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Comparative *In Vitro* Antihaemolytic Activity of *Uvaria chamae* Leave and Stem Fractions Against *Echis ocellatus* and *Naja nigricollis* Venoms

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ABSTRACT
Snakebite envenomation remains a major public health concern globally. This study evaluated the in vitro antihaemolytic activity of methanol extract of *Uvaria chamae* leaves and stem fractions (n-hexane, ethyl acetate, n-butanol, and aqueous) against *Echis ocellatus* and *Naja nigricollis* venoms. Antihaemolytic activity was evaluated using the fractions at concentrations of 100, 50, and 25 mg/mL. The ethyl acetate fraction showed strong qualitative reactions for tannins, phenolic compounds, and flavonoids. The LD_{50} of the crude methanol extract exceeded 5,000 mg/kg body weight, with no mortality observed at the tested dose. Against *E. ocellatus* venom, the n-hexane fraction demonstrated antihaemolytic activities of 89%, 76%, and 83% at 100, 50, and 25 mg/mL, respectively. The butanol fraction recorded 86%, 22%, and 46%, while the ethyl acetate fraction exhibited 85%, 76%, and 76%, respectively. The aqueous fraction demonstrated 87%, 84%, and 55% activity at the corresponding concentrations. Against *N. nigricollis* venom, the aqueous fraction exhibited the highest antihaemolytic activity (75 %) at 100 mg/mL, followed by the ethyl acetate (63%), n-hexane (54 %), and butanol (11 %) fractions. At 50 and 25 mg/mL, the aqueous fraction recorded 55 % and 43%, respectively, while the ethyl acetate fraction demonstrated 55 % and 44%. The n-hexane fraction recorded 53 % and 52 %, whereas the butanol fraction exhibited 47% and 40%, respectively. The findings demonstrate varying in vitro antihaemolytic activities of *U. chamae* leave and stem fractions against both venoms, potentially associated with differences in phytochemical composition.

Keywords: *Uvaria chamae*, *Echis ocellatus*, *Naja nigricollis*, Antihaemolytic Activity, Phytochemical Screening, Acute Toxicity, Snakebite Envenomation

INTRODUCTION

Snakebite envenoming remains an important public health problem in tropical and subtropical regions, particularly in rural communities where agricultural activities, limited access to healthcare facilities, and inadequate availability of effective antivenoms increase the risk of severe complications and death (Truong *et al.*, 2026; Ediriweera *et al.*, 2026). The World Health Organization recognises snakebite envenoming as a neglected tropical disease because of its contribution to preventable mortality, permanent disability, and socioeconomic hardship (Stienstra *et al.*, 2026). Envenoming occurs when a venomous snake injects venom through its fangs, introducing a complex mixture of biologically active proteins, enzymes, and peptides into the victim's body (Migliorisi *et al.*, 2026). These toxins interfere with normal physiological processes and may cause haemorrhage, coagulopathy, neurotoxicity, cardiovascular disturbances, cytotoxicity, and local tissue damage (Alshammari, 2022). The severity and clinical manifestations depend on several factors, including snake species, venom composition, quantity injected, bite location, environmental conditions, and the victim's physiological status (Mao *et al.*, 2025). In tropical Africa, individuals whose occupations involve frequent contact with natural snake habitats, particularly farmers, pastoralists, and herders, are at increased risk of envenoming (Dine *et al.*, 2026). Effective management is further complicated by delays in seeking medical attention, poor transportation networks, inadequate antivenom availability, and reliance on traditional remedies and non-medical interventions, including prayer houses (Isaac *et al.*, 2026).

Nigeria is among the African countries where snakebite envenoming constitutes a significant medical and veterinary concern. Several medically important venomous snake species occur across the country, including *Echis ocellatus* and *Naja nigricollis* (Habib, 2015). *Echis ocellatus*, commonly known as the West African carpet viper, possesses venom associated predominantly with haemotoxic effects, including disturbances of blood coagulation, vascular injury, and haemorrhage (Dingwoke *et al.*, 2024). In contrast, *Naja nigricollis*, commonly known as the black-necked spitting cobra, has venom containing cytotoxic components that contribute to cell membrane damage, blistering, local tissue

destruction, and necrosis (Adeyi *et al.,* 2026). Although these species exhibit different predominant pathological effects, their venoms contain complex toxin mixtures capable of affecting multiple physiological systems.

