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Effect of Roselle-Beetroot-Fig Blended Infusions on Blood Glucose and Glycemic Response in Rats

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Abstract
This study evaluated the antihyperglycemic effects and glycemic index (GI) of roselle (*Hibiscus sabdariffa*), fig (*Ficus carica*), and beetroot (*Beta vulgaris*) infused beverages in rats. Formulations included RBF (20% fig, 36.29% beetroot, 43.71% roselle), RFF (45% fig, 55% roselle), BFF (42.5% fig, 57.5% beetroot), and FBR (35% beetroot, 65% roselle). Beverages were prepared at 2.5%, 5.0%, and 7.5% concentrations. Glucose and a commercial herbal tea served as reference and comparator, respectively. All formulations significantly ($p < 0.05$) reduced postprandial blood glucose (PBG) compared to the glucose control, which exhibited a sharp spike (>300 mg/dL). A dose-dependent response was observed, with 7.5% concentrations producing the greatest glycemic attenuation. Notably, FBR and RBF reduced peak glucose levels by up to ~70%. All beverages were classified as low GI (≤55), with values ranging from ~30% to 47%. The lowest GI was observed in FBR (30%) and RBF (35%) at 7.5%, outperforming the commercial herbal tea. These findings highlight the potential of optimized botanical infusions as functional, low-GI dietary interventions for managing hyperglycemia, metabolic syndrome, and type 2 diabetes.

Keywords: Postprandial glycemia, Glycemic index, Functional beverages, Antihyperglycemic activity, Botanical blends

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Introduction

Postprandial hyperglycemia, defined as elevated blood glucose after eating, is an important predictor of metabolic dysfunction and a risk factor for type 2 diabetes mellitus (T2DM) and its complications (Kaur *et al*., 2020). The glycemic index (GI), which quantifies the blood glucose-raising potential of carbohydrate-containing foods relative to a glucose reference, is widely used to assess postprandial glycemic response and to inform dietary interventions aimed at improving glycemic control (Kaur *et al*., 2020). GI is typically determined by measuring blood glucose at multiple time points after ingestion and calculating the incremental area under the glucose response curve, which provides an integrated measure of glycaemic impact over time (Kaur *et al*., 2020; Röhling *et al*., 2019).

Functional beverages formulated from plant materials rich in bioactive compounds, such as phenolic compounds, flavonoids, and anthocyanins, can influence carbohydrate digestion, glucose absorption, and metabolic signaling pathways (Li *et al*., 2017), resulting in differential postprandial glycemic and insulinemic responses depending on carbohydrate content.

Many traditional food plants contain phytochemicals with bioactive properties relevant to glycemic regulation. *Hibiscus sabdariffa* (roselle) is rich in polyphenols and anthocyanins that exhibit antioxidant activity and have been associated with antidiabetic effects in experimental models (Banwo *et al*., 2022; Jamrozik *et al*., 2022). Scientific reviews indicate that roselle extracts can exert hypoglycemic activities and antioxidant effects in vivo, suggesting potential roles in modulating glucose metabolism (Jamrozik *et al*., 2022). *Beta vulgaris* (beetroot), another common plant used to prepare functional infusions, contains phenolic compounds such as epicatechin, gallic acid, and rutin that confer antioxidant properties (Arjeh *et al*., 2022). Although limited, evidence also suggests that plant decoctions, including those from fig (*Ficus carica*) and related species, may influence postprandial blood glucose levels, possibly through mechanisms distinct from insulin secretion (Ahmadi *et al*., 2016).

Despite the recognized importance of GI and the growing interest in plant-based beverages, there remains limited evidence on the combined effects of roselle, beetroot, and fig infusions on in vivo glycemic response. Therefore, this study aimed to evaluate the blood glucose response and glycemic index of rats administered beverage infusions formulated from roselle calyx, beetroot, and fig fruit blends at varying concentrations. These findings provide foundational insight into the potential of such botanical beverages to modulate postprandial glycemic response.

