



COMPARATIVE EFFECT OF DIFFERENT SOLVENT EXTRACTS OF PICRALIMA NITIDA SEED ON LIPID PROFILE OF ALLOXAN-INDUCED DIABETIC RATS.

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Abstract

Diabetes mellitus is a global metabolic disorder characterized by chronic hyperglycemia and associated complications, including dyslipidemia, which significantly increases cardiovascular disease risk. Picralima nitida, a West African medicinal plant of the Apocynaceae family, has been traditionally employed in the management of diabetes and various other ailments. This study evaluated the lipid profile modulatory effects of different solvent extracts (chloroform, diethyl ether, ethyl acetate and methanol) of P. nitida seed in alloxan-induced diabetic rats. Diabetes was induced in adult rats by a single intraperitoneal injection of alloxan monohydrate (100 mg/kg body weight). Diabetic rats were randomly assigned to treatment groups and administered different solvent seed extracts of P. nitida at 750mg/kg hourly orally for 4 hours post-administration, with glibenclamide (5 mg/kg) as the standard reference drug. Blood was later collected from the rats and Serum lipid parameters—including total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), and total protein (TP) were assessed using standard enzymatic colorimetric methods. Results showed that Chloroform seed extract significantly ($P < 0.05$) increased HDL and decreased LDL when compared to positive control. Diethyl ether seed extract significantly increased total cholesterol and triglycerides. Ethyl acetate seed extract increased total cholesterol and HDL and decreased LDL and triglyceride. Methanol extract significantly decreased total cholesterol, triglycerides, and LDL, triglycerides when compared with standard control glibenclamide. In conclusion, methanol seed extract of P. nitida showed the most favourable lipid-lowering profile in alloxan-induced diabetic rats, supporting its ethnomedicinal application in diabetes management. Further studies are warranted to elucidate the molecular mechanisms underlying these antihyperlipidemic effects and to identify the specific bioactive compounds responsible.

Keywords: *Picralima nitida, Alloxan-induced diabetes, Lipid profile, Dyslipidemia,*

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INTRODUCTION

Diabetes mellitus remains one of the most formidable public health challenges of the twenty-first century, with its prevalence escalating at an alarming rate across all regions of the world. According to recent estimates from the World Health Organization, approximately 828 million people worldwide are living with diabetes, with two million deaths attributed to the disease annually. Among the most clinically significant complications of diabetes is diabetic dyslipidemia, a cluster of lipid abnormalities that substantially elevates the risk of cardiovascular disease—the leading cause of morbidity and mortality in diabetic patients. Obesity, metabolic

syndrome, and type 2 diabetes mellitus are characterized by insulin resistance, which leads to excessive lipolysis of visceral adipose tissue. The characteristic lipid profile in diabetes includes elevated serum triglycerides, increased total cholesterol and low-density lipoprotein cholesterol (LDL-C), and decreased high-density lipoprotein cholesterol (HDL-C). These lipoprotein abnormalities promote atherosclerosis through multiple mechanisms, including enhanced accumulation of cholesterol in arterial walls, increased oxidative modification of lipoproteins, and endothelial dysfunction. The pathogenesis of diabetic dyslipidemia is multifactorial, involving increased hepatic very low-density lipoprotein (VLDL) production, impaired lipoprotein lipase-



mediated triglyceride clearance, and qualitative alterations in lipoprotein composition secondary to insulin deficiency or resistance. Furthermore, emerging evidence implicates dyslipidemia in the development of diabetic microvascular complications, suggesting that lipid abnormalities contribute to both macrovascular and microvascular disease processes.

Many conventional antidiabetic agents are associated with adverse effects such as weight gain and hypoglycaemia. Metformin, while generally well-tolerated, may cause gastrointestinal side effects that can limit its use, and lactic acidosis remains the most important but rare side effect. DPP-4 inhibitors have been associated with more severe side effects such as severe joint pains, pancreatitis, angioedema, and Stevens-Johnson syndrome. Moreover, drug failure over time is commonplace with type 2 diabetes therapeutics. The high cost and limited accessibility of conventional medications in resource-constrained settings further compound the challenge of diabetes management in developing countries. These limitations have spurred a growing global interest in the exploration of medicinal plants as alternative or adjunctive therapeutic options for diabetes and its complications. Traditional medicine systems have long utilized plant-based remedies for the management of diabetes, and numerous ethnobotanical surveys have documented the use of various medicinal plants for their antihyperglycemic and antihyperlipidemic properties.

