

Original Article

CO-ADMINISTRATION OF *V. amygdalina* LEAF AND *P. biglobosa* SEED EXTRACTS EXERTS ANTIDIABETIC EFFECTS AND AMELIORATES ORGAN DYSFUNCTION IN DIABETIC RATS

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ABSTRACT

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V. amygdalina (VA) and *P. biglobosa* (PB) have independently demonstrated antihyperglycemic and tissue-protective effects, but evidence regarding their combined activity remains limited. This study evaluated the antidiabetic effects of co-administration of VA leaf and PB seed extracts in diabetic rats. Forty-eight male Wistar rats were allocated into six groups: Groups 1 (a non-diabetic control), 2 (untreated diabetic), 3 (glibenclamide-treated diabetic, 2.5 mg/kg), 4 to 6 (diabetic groups treated with VA (200 mg/kg), PB (200 mg/kg), and their combination (200 mg/kg). Fasting blood glucose (FBG), insulin, lipid profile, liver and kidney function parameters were assayed. Secondary metabolites were also quantified. Results showed the presence of tannins, cardiac glycosides, anthocyanins, flavonones, flavones and oxalates amongst others in both plants. Compared with untreated diabetic rats, VA, PB, and glibenclamide significantly reduced FBG ($p < 0.05$). VA + PB produced the fastest early glucose reduction (33.6% by Day 9). Serum insulin remained unchanged ($p > 0.05$) in all the experimental groups. The combined treatment produced the greatest overall improvement in lipid profile, reducing triglycerides by 18.8%, total cholesterol by 21.3%, LDL-cholesterol by 91.9%, and VLDL-cholesterol by 18.8%. It also reduced total bilirubin by 73.9%, direct bilirubin by 44.9%, and serum uric acid by 53.7%, although alkaline phosphatase remained elevated. These findings demonstrated that VA and PB possess significant antihyperglycaemic, hypolipidaemic, hepatoprotective, and renoprotective activities, with combined administration providing the broadest overall metabolic benefits.

Keywords: *V. amygdalina*, antidiabetic, *P. biglobosa*, co-administration, organ dysfunction, diabetic rats

1. INTRODUCTION

Vernonia amygdalina Del. is a small- to medium-sized shrub widely distributed throughout tropical Africa (Ijeh, 2011). Commonly known as bitter leaf, it is consumed as both a vegetable and a traditional medicinal plant across West Africa. In Nigeria, particularly among the Igbo people, the leaves are widely used for culinary purposes after washing to reduce their characteristic bitterness. Traditionally, the expressed leaf extract has been used to manage stomach disorders, while the leaves have also served as a substitute for hops in beer production (Pieroni, 2005). Phytochemical studies have identified flavonoids, sesquiterpene lactones, saponins, tannins, and other phenolic compounds that exhibit antioxidant and antihyperglycaemic activities (Soji-Omoniwa *et al.*, 2025). Experimental studies have consistently demonstrated that *V. amygdalina* lowers fasting blood glucose, improves dyslipidaemia, and attenuates oxidative stress in diabetic animal models. These effects have been attributed to enhanced glucose utilization, inhibition of carbohydrate-digesting enzymes, antioxidant activity, and, in some studies, stimulation of insulin secretion (Degu *et al.*, 2024; Soji-Omoniwa *et al.*, 2025).

Parkia biglobosa (Jacq.) R.Br. ex G.Don (African locust bean) is an important indigenous food plant widely consumed in West Africa.

Its fermented seeds are commonly used as a traditional condiment and constitute a valuable source of proteins, polyphenols, dietary fibre, and other bioactive constituents (Masters & Kelly, 2024). Experimental studies have shown that seed extracts and protein isolates possess antioxidant, antihyperglycaemic, and lipid-modulating activities, improving glucose tolerance, reducing hyperglycaemia, and attenuating oxidative stress and tissue injury in diabetic animal models (Ogunyinka *et al.*, 2019; Masters & Kelly, 2024). These findings highlight the potential of *P. biglobosa* as a functional food ingredient and a candidate for adjunctive diabetes management.

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycaemia resulting from defects in insulin secretion, insulin action, or both (Hossain *et al.*, 2024). It remains a major cause of morbidity and mortality worldwide. The global burden of diabetes has increased substantially, with an estimated 828 million adults living with the disease in 2022 and projections exceeding 1.31 billion cases by 2050 (Zhao *et al.*, 2026). Furthermore, approximately 59% of adults aged 30 years and above with diabetes in low- and middle-income countries remain untreated (Zhao *et al.*, 2026). The escalating prevalence of diabetes, together with its associated health and economic burdens, underscores the need for safe, affordable, and effective therapeutic strategies (Anushree *et al.*, 2025).

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Although current antidiabetic therapies improve glycaemic control and reduce diabetes-related complications, their effectiveness may be limited by adverse effects, cost, restricted accessibility, and incomplete preservation of pancreatic β -cell function. Consequently, there is growing interest in plant-derived therapies that target multiple pathogenic pathways. Polyherbal formulations are widely used in traditional medicine and may provide additive or synergistic therapeutic effects by combining complementary bioactive constituents. Recent preclinical studies have demonstrated that polyherbal interventions can improve glycaemic control, lipid metabolism, oxidative stress, and inflammation more effectively than individual plant extracts (Jain *et al.*, 2025). However, evidence supporting the combined use of culturally accepted edible plants with established medicinal value remains limited.

Although *V. amygdalina* and *P. biglobosa* have each demonstrated antihyperglycaemic, antioxidant, and tissue-protective activities in experimental diabetes, their combined effects have received little investigation. Therefore, this study evaluated the antidiabetic effects of co-administered *V. amygdalina* leaf and *P. biglobosa* seed extracts in nicotinamide/streptozotocin-induced diabetic rats, with particular emphasis on glycaemic control, lipid metabolism, and hepatic and renal function.

