Harnessing the potency of home-garden herbs: Natural beverages for managing postprandial hyperglycaemia

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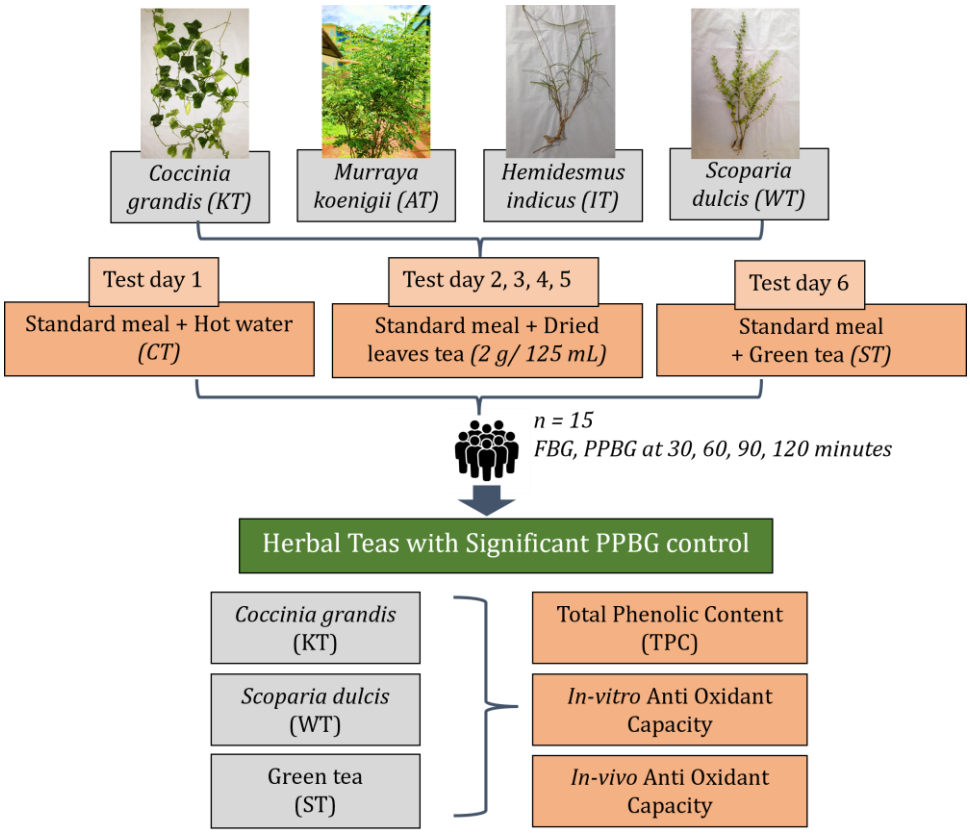

Abstract

Postprandial hyperglycaemia and high oxidative stress increase the risk of developing non-communicable diseases and complications. Polyphenolic compounds present in herbal leaves, can control the postprandial blood glucose while increasing the antioxidant capacity. In this study the effects of selected herbal beverages [*Coccinia grandis* (KT), *Murraya koenigii* (AT), *Hemidesmus indicus* (IT), and *Scoparia dulcis* (WT)] on postprandial blood glucose (PPBG), total phenolic content (TPC), *in-vitro* antioxidant capacity (AOC), and *in-vivo* antioxidant effect were assessed. To assess

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the effects on PPBG, blood glucose levels were analyzed in healthy human volunteers (n=15) at every 30 minutes over 2 hours, after consumption of above beverages (2 g leaf powder brewed in 125 mL for 2 minutes) or hot water (control-CT) or green tea (standard-ST) with a standard meal, on separate days. TPC and *in-vitro* AOC of two beverages (KT and WT) with the highest PPBG control were analyzed by Folin-Ciocalteu method [expressed in Gallic acid Eq/g (GAE/g)] and ABTS free radical scavenging assay [expressed in TroloxEq/g] respectively. *In-vivo* AOC of healthy individuals (n=13) was assessed in serum immediately before and one hour after consumption of the beverages. Compared to CT, significantly low PPBG levels were observed in KT, WT, and ST ($p<0.05$). The highest TPC was in ST (107.5±5.7GAE/g) followed by WT (18.7±0.8GAE/g). In-vitro AOC was significantly high ($p<0.05$) in ST (7.3±0.3TroloxEq/g) compared to WT (0.6±0.04TroloxEq/g) and KT (0.2±0.03TroloxEq/g). *In-vivo* AOC increment after one hour from consumption of KT (0.013 ±0.119TroloxEq/g) was comparable to ST (0.005±0.063TroloxEq/g), while WT (-0.066 ±0.071TroloxEq/g) elicited no AOC increment after one hour from consumption. Hence, it can be concluded that WT and KT possess considerable PPBG controlling effects.

