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Phytochemical Screening And Anti-Diabetic Effect Of *Vernonia Amygdalina*

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Abstract: Background: *Diabetes mellitus continues to impose a heavy health burden, and medicinal plants such as Vernonia amygdalina are widely employed in traditional disease management. However, the relationship between extraction solvent, phytochemical composition and glucose-lowering activity remains insufficiently characterized.* ***Objective:*** *This study profiled the secondary metabolites of aqueous, ethanolic and n-hexane leaf extracts of V. amygdalina and evaluated the anti-diabetic effect of the aqueous extract in alloxan-induced diabetic rats.****Methods:*** *Leaf powder was extracted with each solvent and screened qualitatively for twenty phytochemical classes using standard procedures. For the in vivo component, eighteen albino rats were allocated to six groups of three. Diabetes was induced with a single intraperitoneal dose of alloxan monohydrate (150 mg/kg), and animals with blood glucose of at least 225 mg/dl were retained. Diabetic rats received the aqueous extract orally at 100, 200, 300 or 400 mg/kg body weight for four weeks. Glucose was measured on days 2, 14 and 28 and analyzed by repeated-measures ANOVA ($p \leq 0.05$).****Results:*** *All twenty phytochemical classes occurred in the ethanolic extract, compared with fourteen in the aqueous extract and eleven in the n-hexane extract. Treatment produced a time- and dose-related fall in glucose, with reductions of approximately 49%, 53%, 64% and 63% by day 28 in the 100, 200, 300 and 400 mg/kg groups, respectively, whereas glucose in untreated diabetic rats rose by about 5%. Day-28 values did not differ significantly between the 200 and 300 mg/kg groups ($p > 0.05$).* ***Conclusion:*** *Ethanol was the most versatile solvent for extracting the screened metabolites, and the aqueous leaf extract showed marked glucose-lowering activity in this model. These preliminary findings justify further mechanistic, histopathological and toxicological investigation.*

Keywords: *Vernonia amygdalina*, phytochemical screening, anti-diabetic activity, alloxan, aqueous extract

Introduction

Diabetes mellitus is among the most pressing chronic conditions of the present era. The International Diabetes Federation (2025) estimates that about 589 million adults aged 20–79 years were living with the disease in 2024, a figure projected to climb to roughly 853 million by 2050. More than four in ten affected adults are unaware of their status, and over four-fifths reside in low- and middle-income countries. Nigeria mirrors this trajectory. A recent systematic review and meta-analysis reported a pooled type 2 diabetes prevalence of 7.0%, which is nearly twice the 3.7% previously suggested by international estimates (Olamoyegun *et al.,* 2024). Such figures underscore the need for accessible, affordable and effective strategies for glycemic control. Although synthetic glucose-lowering agents are available, their adverse effects have prompted continued interest in alternative therapeutic sources, particularly natural products with a long record of ethnomedicinal use (Talwar *et al.,* 2024). Plants are attractive in this regard because they supply structurally diverse bioactive compounds that interact with multiple biological targets. Their medicinal value, however, can be established only through systematic chemical and pharmacological evaluation.

Vernonia amygdalina Delile, commonly known as bitter leaf, is a member of the *Asteraceae* family and serves as both a food and a medicinal plant across Africa and Asia (Atolani *et al.,* 2024). Its leaves contain glycosides, saponins, tannins, terpenoids and other constituents to which numerous pharmacological properties, including anti-diabetic, antioxidant and hepatoprotective actions, have been attributed. Toxicological appraisals further suggest that the plant is tolerated at comparatively high concentrations (Atolani *et al.,* 2024). Experimental work has also shown that its extracts can lower glycemia in both in vitro and in vivo systems, and that individual phytoconstituents may account for part of this activity (Talwar *et al.,* 2024). The chemical profile obtained from a plant, and consequently its biological activity, depends heavily on the solvent used for extraction. Differences in solvent polarity change both the yield and the antioxidant capacity of the recovered compounds (Palaiogiannis *et al.,* 2023). Comparing solvents of contrasting polarity, such as water, ethanol and n-hexane, therefore provides a more complete picture of the metabolites present and helps to identify the most suitable medium for their recovery.

