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QUANTITATIVE PHYTOCHEMICAL PROFILING AND ANTIVENOM ACTIVITY OF *Solanecio mannii* AGAINST *Bitis arietans* VENOM

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ABSTRACT

Snakebite envenomation remains a major public health challenge, particularly in low- and middle-income countries where access to effective and affordable antivenoms is constrained by cost, scarcity, stringent storage requirements, and hypersensitivity reactions. Consequently, medicinal plants are increasingly explored as sources of novel antivenom agents. This study investigated phytochemical composition and *in vivo* antivenom activity of the ethanolic leaf extract of *S. mannii* against *B. arietans* venom. Fresh leaves of *S. mannii* were collected, authenticated, shade-dried, pulverized and extracted using absolute ethanol. Phytochemicals were characterized using gas chromatography–mass spectrometry (GC–MS). Antivenom activity was evaluated in female Swiss albino mice by determining the median lethal dose (LD_{50}) of *B. arietans* venom and the median effective dose (ED_{50}) of the extract using a pre-incubation neutralization assay. Data were analyzed using probit regression at $p < 0.05$. GC–MS identified fourteen phytochemicals, predominantly 2-methoxy-4-(prop-2-en-1-yl) phenol (16.8% peak area), together with phenolics, flavonoids, terpenoids, benzopyrones, aldehydes, and diterpene alcohols. The venom LD_{50} was estimated at 175 mg/kg, while extract treatment significantly increased survival from 20% at 200 ppm to 80% at 600–1000 ppm, with an ED_{50} of 452.40 ppm ($p = 0.024$). Identified phytochemical classes, particularly phenolics, flavonoids, and terpenoids, may have contributed to venom neutralization through their collective biological activities, providing a plausible basis for the observed concentration-dependent protection. Further studies should isolate the bioactive constituents, elucidate their mechanisms of venom neutralization, establish safety, efficacy, and pharmacological potential through comprehensive preclinical trials.

Keywords: Antivenom activity, *B. arietans*, GC–MS, Phytochemical profiling, Snakebite envenomation, *S. mannii*.

1 INTRODUCTION

Snakebite envenomation (SBE) remains a major but neglected public health problem, particularly among rural and agricultural populations in low- and middle-income countries [1]. Globally, approximately 4.5–5.4 million people are bitten by snakes annually, of whom an estimated 1.8–2.7 million develop clinical envenomation and 81,000–138,000 die from snakebite-related complications [2]. In addition, more than 400,000 survivors may experience permanent

physical disabilities, including limb amputation and other chronic sequelae [3]. These consequences can substantially affect quality of life and socioeconomic productivity, particularly among populations dependent on agricultural activities. The pathological manifestations of snakebite are largely determined by the biochemical complexity of snake venoms, which are complex mixtures of proteins, peptides, enzymes, and other bioactive molecules that exert diverse toxic effects [3]. Major venom components include snake venom metalloproteinases (SVMPs), phospholipase A_2, serine proteinases, L-amino acid oxidases, hyaluronidases, disintegrins, C-type lectins, and phosphodiesterases. These toxins can act through multiple mechanisms to disrupt haemostasis, damage blood vessels and extracellular matrix components, and induce local and systemic tissue injury [3, 4]. Consequently, envenomation may result in haemorrhage, coagulopathy, oedema, myonecrosis, neurotoxicity, cardiovascular dysfunction, acute kidney injury, and extensive tissue destruction [3]. Among African venomous snakes, *Bitis arietans* (puff adder) is of considerable medical importance because of its wide distribution and the severity of the local and systemic effects associated with its venom. Viperid venoms containing haemorrhagic metalloproteinases can cause disruption of the microvasculature through degradation of components of the vascular basement membrane, resulting in blood extravasation and tissue damage [4]. The venom of *B. arietans* is also associated with haemorrhagic and tissue-damaging effects, making envenomation by this species an important clinical concern in sub-Saharan Africa [3]. Immunoglobulin-based antivenom remains the principal specific treatment for snakebite envenomation. Antivenoms are produced from immunoglobulins or immunoglobulin fragments obtained from animals hyperimmunized with snake venoms [5]. Despite their established therapeutic value, conventional antivenoms face challenges related to accessibility, affordability, storage requirements, geographical variation in venom composition, and the breadth of toxin recognition [5]. Furthermore, antivenoms may have limited capacity to reverse local tissue damage once venom-induced pathological processes have become established. These limitations have stimulated research into complementary therapeutic approaches, including natural and synthetic toxin inhibitors that could be used alongside conventional antivenom therapy [6]. Medicinal plants represent an important source of bioactive compounds that may have applications in snakebite management. Traditional medical systems have long employed plant preparations for snakebite treatment, and numerous medicinal plants and their constituents have been investigated for potential antivenom properties [7]. Experimental studies have demonstrated that certain plant-derived compounds can inhibit venom-associated enzymes and pathological activities. For example, flavonoids isolated from *Schizolobium parahyba* inhibited haemorrhagic and fibrinogenolytic activities of Bothrops venoms and isolated venom metalloproteinases [8]. Such findings support continued investigation of plant-derived compounds as potential complementary agents in snakebite therapy [6]. *Solanecio mannii* (Asteraceae) is an East African medicinal plant associated with traditional medicinal applications. Previous phytochemical investigations have demonstrated that the species contains diverse secondary metabolites. GC–MS analysis of hydrodistilled leaf essential oil of *S. mannii* identified volatile constituents including 3-carene, limonene, 1-undecanol, β-pinene, menthol, β-cymene, longifolene, and α-terpinolene [9]. Recent studies have also investigated the chemical composition and biological activities of *S. mannii* extracts and essential oils [10, 11]. Despite these findings and the reported medicinal importance of *S. mannii*, there is insufficient experimental evidence regarding the phytochemical composition of its ethanolic leaf extract and its *in vivo* activity against *B. arietans* venom. Specifically, the relationship between the phytochemical constituents present in the extract and its capacity to attenuate venom-induced lethality remains inadequately established. This knowledge gap limits the scientific evaluation of *S. mannii* as a potential source of bioactive compounds for snakebite management. Therefore, the present study aimed to systematically characterize the quantitative phytochemical profile of the ethanolic leaf extract of *S. mannii* using gas chromatography–mass spectrometry (GC–MS) and to evaluate its *in vivo* antivenom efficacy against *B. arietans* venom in female Swiss albino mice.