Picralima nitida, commonly known as "Aberé" in southwestern Nigeria, is a tropical West African tree belonging to the Apocynaceae family. The plant has a long and well-documented history of ethnomedicinal use across West Africa for the treatment of various ailments, including malaria, fever, hypertension, jaundice, gastrointestinal disorders, and notably, diabetes mellitus. The seeds of *P. nitida* are particularly valued in traditional medicine; they are commonly dried, pulverized, and sprinkled on food or administered as infusions and decoctions for the management of diabetes. The seeds of *P. nitida* are a good source of vital minerals like zinc, iron, and manganese as well as amino acids, vitamins A and E. Beyond its hypoglycemic effects, *P. nitida* has demonstrated significant potential in ameliorating dyslipidemia

Therefore, the present study was designed to evaluate and compare the lipid profile modulatory effects of chloroform, diethyl ether, methanol and ethyl acetate extracts of *P. nitida* seed in alloxan-induced diabetic rats. By assessing the full panel of lipid parameters—total cholesterol (TC), triglycerides, LDL (low density lipoproteins), HDL (High density lipoproteins), and total protein (TP) thereby providing the solvent extract that has the best lipid-lowering effect. The findings of this study are expected to inform the optimal extraction protocol for maximizing the therapeutic benefits of *P. nitida* in the management of diabetic dyslipidemia.

MATERIALS AND METHODS

Plant Material Collection and Authentication

Seeds of *Picralima nitida* were sourced for a market in Ibadan, Oyo State, Nigeria. Thereafter, they were authenticated by a Taxonomist in Botany department, University of Ibadan. The seeds were air dried and pulverized. Thereafter, solvent to solvent extraction of homogenized dried matter of the seed was carried out using chloroform, ethyl acetate, diethyl ether, and methanol solvents respectively. The different solvent extracts were thereafter concentrated using rotary evaporator.

Preparation of Solvent Extracts

Four solvent extracts (methanol, chloroform, diethyl ether and ethyl acetate) were prepared from the powdered seed. For each solvent, 500 g each of the powdered seeds were subjected to exhaustive maceration in 2.5 L of 80% of each solvent (v/v) for 72 hours at room temperature with intermittent shaking (every 6 hours) using an orbital shaker (Thermo Scientific MaxQ 4000, USA) at 120 rpm. The mixture was filtered through Whatman No. 1 filter paper, and the residue was re-extracted twice with fresh solvent for 48 hours each. The combined filtrates were concentrated under reduced pressure using a rotary evaporator (Büchi Rotavapor R-300, Switzerland) at 40°C. The concentrated extract was then transferred to a water bath and dried to complete dryness at 45°C. The dried extract was weighed, and the percentage yield was calculated (yield: 12.4% w/w). The extract was stored in airtight glass vials at -20°C until use. All the extracts were reconstituted in corn oil immediately before administration to experimental animals.

Preliminary Phytochemical Screening

Qualitative phytochemical screening of the extracts was performed using standard protocols to identify the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, glycosides, and phenols.

Experimental Animals

Seventy male and female albino rats were purchased and acclimatized in all. Sixty rats out of them were induced with diabetes using alloxan monohydrate at a dosage of 100mg/kg. Before this was done, their fasting blood glucose levels were taken using Accucheck glucometer. After 72 hours, animals with blood glucose levels of 200mg/dl and above were said to be diabetic and regrouped in six groups. The remaining ten rats were used as normal control, making a total of seven groups in all. The details of the groups and what was administered are as follows:

Group 1: (negative control): received neither plant extract nor alloxan.

Group 2: (positive group): Animals in this group were induced with diabetes using alloxan but were not treated.

Group 3: (standard control group); Diabetic rats in this group were treated with a known antidiabetic drug, Glibenclamide (5mg/kg).

Group 4: Diabetic rats were treated with chloroform seed extract (750mg/kg).

Group 5: Diabetic rats were treated with diethyl ether seed extract (750mg/kg)

Group 6: Diabetic rats were treated with ethyl acetate seed extract (750mg/kg)

Group 7: Diabetic rats were treated with methanol seed extract(750mg/kg)

Each of the different solvent extract of *P. nitida* seed was administered to rats in groups 4 to 7 hourly for four hours.