The efficacy of candidate anti-diabetic plant preparations is commonly tested in rodents rendered diabetic with alloxan. This compound selectively injures pancreatic β-cells, chiefly through the generation of reactive oxygen species by redox cycling and through inhibition of glucokinase, the cellular glucose sensor (Kottaisamy *et al.,* 2021). The resulting insulin deficiency yields a

sustained hyperglycemic state that permits the glucose-lowering potential of an extract to be quantified over time. Against this background, the present study sought to screen aqueous, ethanolic and n-hexane leaf extracts of *V. amygdalina* for twenty classes of secondary metabolites and to determine the effect of graded oral doses (100–400 mg/kg body weight) of the aqueous leaf extract on blood glucose in alloxan-induced diabetic rats over a four-week period.

Materials and Methods

Collection of Plant Samples

Fresh leaves of *V. amygdalina* were collected from Kano Zoological Garden, Kano State, Nigeria. The sample was immediately taken to the Laboratory of the Department of Life Sciences, Kano State Polytechnic, Nigeria, for storage. The plant sample was identified and authenticated at the Herbarium Unit of the Department of Plant Biology, Bayero University Kano. The identification voucher number assigned is BUKHAN 0143, and a specimen was deposited at the herbarium for future reference.

Extract preparation for Phytochemical Screening

A 30 g portion of fine powder of *V. amygdalina* was extracted with 300 mL of the appropriate solvents (distilled water, ethanol and n-hexane) (1:10 w/v) in a round-bottom flask with a magnetic stirrer for 24 h at room temperature.

The extracts were then centrifuged at 5000 rpm for 15 min. An external magnetic field was applied to the magnetic stirrer to mix the solutions, which facilitated the rotation of the small magnetic bar placed in the mixture of interest (Yashashri *et al.*, 2017).

Preparation of Aqueous Extract of *V. amygdalina* for Determination of Hypoglycemic Effect

The extraction was carried out by soaking 30 g of the leaf powder of *V. amygdalina* in 250 mL of distilled water in a separate container. The volume of the extract and the difference between the weight of the powder and the weight of the residue were used to calculate the concentration of the extract. The concentration of the extract was used to calculate the doses given to the experimental groups (Groups III, IV, V and VI).

Experimental Animals

Male and female albino rats were used for the experiment. They were procured from the Department of Biological Sciences, Bayero University Kano. The animals were housed in plastic cages in a well-ventilated room and had free access to water throughout the experiment. They were fed Vital Feed Growers Mash, manufactured by Grand Cereals Ltd., a subsidiary of UAC of Nigeria.

Induction of Hyperglycemia

Hyperglycemia was induced according to the protocol of Aruna *et al.* (1999) as described by Okoli *et al.* (2010) with slight modification. A single intraperitoneal injection of alloxan monohydrate (150 mg/kg body weight) was given to Group II (positive control) and the treatment groups (Groups III, IV, V and VI). After two days, blood was drawn from the tail vein of the rats in all the groups by snipping to determine the blood glucose level with an automated glucose analyzer device (ACCU-CHEK Active Blood Glucose Monitoring System, Roche Diabetes Care GmbH, Mannheim, Germany). Animals with a blood glucose level ≥ 225 mg/dl were considered diabetic and were used for the study.

Experimental Design

Eighteen albino rats were divided into six groups of three rats each. Group I served as the negative control, while Group II served as the positive control, in which hyperglycemia was induced.
Diabetic rats in Groups III, IV, V and VI were orally administered 100, 200, 300 and 400 mg/kg body weight of aqueous leaf extract of *V. amygdalina*, respectively, for up to four weeks. A glucometer was used to measure the glucose level in the animals at two and four weeks, 24 h after the last administration, respectively.

