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Phytochemical and Antiplasmodial Studies of Methanol Extract from Aerial Parts of *Scadoxus multiflorus*

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Abstract: Scadoxus multiflorus, a fleshy herbaceous plant with a large bulb, is traditionally used in ethno-medicine for managing malaria, treating ulcers, cardiotonic activity and stimulant in debility. This study aimed to conduct phytochemical screening and evaluate the antiplasmodial activity of its aerial parts. The plant material was extracted with methanol using maceration process, and the crude extract was partitioned into hexane, chloroform, ethyl acetate, and butanol fractions. Qualitative phytochemical screening revealed the presence of tannins, flavonoids, alkaloids, terpenoids, steroids, saponins, phenols, and cardiac glycosides in the crude extract and fractions. Quantitative analysis revealed that phenolic compounds were the most abundant in the crude extract (198.32 mg/g), while alkaloids were the least (51.14 mg/g). The n-hexane fraction, however, had the highest tannin content (215 mg/g). Acute toxicity testing, following OECD 2008 guidelines, showed that the median lethal dose (LD_{50}) was greater than 5000 mg/kg, indicating the extract's safety. Antiplasmodial activity was evaluated using both suppressive and curative models in Plasmodium berghei-infected albino mice. The extract at 1000 mg/kg significantly suppressed parasitemia by 58.8% in the early infection (suppressive test) and reduced parasitemia by 61.8% in the established infection (curative test). Chloroquine, the standard drug at 5 mg/kg, produced higher parasite suppression (84.52%) and curative effects (84.50%) compared to the extract. The results suggest that the methanol extract of S. multiflorus aerial parts possesses antiplasmodial activity, supporting its traditional use in malaria management.

Introduction

Plants have long been used for nutritional support and medicine for humankind. Plant-based natural products provide important nutritional benefits, and many people globally also rely on botanical remedies for healthcare, using traditional medicine or complementary and alternative therapies (1). The medicinal use of herbs dates back to ancient times, as humans developed plant-based treatments for ailments such as headaches, fevers, and pain (2).

Malaria is a parasitic disease caused by *Plasmodium* parasites, including *Plasmodium falciparum* and *Plasmodium vivax* (3). It remains a significant health issue in regions such as sub-Saharan Africa, Asia, and Latin America, contributing to high levels of morbidity and mortality (4). The risk of malaria transmission varies across regions and is influenced by several factors, such as the local mosquito species and seasonal conditions, with the highest risk typically occurring during the rainy season in tropical areas (5).

The Amaryllidaceae family is a globally recognized group of bulbous plants, appreciated for their ornamental beauty, medicinal uses, and unique alkaloid compounds (6). *Scadoxus multiflorus*, a member of this family, also known as the fireball lily, African blood lily, or powderpuff lily, was first introduced by Thomas Martyn in 1795 as *Haemanthus multiflorus*. In 1838, Constantine Samuel Rafinesque changed the genus of *H. multiflorus* to a new genus, *Scadoxus* (7). It is a herbaceous plant with a large bulb and broad leaves that appear during the rainy season (8). However, it poses a toxicity risk to animals, as its bulbs and leaves contain potent toxins that can be fatal if ingested. In tropical Africa, it has been used as an arrow poison and fish hunting (7).

S. multiflorus has been used in traditional medicine for various ailments, such as treating dropsy, scabies, wounds, diarrhea, inflammation, and bacterial infections. It has also been applied as an antimalarial, cardiotonic, and stimulant treatment (8). Recent studies have isolated ten compounds from the plant's bulb, including six new phenolic glycosides, one of which demonstrated moderate cytotoxicity against leukemia cells (9). Additionally, aqueous leaf extracts of *S. multiflorus* have shown significant toxic effects and anti-inflammatory, membrane-stabilizing, and anti-thrombolytic activities (7). Phytochemical analysis of the leaf extracts has

reportedly revealed several secondary metabolites, including alkaloids, tannins, and flavonoids, which support its therapeutic potential (7).

