigated for their metabolic and glycemic regulatory effects to diabetes management. (Thenmozhi et al., 2022).

Cinnamomum verum (cinnamon) has been used for centuries due to its culinary therapeutic properties, primarily attributed to cinnamaldehyde, responsible for antidiabetic, antimicrobial, antioxidant, cardioprotective, and lipid-lowering effects (Urooj et al., 2026a). Different plant parts show compositional variability, where bark and leaves are enriched in eugenol, indicating tissue-specific pharmacological potential. Experimental studies have reported that antidiabetic action is linked to insulin-mimetic properties (Al-Qulaly et al., 2021). Supplementation associated with improved glucose and lipid, supporting its potential as a complementary agent alongside conventional antidiabetic drug (Senevirathne et al., 2022).

Trigonella foenum-graecum L. (fenugreek), commonly known as *methi* in Pakistan, is an annual leguminous plant, it's used as a superfood in everyday recipes (Hadi et al., 2020; Tavakoly et al., 2018). Its five active components grown globally and extensively used in traditional diets are derived after HPLC including tri-gonelline, iso-orientin, orientin, vitexin, and iso-vitexin (Yao et al., 2020; Fatima et al., 2018). Its historically utilized in traditional medicine across Ayurveda, Chinese medicine, and ancient Egyptian practices

(Khan et al., 2018; Rashid et al., 2019). Nutritionally, seeds are rich in proteins (20-30%), carbohydrates (40-60%), and lipids (5-10%), along with significant amounts of dietary fiber and phytochemicals (HeshmatGhahdarijani et al., 2020; Ayati et al., 2022). Contemporary research has been studied for its therapeutic applications as supplementation improved in fasting and postprandial glucose levels, and insulin resistance markers in diabetic and prediabetic populations (Ahmad et al., 2021; Alsieni et al., 2021).

Gymnema sylvestre, commonly known as *gurmar* (sugar destroyer) in Pakistan, belonging to the family *Apocynaceae* (Khan et al., 2019; Ahmad et al., 2020). Its traditional medicine like Ayurveda for its use as a functional food and nutraceutical used as standardization markers in commercial formulations (Devangan et al., 2021; Adi et al., 2020). Gymnemic acids are considered pharmacologically active constituents responsible for its antidiabetic and anti-sweet properties which interacts with taste receptors, thereby reducing sweet taste perception and inhibiting glucose uptake in the gastrointestinal tract (Laha and Paul, 2019; Venkatesan et al., 2020). *Gymnema sylvestre* exerts its hypoglycemic effect through multiple mechanisms, like stimulation of insulin secretion, regeneration of pancreatic β-cells, enhancement of glucose utilization, and inhibition of intestinal glucose absorption (Pham et al., 2018; Indumathi et al., 2021).

Early intervention in insulin resistance is an important approach alongside conventional pharmacotherapy. In recent years, phytoconstituents have gained considerable interest due to their multifaceted antidiabetic potential. Medicinal plants such as cinnamon, fenugreek, and *Gymnema sylvestre* have demonstrated significant glucose-lowering effects in both experimental and clinical studies (Akbari-Alavijeh et al., 2020; Ibrahim et al., 2017). In Pakistan, snack bars are commonly consumed dietary products and may serve as convenient functional foods for improving glycemic stability in diabetic individuals. The incorporation of therapeutic ingredients into such products has led to the development of protein bars as suitable dietary options for individuals with diabetes. Therefore, the present study was designed with the following objectives: i. To develop and characterize plant-based antidiabetic protein bars, ii. To evaluate the product's potential efficacy against diabetes mellitus, iii. To assess functional outcomes through experimental trials focusing on glycemic control in diabetic subjects.

2- Materials and Methods

Procurement of raw materials

Cinnamon and fenugreek were collected from the local market of Faisalabad, whereas *Gymnema sylvestre* was purchased from the Ayub Agriculture Research Institute. Chemicals thar were used in these experiments were bought from certified and reputable suppliers. Prior to being used in the study, raw materials were manually cleaned to remove foreign particles, such as dust and dirt and stored under controlled laboratory conditions until further use.

Preparation of high-protein composite flour

High-protein composite flour was prepared using chickpea, white kidney bean, and red kidney bean flours, along with cinnamon, fenugreek, and *Gymnema sylvestre*. Flours used in the study were already pre-processed, while stevia was used as a natural low-calorie sweetener. Composite flour was developed following a total of four formulations of treatments for all ingredients used. Proper mixing was done using procedures as described by Samakradhamrongthai et al. (2021) and Iqbal et al. (2026). Finally, composite flours were packed into polypropylene bags and stored for further analysis.

Preparation of protein bars

Preparation of protein bars was done using the method outlined by Abdel-Salam et al. (2022) where the development and preparation of bars with high energy content were discussed using the steps of mixing, molding, baking, and cooling. T_0 served as the control formulation without functional herbal additives, while T_1, T_2, and T_3 contained increasing concentrations of cinnamon, fenugreek, and *Gymnema sylvestre*. All ingredients were mixed to form a uniform dough, which was moulded into bar shapes. Bars were baked at 225°C for 20-25 min in a laboratory baking oven. After baking, the samples were cooled at room temperature (25°C), packaged and stored in airtight containers for further analyses.