2. MATERIALS AND METHODS

Plant Materials:

Freshly collected *V. amygdalina* leaf (Voucher No. UILH|001|2024|1034) and seed of *P. biglobosa* (Voucher No. UILH|002|2024|1309) were collected from Ilorin West Local Government Area, Kwara State, Nigeria. The plant materials were authenticated and taxonomically identified at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria, where voucher specimens were deposited for future reference.

Animals:

Forty-eight (48) male Wistar rats (*Rattus norvegicus*), weighing between 145g-155g, were utilized in this research. Male rats were chosen because they lack the estrous cycle seen in females, which can influence this study. Rats were gotten from the Animal Holding Unit of the Department of Biochemistry in a Federal University. They were housed in well-ventilated cages under standard laboratory conditions with ad libitum access to standard rat chow and water. The rats were left to acclimatize for one week to the laboratory environment before commencing the study.

Extraction of Plant Materials:

Fresh leaves of *Vernonia amygdalina* were collected, thoroughly washed with clean water, and air-dried at 25 °C until a constant weight was attained. The dried leaves were milled into a fine powder using a commercial mill and stored in airtight containers until extraction. A 2,000 g portion of the powdered leaves was extracted by maceration in 12 L of 80% hydromethanol (methanol, 20:80, v/v) for 48 hours at room temperature. The extract was filtered sequentially through muslin cloth and Whatman No. 1 filter paper to obtain a clear filtrate, which was concentrated to dryness in a water bath maintained at 40 °C until a constant weight was achieved. The extraction yielded 11.84% (w/w) crude hydromethanolic extract.

Dried seeds of *Parkia biglobosa* were milled into a fine powder using a commercial blender. A 1,526 g portion of the powdered sample was extracted by maceration in 14 L of 80% hydromethanol (methanol, 20:80, v/v) for 48 hours at room temperature. The extract was filtered through muslin cloth followed by Whatman No. 1 filter paper, and the filtrate was concentrated to dryness in a water bath at 40 °C until a constant weight was obtained. The extraction yielded 9.21% (w/w) crude hydromethanolic extract.

The dried extracts were transferred into airtight amber bottles and stored at 4 °C, protected from light until use. All extracts were used within two weeks of preparation to minimize degradation of phytochemical constituents and maintain extract stability.

High-Performance Liquid Chromatography (HPLC) Analysis of *V. amygdalina* Leaf and *P. biglobosa* Seed Extracts:

Leaf of *V. amygdalina* and seed of *P. biglobosa* were dried, pulverized, and extracted with 70% methanol. Extracts were sonicated, centrifuged, and filtered through a 0.45 μ m membrane filter, then stored at 4 °C in amber vials until analysis. Chromatographic separation was carried out using a High-Performance Liquid Chromatography system fitted with a C18 column. The mobile phase consisted of acidified water and acetonitrile under gradient conditions at a flow rate of 1.0 mL/min. Detection was performed at 280 nm and 340 nm. Identification and quantification of secondary metabolites were achieved using reference standards (Quercetin and Gallic acid) (Stoene-scu *et al.*, 2022).

Induction and Confirmation of Type 2 Diabetes:

Experimental diabetes was induced using a nicotinamide-streptozotocin (NAD-STZ) protocol, using the method of Masiello *et al.* (1998). Nicotinamide (NAD) was freshly dissolved in normal saline and administered intraperitoneally at a dose of 120 mg/kg body weight to all rats except those in the normal (non-diabetic) control group. Before induction, the animals were fasted overnight, and fasting blood glucose levels were measured using an Accu-Chek® glucometer with compatible test strips.

Fifteen (15) minutes after nicotinamide administration, streptozotocin (STZ) was freshly prepared in ice-cold 0.1 M sodium citrate buffer (pH 4.5). The pH of the buffer was verified using a calibrated pH meter before preparation. Streptozotocin was administered intraperitoneally at a dose of 60 mg/kg body weight to rats in Groups 2–6.

Seven days after NAD-STZ administration, fasting blood glucose levels were re-evaluated using an Accu-Chek® Active glucometer and compatible test strips. Blood samples were obtained from the caudal tail vein after an overnight fast. Rats with fasting blood glucose concentrations >200 mg/dL were considered diabetic and included in the subsequent experimental procedures (Soji-Omoniwa *et al.*, 2025).

Animal Grouping and Experimental Design:

Forty-eight (48) rats were assigned randomly into six experimental groups (n = 8 per group) and extracts were reconstituted in distilled water and administered orally. Group 1: Non-diabetic rats administered distilled water and standard feed. Group 2: Diabetic rats untreated throughout the experimental period. Group 3: Diabetic rats dosed with 2.5 mg/kg body weight of Glibenclamide. Group 4: Diabetic rats dosed with 200 mg/kg body weight of *V. amygdalina* leaf extract. Group 5: Diabetic rats dosed with 200 mg/kg body weight of *P. biglobosa* seed extract. Group 6: Diabetic rats dosed a combination of 200 mg/kg body weight each of *V. amygdalina* leaf and *P. biglobosa* seed extracts.

Treatment Administration:

Treatment commenced immediately after confirmation of diabetes, orally, for 21 days once daily. Extracts were administered orally at a volume of 2 mL/kg body weight using an oral gavage. Throughout the treatment period, rats had ad libitum access to food and water.

Animal sacrifice and serum collection:

Rats were sacrificed after the final day of treatment. Animals were briefly exposed to trichloromethane vapor to induce light anesthesia prior to sacrifice, minimizing handling stress and discomfort. They were sacrificed by incising the jugular veins with scalpel. Blood samples were collected into plain sample bottles, allowed to coagulate and serum collected for biochemical analysis.