The principal specific treatment for snakebite envenoming is the administration of an appropriate antivenom, supported by symptomatic and other necessary medical interventions (Nanyonga *et al.,* 2025). Antivenoms are produced by immunising animals with selected snake venoms and subsequently extracting and purifying antibodies or antibody fragments capable of recognising and neutralising venom toxins. Despite their importance, their effectiveness depends on factors such as the specificity of the antivenom, the offending snake species, the timing of administration, and the distribution of venom toxins within the body. Furthermore, antivenoms may not adequately reverse tissue damage that has already occurred following envenoming. These limitations have stimulated research into complementary therapeutic agents, particularly natural products capable of inhibiting specific venom activities.

Medicinal plants represent important sources of pharmacologically active compounds and have attracted considerable interest in the search for potential antivenom agents. Plant-derived compounds have demonstrated various biological activities, including antioxidant, anti-inflammatory, antimicrobial, and enzyme-inhibitory effects. Some of these compounds may interfere with venom toxicity through enzyme inhibition, interactions with toxic proteins, scavenging of reactive oxygen species, or modulation of cellular responses to tissue injury. However, their biological activities vary according to plant species, plant parts, extraction methods, chemical composition, and the specific venom activities investigated. Consequently, systematic experimental studies are necessary to establish their potential efficacy against particular snake venoms (Opiyo and Njoroge, 2024).

Uvaria chamae, a medicinal plant belonging to the family Annonaceae, is widely distributed in tropical Africa and has been used traditionally for the management of various health conditions. Different parts of the plant, including its roots, stem bark, and leaves, have attracted scientific interest because of their potential pharmacological properties. Its reported biological activities include antimicrobial, antioxidant, and anti-inflammatory effects. These properties have encouraged further investigation into its phytochemical composition and possible therapeutic applications.

The pharmacological activities of *Uvaria chamae* may be associated with secondary metabolites such as flavonoids, alkaloids, tannins, phenolic compounds, and other potentially bioactive constituents (Hounsou *et al.,* 2026). These phytochemicals have been investigated for their ability to interact with proteins, inhibit enzymes, reduce oxidative damage, and protect cellular membranes under certain experimental conditions (Singh, *et al.,* 2026). Their distribution and concentration may vary according to geographical origin, harvesting conditions, plant parts, and extraction solvents (Kokab *et al.,* 2026). Furthermore, fractionation using solvents of different polarities may produce fractions with distinct phytochemical compositions and biological activities. Comparative evaluation of these fractions may therefore help identify those with greater antihaemolytic potential and provide a basis for further phytochemical and pharmacological characterisation. Nevertheless, the presence of these secondary metabolites does not independently establish antihaemolytic activity against *E. ocellatus* or *N. nigricollis* venoms; this requires experimental verification.

Haemolysis refers to the disruption of erythrocytes (red blood cells), resulting in the release of intracellular haemoglobin into the surrounding medium. Under normal physiological conditions, erythrocytes maintain membrane integrity and facilitate oxygen transport to body tissues. However, exposure to certain toxins, enzymes, chemicals, and physical conditions may compromise membrane stability and cause haemoglobin leakage. Snake venom-associated haemolysis may result from direct membrane-disrupting effects or indirect mechanisms involving venom enzymes and other biological processes. The extent of erythrocyte damage depends on venom composition and concentration, erythrocyte susceptibility, incubation conditions, and the experimental system employed.

Snake venoms contain several toxin families with different biological activities. In *E. ocellatus*, haemorrhagic and haemotoxic effects are primarily associated with toxins that interfere with vascular integrity and haemostasis. These effects should, however, be distinguished from direct erythrocyte lysis. Similarly, the cytotoxic activity of *N. nigricollis* venom is associated particularly with membrane-active toxins, including cytotoxins, which can disrupt cellular membranes and contribute to local tissue destruction. The ability of these venoms to induce direct erythrocyte haemolysis must therefore be established experimentally under defined conditions. Investigating this activity provides a measurable laboratory endpoint for evaluating one aspect of venom toxicity and the potential protective effects of test substances (Alvitigala *et al*., 2025).