The present study aims to further investigate the qualitative and quantitative phytochemical properties of the methanol extract and partitioned fractions of the aerial parts of *S. multiflorus* and evaluate its antiplasmodial activity to scientifically validate its ethnomedicinal uses against malaria. This is the first report on quantitative phytochemical screening and antiplasmodial activities on the aerial parts of *S. multiflorus.*

Material and Methods

Plant Material

The aerial parts of *S. multiflorus* comprising (flowers, leaves, and stems) were collected in September, 2022 from Kundugi Village, Sabon Gari Local Government, Kaduna State, Nigeria. The plant was identified by Mr. Namadi Sanusi at the Herbarium Unit of Botany Department, Ahmadu Bello University Zaria, Kaduna State, compared with an existing voucher specimen (ABU019006). The aerial parts were air-dried in the shade at room temperature until they reached a constant weight, after which they were ground. A total of 1.5 kg of the powdered plant material was stored at room temperature.

Solvents, Reagents, and Standard drugs

The solvents used were of analytical grade, including methanol, *n*-butanol, ethyl acetate, chloroform, *n*-hexane, and deionized water. Reagents used were freshly prepared, including those for phytochemical screening, such as Baljet's and Folin-Ciocalteu reagents, and chloroquine (Parker-Davis and Co Ltd. Detroit) was used as a standard drug.

Source and Maintenance of Experiment Animals

Adult Swiss Albino mice (19-30 g) of both sexes were sourced from the Animal House Facility, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. They were housed in standard laboratory conditions, fed with rodent feed, and given water *ad libitum*. The study adhered to Ahmadu Bello University's Committee on Animal Use and Care (ABUCAUC) guidelines and followed the "principle of laboratory animal care" (NIH publication No. 85-23, 1985).

Malaria Parasite

The Mouse-infected chloroquine-sensitive *Plasmodium berghei (NK-65)* strain was obtained from the National Institute of Medical Research, Lagos (NIMR). The parasites were kept alive by continuous intra-peritoneal passage into fresh mice at the Department of Pharmacology and Therapeutics, Animal House Facility Ahmadu Bello University Zaria, Nigeria.

Extraction and Partitioning

The pulverized plant material (1 kg) of *S. multiflorus* was extracted with 15 L of methanol using the maceration method for four days with occasional shaking and swirling. The extract was concentrated *in-vacuo* using a rotary evaporator at 40 °C to get a greenish-brown crude methanol extract. The crude extract (72 g) was suspended in distilled water (10 L) and filtered to obtain water-soluble and insoluble portions. The water-insoluble portion was washed with hexane (5 L), chloroform (2.5 L), and ethyl acetate (1 L) to obtain the water-insoluble hexane fraction (18.43 g), water-insoluble chloroform fraction (9.25 g) and water-insoluble ethyl acetate fraction (1.75 g) respectively. The filtrate was also fractionated with chloroform (500 mL), ethyl acetate (1500 mL), and n-butanol (1 L), yielding a water-soluble chloroform fraction (1.27 g), a water-soluble ethyl acetate fraction (7.33 g), a water-soluble butanol fraction (15.79 g), and a residual aqueous fraction (10 g).

Phytochemical Screening

The crude methanol extract and partitioned fractions were qualitatively analyzed using specific chemical reagents to detect phytochemicals such as alkaloids, terpenoids, anthraquinones, flavonoids, cardiac glycosides, tannins, steroids, and saponins. This was done by observing characteristic color changes according to standard methods described by (11) and modified by (10, 12, 13). Quantitative phytochemical analysis of the crude methanol extract and fractions was performed using standard spectrophotometric methods as follows.

Estimation of Total Cardiac Glycosides

For the analysis, 1 mL of the extract was mixed with 10 mL of freshly prepared Baljet's reagent (a mixture of 95 mL of 1% picric acid and 5 mL of 10% NaOH). After one hour, the mixture was diluted with 20 mL of distilled water, and absorbance was measured at 495 nm using a UV/VIS spectrophotometer. A standard curve was prepared using 1 mL of varying securidaside concentrations (20-100 mg/L), isolated from *Securigera securidaca* seed extract. Based on triplicate measurements, the total glycoside content was expressed as mg of securidaside/Digoxin per gram of dried extract (14).

Estimation of Total Steroids

The Liebermann-Burchard reaction was employed to detect sterols and terpenoids, which produce a dark pink to green color due to the reaction of the hydroxyl group with acetic anhydride and sulfuric acid (H_2SO_4). A standard calibration curve was prepared using varying cholesterol concentrations (10-100 µg/mL) and measured spectrophotometrically at 640 nm. The concentration of steroids in the crude extract was expressed in milligrams per gram (15).