Phytochemical Screening of the Extract Samples

Phytochemical screening was carried out for the aqueous, ethanol and n-hexane extracts using standard methods (Tiwari *et al.*, 2011).

Test for Saponins (Foam Test): 0.5 g of each extract of *V. amygdalina* was mixed and shaken with 2 mL of water. Foam that persists for 10 min indicates the presence of saponins.

Test for Tannins (Gelatin Test): To each extract, 1% gelatin solution containing sodium chloride was added. The formation of a white precipitate indicates the presence of tannins.

Test for Phenols (Ferric Chloride Test): *V. amygdalina* extracts were treated with 3–4 drops of ferric chloride solution. The formation of a bluish-black colour indicates the presence of phenols.

Test for cardiac glycosides (Legal's Test): Extracts of *V. amygdalina* were treated with sodium nitroprusside in pyridine and sodium hydroxide. The formation of a pink to blood-red colour indicates the presence of cardiac glycosides.

Test for Anthranol Glycosides (Modified Borntrager's): The extracts of *V. amygdalina* were treated with ferric chloride solution and immersed in boiling water for about 5 min. The mixture was cooled and extracted with an equal volume of benzene. The benzene layer was separated and treated with ammonia solution. The formation of a rose-pink colour in the ammoniacal layer indicates the presence of anthranol glycosides.

Test for Anthraquinone: A few drops of magnesium acetate solution were added to the *V. amygdalina* extracts. The formation of a pink colouration is indicative of anthraquinones.

Test for Flavonoids (Alkaline Reagent Test): *V. amygdalina* extracts were treated with a few drops of sodium hydroxide solution. The formation of an intense yellow colour, which becomes colourless on the addition of dilute acid, indicates the presence of flavonoids.

Test for Alkaloids (Dragendorff's Test): The *V. amygdalina* extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were treated with Dragendorff's reagent (potassium bismuth iodide solution). The formation of a red precipitate indicates the presence of alkaloids.

Test for Terpenoid (Noller's Test): *V. amygdalina* extracts were warmed with a piece of tin and a few drops of thionyl chloride. The appearance of a violet or purple colouration indicates the presence of terpenoids.

Test for Diterpenes (Copper acetate Test): Extracts of *V. amygdalina* were dissolved in water and treated with 3–4 drops of copper acetate solution. The formation of an emerald-green colour indicates the presence of diterpenes.

Test for Triterpenes (Salkowski's Test): *V. amygdalina* extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of concentrated sulphuric acid, shaken and allowed to stand. The appearance of a golden-yellow colour indicates the presence of triterpenes.

Test for Reducing Sugars (Benedict's test): Filtrates from the *V. amygdalina* extracts were treated with Benedict's reagent and heated gently. An orange-red precipitate indicates the presence of reducing sugars.

Test for Phytosterols (Liebermann–Burchard Test): *V. amygdalina* extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added. The formation of a brown ring at the junction indicates the presence of phytosterols.

Test for Coumarin: To 2 mL of each *V. amygdalina* extract, a few drops of alcoholic sodium hydroxide were added. The appearance of a yellow colour indicates the presence of coumarins.

Test for Steroid (Liebermann–Burchard Test): To 2 mL of *V. amygdalina* extract, a few drops of chloroform, 3–4 drops of acetic anhydride and one drop of concentrated sulphuric acid were added. The appearance of a purple colour, which changes to blue or green, shows the presence of steroids.

Test for Quinone: Each *V. amygdalina* extract was treated with a few drops of concentrated sulphuric acid or aqueous sodium hydroxide solution. Colour formation indicates the presence of quinoid compounds.

Test for Essential oil (Spot test): A small quantity of dried powdered *V. amygdalina* extract was pressed between two filter papers. The formation of a grease spot indicates the presence of essential oil.