Biochemical Assays

Determination of Fasting Blood Glucose (FBG) Concentration:

Fasting blood glucose (FBG) was measured using an Accu-Chek® Active glucometer with compatible test strips. Measurements were obtained at baseline (Day 0) and on Days 1, 5, 9, 13, 17, and 21 of the experimental period. Before each measurement, the rats were fasted overnight. Blood samples were collected from the caudal tail vein by making a small incision and obtaining the minimum volume required for glucose determination. Changes in FBG were monitored throughout the treatment period to evaluate the antihyperglycaemic effects of the experimental interventions.

Determination of Insulin Concentration of Experimental Rats:

Quantification of serum insulin was performed with the Calbiotech insulin ELISA kit (Cat No: IS130D) according to the provided protocol. Before beginning the insulin ELISA assay, all chemicals were at ambient temperature and carefully stirred to ensure uniform distribution. The necessary number of coated strips were prepared, placing them carefully into the holder. Next, the samples were carefully dispensed into their designated wells using a pipette; 25 microliters of insulin standards, control samples, and serum samples. With precision, 100 microliters of Insulin Conjugate Reagent were added to each well. To ensure the contents were thoroughly mixed, the plate was gently swirled for 20 seconds. Once mixed, the plate was incubated at 25 °C for 1 hour. After the incubation, the liquid from all the wells was removed. The wells were then washed three times with 300 microliters of 1X wash buffer to remove any unbound substances. Next, 100 microliters of Tetramethylbenzidine (TMB) substrate were added to each well. The plate was again incubated at 25 °C for 15 minutes. To stop the reaction and stabilize the color, 50 microliters of stop solution was added to each well. The plate was gently shaken to mix the solutions thoroughly. Finally, the absorbance of each well was read on microplate reader (Model: 550, wavelength 450 nm). This was done within 15 minutes of adding the stop solution.

Calculation of HOMA-IR and HOMA-β scores:

Homeostatic model assessment for insulin resistance (HOMA-IR) and homeostatic model assessment for beta score (HOMA-β scores) were calculated using the formulas by Matthews *et al.*, 1985

$$\text{HOMA-IR} = \frac{\text{Fasting Insulin (mIU/L)} \times \text{Fasting Glucose (mmol/L)}}{22.5} \quad (1)$$

$$\text{HOMA-}\beta = \frac{20 \times \text{Fasting Insulin (mIU/L/mL)}}{\text{Fasting Glucose (mmol/L)} - 3.5} \quad (2)$$

Conversion Factor 1 Mg/dL is 0.0555 mmol/L

Determination of Total Lipid Profile, Liver and Kidney Function Indices of Diabetic Rats Treated with *V. amygdalina* Leaf and *P. biglobosa* Seed Extracts:

Total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein (HDL) liver function parameters, including total protein, albumin, globulin, total and direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), as well as renal function indices comprising urea, creatinine, uric acid, and serum electrolytes, were analyzed according to the standard procedures outlined in the assay kit protocols provided by Fortress Diagnostics Ltd. (United Kingdom).

Low-density lipoprotein cholesterol (LDLc) and Very low-density lipoprotein cholesterol (VLDLc) concentrations were calculated by Friedewald's formula:

$$\text{LDLc (mmol/l)} = \text{TC} - \text{HDLc} - \frac{\text{Triglyceride}}{2.2} \quad (3)$$

$$\text{VLDL (mmol/l)} = \frac{\text{Triglyceride}}{2.2} \quad (\text{Friedewald et al., 1972}) \quad (4)$$

Statistical Analysis:

Data were analysed using SPSS (version 27) and one-way ANOVA with Duncan's multiple range tests were used for multiple comparison. Differences were taken as statistically significant at $p < 0.05$. Values were represented as means of 8 determinations $\pm$ standard error mean (SEM).

RESULTS

Secondary metabolites constituents of *V. amygdalina* leaf extract:

The phytochemical composition of *V. amygdalina* leaf extract is presented in Table 1. A wide range of secondary metabolites was identified at varying concentrations. Among the detected compounds, tannin (11.0566 µg/mL), sparteine (9.2781 µg/mL), and cardiac glycosides (7.8816 µg/mL) were the most abundant constituents. Anthocyanin (7.8049 µg/mL) and flavonones (6.3411 µg/mL) were also present in relatively high concentrations. Moderate levels of Kaempferol (4.4926 µg/mL), Catechin (3.5605 µg/mL), flavone (3.6569 µg/mL), and dihydrocytisine (3.1593 µg/mL) were observed. Lower concentrations were recorded for sapogenin (1.7930 µg/mL), ephedrine (1.7854 µg/mL), cyanogenic glycosides (1.4510 µg/mL), and ammondenrine (0.6006 µg/mL). Other constituents such as ribalinidine (5.0940 µg/mL), phytate (4.2924 µg/mL), proanthocyanidin (2.4434 µg/mL), and oxalate (1.5898 µg/mL) were also detected, indicating a diverse phytochemical profile of the extract.

Secondary metabolites constituents of *P. biglobosa* seed extract:

The phytochemical profile of the extract is presented in Table 2, revealing the presence of diverse secondary metabolites in varying concentrations. Among the identified compounds, tannin (18.3554 µg/mL) and oxalate (17.8919 µg/mL) were the most abundant constituents. Flavonones (9.3773 µg/mL), sapogenin (6.7594 µg/mL), flavone (6.7687 µg/mL), and aphyllidine (6.3324 µg/mL) were also present in relatively high concentrations. Moderate levels of Catechin (3.9716 µg/mL), dihydrocytisine (3.9127 µg/mL), cyanogenic glycosides (3.2998 µg/mL), and steroids (2.9950 µg/mL) were observed. Lower concentrations were recorded for Kaempferol (1.5279 µg/mL), ephedrine (1.9677 µg/mL), naringenin (2.0459 µg/mL), ribalinidine (2.0818 µg/mL), and anthocyanin (2.1245 µg/mL). Sparteine (5.7222 µg/mL) and proanthocyanidin (4.0698 µg/mL) were present in moderate amounts, while phytate (0.0130 µg/mL) was detected at a very low concentration. Overall, the results indicate a rich and varied phytochemical composition of the extract.