2 MATERIALS AND METHODS

2.1 Study Site

The study was conducted in Marani, Kisii County, Kenya where the plant leaf materials were collected from. Kisii County is located in the southwestern highlands of Kenya at approximately 0.746° S and 34.779° E. Marani is situated within Kisii Central Sub-County, a predominantly rural and agricultural area characterized by fertile soils, high rainfall, and high population density. Kisii County is among the most densely populated counties in Kenya, with an average population density of over 1,000 people per square kilometer [12].

2.2 Study Design

In determining the snake-antivenom activity of the ethanolic leaf extract of *S. mannii*, a Completely Randomized Design (CRD) was adopted, comprising six venom-dose treatments with five replicates per treatment for LD_{50} determination and seven treatment groups with five replicates per group for venom-neutralization assessment.

2.3 Collection and Extraction of Plant Material

Leaves of *S. mannii* were collected from their natural habitat from Marani, with the assistance of a local herbalist who facilitated identification using the plant's local name. The collected plant material was transported to the Chuka University Biochemistry and Biomedical Sciences and Technology Laboratory for processing. The leaves were shade-dried for 21 days away from direct sunlight to minimize degradation of thermolabile and photosensitive constituents.

The dried leaves were pulverized into a fine powder using a heavy-duty blender. 600 g of the powdered leaf material was macerated in 5,000 mL of absolute ethanol and maintained in a water bath at 60°C for 24 h to facilitate extraction of the bioactive constituents. The extract was initially filtered through a muslin cloth and subsequently re-filtered using Whatman No. 1 filter paper to remove particulate matter. The filtrate was concentrated under reduced pressure using a rotary evaporator to remove the excess solvent. A total of 20.5 g of concentrated ethanolic leaf extract was obtained and stored at 4°C until further phytochemical analysis and snake-antivenom evaluation.

2.4 Venom and Experimental Animals

Lyophilized *B. arietans* venom was obtained from Bio-Ken Snake Farm, Kenya. The venom was reconstituted in sterile normal saline and stored at 4°C until use. A total of 65 female Swiss albino mice were obtained from the Department of Biological Sciences, Jomo Kenyatta University of Agriculture and Technology (JKUAT), and used as experimental animals. The mice were acclimatized to the laboratory environment for seven days prior to experimentation.

Throughout the experimental period, the animals were maintained under standard laboratory conditions, with free access to standard pellet diet and clean drinking water *ad libitum*. The animals were maintained under a 12 h light/12 h dark photoperiod, an ambient temperature of 22–25°C, and relative humidity of 50–60%. These conditions were maintained to minimize environmental stress and ensure consistency of the experimental responses. All procedures involving experimental animals were conducted in accordance with established ethical guidelines for the care and use of laboratory animals.

2.5 Phytochemical Analysis Using Gas Chromatography–Mass Spectrometry

The phytochemical composition of the ethanolic leaf extract of *S. mannii* was determined using the method described by Ouandaogo *et al*. [13], with modifications where necessary. A portion of the extract was transferred into a clean reaction vial and evaporated to dryness to remove the residual solvent and moisture. The dried residue was then treated with excess N, O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), tightly capped, and heated at 70°C for 30 min to derivatize compounds containing hydroxyl, carboxyl, and amino functional groups. The derivatized sample was cooled to room temperature and transferred into a clean autosampler vial for GC-MS analysis.