Keywords: *Herbal tea beverages; Postprandial blood glucose; Antioxidant capacity*

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1. Introduction

Glucose is an essential energy source; however, tight regulation of blood glucose levels throughout the day is essential in maintaining the normal metabolic functions of the body. Defects in insulin secretion, peripheral insulin resistance, or both can break the normal blood glucose homeostasis (Magkos et al., 2022) resulting in short- and long-term complications associated with hyperglycaemia. At present, chronic hyperglycaemia or diabetes mellitus (DM) has become a pandemic, with a global prevalence of 10.5% in 2021(International diabetes Federation, 2021). Furthermore, it is projected that the number of individuals affected will rise to 537 million, accounting for 11.3% of the global population by 2030 (Ong et al., 2023). Developing countries are anticipated to bear the highest burden of this increase in prevalence (International diabetes Federation, 2021).

Persistent elevation of blood sugar following meals is one of the main reasons for most of the complications associated with DM. Thus, control of postprandial blood glucose (PPBG) is pivotal in the management of DM (Nathan, 1993).The use of herbal preparations has become a common methods to control sudden surges of PPBG (Munasinghe et al., 2011; Shibly, Zohora and Islam, 2015). The use of herbal medicine in treating DM or controlling hyperglycaemia has been a practice in many Asian countries including Sri Lanka since ancient times (Munasinghe et al., 2011; Utomo et al., 2022). A recent study conducted in Sri Lanka in 2015, revealed the use of herbal remedies to manage the blood glucose levels by about 48% of individuals with type 2 DM particularly in areas with restricted access to medical resources (Waisundara, Watawana and Jayawardena, 2015). Furthermore, another study conducted in Sri Lanka revealed the consumption of herbal remedies controlling hyperglycaemia along with the treatments given by Western medicine by more than 90% of patients with DM (Edussuriya et al., 2021).

About 800 plants have been identified as having anti-diabetic potential (Ponnusamy et al., 2011). However, only a limited number of plants have been researched scientifically to identify the anti-diabetic effects and to isolate the phytochemicals responsible for blood glucose-lowering effects (Patel et al., 2012). *Scoparia dulcis*, *Allium sativum, Gymnema sylvestre, Citrullus colocynthis, Trigonella foenumgreacum, Momordica charantia* and *Ficus bengalensis*are are some herbal leaves used to control blood glucose by different nations across the world (Patel et al., 2012). The consumption of herbal tea is emerging as a notable trend worldwide and holds a substantial position in the global tea market. Most of the anti-hyperglycemic effects elicited by herbal teas are due to the presence of phytonutrients, especially antioxidants. Flavonoids, tannins, alkaloids, and terpenoids are the main active antioxidants present in plant leaves as polyphenolic compounds. Green tea is a common herbal beverage that is used to reduce PPBG. Catechins in green tea inhibit amylase, sucrase, and the sodium-dependent glucose transporter, which decreases PPBG by slowing down dietary glucose absorption. Green tea showed a reduction in PPBG in both patients with DM and healthy adolescents (Lahirin et al., 2015).

Coccinia grandis, *Murraya koenigii*, *Hemidesmus indicus*, and *Scoparia dulcis* are some of the herbs widely grown in Sri Lanka and used as herbal remedies to lower the blood glucose since ancient times (Muralitharan et al., 2007; Waisundara, Watawana and Jayawardena, 2015). Although dried leaves of these plants are available as ready-to-use tea bags, the effectiveness and the potential health effects of the dose in a tea bag have not yet been scientifically evaluated. Therefore, the present study profiled the effect of those herbal leaves on PPBG control that can cause significant PPBG lowering effects when consumed as beverages. Herbal beverages that showed significant effects on PPBG were further evaluated to assess their total polyphenolic content, and *in-vitro* and *in-vivo* antioxidant capacity to identify their potential blood glucose lowering effects in healthy young adults.

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2. Methodology

2.1 Study setting

The study was conducted at the Department of Biochemistry, Faculty of Medicine and Allied Sciences (FMAS), Rajarata University of Sri Lanka (RUSL) and the Department of Animal and Food Sciences, Faculty of Agriculture, RUSL from May 2022 to December 2023. The ethical clearance (ERC/2021/05 and ERC/2023/37) was obtained from the Ethics Review Committee, FMAS, RUSL. For human studies, healthy adults between 20-30 years of age, who were not on any medication or supplements and had normal body mass index (18-23 kg/m^2), and normal fasting blood glucose (FBG) were recruited after obtaining informed written consent for participation.