Fasting blood glucose concentration of experimental rats:

The effect of treatments on fasting blood glucose levels over a 21-day period is presented in Table 3. At baseline (Day 0), there was no significant difference ($p > 0.05$) in blood glucose levels across all groups, indicating uniformity prior to induction. Following induction, the untreated diabetic group showed a significant increase ($p < 0.05$) in glucose levels from Day 1 onward, reaching peak values at Day 13 and remaining persistently elevated throughout the study period. In contrast, the standard drug-treated group (Group 3, Glibenclamide) demonstrated a progressive and significant reduction ($p < 0.05$) in blood glucose levels, with marked improvement observed from Day 13 to Day 21. Similarly, treatment groups administered with *Vernonia amygdalina* and *Parkia biglobosa* extracts, either individually or in combination, showed gradual reductions in glucose levels compared to the diabetic control group. Notably, the combination group (Group 6) exhibited a steady decline from Day 9 onwards, indicating enhanced glycemic control, although values remained slightly elevated relative to the normal control.

Table 1: HPLC quantification of secondary metabolites in *V. amygdalina* leaf extract

Constituents	Retention	Area	Height	External	Units
Sapogenin	0.140	4083.6304	389.518	1.7930	ug/ml
Narigenin	3.473	4093.0642	320.769	2.8958	ug/ml
Anthocyanin	4.613	6355.7564	494.097	7.8049	ug/ml
Epilhedrine	9.480	4859.7778	380.588	1.7854	ug/ml
Dihydrocytisine	10.866	4880.9656	366.718	3.1593	ug/ml
Kaempferol	13.126	6488.6312	507.928	4.4926	ug/ml
Cyanogenic glycoside	14.886	5010.1896	392.627	1.4510	ug/ml
Aphyllidine	16.956	3363.0461	263.532	1.5098	ug/ml
Steroid	19.440	4580.4180	359.146	3.1713	ug/ml
Tannin	22.883	8235.4138	642.944	11.0566	ug/ml
Flavonones	25.713	7931.5974	618.930	6.3411	ug/ml
Catechin	28.606	2956.9610	231.988	3.5605	ug/ml
Flavone	30.280	5551.2211	435.394	3.6569	ug/ml
Proanthocyanidin	32.730	4433.1010	347.266	2.4434	ug/ml
Ribalinidine	34.633	4104.5175	321.457	5.0940	ug/ml
Sparteine	36.896	7473.4783	583.325	9.2781	ug/ml
Oxalate	38.526	4454.0796	349.461	1.5898	ug/ml
Cardiac glycoside	39.326	8756.4876	683.810	7.8816	ug/ml
Phytate	40.326	6201.0704	486.517	4.2924	ug/ml
Ammodendrine	40.953	3567.9760	280.808	0.6006	ug/ml

Table 2: HPLC quantification of secondary metabolites in *P. biglobosa* seed extract

Constituents	Retention	Area	Height	External	Units
Kaempferol	0.386	2631.0434	206.270	1.5279	ug/ml
Steroid	0.873	4824.9767	377.473	2.9950	ug/ml
Epilhedrine	3.093	3387.3150	265.781	1.9677	ug/ml
Catechin	5.483	2954.8422	231.937	3.9716	ug/ml
Anthocyanin	7.873	4968.0357	389.418	2.1245	ug/ml
Dihydrocytisine	9.350	3057.1151	240.132	3.9127	ug/ml
Cyanogenic glycoside	14.036	3250.6604	255.555	3.2998	ug/ml
Aphyllidine	19.973	4928.6840	387.165	6.3324	ug/ml
Narigenin	20.616	3419.6849	268.983	2.0459	ug/ml
Tannin	23.363	6921.5652	536.497	18.3554	ug/ml
Flavonones	25.960	7289.2832	570.180	9.3773	ug/ml
Flavone	29.326	5263.8132	410.175	6.7687	ug/ml
Proanthocyanidin	31.653	6802.6676	532.675	4.0698	ug/ml
Ribalinidine	34.150	3084.5690	241.227	2.0818	ug/ml
Sparteine	36.773	4449.9298	348.939	5.7222	ug/ml
Oxalate	38.640	8023.9062	624.600	17.8919	ug/ml
Sapogenin	43.536	5031.2166	394.788	6.7594	ug/ml
Phytate	45.066	10.1155	0.225	0.0130	ug/ml

Table 3: Fasting Blood Glucose Concentration of Nicotinamide and Streptozotocin- induced diabetic and normal rats treated with *V. amygdalina* leaf and *P. biglobosa* seed extract