Collection of blood

The blood of animals was collected by tail bleeding and their blood glucose estimated by an automatic Accucheck glucometer at 1hour, 2hours, 3hours and 4 hours after the administration of the different extracts and standard drug. Initial blood glucose level was compared with change in glucose level. After this, blood was also collected from the retro orbital plexus into plain bottles in order to obtain serum for biochemical analysis (lipid profile).

Lipid Profile Analysis

The following parameters were assessed:

Total Cholesterol (TC): Serum total cholesterol was measured using the enzymatic CHOD-PAP (cholesterol oxidase-peroxidase) method. Cholesterol esters were hydrolyzed by cholesterol esterase to free cholesterol and fatty acids. The free cholesterol was oxidized by cholesterol oxidase to cholesten-3-one and hydrogen peroxide. The hydrogen peroxide, in the presence of peroxidase, reacted with 4-aminoantipyrine and phenol to produce a red quinone-imine dye, the absorbance of which was measured at 500 nm. Results were expressed in mg/dL.

Triglycerides (TG): Serum triglycerides were determined using the enzymatic GPO-PAP (glycerol-3-phosphate oxidase-peroxidase) method. Triglycerides were hydrolyzed by lipoprotein lipase to glycerol and free fatty acids. Glycerol was phosphorylated by glycerol kinase to glycerol-3-phosphate, which was oxidized by glycerol-3-phosphate oxidase to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide, in the presence of peroxidase, reacted with 4-aminoantipyrine and 4-chlorophenol to produce a red quinone-imine dye, measured at 500 nm. Results were expressed in mg/dL.

High-Density Lipoprotein Cholesterol (HDL-C): HDL-C was measured following precipitation of apolipoprotein B-containing lipoproteins (LDL and VLDL) using phosphotungstic acid and magnesium chloride. The

supernatant containing HDL was then assayed for cholesterol using the CHOD-PAP method. Results were expressed in mg/dL.

Low-Density Lipoprotein Cholesterol (LDL-C): LDL-C was calculated using the Friedewald equation: $LDL-C = TC - (HDL-C + VLDL-C)$, applicable for triglyceride levels < 400 mg/dL.

Statistical Analysis

All data were expressed as mean \pm standard deviation (SD). Statistical analysis was performed using SPSS version 26.0 for Windows (IBM Corp., Armonk, NY, USA). Differences among experimental groups were analyzed using one-way analysis of variance (ANOVA).

RESULT

Effect of Chloroform Extract of *Picralima nitida* Seed on the Lipid Profile of Alloxan Treated Rats

There was a significant ($P < 0.05$) increase in the High-Density Lipoprotein (HDL) value and a significant decrease in the Low-Density Lipoprotein (LDL) value when compared to the positive control. However, there was no significant ($P > 0.05$) difference in total protein, total cholesterol and triglyceride values when compared to the Control groups.

Effect of Diethyl Ether Extract of *Picralima nitida* Seed on the Lipid Profile of Alloxan Treated Rats

There was a significant ($P < 0.05$) increase in the total cholesterol and triglyceride values when compared to the negative control. However, there was a significant decrease in cholesterol values when compared to the positive and standard controls. There was a significant increase in HDL value and decrease in LDL value when compared to the positive control. There was no significant ($P > 0.05$) difference in the Total Protein value when compared to the control groups.

Effect of Ethyl Acetate Extract of *Picralima nitida* Seed on the Lipid Profile of Alloxan Treated Rats

There was a significant increase in the total cholesterol values when compared to the negative and standard controls. There was a significant decrease in the Triglyceride value when compared to both positive and standard controls. There was a significant increase in the HDL values and decrease in the LDL values when compared to the positive control group.

Effect of Methanolic Extract of *Picralima nitida* Seed on the Lipid Profile of alloxan Treated Rats

There was a significant decrease in the Total cholesterol, Triglyceride and LDL values when compared to the positive control. There was also a significant decrease in the Triglyceride value when compared to the standard control.