2.2 Preparation of herbal leaf powder tea bags

The leaves of selected four herbs namely *Coccinia grandis* (KT), *Murraya koenigii* (AT), *Hemidesmus indicus* (IT) and *Scoparia dulcis* (WT), were obtained from the local market Anuradhapura, Sri Lanka, and authenticated by the National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka. After cleaning and homogenizing, the leaves were air-dried at room temperature for 3-5 hours followed by drying in an oven for 24 hours at a temperature of 50 ^{0}C. Dried leaves were finely powdered and the leaf powder was separated from the particles using a mesh sieve (Wang et al., 2020). Tea bags containing 2 g of the herbal leaf powder were prepared and stored at room temperature in tightly sealed containers.

2.3 Assessment of organoleptic properties of prepared herbal tea varieties

The palatability of the four tea varieties was tested by evaluating their organoleptic properties using 33 untrained sensory panellists. For the sensory assay, the beverages were prepared by brewing the pre-prepared tea bags in 125 mL of boiling water for 5 minutes. Teabag containing one herb was brewed at a time to maintain uniformity in the preparation process of herbal beverages. The appearance, colour, flavour, after taste, aroma and overall acceptability were tested. The panellists who participated in the sensory evaluation were asked to rate each beverage on a five-point hedonic scale.

2.4 Effect of herbal beverages on PPBG

Method described by Harrison *et al.*, 2009 was used to assess the effect of the prepared herbal beverages on PPBG (Harrison et al., 2009). Accordingly, the participants who consented to take part in the study were advised to have a normal routine dinner, refrain from heavy exercise, alcohol, and smoking, and fast for 8-10 hours on the night before each test day. On each of the test dates, after giving ~ 10 minutes rest in the laboratory, ~ 0.5 mL of capillary blood was collected by finger prick method into sodium fluoride containing 1.5 mL microcentrifuge tube for fasting blood glucose (FBG) estimation. To obtain the control values (CT), on test day 01, each participant was given a standard meal (873 kcal) consisting of 5 white bread slices (335 kcal), 4 milk shortcake biscuits (482 kcal), and 20 g of mixed fruit jam (56 kcal) (Wolever and Bolognesi, 1996; Harrison *et al.*, 2009). A volume of 200 mL of hot water was given to drink within 5 minutes, and 15 minutes later the above-mentioned standard meal to be consumed within 10 minutes. Using the same above-mentioned method, ~0.5 mL of capillary blood was taken at 30, 60, 90, and 120 minutes from the first bite of the meal to estimate blood glucose level (Wolever et al., 2008). To assess the effect of the prepared herbal beverages on PPBG, the same procedure was repeated using the same group of volunteer participants by replacing the hot water cup with herbal beverages. One herbal beverage was tested on a given date and a 3-4 day interval was given in between two test days. Finally, the same procedure was repeated using a commercially available green tea (standard- ST) to compare the effects of the tested teas with green tea. Blood glucose levels at each time point were estimated using a glucose oxidase kit (BIOLABO,

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France) following the standard protocol in the assay kit. Light absorbance was measured at 500nm by UV-visible spectrophotometer (Wolever et al., 2008).

2.5 In vitro antioxidant capacity of herbal tea beverages

2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonicacid (ABTS) cation free radical decolouration assay was used to determine the *in-vitro* free radical scavenging activity (Re et al., 1999). Herbal leaf samples for the assay were prepared by homogenizing 0.5 g of dried herbal leaf powder in 10 mL of phosphate-buffered saline (PBS, pH-7.4) using a mortar and pestle. The mixture was filtered through a Whatman No.1 filter paper, and the filtrate was taken for antioxidant assay. ABTS (EC No. 250-396-6, Sigma-Aldrich, USA) free radical solution (stock solution) was prepared by completely dissolving one ABTS tablet (10 mg) in 200 µL of ethanol, 2.6 mL of PBS and $K_2S_2O_8$ (2.6 mL, 7 mmol/L). The solution was kept in the dark for 12-16 hours until the development of a dark blackish-green colour, and then diluted with deionized water until an absorbance of 0.700±0.02 at 734 nm against a buffer blank was obtained. Diluted ABTS solution (2.9 mL), PBS (85 µL), and a prepared tea sample (15 µL) were mixed in a cuvette (total volume = 3 mL) and the initial absorbance was measured at 734 nm. Reduction in absorbance was recorded every 30 seconds up to 6 minutes (Re et al., 1999). All test samples were replicated four times.