Groups	0	1	5	Days 9 (mg/dL)	13	17	21
1	64.50 ± 1.65 ^a	67.50 ± 0.28 ^a	65.00 ± 0.57 ^a	74.00 ± 0.57 ^a	81.00 ± 1.73 ^a	82.00 ± 1.15 ^a	64.00 ± 0.57 ^a
2	63.53 ± 1.44 ^a	287.0 ± 0.57 ^b	293.50 ± 1.44 ^b	224.00 ± 0.57 ^b	348.50 ± 4.33 ^b	323.00 ± 1.73 ^b	309.50 ± 4.33 ^b
3	68.00 ± 1.22 ^a	287.00 ± 0.57 ^b	286.50 ± 1.84 ^b	288.00 ± 1.00 ^c	117.50 ± 0.28 ^c	101.00 ± 0.57 ^c	121.50 ± 1.25 ^c
4	68.00 ± 0.57 ^b	295.50 ± 0.28 ^b	275.00 ± 1.50 ^c	255.00 ± 1.32 ^d	117.50 ± 0.28 ^c	174.00 ± 1.00 ^d	90.00 ± 1.00 ^d
5	71.00 ± 1.73 ^a	247.0 ± 5.77 ^c	252.00 ± 1.00 ^d	217.50 ± 1.10 ^e	116.50 ± 0.28 ^c	145.75 ± 1.75 ^e	106.50 ± 7.79 ^c
6	72.50 ± 2.59 ^a	286.00 ± 1.73 ^b	264.50 ± 2.59 ^d	190.00 ± 2.88 ^f	142.00 ± 1.10 ^d	122.00 ± 1.73 ^f	128.00 ± 1.39 ^c

Values are expressed as mean ± SEM, n=8. Groups with varying alphabets down the column are different (p < 0.05); day 0 = FBG pre-induction

Effects of *V. amygdalina* leaf and *P. biglobosa* seed extract on insulin levels, insulin resistance (HOMA-IR), and β-cell function (HOMA-β) of nicotinamide and streptozotocin-induced diabetic rats

The effects of treatment on insulin levels, insulin resistance (HOMA-IR), and β-cell function (HOMA-β) are presented in Table 4. There was no significant difference (p > 0.05) in insulin levels across all groups. The untreated diabetic group showed a significant increase (p < 0.05) in HOMA-IR and a significant reduction (p < 0.05) in HOMA-β compared to the normal control. Treatment groups exhibited further increases in HOMA-IR values relative to the diabetic

control, with Group 5 showing the highest level of insulin resistance, while the combination group (Group 6) showed a comparatively lower HOMA-IR than some treated groups but remained elevated above normal. Similarly, HOMA-β scores were significantly reduced in all diabetic and treated groups compared to the normal control.

Effects of *V. amygdalina* leaf and *P. biglobosa* seed extract on lipid parameters of nicotinamide and streptozotocin-induced diabetic rats:

The effects of treatment on lipid profile parameters are presented in Table 5. The untreated diabetic group exhibited significant ele-

variations ($p < 0.05$) in triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL), along with an alteration in high-density lipoprotein (HDL), compared to the normal control group, indicating dyslipidemia associated with Diabetes Mellitus. Treatment with the standard drug (Group 3, Glibenclamide) and plant extracts resulted in significant modulation of lipid parameters. Notably, the combination group (Group 6) showed marked improvement, with TG, TC, LDL, and VLDL levels significantly reduced ($p < 0.05$) compared to the diabetic control and approaching normal values. Additionally, HDL levels were elevated in treated groups relative to the normal control, with individual extract-treated groups (Groups 4 and 5) showing higher HDL values than the combination group.

Effects of *V. amygdalina* leaf and *P. biglobosa* seed extract on liver function parameters of nicotinamide and streptozotocin- induced diabetic rats:

The effects of treatment on liver function parameters are presented in Table 6. The untreated diabetic group showed significant increase ($p < 0.05$) in total and direct bilirubin levels compared to the normal control, indicating hepatic dysfunction associated with Diabetes Mellitus. Total protein, albumin, and globulin levels were also increased in the diabetic group. Treatment with the standard drug (Group 3, Glibenclamide) and plant extracts resulted in varying degrees of modulation of these parameters. Notably, the combination group (Group 6) exhibited a significant reduction ($p < 0.05$) in total and direct bilirubin levels compared to the diabetic control, with values approaching those of the normal group. However, total protein

and globulin levels in the combination group were lower than those of the diabetic control and comparable ($p > 0.05$) to the normal control, while albumin levels remained relatively stable across groups. Alkaline phosphatase (ALP) activity did not show significant differences across the groups ($p > 0.05$).

Effects of *V. amygdalina* leaf and *P. biglobosa* seed extract on kidney function parameters of nicotinamide and streptozotocin- induced diabetic rats:

The effects of treatment on renal function and electrolyte parameters are presented in Table 7. The untreated diabetic group exhibited significant elevations ($p < 0.05$) in uric acid, creatinine, and urea levels compared to the normal control, indicating renal impairment associated with Diabetes Mellitus. Bicarbonate (HCO_3^-) levels were also increased in the diabetic group, while potassium, chloride, and sodium levels showed no marked deviation ($p > 0.05$) from the control. Treatment with the standard drug (Group 3, Glibenclamide) and plant extracts resulted in significant improvements in renal biomarkers. Notably, uric acid levels were reduced to values comparable to the normal control in all treated groups. Creatinine levels were also significantly ($p < 0.05$) decreased in the treated groups compared to the diabetic control, with Group 5 showing the greatest reduction. Urea levels showed partial normalization, particularly in Groups 4 and 5. Electrolyte parameters exhibited variable responses, with bicarbonate levels remaining elevated in treated groups, while potassium and sodium levels showed slight increases in some groups but remained within a comparable range to the control.