Figure 1: Pictorial representation of raw ingredients and formulated protein bars

Nutritional composition

Nutritional composition of protein bars was determined according to standard methods described by AOAC (2019) procedure. Moisture content was determined using the oven-drying method. 10 g of sample was dried in a hot air oven at 105 °C for 24 hours, and moisture percentage was calculated from the loss in weight. Crude fat content was determined using the Soxhlet extraction method, with hexane as the extraction solvent. Fat percentage was calculated based on the weight of hexane extract relative to the initial sample weight. Crude fiber content was determined using the acid–alkali digestion method. Fiber percentage was calculated based on the weight loss after ignition of the dried residue in a muffle furnace relative to the initial sample weight. Crude protein content of bars was determined using the Kjeldahl method. Nitrogen content obtained after digestion, distillation, and titration was multiplied by a conversion factor of 6.25 to calculate crude protein percentage. Ash content of the protein bars was determined by incinerating the sample in a muffle furnace at 550

°C until white ash was obtained. Ash percentage was calculated based on the weight of the residue relative to the initial sample weight. Nitrogen free extract was determined by subtracting the sum of crude protein, crude fat, crude fiber, ash, and moisture from 100.

Antioxidants activity

Extract preparation

Ethanol extract of protein bars was prepared following the method described by AlJaloudi et al. (2024) with slight modifications. 10 g of finely ground bar sample was macerated in 50 mL of absolute ethanol for 15 min to ensure efficient extraction. Mixture was then filtered and filtrate was adjusted to 100 mL using absolute ethanol to obtain a uniform extract solution. The prepared extract was used for determination of total phenolic content, total flavonoid content, and antioxidant activity analyses.

Total phenolic content (TPC)

Phenolic content of bars extracts was analyzed using the Folin Ciocalteu (FC) colorimetric method as mentioned by AlJaloudi et al. (2024) with gallic acid as the standard. The diluted sample extract was centrifuged at 6000 rpm for 5 min, and 0.05 mL of the supernatant was mixed with 0.5 mL of FC-reagent followed by the addition of 2.5 mL of 7.5% sodium carbonate solution. The mixture was incubated and the absorbance was measured at 765 nm using a spectrophotometer. Total phenolic content was quantified from the gallic acid calibration curve and expressed as gallic acid equivalents (GAE).

Total flavonoid content (TFC)

Total flavonoid in samples were determined using colorimetric method as outlined by Abdel-Salam et al. (2022) using catechin as a standard. Calibration curve was plotted using catechin solution (0.1 mg mL^{-1}) at various concentrations ranging from 0 to 0.05 mL. For sample preparation, bar extracts were diluted 10-fold with distilled water followed by centrifugation at 6000 rpm for 5 min at 15 °C. 0.25 mL of centrifuged extract was diluted with 0.5 mL of distilled water. Diluted extract was then mixed with 0.3 mL of 5% sodium nitrite ($NaNO_2$) solution and incubated for 5 min. Then, 0.3 mL of 10% aluminum chloride (AlCl3) solution was added and incubated for another 6 minutes at ambient temperature (26 ± 2°C). Thereafter, 1 M sodium hydroxide (NaOH) solution was added to make final volume 1 mL followed by incubation for 5 min. Absorbance of the reaction mixture was measured at 510 nm using a spectrophotometer. Total flavonoid content was calculated from calibration curve and expressed as catechin equivalent (CE) per mL of sample.

DPPH radical scavenging activity

Antioxidant activity of bar samples was determined using the DPPH radical scavenging assay according to the method described by Tomassi et al. (2025) with slight modifications. For sample preparation, the extracts were centrifuged at 5000 rpm for 25 min at 15 °C, and the supernatant was collected by filtration. A DPPH solution was prepared by dissolving 4 mg of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in 80 mL of methanol. Subsequently, 1 mL of the extract was mixed with 3 mL of the DPPH solution, and the reaction mixture was kept in the dark for 40 min at room temperature. After incubation, the absorbance of the mixture was measured at 517 nm using a spectrophotometer. A blank solution containing methanol and DPPH without sample extract was also prepared. The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\text{DPPH Scavenging Activity (\%)} = \left(\frac{A1-A2}{A1}\right)\times 100$$

Where:

A_1 = absorbance of the blank sample

A_2 = absorbance of the test sample at 517 nm

Sensory evaluation

Sensory evaluation of anti-diabetic bars was done according to the standardized procedures reported by You et al. (2024) and Samakradhamrongthai et al. (2021) for protein bars and cereal-based bars. Freshly made bars were sliced to form cuboid shapes (5 cm × 3.4 cm × 2.2 cm). Each bar was coded with a randomly 3-digits. They were then individually served on disposable plates within the laboratory environment. 20-trained and semitrained judges from the National Institute of Food Science and Technology (NIFSAT) were recruited to evaluate the sensory properties of the bars. This was done according to the sensory evaluation process explained by Butt et al. (2026). Prior to testing, the panelists were briefly trained regarding the sensory attributes to ensure uniform understanding of evaluation criteria. Sensory characteristics evaluated included color, flavour, taste, texture, and overall acceptability. Samples were evaluated using a 9-point hedonic scale, where 9 indicated "like extremely" and 1 indicated "dislike extremely" (Urooj *et al.* 2026b). Panelists were advised to rinse their mouths with water before evaluating each bar.