Materials and Methods

Sources of materials

Fig fruits (*Ficus carica* L.) were freshly collected from Osun State University, Osogbo, Nigeria, while beetroot (*Beta vulgaris* L.) and roselle calyces (*Hibiscus sabdariffa* L.) were sourced from Igbona Market, Osogbo, Osun State, Nigeria.

Sample preparation

Production of sample powder

Fig fruits and beetroots were carefully inspected to remove damaged, discolored, or extraneous materials, followed by thorough washing with distilled water to eliminate impurities. The cleaned samples were then shredded into smaller pieces and dried in a hot air oven at 40 °C for three days before being milled into powder. Dried roselle calyces were prepared following the method of Famurewa *et al*. (2017) with minor modifications. The calyces were sorted to remove foreign materials, winnowed, and hot air-dried at 40 °C for 12 hours until a constant weight was reached, after which they were ground into powder. All resulting powders were stored in airtight polyethylene bags at room temperature (approximately 27 ± 2 °C) until required for further use.

Sample mixture design

The proportions of the powder mixtures were determined using Response Surface Methodology (RSM) to assess total phenolic content across 33 different combinations. From these, the mixture exhibiting the highest total phenolic content, along with the three individual powders, was selected, resulting in a total of seven samples. This approach ensures that the selected proportions reflect both the optimal bioactive blend and the contribution of each individual component, providing a clear rationale for the mixture design.

Beverage formulation and preparation

Each powder sample was accurately weighed and blended using a Kenwood blender according to the proportions presented in Table 1 to obtain four composite formulations. The individual powders (roselle, beetroot, and fig) were also retained as control samples. Each mixture was blended for 3 minutes to ensure homogeneity. The blended powders were subsequently packed into non-drip tea bags using a suitable filter material and sealing machine, with each tea bag containing approximately 2.5 g of powder. To evaluate the effect of concentration on glycemic response, the infusions were prepared as presented in Figure 1 at three different concentrations of 2.5%, 5.0%, and 7.5% (w/v) by varying the amount of powder relative to the volume of water. Each tea bag was infused in 100 mL of distilled water at 90 °C for 5 minutes and then allowed to cool to room temperature before administration. A glucose solution (50% w/v) was prepared and used as the reference for glycemic index determination. In addition, a commercial control infusion (COM) was prepared according to label instructions by adding 300 mL of boiling water to two tea bags and allowing it to infuse for 10–15 minutes before cooling.

Ethical approval

The animal experiments adhered to the applicable laws and regulations governing animal use. They were conducted with approval from the Federal University of Technology Akure, Nigeria, Centre of Research and Development Ethical Committee with ethical number FUTA/ETH/25/256.

Experimental animals and fasting protocol

Male Wistar rats (180–220 g) were obtained from the animal facility at the Department of Biochemistry, University of Ibadan, Oyo State, Nigeria. A total of 24 rats were used and randomly assigned to six treatment groups (n = 4 per group), corresponding to the different infusion formulations and control treatments: RBF, RFF, BFF, FBR, commercial herbal tea, and glucose control. Rats were housed under standard laboratory conditions (22–25 °C, 12 h light/dark cycle) with free access to standard rat chow and water. They were fasted overnight (12 h) before testing to ensure baseline stabilization of blood glucose, a standard procedure in rat glycemic response studies (Li and Hu, 2022). Each rat received the assigned infusion (2 mL) by oral gavage prior to the first blood glucose measurement.

Blood glucose response determination

Blood glucose concentrations were recorded using a validated glucometer immediately at each indicated time point. Blood was sampled from the tail vein at designated time points (0, 15, 30, 60, 90, and 120 min) after oral administration of test beverages and the glucose reference, in line with established protocols for evaluating postprandial glycemic response in rats (Boaventura *et al*., 2023). Blood glucose values were obtained as single measurements per time point. No replicates were performed.