A standard curve of Trolox was plotted according to the above procedure with the mean reduction absorbance vs quantity of Trolox in µg [5 µL (1.56 µg Trolox), 10 µL (3.12 µg Trolox), 15 µL (4.68 µg Trolox), 20 µL (6.24 µg Trolox) and 25 µL (7.80 µg Trolox)] using a stock solution of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, 0.0161g in 50 mL of deionized water). Five replicates were carried out for each concentration. Antioxidant activity was expressed as (Trolox Eq.µ mol/g) Trolox equivalent antioxidant capacity (TEAC) and (Vit C Eq.µg/10mg) Vitamin C equivalent antioxidant capacity considering Trolox and vitamin C were as the standards.

2.6 Total polyphenol content of herbal tea beverages

Total polyphenol content was measured by the method described by Waterhouse, 2003 (Waterhouse, 2003). Briefly, 0.1 mL of tea was mixed with 2 mL of deionized water and 0.2 mL of diluted Folin Ceocalteu (BDH, England) reagent (reagent: deionized water 1:1) solution and incubated at room temperature for 10 minutes. Then, 1 mL of Na_2CO_3 was added and incubated in the dark for 2 hours and absorbance was measured at 765 nm. To plot the standard curve of gallic acid, 10, 20, 50, 100, 250, and 500 mg/L standard solutions were prepared. The above procedure was repeated in which the standard solution was added instead of the sample. Six replicates were carried out from each concentration. Results were interpreted in mg/L/10 mg gallic acid equivalents.

2.7 Effect of consumption of herbal tea beverages on serum antioxidant capacity

The healthy volunteers (n=13) who were selected for the analysis of serum antioxidant capacity were asked to consume their regular meals on the previous day. On the test day morning, after 15 minutes of rest, ~1 mL of venous blood sample was obtained from each participant by a phlebotomist. A cup of herbal tea prepared according to the above-described method was offered and requested to be consumed within 5 minutes. From each participant, ~1 mL of venous blood was obtained after one hour from the consumption of the tea. To separate serum, blood samples were centrifuged at 1000 g for 10 minutes and the antioxidant capacity was assessed by the ABTS cation free radical scavenging assay as described above in which 15 µLof serum samples instead of tea samples was used. From all the samples three replicates were analyzed.

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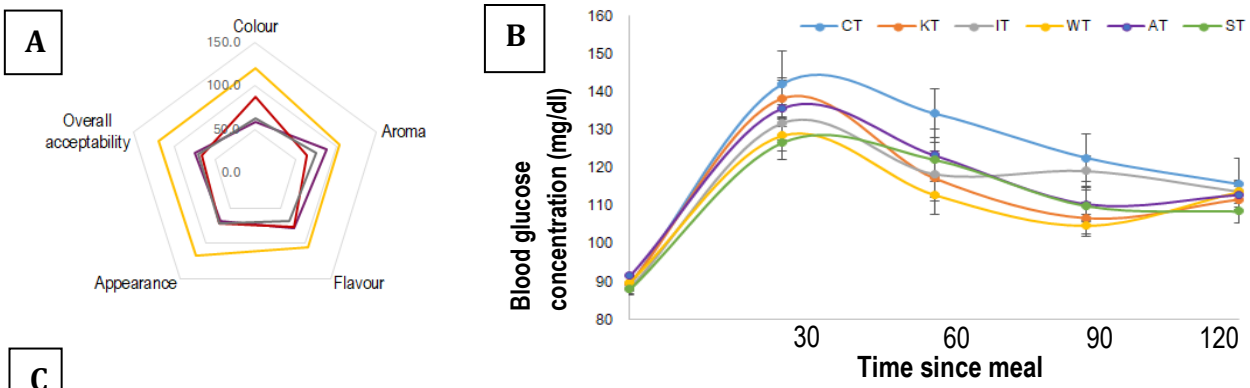
2.8 Statistical analysis

In PPBG analysis, glucose concentration vs time was plotted separately for each individual and for each meal, to estimate the incremental area under the curve (IAUC). Participants who had IAUC values 2 or more times above or below the standard deviation (SD) were excluded and removed from the final data analysis. The presence of any significant difference in IAUC among all the groups was analyzed by ANOVA Tukey's posthoc test while paired t-test was used to assess the presence of any significant difference (CI 95%) in PPBG values at a particular time interval. Furthermore, the antioxidant capacities and TPC of the tested tea varieties were statistically compared using Student's t-test. In all statistical analyses, $p<0.05$ was considered statistically significant.