Selection of optimized formulation

For the efficacy trial, three optimized formulations (T_1-T_3) were selected based on their compositional and functional properties and designated as PB-I, PB-II, and PB-III, respectively. Thus, PB-I corresponded to formulation T_1 (low concentration), PB-II to T_2 (medium concentration), and PB-III to T_3 (high concentration of bioactive ingredients). These formulations were used for in vivo evaluation in diabetic patients.

Efficacy study

In total of twenty (n = 20) subjects were chosen as diagnosed diabetics from Mutahir Memorial Hospital, Attock. Participants were randomly allocated into 5 groups, 4 in each. Normal control group (G_0), consuming a standard diet without being diabetic, and diabetic control group (G_1), which receiving usual diet without any intervention. The other 20 diabetic patients were evenly distributed into 3 treatment groups (G_2, G_3, and G_4). These groups received their regular diet along with various preparations of protein bars (PB-I, PB-II, and PB-III, respectively). All selected participants were clinically diagnosed with diabetes mellitus and were receiving prescribed medication, including insulin injections where necessary. Pre-diabetic patients were excluded from the experiment to achieve homogeneity and ensure accurate assessment of the intervention effect. Baseline counselling was provided to all participants on day 1st of the study regarding diet consumption and monitoring procedures.

Table 1: Treatment plan for efficacy study

Groups	Description	Treatments
G_0	Normal control	Normal Diet
G_1	Diabetic control	Normal Diet
G_2	Diabetic	Normal + PB-I
G_3	Diabetic	Normal + PB-II
G_4	Diabetic	Normal + PB-III

Biochemical analysis

Random blood glucose (RBG)

Capillary blood glucose measurement using a glucometer is a reliable and rapid method as reported by Nayeri et al. (2014). RBG was evaluated to assess glycemic control and the body's ability to regulate blood glucose levels under non-fasting conditions. Blood glucose levels were measured using a calibrated digital glucometer based on biosensor technology, as described in previous studies, with slight modifications. For sample collection, a capillary blood drop was obtained from the fingertip using a sterile lancet under aseptic conditions. A trained registered nurse performed all sampling procedures to ensure accuracy and safety. Sample was immediately applied to a test strip inserted into the glucometer, and glucose concentration was displayed directly on the digital screen. All readings were recorded for further statistical analysis.

Glycated hemoglobin (HbA1c)

According to Yun et al. (2023), it is vital to consider that HbA1c testing methods are critical in managing diabetes cases, was performed to evaluate the average blood glucose levels. HbA1c estimation involved the collection of venous blood from each participant under sterile conditions and analyzed in a clinical laboratory using standard diagnostic procedures. The findings obtained from this test were useful in determining the glycemic status and confirming the presence and degree of diabetes. According to standard diagnostic criteria, HbA1c values lower than 5.7% were indicative of a normal glycemic status, while values ranging from 5.7% to 6.4% indicated prediabetes, while values equal to or greater than 6.5% confirm diabetes mellitus.

Urine glucose test

Urinary glucose testing was carried out to examine the presence of glucose excretion in urine. Lakhera et al. (2025) and Pighi et al. (2023) reviewed modern researches conducted on urinary glucose excretion during normal physiological functions, when there is no glucose found in the urine; but in uncontrolled cases of diabetes mellitus, hyperglycemia can result in the development of glucosuria, due to decreased ability of kidneys to absorb the sugar. Fresh urine samples from individuals and obtained, using standard qualitative methods. Presence of glucose in urine was assessed using a semi-quantitative scale, where results were expressed as negative, traces, +, ++, or +++, indicating increasing levels of glucose concentration in urine.

Statistical analysis

All collected data were subjected to statistically analysis using a Completely Randomized Design (CRD). For significant ($p<0.05$) differences between treatments, one-way ANOVA test was used as indicated by Butt et al. (2026). Biochemical analysis involved two-way factorial, as noted by Urooj et al. (2026c).

3- Results and Discussions

Nutritional composition

Nutritional composition is an important indicator of nutritional quality and suitability of food; composition of protein bars as shown in Table 2. Moisture content is an essential affecting storage life, texture, and microbial stability of foods. Higher moisture increases susceptibility to microbial activity and reduces shelf-life. Moisture content of the bars ranged from 16.9% to 21.9%. T_0 sample showed the lowest moisture content (16.9 ± 0.66%), followed by T_1 (18.0 ± 0.56%), while T_2 (21.4 ± 0.53%) and T_3 (21.9 ± 0.42%) showed higher contents. The increased moisture in T_3 may be attributed to the higher levels of fenugreek and gymnema, which possess greater water-holding capacity. Crude fat is an important component contributing to energy, flavor, and texture in food products. The highest crude fat content was measured in T_3 (20.8 ± 0.79%), while lowest was observed in T_1 (6.3 ± 0.87%). The control sample T_0 showed a moderate value (13.0 ± 0.65%), whereas T_2 showed a lower level (8.09 ± 0.66%). It could be due to added quantities of cinnamon, fenugreek, and gymnema, which contribute to fat content, as presented by Gul et al. (2016).

