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Antidiabetic and antioxidant potential of powder blend of *Vigna subterranea*, *Curcuma longa*, and *Piper nigrum* in streptozotocin-induced rats

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Abstract

Background: Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia and frequently associated with oxidative stress and metabolic complications. The present study evaluated the antidiabetic and antioxidant potential of a powder blend composed of *Vigna subterranea*, *Curcuma longa*, and *Piper nigrum* in streptozotocin-induced diabetic rats.

Methods: A mixture design approach was used to optimize the formulation of the powder blend based on total phenolic content, flavonoid content, DPPH radical scavenging activity, and α-amylase inhibition. Phytochemical screening and antioxidant assays were performed to characterize the optimized mixture and compare it with individual plant components and binary combinations. Diabetes was induced in Wistar rats using streptozotocin (50 mg/kg), and treatments were administered orally for 14 days.

Results: The optimized mixture exhibited significantly higher total phenolic content (135.65 ± 3.96 mg GAE/g) and flavonoid content (89.62 ± 2.71 mg CE/g) compared with individual plant extracts. Strong antioxidant activity was observed, with ferric reducing antioxidant power (67.42 ± 0.54 µg Trolox/g) and DPPH radical scavenging activity of 59.11 ± 1.58%. In diabetic rats, treatment with the optimized mixture significantly reduced blood glucose levels from 287 mg/dL on day 0 to 173 mg/dL on day 14 ($p<0.05$). Additionally, the mixture improved lipid profile parameters and reduced serum levels of transaminases, urea, creatinine, and uric acid. Antioxidant enzyme activities, including catalase and superoxide dismutase, were significantly increased, while lipid peroxidation decreased.

Conclusion: These findings demonstrate that the combination of *Vigna subterranea*, *Curcuma longa*, and *Piper nigrum* possesses significant antidiabetic and antioxidant properties and may represent a promising nutraceutical strategy for the management of type 2 diabetes.

Keywords: antioxidant activity; *Curcuma longa*; Diabetes mellitus; *Piper nigrum*; streptozotocin; *Vigna subterranea*.

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Background

Metabolic disorders such as obesity, hypertension, and diabetes mellitus have become major global health challenges in recent decades. According to the World Health Organization (WHO), the global prevalence of diabetes has increased dramatically, rising from approximately 200 million cases in 1990 to more than 830 million cases in 2022 [1]. Diabetes and its associated complications, including diabetic nephropathy and cardiovascular diseases, were responsible for more than two million deaths worldwide in 2021 [2]. Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting either from an absolute deficiency in insulin secretion (type 1 diabetes) or from a combination of insulin resistance and impaired insulin secretion (type 2 diabetes). Chronic hyperglycemia leads to several metabolic disturbances and is frequently associated with complications such as dyslipidemia, oxidative stress, cardiovascular diseases, and renal dysfunction [3]. Oxidative stress plays a crucial role in the progression of diabetes by promoting cellular damage through excessive production of reactive oxygen species, particularly affecting pancreatic β-cells and insulin-sensitive tissues. Although several pharmacological treatments, including metformin and sulfonylureas, are currently available for diabetes management, their long-term use may be associated with adverse effects and economic burden, especially in developing countries. Consequently, increasing attention has been directed toward medicinal plants and functional foods as alternative or complementary therapeutic strategies [4]. Many plant species have demonstrated antidiabetic properties through multiple mechanisms, including inhibition of carbohydrate-digesting enzymes, improvement of insulin sensitivity, and reduction of oxidative stress. For example, *Vernonia amygdalina* Del. (Asteraceae) and *Ocimum gratissimum* L. (Lamiaceae) have been reported to exhibit significant antihyperglycemic and antioxidant effects in experimental models [5, 6]. Similarly, *Vigna subterranea* (L.) Verdc. (Bambara groundnut; Fabaceae) has been shown to reduce hyperglycemia and improve metabolic parameters in diabetic models [7, 8]. However, some studies suggest that aqueous extracts of *Vigna subterranea* alone may have limited effects on certain metabolic biomarkers, indicating the need for combination strategies to enhance therapeutic efficacy. Combining medicinal plants with complementary bioactive compounds may enhance therapeutic outcomes through synergistic interactions. *Curcuma longa* L. (turmeric; Zingiberaceae) is widely recognized for its strong antioxidant and anti-inflammatory properties, largely attributed to curcuminoids [9]. In addition, *Piper nigrum* L. (black pepper; Piperaceae) contains piperine, an alkaloid known to enhance the bioavailability of several phytochemicals, including curcumin. Previous studies have demonstrated that piperine significantly increases the intestinal absorption and metabolic stability of curcumin, thereby enhancing its biological activity [10]. Despite the well-documented individual properties of these plants, limited information is available regarding the synergistic effects of their combined use, particularly using optimized mixture formulations. Therefore, the present study aimed to evaluate the antidiabetic and antioxidant potential of an optimized powder blend composed of *Vigna subterranea*, *Curcuma longa*, and *Piper nigrum* using a simplex lattice mixture design approach in streptozotocin-induced diabetic rats.

