Toxicon xxx (xxxx) 107035

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Pre-clinical efficacy of African medicinal plants used in the treatment of snakebite envenoming: A systematic review

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ARTICLE INFO

Handling Editor: Ray Norton

Keywords:
African medicinal plants
Snakebite
Venom
Systematic review
Envenoming

ABSTRACT

The World Health Organization has listed Snakebite Envenoming (SBE) as a priority neglected tropical disease, with a worldwide annual snakebite affecting 5.4 million people and injuring 2.7 million lives. In many parts of rural areas of Africa and Asia, medicinal plants have been used as alternatives to conventional antisnake venom (ASV) due in part to inaccessibility to hospitals. Systemic reviews (SR) of laboratory-based preclinical studies play an essential role in drug discovery. We conducted an SR to evaluate the relationship between interventional medicinal plants and their observed effects on venom-induced experiments. This SR was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses. The Modified collaborative approach to meta-analysis and review of animal data from experimental studies (CAMARADES) and SYRCLE's risk of bias tools were used to appraise the included studies. Data were searched online in Medline via PubMed, Embase via OVID, and Scopus. Studies reporting *in vivo* and *in vitro* pharmacological activities of African medicinal plants/extracts/constituents against venom-induced pathologies were identified and included for screening. Data from the included studies were extracted and synthesized. Ten studies reported statistically significant percentage protection (40–100%) of animals against venom-induced lethality compared with control groups that received no medicinal plant intervention. Sixteen studies reported significant effects ($p \leq 0.05$) against venom-induced pathologies compared with the control group; these include hemolytic, histopathologic, necrotic, and anti-enzymatic effects. The plant family Fabaceae has the highest number of studies reporting its efficacy, followed by

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https://doi.org/10.1016/j.toxicon.2023.107035
Received 30 October 2022; Received in revised form 19 January 2023; Accepted 19 January 2023
0041-0101/

Annonaceae, Malvaceae, Combretaceae, Sterculiaceae, and Olacaceae. Some African medicinal plants are preclinically effective against venom-induced lethality, hematotoxicity, and cytotoxicity. The evidence was extracted from three *in vitro* studies, nine *in vivo* studies, and five studies that combined both *in vivo* and *in vitro* models. The effective plants belong to the Fabaceae family, followed by Malvaceae, and Annonaceae.

1. Introduction

The World Health Organization (WHO) has listed Snakebite Envenoming (SBE) as a priority neglected tropical disease, with a worldwide annual snakebite affecting 4.5 million people and injuring 2.7 million lives (WHO, 2019). Snakebite incidence is grossly underreported in Sub-Saharan Africa because case-fatality alone is about 3557–5450 in West Africa. However, more than a million people are victims of snakebites, with estimates of 7000–20,000 deaths annually (WHO, 2022). The SBE burden has reached a disturbing level, particularly in Asia and sub-Saharan Africa, primarily due to poor access to appropriate treatment and scarcity of antisnake venoms (Habib and Brown, 2018; World Health Organization, 2017). SBE cases are attributed to three families of venomous snakes belonging to the front-fanged snakes, including Viperidae, Elapidae, and Actraspididae. In sub-Saharan Africa, SBE causes the loss of approximately 1.03 million Disability Adjusted Life Years (DALYs) (Yusuf et al., 2015; Habib et al., 2015; Halilu et al., 2019). Antisnake Venom (ASV) remains the only approved cornerstone of treatment for SBE. However, scarcity of ASV, high cost of ASV, and poor access to healthcare services in most African and Asian settings often result in poor treatment outcomes with substantial morbidity and mortality.Therefore, some SBE victims seek alternative care from traditional snake charmers who mainly utilize medicinal plants to treat victims (Ameen et al., 2015; World Health Organization, 2017).

Medicinal plants have been used to treat many diseases in Africa and around the globe. They have also been the most productive sources for developing efficacious and safe drugs in orthodox medicine today. Herbal treatments using medicinal plants are the most popular form of traditional medicine (World Health Organization WHO, 2013). According to WHO, 80% of people worldwide rely on herbal medicines for some aspect of their primary health care needs. Further to this, around 21,000 plant species have the potential to be used as medicinal plants. Owing to this prospect, the efficacy and safety of medicinal plants have gone through rigorous scientific methods for developing conventional drugs (Tor-Anyiin et al., 2013).