The derivatized sample (1.0 µL) was injected into the GC-MS in split mode at a 15:1 split ratio. Separation was performed using an Rtx-5MS fused-silica capillary column (30 m × 0.25 mm × 0.25 µm), with helium as the carrier gas at a constant linear velocity of 36.8 cm/s. The injector temperature was maintained at 250°C. The oven temperature was initially set at 60°C for 2 min, increased at 10°C/min to 200°C, and subsequently increased at 5°C/min to 280°C, where it was held for 5 min, giving a total run time of 31 min.

Following chromatographic separation, the eluting compounds were transferred to the mass spectrometer through the heated interface maintained at 250°C. Compounds were ionized by electron ionization at 70 eV, and the resulting ions were analyzed over a mass-to-charge range of m/z 45–600 in full-scan mode. The mass spectra generated for the separated compounds were recorded and compared with reference spectra in established mass spectral libraries for compound identification. The resulting chromatographic peaks and corresponding mass spectra were used to establish the phytochemical profile of the ethanolic *S. mannii* leaf extract.

2.6 *In vivo* Determination of Snake Antivenom Activity

Snake-antivenom activity of the ethanolic leaf extract of *S. mannii* was evaluated using a modified method of Theakston and Reid [14], with reference to Premendran *et al*. [15], Rajesh *et al*. [16], Adrião *et al*. [17], and WHO recommendations [18]. The assay was conducted in two phases.

2.7 Determination of LD_{50}

Thirty mice were randomly allocated into six groups of five animals each and administered *B. arietans* venom at 50, 100, 150, 200, 250 and 300 mg/kg body weight, respectively. Animals were observed for clinical signs of envenomation and mortality during the observation period. The number of deaths in each group was recorded and used to estimate the median lethal dose (LD_{50}) of the venom by probit analysis.

2.8 Venom-neutralization assay

The antivenom activity of the extract was evaluated using a pre-incubation model. Thirty-five mice were randomly assigned to seven groups of five animals each. Group F served as the normal control and received 0.2 mL of 0.9% normal saline, while Group G served as the venom control and received $2LD_{50}$ of *B. arietans* venom. Groups H–L received 200, 400, 600, 800 and 1000 ppm of the ethanolic *S. mannii* leaf extract, respectively, each pre-incubated with $2LD_{50}$ of venom for 30 min before administration. The venom–extract mixtures were administered intraperitoneally, and the animals were monitored for clinical signs and mortality. Mortality and survival were recorded for each group, and the protective effect of the extract was evaluated relative to the venom control. The neutralizing activity was assessed using probit analysis.

2.9 Statistical Analysis

Data obtained from all experiments were recorded, entered into Microsoft Excel for cleaning and systematic organization, and subsequently exported to Minitab Statistical Software for analysis. Mortality and survival responses obtained from the *B. arietans* venom toxicity and venom-neutralization experiments were summarized as frequencies and percentages. Probit regression analysis was used to establish the dose–response relationship and estimate the median lethal dose (LD_{50}) of *B. arietans* venom and the median effective dose (ED_{50}) of the ethanolic leaf extract of *S. mannii*, with estimates presented together with their 95% fiducial confidence limits.