3. Results

Organoleptic properties of all four herbal tea beverages were accepted by the 33 untrained panelists, where the highest overall preference was for WT (Figure 1A). The 15 young healthy volunteers who participated in the study were between 20-30 years old and among them 11 were males. All the participants had normal FBG (mean = 89, SD = 5.6mg/dL), and the mean blood glucose concentrations obtained at fasting, 30 min, 60 min, 90 min, and 120 min following the standard meal with either hot water (CT), test beverages or ST are shown in Figure 1B and 1C.



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Time (minutes)	CT		KT		IT		WT		AT		ST	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Fasting	89	1.56	89	1.95	88	1.66	90	1.15	91	0.81	88	1.34
30	142	8.62	138	5.23	132	4.66	129	4.17	136	7.22	127	4.35
60	134	6.50	117	5.96	118	6.07	113	5.07	123	6.89	122	4.45
90	123	6.21	107	4.16	119	3.97	105	2.92	110	4.24	110	4.18
120	116	6.71	112	3.27	114	3.15	114	2.25	113	3.40	109	3.03

Figure 1. Sensory evaluation of herbal tea beverages and assessment of their effect on post-prandial blood glucose **A.** Comparison of different sensory characters of different herbal beverages **B.** Mean postprandial glucose concentrations (mg/dL) following different treatments (Error bars represent standard error of mean) **C.** Mean and SEM of blood glucose values

All the beverages caused a reduction in PPBG compared to the CT. Amongst, ST showed the lowest blood glucose surge following the meal (peak blood glucose in 60 min) and the slowest blood glucose rise over the 2 hours. Consumption of all the test beverages caused a peak blood glucose surge in 30 minutes. Of the tested beverages the slowest blood glucose rise was observed with the WT and was followed by IT, AT, and KT respectively.

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The effect of different herbal beverages on PPBG was further compared using the IAUC values (Table 1). The lowest IUAC value (2667±199) was obtained for WT. Importantly, this value was even lower than the IAUC obtained for the ST (3076±332). In comparison with the IAUC value of the CT, statistically significant ($p<0.05$), low mean IAUC values were observed only for WT ($p=0.02$), KT ($p=0.04$), and ST ($P=0.03$). The p values among the tea varieties for IAUC are shown in Table 2. None of the tea varieties showed a statistically significant difference at 95% CI with the standard green tea (ST). However, WT showed a significantly lower IAUC compared to IT ($p<0.05$).

Table 1. Postprandial blood glucose incremental area under the curve (IAUC) for different tea varieties

Test	Mean (IAUC)	SD	*p*-value with CT
SM+CT	4356	±558	-
SM+KT	3268	±327	0.04
SM+IT	4025	±542	0.69
SM+WT	2667	±199	0.02
SM+AT	3215	±406	0.09
SM+ST	3076	±332	0.03

Table 2. P values for the IAUC among tea varieties

Treatment	KT	IT	WT	AT	ST
Control	0.04*	0.69	0.02*	0.09	0.03*
KT		0.23	0.15	0.91	0.62
IT			0.02*	0.15	0.14
WT				0.25	0.23
AT					0.76

* *Significant difference at 95% CI*

3.1 Total phenolic content (TPC) and antioxidant capacity (AOC)

The TPC, *in-vitro* AOC and change in *in-vivo* AOC after 1 hour of WT and KT were compared with green tea (ST) and the values are shown in Table 3. Green tea showed a significantly higher ($p<0.05$) TPC and an *in-vitro* AOC compared to WT and KT. Compared to KT, WT showed significantly high TPC, however *in-vitro* AOC of the two beverages were not significantly different ($p>005$).

The increase of serum antioxidant capacity after consumption of the beverage was highest in KT and it is comparable to the *in-vivo* AOC of ST (green tea). Despite having significantly high TPC and in-vitro AOC, a significantly higher *in-vivo* AOC has not been elicited by ST. *In-vivo* AOC of WT was the lowest (negligible) even with a considerable TPC compared to KT.

