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Exploring *Clitoria ternatea* (Blue pea) herbal tea: A potent beverage with antioxidant and α- amylase inhibitory activity

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Diabetes is a global health challenge, with oxidative stress playing a crucial role in its development. Incorporating functional foods derived from medicinal plants is an effective approach to diabetes management. This study aimed to formulate and evaluate a composite herbal tea using *Clitoria ternatea* (Bluepea) as the primary ingredient, combined with *Mentha arvensis* (Mint), *Cymbopogon citratus* (Lemongrass), and *Rosa centifolia* (Rose petals) known for their pharmacological properties. Three herbal tea formulations were developed and subjected to sensory analysis to select the most acceptable formulation, which was then subjected to proximate nutrient analysis, phytochemical analysis, DPPH assay, microbial analysis, and *in vitro* α-amylase inhibitory activity. Statistical analysis was performed using SPSS version 26. Blue pea and lemongrass formulation was the most acceptable formulation, with one-way ANOVA results reporting statistically significant differences (P <0.001). Nutrient analysis showed low moisture content (2.95%) with a product acceptability index of 91.60%. It also exhibited a strong antioxidant activity with an IC50 value of 0.385 µg/g and demonstrated dose-dependent *in vitro* α-amylase inhibitory activity, indicating the potential for glycemic control. Microbial analysis confirmed the product's safety for consumption. The composite herbal tea is a promising functional beverage for diabetes management, with potent antioxidant properties and alpha-amylase inhibitory activity.

Keywords: Antioxidant, *Clitoria ternatea*, Diabetes management, Functional food, Herbal tea

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Introduction

Diabetes mellitus is a global health concern, with over 500 million individuals affected worldwide. The prevalence is expected to rise by 30% by 2045[1], emphasising the need for preventive and management strategies. Oxidative stress is a major contributing factor in the development of type 2 diabetes mellitus (T2DM) and its complications[2]. Although the human body has an intrinsic antioxidant defence system[3], excessive exposure to Reactive oxygen species (ROS) weakens the system, creating a need for external sources of antioxidants[2].

Current management of T2DM focuses on medication and lifestyle modifications, with diet playing a major role[4,5]. This has led to the growing interest in using functional foods, particularly derived from medicinal plants, due to their bioactive properties and minimal side effects[6]. Among these, *Clitoria ternatea*, an underutilised medicinal plant commonly found in the southern states of India[7], is rich in bioactive compounds scientifically validated to counteract oxidative stress and improve glycaemic control[8,9]. Despite its therapeutic value, no novel products have been derived from *C. ternatea*. Based on folklore claims, we hypothesised *C. ternatea* could be a valuable source for developing a herbal tea with enhanced antioxidant properties. Additionally, we predicted that *C. ternatea* could add functional values, such as inhibition of carbohydrate-digesting enzymes responsible for elevated postprandial glucose levels. Therefore, we developed a composite herbal tea by blending *C. ternatea* petals with three other medicinal plants, namely, *Mentha arvensis*, *Cymbopogon citratus*, and *Rosa centifolia*, commonly used in the region for their diverse pharmacological properties. Combining these medicinal plants in a standardised herbal tea formulation is an effective approach to supplement the body with external antioxidants.

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This study formulated and evaluated a functional composite herbal tea using *C. ternatea* as the primary ingredient, combined with these plants in three formulations. The most acceptable combination, determined through sensory evaluation, was further analysed for its nutrient composition, phytochemical profile, antioxidant capacity (DPPH assay), microbial safety, and *in vitro* α-amylase inhibitory activity to assess its potential as a natural dietary intervention for glycaemic control.

***Clitoria ternatea* (Blue pea)**

C. ternatea, commonly known as blue pea, is an edible flower belonging to the family Fabaceae with diverse applications in medicine and agriculture. Its medicinal properties include antioxidant, antidiabetic, and hepatoprotective properties[10,11]. The flower's phytochemical profile includes quercetin glycosides, flavonol glycosides, kaempferol glycosides, terpenoids, myristetin glycosides, and tannins, with a particularly high content of anthocyanin ternatins[10]. These bioactive compounds contribute to the flower's ability to combat oxidative stress and regulate blood sugar levels. The main antidiabetic mechanism of blue pea is attributed to its inhibition of enzymes such as pancreatic α-amylase and α-glucosidase, thus delaying the postprandial blood glucose levels[8,12]. Moreover, quercetin and kaempferol enhance insulin sensitivity, stimulate insulin secretion and facilitate glucose transport to peripheral tissues[13].