3 RESULTS

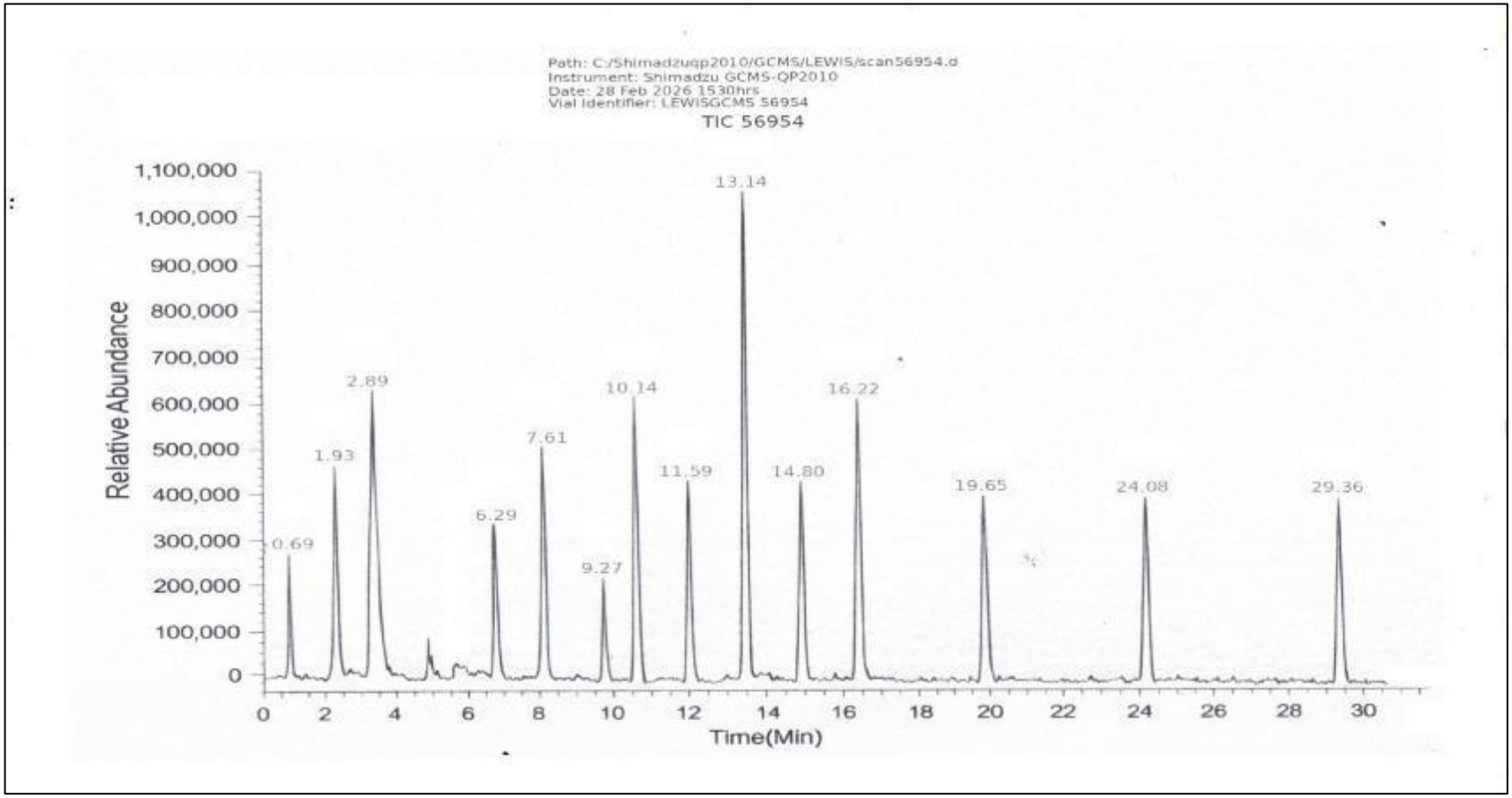
3.1 GC–MS Phytochemical Profile of the Ethanolic Leaf Extract of *S. mannii*

GC–MS analysis of the ethanolic leaf extract of *S. mannii* identified 14 phytochemical constituents, with retention times ranging from 0.69 to 29.36 min (Table 1). Compound identification was based on retention time and mass spectral fragmentation patterns, with spectral matching against The National Institute of Standards and Technology (NIST) libraries.

The most abundant constituent was 2-methoxy-4-(prop-2-en-1-yl) phenol (16.8%; 13.14 min), followed by (2E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol (8.8%; 24.08 min), (1R,4E,9S)-4,11,11-trimethyl-8-methylidenebicyclo [7.2.0] undec-4-ene (8.3%; 16.22 min), 1-methyl-4-(prop-1-en-2-yl) cyclohex-1-ene (7.8%; 7.61 min), 3,7-dimethylocta-1,6-dien-3-ol (7.5%; 10.14 min), and 4,12,12-trimethyl-9-methylidene-5-oxatricyclo[$8.2.0.0^{4,6}$]dodecane (7.0%; 19.65 min). Hexanal was the least abundant constituent (3.4%). The identified compounds represented diverse chemical classes, including phenylpropanoids, monoterpenes, oxygenated monoterpenes, sesquiterpenes, oxygenated sesquiterpenes, diterpene alcohols, flavonoids, benzopyrones, and aldehydes. The GC–MS chromatogram is presented in Figure 1.

Table 1: Phytochemical Compounds Identified in the Ethanolic Leaf Extract of *S. mannii*