Methods

Plant Material and Extraction

The plant material used in this study included seeds of *Vigna subterranea* (Bambara groundnut) collected in Bongor, Chad, rhizomes of *Curcuma longa* (turmeric) and fruits of *Piper nigrum L.* (black pepper), both purchased from Dschang local market, Menoua Division, West Cameroon. Air-dried and powdered plant material was macerated in methanol for 48 h to give a solution which was evaporated until dryness under reduced pressure, to give the crude extract.

Laboratory animals

Male *Wistar* rats, aged one month, were bred at the Animal Faculty of the Biochemistry Department, University of Dschang, under standard laboratory conditions in accordance with OECD guidelines [11]. A hypercaloric diet model, as described by Al-Okbi et al. [12], was used for diabetes induction.

Ethical consideration

Animal experiments were performed in this study according to the guidelines set for the care and use of laboratory animals and with the rules formulated under the Animal Welfare Act by the United States Department of Agriculture (USDA) and by adopting ARRIVE guidelines [13], and in agreement with the Ethics and Animal Experimentation Committee of the University of Dschang.

Chemicals

Alpha-amylase, DPPH, and streptozotocin were purchased from Sigma-Aldrich (Germany). Extracts stock solutions and reagents were prepared on the day of the experiments. Streptozotocin was administered intraperitoneally (i.p.) as a disease inducer, while the extracts and extract mixture were delivered via oral gavage.

Preparation of plant powders and extracts

Bambara groundnut seeds were processed according to the method described by [14]. Turmeric rhizomes were peeled, sliced, and dried at 45°C for 48 h, while black pepper fruits were cleaned and dried at 35°C for 24 h [15]. The dried materials were ground separately using a laboratory grinder and sieved to obtain fine powders. The powders were stored in airtight containers protected from light and moisture until further use.

Aqueous extracts were prepared by dissolving the powder samples in distilled water at a concentration of 1000 mg/mL. The mixtures were homogenized thoroughly and filtered using Whatman No. 1 filter paper. The filtrates were freshly prepared prior to administration of experimental animals.

Experimental design and optimization of mixture

A simplex lattice mixture design was used to optimize the proportions of *Vigna subterranea*, *Curcuma longa*, and *Piper nigrum*. The experimental design and statistical analysis were performed using Minitab software. The responses evaluated during optimization included: Total phenolic content (TPC), Total flavonoid content (TFC), DPPH radical scavenging activity, and α-amylase

inhibition activity. Optimal mixture proportions were selected based on the desirability function to achieve maximum biological activity.

Phytochemical Analysis

Total phenolic content (TPC)

Total phenolic content was determined using the Folin–Ciocalteu method as described by [16]. Briefly, 10 µL of extract was mixed with 1.39 mL distilled water and 200 µL Folin–Ciocalteu reagent (1:10 dilution). After 30 min, 40 µL sodium carbonate (20%) was added. The mixture was incubated at 40°C for 20 min, and absorbance was measured at 760 nm using a spectrophotometer. Results were expressed as mg gallic acid equivalents per gram of extract (mg GAE/g) using a freshly prepared gallic acid calibration curve (0.2 g/L).

Total flavonoid content (TFC)

Total flavonoids were quantified using the aluminum chloride colorimetric assay [17]. 100 µL of extract was mixed with 1.4 mL distilled water, 30 µL of sodium nitrite (5%), then 200 µL of AlCl3 (10%), 200 µL NaOH (10%), and 240 µL water. After 10 min, absorbance was measured at 510 nm. Flavonoid content was calculated using a catechin standard curve and expressed as mg catechin equivalent per gram of extract (mg CE/g).