Estimation of Total Triterpenoid

A 1 mL extract solution was combined with 1.5 mL of a 5% (w/v) vanillin-glacial acetic acid solution and 5 mL of perchloric acid. The mixture was heated for 45 min at 60 °C, then cooled in an ice-water bath to room temperature. After adding 2.25 mL of glacial acetic acid, the absorbance was measured at 548 nm using a UV-visible spectrophotometer. Ursolic acid or cholesterol (20–100 µg/mL in methanol) served as the standard, and results were expressed in milligrams of ursolic acid/cholesterol equivalents per gram of extract (15).

Estimation of Total Phenolic Content

Total phenol content was estimated spectrophotometrically using the Folin–Ciocalteu colorimetric method, with gallic acid as the standard, and results were expressed as gallic acid equivalent (GAE) per gram of sample. Gallic acid solutions (0.01-0.1 mg/mL) were prepared in methanol. A 0.5 mL aliquot of the test sample and each standard solution was mixed with 2 mL of Folin–Ciocalteu reagent (diluted 1:10

in deionized water) and 4 mL of saturated sodium carbonate solution (7.5% w/v). The mixtures were covered with foil and incubated for 30 min at room temperature with intermittent shaking. Absorbance was measured at 765 nm using methanol as the blank. All samples were analyzed in triplicate, and total phenol content was determined using a standard curve generated from pure gallic acid (10, 16).

Estimation of Total Flavonoid

The total flavonoid content (TFC) was determined using the aluminum chloride colorimetric assay. In brief, 0.5 mL aliquots of the sample and quercetin standard solutions (0.01-1.0 mg/mL) were mixed with 2 mL of distilled water and 0.15 mL of 5% sodium nitrite ($NaNO_2$) solution. After 6 min, 0.15 mL of 10% aluminum chloride ($AlCl_3$) solution was added. Following another 6-minute incubation, 2 mL of 4% sodium hydroxide (NaOH) solution was added, and the final volume was adjusted to 5 mL by adding distilled water. The mixture was thoroughly mixed and left to stand for 15 min. Absorbance was measured at 510 nm. TFC was calculated as mg quercetin equivalent per gram of sample using a calibration curve of quercetin, with all measurements performed in triplicate (17).

Estimation of Total Alkaloid

Total alkaloid content (TAC) was quantified using a reaction between alkaloids and bromocresol green (BCG). The plant extract and fractions (1 mg/mL) were dissolved in 2 N HCl and filtered. The pH of a phosphate buffer solution was adjusted to neutral using 0.1 N NaOH. A 1 mL portion of this solution was placed in a separatory funnel, adding 5 mL of BCG solution and 5 mL of phosphate buffer. The mixture was shaken, and the resulting complex was extracted with chloroform through vigorous shaking. The extract was transferred into a 10 mL volumetric flask and diluted to the mark with chloroform. The absorbance of the alkaloid-BCG complex in chloroform was measured at 470 nm. TAC was expressed as mg atropine equivalent per gram of sample using a calibration curve of atropine, with all experiments conducted in triplicate (12, 18).

Estimation of Total Tannin

Tannins were quantified using the Folin-Ciocalteu method. A 0.1 mL aliquot of the sample extract was placed into a 10 mL volumetric flask containing 7.5 mL of distilled water, 0.5 mL of Folin-Ciocalteu phenol reagent, and 1 mL of 35% sodium carbonate (Na_2CO_3) solution, then diluted to 10 mL with distilled water. The mixture was thoroughly shaken and allowed to sit at room temperature for 30 min. A set of gallic acid standard solutions (20, 40, 60, 80, and 100 µg/mL) was prepared similarly. The absorbance of the test and standard solutions was measured at 725 nm using a UV/Visible spectrophotometer, and the tannin content was expressed as mg of gallic acid equivalent (GAE) per gram of extract (19).

Estimation of Total Saponins

A known amount of freeze-dried extract was dissolved in 50% aqueous methanol, and a suitable aliquot (5 mg/mL) was taken for analysis. Vanillin reagent (0.25 mL, 8%) was added, followed by 2.5 mL of sulfuric acid (72% v/v). The reaction mixture was thoroughly mixed and incubated in a water bath at 60 °C for 10 min. After incubation, the mixture was cooled on ice, and the absorbance was measured at 544 nm using a UV-visible spectrophotometer, with a blank lacking the extract. A standard calibration curve was prepared using aliquots of diosgenin (0.5 mg/mL in 50% aqueous methanol). The total saponin content was expressed as mg diosgenin equivalents (DE) per gram of dry weight (20).