Retention time (min)	IUPAC Name	Molecular formula	Molecular weight (g/mol)	Peak area (%)	Classification
0.69	Hexanal	$C_6H_{12}O$	100.16	3.4	Aldehyde
1.93	2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	$C_{10}H_{16}$	136.24	5.5	Monoterpene hydrocarbon
2.89	1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane	$C_{10}H_{18}O$	154.25	6.3	Oxygenated monoterpene
6.29	1-Methyl-4-(propan-2-yl)benzene	$C_{10}H_{14}$	134.22	5.1	Monoterpene aromatic hydrocarbon
7.61	1-Methyl-4-(prop-1-en-2-yl)cyclohex-1-ene	$C_{10}H_{16}$	136.24	7.8	Monoterpene hydrocarbon
9.27	2H-Chromen-2-one	$C_9H_6O_2$	146.14	6.0	Benzopyrone
10.14	3,7-Dimethylocta-1,6-dien-3-ol	$C_{10}H_{18}O$	154.25	7.5	Oxygenated monoterpene alcohol
11.59	5,7-Dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydrochromen-4-one	$C_{15}H_{12}O_5$	272.25	6.2	Flavonoid
13.14	2-Methoxy-4-(prop-2-en-1-yl)phenol	$C_{10}H_{12}O_2$	164.20	16.8	Phenylpropanoid
14.80	(E)-3-Phenylprop-2-enal	C_9H_8O	132.16	5.9	Phenylpropanoid aldehyde
16.22	(1R,4E,9S)-4,11,11-Trimethyl-8-methylidenebicyclo [7.2.0] undec-4-ene	$C_{15}H_{24}$	204.35	8.3	Sesquiterpene hydrocarbon
19.65	4,12,12-Trimethyl-9-methylidene-5-oxatricyclo[8.2.0.0^{4},^{6}]dodecane	$C_{15}H_{24}O$	220.35	7.0	Oxygenated sesquiterpene
24.08	(2E,7R,11R)-3,7,11,15-Tetramethylhexadec-2-en-1-ol	$C_{20}H_{40}O$	296.53	8.8	Diterpene alcohol
29.36	2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one	$C_{15}H_{10}O_7$	302.24	5.4	Flavonoid



Legend: The chromatogram shows the gas chromatography–mass spectrometry profile of *S. mannii* ethanolic leaf extract. The x-axis represents retention time in minutes, while the y-axis represents relative abundance. Each labelled peak corresponds to a phytochemical compound detected in the extract at a specific retention time, with the most abundant peak observed at 13.14 minutes.

Figure 1: Ethanolic Leaf Extract of *S. mannii* GC-MS Chromatogram

3.2 Determination of the Median Lethal Dose (LD_{50}) of *B. arietans* Venom

Administration of graded doses of *B. arietans* venom (50–300 mg/kg body weight) to female Swiss albino mice resulted in a progressive, dose-dependent increase in mortality (Table 2). No mortality was recorded at 50 mg/kg, whereas mortality increased to 20%, 40%, 60%, and 80% following administration of 100, 150, 200, and 250 mg/kg body weight, respectively. Complete mortality (100%) was observed at 300 mg/kg body weight. The observed clinical signs of envenomation included weakness, respiratory distress, tremors, and bleeding, with the severity of these manifestations increasing with venom dose.

Table 2: Mortality response of mice following administration of graded doses of *B. arietans* venom

Group	Dose (mg/kg) weight)	Number of mice	Number of Mice dead	Percentage mortality (%)
N	50	5	0	0
O	100	5	1	20
P	150	5	2	40
Q	200	5	3	60
R	250	5	4	80
S	300	5	5	100

Probit regression analysis demonstrated a positive dose–response relationship between *B. arietans* venom dose and mortality. The median lethal dose (LD_{50}) was estimated at 175 mg/kg body weight, with 95% fiducial confidence limits of 126.491–223.509 mg/kg body weight (Figure 2). The progressive increase in mortality across the tested venom doses confirmed the suitability of the dose range for subsequent evaluation of the venom-neutralizing activity of the plant extract.

