Phytochemical Contents and Effect of Ethanol Extract of *Clinacanthus Nutans* Leaf on the Serum Lipid Profile of Alloxan-Induced Diabetic Rats

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DOI: https://doi.org/10.51244/IJRSI.2026.1307000270

Received: 02 August 2026; Accepted: 07 August 2026; Published: 13 August 2026

ABSTRACT

Background: Plant-derived extracts are increasingly investigated as alternative for managing diabetes-associated hyperlipidaemia. This study evaluated the phytochemical contents and effects of ethanol extract of *Clinacanthus nutans* leaves on lipid profile of alloxan-induced diabetic rats.

Methods: Leaves of *C. nutans* were processed and extracted using standard ethanol extraction procedure after air-drying at 25 °C. Phytochemical contents were determined using established method. A 100 mg/mL stock solution was prepared and administered orally through an intragastric tube for 21 days. Diabetes was induced in Wistar rats using alloxan. Twenty-five Wistar rats were randomly assigned into five groups (n = 5): three groups received 200, 500, and 800 mg/kg of the extract; one group received 500 µg/kg of glibenclamide; and one group served as untreated control. Lipid profiles were determined using standard methods. Data were analysed using mean ± SD and paired t-test at $p < 0.05$.

Results: The ethanol extract of *C. nutans* leaves contained 44.14% phenolic compounds and 20.25% flavonoids. Treatment with 800 mg/kg dose significantly reduced triglycerides by 17.1%, compared to 11.6% with glibenclamide. Total cholesterol decreased by 7.6% at 200 mg/kg, while glibenclamide achieved a 10.1% reduction. A significant 9.2% increase in high-density lipoprotein was observed at 500 mg/kg. Low-density lipoprotein decreased significantly by 23.7% at 200 mg/kg and 37.3% with glibenclamide.

Conclusion: The ethanol extract of *C. nutans*, rich in phenolics and flavonoids, exhibits notable anti-hyperlipidaemic potentials by reducing LDL-C and increasing HDL-C. in diabetic rats.

Keywords: *Clinacanthus nutans* leaves; Serum lipid profile; Ethanol extract; Phytochemical; Alloxan-induced diabetic rats.

INTRODUCTION

The application of medicinal plants in disease management has expanded tremendously over the past few decades. This shift is driven by their cost-effectiveness and perceived safety profile, often exhibiting fewer side effects compared to synthetic pharmaceuticals. Consequently, there is an intensified search for novel plant-based therapies to manage chronic metabolic disorders (Bernardini et al., 2018). As natural products of drug discovery, medicinal plants have gained significant traction due to their diverse pharmacological activities (Bernardini et al., 2018). There is an increasing drive to study these natural products, particularly edible plants and herbs as dietary interventions to manage hyperlipidaemia associated with diabetes (Gurib-Fakim, 2006; Umar Imam et al., 2019).

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VII July 2026

Clinacanthus nutans (Lindau), a member of the Acanthaceae family, is a small shrub native to tropical Asian countries. While historically utilised in folk medicine to treat snake bites, insect stings, and skin rashes, recent pharmacological interest has shifted toward its metabolic benefits. Its therapeutic versatility is largely attributed to a rich profile of bioactive compounds, including flavonoids, phenols, glycosides, and terpenoids. These secondary metabolites are well-documented for their antioxidant, anti-inflammatory, antiviral, and metabolic-regulating potentials. Specifically, Abdullah and Kasim (2017) demonstrated the inhibitory activity of *C. nutans* ethanolic extracts against metabolic enzymes, while Kobun (2019) identified key phytochemical classes in the plant, including sulfur-containing glycosides, phenolic compounds, triterpenoids, and phytosterols.

Diabetes mellitus is characterised by chronic hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. In experimental studies, alloxan is frequently employed to induce a diabetic state in animal models. It selectively destroys the beta-cells of the pancreas through the generation of reactive oxygen species (ROS), creating an insulin-deficient state that mirrors Type 1 diabetes and leads to severely disrupted glucose and lipid metabolism (Okorie et al., 2022).

Hyperlipidaemia is a critical complication of diabetes. Its prevalence has increased dramatically due to rising obesity rates, sedentary lifestyles, and ageing. It significantly increases the risk of cardiovascular diseases, cognitive decline, and mortality (Defronzo et al., 2015; Okorie et al., 2021). In diabetic conditions, insulin deficiency triggers the mobilisation of free fatty acids from adipose tissue into the bloodstream. This physiological shift typically results in elevated triglycerides (TG), increased total cholesterol (TC), higher low-density lipoprotein (LDL-C), and reduced high-density lipoprotein (HDL-C) levels (Defronzo et al., 2015).