In many parts of rural areas of Africa and Asia, medicinal plants have been used as alternatives to ASV due in part to inaccessibility to hospitals and poor storage facilities for ASV (Gomes et al., 2010). The pharmacological activities of many herbs used traditionally in the treatment of SBE have been validated, with some undergoing preclinical scientific validation (Abubakar et al., 2000; Alam and Gomes, 2003). In addition, crude extracts, fractions, and compounds with pharmacological activities against snake venom-induced pathologies and lethality factors have been isolated from plants originating in Africa and Asia (Haruna and Choudhury, 1995; Mors et al., 2000; Abubakar et al., 2006; Ameen et al., 2015). The availability of medicinal plant collections worldwide, particularly in Africa, might provide an exciting opportunity for toxinologists and researchers to search for compounds from natural sources with inhibitory activity on snake venom toxins. According to the WHO's SBE Working Group roadmap document, the death and disability burden caused by SBE is targeted to be reduced by half by 2030. This ensures that cheap and effective antisnake venom is available as one of the critical foundations for reducing mortalities from snakebites (Williams et al., 2019).

Systemic reviews of laboratory-based preclinical studies play an essential role in drug discovery and understanding of the physiologic and pathologic mechanisms of diseases (de Vries et al., 2014). Systematic reviews of preclinical studies have recently been used to identify, synthesize and appraise *in vitro* and *in vivo* studies, thereby prompting scientists to recognize and explore the importance of rigor and reproducibility of preclinical systematic reviews, which will ultimately provide relevant data that will inform critical decisions in drug discovery (Peters et al., 2006; Sena et al., 2014; de Vries et al., 2014). However, despite the preclinical studies reporting the everyday use and efficacy of medicinal plants against venom-induced pathologies in Africa, there is no systematic review summarising their findings and level of evidence in efficacy. Therefore, we conducted a systematic review to address this gap and to provide a good understanding and a reliable conclusion regarding the ethnopharmacological relationship between interventional medicinal plants and their observed effects on laboratory-based snake venom-induced experiments.

2. Methods

2.1. Reporting strategy

This systematic review was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Hunniford et al., 2021). The Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies (CAMARADES 2020) was slightly modified by a 5-member panel of experts to include studies reporting *in vivo* and *in vitro* models, and studies that combine both models as reported in our registered (PROSPERO CRD42021247711) and published protocol (Bala et al., 2022).

2.2. Database search strategy

The data were searched online in the following databases: Medline via PubMed, Embase via OVID, and Scopus. The search was limited to articles published in English from 2000 to 2021. The following keywords were used in different formats depending on the database; Herbal medicine, medicinal plants, snake venom, snake, snakebite, snake envenoming, snake envenomation, snake antidotes, antisnake venom, neutralization, snake venom inhibition, pharmacological activity, Viperidae, Crotalina, Elapidae, Echis, Cobra, Naja, Bitis, Africa, African, African's, sub-Saharan Africa, South Africa, West Africa, East, and North Africa.

2.3. Study eligibility

African studies reporting *in vivo* biological activities of medicinal plants/extracts/constituents used in treating SBE or pathologies due to envenoming in rodents (mice, rats and rabbits) were identified and included for screening. *In vitro* models of rodents' plasma/serum, whole blood, cell lines, and isolated tissues/organs were also included for screening. The abstracts and full-text articles that passed these criteria were considered for data extraction and synthesis.

2.4. Data screening

Articles searched from Medline, Embase, and Scopus were pooled using EndNote to remove duplicates (Gotschall, 2021). Three independent reviewers (Auwal Bala, Alkassim Mohammed, and Mustapha Mohammed) screened the articles for eligibility, and discrepancies were resolved through consensus. Full-text articles of included studies were identified for quality and risk of bias assessments.

2.5. Quality assessment

The quality and reliability of the included studies were assessed using a modified tool from the CAMARADES and A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data (Klimisch et al., 1997; Macleod et al., 2004). To accommodate both *in vivo* and *in vitro* studies, minor modifications of items were validated by a five-member panel of experts. Fifteen items were assigned to the modified tool. Each item in the tool carries one mark of quality. The qualities of the included studies were determined based on the total scores, from 1 - 15 marks (lowest to the highest quality).