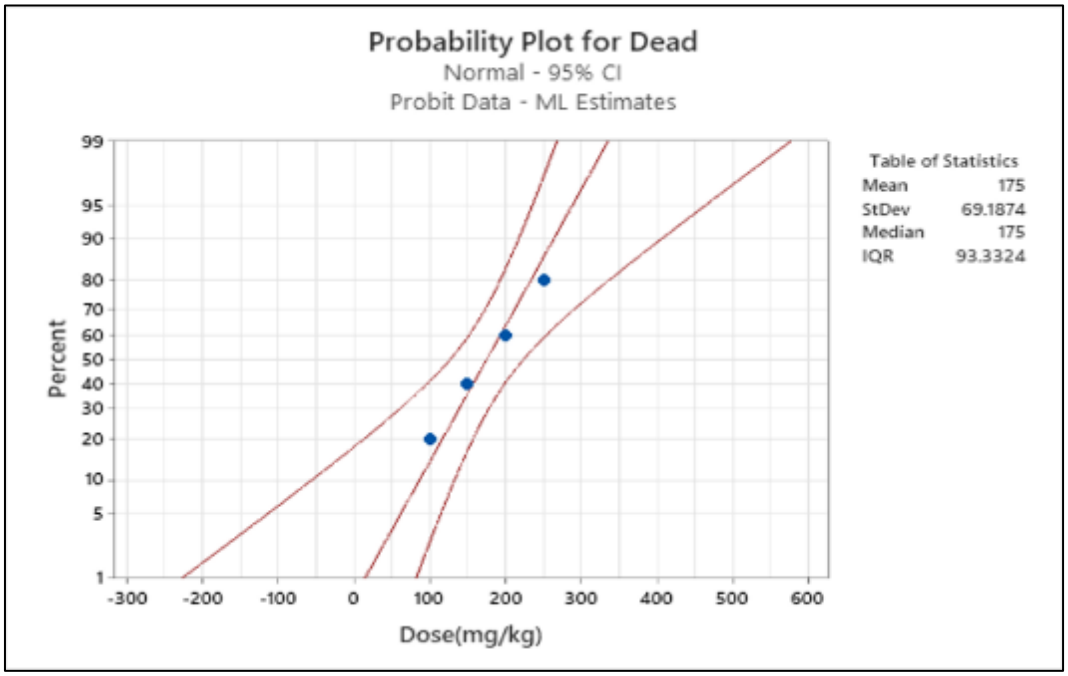


Figure 2: Dose-response probit plot for determination of LD_{50} of *B. arietans* venom

Legend: The blue diamonds represent the observed percentage mortality of mice at each tested dose of *B. arietans* venom. The central red line represents the fitted probit regression line used to estimate the median lethal dose. The two outer red lines represent the 95% confidence limits around the fitted regression model. The plot shows a dose-dependent increase in mortality, with the median lethal dose estimated at 175 mg/kg body weight.

3.3 *In vivo* Venom Neutralization by the Ethanolic Leaf Extract of *S. mannii*

The venom-neutralizing activity of the ethanolic leaf extract of *S. mannii* was evaluated against a fixed challenge dose of 350 mg/kg body weight of *B. arietans* venom. The normal saline control group recorded complete survival (100%), whereas the venom control group recorded complete mortality (0% survival). Pre-incubation of the venom with the plant extract resulted in an improvement in survival across the tested extract concentrations (Table 3).

Survival increased from 20% at 200 ppm to 60% at 400 ppm, while treatment with 600, 800, and 1000 ppm resulted in 80% survival in each group. The observed increase in survival following treatment with the extract indicates a protective effect against *B. arietans* venom under the experimental conditions. Clinical manifestations, including weakness, respiratory distress, tremors, and bleeding, were observed among the challenged animals; however, their apparent severity decreased with increasing extract concentration.

Table 3: Survival response of mice treated with ethanolic leaf extract pre-incubated with 350mg/kg of *B. arietans* venom

Group	Treatment	Number of mice	Number alive	Percentage survival (%)
F	0.2 mL normal saline	5	5	100
G	350 mg/kg venom	5	0	0
H	200 ppm + 350 mg/kg venom	5	1	20
I	400 ppm + 350 mg/kg venom	5	3	60
J	600 ppm + 350 mg/kg venom	5	4	80
K	800 ppm + 350 mg/kg venom	5	4	80
L	1000 ppm + 350 mg/kg venom	5	4	80

The median effective dose (ED_{50}) of the ethanolic leaf extract was subsequently estimated using probit regression analysis based on the relationship between extract concentration and survival. The fitted model yielded an ED_{50} of 2.65552 on the logarithmic concentration scale, corresponding to 452.40 ppm after back-transformation to the original concentration scale. The estimated 95% fiducial confidence limits were 97.26–852.92 ppm (Figure 3).