The antihaemolytic activity of a substance refers to its ability to reduce or prevent erythrocyte destruction under defined experimental conditions. In an in vitro haemolysis assay, erythrocytes are exposed to specific venom concentrations, and the extent of haemolysis is determined by measuring the haemoglobin released into the surrounding medium. The protective potential of a plant fraction can subsequently be evaluated by comparing haemolysis in venom-exposed erythrocytes treated with the fraction against appropriate venom-only and control groups. A reduction in haemolysis may indicate that the fraction interferes with the haemolytic process through interactions with venom components or protection of erythrocyte membranes. However, such an observation does not necessarily demonstrate direct venom neutralisation or protection against other manifestations of envenoming, including coagulopathy, neurotoxicity, and tissue necrosis.

A comparative investigation of the antihaemolytic activities of *Uvaria chamae* fractions against *E. ocellatus* and *N. nigricollis* venoms may provide useful information about the potential biological activities of the plant. Since the two venoms differ in their toxin composition and predominant pathological effects, the fractions may exhibit varying degrees of inhibitory activity against each venom (Tong *et al.,* 2026). A fraction that reduces haemolysis induced by one venom may not necessarily demonstrate similar activity against the other. Such differences may be associated with variations in venom-mediated erythrocyte damage, phytochemical composition, and interactions between plant constituents and venom components.

In view of the continuing burden of snakebite envenoming and the need to identify additional sources of potentially useful pharmacological compounds, investigating the antihaemolytic properties of *Uvaria chamae* fractions represents an important area of natural products research. Therefore, this study aims to evaluate and compare the antihaemolytic activities of selected fractions of *Uvaria chamae* against *Echis ocellatus* and *Naja nigricollis* venoms

under controlled in vitro conditions. The study will determine whether the fractions possess measurable antihaemolytic activity, establish differences in their effectiveness against the two venoms, and identify fractions that may warrant further phytochemical and pharmacological investigation. The findings may provide preliminary scientific evidence on the potential of *Uvaria chamae* as a source of bioactive compounds for further research into venom-associated cellular injury.

MATERIAL AND METHODS

Location of Experiment

The research was conducted at the Department of Veterinary Pharmacology and Toxicology, Department of pharmacognosy and Drugs development, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Collection of Plant Material

The leaves of *Uvaria chamae* were collected in February 2026 from Agatu Local Government Area of Benue State, Nigeria. The plant was identified and authenticated at the Herbarium of the Department of Biology, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria. A voucher specimen (V/N: 819) was deposited at the herbarium for future reference.

Plant Extraction

The leaves and stem of *Uvaria chamae* were collected, cleaned, and shade-dried before being pulverized into a fine powder using Motter and pestle. Approximately 5000 g of the powdered plant material was macerated in 95% methanol at room temperature for 48 hours. The resulting extracts were pooled and filtered through Whatman No. 1 filter paper. The filtrate was subsequently concentrated in a water bath maintained at 40°C. The resulting crude extract was weighed and stored in an airtight container at 4°C until required for subsequent use.