***Cymbopogon citratus* (Lemon Grass)**

C. citratus, commonly known as lemongrass, is a member of the family Gramineae and has a long history of application in traditional and ayurvedic medicine. It is known for its antibacterial, anti-inflammatory, and antioxidant activities, which have additional benefits in managing anxiety, pneumonia, gastrointestinal infections, and diabetes[14]. The key bioactive compounds include ketones, alcohols, phenols, terpenes, flavonoids, saponins, tannins, and alkaloids, which contribute to its antioxidant properties[15]. Its antidiabetic effects are linked to its ability to modulate insulin-regulating gene expression, and specifically, it enhances insulin secretion and sensitivity by activating genes TGR5, PPAR-γ, GLP-1, Glucokinase, and GLUT2 while simultaneously reducing inflammation[16].

***Mentha arvensis* (Field mint leaves)**

M. arvensis, commonly referred to as field mint, belongs to the Lamiaceae family and is a medicinal plant used for tea preparations in different areas of the World. This plant is rich in bioactive compounds such as flavonoids, phenolic acid, polyphenols, tannins, sterols, triterpenes, glycosides and terpenes, contributing to its therapeutic properties. It has been traditionally used to treat ailments like headaches, flatulence, and skin diseases. Animal studies have reported the antidiabetic potential of *M. arvensis* leaves[17].

***Rosa centifolia* (Rose petals)**

R. centifolia, commonly referred to as Gulab, belongs to the Rosaceae family and has been traditionally used in Ayurveda to treat various disorders. It has been validated to have analgesic, antioxidant, anti-tussive and anti-depressant activities. Its essential oil is known for its calming properties in treating insomnia and blood pressure. The therapeutic properties, specifically the antioxidant activity, are attributed to the rich presence of phytoconstituents, namely terpenoids, glycosides, flavonoids, phenolic compounds and tannins[18].

Materials and Methods

Study Design

This study followed an experimental design to formulate and evaluate a composite herbal tea using *C. ternatea* (CT) combined with three medicinal plants in three different combinations: T1 (CT and Mint leaves), T2 (CT and Lemon grass), and T3 (CT and Rose petals). Based on sensory evaluation, the most preferred formulation was selected for further analysis.

Collection of plant materials

Fully bloomed, healthy and undamaged flowers of *C. ternatea* and *R. centifolia* were collected in the morning hours from a local market in Tamil Nadu, India. The flower samples were taxonomically identified and authenticated by the Botanical Survey of India (Ref. No. BSI/SRC/5/23/2022/Tech/566, dated 10/11/2022). Mint leaves and dried lemon grass were also procured from a local market in Tamil Nadu, India. All samples were packed into polyethylene bags for transport to the Institute Food Science Laboratory.

Preparation of herbal tea powders

The freshly collected flowers and leaves were gently washed with potable tap water to ensure they were free from contaminants. They were then sundried at temperatures between 30–40°C until the plant materials were free from residual moisture.

The samples were spread in single layers on clean aluminium trays and covered with a mesh net to prevent contamination. The dried materials were then individually ground using a domestic grinder (Model MX-AC400, Panasonic, India) for 3 minutes, sieved (1 mm sieve) and stored in a sealed low-density polyethylene (LDPE) bag in a dark place at room temperature until further analyses and formulation of the tea.

Infusion preparation

The formulation process involved developing three distinct combinations: T1 (CT and Mint leaves), T2 (CT and Lemon grass), and T3 (CT and Rose petals). For each formulation, 2g of ground dried CT petals were mixed with 1 g of a single additional ingredient: ground mint leaves (T1), ground lemon grass (T2) and ground rose petals (T3). The prepared tea formulations were portioned into cotton tea bags with drawstrings sourced from a reliable online supplier, with each tea bag containing 3 g of the herbal tea mix. The tea bags were steeped in 200 mL of hot water (70–90°C) for 3 minutes. This brewing time and temperature were determined from previous studies, which established that the half-time required to extract 50% of the total polyphenols in true teas ranges from 100 to 150 seconds, supporting the common practice of steeping tea for two to three minutes at these temperatures[19,20]. Sensory evaluation was conducted for all three formulations.