Proteins are the primary components responsible for body growth, development, and repair and ranged from 20.16% to 22.26%. In control sample T_0, the lowest amount of protein was measured (20.16 ± 0.37%), followed by T_1 (20.79 ± 0.17%), T_2 (21.62 ± 0.39%), and T_3 (22.26 ± 0.34%). With increasing amounts of cinnamon, fenugreek, and gymnema, there is an increase in the content of proteins in the food product. These observations have also been made by Gorissen et al. (2018) regarding the importance of plant-based ingredients in food products. Fiber content of crude bars was in the range of 14.5% to 16.03%. The maximum fiber content was recorded in T_2 (16.03 ± 0.15%), while the minimum value was recorded in T_3 (14.5 ± 0.40%). T_0 (15.8 ± 0.10%) and T_1 (15.5 ± 0.25%) showed relatively higher values than T_3. The increase in content in fortified bars could be attributed to the presence of dietary fibers in fenugreek. Sun et al. (2021) also reported an increased fiber content in fortified bars made from cereal-based diets with fenugreek. Mineral content present in food products is represented by ash content, which increases gradually with the increase in the addition of cinnamon, fenugreek, and gymnema. From Table 2, ash content ranged from 1.96 ± 0.04% in T_0 to 2.70 ± 0.05% in T_3. Increased ash content in T_3 suggests higher mineral content due to additional plant ingredients, consistent with Samuel et al. (2020). Nitrogen-free extract (NFE), showing carbohydrate content and a key energy source, varied between 18.69% to 37.25%. The maximum NFE was observed in T_1 (37.25 ± 0.82%), followed by T_0 (32.18 ± 0.75%) and T_2 (31.25 ± 0.70%), while the minimum value was recorded in T_3 (18.69 ± 0.64%). The reduction in NFE with increasing concentrations of cinnamon, fenugreek, and gymnema could be due to higher proportions of fiber and other non-carbohydrate constituents in the formulation.

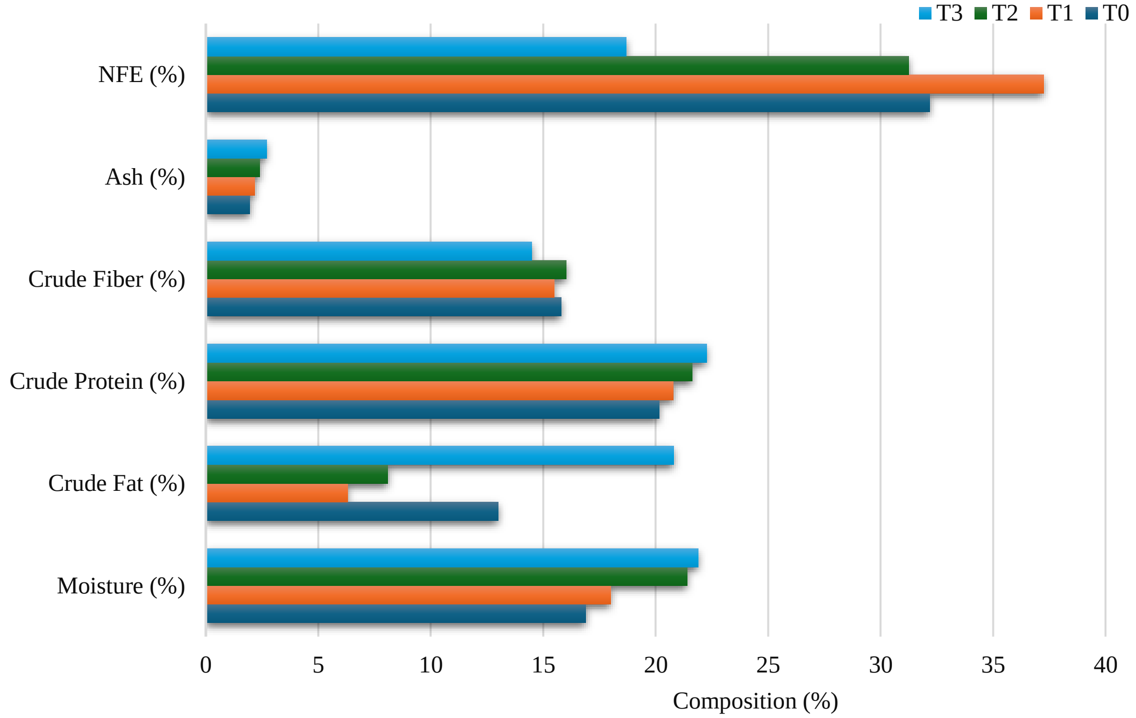

Figure 2: Graphical representation of effect of different treatments on nutritional composition of protein bars