Table 4: Insulin, Homeostatis model Assessment for Insulin Resistance and Pancreatic β -score of Nicotinamide and Streptozotocin-induced diabetic and normal rats treated with *V. amygdalina* leaf and *P. biglobosa* seed extract

Groups	TG	TC	HDL (mmol/L)	LDL	VLDL
1	1.56 $\pm$ 0.11 ^a	2.24 $\pm$ 0.09 ^a	1.08 $\pm$ 0.10 ^a	0.45 $\pm$ 0.03 ^a	0.71 $\pm$ 0.08 ^a
2	1.86 $\pm$ 0.08 ^b	3.62 $\pm$ 0.25 ^b	1.42 $\pm$ 0.00 ^b	1.35 $\pm$ 0.06 ^b	0.85 $\pm$ 0.05 ^{ab}
3	2.05 $\pm$ 0.15 ^b	3.84 $\pm$ 0.12 ^b	2.31 $\pm$ 0.07 ^c	0.60 $\pm$ 0.04 ^c	0.93 $\pm$ 0.07 ^b
4	2.75 $\pm$ 0.00 ^c	3.88 $\pm$ 0.00 ^b	2.37 $\pm$ 0.00 ^c	0.26 $\pm$ 0.03 ^d	1.25 $\pm$ 0.03 ^c
5	1.97 $\pm$ 0.03 ^b	3.54 $\pm$ 0.07 ^b	2.37 $\pm$ 0.06 ^c	0.27 $\pm$ 0.08 ^d	0.90 $\pm$ 0.03 ^b
6	1.51 $\pm$ 0.14 ^a	2.85 $\pm$ 0.31 ^c	2.05 $\pm$ 0.08 ^d	0.11 $\pm$ 0.01 ^e	0.69 $\pm$ 0.09 ^a

Values are expressed as mean $\pm$ SEM, n=8. Groups with varying alphabets down the column are different ($p < 0.05$) while those with same alphabets are not significantly different ($p > 0.05$)

Table 5: Lipid Parameters of Nicotinamide and Streptozotocin- induced diabetic and normal rats treated with *V. amygdalina* leaf and *P. biglobosa* seed extract

Groups	Insulin (mIU/L)	HOMA-IR	HOMA β SCORE
1	4.27 $\pm$ 0.08 ^a	0.71 $\pm$ 0.04 ^a	125.22 $\pm$ 0.00 ^a
2	4.80 $\pm$ 0.31 ^a	2.06 $\pm$ 0.31 ^{ab}	11.75 $\pm$ 1.18 ^b
3	4.59 $\pm$ 0.16 ^a	2.97 $\pm$ 0.48 ^b	7.22 $\pm$ 0.93 ^c
4	4.08 $\pm$ 0.00 ^a	2.95 $\pm$ 0.00 ^b	6.40 $\pm$ 0.00 ^c
5	4.37 $\pm$ 0.15 ^a	5.13 $\pm$ 0.62 ^c	4.30 $\pm$ 0.27 ^c
6	4.32 $\pm$ 0.17 ^a	3.61 $\pm$ 0.58 ^{bc}	6.57 $\pm$ 0.69 ^c

Values are expressed as mean $\pm$ SEM, n=8. Groups with varying alphabets down the column are different ($p < 0.05$)

Table 6: Liver function parameters of Nicotinamide and Streptozotocin-induced diabetic and normal rats treated with *V. amygdalina* leaf and *P. biglobosa* seed extract

Groups	Total bilirubin ($\mu\text{mol/l}$)	Direct Bilirubin ($\mu\text{mol/l}$)	Total Protein (g/l)	Albumin (g/dl)	Globulin (g/dl)	ALP (U/L)
1	86.58 $\pm$ 0.98 ^b	66.42 $\pm$ 1.82 ^{bc}	65.81 $\pm$ 1.17 ^{ab}	3.13 $\pm$ 0.05 ^{ab}	2.71 $\pm$ 0.11 ^a	77.61 $\pm$ 2.28 ^b
2	215.17 $\pm$ 0.00 ^c	92.35 $\pm$ 0.00 ^c	80.97 $\pm$ 0.00 ^d	4.02 $\pm$ 0.00 ^c	4.06 $\pm$ 0.00 ^{bc}	305.37 $\pm$ 3.83 ^b
3	90.01 $\pm$ 1.97 ^b	74.48 $\pm$ 2.49 ^d	79.46 $\pm$ 1.83 ^{cd}	3.20 $\pm$ 0.09 ^b	4.59 $\pm$ 0.23 ^{cd}	292.07 $\pm$ 0.02 ^b
4	88.29 $\pm$ 0.00 ^b	71.48 $\pm$ 0.00 ^{cd}	78.49 $\pm$ 0.00 ^d	2.82 $\pm$ 0.00 ^a	5.02 $\pm$ 0.00 ^d	396.47 $\pm$ 0.00 ^b
5	86.15 $\pm$ 0.74 ^b	60.72 $\pm$ 1.09 ^b	69.53 $\pm$ 4.68 ^{bc}	3.22 $\pm$ 0.12 ^b	3.75 $\pm$ 0.08 ^b	286.50 $\pm$ 3.17 ^b
6	56.15 $\pm$ 1.23 ^a	50.92 $\pm$ 0.18 ^a	56.28 $\pm$ 3.38 ^a	3.15 $\pm$ 0.04 ^a	2.51 $\pm$ 0.09 ^a	281.12 $\pm$ 3.92 ^b

Values are expressed as mean $\pm$ SEM, n=8. Groups with varying alphabets down the column are different ($p < 0.05$)

Table 7: Kidney function parameters of Nicotinamide and Streptozotocin- induced diabetic and normal rats treated with *V. amygdalina* leaf and *P. biglobosa* seed extract