Table 1: Sample Formulation

Sample code	Fig fruit powder (%)	Beetroot powder (%)	Roselle powder (%)
RBF	43.712	36.288	20
BFF	42.5	57.5	0
RFF	45	0	55
FBR	0	35	65
FFF	100	0	0
BTF	0	100	0
RCF	0	0	100

Note: RBF (20% fig + 36.288% beetroot + 43.712% roselle), RFF (45% fig + 55% roselle), BFF (42.5% fig + 57.5% beetroot), FBR (35% beetroot + 65% roselle), RCF (100% roselle), FFF (100% fig), BTF (100% beetroot) powder blends.

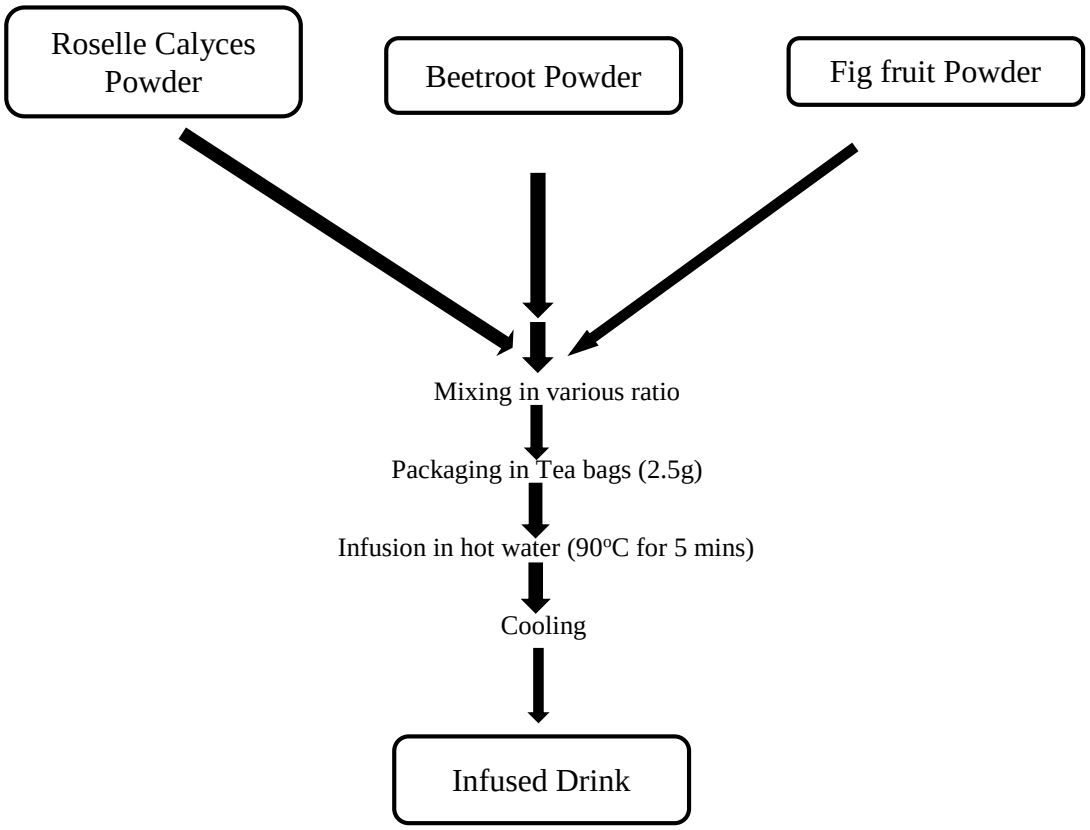

Figure 1: Flow chart of beetroot-fig fruits-roselle calyces infused beverage

Glycemic index determination

The incremental area under the blood glucose response curve (iAUC) was calculated using the trapezoidal rule, considering only areas above baseline (fasting glucose) (2, 4). Glycemic index (GI) for each beverage was computed by comparing the iAUC of the test beverage with that of the glucose reference and expressing it as a percentage (glucose reference GI = 100) (Li and Hu, 2022) as shown in the formula below:

$$GI = \frac{\text{iAUC for test beverage}}{\text{iAUC for glucose reference}} \times 100$$

Statistical analysis

All results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using (Version 25.0, IBM Corp., Armonk, NY, USA). Differences among the means of glycemic index values for the various beverage formulations were analyzed using one-way analysis of variance (ANOVA). Where significant differences were observed, Duncan's Multiple Range Test (DMRT) was used for post hoc comparison of means. Statistical significance was accepted at $p < 0.05$.