α-Amylase inhibition

The α-amylase inhibition assay was performed according to [18]. 100 µL of extract was incubated with 100 µL of freshly prepared porcine pancreatic α-amylase (0.5 mg/mL) in phosphate buffer (0.02 M, pH 6.9) at 25 °C for 10 min. Then, 100 µL of starch solution (1% w/v) was added, and the mixture incubated for 10 min. The reaction was stopped by adding 200 µL of DNS and boiling for 15 min. Absorbance was measured at 540 nm. Percentage inhibition was calculated as:

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

Antioxidant activity

DPPH Radical Scavenging

Radical scavenging activity was measured using DPPH, as described by [19]. 100 µL of extract was mixed with 900 µL of methanolic DPPH solution and incubated for 30 min in the dark. Absorbance was measured at 517 nm, and inhibition percentage calculated as:

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

FRAP assay

Ferric reducing antioxidant power (FRAP) was assessed following [20]. 100 µL of sample was mixed with 900 µL FRAP reagent, incubated at room temperature for 30 min, and absorbance was measured at 595 nm. Trolox was used as standard.

Experimental animals

Male Wistar rats aged one month were obtained from the Animal Facility of the Department of Biochemistry, University of Dschang. Animals were maintained under standard laboratory conditions: temperature of 22 ± 2°C, relative humidity between 50 and 60%, and light/dark cycle of 12 h/12 h. Animals were provided with standard laboratory feed and water ad libitum.

Induction of experimental diabetes

Thirty-five male rats (≥1 month old) were maintained on a high-fat hypercaloric diet for 8 weeks. Rats weighing 290-400 g with Lee index ≥ 300 were selected. Type 2 diabetes was induced by a single intraperitoneal injection of streptozotocin (50 mg/kg) dissolved in 0.1 M citrate buffer (pH 4.5) [19]. Three days post-injection, blood glucose was measured using Accu-Chek. Rats with blood glucose ≥ 250 mg/dL were considered diabetic.

Animal grouping and treatment

Rats were randomly assigned to six groups of five animals for 14 days:
Healthy control: standard diet + water
Diabetic control: standard diet + water
Diabetic + Metformin: 250 mg/kg
Diabetic + mixture: 1 mL/100g body weight (BW)
Diabetic + Bambara groundnut: 1 mL/100g BW
Diabetic + turmeric + black pepper: 1 mL/100g BW

Biochemical analysis

Biochemical analyses were performed as previously described by Tekou et al [5]. After the treatment period, blood samples were collected and centrifuged at 3500 rpm for 30 min to obtain serum. The following parameters were measured: blood glucose, lipid profile (triglycerides, total cholesterol, HDL, LDL), Liver enzymes (ALT, AST), kidney markers (urea, creatinine), Total protein, and oxidative stress markers (MDA, catalase, SOD).

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). Analysis of variance (ANOVA) followed by Fisher's test was performed using SPSS v26.0. Differences were considered statistically significant at $p < 0.05$.

Results

Effect of factors on responses

Table 1 presents nine experimental runs combining *Vigna subterranea* (67.00–70.00%), *Curcuma longa* (27.00–30.00%), and *Piper nigrum* (2.00–3.00%). α-amylase inhibition ranged from 74.80% to 81.44%, while reducing sugars varied between 4.48 and 6.05 mg/100 g. Total phenols ranged from 103.70 to 132.85 mg GAE/g, flavonoids from 71.40 to 91.17 mg CE/g, and DPPH scavenging activity from 52.74% to 59.56% across runs.

Validation of models and assays

Model validation parameters presented in Table 2 show R^2 values between 0.8803 and 0.9673 across responses. The highest R^2 was observed for α-amylase inhibition (0.9673), while flavonoids showed 0.8803. Mean absolute deviation (MAD) values were approximately 0 for all parameters. Bias factor (Bf) values remained constant at 1.00, falling within the required 0.75–1.00 range for all measured responses.

Optimal conditions of different responses

Optimal conditions of different responses are depicted in Table 3. *V. subterranea* ranging from 67.00% to 68.70%, *C. longa* from 28.29% to 30.00%, and *P. nigrum* from 2.00% to 3.00%. Predicted α-amylase inhibition reached 91.80%, reducing sugars were 4.40 mg/100 g, total phenols were 135.21 mg GAE/g, flavonoids 88.86 mg CE/g, and DPPH scavenging 59.94%, with desirability values equal to 1.00 for all responses.

Phytochemical characterisation and in vitro antioxidant properties

Phytochemical characterisation and in vitro antioxidant properties shown in Table 4 indicate phenolic content ranging from 86.88 ± 0.79 to 135.65 ± 3.96 mg GAE/g, with the optimized mixture recording 135.65 mg GAE/g. Flavonoids varied between 46.50 ± 0.77 and 89.62 ± 2.71 mg CE/g. DPPH scavenging values ranged from 32.38 ± 2.15% to 65.32 ± 0.67%, while FRAP values ranged between 59.84 ± 0.22 and 67.42 ± 0.54 µg Trolox/g. Reducing sugars ranged from 4.34 to 7.74 mg/100 g.