Groups	Uric acid (mg/dl)	Creatinine (μmol/L)	Urea (mg/dl)	HCO ₃ ⁻ (mmol/l)	K (mmol/l)	Cl ⁻ (mmol/l)	Na (mmol/l)
1	16.69 ± 0.60 ^a	70.80 ± 0.10 ^a	26.10 ± 0.97 ^a	19.92 ± 0.62 ^a	3.19 ± 0.20 ^a	71.47 ± 3.20 ^a	172.28 ± 2.83 ^{ab}
2	24.92 ± 0.52 ^b	132.75 ± 0.21 ^b	29.20 ± 1.96 ^b	23.02 ± 0.16 ^b	3.39 ± 0.12 ^{ab}	83.34 ± 4.56 ^a	167.54 ± 0.44 ^{ab}
3	15.24 ± 1.75 ^a	88.50 ± 0.21 ^c	32.50 ± 2.16 ^b	21.64 ± 0.62 ^b	3.02 ± 0.05 ^a	78.77 ± 3.30 ^a	174.29 ± 2.70 ^{ab}
4	15.56 ± 0.02 ^c	88.50 ± 0.02 ^c	26.40 ± 0.12 ^a	21.86 ± 0.02 ^b	3.54 ± 0.00 ^{ab}	100.89 ± 0.00 ^b	196.49 ± 0.00 ^b
5	15.63 ± 0.29 ^c	78.32 ± 1.27 ^d	27.80 ± 1.50 ^{ab}	25.70 ± 0.86 ^c	4.39 ± 0.28 ^b	82.40 ± 0.58 ^a	183.94 ± 2.74 ^{ab}
6	11.54 ± 0.08 ^d	92.95 ± 1.37 ^e	27.70 ± 1.13 ^{ab}	23.38 ± 1.40 ^{bc}	2.13 ± 0.31 ^a	79.58 ± 1.69 ^a	161.57 ± 2.35 ^a

Values are expressed as mean ± SEM, n=8. Groups with varying alphabets down the column are different (p < 0.05); HCO₃⁻ - bicarbonate; K - Potassium; Cl⁻ - Chloride; Na - Sodium; ALP - Alkaline phosphatase

3. DISCUSSION

Findings from this study revealed that both *V. amygdalina* leaf and *P. biglobosa* seed have a diverse range of bioactive secondary metabolites, including alkaloids, flavonoids, tannins, glycosides, steroids, and phenolic-related compounds, at varying concentration. This confirms that the plant materials are rich sources of structurally and pharmacologically active constituents that may contribute to their observed biological effects in diabetes mellitus. Among the identified compounds, tannins, cardiac glycosides, anthocyanins and flavonones, flavones and oxalates were among the most abundant constituents in both plants. These compounds are well known for their strong antioxidant, anti-inflammatory, and metabolic regulatory activities. Tannins, for instance have been reported to contribute to inhibition of α-amylase and lipase, thereby modulating glucose and lipid metabolism (Ansari et al., 2024). Anthocyanins and flavonoids are potent free radical scavengers, reducing oxidative stress linked to diabetic complications. Cardiac glycosides, though bioactive at low concentrations, may influence cellular ion transport and metabolic signaling pathways. The key flavonoids detected include kaempferol, catechin, naringenin and flavone. These compounds are known to enhance insulin sensitivity, improve glucose uptake in peripheral tissues, modulate lipid metabolism and protect pancreatic β-cells from oxidative damage (Al-Ishaq et al., 2019; Ansari et al., 2024; Bouyahya et al., 2024). Their presence strongly supports the antidiabetic potential of the plant extracts. Alkaloids such as sparteine, dihydrocytisine, and ribalinidine were also detected. These compounds are associated with modulation of neurotransmission, enzyme inhibition, possible influence on appetite and metabolic regulation (Bouyahya et al., 2024). However, some alkaloids require careful consideration due to potential toxicity at high concentrations.

Compounds such as cyanogenic glycosides, phytate, and oxalate were present at relatively low levels in *V. amygdalina* leaf. While these are classified as anti-nutritional factors, their concentrations in this study are within low ranges and are unlikely to cause significant toxicity under controlled dosage conditions. In *P. biglobosa* seed, oxalate and phytate were also detected. While oxalate levels appear relatively high, their physiological impact depends on bioavailability and ingestion levels. Phytate levels were very low, suggesting minimal interference with mineral absorption under experimental conditions (Al-Ishaq et al., 2019).

Induction of diabetes with nicotinamide and streptozotocin resulted in a significant elevation of fasting blood glucose levels in untreated diabetic rats (Group 2), confirming successful establishment of a type-2-like diabetic model. Persistent hyperglycemia observed in this group throughout the experimental period is consistent with impaired insulin action and pancreatic β-cell dysfunction. Treatment with *V. amygdalina* alone (Group 4) and *P. biglobosa* alone (Group 5) produced significant reductions in fasting blood glucose levels over time compared with untreated diabetic rats. However, the combined extract treatment (Group 6) produced a more pronounced and sustained glucose-lowering effect, approaching normoglycemic values by day 9. This effect was comparable to, and in some instances better than, the standard drug glibenclamide. The hypoglycemic effect of the combined treatment may be attributed to the complementary actions of flavonoids, alkaloids, and glycosides present in both plants, which may collectively enhance peripheral glucose uptake, suppress hepatic

gluconeogenesis, and protect pancreatic β-cells from oxidative damage (Singh et al., 2022). Anthocyanins, kaempferol, and catechins, are known to mitigate oxidative stress-induced β-cell damage and improve insulin signaling pathways (Clemente-Suárez et al., 2025; Situmorang et al., 2025).

Despite hyperglycemia, serum insulin levels did not differ significantly among groups, reflecting the partial β-cell protection conferred by nicotinamide in the diabetic model. The observed hypoglycemic effects may therefore be mediated through insulin-independent mechanisms, including enhanced peripheral glucose utilization, inhibition of intestinal glucose absorption, or modulation of carbohydrate-metabolizing enzymes. These findings suggest that the extracts exert their antidiabetic effects without directly stimulating insulin release.