Results

Blood glucose response of rats fed with the infused beverages

The effects of the infused roselle–beetroot–fig beverages on postprandial blood glucose (PBG) concentrations are illustrated in Figures 2–4. The glucose control group exhibited a classic glycemic spike, peaking between 15 and 30 minutes (>300 mg/dL) and remaining elevated before a late-phase decline, consistent with the rapid intestinal absorption of monosaccharides.

In contrast, all experimental formulations (RBF, BFF, RFF, and FBR) significantly attenuated postprandial hyperglycemia, maintaining blood glucose concentrations substantially lower than the glucose reference ($p<0.05$). The PBG curves demonstrated a clear, dose-dependent modulation of glucose homeostasis. At the 2.5% concentration, a distinct but controlled rise in glucose was observed across all blends, peaking at 15 minutes followed by a gradual decline toward baseline over 120 minutes. However, at the 7.5% concentration, the infusions exhibited a remarkably blunted profile. Specifically, the curves for RBF and FBR remained significantly lower than the 2.5% dosages, representing a near-total suppression of the typical glycemic spike. The commercial herbal tea (COM) produced intermediate glucose responses, demonstrating moderate glycemic modulation that was less effective than the 7.5% experimental formulations.

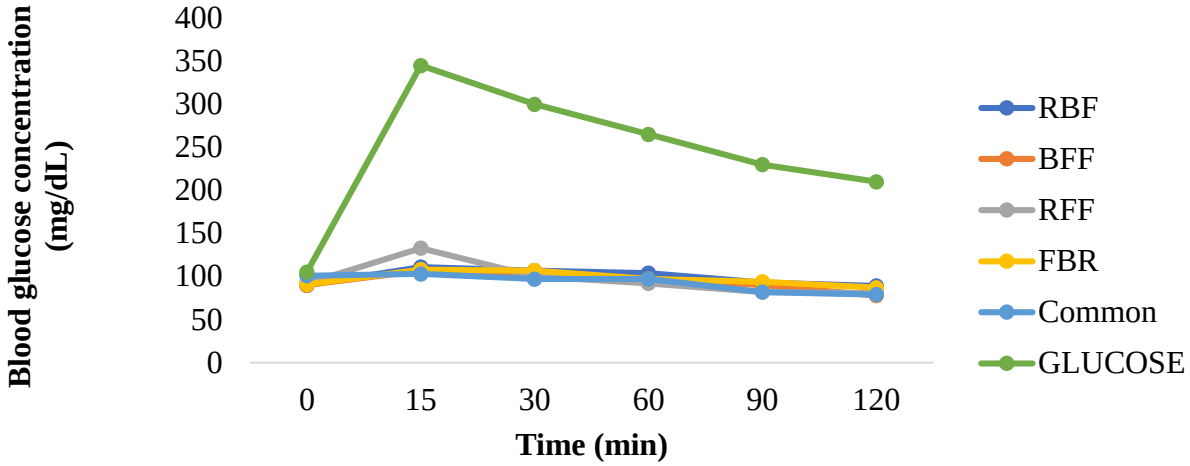

Figure 2: 2.5% Postprandial blood glucose response of rats fed with the infused beverages
Note: RBF (20% fig + 36.288% beetroot + 43.712% roselle), RFF (45% fig + 55% roselle), BFF (42.5% fig + 57.5% beetroot), FBR (35% beetroot + 65% roselle), Common (commercial herbal tea).