Sensory evaluation

The brewed tea formulations were decanted into tea-tasting cups labelled with random two-digit codes. A sensory analysis was performed with 30 untrained panellists to determine the best CT-incorporated tea formulation. It was conducted at the University's Food Science Laboratory, which was equipped with specialised lighting and controlled conditions for unbiased evaluation. The laboratory was well-lit and air-conditioned to create an ambience without distractions. The samples were given to the panellists in odourless containers to avoid any external influences. The primary objective of this sensory evaluation was to determine the most palatable combination among the three herbal drink formulations. Since the focus was on comparative assessment, no control beverage was included for standardisation or reference. Consent was obtained from panellists prior to the sensory evaluation, and the study was approved by the Institutional Ethics Committee (REF: IEC/22/SEP/174/58). Panellists assessed the appearance, colour, taste, aroma, flavour, mouthfeel and overall acceptability on a 9-point hedonic scale ranging from 1 (Dislike extremely) to 9 (Like extremely). Panellists had no prior information about the products to prevent bias. About 50 mL of each combination was given to the panellists, and infusions were around 50–60°C at the time of evaluation. After tasting each combination, panellists were asked to rinse their mouths with lukewarm water and waited 10 minutes before testing the next combination. Scores were recorded for further statistical analysis.

The product acceptability index (PAI %) was then calculated using the scores from sensory evaluation for all three combinations to measure the overall acceptability using this formula.

PAI = (Mean Score obtained for the product/ Maximum Possible Score) ×100

The preferred combination selected through sensory evaluation was packed in sealed airtight LDPE bags at ambient conditions (27°C) until further use for the analysis.

Proximate analysis

The herbal tea mix was subjected to proximate nutrient analysis to assess its nutrient composition. The analysis was performed in triplicates for accuracy, following the standard procedures outlined by the Association of Official Analytical Chemists[21]. The sample was analysed for moisture, carbohydrates, protein, total fat, sugar and crude fibre. The energy was done by calculation method. Carbohydrates were analysed by the difference method, which involves determining the total carbohydrate content directly and calculating it by subtracting the sum of other constituents (protein, fat, water, alcohol, ash) from the total weight of the food. Protein content was determined based on total nitrogen using the Kjeldahl method. Fat content was analysed using a gravimetric method. Sugar content and moisture were analysed using standard FSSAI procedures[22]. The analysis was conducted at an NABL-accredited food laboratory to ensure the precision and reliability of the results.

The nutrient requirements vary according to gender and age group. Hence, the nutritional content of the developed herbal tea mix was compared with the Recommended Dietary Allowance (RDA) provided by the Indian Council of Medical Research[23]. The comparison focuses on the RDA of a 65 kg adult man and a 55 kg adult woman with sedentary activity levels.

Phytochemical analysis

The herbal tea was subjected to preliminary phytochemical screening to identify the presence of phenols, reducing sugars, flavones, glycosides, saponins, alkaloids, anthraquinones, quinones, proteins, tannins, and steroids[24]. Quantitative analysis was done for key phytochemicals, including total polyphenols, tannins, flavonoids, and Vitamin E. Total polyphenol content was measured using the Folin-Ciocalteu method with gallic acid as standard, absorbance read at 700 nm in the UV-Vis spectrophotometer. Flavonoids were determined using the aluminium chloride colourimetric method with quercetin as the standard absorbance at 415 nm. Tannins were estimated using gallic acid as a reference using the Schanderl method, with absorbance reading at 640 nm. Vitamin E was estimated using a Bio-Rad UV/visible spectrophotometer at 695 nm.

Determination of antioxidant activity: DPPH free radical-scavenging assay

The capability to scavenge free radicals of the herbal tea was assessed using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. In methanol solution, DPPH gives a purple colour, which diminishes to yellow in the presence of antioxidants. To perform the assay, 2.4 mL of 0.1 mM DPPH solution was combined with 1.6 mL of the extract in methanol at varying concentrations (12.5–150 µg/mL). The absorbance of the mixture was measured at 517 nm using a spectrophotometer after 20 minutes. Ascorbic acid was utilised as the positive control.