Untreated diabetic rats demonstrated a marked elevation in HOMA-IR and a drastic reduction in HOMA-β score, suggesting insulin resistance with impaired β-cell function. The treatments however, did not improve the values of HOMA-IR and HOMA-β score respectively. The observed increase in HOMA-IR and altered HOMA-β indices in treated groups compared to diabetic controls may be attributed to partial restoration of insulin secretion without a corresponding reduction in blood glucose levels. Since HOMA indices depend on both insulin and glucose concentrations, even modest increases in insulin in the presence of persistent hyperglycemia can result in apparently elevated HOMA-IR values (Wallace et al., 2004). Furthermore, the Streptozotocin-induced diabetic model is characterized by severe β-cell destruction, which may limit the reliability of HOMA indices in this context. This result reflects the mathematical behavior of HOMA, not true worsening, hence, a limitation of using HOMA in STZ models. Furthermore, HOMA indices were originally developed for clinical assessment in humans and have limited validation in streptozotocin-induced diabetic rodent models. Consequently, these indices should be interpreted cautiously in the present study.

Dyslipidemia is a common metabolic complication of diabetes, characterized by elevated triglycerides, total cholesterol, LDL-cholesterol, and reduced HDL-cholesterol (Abdissa and Hirpa, 2022; Strikić et al., 2023). Untreated diabetic rats exhibited marked lipid abnormalities, confirming metabolic derangement. Administration of *V. amygdalina* leaf and *P. biglobosa* seed elicited significant hypolipidemic properties, with enhanced efficacy for most of the lipid parameters when combined, however, individual extracts administration showed significant HDL elevation. These improvements suggest enhanced lipid metabolism and reduced cardiovascular risk. The hypolipidemic effects may be attributed to phytochemicals such as saponins, flavonoids, and tannins, which are known to inhibit intestinal lipid absorption, enhance bile acid excretion, and regulate lipid-metabolizing enzymes (Hu et al., 2022; Liu et al., 2022).

Although the combined treatment produced the greatest overall improvement in the lipid profile, HDL-cholesterol was higher in the groups receiving individual extracts. The mechanism underlying this observation was not investigated and therefore requires further study. Nevertheless, the overall improvements in triglycerides, total cholesterol, LDL-cholesterol, and VLDL-cholesterol indicate a favourable effect of the treatments on diabetic dyslipidaemia.

Findings from the liver function parameters showed that untreated diabetic rats (group 2) exhibited marked hepatic dysfunction, with significantly elevated total bilirubin, direct bilirubin, total protein, albumin, globulin, and ALP. Treatment groups 3 and 5 showed im-

improvements in bilirubin and protein indices compared with Group 2, indicating partial hepatoprotection; however, ALP remained significantly elevated, suggesting incomplete hepatic recovery. The combined treated groups (group 6) produced the greatest improvement, with total and direct bilirubin returning close to or below control values and globulin levels comparable to the control. Nevertheless, ALP remained significantly higher than the normal control, indicating that although glucose homeostasis and several liver function markers improved substantially, complete restoration of hepatic architecture and biliary function had not yet occurred. Hepatic recovery generally lags behind improvements in glycaemic control (Zhou *et al.*, 2026). Chronic hyperglycaemia induces oxidative stress, inflammation, lipid accumulation, and structural damage to hepatocytes (Zhao *et al.*, 2026), all of which require a longer period to resolve than blood glucose abnormalities.

The persistent elevation of alkaline phosphatase (ALP) in the treated groups suggests that mild hepatobiliary dysfunction or ongoing hepatic tissue remodeling may still have been present. ALP is a relatively slow-responsive marker and often remains elevated even after metabolic improvement because regeneration of damaged liver cells and restoration of bile canaliculi function require additional time. Overall, these findings suggest that the intervention effectively improved metabolic control and provided partial hepatoprotection, but complete recovery of liver function likely requires a longer treatment period.

Diabetes-induced renal dysfunction was evident in untreated diabetic rats, as reflected by altered creatinine, urea and electrolyte imbalance. Treatment with individual plant extracts moderately improved renal indices, while the combination treatment exhibited significant renoprotective effects, evidenced by a reduction in renal biomarkers compared to diabetic controls, although values did not fully return to normal levels. The partial nephroprotective action may result from reduced oxidative stress, improved glycemic control, and stabilization of electrolyte balance. Bioactive compounds such as flavonoids, proanthocyanidins, and saponins are known to protect renal tissues by attenuating inflammation and oxidative injury (Ranasinghe *et al.*, 2023). Overall, the treatments demonstrated renoprotective effects, as evidenced by the improvement in key renal biomarkers, although complete normalization of all parameters was not achieved.

4. CONCLUSION

The study findings provide preclinical evidence that *Vernonia amygdalina* and *Parkia biglobosa* possess antihyperglycaemic, hypolipidaemic, hepatoprotective, and renoprotective activities in streptozotocin-induced diabetic rats, with combined administration producing the broadest overall metabolic benefits. However, persistent impairment of β -cell function and incomplete normalization of some hepatic and renal indices indicate that the extracts did not fully reverse diabetes-associated metabolic dysfunction. Consequently, these findings should be interpreted as preclinical evidence only. Further studies are required to establish optimal dose-response relationships, evaluate long-term efficacy and chronic toxicity, and elucidate the molecular mechanisms underlying the observed effects before these extracts can be considered as potential therapeutic agents for diabetes management.

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Ethical Statement:

All experimental procedures involving animals were conducted in accordance with the guidelines for the care and use of laboratory animals as approved by the Institutional Animal Ethical Review Committee of the University of Ilorin, Ilorin, Nigeria (UERC/LSC 110).

Conflict of Interests:

The authors report no financial or personal relationships that could inappropriately influence this work.

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O.S. designed and supervised the study. O.A.O., A.R.F., O.A.A., O.M.A., A.A.G., O.O.E. and O.A., conducted the laboratory work. O.A.O. and A.R.F. ran statistical analysis. O.S. and O.A.O. wrote the manuscript. All authors read and gave approval for this draft.

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