Acute Toxicity Studies

The LD50 of *S. multiflorus* extract was determined using Swiss Albino mice at 2000 and 5000 mg/kg doses, following OECD guideline (OECD test guideline 425, 2008). Mice were observed for toxicity and mortality over 14 days.

In Vivo Determination of Antimalarial Activity

The suppressive and curative antiplasmodial test was carried out on the crude methanol extract using animal model methods, as illustrated below.

Test on Early Malaria Infection (4-day Suppressive Test)

Three h after the parasite infection, 25 mice were randomly selected and divided into five treatment groups, with five mice in each group. On the first day of the experiment, mice in groups 1, 2, and 3 received the plant extract at doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg body weight, respectively. The fourth group received 5 mg/kg of chloroquine as a positive control, while the fifth group was given 2 mL/kg of distilled water as a negative control. The treatments were administered orally once daily for four consecutive days. On day five, a thin blood smear was prepared by collecting a drop of blood from the caudal vein of each mouse. The smears were air-dried, fixed in methanol, stained with 3% Giemsa solution at a pH of 7.2, and examined microscopically (21). The average percentage (%) of parasitemia suppression was evaluated using **Equation 1**.

Test on Established Infection (Curative Test)

Mice were infected with the parasite, and 72 h were allowed for parasitemia to develop before being randomly assigned to five groups, each containing five mice. On day 4 (72 h post-infection), groups 1, 2, and 3 received the plant extract at doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg body weight, respectively. The fourth group received 5 mg/kg of chloroquine as a positive control, while the fifth group was given 2 mL/kg of distilled water as a negative control. The treatments were administered orally once daily for four consecutive days. On day five, thin blood smears were prepared by collecting a drop of blood from the caudal vein of each mouse. The smears were air-dried, fixed in methanol, stained with 3% Giemsa solution at a pH of 7.2, and examined microscopically (22). Percentage (%) parasitemia curative was also calculated using **Equation 1**.

Results

Qualitative and Quantitative Phytochemical Screening

Tannins were present in all water-soluble fractions (Crude, CF, EF, BF, HF) and in the EF of the water-insoluble fractions, but absent in the CF. Triterpenes were detected in the Crude, CF, and HF water-soluble fractions, and in the CF of the water-insoluble fractions. Steroids showed a similar pattern, being present in the Crude, CF, and HF water-soluble

$$\text{Suppression/Curation}\ (\%) = \frac{\text{AP}_{\text{control}} - \text{AP}_{\text{group}}}{\text{AP}_{\text{control}}} \times 100$$

Equation 1 | AP = average number of parasitemia.

Table 1. Qualitative phytochemical constituents of the crude and partition fractions (water soluble and insoluble portions) of the aerial parts of *S. multiflorus*.

Constituent	Test	Inference						
		Water Soluble				Water Insoluble		
		Crude	CF	EF	BF	HF	CF	EF
Tannins	Lead acetate	+	+	+	+	+	-	+
Triterpenes	Liebermann-Burchard	+	+	-	-	+	+	-
Steroids	Salkowski	+	+	-	-	+	+	-
Saponins	Frothing	+	-	+	+	-	-	+
Flavonoids	NaOH	+	+	+	+	-	+	+
Anthraquinones	Bontrager	-	-	-	-	-	-	-
Alkaloids	Dragendoff	+	+	+	+	+	+	+
Cardiac glycoside	Keller-Killiani	+	-	+	+	-	-	+
Phenols	Ferric Chloride	+	+	+	+	+	+	+

Note: (+) means present and (-) means absent. HF = Hexane Fraction, CF = Chloroform Fraction, EF = Ethylacetate Fraction, BF = Butanol Fraction.

Table 2. Quantitative phytochemical constituents of the crude and partition fractions (water soluble and insoluble portions) of the aerial parts of *S. multiflorus*.