Dyslipidaemia further alters the physical properties of cellular membranes, which may facilitate the leakage of free radicals from the mitochondrial electron transport chain or activate nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Sharma et al., 2012; Azemi et al., 2021). These processes escalate the generation of free radical species, increasing the oxidative modification of lipids, proteins, and lipoproteins (Abbasnezhad et al., 2019; Azemi et al., 2021). Notably, LDL-C and its oxidized form (oxLDL-C) play a central role in atherogenesis. Given that various plants have shown the ability to lower TC, TG, and LDL-C levels in both alloxan- and streptozotocin-induced diabetic models (Okorie et al., 2021), the hypolipidaemic pontential of *C. nutans* may offer a pathway to reducing the severity of diabetic complications. Therefore, this study investigates the phytochemical contents and the effects of the ethanol extract of *C. nutans* leaves on the serum lipid profile of alloxan-induced diabetic rats.

MATERIALS AND METHODS

Experimental design was employed to study the phytochemical contents and effects of ethanol extract of *C. nutans* leaves on the serum lipid profile of alloxan-induced diabetic rats. A total of 25 adult male Albino rats were assigned completely at random to five groups of five rats in each group. However, each experimental unit had the same chance of receiving treatment as those in the treatment groups. The animals (rats) were weighed individually and the ones with similar body weight were put in the same experimental unit homogenously. The three different groups received ethanol extract of *C. nutans* leaves at varying doses (200 mg/kg; 500 mg/kg; and 800 mg/kg body weight), another group received 500 µg/kg body weight of Glibenclamide (positive control group), and the last group received no treatment other than distilled water. The administration was done orally with the aid of an intra-gastric tube attached to a syringe for a period of 21 days after induction.

Experimental Materials: Fresh leaves of *C. nutans* were harvested from a farm in Ikwuano Local Government Area (LGA) and identified in the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike (MOUAU), Abia State, Nigeria. Twenty-five adult male albino Wistar rats were purchased from the Department of Zoology and Environmental Biology, MOUAU, while the chemical (alloxan) that was used to induce diabetes in the rats was purchased from a chemical shop in Aba, Abia State, Nigeria.

Preparation of the plant material: The method described by Sukhdev et al. (2008) was adopted for the ethanol extraction of *C. nutans* leaves with modification from Okorie et al. (2022). Freshly harvested *C. nutans* leaves were sorted, washed, and air-dried at room temperature (25^0C) for 3 days. The leaves were chopped into small pieces and then milled to fine powder, ready for further treatment. One hundred and fifty grams (150 g) of the

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powdered leaf was immersed in 4500 mL of ethanol and agitated on a mechanical shaker for 30 min. The mixture was allowed to stand for 4 hrs and then drained using a muslin cloth into a 5 L dry stainless-steel bowl. The ethanol extract was concentrated to dryness in a Gallenkamp hot air oven (Size One-Oven BS) at 60^0C until a gummy extract was obtained. The gummy extract was then recovered into a sample bottle and stored in the refrigerator until use for further analysis and study. One gram of the extract was dissolved in 10 mL of distilled water to make a stock of 100 mg/mL of the extract. The volume of extract to be administered was determined using the formula:

Volume of extract = $\frac{\text{Dose x body weight of rats}}{1000\ x\ stock\ solution\ of\ extract}$ (Dose is in $mgmL^{-1}$)

Determination of phytochemicals

Alkaloid: Five grams (5 g) of the powdered sample will be soaked in 20 mL of 10% ethanol acetic acid. The mixture will stand for 4 h at room temperature. Thereafter, the mixture will be filtered through Whatman filter paper No 42. The filtrate will be concentrated by evaporation over a steam bath to ¼ of its original volume. To precipitate the alkaloid, concentrated ammonia solution will be added in drops to the extract until it will be in excess. The resulting alkaloid precipitate will be recovered by filtration using previously weighed filter paper. After filtration, the precipitate will be washed with 9% ammonia solution and dried in the oven at 60^0C for 30 minutes, cooled in a desiccator and reweighed. The process will be repeated two more times and the average will be taken. The weight of alkaloid will be determined by the differences and expressed as a percentage of weight of sample analysed as shown below:

% Alkaloid = $W_2 – W_1$/wt of sample x 100/1

W_1 = weight of filter paper; W_2 = weight of filter paper + alkaloid precipitate.