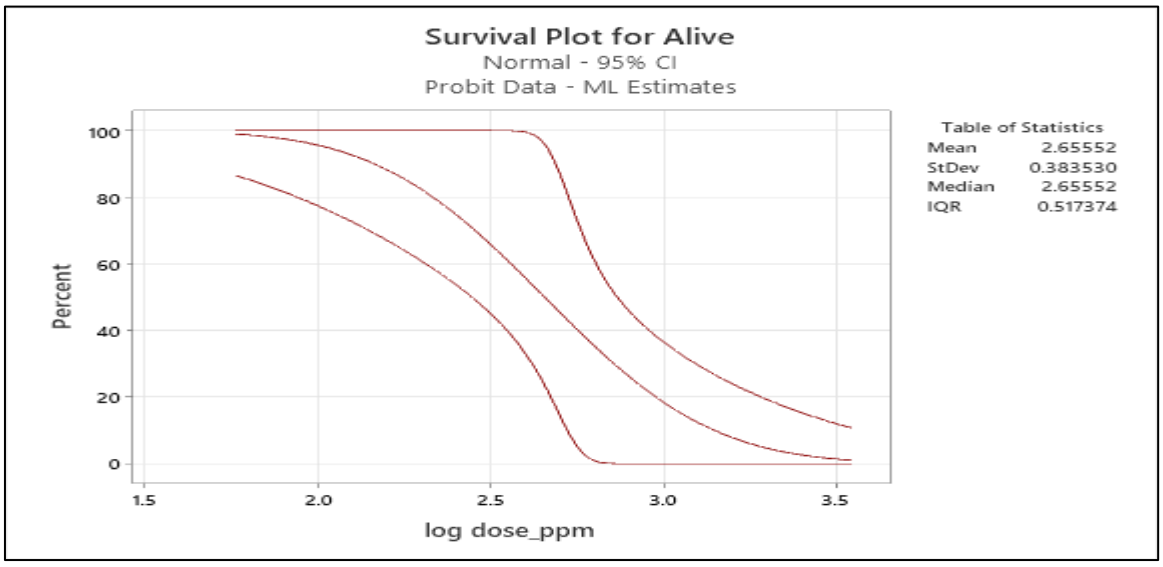


Figure 3: Probit plot for estimation of the median effective dose (ED_{50}) of the ethanolic leaf extract against *B. arietans* venom using log-transformed extract concentrations

Legend: The central red curve represents the fitted probit regression model describing the relationship between the log transformed concentration of *S. mannii* extract and survival among mice exposed to *B. arietans* venom. The two outer red curves indicate the 95% confidence intervals for the fitted model. The x-axis represents the log transformed extract concentration in parts per million, while the y-axis represents the percentage of surviving mice.

4 DISCUSSION

The present study demonstrated that the ethanolic leaf extract of *S. mannii* contains a diverse range of phytochemical constituents and exhibits *in vivo* protective activity against *B. arietans* venom in mice. The most important finding was the concentration-related increase in survival following pre-incubation of venom with the extract, with survival increasing from 20% at 200 ppm to 80% at 600 ppm and remaining at 80% at 800 and 1000 ppm. In addition, the estimated ED_{50} of 452.40 ppm indicates that the extract was capable of providing measurable protection against a venom challenge equivalent to twice the experimentally determined LD_{50}. These findings suggest that constituents present in *S. mannii* may interfere with one or more biological activities of *B. arietans* venom.

The GC–MS analysis identified fourteen constituents representing several chemical classes, including phenylpropanoids, monoterpenes, sesquiterpenes, oxygenated terpenoids, diterpene alcohols, flavonoids, benzopyrones and aldehydes. The predominance of 2-methoxy-4-(prop-2-en-1-yl) phenol, which accounted for 16.8% of the total peak area, indicates that phenolic constituents represented an important component of the extract. This finding is relevant because phenolic phytochemicals have been associated with antioxidant and anti-inflammatory activities. Forni *et al*. [19] demonstrated that plant-derived phytochemicals can modulate oxidative stress through free-radical scavenging and regulation of cellular antioxidant mechanisms. However, the present study did not directly evaluate oxidative stress or establish the activity of the individual phenolic constituent; consequently, its contribution to venom neutralization cannot be confirmed from the current data alone.

The phytochemical diversity observed in the present investigation is consistent with previous chemical investigations of *S. mannii*. Njuguna [9] identified several volatile constituents in the essential oil of *S. mannii*, while Kiragu *et al*. [11] reported diverse compounds in the essential oil and hydrosol of the species. Elbasyouni *et al*. [10] also reported a diverse metabolomic profile in *S. mannii* root extracts. Although differences in plant part, geographical origin and extraction method make direct comparison of compound abundance inappropriate, these studies collectively support the presence of chemically diverse secondary metabolites in the species. The occurrence of such metabolites in the ethanolic leaf extract provides a reasonable basis for investigating its pharmacological activity.

Of particular relevance to the antivenom activity is the occurrence of flavonoid-related constituents. Flavonoids have been investigated as potential inhibitors of snake venom enzymes because of their ability to interact with proteins and, in some cases, interfere with enzymatic activity. Vale *et al*. [8] reported protective effects of flavonoids from *Schizolobium parahyba* against snake venoms and isolated toxins. Similarly, Shivashankar *et al*. [20] demonstrated inhibition of phospholipase A_2 from *Daboia russelii* venom by phytochemicals from *Andrographis paniculata*. These findings provide a plausible basis for considering flavonoid and other polyphenolic constituents among the compounds that may contribute to the activity observed with *S. mannii*. Nevertheless, because the present study did not perform direct venom-enzyme inhibition assays, the specific mechanism by which the extract produced protection remains to be established.

The determination of the LD_{50} demonstrated a clear dose-dependent increase in mortality following administration of *B. arietans* venom. Mortality increased from 0% at 50 mg/kg to 100% at 300 mg/kg, giving an estimated LD_{50} of 175 mg/kg body weight. The progressive mortality response confirms the biological activity of the venom preparation and provided an experimental basis for selecting 350 mg/kg, equivalent to $2LD_{50}$, as the challenge dose for the neutralization assay. The associated clinical signs, particularly bleeding and progressive weakness, are consistent with the known toxic effects of viperid venoms. Gutiérrez *et al*. [3] described snake venoms as complex mixtures of toxins capable of producing haemorrhage, coagulopathy, inflammation, tissue destruction and systemic toxicity. In particular, snake venom metalloproteinases are important mediators of haemorrhage because they degrade components of the vascular basement membrane and extracellular matrix [4].