2.6. Risk of bias assessment

The risk of bias was assessed using the SYRCLE's risk of bias tool for animal studies (Hooijmans et al., 2014). We used Rooney's (2015) "Extending a Risk of Bias Approach to Address *In Vitro* Studies" to slightly modify and accommodate the *in vitro* studies included in our SR. Twelve queries were asked in the modified tool validated by a five-member panel of experts. Each study was identified to have a high, medium, or low risk of bias.

2.7. Data extraction

Four independent groups of authors extracted the following details from the included studies: (1) Publication year and the first author, (2) Type of study (*in vivo* or *in vitro*), (3) Animal population, (4) Specimen/sample for *in vitro* studies, (5) Animal sample size, (6) Snake species, (7) Study Design, (8) Origin of plants, (9) Test method, (10) Intervention (Medicinal plant), (11) venom dose/concentration, (12) Primary Outcome, and (13) Secondary Outcome.

3. Results

3.1. Data search and screening

A total of 614 articles were returned from a search on the three databases, and 413 were retained after removing duplicates. At first, 35 studies met the inclusion criteria. However, after resolving discrepancies from the screening panel, only 17 studies were included in the final data extraction, study quality, and assessment of the risk of bias as shown in Fig. 1. Additionally, Fig. 2 represents the graphical illustration of the systematic review.