Fractionation of Extract

The crude methanolic extract of *Uvaria chamae* leaves was dissolved in 500 mL of distilled water and transferred into a 1 Letter separating funnel. An equal volume of n-hexane was slowly added, after which the funnel was securely closed and gently shaken to facilitate proper solvent partitioning. Accumulated pressure was released periodically by inverting the separating funnel and briefly opening the stopcock. This procedure was repeated three times, and the mixture was allowed to stand for approximately 10 minutes to facilitate phase separation. The lower aqueous layer was subsequently collected in a clean container, while the n-hexane layer was separated and concentrated to dryness under a continuous stream of air. The aqueous fraction was extracted twice with n-hexane, followed by exposure to a continuous stream of air for approximately 2 hours to eliminate residual traces of the solvent. Subsequently, the aqueous fraction was mixed with an equal volume of ethyl acetate in the separating funnel and gently shaken to facilitate the extraction of ethyl acetate-soluble biochemical constituents. The separating funnel was then inverted, and the stopcock was briefly opened to release the pressure generated during mixing. The mixture was allowed to stand for approximately 15 minutes to facilitate phase separation. The lower aqueous phase was carefully decanted and collected separately from the upper ethyl acetate phase. The extraction procedure was repeated, and the resulting fractions were individually concentrated to dryness under a continuous stream of air. All fractions (n-hexane, ethyl acetate, n-butanol, and aqueous) were separately concentrated over a water bath at 35 ℃ using the method described by Abubakar and Haque (2020). The concentrated fractions were transferred into properly labelled sample bottles and stored for further analysis.

Collection and Crystallization of Crude Venom

Crude venom of *Echis ocellatus* and *Naja nigricollis* was collected using the method described by Markfarlane (1967), with a modification involving the placement of cellophane over the opening of the collection beaker. The venom-containing beaker was subsequently placed in a desiccator containing activated silica gel and maintained at 4°C to facilitate crystallization. Following crystallization, the dried venom was transferred into appropriately labelled Eppendorf tubes and stored at -18°C until required for subsequent analyses.

Determination of Median Lethal Dose (LD50) of Crude Extract of *Uvaria chamae* Leaves and Stem

The Organisation for Economic Co-operation and Development (OECD 2008), was adopted to assess the acute toxicity (LD50) of the crude methanol extract of *Uvaria chamae* in mice via the intraperitoneal route. The results indicated that the LD_{50} was greater than 5,000 mg/kg body weight, as no mortality was recorded at this dose within 24 hours of administration under the conditions of the study.

Evaluation of the Modulatory Activity of *Uvaria chamae* Methanol Fractions on *Naja nigricollis* and *Echis ocellatus* Venoms

Anti-haemolytic Activity of *Uvaria chamae* Fractions

The method described by Gomes and Pallabi (1999) was adopted, with the slight modifications, to evaluate the anti-haemolytic activity of the extract fractions against *Naja nigricollis* and *Echis ocellatus* venom. Briefly, 20 mL of bovine blood was collected through jugular vein into a container containing sodium citrate as an anticoagulant. The blood sample was centrifuged at 3,000 rpm for 10 minutes, and the supernatant plasma was subsequently removed. The packed erythrocytes were washed with 5 mL of normal saline and centrifuged again at 3,000 rpm for 10 minutes. The resulting supernatant was discarded, and the washing procedure was repeated ten times to obtain plasma-free packed erythrocytes.

Crude *N. nigricollis* and Echis ocellatus venoms (10 mg/mL) each was mixed with the crude extract fractions (100 mg/mL, 50mg/ml, and 25mg/ml comprising the control (A), n-hexane fraction (B), n-butanol fraction (C), aqueous residue (D), and ethyl acetate fraction (E). The mixtures were then added to 10 mL of a 1% erythrocyte suspension prepared in normal saline and incubated at 37 +/- 1 ^{0}C for 60 minutes. The reaction was terminated by adding 3 mL of chilled phosphate-buffered saline (PBS). The mixtures were subsequently centrifuged at 2,000 rpm for 10 minutes, and the absorbance of the resulting supernatant was measured at 540 nm using a Spectral 23A spectrophotometer. For the 100% haemolysis reference, the supernatant from the experimental tube was treated with 3 mL of chilled distilled water. The anti-haemolytic activity was determined spectrophotometrically and expressed as a percentage using the following formula: Percentage anti-haemolysis = (Absorbance of the treated sample / Absorbance of the control) x 100.

Data Analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical comparisons among groups were

performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. All statistical analyses were conducted using GraphPad Prism version 5.0. Differences were considered statistically significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Results

Percentage of Extraction Yield of U. chamae Leave and Stem Yield

The following were obtained from the maceration of 5000g of the *Uvaria chamae* leaves and stem using various solvents.