Table 3. Total phenolic content and antioxidant capacity

	WT	KT	ST (green tea)
TPC (GAE/g)±SD	18.726 ±0.769^{a}	5.004 ±0.109 ^{b}	107.469 ±5.756^{c}
In-vitro AOC (TroloxEq/g) ±SD	0.614 ±0.037^{a}	0.213 ±0.029^{a}	7.275 ±0.322^{b}
Change in in-vivo AOC after 1 hour (TroloxEq/g) ±SD	-0.066 ±0.071^{a}	0.013±0.119 ^{b}	0.005 ±0.063^{b}

Same superscript within a row indicates no significant difference at 95% CI.

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4. Discussion

Since ancient times, many herbs have been used to treat hyperglycaemia by different nations across the globe owing to the presence of various active compounds that possess a variety of blood glucose-lowering effects. Despite their wide usage, limited studies have been carried out to scientifically assess their hypoglycaemic effects on the human body. In this study, four herbal leaves which have been used to treat hyperglycaemia since ancient times were tested to identify their effects on postprandial blood glucose regulation in healthy humans. The study revealed a significant reduction in PPBG levels following the consumption of herbal tea prepared by dried leaves of *Scoparia dulcis* (WT) and *Coccinia grandis* (KT) as tea beverages (2g). The results indicated potential use of these herbal leaves as a beverage in controlling postprandial hyperglycaemia. However, any significant blood glucose lowering effects of the herbal beverage prepared by *Murraya koenigii* (AT) and *Hemidesmus indicus* (IT) leaves were not observed.

Of the tested tea beverages, the best PPBG control was achieved with the beverage prepared by *Scoparia dulcis* (WT). A decoction prepared using the whole plant of *Scoparia dulcis* is frequently used by traditional medical practitioners in Sri Lanka to treat DM (Perera, Ekanayake and Ranaweera, 2020).The molecule, scoparic acid D isolated from *Scoparia dulcis*is known to cause hypoglycemic effects through inhibiting two main enzymes involved in carbohydrate digestion namely, alpha-amylase and glucosidase (R. Saikia, M. Choudhury Dutta, A. Talukdar Das, 2012). Furthermore, previous studies revealed a decrease in oxidative stress in RINm5F pancreatic cells (Latha et al., 2004), glucose 6-phosphatase, fructose 1, 6-bisphosphatase, and sorbitol dehydrogenase enzyme activities (Latha et al., 2004; Pari and Latha, 2005), and an increase in hexokinase, glucose 6-phosphate dehydrogenase and glycogen synthase enzyme activities (Pari and Latha, 2005). It also seems to increase cellular glucose uptake by increasing the expression, translocation, and activity of GLUT4 receptors (Beh et al., 2010).These enzyme regulations decrease dietary carbohydrate digestion, gluconeogenesis, and glycogenolysis while increasing glycogen synthesis, cellular glucose uptake, and glycolysis leading to a summative effect of blood glucose lowering, especially in the postprandial state.

IAUC of WT was the lowest among all tested teas indicating the high possibility of *Scoparia dulcis* leaves to be used as an herbal beverage to reduce PPBG. Furthermore, *Scoparia dulcis* leaves had the highest polyphenolic content. Green tea, one of the beverages extensively studied for its effects on blood glucose control (Cabrera, Artacho and Giménez, 2006), is rich in catechins and phenolic compounds (EGCG, ECG, EGC, and EC) than black tea (Cabrera,, Artacho and Giménez, 2006). The phenolic compounds in green tea inhibit amylase and glucosidase, two essential enzymes in the digestion of carbohydrates (Hara and Honda, 1990). Additionally, polyphenols reduce the uptake of glucose by intestinal Caco-2 cells (Johnston, 2005). Tannic acid, which promotes the translocation of glucose transporter GLUT 4 and phosphorylation of insulin receptors significantly involved in circulating glucose clearance. Thus, similar effects can be expected by the polyphenolic compounds present in *Scoparia dulcis* leaves as well. Although WT showed high *in vitro* antioxidant capacity, our *in-vivo* study did not demonstrate an elevation of serum antioxidant levels. This might be due to the rapid reduction of antioxidants/ polyphenolic compounds within one hour of consumption or due to the absence of a significant antioxidant capacity in many polyphenolic compounds present in *Scoparia dulcis* leaves. Thus, the PPBG lowering effect of WT cannot be merely attributed to the antioxidant activity. However, further studies that profile the polyphenolic compound composition of *Scoparia dulcis* leaves and their cellular effects which can enhance the blood glucose lowering effects will pave the way for novel therapeutic interventions via herbal remedies, to combat hyperglycaemia.