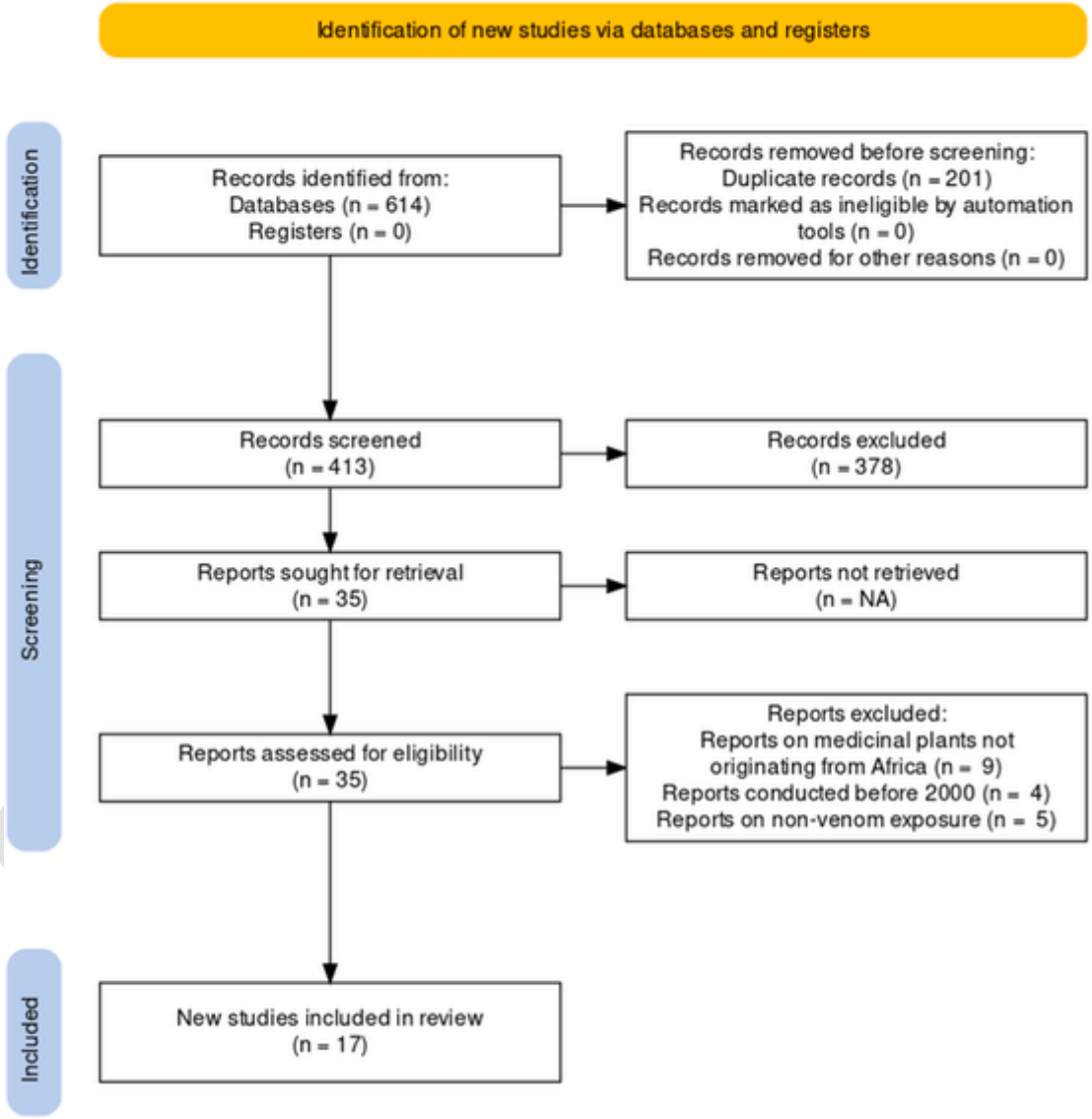

Fig. 1. Schematic illustration of data search, extraction and screening.

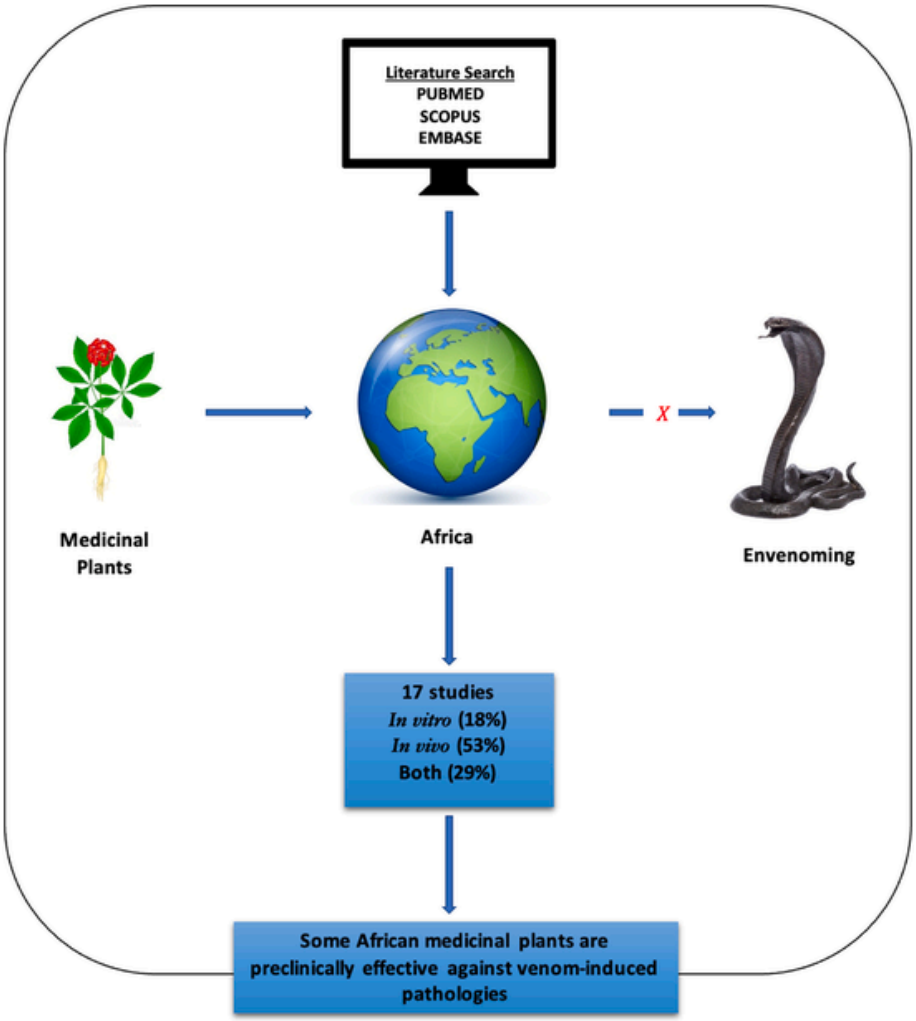

Fig. 2. Graphical presentation of the systematic review.