Table 1: Percentage of Extraction Yield of *U. chamae* Leave and Stem Yield

S/N	Solvents	Weight (g)	Percentage (%) Yield	Colour
1	n-hexane (NHF)	150.0	3.00	Yellowish-brown
2	n-butanol (NBF)	42.3	0.85	Yellow
3	Ethyl acetate (EAF)	57.70	1.154	Reddish-brown
4	Aqueous residues (AQR)	34.5	0.69	Reddish-brown

Phytochemical Constituents

The preliminary phytochemical screening of the combined stem and leaf fractions of *Uvaria chamae*, using n-hexane, n-butanol, and ethyl acetate solvents, is shown in Table 4.1. All fractions were found to contain steroids and triterpenes. However, alkaloids, tannin and phenolic compounds, anthraquinones, and flavonoids were detected only in the ethyl acetate, *n*-butanol, and aqueous fractions, but were absent in the *n*-hexane fraction.

Table 2: The Phytochemical Constituents of *Uvaria chamae* Leave and Stem Extract

S/No.	Tests	Constituent	CME	NHF	EAF	NBF	AQR
1	Lieberman Bunchand	Steroid and Triterpene	+	+	+	+	+
2	Dragendorff	Alkaloids	+	-	+	+	+
3	$FeCl_3$	Tannins/Phenolic compound	+	-	+++	+	+
4	Bontragers	Anthraquinones	+	-	+	+	+
5	$AlCl_3$	Flavanoids		-	+++		-

+++ appreciating amount, ++ moderate amount, + trace amount and – absence
CME=Crude Methanol extract; NHF=N-Hexane fraction; EAF= Ethyl acetate fraction;
NBF=N-Butanol fraction; AQR=Aqueous residues

LD_{50} of the Snake Venoms

The Organisation for Economic Co-operation and Development (OECD 2008) technique was used to assess the acute toxicity (LD50) of the *Naja nigricollis* and *Echis ocellatus* venoms, through the intraperitoneal route in mice, and the results indicated that the LD50 was 0.45 and 1mg/kg

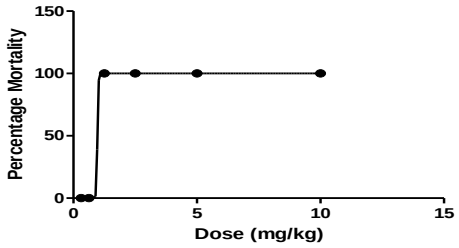


Figure 1: LD50 for *Echis ocellatus* is 0.950 mg/kg



Figure 2: LD50 of Naja Nigricollis is 0.45mg/kg

In vitro Anti-haemolytic Potential of *Uvaria Chamae* leaf and stem Fractions Against *Echis ocellatus* and Naja nigricollis Venoms

Table 3: Antihaemolytic Assay of *Uvaria Chamae* Leave and Stem Fractions Against the *Echis ocellatus* Venom

	% Antihaemolytic Activity			
Extract/Conc.	**100mg/ml**	**50mg/ml**	**25mg/ml**	**Venom+RBC**
N-hexane	89±0.58	76±1.2	83±0.95	0.0±0.0
Butanol	86±0.88	22±0.85	46±1.2	0.0±0.0
EA	85±0.61	76±0.84	76±1.4	0.0±0.0
Aqueous	87±1.4	84±0.88	55±1.2	0.0±0.0

Table 4: Antihaemolytic Assay of *Uvaria Chamae* Leave and Stem Fractions Against the Venom of *Naja nigricollis*

	% Antihaemolytic Activity			
Extract/Conc.	**100mg/ml**	**50mg/ml**	**25mg/ml**	**Venom+RBC**
N-hexane	54±0.92	53±0.72	52±0.98	0.0±0.0
Butanol	11±0.72	47±0.36	40±0.52	0.0±0.0
EA	63±1.4	55±1.5	44±1.6	0.0±0.0
Aqueous	75±1.3	55±1.7	43±1.5	0.0±0.0