Effect of treatments on lipid profile

Table 5 presents triglyceride levels ranging from 44.58 ± 7.08 mg/dL in healthy controls to 97.41 ± 4.72 mg/dL in untreated diabetic rats. Total cholesterol values varied between 57.32 ± 7.93 and 129.88 ± 8.10 mg/dL. HDL cholesterol ranged from 43.70 ± 8.06 to 78.86 ± 9.74 mg/dL, while LDL cholesterol values ranged between 3.60 ± 3.03 and 36.68 ± 6.44 mg/dL across treatment groups.

Effect of treatments on serum liver enzyme levels (ALT and AST)

The effects of treatments on liver enzyme levels are presented in Table 6. Results display ALT values ranging from 39.21 ± 12.40 U/L in healthy controls to 242.52 ± 10.21 U/L in untreated diabetic rats. AST levels ranged from 50.57 ± 8.75 U/L to 336.95 ± 5.84 U/L. Treated groups showed ALT values between 84.62 ± 10.21 and 103.20 ± 2.92 U/L, while AST values ranged from 76.37 ± 10.94 to 89.26 ± 2.92 U/L.

Effect of treatments on kidney function markers

Table 7 shows the effects of treatments on kidney function markers. Urea levels ranged from 1.28 ± 1.01 to 3.70 ± 0.64 mg/dL among groups. Serum creatinine values varied between 0.07 ± 0.02 and 0.17 ± 0.06 mg/dL, while urinary creatinine ranged from 1.39 ± 0.21 to 2.36 ± 0.10 mg/dL. Healthy controls showed 1.61 ± 0.51 mg/dL urea, while untreated diabetic rats recorded 3.70 ± 0.64 mg/dL.

Effect of treatments on oxidative stress markers

The effects of treatments on oxidative stress markers are presented in Table 8. MDA values ranged from 0.028 ± 0.007 to 0.069 ± 0.020 nmol/mg protein. Catalase activity varied between 2.42 ± 2.31 and 12.57 ± 3.96 U/mg protein, while SOD activity ranged from 1.12 ± 0.59 to 1.42 ± 0.70 U/mg protein. Healthy controls showed 12.57 U/mg catalase, whereas untreated diabetic rats recorded 2.42 U/mg.

Effect of treatments on the animal's blood sugar level

The results of the effect of treatments on the animal's blood sugar level are presented in Figure 1. Fasting blood glucose levels remained stable in the healthy control group, ranging from 71 mg/dL at Day 0 to 72 mg/dL on Day 14. In untreated diabetic rats, glucose levels increased from 326 mg/dL to 410 mg/dL over the same period. Treatment with metformin reduced glucose levels from 323.5 mg/dL to 94 mg/dL, while the optimized mixture decreased glucose from 287 mg/dL to 173 mg/dL by Day 14.

Effect of treatments on serum level of total protein

The results of the effect of treatments on the serum level of total protein are presented in Figure 2. Serum total protein levels varied among experimental groups. Untreated diabetic rats showed the highest protein level (1.079 g/dL) compared with healthy controls (0.814 g/dL). Treatment with metformin and the optimized mixture reduced protein levels to 0.886 g/dL and 0.930 g/dL, respectively. Values observed in treated groups were lower than those of untreated diabetic rats.

Discussion

The present study demonstrated that the optimized blend of *Vigna subterranea*, *Curcuma longa*, and *Piper nigrum* exhibited significantly higher levels of total phenolic compounds and flavonoids compared with the individual plant extracts. This increase suggests a potential synergistic interaction between the phytochemicals present in the different plant species [21]. Phenolic compounds and flavonoids are well known for their strong antioxidant properties and their ability to prevent or delay oxidative stress–related diseases, including diabetes mellitus [22]. These compounds act as free radical scavengers and metal chelators, thereby protecting biological systems against oxidative damage [23]. Oxidative stress plays a major role in the pathogenesis of diabetes and its complications through the overproduction of reactive oxygen species that damage pancreatic β-cells and other tissues [24]. The high antioxidant activity observed in the optimized mixture, confirmed by the DPPH and FRAP assays, could therefore be attributed to the elevated levels of these bioactive compounds. Previous studies have reported that turmeric contains curcuminoids with strong antioxidant properties capable of neutralizing free radicals and improving antioxidant defenses [25, 26]. Experimental studies in diabetic rats have shown that curcuminoids can significantly reduce oxidative stress markers while enhancing antioxidant enzyme activities [27]. Furthermore, black pepper contains piperine, an alkaloid that is known to enhance the bioavailability of many phytochemicals. Piperine increases intestinal absorption and inhibits hepatic metabolism of several compounds, including curcumin [28]. Studies have demonstrated that the co-administration of piperine with curcumin can increase the bioavailability of curcumin by up to 2000%, thereby enhancing its biological activity [10]. The results of the present study showed that treatment with the optimized mixture significantly reduced blood glucose levels in streptozotocin-induced diabetic rats. This antihyperglycemic effect may be explained by several biological mechanisms. Streptozotocin induces experimental diabetes by selectively destroying pancreatic β-cells, leading to decreased insulin secretion and persistent hyperglycemia [29]. Plant extracts rich in polyphenols may exert protective effects on these cells by reducing oxidative stress and inflammation [30]. Curcumin, the principal bioactive compound of