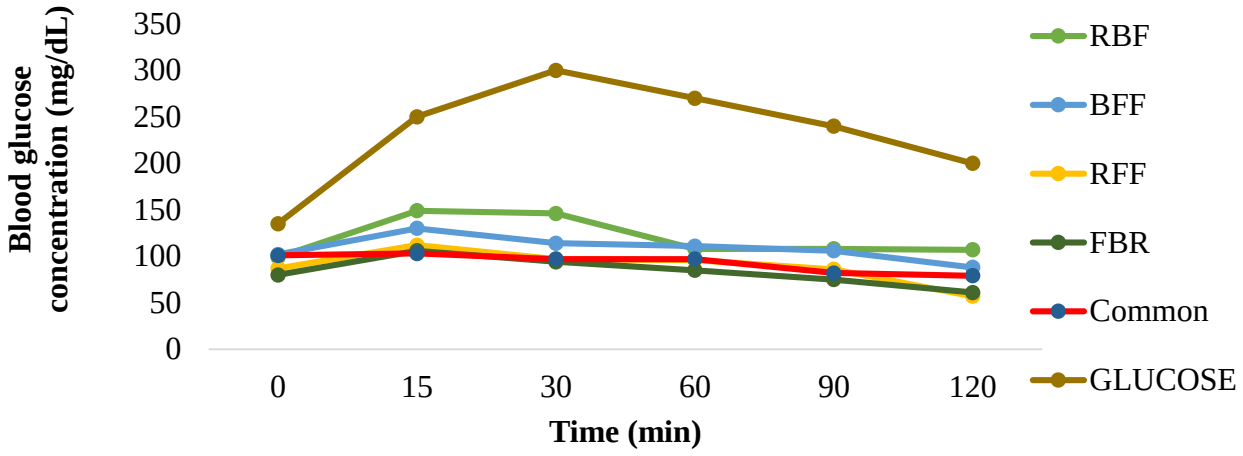

Figure 3: 5.0 % Postprandial blood glucose response of rats fed with the infused beverages
Note: RBF (20% fig + 36.288% beetroot + 43.712% roselle), RFF (45% fig + 55% roselle), BFF (42.5% fig + 57.5% beetroot), FBR (35% beetroot + 65% roselle), Common (commercial herbal tea).

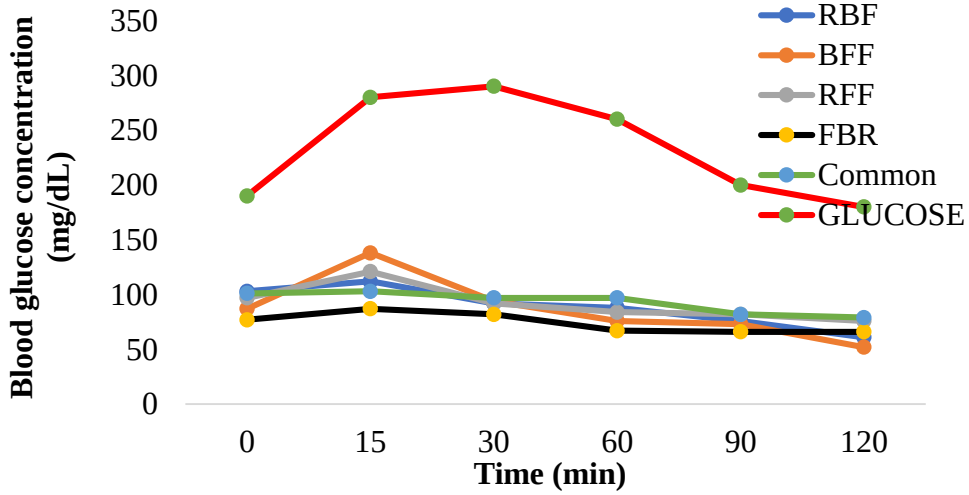

Figure 4: 7.5% Postprandial blood glucose response of rats fed with the infused beverages
Note: RBF (20% fig + 36.288% beetroot + 43.712% roselle), RFF (45% fig + 55% roselle), BFF (42.5% fig + 57.5% beetroot), FBR (35% beetroot + 65% roselle), Common (commercial herbal tea).