3.2. Study quality

All the included studies scored more than ten points on the 15-item quality assessment tool, out of which ten have the highest quality, scoring between 12 and 14 points. All seventeen studies were published in peer-review journals. All articles described the test methods and origin of the intervention, the dose of venom, and the intervention (medicinal plant). However, only one study declared a statement on conflict of interest (Adeyemi et al., 2021), as shown in Table 1.

3.3. Risk of bias

The risk of bias in each study was assessed using a modified SYRCLE's risk of bias tool for animal studies (Hooijmans et al., 2014) containing 12 queries. Thirteen studies were found to have a low risk of bias, while 4 studies had a medium risk of bias, as shown in Table 2.

3.4. Study characteristics and efficacy reports

A total of seventeen studies that reported the biological activities of 240 plants from 34 families against the venom of 15 snake species were reviewed (Fig. 3). Three studies were conducted using *in vitro* models (18%), nine studies employed *in vivo* models (53%), and five studies used both *in vitro* and *in vivo* models (29%).

3.5. Animals and in vitro test specimens

The reviewed studies were performed using rodents, including rats (Adzu et al., 2005; Fung et al., 2009; Tan et al., 2009; Fung et al., 2011; Omale et al., 2013; Fung et al., 2014; Nasser et al., 2018; Fasuba et al., 2019; Gabriel et al., 2020; Adeyemi et al., 2021) and mice (Abubakar et al., 2000; Asuzu and Harvey, 2003; Abubakar et al., 2006; Ode and Asuzu, 2006) while the *in vitro* studies were conducted using pig muscle cell lines, mice muscle cell lines, and egg yolk (Abubakar et al., 2006, 2020; Fung et al., 2014; Molander et al., 2014, 2015).

3.6. Primary outcome

Protection against venom-induced lethality is considered the primary outcome of this review as recommended by World Health Organization (2017). Ten studies reported statistically significant percentage protection of animals against venom-induced lethality by the tested medicinal plants compared with control groups that received no medicinal plant intervention; 80% protection (Abubakar et al., 2000), 40% protection (Asuzu and Harvey, 2003), 50% protection (Adzu et al., 2005), 44.4% protection (Abubakar et al., 2006), 100% protection (Ode and Asuzu, 2006), 67% protection (Abubakar et al., 2000), and significant protection ($p \leq 0.05$) (Fasuba et al., 2019; Gabriel et al., 2020). Other studies (Abubakar et al., 2000; Molander et al., 2014; Fung et al., 2014; Adeyemi et al., 2021) reported significant ($p \leq 0.05$) efficacy on specific pathologies induced by snakebite envenoming, including neu-

Table 1
Quality assessment of included studies.

S/N	Study and year of publication	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15	Total Score/15
1.	Abubakar et al. (2000)	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	11
2.	Asuzu and Harvey, 2003	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	No	11
3.	Adzu et al. (2005)	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	12
4.	Abubakar et al. (2006)	Yes	Yes	Yes	Yes	–	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	12
5.	Ode and Asuzu, 2006	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	14
6.	Fung et al. (2009)	Yes	Yes	Yes	Yes	–	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	No	13
7.	Tan et al. (2009)	Yes	Yes	Yes	Yes	–	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	No	11
8.	Fung et al. (2011)	Yes	No	Yes	Yes	–	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	No	10
9.	Omale et al., 2013	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	12
10.	Fung et al. (2014)	Yes	Yes	Yes	Yes	–	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	12
11.	Molander et al. (2014)	Yes	Yes	–	–	–	Yes	Yes	Yes	Yes	Yes	Yes	–	Yes	Yes	No	10
12.	Molander et al. (2015)	Yes	Yes	–	–	–	Yes	Yes	Yes	Yes	Yes	Yes	–	Yes	Yes	No	10
13.	Nasser et al. (2018)	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	13
14.	Fasuba et al. (2019)	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	13
15.	Abubakar et al. (2020)	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	No	–	Yes	Yes	No	11
16.	Gabriel et al. (2020)	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	14
17.	Adeyemi et al. (2021)	Yes	Yes	No	Yes		No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	13

Modified Tool: (Q1) publication in a peer-reviewed journal; (Q2) statement of temperature control; (Q3) random allocation to groups (Q4) allocation concealment (Q5) blinded assessment of outcome; (Q6) description of the test methods (Q7) Description/origin of the intervention/venom (Q8) reports on the dose/concentration of venom and intervention (Q9) appropriate animal/test model (Q10) appropriate control group/test as part of the method (Q11) report of exposure period (Q12) sample size calculation; (Q13) reports of statistical methods employed (Q14) compliance with animal welfare regulations; (Q15) statement of potential conflict of interests.