The scavenging activity was calculated using the formula:

$$\text{Scavenging activity (\%)} = (A_{control} - A_{sample}/A_{control}) \times 100$$

Where: $A_{control}$ is the absorbance without the herbal tea extract and

A_{sample} is the absorbance mixed with the extract

***In vitro* α-amylase inhibitory activity**

The *in vitro* α-amylase inhibition of the selected herbal tea combination was assessed using standard protocol. This test evaluates the ability of the developed product to inhibit α-amylase (Hi media RM 638), an enzyme involved in starch breakdown. For the assay, 100 µL of various concentrations of the tea extract (5, 10, 20, 40, 80 & 160 µL) was mixed with 200 µL of α-amylase enzyme and 100 µL of 2 mM of phosphate buffer. After incubation, 100 µL of 1% starch solution was added. Controls were prepared similarly, except that the enzyme was replaced with buffer. Following the incubation, 500 µL of Dinitrosalicylic acid reagent was added to control and test samples, which were kept in a boiling water bath for 5 minutes, followed by measurement of absorbance at 540 nm using a spectrophotometer.

The α-amylase inhibition was calculated with the formula:

$$\text{Inhibition activity (\%)} = (A_{control} - A_{sample}/A_{control}) \times 100$$

Where: $A_{control}$ is the absorbance of the control, and A_{sample} is the absorbance of the tea extract.

Microbial safety analysis

Microbiological parameters play a significant role in the safety and quality of the product. The selected combination of herbal tea powder was stored in airtight LDPE pouches and assessed for the presence of any harmful microorganisms that may affect the quality and safety of the product. The microbial culture was performed to detect Total aerobic plant (FSSAI 15.001:2024) count, yeast and mould (FSSAI 15.023:2024), and the presence of *E. coli*, (FSSAI 15.012:2024) Coliforms (FSSAI 15.025:2024), and Salmonella (FSSAI 15.016:2024) following the methods described in the Association of Official Agricultural Chemists[25].

Statistical analysis

The mean scores of the sensory analysis and one-way ANOVA were analysed using Statistical Package for Social Sciences (SPSS) software version 26.

Results

Sensory evaluation

A sensory evaluation (9-point hedonic scale) was conducted with 30 untrained panellists to quantify the sensory attributes of the developed herbal tea formulations. The mean and standard deviation of the total scores are presented in Table 1, and the ANOVA results are used to evaluate differences among the samples. One-way ANOVA revealed a significant difference between the three formulations across all evaluated attributes (p <0.001). Product T2 (CT and lemongrass) showed the highest mean value and was the most preferred formulation. Panellists reported that T1 has an overpowering mint flavour, while T3

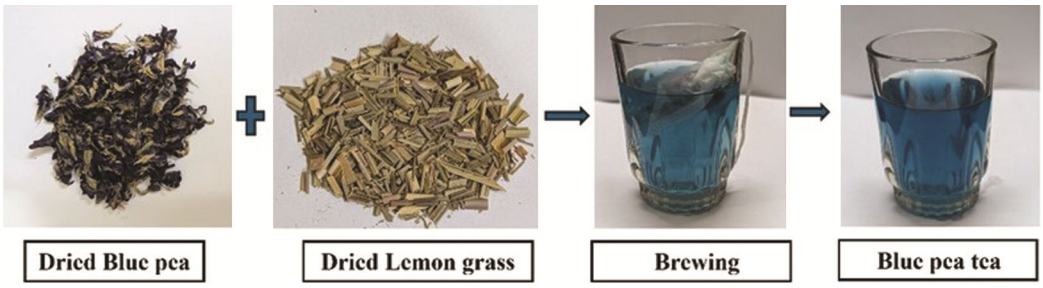

Fig. 1 — Developed herbal tea with blue pea and lemon grass combination.