Among the test beverages, the roselle–fig blend (RFF) and roselle–beetroot blend (FBR) consistently produced the lowest postprandial glucose levels across both dosages. For instance, at 7.5% concentration, FBR reduced the glucose peak by approximately 70% relative to the pure glucose reference. In contrast, fig-only (FFF) and beetroot–fig (BFF) formulations

exhibited relatively higher glucose responses, though still below the commercial tea and glucose controls. These differences reflect both formulation composition and the dosage effect, with higher concentrations (7.5%) achieving more pronounced glycemic control.

In-vivo glycemic index of the beverages

The in vivo glycemic index (GI) of roselle–fig–beetroot infused beverages is presented in Figure 5. Glucose (100%) served as the reference standard and produced the highest glycemic response (~100%), significantly higher ($p < 0.05$) than all formulated beverages.

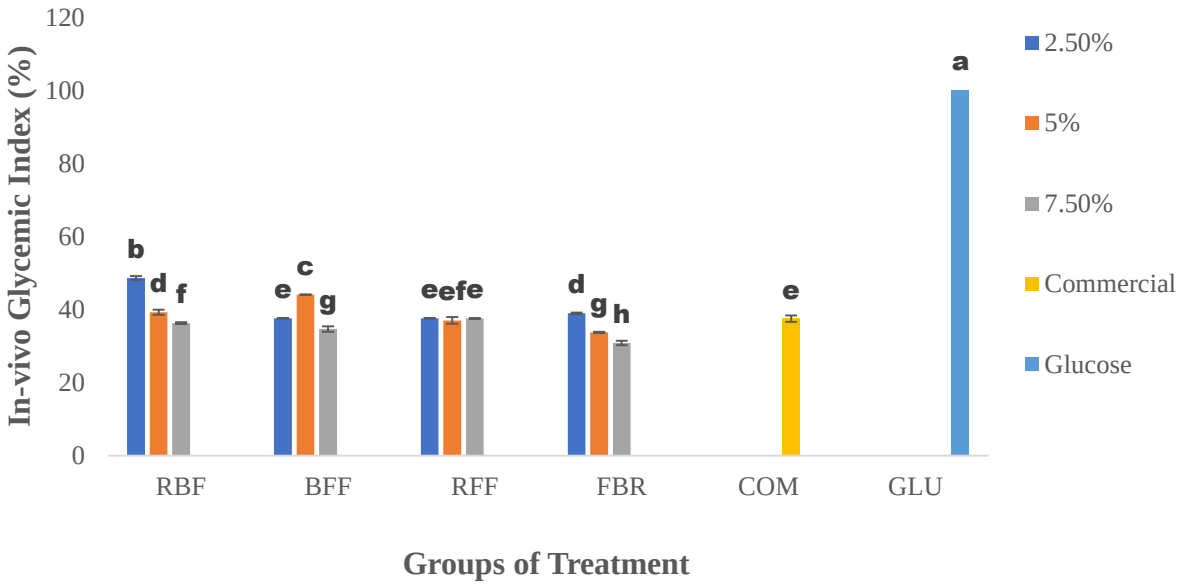

Figure 5: In-vivo glycemic index of the beverages

Bars are presented as means with error bars from the standard deviation (n=3). Bars with different superscripts are significantly different ($p \leq 0.05$) according to Duncan multiple comparison.

Note: RBF (20% fig + 36.288% beetroot + 43.712% roselle), RFF (45% fig + 55% roselle), BFF (42.5% fig + 57.5% beetroot), FBR (35% beetroot + 65% roselle), RCF (100% roselle), FFF (100% fig), BTF (100% beetroot) infused drinks blends.