Curcuma longa, has been widely reported to exhibit antihyperglycemic properties. It improves insulin sensitivity and modulates key enzymes involved in glucose metabolism [31]. Experimental studies have shown that curcumin administration significantly reduces blood glucose levels and improves insulin signaling pathways in diabetic animal models [32, 33].

Another mechanism that may contribute to the reduction of blood glucose observed in this study is the inhibition of carbohydrate-digesting enzymes such as α-amylase. Inhibition of these enzymes delays carbohydrate digestion and reduces intestinal glucose absorption, thereby contributing to improved glycemic control [34]. Several polyphenolic compounds have been reported to exhibit strong inhibitory effects on α-amylase and α-glucosidase enzymes.

Diabetes mellitus is frequently associated with dyslipidemia characterized by elevated levels of triglycerides, total cholesterol, and low-density lipoprotein cholesterol (LDL-C), as well as decreased levels of high-density lipoprotein cholesterol (HDL-C) [35]. In the present study, diabetic untreated rats showed significant alterations in lipid profile parameters, confirming the metabolic disturbances associated with diabetes. Treatment with the plant mixture significantly improved these lipid parameters, suggesting a hypolipidemic effect. Polyphenols and flavonoids are known to modulate lipid metabolism by reducing cholesterol synthesis and increasing lipid clearance from the bloodstream [36]. Curcumin has been shown to decrease triglycerides and LDL cholesterol while increasing HDL cholesterol in experimental models and clinical studies [37]. The improvement in lipid profile observed in this study may therefore be attributed to the combined action of the bioactive compounds present in the three plants, particularly curcuminoids and phenolic compounds.

Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are widely used as biomarkers of hepatic injury. Elevated levels of these enzymes in diabetic conditions are usually associated with hepatic damage caused by oxidative stress and metabolic disturbances [38]. In this study, diabetic rats showed significantly increased levels of ALT and AST, indicating liver damage. However, treatment with the plant mixture significantly reduced these enzyme levels, suggesting a hepatoprotective effect. Previous studies have demonstrated that curcumin possesses strong hepatoprotective properties due to its antioxidant and anti-inflammatory activities [39]. Curcuminoids have been shown to reduce liver enzyme levels and improve liver histology in diabetic animal models [40].

The kidney is one of the organs most affected by chronic hyperglycemia. Diabetic nephropathy is characterized by increased serum levels of urea and creatinine due to impaired renal function [41]. In the present study, diabetic untreated rats exhibited significantly elevated levels of these biomarkers, indicating renal impairment. Treatment with the optimized mixture significantly reduced serum urea and creatinine levels, suggesting a protective effect on renal function. This protective effect may be explained by the antioxidant properties of the phytochemicals present in the mixture, which reduce oxidative stress and inflammation in renal tissues [42].

Oxidative stress is a key factor in the development and progression of diabetes and its complications. Increased lipid peroxidation and reduced antioxidant enzyme activity are commonly observed in diabetic conditions [43]. In the present study, untreated diabetic rats exhibited elevated levels of malondialdehyde (MDA), a marker of lipid peroxidation, along with reduced activities of antioxidant enzymes such as catalase and superoxide dismutase (SOD). These findings confirm the presence of oxidative stress induced by diabetes. Treatment with the plant mixture significantly reduced MDA levels while increasing catalase and SOD activities, indicating an improvement in the antioxidant defense system. Curcumin and other phenolic compounds have been reported to enhance antioxidant enzyme activities and reduce oxidative damage in diabetic models [44]. These findings support the hypothesis that the antidiabetic effect of the mixture may partly be mediated through its antioxidant properties.