Antioxidant activity

Phytochemicals are plant-derived secondary metabolites that indicate the functionality and health properties of food products. As shown in Table 3, phenolic have a strong antioxidant and free radical scavenging activity that helps to reduce oxidative damage. The total phenolic content in protein bars ranged from 325-447 mg GAE/100 g. The highest value was noted in T_0 (447 ± 18.1 mg GAE/100g) followed by T_1 (412 ± 18.1 mg GAE/100g), and T_2 (369 ± 14.7 mg GAE/100g), while the lowest was determined in T_3 (325 ± 12.8 mg GAE/100g). The decrease in phenolic content with increasing herbal additives may be due to interactions among phytonutrients, which are consistent with Getachew et al. (2017), who concluded that plant additives significantly influence phenolic composition in functional foods. Flavonoid content varied significantly among treatments, with the highest value in T_0 (276 ± 24 mg QE/100g), followed by T_1 (238 ± 20.8 mg QE/100g), and T_2 (193 ± 12.3 mg QE/100g), while the lowest level was found in T_3 (161 ± 18.7 mg QE/100g). The decrease in fortified treatments may be attributed to processing conditions and interaction between herbal components and the food matrix, as reported by Li et al. (2021). Activity was assessed using the DPPH assay, was determined via % inhibition as depicted in Table 3. The highest activity was observed in T_1 (23.5 ± 1.4%), followed by T_0 (20.0 ± 1.2%), and T_2 (16.0 ± 0.9%), whereas the lowest was recorded in T_3 (14.4 ± 0.8%), as such variations in antioxidant activity were reported by Huneif et al. (2022).

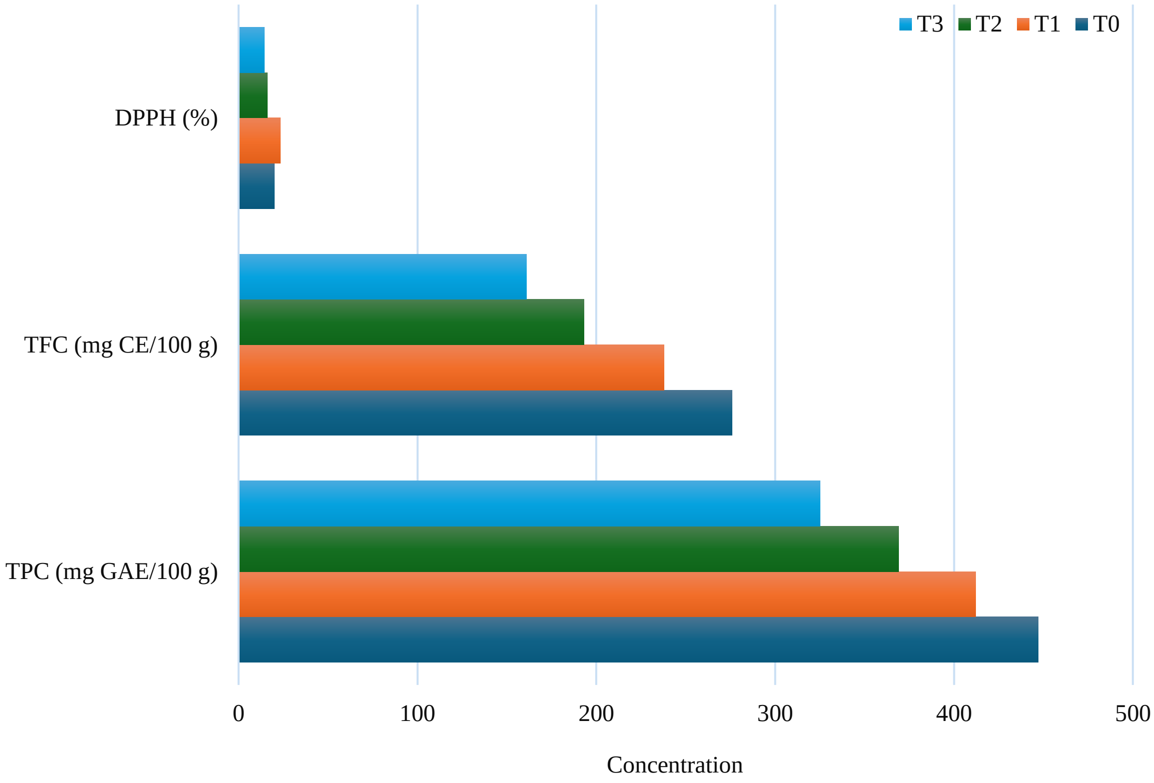

Figure 3: Graphical representation shows the effect of various treatments on phytochemical profile and antioxidant activity of protein bars

Sensory evaluation

Sensory evaluation of bars was conducted to assess consumer acceptability. Color plays an essential role in consumer perception, marketability, and taste. Color scores ranged from 8.7 ± 0.05 (T_0) to 6.7 ± 0.15 (T_3), suggesting a significant decrease with increasing levels of cinnamon, fenugreek, and *Gymnema sylvestre*. The highest acceptability was recorded in T_0 due to the absence of herbal additives, while T_3 showed the lowest value because of high concentrations of plant powders. Similar findings were provided by Malecki et al. (2022), where a drop in color acceptability in fortified bars than the control formulation due to higher pigment and fiber content.

Flavor is the combined perception of taste and aroma ranged from 6.7 ± 0.05 to 6.8 ± 0.1, showing no insignificant differences among the treatments ($p > 0.05$). Slight reductions at higher supplementation levels may be linked to bitterness. These results are consistent with Samakradhamrongthai et al. (2021), who stated lower flavor acceptability in functional bars containing higher proportions of bioactive ingredients owing to phytochemical bitterness.