All beverage formulations produced GI values below 55%, classifying them as low glycemic index foods (American Diabetes Association, 2022). The glycemic index (GI) values of the roselle–fig–beetroot infused beverages varied across formulations and concentrations (Figure 8). For the RBF formulation, GI decreased significantly ($p < 0.05$) with increasing concentration, declining from approximately 47% at 2.5% to 38% at 5%, and further to 35% at 7.5%.

In the BFF formulation, the 2.5% concentration recorded a GI of approximately 37%, which increased significantly ($p < 0.05$) to 43% at 5%, before decreasing to 34% at 7.5%, representing the lowest value within this group. The RFF formulation exhibited minimal variation across concentrations, with GI values ranging from approximately 36% to 37%, and no significant difference ($p > 0.05$) observed among the inclusion levels. For the FBR formulation, a significant concentration-dependent reduction ($p < 0.05$) was observed, with GI values decreasing from approximately 38% at 2.5% to 32% at 5%, and further to 30% at 7.5%, which was the lowest value recorded among all formulations. The commercial herbal tea recorded a GI value of approximately 36%, comparable to several of the formulated beverages. Overall, all formulations produced GI values substantially lower than the glucose reference and within the low-glycemic range.

Discussion

Postprandial blood glucose response

The postprandial glycemic profiles demonstrate that the formulated infusions (RBF, BFF, RFF, and FBR) significantly altered the kinetics of glucose absorption compared to the oral glucose load. In the control group, the rapid escalation of blood glucose levels—peaking at approximately 30 minutes—reflects the immediate breakdown and absorption of simple sugars in the upper gastrointestinal tract. In contrast, the infusion groups exhibited a "flattened" curve, which is a hallmark of delayed gastric emptying and inhibited intestinal glucose transport. As the concentration increased from 2.5% to 7.5%, the Cmax (peak concentration) was not only lowered but also slightly delayed in several blends. This suggests that the higher concentrations of *Hibiscus sabdariffa* and *Ficus carica* create a more viscous or inhibitory environment in the gut. According to Kim and Kim, (2024), the soluble fibers and mucilaginous compounds found in botanical infusions can increase the viscosity of the digesta, thereby creating a physical barrier that slows the interaction between digestive enzymes and substrates.

The profound suppression of the 15–60 minute glucose spike by the 7.5% FBR and RBF blends can be mechanistically explained by the presence of specific polyphenols. Roselle (*Hibiscus sabdariffa*) is rich in hibiscus acid and anthocyanins, which have been clinically shown to act as potent inhibitors of α-amylase and α-glucosidase (Banwo *et al.*, 2022). By

blocking these enzymes, the rate at which complex carbohydrates are hydrolyzed into absorbable glucose is significantly reduced. Furthermore, the Fig (*Ficus carica*) components likely contribute via the modulation of glucose transporters.

Recent studies by Atkinson *et al*. (2019) suggest that flavonoids like quercetin, present in fig extracts, can downregulate the expression of Sodium-Glucose Linked Transporter 1 (SGLT1) and Glucose Transporter 2 (GLUT2) in the intestinal brush border membrane. This dual action—slowing digestion while simultaneously limiting transport—explains why the 7.5% concentration maintained blood glucose at levels near the baseline (0-minute) value throughout the 120-minute period.

The difference between 2.5% and 7.5% concentrations highlights a dose-dependent threshold, where higher phytochemical content achieves near-complete suppression of glycemic spikes. This indicates a dose-dependent efficacy where higher concentrations of beetroot-derived betalains and roselle anthocyanins saturate the metabolic pathways involved in glucose regulation.

This finding is supported by Wootton-Beard *et al*. (2011), who noted that the antioxidant capacity of beetroot-based functional drinks has a direct correlation with the reduction of postprandial oxidative stress, which in turn prevents the rapid glucose fluctuations seen in high-GI diets. The superior performance of the 7.5% FBR blend over the commercial herbal tea (COM) highlights that the specific ratio of 35% beetroot to 65% roselle provides a more effective phytochemical matrix for stabilizing the metabolic response than standard commercial formulations.