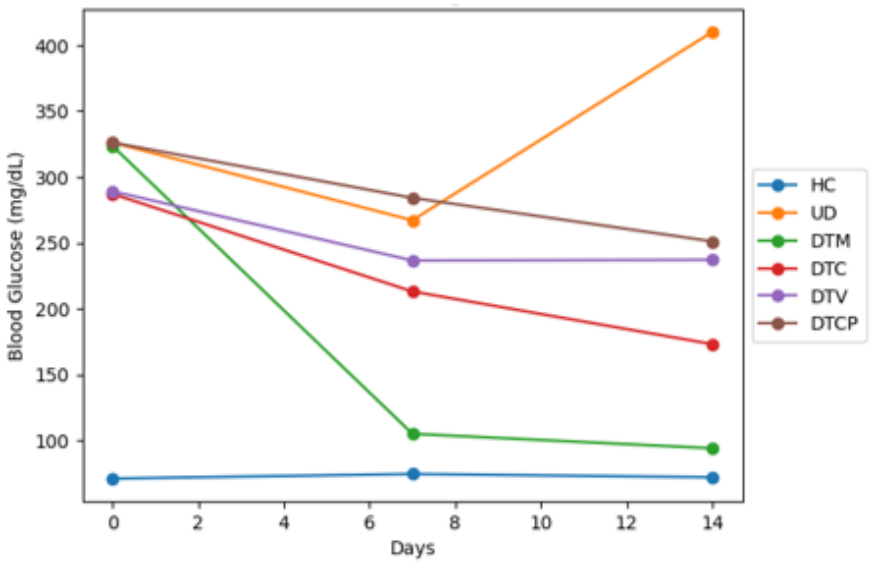

Figure 1. Effect of treatments on fasting blood glucose levels in streptozotocin-induced diabetic rats measured on day 0, 7, and 14.
Values are expressed as mean ± SEM (n = 5). Differences among groups were considered statistically significant at $p < 0.05$.

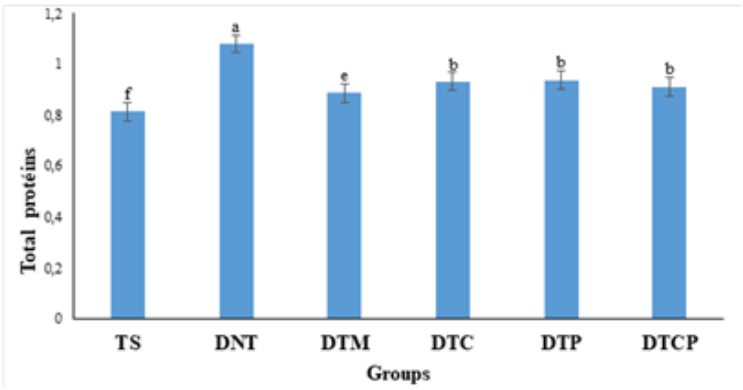

Figure 2. Effect of treatments on serum total protein levels in streptozotocin-induced diabetic rats.
Values are expressed as mean ± SEM (n = 5). Differences among groups were considered statistically significant at $p < 0.05$.

Table 1. Experimental runs and measured responses of the simplex lattice mixture design

Run	*Vs* (%)	*Cl* (%)	*Pn* (%)	α-Amylase inhibition (%)	Reducing sugars (mg/100 g)	Phenols (mg GAE/g)	Flavonoids (mg CE/g)	DPPH (%)
1	67.00	30.00	3.00	81.44 ± 0.92	6.05 ± 0.08	132.85 ± 3.17	86.78 ± 4.27	52.85 ± 2.33
2	70.00	28.00	2.00	79.36 ± 1.96	5.14 ± 0.60	103.70 ± 1.58	76.89 ± 0.39	54.36 ± 0.31
3	70.00	27.00	3.00	78.76 ± 0.71	5.09 ± 0.69	128.36 ± 2.38	84.40 ± 2.72	54.75 ± 1.58
4	68.00	30.00	2.00	74.80 ± 0.50	5.09 ± 0.52	123.87 ± 9.53	91.17 ± 2.72	59.56 ± 1.31
5	68.75	28.75	2.50	75.82 ± 1.14	4.74 ± 0.57	110.42 ± 1.58	73.41 ± 0.38	53.86 ± 2.70
6	67.88	29.38	2.75	75.82 ± 0.30	4.93 ± 0.65	127.24 ± 0.79	79.09 ± 1.16	52.74 ± 4.44
7	69.38	28.38	2.25	75.82 ± 0.87	4.48 ± 0.01	105.94 ± 1.58	73.05 ± 1.16	54.67 ± 1.30
8	69.38	27.88	2.75	75.82 ± 1.07	4.56 ± 0.60	107.06 ± 7.92	76.89 ± 3.10	54.04 ± 0.63
9	68.38	29.38	2.25	75.82 ± 0.51	5.02 ± 0.70	109.30 ± 7.92	71.40 ± 0.77	57.74 ± 0.11

Vs: Vigna subterranea, Cl: Curcuma longa, Pn: Piper nigrum.