Taste acceptability revealed slight differences between treatment groups, with scores of 6.80 ± 0.10 (T_0) and 6.82 ± 0.29 (T_2). An evident decrease in the score is observed with the increase of herbal content, especially in case of T_3. Such a trend may be associated with the intense phytochemicals of fenugreek and *Gymnema sylvestre* that might cause a slight bitterness at high concentrations. These conclusions by Sahni et al. (2022) who stated that an increase of functional ingredient concentration resulted in decreased taste acceptability of cereal-based bars. Texture contributes greatly to mouthfeel that varied slightly from 7.50 ± 0.01 (T_0) to 7.44 ± 0.01 (T_3). This stability of scores could be associated with stable baking parameters and base composition used in all treatments. The observations are in line with those of Parit et al. (2018) who stated that processing conditions played a more important role than ingredient changes in maintaining texture stability of baked products. Overall acceptability perception of the product as the highest in T_0 (8.2), followed by in T_1 (7.6), T_2 (7.2), and T_3 (6.8). The decline in acceptability due to increase in concentration of herb indicates that while functional ingredients help in increasing the nutrition content, they slightly affect sensory characteristics when used in larger concentrations. The same results were reported by Ramírez-Jiménez et al. (2018) in protein-enriched biscuits using plant ingredients.

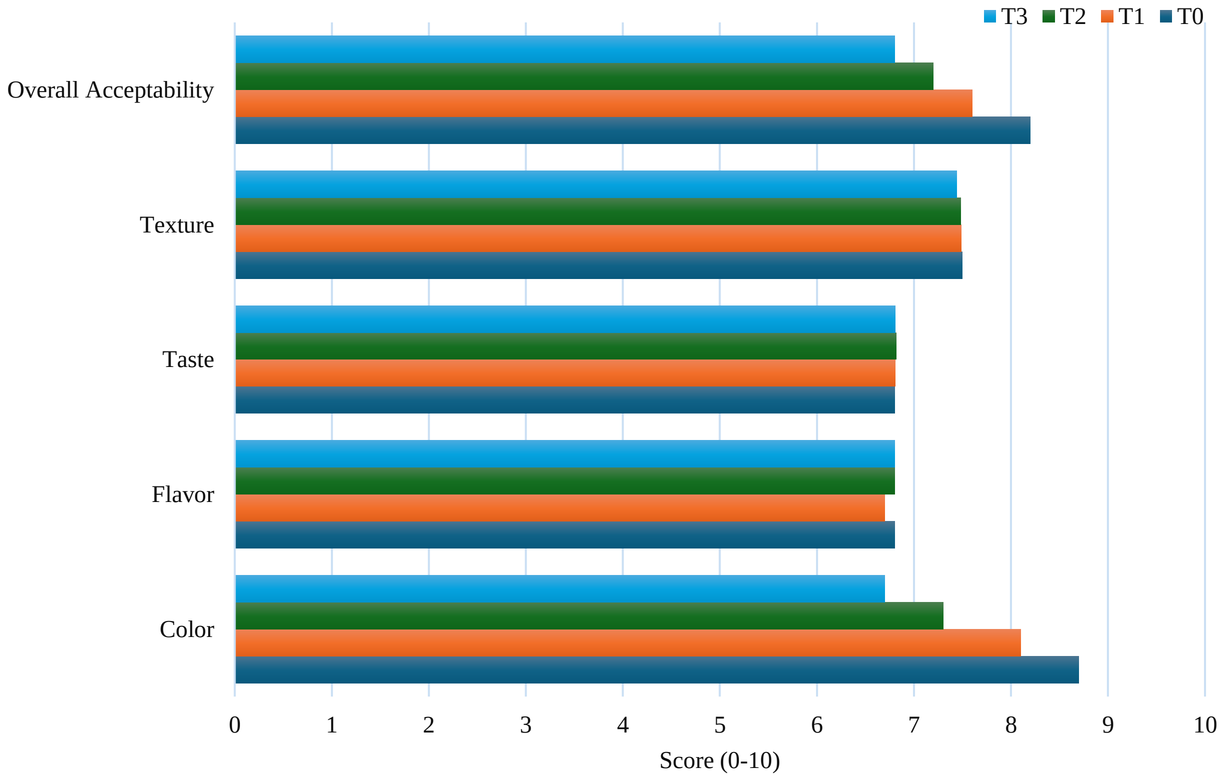

Figure 4: Graphical representation of sensory evaluation of protein bars across treatments

Hemoglobin A1c (HbA1c) test

HbA1c is a widely recognized biomarker for monitoring glycemic control in individuals, showing average glucose concentration in the blood. Based on clinical standards, HbA1c concentrations of less than 5.7%, 5.7-6.4%, and more than 6.5% denote normal glycemic profile, prediabetes, and diabetes mellitus, respectively (English et al., 2018). Its one of the primary markers for assessing the effectiveness of formulated functional bars containing plant bioactive compounds. At baseline, all experimental subjects had remarkably high HbA1c concentrations (12.5-30.3%), implying insufficient glycemic control. Notably, no changes in the HbA1c concentration were recorded in control group (G_1) over the experiment duration, which shows that hyperglycemia persists even when the patients are adhering to the regular diet. In comparison groups (G_2-G_4) experienced gradual decreases in HbA1c concentrations. A dose-dependent response was observed, with PB-I (G_2) showing mild improvement, PB-II (G_3) demonstrating moderate reduction, and PB-III

(G_4) producing the most significant decline in HbA1c levels. This suggests that increasing concentrations of functional ingredients such as cinnamon, fenugreek, and *Gymnema sylvestre* enhance antihyperglycemic efficacy. These results are in line with Yang et al. (2022) found that modulation of the gut microbiome through bioactive compounds improved glucose metabolism and alleviation of hyperglycemia. Likewise, Khalaf et al. (2023) observed that herbal extracts lowered insulin resistance and blood glucose levels in obese rat models. The observed antihyperglycemic synergy of functional herbal ingredients also contributes to reduced glycemic load, enhanced satiety, and improved post-prandial glucose response. Previous studies have shown that consumption of such functional foods markedly decreases HbA1c levels.