The ability of these infusions to attenuate rapid postprandial glucose fluctuations ("glucose roller coaster") is of considerable clinical relevance. Acute glucose excursions are strongly associated with oxidative stress through the overproduction of reactive oxygen species (ROS), which contributes to endothelial dysfunction and the progression of diabetic complications. Sustained postprandial glycemic control has therefore been identified as a critical target in diabetes management (Augustin *et al*., 2015; Reynolds *et al*., 2019). By maintaining postprandial blood glucose (PBG) within a relatively narrow physiological range (70–140 mg/dL), as observed in the 7.5% RBF and FBR treatments, these functional beverages may represent an effective dietary approach for minimizing glycemic variability and improving long-term glycemic indices such as HbA1c.

In-vivo Glycemic Index (GI)

The in-vivo Glycemic Index (GI) values provide a robust evaluation of the metabolic quality of the formulated beverages. All experimental infusions (RBF, BFF, RFF, and FBR) were classified as **low GI (≤55)**, which is consistent with established classification frameworks used in clinical nutrition research (Augustin *et al*., 2015).

A significant ($p < 0.05$) dose-dependent reduction in GI was observed in the RBF and FBR blends, with GI values decreasing from the mid-40s at 2.5% concentration to the low-30s at 7.5%. This trend suggests enhanced physiological activity of bioactive compounds at higher concentrations.

This reduction in GI can be attributed to the inhibitory effects of dietary polyphenols on carbohydrate-digesting enzymes. Polyphenols have been widely reported to inhibit α-amylase and α-glucosidase, thereby slowing carbohydrate digestion and reducing glucose absorption. Additionally, recent mechanistic studies confirm that polyphenols can also suppress intestinal glucose transporters such as SGLT1 and GLUT2, further attenuating postprandial glycemia.

The remarkably low GI observed in the 7.5% FBR infusion highlights the synergistic effects of phytochemicals from *Hibiscus sabdariffa*, *Ficus carica*, and *Beta vulgaris*. These plant-derived bioactives—including anthocyanins, flavonoids, and betalains—have been shown to exert complementary antidiabetic mechanisms, including enzyme inhibition and modulation of glucose transport pathways.

Recent studies further demonstrate that polyphenol-rich extracts exhibit strong α-glucosidase inhibitory activity through molecular interactions at enzyme active sites, thereby reducing glucose release into circulation. This supports the observed decrease in GI with increasing infusion concentration.

A key finding is that the optimized 7.5% formulations outperformed the commercial herbal tea (COM), suggesting that **phytochemical synergy and optimized blending ratios** play a more critical role than single-source formulations in glycemic regulation. Maintaining a GI in the range of 30–35, as observed in the 7.5% RBF and FBR blends, is clinically significant. Diets based on low-GI foods are consistently associated with improved glycemic control, reduced insulin demand, and lower risk of cardiometabolic complications. This positions the formulated beverages as promising functional foods capable of addressing both hyperglycemia and oxidative stress in metabolic syndrome.

Conclusion

This study shows that botanical infusions of *Hibiscus sabdariffa* (roselle), *Ficus carica* (fig), and *Beta vulgaris* (beetroot) significantly reduce postprandial glycemic response in a dose-dependent manner. All formulations were classified as low glycemic index (≤55), with the 7.5% FBR and RBF blends showing the strongest glucose-lowering effects. The enhanced efficacy at higher concentrations is likely due to increased phytochemical content, which may inhibit carbohydrate-digesting enzymes and improve insulin activity. Notably, the 7.5% formulations outperformed a commercial herbal tea, highlighting their potential as functional, low-GI alternatives for managing hyperglycemia, metabolic syndrome, and type 2 diabetes.

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Conflict of Interest
The authors declare no conflicts of interest related to this study.

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