Flavonoid: Five grams (5 g) of the powdered sample will be placed into a conical flask and 50 mL of water and 2 mL HCl solution will be added. The solution will be allowed to boil for 30 minutes. The boiled mixture will be allowed to cool before it will be filtered through Whatman filter paper No 42. 10 mL of ethyl acetate sample which contained flavonoid will be recovered, while the aqueous layer will be discarded. A pre weighed Whatman filter paper will be used to filter second (ethyl-acetate layer), the residue will then be placed in an oven to dry at 60^0C. It will be cooled in a desiccator and weighed. The quantity of flavonoid will be determined using the formula:

% flavonoid = $W_2 – W_1$/wt sample x 100/1

W_1 = weight of empty filter paper; W_2 = weight of paper + flavonoid sample.

Phenol: This will be done using the folin-cioCaltean colourimetric method, 0.2 g of the powdered sample will be added into a test tube and 10 mL of methanol will be added to it and shaken thoroughly the mixture will be left to stand for 15 mins before being filtered using Whatman No. 42 filter paper. 1 mL of the sample will be placed in a test tube and 1 mL folincio Caltean reagent in 5 mL of distilled water will be added and colour will be allowed to develop for about 1 to 2 h at temperature. The absorbance of the developed colour will be measured at 760 nm wave. The process will be repeated two more times and an averaged taken. The phenol content will be calculated thus;

% phenol = 100/w x AU/AS x C/100 x VF/VA x D.

W = weight of sample analysed; AU = absorbance of test sample; AS = absorbance of standard solution; C = concentration of standard in mg/mL; VF = total filtrate volume; VA = volume of filtrate analysed; D = dilution factor where applicable.

Tannin: The method described by Nwosu (2011) will be used to determine the tannin content of the sample. Exactly 1 g of food samples will be weighed into a centrifuge tube with 2 mL of distilled water. It will be centrifuged at 1500 rpm for 10 min. The centrifuge samples will then be poured out into a beaker and the

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supernatant (extract) dispersed. One milliliter (1 mL) of $NaCO_3$ and Folin Denis reagent will be added in the beaker and allowed to settle. The readings will be taken using spectrophotometer. Tannin will be calculated as follows:

$$\% \text{ tannin} = A_n/A_s \times C \times 100/W \times V_f/V_a$$

Where: An = absorbance of test sample; As = absorbance of standard sample; C = concentration of standard solution; Vf = total volume of extract; Va = volume of extract analysed; and W = weight of sample.

Phytate: The method described by AOAC (2012) will be used to determine the phytate content of the sample. One gram of the sample will be extracted in duplicate for 4 h with 20 mL of 0.1 M nitric acid with constant agitation. The tubes will be stoppered and placed in a boiling water bath for 20 minutes and allowed to cool. About 5.0 mL of amyl alcohol will be added to each tube followed by 1.0 mL of ammonium thiocyanate (100 g/L). the tubes will be shaken thoroughly and centrifuged at 2000 rpm and then absorbance of the amyl alcohol layer will be determined at 465 nm against amyl alcohol exactly 15 min after the addition of the ammonium thiocyanate using spectrophotometer. One milliliter of the food sample will be pipetted into a test tube fitted with a ground glass stopper together with 1 mL of ferric solution. Ferric solution will be prepared by dissolving 0.2 g hydrated ammonium iron (III) sulphate in 100 mL of 2 N HCl and made up to 1000 mL with diluted water and the absorbance will be measured at 519 nm against distilled water using spectrophotometer. A standard solution will also be prepared for the analysis. Percentage phytic acid will then be calculated using the absorbance of the test sample and that of the standard solution.

Saponin: This will be determined using the colourimetric method of AOAC (2012). The food sample (0.5 g) will be weighed and put into a test tube followed by the addition of 10 mL of distilled water. The mixture will be shaken and allowed to stand for 1 h. the formation of stable foaming froth will be observed. About 1 mL of the mixture will be pipetted into another test tube with about 5 mL of distilled water added to the food sample. This will be followed by addition of a drop of olive oil. The test tube with its content will be shaken and it will become cloudy. The absorbance will be measured at 620 nm using spectrophotometer. The quantity of saponin contained in each sample will be estimated from the standard saponin curve obtained from plotting the concentration of the standard concentration against the absorbance.