Table 2. Statistical validation parameters of the predictive models

Parameter	Abbreviation	Standard requirement	α-Amylase inhibition	Reducing sugars	Phenols	Flavonoids	DPPH
Coefficient of determination	R^2	≥ 0.75	0.9673	0.9232	0.8940	0.8803	0.9267
Mean absolute deviation	MAD	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0
Bias factor	Bf	0.75–1.00	1.00	1.00	1.00	1.00	1.00

Table 3. Optimal proportions of mixture components predicted by the model

Response	*V. subterranea* (%)	*C. longa* (%)	*P. nigrum* (%)	Predicted optimal value	Desirability
α-Amylase inhibition	68.50	28.50	3.00	91.80 %	1.00
Reducing sugars	68.70	28.29	3.00	4.40 mg/100 g	1.00
Total phenols	67.00	30.00	3.00	135.21 mg GAE/g	1.00
Flavonoids	68.00	30.00	2.00	88.86 mg CE/g	1.00
DPPH scavenging	68.00	30.00	2.00	59.94 %	1.00

Table 4. Phytochemical content and antioxidant activity of individual components and mixtures

Parameter	*V. subterranea*	*C. longa* + *P. nigrum*	Optimized mixture (*Vs* +*Cl* + *Pn*)	*C. longa*
Phenols (mg GAE/g)	112.67 ± 1.58^{a}	86.88 ± 0.79^{b}	135.65 ± 3.96^{c}	100.89 ± 6.34^{d}
Flavonoids (mg CE/g)	64.90 ± 0.38^{a}	46.50 ± 0.77^{b}	89.62 ± 2.71^{c}	54.47 ± 1.94^{d}
DPPH (%)	32.38 ± 2.15^{a}	65.32 ± 0.67^{b}	59.11 ± 1.58^{c}	58.72 ± 3.35^{c}
FRAP (µg Trolox/g)	59.84 ± 0.22^{a}	65.58 ± 0.97^{b}	67.42 ± 0.54^{c}	65.97 ± 1.08^{b}
Reducing sugars (mg/100 g)	4.34 ± 0.17^{a}	7.62 ± 0.16^{b}	5.66 ± 0.15^{c}	7.74 ± 0.10^{b}

Vs: Vigna subterranea, Cl: Curcuma longa, Pn: Piper nigrum. Values are expressed as mean ± SEM (n = 3). Values with different superscript letters (a–d) within the same row differ significantly ($p < 0.05$).

Table 5. Effect of different treatments on serum lipid profile in streptozotocin-induced diabetic rats

Group	Triglycerides (mg/dL)	Total Cholesterol (mg/dL)	HDL Cholesterol (mg/dL)	LDL Cholesterol (mg/dL)
Healthy Control (HC)	44.58 ± 7.08^{f}	57.32 ± 7.93^{f}	78.86 ± 9.74^{a}	10.02 ± 3.46^{e}
Untreated Diabetic (UD)	97.41 ± 4.72^{a}	129.88 ± 8.10^{a}	43.70 ± 8.06^{f}	36.68 ± 6.44^{a}
Diabetic + Metformin (DTM)	60.38 ± 6.03^{c}	61.22 ± 6.90^{e}	52.85 ± 2.18^{e}	3.60 ± 3.03^{f}
Diabetic + Mixture (DTC)	78.18 ± 13.48^{b}	81.58 ± 11.21^{b}	54.39 ± 9.74^{d}	18.44 ± 1.23^{b}
Diabetic + *Vs* (DTV)	57.37 ± 1.01^{d}	66.58 ± 0.34^{d}	57.48 ± 0.10^{c}	11.97 ± 9.39^{d}
Diabetic + *Cl* + *Pn* (DTCP)	47.20 ± 0.67^{e}	67.93 ± 3.27^{c}	58.43 ± 9.40^{b}	15.77 ± 9.48^{c}

Vs: Vigna subterranea, Cl: Curcuma longa, Pn: Piper nigrum. Values are expressed as mean ± SEM (n = 5). Values with different superscript letters within the same column differ significantly ($p < 0.05$). Lipid parameters were measured using MONLAB diagnostic kits.