Discussion

The preliminary phytochemical screening of the crude methanolic extract and its solvent fractions revealed differences in the distribution of secondary metabolites among the different fractions of *Uvaria chamae*, as presented in (Table 2.0). The identified phytochemical groups included steroids and triterpenes, alkaloids, tannins and phenolic compounds, anthraquinones, and flavonoids are in agreement with the work of Enin, *et al.* (2021). The observed differences in their distribution may be attributed to variations in the polarity of the extraction solvents and the chemical properties of the constituent compounds. Steroids and triterpenes were detected in the crude methanol extract (CME), n-hexane fraction (NHF), ethyl acetate fraction (EAF), n-butanol fraction (NBF), and aqueous residue (AQR). Their presence across all the tested samples suggests that compounds belonging to these phytochemical groups were distributed throughout the extract and its fractions. Steroids and triterpenes are structurally diverse natural products that have been investigated for several biological activities Anjali *et al.* (2026). However, their detection in the present study does establish their specific antiheamolytic contribution to the biological effects of *U. chamae* is in agreement with the work of Basyuni *et al.* (2026). Alkaloids were detected in the crude methanol extract, ethyl acetate fraction, n-butanol fraction, and aqueous residue but were absent from the n-hexane fraction. This distribution suggests that the alkaloids detectable by the screening method were preferentially retained in the more polar fractions rather than the non-polar n-hexane fraction. Alkaloids constitute a diverse group of nitrogen-containing natural products with a range of biological activities. Their presence may therefore provide a basis for further investigation of the pharmacological properties of *U. chamae*. Screening for tannins and phenolic compounds revealed their presence in the crude methanol extract, ethyl acetate fraction, n-butanol fraction, and aqueous residue, but not in the n-hexane fraction. This finding is consistent with the report of Ugwu *et al.* (2026). The ethyl acetate fraction recorded the highest qualitative abundance (+++), whereas the other positive samples showed only trace amounts (+). This finding suggests that the ethyl acetate fraction may be enriched in compounds that react positively in the ferric chloride test. Phenolic compounds are of particular interest in medicinal plant research because many exhibit antioxidant activity through mechanisms such as free-radical scavenging and metal chelation. However, the intensity of a qualitative colour reaction does not directly quantify total phenolic content or demonstrate antioxidant efficacy. Quantitative assays would be required to confirm the relative abundance and biological activity of these compounds. Anthraquinones were detected in the crude methanol extract, ethyl acetate fraction, n-butanol fraction, and aqueous residue but were absent from the n-hexane fraction. This pattern suggests that the detectable anthraquinone-related compounds were more readily recovered in the fractions other than the non-polar n-hexane fraction. Anthraquinones comprise a diverse class of aromatic compounds associated with various biological properties, depending on their chemical structures. Further chromatographic and spectroscopic analyses would be necessary to identify the individual compounds present in *U. chamae* and assess their potential pharmacological relevance. Flavonoids were recorded at a high qualitative abundance (+++) in the ethyl acetate fraction, while they were absent from the n-hexane fraction. The results for the crude methanol extract, n-butanol fraction, and aqueous residue are not clearly indicated in the supplied table and should therefore be verified before drawing conclusions about their flavonoid content. The apparent enrichment of flavonoids in the ethyl acetate fraction is consistent with the ability of intermediate-polarity solvents to extract certain flavonoid aglycones and related phenolic compounds. Nevertheless, the distribution of flavonoids depends on their individual structures, including the presence of sugar groups and other functional groups. Quantitative flavonoid determination and compound-specific analysis would be needed to establish the actual concentrations present in each fraction. The phytochemical screening demonstrates that *Uvaria chamae* leave and stem contains several classes of secondary metabolites that may necessitate further investigation in relation to its reported medicinal uses. The ethyl acetate fraction showed particularly strong qualitative reactions for tannins and phenolic compounds and flavonoids, suggesting that it may be a useful fraction for subsequent phytochemical characterization, this result agrees with the works of Enin, *et al.* (2021). The results obtained in Tables 3.0 and 4.0 demonstrate that the different solvent fractions of *Uvaria chamae* leaves and stem exhibited varying degrees of anti-haemolytic activity against *Echis ocellatus* and *Naja nigricollis* venoms. The observed activities differed according to the solvent fraction, venom