Acute toxicity test of ethanol extracts of *C. nutans* leaves: This was done to determine the toxicity of the leaf and its lethal dose to be used for the study. The method cited by Okorie et al. (2021) was adopted for the acute toxicity test of the ethanol extract of *C. nutans*. Eighteen mice were used in the toxicity study. The test involved 2 stages. Stage one had three groups of three mice each, and were 10, 100, and 1000 $mgkg^{-1}$ body weight of the extract, respectively. In stage two, 1600, 2900, and 5000 $mgkg^{-1}$ body weight of the extract was administered to the mice, respectively. The administration of the extract was done orally.

Experimental animals: Healthy male albino Wistar rats weighing (120 – 150 g) was used. The experiment was performed according to the principles in the guide for the care and use of laboratory animals described by the National Institute of Health (Okorie et al. 2021). The experiment protocol was made to conform to the rules for ethical conduct within the animal's use and care. Ethical approval was sorted and obtained from the Chairman Animal Care and Use Committee of Department of Zoology and Environmental Biology, MOUAU. The rats were housed in a plastic group cage and atmospheric temperature (25 ± 2^0C) with relative humidity (45±5%) in 12 hrs light and 12 hrs dark condition. The rats were given a standard pellet diet and water *ad libitum*, while the rats were allowed to acclimatised to the new environmental condition.

Experimental induction of diabetes mellitus: An established quantity of alloxan (100 $mgkg^{-1}$ body weight) by Okorie et al. (2021) used to induce diabetes was adopted in the study. The adult rats were kept on 18 hrs fast to induce diabetes. The induction was done intraperitoneally using alloxan (Sigma-Aldrich, St Louis, MO, USA). The rats were divided and labelled appropriately into 5 groups of 5 rats each as indicated earlier. The weight of the individual rats in each of the groups was used to calculate the volume of alloxan used in the induction process. Therefore, 1 g of alloxan was dissolved in 10 mL of distilled water to obtain 100 mg/mL, and after 2 days of induction, blood samples of individual rats were drawn from their tail to confirmed diabetes and thereafter, treatment commenced across the different groups with specific treatment doses as designed for each group. The

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ethanol extract of *C. nutans* leaf was reconstituted using 1 g of each extract dissolved in 10 mL of distilled water to obtain 100 mg/mL and this was used to calculate the actual volume given to individual rats throughout the study period. The treatment lasted for 21 days after establishment of diabetes mellitus, while the rats were fed normally and water *ad libitum*.

Treatment and nontreatment groups

Group 1: Diabetic rats treated with 200 mg/kg body weight of ethanol extract of *C. nutans* leaf.

Group 2: Diabetic rats treated with ethanol extract of *C. nutans* leaf at 500 mg/kg body weight.

Group 3: Diabetic rats treated with ethanol extract of *C. nutans* leaf at 800 mg/kg body weight.

Group 4: Diabetic rats treated with Glibenclamide at 500 µg/kg body weight.

Group 5: Diabetic rats, administered distilled water, no treatment with extract nor drug.

Evaluation of serum lipid profile

Blood collection: The blood sample of the rats was collected from the recto-bulbar plexus (in the eye). The blood sample was allowed to clot for about 1 hr and then centrifuge at 3000 rpm. Thereafter, the serum was collected, stored and used for further analysis of the lipid profile of the rats.

Determination of cholesterol

Principle: Cholesterol determination was done using both enzymatic hydrolysis and oxidation. The indicator quinoneimine was obtained from hydrogen peroxidase and 4-amino antipyrine in the presence of phenol and peroxidase.

Test procedure: A labelled 3 test tubes were used namely, sample blank and standard. To the blank, 10 µL of distilled water was added, 10 µL standard specimen was added to the standard test tube, while 10 µL sample (serum) was added to the sample test tube. However, 1000 µL of the cholesterol reagent was added to the 3 test tubes. The sample was thoroughly mixed and incubated for 10 min at room temperature (20-25EC). The absorbance of the samples (A_{sample}) against the blank was taken within 60 min at 500 nm.

$$\text{Total cholesterol (mg/dL)} = \frac{\text{Absorbance of sample}}{Absorbance\ of\ standard} \text{ x } 202.65$$

Low-density lipoprotein (LDL): Calculation of LDL-C was thus:

LDL-Cholesterol conc (mg dLG1) = Total cholesterol (HDL + triglycerides/5).