Table 6. Effect of treatments on serum liver enzyme levels (ALT and AST) in streptozotocin-induced diabetic rats

Group	ALT (U/L)	AST (U/L)
Healthy Control (HC)	39.21 ± 12.40^{f}	50.57 ± 8.75^{f}
Untreated Diabetic (UD)	242.52 ± 10.21^{a}	336.95 ± 5.84^{a}
Diabetic + Metformin (DTM)	84.62 ± 10.21^{e}	76.37 ± 10.94^{e}
Diabetic + Mixture (DTC)	99.07 ± 1.46^{c}	88.23 ± 8.03^{c}
Diabetic + *V. subterranea* (DTV)	103.20 ± 2.92^{b}	89.26 ± 2.92^{b}
Diabetic + *C. longa* + *P. nigrum* (DTCP)	91.84 ± 6.56^{d}	82.56 ± 4.38^{d}

Values are expressed as mean ± SEM (n = 5). Values with different superscript letters within the same column differ significantly ($p < 0.05$).

Table 7. Effect of treatments on kidney function parameters in streptozotocin-induced diabetic rats

Group	Serum Urea (mg/dL)	Serum Creatinine (mg/dL)	Urinary Creatinine (mg/dL)
Healthy Control (HC)	1.61 ± 0.51^{d}	0.14 ± 0.03^{c}	1.85 ± 0.25^{c}
Untreated Diabetic (UD)	3.70 ± 0.64^{a}	0.17 ± 0.06^{a}	1.39 ± 0.21^{f}
Diabetic + Metformin (DTM)	2.21 ± 0.73^{c}	0.09 ± 0.02^{d}	2.36 ± 0.10^{a}
Diabetic + Mixture (DTC)	2.54 ± 0.88^{b}	0.08 ± 0.01^{e}	1.51 ± 0.50^{e}
Diabetic + *V. subterranea* (DTV)	1.45 ± 0.41^{e}	0.15 ± 0.01^{b}	1.75 ± 0.43^{d}
Diabetic + *C. longa* + *P. nigrum* (DTCP)	1.28 ± 1.01^{f}	0.07 ± 0.02^{f}	2.06 ± 0.68^{b}

Values are expressed as mean ± SEM (n = 5). Values with different superscript letters within the same column differ significantly ($p < 0.05$).

Table 8. Effect of treatments on oxidative stress parameters in streptozotocin-induced diabetic rats.

Group	MDA (nmol/mg protein)	Catalase (U/mg protein)	SOD (U/mg protein)
Healthy Control (HC)	0.028 ± 0.007^{f}	12.57 ± 3.96^{a}	1.35 ± 1.06^{b}
Untreated Diabetic (UD)	0.069 ± 0.020^{a}	2.42 ± 2.31^{f}	1.12 ± 0.59^{f}
Diabetic + Metformin (DTM)	0.036 ± 0.010^{c}	8.76 ± 3.76^{d}	1.18 ± 0.16^{d}
Diabetic + Mixture (DTC)	0.043 ± 0.050^{d}	8.79 ± 1.23^{c}	1.42 ± 0.70^{a}
Diabetic + *V. subterranea* (DTV)	0.050 ± 0.050^{c}	5.17 ± 1.67^{e}	1.14 ± 0.66^{e}
Diabetic + *C. longa* + *P. nigrum* (DTCP)	0.051 ± 0.039^{b}	11.47 ± 4.30^{b}	1.23 ± 0.52^{c}

MDA = Malondialdehyde, SOD = Superoxide dismutase. Values are expressed as mean ± SEM (n = 5). Values with different superscript letters within the same column differ significantly ($p < 0.05$).

Conclusion

The results of this study demonstrate that the combination of *Vigna subterranea, Curcuma longa,* and *Piper nigrum* exhibits significant antidiabetic, hypolipidemic, and antioxidant effects in streptozotocin-induced diabetic rats. These beneficial effects may be attributed to the synergistic interaction of bioactive compounds such as polyphenols, flavonoids, curcuminoids, and piperine. The mixture appears to exert its effects through multiple mechanisms, including inhibition of carbohydrate-digesting enzymes, improvement of glucose metabolism, regulation of lipid profile, protection of liver and kidney functions, and enhancement of antioxidant defense systems. These findings suggest that this plant combination may represent a promising nutraceutical strategy for the management of type 2 diabetes.

Abbreviations

ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
DPPH: 2,2-Diphenyl-1-picrylhydrazyl
FRAP: Ferric reducing antioxidant power
GAE: Gallic acid equivalent
HDL: High-density lipoprotein
LDL: Low-density lipoprotein
MDA: Malondialdehyde
SEM: Standard error of the mean
SOD: Superoxide dismutase
TPC: Total phenolic content
TFC: Total flavonoid content
WHO: World Health Organization

Authors' Contribution

EAH conducted experiments, analyzed the data, and drafted the manuscript. JPD designed the study, supervised the research, and revised the manuscript. CTM conceived the study, supervised the experiments, and approved the final version. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare no conflict of interest

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