species, and concentration of the extracts, suggesting that the distribution and concentration of potentially active constituents may influence their ability to protect erythrocytes against venom-induced haemolysis (Tong *et al.,* 2026). As presented in (Table 3.0), all four fractions of *Uvaria chamae* exhibited substantial anti-haemolytic activity against *Echis ocellatus* venom, with percentage activities ranging from 22% to 89% across the tested concentrations. At 100 mg/mL, the n-hexane fraction demonstrated the highest anti-haemolytic activity (89%), followed by the aqueous fraction (87%), butanol fraction (86%), and ethyl acetate (EA) fraction (85%). These findings indicate that all the tested fractions exhibited considerable activity at the highest concentration, although the n-hexane fraction produced the highest observed value (Singh, *et al.,* 2017). At 50 mg/mL, the aqueous fraction showed the highest activity (84%), followed by the n-hexane and EA fractions, which both recorded 76%. The butanol fraction exhibited a markedly lower activity of 22%. At 25 mg/mL, the n-hexane and EA fractions maintained relatively high activities of 83% and 76%, respectively, while the aqueous and butanol fractions recorded 55% and 46%, respectively. The relatively high activity of the n-hexane fraction across the three concentrations suggests that its constituents may possess properties that interfere with venom-induced erythrocyte damage. Similarly, the EA fraction demonstrated consistently high activity, with values ranging from 76% to 85%. The aqueous fraction also showed appreciable activity, particularly at 100 and 50 mg/mL. However, the butanol fraction exhibited considerable variation across concentrations, suggesting that its observed activity may be influenced by concentration-dependent effects, experimental variability, or interactions among its constituents. The absence of detectable anti-haemolytic activity in the venom-plus-erythrocyte control, as reported in the table, requires clarification because this control may represent a venom-induced haemolysis reference rather than an untreated negative control. The interpretation of this result depends on the precise experimental design and the method used to calculate percentage anti-haemolysis. Table 4.0 shows that the *Uvaria chamae* leaf fractions also exhibited anti-haemolytic activity against *Naja nigricollis* venom, although the magnitude and concentration-related patterns differed from those observed against *Echis ocellatus* venom. At 100 mg/mL, the aqueous fraction produced the highest activity (75%), followed by the EA fraction (63%) and n-hexane fraction (54%). The butanol fraction exhibited the lowest activity at this concentration (11%). At 50 mg/mL, the EA fraction recorded the highest anti-haemolytic activity (55%), followed by the aqueous fraction (55%), n-hexane fraction (53%), and butanol fraction (47%). At 25 mg/mL, the n-hexane fraction showed the highest activity (52%), followed by the butanol fraction (40%), aqueous fraction (43%), and EA fraction (44%). The values for the aqueous and EA fractions at this concentration were relatively similar, with the aqueous fraction recording 43% and the EA fraction recording 44%. The aqueous fraction demonstrated the highest activity at 100 mg/mL, suggesting that constituents preferentially extracted into water may contribute to the observed protection against *N. nigricollis* venom-induced erythrocyte damage. In contrast, the EA fraction showed a progressive reduction in activity from 63% at 100 mg/mL to 44% at 25 mg/mL. The n-hexane fraction maintained relatively similar activity across the tested concentrations, with values ranging from 52% to 54%. The butanol fraction showed an unusual pattern, recording 11% at 100 mg/mL before increasing to 47% and 40% at 50 and 25 mg/mL, respectively. This non-linear response warrants further investigation and should not be interpreted as evidence of a specific mechanism without additional experiments. The differences in activity among the fractions may reflect variations in their phytochemical composition and the interactions of their constituents with venom components or erythrocyte membranes. However, the present results do not establish the specific compounds responsible for the observed effects or demonstrate direct neutralisation of venom toxins. A comparison of Tables 3.0 and 4.0 indicates that the fractions generally exhibited higher anti-haemolytic activity against *Echis ocellatus* venom than against *Naja nigricollis* venom at the same nominal concentrations. At 100 mg/mL, the activities against *E. ocellatus* ranged from 85% to 89%, whereas those against *N. nigricollis* ranged from 11% to 75%. At 50 mg/mL, the corresponding ranges were 22%-84% and 47%-55%, respectively. At 25 mg/mL, the fractions showed activities ranging from 46% to 83% against *E. ocellatus* and from 40% to 52% against *N. nigricollis*. These differences suggest that the protective effects of the fractions may vary according to the venom tested. Such variation is biologically plausible because snake venoms differ in their composition and relative abundance of haemolytic, cytotoxic, membrane-damaging, and other biologically active components. The venom of *Echis ocellatus*, a viper, and that of *Naja nigricollis*, an elapid, have distinct toxin profiles that may contribute to differences in their effects on erythrocytes and the apparent activities of plant-derived fractions. Nevertheless, the observed differences should be interpreted cautiously. The data alone cannot establish that *U. chamae* is intrinsically more effective against *E. ocellatus* venom. Such a conclusion would require confirmation that the venom concentrations, assay conditions, experimental controls, and calculation methods were comparable between the two venom groups. Statistical analysis would also be necessary to determine whether the observed differences are significant. The findings provide preliminary evidence that the leaf fractions of *Uvaria chamae* possess in vitro anti-haemolytic potential against both *E. ocellatus* and *N. nigricollis* venoms. The n-hexane and EA fractions showed relatively consistent activity against *E. ocellatus*, while the aqueous fraction exhibited the highest activity against *N. nigricollis* at 100 mg/mL. These findings support further investigation of the fractions to identify their active constituents and establish their mechanisms of action. Additional studies should include appropriate haemolysis controls, extract-only controls, concentration-response analysis, and independent confirmation of venom-neutralising activity before therapeutic implications are drawn.