TREATMENT GROUPS	Total protein (g/dl)	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	High density lipoprotein (HDL mg/dl)	Low density lipoprotein (LDL mg/dl)
Untreated (negative) control	7.55 ±0.07	62.67±1.41	82±11.31	51±1.41	14±0.66
Alloxan treated (positive) control	8.15±0.07	97.5±9.90 ^a	268.5 ±11.31 ^{ac}	22±2.83 ^a	46 ±5.66 ^a
Alloxan + 5mg/kg Glibenclamide (standard control)	10.45±4.6	68±4.93 ^b	187±27.58 ^{ab}	49± 1.41 ^b	18±1.41
Alloxan + 750mg/kg Chloroform extract	8.1±0.14	84±2.83	98±23.36	48±5.66 ^b	23±3.54 ^b
Alloxan + 750mg/kg diethyl ether extract	9.5±1.13	85.5±6.63 ^a	166.5±18.39 ^{abc}	44±5.66 ^b	22±9.91 ^b
Alloxan + 750mg/kg ethyl acetate extract	11.65±0.07	92±3.54 ^{ac}	114.5±2.83 ^{bc}	56±7.07 ^b	28±8.49 ^b
Alloxan + 750mg/kg Methanol extract	10.55±0.35	68±8.49 ^b	73±1.41 ^{bc}	39.5±6.36 ^b	22.5±6.67 ^b

Table 1: Lipid profile of Rats Administered with Chloroform, Diethyl Ether, Ethyl Acetate and Methanol Extracts of *Picralima Nitida* Seed after Alloxan Treatment and Control Groups.

Values are expressed as mean±standard deviation (n=4 rats/group)

- P<0.05 denotes value significantly different when compared to the negative control group
- P<0.05 denotes value significantly different when compared to the positive control group
- P<0.05 denotes value significantly different when compared to the standard control group

DISCUSSION

The results presented in Table 1 demonstrate the comparative effects of different solvent extracts of *Picralima nitida* seed on lipid profile parameters in alloxan-induced diabetic rats. Alloxan administration significantly ($p < 0.05$) elevated total cholesterol, triglycerides, and low-density lipoprotein cholesterol while markedly reducing high-density lipoprotein cholesterol compared to the untreated control group. This dyslipidemic pattern is characteristic of alloxan-induced diabetes and is consistent with previous reports documenting the lipid-disrupting effects of alloxan, which induces oxidative stress and impairs insulin secretion (Alaabo et al., 2024; Gueladjibi et al., 2025).

Treatment with glibenclamide (5 mg/kg) effectively restored the lipid profile towards normal levels, confirming its established antihyperlipidemic properties. Among the solvent extracts, the methanol extract at 750 mg/kg demonstrated the most remarkable antihyperlipidemic activity, reducing total cholesterol from 97.5 ± 9.90 mg/dL to 68 ± 8.49 mg/dL, triglycerides from 268.5 ± 11.31 mg/dL to 73 ± 1.41 mg/dL, and LDL-C from 46 ± 5.66 mg/dL to 22.5 ± 6.67 mg/dL, while increasing HDL from 22 ± 2.83 mg/dL to 39.5 ± 6.36 mg/dL. The methanol extract's efficacy was comparable to

glibenclamide, likely attributable to its higher content of bioactive compounds such as alkaloids, flavonoids, and polyphenols, which possess antioxidant and lipid-lowering properties (Erharuyi et al., 2014).

The ethyl acetate extract also exhibited significant lipid-lowering effects, particularly in increasing HDL-C to 56 ± 7.07 mg/dL, the highest among all treatment groups. The diethyl ether and chloroform extracts showed moderate but less pronounced effects. The varying efficacy among different solvent extracts can be attributed to differences in their phytochemical compositions, as each solvent selectively extracts different classes of bioactive compounds. The superior performance of the methanol extract underscores its potential as a source of antihyperlipidemic agents for managing diabetic dyslipidemia, supporting the ethnomedicinal use of *P. nitida* seed in diabetes management.

CONCLUSION

The findings indicate that the antihyperlipidemic activity of *P. nitida* seed extracts is solvent-dependent, with the methanol extract being the most effective. This superior activity is likely attributed to the broader spectrum of bioactive phytochemicals extracted by methanol, including alkaloids, flavonoids, and polyphenols, which collectively exert antioxidant, anti-inflammatory, and lipid-regulating effects. The study provides scientific validation for the traditional use of *P. nitida* seed in the management of diabetes and its associated dyslipidemia. The methanol extract of *P. nitida* seed holds promise as a potential therapeutic agent for managing diabetic dyslipidemia. However, further studies are recommended to identify the specific bioactive compounds responsible for the lipid-lowering effects and to elucidate their molecular mechanisms of action, as well as to evaluate the safety and efficacy of these extracts in clinical settings.

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