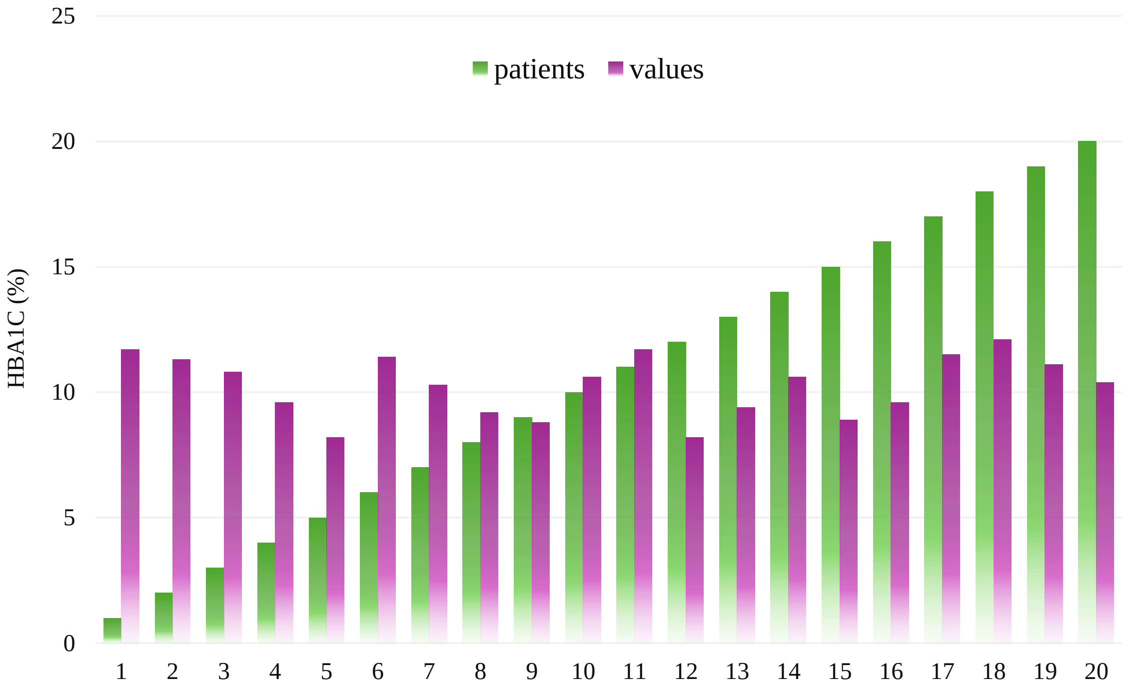

Figure 5: Graphical representation HbA1c test values of patients

Random blood glucose (RBG)

Random blood glucose (RBG) was assessed to evaluate changes in blood glucose levels. In healthy individuals, RBG typically ranges from 80-160 mg/dL, while values between 160-200 mg/dL indicate a pre-diabetic condition (Lazarus et al., 2021). The results showed that the control group (G_0) maintained relatively stable glucose levels throughout the study period (139 ± 0.25 to 136 ± 0.42 mg/dL). The diabetic control group (G_1) showed consistently elevated glucose levels ranging from 197 ± 0.21 to 206 ± 0.20 mg/dL. In contrast, treatment groups receiving bars showed varying responses to the intervention. G_2 showed an increase in RBG from 256 ± 0.12 to 323 ± 0.12 mg/dL, while G_3 demonstrated fluctuation with values of 298 ± 0.25, 251 ± 0.10, and 302 ± 0.42 mg/dL. Similarly, G_4 exhibited higher variability with values ranging from 263 ± 0.21 to 365 ± 0.20 mg/dL. The highest improvement in glycemic control was observed in the group receiving Protein Bar III (G_4 formulation), which contained the highest concentration of cinnamon (6%), fenugreek (7%), and *Gymnema sylvestre* (3%). The findings of the present study are consistent with Perry et al. (2021), who reported that plant-based bioactive compounds significantly influence blood glucose regulation in diabetic models.