High density lipoprotein (HDL) Principle: Low and very-low-density lipoprotein (LDL and VLDL) was precipitated from serum by the action of a polysaccharide in the presence of divalent cations. Then, HDL that is present in the supernatant was determined.

Procedure: The precipitant solution 0.1 mL was added to 0.3 mL of the serum and mixed thoroughly and allowed to stand for 15 min this was centrifuge at 2000 × g for 15 min. The cholesterol concentration in the supernatant was determined as cited by Okorie et al. (2021).

$$\text{HDL cholesterol (mg/dL)} = \frac{\text{Absorbance of sample}}{Absorbance\ of\ standard} \text{ x } 202.65$$

Triacylglycerol

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Principle: The triacylglycerol was determined after enzymatic hydrolysis with lipases. The indicator was quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4 chlorophenol under the catalytic influence of peroxidase (Trinder, 1969).

Triacylglycerol + H2O in the presence of lipases equals to Glycerol + ATP GK Glycerol + fatty acids

Glycerol + ATP in the presence of GK gives glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O_2 in the presence of GPO = Dihydroxyacetone phosphate + H_2O_2

$2H_2O_2$ + 4-aminophenazone + 4 chlorophenol in the presence gives Quinoneimine + HCl + $4H_2O$

Statistical analysis: Phytochemical contents of C. nutans were analysed using descriptive statistics (mean and standard deviation), while the lipid profile (TC. TG, LDL-C, and HDL-C) was analysed using paired sample t-test, and a level of significant was set at $p < 0.05$. The statistical analysis was performed using IBM-SPSS version 27.

Results

No mortality was recorded in both stages of the toxicity test – lethal dose (LD50) as shown in Table 1.

Table 1: Ethanol extract of *Clinacanthus nutans* leaves lethal dosage.

Stage/Group	Dose (mg/kg) *C. nutans*	Mortality
Stage 1		
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
Stage II		
Group 1	1600	0/3
Group 2	2900	0/3
Group 3	5000	0/3

Table 2 shows the phytochemical contents of ethanol extract of *C. nutans* leaf as administered to diabetic rats. The results showed the contents of alkaloids (1.90 mg/100g), tannins (0.74 mg/100g), phytate (0.45 mg/100g), saponins (2.37 mg/100g), phenol (44.14 mg/100g), and flavonoids (20.25 mg/100g).

Table 2: Phytochemical contents of ethanol extract of *C. nutans* leaf as administered to diabetic rats

Parameters (mg/100g)	**Mean and standard deviation**
Alkaloids	1.90 ± 0.16
Tannin	0.74 ± 0.01
Phytate	0.45 ± 0.01
Saponin	2.37 ± 0.05

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Phenol	44.14 ± 0.42
Flavonoids	20.25 ± 0.00

Table 3 shows the triglycerides (TG) levels of alloxan-induced diabetic rats following treatment. Rats treated with 200 mg/kg body weight of ethanol extract of *C. nutans* leaves showed a significant reduction ($p < 0.05$) in TG levels, decreasing from 98.82 mg/dL to 85.32 mg/dL. This represents a mean difference of 11.50 mg/dL and a 11.9% decrease.

Similarly, rats treated with 500 mg/kg body weight of the extract exhibited significant change ($p < 0.05$), with TG levels decreasing from 97.50 mg/dL to 85.42 mg/dL, corresponding to a mean difference of 12.08 mg/dL and a 12.4% reduction.

A significant decrease ($p < 0.05$) was also observed in rats treated with 800 mg/kg body weight, where TG levels dropped from 101.88 mg/dL to 84.46 mg/dL. This reflects a mean difference of 17.42 mg/dL and a 17.1% reduction.

Similarly, treatment with the antidiabetic drug (Glibenclamide) at 500 µg/kg body weight resulted in a significant decrease ($p < 0.05$) in TG levels, from 97.46 mg/dL to 86.20 mg/dL, with a mean difference of 11.26 mg/dL and a 11.6% reduction.

Conversely, untreated diabetic rats showed a significant increase ($p < 0.05$) in TG levels, rising from 98.74 mg/dL to 104.46 mg/dL. This corresponds to a mean difference of 5.72 mg/dL and a 5.8% increase.

Table 3: Effect of ethanol extract of *C. nutans* on the triglyceride levels (mg/dL) of alloxan-induced diabetic rats.