Test for Phlobatannins (hydrochloric acid test): To 2 mL of the crude solution of each extract, dilute hydrochloric acid was added and the mixture was observed for a red precipitate, which indicates the presence of phlobatannins.

Test for Ketone: A few crystals of resorcinol and an equal volume of concentrated hydrochloric acid were added to 2 mL of the crude solution of each *V. amygdalina* extract and then heated over a spirit lamp flame and observed for a rose colouration, which shows the presence of ketones.

Test for Pentoses: To 2 mL of the solution of each extract of *V. amygdalina*, an equal volume of concentrated hydrochloric acid containing a little phloroglucinol was added. This was then heated over a spirit lamp flame and observed for red colouration, indicative of the presence of pentoses.

Statistical Analyses

Data collected on the various parameters measured for *V. amygdalina* were presented as mean ± standard deviation. Sample means were subjected to repeated-measures analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS, Version 23.0; IBM Corp.). Duncan's multiple range test (DMRT) was used as a post hoc test following ANOVA to identify which means differed significantly ($p \leq 0.05$).

Results

The results of the qualitative phytochemical screening are presented in Table 1. Saponins, tannins, phenols, cardiac glycosides, anthranol glycosides, flavonoids, alkaloids, terpenoids, diterpenes, triterpenoids, reducing sugars, phytosterols, coumarins, steroids, quinones, essential oils, phlobatannins, ketones, pentoses and anthraquinones were detected in at least one of the three solvent extracts (aqueous, ethanolic and n-hexane) of *V. amygdalina* leaf. All twenty phytochemicals screened were present in the ethanolic extract, making ethanol the most versatile solvent in this screening. Saponins, tannins, phenols, cardiac glycosides and anthranol glycosides were detected in all three extracts. In contrast, the aqueous extract lacked terpenoids, diterpenes, triterpenoids, phytosterols, steroids and essential oils, while the n-hexane extract lacked flavonoids, alkaloids, reducing sugars, coumarins, quinones, phlobatannins, ketones, pentoses and anthraquinones. This distribution reflects the polarity of the solvents: the polar constituents were extracted mainly by the aqueous and ethanolic extracts, whereas the non-polar constituents (terpenoids, sterols and essential oils) were extracted by the ethanolic and n-hexane extracts.

Table 1: Qualitative phytochemical (secondary metabolite) screening of aqueous, ethanolic and n-hexane extracts of *V. amygdalina* leaf.

	Extract		
Parameters	**Aqueous**	**Ethanolic**	**n-Hexane**
Saponin	+	+	+
Tannin	+	+	+
Phenol	+	+	+
Cardiac glycoside	+	+	+
Anthranol glycoside	+	+	+
Flavonoid	+	+	-
Alkaloid	+	+	-
Terpenoid	-	+	+
Diterpenes	-	+	+
Triterpenoids	-	+	+
Reducing sugar	+	+	-
Phytosterol	-	+	+
Coumarin	+	+	-
Steroids	-	+	+
Quinone	+	+	-
Essential oil	-	+	+
Phlobatannin	+	+	-
Ketone	+	+	-
Pentose	+	+	-
Anthraquinone	+	+	-

Key: + = present; – = absent

Table 2 presents the anti-diabetic effect of the aqueous leaf extract of *V. amygdalina* on blood glucose levels in alloxan-induced diabetic rats. By day 2 after alloxan administration, animals in Groups II, III, IV, V and VI had developed diabetes.

The negative control group maintained a steady glucose level, whereas diabetes worsened progressively in the positive control group. The extract-treated groups showed a dose- and time-dependent reduction in blood glucose levels, with the higher doses producing the largest reductions relative to baseline. By day 28, glucose had fallen by approximately 49%, 53%, 64% and 63% in Groups III, IV, V and VI, respectively, whereas it had risen by about 5% in the positive control group.