Constituents	Concentration (mg/g)						
	H2O Soluble				H2O Insoluble		
	Crude	CF	EF	BF	HF	CF	EF
Tannins	169.75	77.04	39.00	62.33	215.93	----	59.00
Triterpenes	95.21	42.65	----	----	91.08	31.02	----
Steroids	84.24	76.24	----	----	121.23	5.74	----
Saponins	121.45	----	34.73	18.06	----	----	28.96
Flavonoids	148.10	151.38	181.54	152.08	----	52.38	23.26
Alkaloids	51.14	26.23	32.42	21.32	76.23	18.41	15.69
Cardiacglycoside	119.90	----	87.41	92.75	----	----	23.13
Phenols	198.32	201.62	360.15	273.83	191.07	227.16	374.12

Note: HF = Hexane Fraction, CF = Chloroform Fraction, EF = Ethylacetate Fraction, BF = Butanol Fraction.

fractions and in the CF of the water-insoluble fractions. Saponins were found in the Crude, EF, and BF water-soluble fractions, and in the EF of the water-insoluble fractions. Flavonoids were observed in all water-soluble fractions except HF, and in both water-insoluble fractions. Anthraquinones were absent in all tested fractions. Alkaloids were present in all fractions across both solubility categories. Cardiac glycosides were detected in the Crude, EF, and BF water-soluble fractions, and in the EF of the water-insoluble fractions. Phenols were found to be present in all fractions. The qualitative phytochemical findings can be seen in **Table 1**.

Tannins showed a broad distribution across most fractions, with notably higher levels in the hexane and crude extracts. Triterpenes and steroids were primarily associated with the crude, chloroform, and hexane fractions, with minimal presence in the ethylacetate and butanol fractions. Saponins were concentrated in the crude, ethylacetate, and butanol extracts, with additional presence in the ethylacetate fraction of the water-insoluble portion. Flavonoids were widely distributed among the water-soluble fractions, especially prominent in the ethylacetate extract, but present at lower levels in the water-insoluble fractions. Alkaloids were detected in all fractions, with moderate variation in concentration. Cardiac glycosides appeared mainly in the crude, ethylacetate, and butanol fractions, and to a lesser extent in the water-insoluble ethylacetate extract. Phenols exhibited consistently high levels across all tested fractions, both water-soluble and water-insoluble. This findings can be seen in **Table 2**.

Antiplasmodial Studies

Treatment with the crude methanol extract of *S. multiflorus* resulted in a dose-dependent reduction in parasitemia levels in mice infected with *P. berghei*. Increasing extract doses corresponded to higher suppression percentages. The highest extract dose exhibited the greatest suppressive effect, although still lower than that of the chloroquine-treated group. In contrast, the normal saline group showed no suppressive activity. **Table 3** shows the supressive effects of methanol aerial extract of *S. multiflorus*.

Discussion

S. multiflorus is a plant known for its rich content of bioactive phytochemicals (7). The preliminary phytochemical

Table 3. Effect of methanol aerial extract of *S. multiflorus* on early *P. berghei* infection in mice.

Treatment (mg/kg)	Number of parasitemia (mean ± SEM)	Percentage Suppressive (%)
CME 250	13.84 ± 0.40*	33.07
CME 500	10.68 ± 0.36*	48.35
CME 1000	8.52 ± 0.30*	58.80
CQ 5	3.52 ± 0.27*	84.52
N/S 10 mL/kg	20.68 ± 1.02	0

Note: Data presented as mean ± SEM (n = 5). *p < 0.05 with Dunnet post hoc test. N/S = normal saline, CME = crude methanol extract, and CQ = chloroquine.

Table 4. Effect of methanol aerial extract of *S. multiflorus* on established infection in *P. berghei* infected mice.

Treatment (mg/kg)	Number of parasitemia (mean ± SEM)	Percentage Suppressive (%)
CME 250	16.4 ± 0.47*	25.0
CME 500	10.12 ± 0.32*	54.0
CME 1000	8.4 ± 0.27*	61.8
CQ 5	4.04 ± 0.21*	84.5
N/S 10 mL/kg	22.00 ± 1.14	0

Note: Data presented as mean ± SEM (n = 5). *p < 0.05 with Dunnet post hoc test. N/S = normal saline, CME = crude methanol extract, and CQ = chloroquine.