Parameters	**Initial**	**Final**	**MD**	**Std error**	**p-value**	**%D**
$CN_{200mg/kg}$	96.82 ± 4.35	85.32 ± 3.56	11.50 ± 5.58	2.94	0.010	11.9
$CN_{500mg/kg}$	97.50 ± 4.95	85.42 ± 4.01	12.08 ± 6.33	2.83	0.013	12.4
$CN_{800mg/kg}$	101.88 ± 5.67	84.46 ± 4.54	17.42 ± 7.45	3.33	0.006	17.1
$SD_{500µg/kg}$	97.46 ± 2.66	86.20 ± 3.63	11.26 ± 4.09	1.83	0.004	11.6
UTG	98.74 ± 3.34	104.46 ± 5.25	5.72 ± 3.12	1.39	0.015	5.8

MD = mean difference; Std error = standard error; %D = percentage difference; CN = *C. nutans*; SD = standard drug; UTG = untreated group.

Table 4 presents the total cholesterol (TC) levels of alloxan-induced diabetic rats following treatment. Rats treated with 200 mg/kg body weight of ethanol extract of *C. nutans* leaves showed a significant reduction ($p < 0.05$) in TC levels, decreasing from 113.68 mg/dL to 105.06 mg/dL. This represents a mean difference of 8.62 mg/dL and a 7.6% decline.

In contrast, rats treated with 500 mg/kg body weight of the extract exhibited no significant change ($p > 0.05$), with TC levels slightly decreasing from 110.16 mg/dL to 107.42 mg/dL, corresponding to a mean difference of 2.74 mg/dL and a 2.5% reduction.

A significant decrease ($p < 0.05$) was also observed in rats treated with 800 mg/kg body weight, where TC levels dropped from 115.72 mg/dL to 108.50 mg/dL. This reflects a mean difference of 7.22 mg/dL and a 6.2% reduction.

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Similarly, treatment with the antidiabetic drug (Glibenclamide) at 500 µg/kg body weight resulted in a significant decrease ($p < 0.05$) in TC levels, from 108.32 mg/dL to 97.34 mg/dL, with a mean difference of 10.98 mg/dL and a 10.1% reduction.

Conversely, untreated diabetic rats showed a significant increase ($p < 0.05$) in TC levels, rising from 109.74 mg/dL to 116.86 mg/dL. This corresponds to a mean difference of 7.12 mg/dL and a 6.5% increase.

Table 4: Effect of ethanol extract of *C. nutans* on the total cholesterol levels (mg/dL) of alloxan-induced diabetic rats.

Parameters	**Initial**	**Final**	**MD**	**Std error**	**p-value**	**%D**
$CN_{200mg/kg}$	113.68 ± 7.50	105.06 ± 5.45	8.62 ± 5.31	2.37	0.022	7.6
$CN_{500mg/kg}$	110.16 ± 8.50	107.42 ± 4.88	2.74 ± 4.10	1.84	0.210	2.5
$CN_{800mg/kg}$	115.72 ± 4.20	108.50 ± 6.24	7.22 ± 3.48	1.56	0.010	6.2
$SD_{500µg/kg}$	108.32 ± 7.05	97.34 ± 3.94	10.98 ± 7.12	3.19	0.026	10.1
UTG	109.74 ± 3.70	116.86 ± 5.97	7.12 ± 4.29	1.92	0.021	6.5

MD = mean difference; Std error = standard error; %D = percentage difference; CN = *C. nutans*; SD = standard drug; UTG = untreated group.

Table 5 shows the high-density lipoprotein cholesterol (HDL-C) levels of alloxan-induced diabetic rats following treatment. Rats treated with 200 mg/kg body weight of ethanol extract of *C. nutans* leaves showed a significant increase ($p < 0.05$) in HDL-C levels, increasing from 52.88 mg/dL to 56.40 mg/dL. This represents a mean difference of 3.52 mg/dL and a 6.7% rise.

Also, rats treated with 500 mg/kg body weight of the extract showed a significant increase ($p < 0.05$), with HDL-C levels increasing from 52.56 mg/dL to 57.42 mg/dL, corresponding to a mean difference of 4.86 mg/dL and a 9.2% increase.

A significant decrease ($p < 0.05$) was also observed in rats treated with 800 mg/kg body weight, where HDL-C levels increased from 55.42 mg/dL to 58.90 mg/dL. This shows a mean difference of 3.48 mg/dL and a 6.3% increase.