Repeated-measures ANOVA revealed a significant main effect of time on blood glucose levels. A significant time × treatment group interaction was also observed, indicating that the pattern of glucose change over time differed significantly among the experimental groups. The positive control group showed progressive worsening of diabetes, whereas the extract-treated groups showed a dose- and time-dependent reduction in glucose, most pronounced at day 28. The 400 mg/kg bw group approached the normoglycemic range by day 28, suggesting strong anti-diabetic potential of the extract. A significant between-subjects effect of treatment group was also observed when glucose was averaged across days 2, 14 and 28, indicating that overall glucose levels differed significantly among the treatment groups.

The mean glucose level of Group I (negative control) differed significantly ($p \leq 0.05$) from those of Group II (positive control) and Groups III, IV, V and VI, which received 100, 200, 300 and 400 mg/kg bw of the aqueous leaf extract of *V. amygdalina*, respectively, for four weeks. Glucose was measured on day 14 and day 28 of extract administration. All doses produced a significant reduction ($p \leq 0.05$) in blood glucose on days 14 and 28 when compared with day 2 (before treatment). However, no significant difference ($p > 0.05$) was observed between the day 28 glucose levels of Group IV (115.00 ± 5.00 mg/dl) and Group V (119.67 ± 5.03 mg/dl), suggesting that increasing the dose from 200 to 300 mg/kg bw gave no further reduction at that time point.

Table 2: Effect of the aqueous leaf extract of *V. amygdalina* (AEVA) on blood glucose level in alloxan-induced diabetic rats.

		Glucose Concentration (mg/dl)		
Group	**Dose**	**Day 2**	**Day 14**	**Day 28**
Group I	Negative Control	137.67 ± 4.16^{Ae}	140.33 ± 2.08^{Ae}	139.00 ± 1.00^{Ab}
Group II	Positive Control	236.00 ± 3.61^{Bd}	239.33 ± 4.04^{Ba}	247.33 ± 2.52^{Aa}
Group III	100 mg/kg bw	246.00 ± 3.61^{Ab}	137.00 ± 6.25^{Bb}	125.33 ± 4.51^{Ce}
Group IV	200 mg/kg bw	245.00 ± 5.00^{Ac}	126.67 ± 6.51^{Bc}	115.00 ± 5.00^{Cd}
Group V	300 mg/kg bw	330.67 ± 4.04^{Ac}	215.00 ± 5.00^{Bd}	119.67 ± 5.03^{Cd}
Group VI	400 mg/kg bw	294.67 ± 8.19^{Aa}	181.11 ± 6.11^{Be}	107.67 ± 2.52^{Ce}

Values are mean ± SD (n = 3). Different lowercase letters (a–e) within the same column indicate significant differences among groups at the same time point; different uppercase letters (A–C) within the same row indicate significant differences across time within the same group (Bonferroni-adjusted, $p \leq 0.05$). bw = body weight.

Discussion

The qualitative screening showed that the leaf of *Vernonia amygdalina* holds a chemically varied pool of secondary metabolites, and that how much of it is recovered depends strongly on the extraction solvent. All twenty phytochemical classes were detected in the ethanolic extract which is similar to the findings of Othman *et al.* (2026). Fourteen were found in the aqueous extract and eleven in the n-hexane extract. Ethanol's intermediate polarity probably allows it to dissolve both hydrophilic and moderately lipophilic constituents, which would explain this breadth. This agrees with evidence that solvent identity significantly alters both the yield and the antioxidant capacity of plant extracts, with ethanol-based systems among the most efficient (Palaiogiannis *et al.*, 2023). The polarity-dependent pattern was clear. Flavonoids, alkaloids, coumarins, quinones, reducing sugars and related polar compounds were absent from the n-hexane extract. Terpenoids, sterols, steroids and essential oils were absent from the aqueous extract. Saponins, tannins, phenols and cardiac and anthranol glycosides appeared in all three solvents. Their wide distribution may reflect the amphiphilic or structurally heterogeneous nature of these groups, and it warrants confirmation by quantitative assays. These findings are consistent with the review literature, which attributes the pharmacological value of the species to its glycosides, saponins, tannins, terpenoids and related constituents (Atolani *et al.,* 2024). Similarly, the findings of the current study agree with those of Muhammad *et al.* (2024), who qualitatively analyzed the aqueous, ethanol and n-hexane extracts of *Hyphaene thebaica* and detected saponins, tannins, phenols, cardiac glycosides, anthranol glycosides, flavonoids, alkaloids, terpenoids, diterpenes, triterpenes, reducing sugars, phytosterols, coumarins, steroids, quinones, essential oils, phlobatannins, ketones, pentoses and anthraquinones, compounds that play important roles in combating certain pathological conditions.