The most significant biological finding was the ability of the *S. mannii* extract to increase survival following exposure to the 350 mg/kg venom challenge. The venom-control group recorded complete mortality, whereas pre-incubation with the extract resulted in survival of 20%, 60% and 80% at 200, 400 and 600 ppm, respectively. Survival remained at 80% at 800 and 1000 ppm. The progressive increase in survival over the lower concentration range indicates a concentration-related protective effect of the extract. This observation is consistent with the broader evidence that selected plant-derived compounds can inhibit venom toxins and thereby complement conventional antivenom therapy. Gutiérrez *et al*. [6] reviewed natural venom inhibitors and demonstrated that several plant-derived compounds have the capacity to inhibit important venom enzymes and toxins.

The increase in protection with increasing extract concentration may reflect increased availability of active constituents capable of interacting with venom components before administration. Such interactions may reduce the activity of enzymatic toxins or interfere with toxin–target interactions. This interpretation is supported by experimental evidence showing that certain plant-derived flavonoids and other phytochemicals can inhibit venom-associated enzymes such as

phospholipase A_2 and metalloproteinases [8, 20]. However, because the present investigation assessed whole-extract protection rather than individual biochemical targets, the observed survival cannot be attributed to any particular compound or venom enzyme.

The absence of a further increase in survival above 600 ppm is also noteworthy. The 80% survival observed at 600, 800 and 1000 ppm suggests that increasing the extract concentration beyond 600 ppm did not result in additional protection under the conditions of the experiment. One possible explanation is that the extract approached its maximum protective capacity against the selected venom challenge at approximately 600 ppm. Another possibility is that some venom components remained unaffected by the constituents present in the crude extract. Snake venom contains multiple toxins with different molecular targets, and inhibition of some components does not necessarily result in complete neutralization of the venom [3]. The observed plateau therefore suggests that the protective effect of the crude extract may be selective rather than complete.

The estimated ED_{50} of 452.40 ppm provides further quantitative evidence of the protective activity of the extract. Under the experimental conditions, this concentration represents the estimated extract concentration associated with 50% survival following the venom challenge. The 95% fiducial limits of 97.26–852.92 ppm indicate considerable uncertainty around the estimate. This relatively broad interval is expected to some extent from a small-group survival experiment in which the response is measured in discrete proportions. Therefore, although the ED_{50} provides useful evidence of extract potency, it should be regarded as a preliminary estimate rather than a definitive therapeutic concentration.

The venom pre-incubation design is important when interpreting these findings. By allowing the extract and venom to interact before administration, the experiment primarily assesses the capacity of the extract constituents to directly interfere with venom activity. This approach is useful for preliminary identification of venom-neutralizing substances, but it does not reproduce the clinical sequence of snakebite, in which venom is injected before treatment is administered. Consequently, the present findings demonstrate venom-neutralizing potential rather than established post-envenomation therapeutic efficacy. Further experiments using treatment administered after venom injection would be necessary to determine whether the extract can inhibit or reverse established systemic and local effects of *B. arietans* venom.

The observed activity is therefore most appropriately interpreted as a property of the chemically complex extract rather than as evidence for a single active compound. The combination of phenolic, flavonoid and terpenoid constituents may produce complementary biological effects, although synergistic activity was not directly demonstrated in the present study. Gupta and Peshin [7] highlighted the potential of medicinal plant preparations to act against snake envenoming through multiple pharmacological mechanisms. In the present case, however, establishing such interactions will require fractionation of the crude extract followed by comparative venom-neutralization and enzyme-inhibition assays.

5 CONCLUSIONS

The findings of this study provide preliminary scientific support for the ethnomedicinal use of *S. mannii* in the management of snakebite-related toxicity. The observed protective effect of the extract in venom-exposed mice suggests that the plant may contain bioactive constituents with antivenom-related activity under the experimental conditions used. However, these findings should be interpreted as preliminary and should not be taken to suggest that *S. mannii* can replace conventional antivenom therapy, which remains the standard treatment for systemic snakebite envenomation

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Statement of ethical approval

All animal experiments were conducted in accordance with established ethical guidelines for the care and use of laboratory animals. Ethical clearance for the study was obtained from the Chuka University Institutional Ethics Review Committee under approval number NACOSTI/NBC/AC-0812. In addition, a research permit was obtained from the National Commission for Science, Technology and Innovation (NACOSTI) under license number NACOSTI/P/26/4185223 These approvals ensured that the study complied with the required ethical and regulatory standards.

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