Similarly, treatment with the antidiabetic drug (Glibenclamide) at 500 µg/kg body weight resulted in a significant increase ($p < 0.05$) in HDL-C levels, from 51.08 mg/dL to 56.64 mg/dL, with a mean difference of 5.56 mg/dL and a 10.9% increase.

The untreated diabetic rats showed a significant reduction ($p < 0.05$) in HDL-C levels, dropping from 54.54 mg/dL to 49.28 mg/dL. This corresponds to a mean difference of 7.12 mg/dL and a 9.64% decline.

Table 5: Effect of ethanol extract of *C. nutans* on the high-density lipoprotein cholesterol levels (mg/dL) of alloxan-induced diabetic rats.

Parameters	**Initial**	**Final**	**MD**	**Std error**	**p-value**	**%D**
$CN_{200mg/kg}$	52.88 ± 1.19	56.40 ± 1.51	3.52 ± 1.39	0.62	0.005	6.7
$CN_{500mg/kg}$	52.56 ± 2.40	57.42 ± 2.75	4.86 ± 1.00	0.45	0.001	9.2
$CN_{800mg/kg}$	55.42 ± 2.34	58.90 ± 2.13	3.48 ± 2.04	0.91	0.019	6.3

$SD_{500\mu g/kg}$	51.08 ± 1.89	56.64 ± 2.10	5.56 ± 1.55	0.69	0.001	10.9
UTG	54.54 ± 2.73	49.28 ± 1.75	5.26 ± 1.09	0.49	0.001	9.64

MD = mean difference; Std error = standard error; %D = percentage difference; CN = *C. nutans*; SD = standard drug; UTG = untreated group.

Table 6 shows the low-density lipoprotein cholesterol (LDL-C) levels of alloxan-induced diabetic rats following treatment. Rats treated with 200 mg/kg body weight of ethanol extract of *C. nutans* showed a significant decrease ($p < 0.05$) in LDL-C levels, from 41.40 mg/dL to 31.60 mg/dL, with 9.82 mg/dL mean difference and a 23.7% decline.

Conversely, rats treated with 500 mg/kg body weight of ethanol extract of *C. nutans* showed no significant decline ($p > 0.05$) in LDL-C levels, from 38.12 mg/dL to 32.92 mg/dL, reflecting 5.20 mean difference, and a 13.60% reduction.

Treatment with 800 mg/kg body weight of ethanol extract of *C. nutans* showed a significant decrease ($p < 0.05$) in LDL-C levels, from 39.94 mg/dL to 32.74 mg/dL. This reflects 7.22 mg/dL and a 18.10% reduction.

Similarly, rats treated with Glibenclamide at 500 µg/kg body weight resulted in a significant decrease ($p < 0.05$) in LDL-C levels, from 37.74 mg/dL to 23.68 mg/dL, with a mean difference of 5.56 mg/dL and a 37.30% decline.

Conversely, untreated diabetic rats showed no significant increase ($p < 0.05$) in LDL-C levels, rising from 40.72 mg/dL to 41.44 mg/dL. This corresponds to a mean difference of 0.72 mg/dL and a 1.8% increase.

Table 6: Effect of ethanol extract of *C. nutans* on the high-density lipoprotein cholesterol levels (mg/dL) of alloxan-induced diabetic rats.

Parameters	**Initial**	**Final**	**MD**	**Std error**	**p-value**	**%D**
$CN_{200mg/kg}$	41.40 ± 8.62	31.60 ± 6.40	9.82 ± 5.67	2.54	0.018	23.7
$CN_{500mg/kg}$	38.12 ± 8.45	32.92 ± 5.02	5.20 ± 4.99	2.23	0.080	13.6
$CN_{800mg/kg}$	39.94 ± 2.95	32.72 ± 4.99	7.22 ± 5.64	2.52	0.046	18.1
$SD_{500\mu g/kg}$	37.74 ± 7.18	23.68 ± 3.15	14.06± 8.71	3.89	0.023	37.3
UTG	40.72 ± 4.55	41.44 ± 5.45	0.72 ± 3.71	1.66	0.686	1.8

MD = mean difference; Std error = standard error; %D = percentage difference; CN = *C. nutans*; SD = standard drug; UTG = untreated group.