Table 1 — Sensory evaluation and product acceptability index of herbal tea formulations

Sample	Mean ± SD	PAI (%)	F test	P value
T1 (CT and mint)	44.2±1.794	81.80	3.158	0.00059*
T2(CT and lemon grass)	49.5±2.039	91.60		
T3 (CT and rose petals)	47.4±1.667	87.70		
*Statistically significant (p <0.001)				

Table 2 — Nutrient composition of herbal tea powder (100 g)

Parameter	Values
Moisture	2.95%
Energy (By calculation)	352.82 kcal/100 g
Carbohydrate (By difference)	65.98 g/100 g
Protein (N × 6.25)	19.5 g/100 g
Sugar	BQL
Total fat	1.34 g/100 g
Crude fibre	12.65 g/100 g

has a pleasant aroma, and the taste of rose petals and blue peas did not blend well. The product acceptability index (PAI) calculated with the average total scores is shown in Table 1. Product T2 (Fig. 1) showed a high level of acceptability based on sensory evaluation. Therefore, T2 was selected for subsequent analysis due to its high sensory attributes and acceptability.

Proximate analysis

Table 2 represents the moisture content and nutrient analysis of the developed herbal tea powder per 100 g. Estimating moisture content is important as it plays a major in the stability of the product. High moisture content makes the plant material susceptible to fungal contamination, compromising product quality and safety. Hence, the moisture content of 2.95% in the developed blue pea and lemon grass herbal tea is within the acceptable range indicating good quality and safety. A single serving of this product delivers 10.56 kcal of energy, 1.97 g of carbohydrates, 0.576 g of protein, 0.004 g of fat, and 0.3795 g of crude fibre.

Table 3 compares the nutrient content with the RDA of an adult man and woman with sedentary activity levels. The developed herbal tea is a low-calorie beverage (0.5-0.637%) with a very low-fat content (0.148-0.18%) suitable for daily consumption to improve overall dietary quality.

Phytochemical screening

The qualitative and quantitative analysis results of the developed blue pea tea are summarised in Tables 4 and 5.

DPPH free radical scavenging assay

The antioxidant activity is measured using the DPPH assay, which assesses hydrogen-donating capability. The ability to neutralise free radicals is essential to combat the detrimental effects of free radicals associated with diabetes mellitus. The IC50 is a measure of the concentration of a substance needed to inhibit or neutralise 50% of the DPPH radicals in the system[26]. The developed blue pea tea demonstrated an IC50 value of 0.385 µg/mL, indicating strong antioxidant activity.

***In vitro* α-amylase inhibitory activity**

Results presented in Table 6 compare the α-amylase inhibition by the herbal tea in comparison with the established standard, acarbose at various concentrations. As the concentration of the herbal tea increases from 5 to 160 µg/mL, the inhibition of α-amylase also increases from 10.11 to 73.38%, showing concentration-dependent inhibition activity.

Microbial safety analysis

To ensure food safety, it is imperative to monitor the presence of certain microbes like *E. coli*, *Staphylococcus*, and *Salmonella*, which can spoil the

Table 3 — Comparison of nutrient content with RDA of adult men and women

Parameter	Nutrient content of herbal tea (100 g)	RDA Adult man	% meets for 100 g (Man)	% meets for a single serving -3 g (Man)	RDA Adult woman	% meets for 100 g (Woman)	% meets for a single serving-3 g (Woman)
Energy	352.82 kcal	2110 kcal	16.70	0.5	1660 kcal	21.25	0.637
Protein	19.2 g	54 g	35.5	1.06	45.7 g	42.01	1.26
Fat	1.34 g	27 g	4.96	0.148	22 g	6.09	0.18
Fibre	12.62 g	30 g	42	1.26	30 g	42	1.26

Table 4 — Qualitative analysis of phytoconstituents in herbal tea

Phyto constituent	Method used	Result
Phenols	Ferric chloride test	+
Reducing sugars	Fehlings test	-
Flavones	Shinoda test	+
Glycosides	Anthrone test	+
Saponins	Frothing test	+
Alkaloids	Dragendroff and Mayer's test	+
Anthraquinones	Borntrager's test	-
Quinone	Sodium hydroxide test	-
Protein	Biuret test	+
Tannins	Lead acetate test	+
Steroids	Libermann and Ferric chloride	-

+ present, - absent

Table 5 — Quantitative analysis of phytoconstituents in herbal tea

Phytoconstituent	Concentration
Vitamin E	33.27±0.21
Flavonoids	74.48±0.18
Tannins	17.22±0.14
Total polyphenols	96.20±0.35

Table 6 — Effect of the developed functional beverage on inhibition of α-amylase compared with standard acarbose