A significant reduction of PPBG was also observed following the consumption of herbal tea prepared by *Coccinia grandis* (KT) leaves. It is another herbal leaf commonly used in traditional medicine to control hyperglycaemia. Its effects on blood glucose control were tested using an interventional study conducted in a cohort of patients with DM. The study revealed a significant

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reduction ($p<0.001$) in hyperglycaemic symptoms like polyuria, polydipsia, and generalized body weakness, by consumption of *Coccinia indica* powder for 60 consecutive days. Further, this study showed a percentage decrease in fasting mean blood glucose, PPBG, and HbA1c by 19.86%, 24.52%, and 8.4%, respectively. These results indicated the possibility of the use of *Coccinia indica* powder for long-term blood glucose control in patients with DM (Mohd Junaid et al 2020). Another double-blind phase I clinical trial conducted in Sri Lanka noted a significant reductionin 1-hour and 2-hour blood glucose values of oral glucose tolerance tests performed after consumption of *Coccinia indica* leaves (Munasinghe et al., 2011). PPBG control exerted by *Coccinia indica* leaves can be attributed to the suppression of glucose-6-phosphatase (Hossain, Shibib and Rahman, 1992) and an increase in glycogen synthesis (Kumar, Sudheesh and Vijayalakshmi, 1993). The current study identified a reduction in PPBG even by consumption of a single dose of tea prepared using 2 g of dried *Coccinia grandi* leaves. This indicated the possible use of *Coccinia grandis* leaves as a herbal beverage to obtain immediate blood glucose lowering effects.

Though some previous studies have reported hypoglycaemic effects of *Hemidesmus indicus* and *Murraya koenigii*, such effects were not identified in the present study with 2-hour blood glucose changes. For example, an *in-vitro* study conducted with *Murraya koenigii* hydro-alcoholic extract showed significant alpha-amylase inhibition (Rani, Kumar and Khar, 2019). Single oral administration of *M. koenigii* leaf water extract (300 mg/kgBW) to normal and mild diabetic rabbits resulted in a reduction in PPBG levels by ~15% and 28% respectively when the researchers observed ~46% and 39% PPBG reduction in sub-diabetic and mild diabetic rabbits respectively by administrating a commonly used oral hypoglycaemic drug; tolbutamide (Kesari, Gupta and Watal, 2005). Thus, this study showed that the leaf extract has the potential to reduce blood glucose in mild diabetic rabbits. Active compounds in *M. koenigii* leaves that can enhance the glucose-lowering effects have also been studied. These studies lead to identifying the presence of potent antioxidants like carbazole alkaloids, glycoside, triterpenoids, and phenolic compounds in ethanolic extract of *M. koenigii* leaves. It is postulated that the potent antioxidant activity (Tembhurne and Sakarkar, 2010) of the leaves and their ability to activate β-cells of the pancreas could be one of the reasons for the antidiabetic effect exerted by *M. koenigii* (Choudhury, and Garg, 2007). Despite the antioxidants in *M. koenigii* leaves, a significant PPBG control based on IAUC was not observed with AT in the present study, indicating the role of other synergistic factors in these leaves which may contribute to or interfere with blood glucose control.

*Hemidesmus indicus*is another reputed beverage used by South Asians from ancient times to treat many disease ailments like rheumatism, leprosy, impotence, urinary tract, and skin infections. Although many studies have proven the anti-hyperglycemic effects of *Hemidesmus indicus* root extracts (Gayathri and Kannabiran, 2008), the effect of leaf extract on blood glucose control is lacking. An *in-vitro* study has elucidated the glucose diffusion inhibition effects of *Hemidesmus indicus.* In this study, *Hemidesmus indicus* aqueous extract and glucose solution were combined in a bio-membrane, and the bio-membrane was submerged in a beaker containing sodium chloride and distilled water. Observation of the amount of glucose in the beaker in every half an hour showed a considerable obstruction to glucose flow through the bio-membrane and efficient glucose diffusion inhibition for 150 minutes (Gayathri and Punnagai, 2014).

The previously published studies have been conducted with powdered alcoholic or aqueous extracts of *Hemidesmus indicus* and *Murraya koenigii* leaves which might extract higher amount of active compounds of the leaves. However, the effective dose of active compounds present in the dose (2 g of dried leaves) used in the current study might not be sufficient to elicit hypoglycaemic effects by *Hemidesmus indicus* and *Murraya koenigii* leaves. The sufficiency of the active compounds in the tea prepared from the dried leaves could be analysed in future studies and will be able to compare these values with the published complete phytochemical profiles of the leaf extracts, obtained by different

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extraction methods (*Hemidesmus indicus* - Mishra et al., 2018; *Murraya koenigii* - Balakrishnan et al., 2020; *Scoparia dulcis* – Jiang et al., 2021; *Coccinia grandis* - Hossain et al., 2024).