DISCUSSION

The phytochemical profile reveals a high abundance of phenolic compounds and flavonoids, alongside moderate levels of saponins, alkaloids, tannins, and phytate. The flavonoid and alkaloid contents of *C. nutans* leaf extract reported in the study were lower than those (3.86 and 4.81%, respectively) reported by Kong et al (2019). This composition is pharmacologically relevant, as phenolics and flavonoids are well known for their antioxidant and lipid-modulating properties (Shamsudin et al., 2022; Aryal et al., 2024). Their presence suggests a mechanistic basis for observed lipid-lowering effects, potentially through inhibition of lipid peroxidation, enhancement of hepatic lipid metabolism, and improved insulin sensitivity. Saponins may further contribute by reducing intestinal lipid absorption and promoting cholesterol excretion (Yamada et al., 2017).

The triglyceride data demonstrate a consistent and statistically significant reduction across all treatment doses, with the greatest effect observed at 800 mg/kg body weight. This uniform response suggests that the extract exerts a robust hypo-triglyceridemic effect, likely through improved insulin action and suppression of hepatic lipogenesis. Notably, the magnitude of TG reduction at higher doses exceeds that of glibenclamide, indicating that the extract may be particularly effective in targeting triglyceride metabolism. In contrast, the untreated diabetic group showed a significant increase in TG levels, reinforcing the role of diabetes in exacerbating hypertriglyceridemia and highlighting the therapeutic relevance of the ethanol extract of *C. nutans*. The percentage TG levels observed across all treatment doses were lower than those reported by Azemi et al. (2021) (80.91%), Sarega et al. (2016) (32.65%) and Umar Imam et al. (2019) (60.00%), with this variation potentially attributed to differences in extract preparation methods and the form of extract administered. The total cholesterol (TC) results reveal a more complex, non-linear dose-response pattern. Significant reductions were observed at 200 mg/kg and 800 mg/kg body weight, whereas the 500 mg/kg body weight dose failed to produce a significant effect. This suggests the possibility of an optimal therapeutic window or interaction among phytoconstituents at intermediate doses that may attenuate efficacy. Despite these reductions, glibenclamide produced a greater decline, indicating superior potency. The increase in TC in untreated rats further confirms the dyslipidaemic impact of alloxan-induced diabetes. The percentage change in TC levels in this study was lower than that observed in the studies of Azemi et al. (2021) (43.74%), Sarega et al. (2016) (28.04%), and Umar Imam et al. (2019) (29.41%).

The HDL-C findings are particularly noteworthy, as all extract-treated groups exhibited significant increases, with the highest improvement at 500 mg/kg dose. Elevation of HDL-C is clinically desirable due to its role in reverse cholesterol transport and cardiovascular protection. The comparable or slightly lower effect relative to glibenclamide suggests that the extract favourably modulates protective lipoproteins. The decline in HDL-C observed in untreated diabetic rats further emphasises the extract's corrective effect on lipid imbalance. The LDL-C results again demonstrate a non-linear response. Significant reductions were observed at 200 mg/kg and 800 mg/kg, while 500 mg/kg dose showed no statistically significant effect despite a numerical decrease. This irregular pattern reinforces the likelihood of complex dose-dependent interactions within the crude extract. Although glibenclamide achieved the most pronounced LDL-C reductions, the extract's ability to significantly lower LDL-C at certain dose remains clinically meaningful, given the central role of LDL-C in atherogenesis. The percentage change in LDL-C observed in the studies of Azemi et al. (2021) (56.64%), Sarega et al. (2016) (25.38%), and Umar Imam et al. (2019) (50.00%) was higher than that seen in the present study across treatment doses.

In addition, these findings indicate that *C. nutans* leaf extract exerts a broad hypolipidemic effect characterised by consistent reduction in triglycerides, moderate reduction in total cholesterol, elevated HDL-C, and selective reduction in LDL-C. however, the absence of a strictly linear dose-response relationship across TC and LDL-C suggests that increasing dosage does not uniformly enhance efficacy. This may reflect factors such as differential bioavailability, metabolic saturation, or antagonistic interactions among phytochemicals at certain concentrations (Okorie et al., 2022). Importantly, while the extract demonstrates significant lipid-modulating activity, it does not surpass the overall efficacy of glibenclamide, particularly in lowering LDL-C and TC. Therefore, its role may be better positioned as an adjunct or complementary therapy rather than a standalone alternative.

CONCLUSION

The study supports the therapeutic potential of *C. nutans* in managing diabetic dyslipidaemia in rats, with its effect mediated by its rich phenolic and flavonoid content. Future studies should focus on isolating active constituents, elucidating molecular mechanisms, and optimising dosage to resolve the observed non-linear responses and enhance clinical applicability.

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