Concentration µL	% inhibition of amylase	
	Herbal tea	Standard acarbose
Control	0.19±0.08	0.42±0.18
5	10.11±0.04	16.89±0.06
10	21.48±0.06	27.39±0.12
20	38.79±0.10	42.22±0.08
40	54.27±0.07	57.94±0.06
80	62.27±0.06	73.18±0.04
160	73.38±0.11	87.82±0.09

food and pose health risks upon consumption[27]. The developed blue pea tea was analysed to detect these microbial indicators, along with coliforms. The analysis showed a total plate count of 9.2 x 10^3 cfu /g, within the acceptable limit of $1*10^6$cfu/g as per reference values[25]. Yeast, mould, *E. coli*, *S. aureus*, and coliforms were found to be less than 10 cfu/g with the absence of *Salmonella spp*. in 25 g. The microbial parameters remain within safe and acceptable limits, indicating their suitability for human consumption.

Discussion

This study investigated the nutraceutical properties of underutilised edible plants and their potential for use as a key ingredient in developing herbal tea with enhanced functional properties. The composite of blue tea and lemon grass was selected as the most acceptable formulation through sensory analysis with a product acceptability index of 91.60%. The moisture content of the herbal tea (2.95%) is within the acceptable limits of 6% for dry tea powders, ensuring good quality and safety. Maintaining low moisture levels (13–15%) is essential for the preservation of herbal plant materials[28]. The microbial safety parameters confirm the absence of significant indicator organisms[29] in the developed herbal tea, implying microbial safety and suitability for human consumption. The developed tea exhibits strong antioxidant activity with an IC50 value of 0.385 µg/g, indicating the ability of the herbal tea to aid in neutralising the free radicals and thereby mitigating oxidative damage[30]. The phytoconstituents present in herbal tea have been reported for their antioxidant and antidiabetic properties[31]. Flavonoids present in the tea contribute to the tea's potential hypoglycaemic effects through regulation of glucose absorption, modulation of gut microbiota, and inhibition of advanced glycation end products (AGEs) formation[32]. Tannins delay intestinal glucose absorption[33], while alkaloids exhibit potent antidiabetic effects through inhibition of digestive enzymes and enhancement of insulin secretion[34]. Substantial Vitamin E content contributes to antioxidant activity by delaying free radical-mediated tissue injuries[35] and delaying the progression of oxidative stress-related diseases[36]. These phytochemicals work synergistically, targeting multiple aspects of diabetic therapy, from delaying carbohydrate digestion to enhancing glucose metabolism. The presence of various phytoconstituents suggests that the benefits of glucose metabolism are likely mediated by their combined action rather than the activity of a single compound. This synergistic effect enhances the

overall antidiabetic potential of the tea, providing comprehensive support for glycaemic control.

Delaying carbohydrate digestion and absorption by inhibiting pancreatic α-amylase and α-glucosidase is important for controlling postprandial hyperglycemia[32,37]. The herbal tea's dose-dependent α-amylase inhibition aligns with previous research highlighting the strong amylase inhibitory activity of plant-based foods. These phytochemicals in the tea suggest their potential to delay postprandial glucose levels. By inhibiting key digestive enzymes and exerting antioxidant effects, this herbal tea provides comprehensive support for glycaemic control and oxidative stress reduction, making it a valuable addition to antidiabetic dietary strategies.

Conclusion

Beverages play an integral role in daily consumption patterns, with tea being widely consumed globally for its diverse flavours and cultural significance. Incorporating herbal tea with a unique combination of blue peas and lemon grass into one's routine is an easy way to include antioxidants in the diet. The developed herbal tea offers a natural and holistic dietary approach to managing diabetes mellitus, boasting rich antioxidant properties that can mitigate oxidative stress associated with chronic illnesses. With a wide range of bioactive compounds that target multiple pathways related to glycaemic control, it highlights its potential as a functional beverage for managing diabetes. Future directions include evaluating the herbal tea's impact on acute plasma antioxidant status and its potential in managing postprandial hyperglycemia. Future research will focus on isolating specific phytochemicals and conducting cell line studies to elucidate the antioxidant and antidiabetic mechanisms at the cellular level.

Conflict of interest

The authors declare no conflict of interest.

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