CONCLUSION

The study demonstrated that the methanolic extract and fractions of *Uvaria chamae* leaves and stems contain various classes of phytochemicals, including steroids, triterpenes, alkaloids, tannins, phenolic compounds, anthraquinones, and flavonoids. These bioactive constituents may contribute to the protective effects observed in the in vitro anti-haemolytic assay, which revealed varying degrees of activity against erythrocyte haemolysis induced by *Echis ocellatus* and *Naja nigricollis* venoms. These findings highlight the potential of *U. chamae* leave and stem fractions as a source of phytochemicals with protective effects against snake venom-induced erythrocyte damage.

RECOMMENDATIONS

Based on the findings of this study, the following recommendations are proposed:

i. 1.Further studies should be conducted to isolate, purify, and characterise the bioactive compounds present in the different fractions of *Uvaria chamae* leaves and stem using analytical techniques such as gas chromatography–mass spectrometry (GC-MS), liquid chromatography–mass spectrometry (LC-MS), and nuclear magnetic resonance (NMR) spectroscopy, as appropriate.
ii. Anti-haemolytic effects of the fractions should be investigated using validated assays, appropriate controls, and clearly defined calculations to establish their actual capacity to protect erythrocytes against venom-induced haemolysis.
iii. Evaluation of venom-neutralising potential: Further in vitro studies should investigate whether the fractions can inhibit other relevant toxic activities of *Echis ocellatus* and *Naja nigricollis* venoms, including proteolytic, phospholipase A_2, and haemorrhagic activities, using appropriate validated methods.
iv. Where justified by the results of additional in vitro studies, appropriately designed in vivo investigations should be considered to evaluate efficacy and safety, subject to institutional ethical approval and applicable animal welfare guidelines.
v. Further research should examine the mechanisms through which the fractions may protect erythrocytes against venom-induced haemolysis and determine whether the observed effects are associated with particular phytochemical constituents.
vi. The findings should be regarded as preliminary evidence for further research rather than proof of clinical efficacy. Any potential application of *Uvaria chamae* in snakebite management should be established through rigorous pharmacological, toxicological, and clinical investigations. The plant fractions should not be used as substitutes for established antivenom treatment.

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