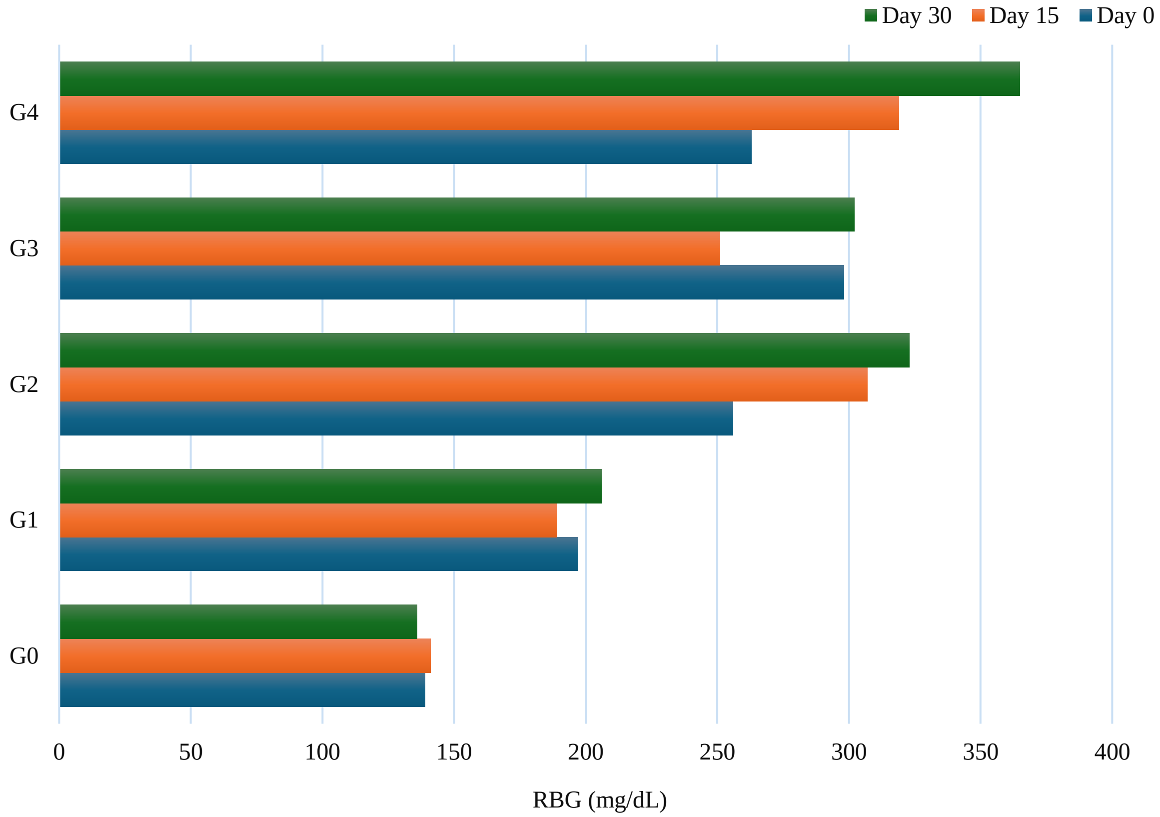

Figure 6: Graphical representation of random blood glucose in groups

Urine glucose test (UGT)

Glucose in urine is a supportive diagnostic test that can help diagnose the presence of glucose in the urine (glycosuria). Normally, the concentrations of glucose in the urine are in the range of 0 to 0.8 mmol/L. Abnormally high concentrations of glucose in the urine are noted when individuals suffer from diabetes due to reduced renal threshold. Chronic hyperglycemia leads to the development of several adverse health effects such as retinopathy, nephropathy, neuropathy, polyphagia, polyuria, and polydipsia (El-Deyarbi et al., 2024). In the current study, urine samples were collected during different time intervals including 1st morning urine and after hydration urine, then analyzed in laboratory areas. The qualitative results based on glucose concentrations was as follows: <1.5 mmol/L were registered as (+), <2.5 mmol/L as (++), and >3.5 mmol/L as (+++), whereas the concentration of glucose in normal controls was nil. It was found out that the normal control group demonstrated no detectable amount of glucose in the urine (Nil). Meanwhile, the diabetic control group possessed high concentrations of glucose in the urine (++), while the diabetic treatment group revealed progressive decrease in urinary glucose levels.

Table 5: Results of urine glucose (mmol/L) tests of diabetic patients

Groups	Urine glucose
G_0	Nil
G_1	++
G_2	+
G_3	Trace

G_4	Nil

4- Conclusion

This study developed and evaluated functional protein bars enriched with cinnamon, fenugreek, and *Gymnema sylvestre* were prepared, tested, and evaluated for their effectiveness in addressing anti-diabetic properties through the use of chickpea, white kidney bean, and black kidney bean composite flour. sensory evaluation tests, it was determined that consumer acceptability was satisfactory among all the different types of formulated samples, but increased herbal fortifications resulted in decreased acceptability, color, and taste, with minimal changes to the flavor and texture of the protein bars. Nutritional assessment showed that the presence of the herbs resulted in improved proximates, especially in terms of protein, fat, and minerals content, thereby proving the efficacy of the herbs incorporated. Phytochemical analysis revealed appreciable levels of phenolics, flavonoids, and antioxidant activity across all treatments, supporting their bioactive potential. Despite a gradual decline in total phenolic content and total flavonoid content as a result of increased fortification, the antioxidant capacities still remained considerable. Clinical tests showed a positive change in the glycemic index, including decreased hemoglobin A1c levels, stabilized random blood glucose, and improved urine glucose levels when the highest level of fortified product was consumed. Overall findings suggest a positive role in glycemic regulation without adverse effects on body composition. Herbal-enriched protein bars possess strong functional, nutritional, and therapeutic potential and may serve as a promising dietary strategy for the management of type 2 diabetes mellitus.

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