screening of its methanol leaf extract revealed various compounds, including alkaloids, tannins, saponins, steroids, glycosides, flavonoids, and reducing sugars (7). In this study, qualitative and quantitative analyses were conducted on the methanol extract and partitioned fractions from the aerial parts of the plant. In addition, acute toxicity and suppressive and curative antiplasmodial activity were evaluated using an *in vivo* model. The phytochemical screening showed that without anthraquinone, the methanol and partitioned fractions contained flavonoids, alkaloids, steroids, tannins, carbohydrates, triterpenes, saponins, phenolics, and cardiac glycosides. These phytochemicals have been reported to exhibit pharmacological properties, including anti-inflammatory, membrane-stabilizing, and antithrombotic effects (7), as well as moderate cytotoxicity against HL-60 human promyelocytic leukemia cells (9).

The methanol extract and all partitioned fractions, except the n-hexane water-insoluble fraction, had high phenolic content, aligning with phenolic compounds' widespread occurrence in plant secondary metabolites (23). Phenolic compounds, including coumarins, flavonoids, tannins, and stilbenes, are known for their pharmacological properties, such as antimalarial, anti-inflammatory, and antioxidant activities (24). They also serve as natural defense agents in plants. The n-hexane water-insoluble fraction exhibited the highest tannin content, possibly due to condensed tannins and their polarity characteristics. Tannins are recognized for their astringent, wound-healing properties, and stable antioxidant activity (25). Differences in the concentrations of secondary metabolites across the fractions and crude extracts are likely attributed to the solubility of the compounds and environmental factors like climate and altitude (26, 27).

Oral acute toxicity (LD_{50}) of the methanol extract of *S. multiflorus* aerial parts was found to be greater than 5000 mg/kg body weight, with no observed mortality or adverse behavioral effects on mice. This indicates that the extract is relatively safe for oral use, particularly for managing malaria. In the suppressive test, the crude methanol extract exhibited a dose-dependent reduction in parasitemia at doses of 250, 500, and 1000 mg/kg, with parasite suppression rates of 33.07%, 48.35%, and 58.8%, respectively. The highest suppression rate was observed with chloroquine at 5 mg/kg, which achieved 84.52% suppression as shown in **Table 3**. These results suggest that the extract has a blood schizonticidal effect, crucial in controlling malaria.

In the curative test, parasitemia levels were significantly reduced in a dose-dependent manner, with cure rates of 25.00%, 54.00%, and 61.80% at doses of 250, 500, and 1000 mg/kg, respectively. Chloroquine, the positive control, exhibited the highest cure rate of 84.54%. The crude extract achieved its highest parasitemia inhibition at 1000 mg/kg (61.80%), while the lowest inhibition was at 250 mg/kg (25.00%), as shown in **Table 4**.

These findings suggest that the crude methanol extract of *S. multiflorus* exhibits significant antiplasmodial activity, supporting its traditional use in treating malaria (8). The antiplasmodial effect may be due to the extract's inhibition of processes associated with high parasitemia and also due to secondary metabolites like coumarin, which has been reportedly isolated from the aerial parts of this plant (29) as coumarins have been identified as the active components in some plants used traditionally against malaria (30). Natural products with antimalarial properties, such as those found in *S. multiflorus*, have been linked to their active phytochemical components, including alkaloids, terpenes, and phenols (28). While the extract's activity was less potent than chloroquine, its dose-dependent efficacy partially validates its traditional use by healers for malaria management.

Conclusion

This study revealed that the crude methanol extract and its partitioned fractions of *S. multiflorus* contained the highest phenolic compounds, except for the n-hexane fraction, which exhibited the highest concentration of tannins. This may be attributed to the presence of condensed tannins, likely

influenced by their polarity index. The study also demonstrated a dose-dependent and statistically significant antiplasmodial activity of the crude methanol extract from the aerial parts of *S. multiflorus* using an *in vivo* model. This shows the first scientific validation of its traditional claim for this purpose. Further research is recommended to isolate and characterize the active bioactive constituents from the crude extract and its partitioned fractions, focusing on the plant parts investigated in this study.

Declarations

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Conflict of Interest

The authors declare no conflicting interest.

Data Availability

The unpublished data is available upon request to the corresponding author.

Ethics Statement

The study adhered to Ahmadu Bello University's Committee on Animal Use and Care (ABUCAUC) guidelines and followed the "principle of laboratory animal care" (NIH publication No. 85-23, 1985).

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Additional Information

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