The in vivo data suggest that the aqueous extract has a substantial anti-diabetic effect (Table 2). Alloxan induces diabetes mainly by generating reactive oxygen species through redox cycling and by inhibiting glucokinase, the glucose sensor of the β-cell, which leads to DNA fragmentation and cell loss (Kottaisamy *et al.,* 2021). Diabetes was established in all alloxan-treated groups by day 2. Glycemia then worsened progressively in the untreated diabetic group, rising by roughly 5% by day 28, while the negative control remained stable. In contrast, every extract-treated group showed a time-dependent decline in glucose. Reductions by day 28 were approximately 49%, 53%, 64% and 63% for the 100, 200, 300 and 400 mg/kg groups, respectively. The significant time × treatment interaction in the repeated-measures analysis confirms that glucose trajectories diverged between groups rather than shifting uniformly. The 400 mg/kg group approached the normoglycemic range by the end of the study. These findings agree with Othman *et al.* (2026), who reported that the aqueous leaf extract of *L. hastata* demonstrated significant anti-diabetic effects in alloxan-induced diabetic rats, possibly because of its high secondary-metabolite content. Our results suggest that *Vernonia amygdalina* has anti-diabetic potential, although these preliminary findings in rats cannot be extrapolated directly to clinical use.

A mechanistic interpretation should remain tentative, because the present design did not assess insulin, oxidative stress markers or islet histology. It is plausible, however, that the phenolic compounds, flavonoids and saponins detected in the aqueous extract contribute through antioxidant protection of surviving β-cells, improved peripheral glucose utilization, or both. This is consistent with reviews that attribute the anti-diabetic activity of the plant to its bioactive constituents (Talwar *et al.,* 2024).

The dose-response relationship was not strictly linear. Glucose at day 28 did not differ significantly between the 200 mg/kg group (115.00 ± 5.00 mg/dl) and the 300 mg/kg group (119.67 ± 5.03 mg/dl). However, increasing the dose to 400 mg/kg produced a further reduction in blood glucose, to 107.67 ± 2.52 mg/dl at day 28. Toxicological appraisals report that the plant is tolerated at relatively high concentrations (Atolani *et al.,* 2024), although toxicity was not evaluated in the present study, and a dose of 200 mg/kg appears to be the most economical effective dose in this experiment.

The anti-diabetic effect of the leaf extract of *V. amygdalina* may be attributable to the secondary metabolites screened in this study or to one or more mechanisms. Possible mechanisms include stimulation of insulin secretion from residual β-cells, enhanced insulin-receptor activity, increased peripheral glucose uptake and inhibition of α-glucosidase activity (Babu *et al.,* 2003), although none of these was investigated here. Administration of the leaf extract of *V. amygdalina* for up to four weeks significantly reduced blood glucose levels compared with day-2 post-alloxan values.

Conclusion

In this study, ethanol was the most versatile solvent for extracting the screened metabolites from *V. amygdalina* leaf, and the aqueous leaf extract of *V. amygdalina* showed marked glucose-lowering activity in this model. Subsequent work should enlarge the cohorts, include a reference drug, assess toxicity under repeated dosing, and relate the glucose-lowering effect to specific phytoconstituents.

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Competing interest statement

The authors declare that there is no conflict of interest.

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