The current study examined only the PPBG regulation following the consumption of a single dose of herbal beverages prepared by four selected herbal leaves and did not assess their long-term effects on PPBG control. Furthermore, as some of the active compounds in herbs are known to cause organ toxicities, especially in long-term consumption it is imperative to conduct randomized controlled trials to assess the long-term effects of the herbal beverages which were identified to have significant post-prandial blood glucose lowering effects. Furthermore, as this study elicited the blood glucose controlling effects of selected herbal beverages using healthy individuals, it is essential to conduct further studies on patients with diabetes mellitus and impaired glucose tolerance to identify their potential uses in such disease conditions.

Increased oxidative stress is an aetiological factor that can contribute to the development and progression of non-communicable diseases (Crisóstomo, Oliveira and Alves, 2022). Use of natural antioxidants in foods and beverages is a reputed dietary intervention in reducing oxidative stress (Pandey and Rizvi, 2009). Green tea is considered as an essence of antioxidants; however, the amount of antioxidant compounds present in a tea bag might not be effective in exerting considerable antioxidant functions. A similar study conducted with 10 healthy human subjects elicited no significant increase (1.1% to 2.1% increase) in plasma antioxidant levels after one hour from the consumption of green tea (2.5g brewed in 150 mL water). However, a significant plasma antioxidant increase has been observed [7.0% increase after 60 min and 6.2% after 120 min ($P<0.0001$)] with 300mL of green tea and [12.0% increase after 60 min and 12.7% after 120 min over baseline value ($P<0.0001$)] with 450mL of green tea (Sung et al., 2000). Despite having a considerable amount of TPC and *in-vitro* AOC, WT also elicited similarly low in-vivo AOC.

Scientific literature indicates that the antioxidant compounds that possess antioxidant capacities *in-vitro* might not possess considerable antioxidant capacities *in-vivo*. The reason is the rapid spread and diffusion of free radicals throughout the body. Moreover, free radicals have an extremely short life span. Thus, the antioxidant compound might not reach the free radical rapidly at the time of the oxidative damage caused by the free radical. Thus the antioxidant capacity depends on the concentration of antioxidant compounds and also the free radicals, the structure of the antioxidant compound, the type of medium, and reaction conditions (Francenia Santos-Sánchez et al., 2019).

The insignificant change in antioxidant capacity within 1 hour in all tested beverages, in the present study could be due to the insufficient concentration of antioxidant compounds in the beverages. As in the previously mentioned study (Sung et al., 2000) consumption of three to four times high amounts of the beverage might cause considerable antioxidant capacities, however, is not commonly practiced. Furthermore, the structure of the phenolic compounds might not scavenge the free radicals in the human body thus eliciting high TPC with low in-vivo AOC (Francenia Santos-Sánchez et al., 2019).

In-vitro AOC is commonly assessed by hydrogen atom transfer (HAT), and single electro-transfer (SET) methods. Scientists suggest that it is recommended to conduct at least three antioxidant capacity assessing methods that evaluate HAT, SET, and a combined method, HAT/ SET. Due to the limited resources and time constraints, we were able to conduct only the ABTS assay and more precise results on antioxidant capacity could be obtained if further studies could have been performed (Francenia Santos-Sánchez et al., 2019).

5. Conclusions

The herbal tea beverages prepared using dried leaves of *Scoparia dulcis* (WT) and *Coccinia grandis* (KT) have demonstrated PPBG-lowering effects comparable to the effects elicited by green tea. Thus, dried leaves of *Scoparia dulcis* (WT) and *Coccinia grandis* (KT) can potentially be used to prepare herbal beverages to control PPBG surge in patients with diabetes mellitus or impaired glucose tolerance. Green tea showed a significantly higher ($p<0.05$) TPC and an in-vitro AOC compared to

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WT and KT. Compared to KT, WT showed significantly high TPC, however in-vitro AOC of the two beverages were not significantly different. A considerable level of *in-vivo* AOC was not shown by all the beverages. Thus, the blood glucose-controlling effects of the beverages cannot be merely attributed to the AOC.

Ethical approval

Ethical clearance (ERC/2021/05 and ERC/2023/37) was obtained from the ethics review committee, FMAS, RUSL. Informed written consent was obtained from all the participants prior to the recruitment.

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