Table 2
Risk of Bias in included studies.

S/N	Study and year of publication	Type of study	Query 1	Query 2	Query 3	Query 4	Query 5	Query 6	Query 7	Query 8	Query 9	Query 10	Query 11	Query 12	RISK
1.	Abubakar et al. (2000)	*In vivo*	Yes	Yes	Yes	Yes	–	No	No	No	Yes	Yes	Yes	Yes	Low
2.	Asuzu and Harvey, 2003	*In vivo* and *In vitro*	Yes	Yes	Yes	–	Yes	No	No	No	Yes	Yes	Yes	Yes	Low
3.	Adzu et al. (2005)	*In vivo*	Yes	Yes	Yes	–	–	No	–	No	Yes	Yes	Yes	Yes	Medium
4.	Abubakar et al. (2006)	*In vivo* and *In vitro*	Yes	Yes	Yes	No	No	No	–	No	Yes	Yes	Yes	Yes	Medium
5.	Ode and Asuzu, 2006	*In vivo* and *In vitro*	Yes	Yes	Yes	Yes	Yes	No	–	No	Yes	Yes	Yes	Yes	Low
6.	Fung et al. (2009)	*In vivo*	Yes	Yes	Yes	–	Yes	–	–	–	Yes	Yes	Yes	Yes	Low
7.	Tan et al. (2009)	*In vivo* and *In vitro*	Yes	Yes	Yes	–	Yes	–	–	–	Yes	Yes	Yes	Yes	Low
8.	Fung et al. (2011)	*In vivo*	Yes	Yes	Yes	–	Yes	–	–	–	Yes	Yes	Yes	Yes	Low
9.	Omale et al., 2013	*In vivo*	Yes	Yes	Yes	–	Yes	No	No	No	Yes	Yes	Yes	Yes	Low
10.	Fung et al. (2014)	*In vivo*	Yes	Yes	Yes	–	Yes	–	–	–	Yes	Yes	Yes	Yes	Low
11.	Molander et al. (2014)	*In vitro*	–	Yes	Yes	–	Yes	–	–	–	Yes	Yes	Yes	Yes	Medium
12.	Molander et al. (2015)	*In vitro*	–	Yes	Yes	–	Yes	–	–	–	Yes	Yes	Yes	Yes	Medium
13.	Nasser et al. (2018)	*In vivo*	Yes	Yes	Yes	Yes	–	–	No	–	Yes	Yes	Yes	Yes	Low
14.	Fasuba et al. (2019)	*In vivo*	Yes	Yes	Yes	–	–	–	No	No	Yes	Yes	Yes	Yes	Low
15.	Abubakar et al. (2020)	*In vitro*	Yes	Yes	Yes	No	Yes	No	No	No	Yes	Yes	Yes	Yes	Low
16.	Gabriel et al. (2020)	*In vivo* and *In vitro*	Yes	Yes	Yes	–	Yes	No	No	No	Yes	Yes	Yes	Yes	Low
17.	Adeyemi et al. (2021)	*In vivo*	Yes	Yes	Yes	–	Yes	No	–	–	Yes	Yes	Yes	Yes	Low

Query 1: Was the allocation sequence adequately generated and applied? **Query 2:** Were the groups/test sample similar at baseline or homogenous? **Query 3:** Was the allocation adequately concealed or homogenous test samples? **Query 4:** Were the animals randomly housed during the experiment? **Query 5:** Were the test specimen appropriately and uniformly stored during the experiment? **Query 6:** Were the investigators blinded from knowing which intervention each animal/test group received during the experiment? **Query 7:** Were animals selected at random for outcome assessment? **Query 8:** Was the outcome assessor-blinded? **Query 9:** Were all major outcomes reported? **Query 10:** Are reports of the study free of selective outcome reporting? **Query 11:** Was the study apparently free of other problems that could result in a high risk of bias?**Query 12:**Was the administered dose uniform or the concentration level of the intervention homogenous?.

rotoxicity, hematotoxicity, cytotoxicity, and effect on cholesterol profile. Notably, two studies (Tan et al., 2009; Fung et al., 2014) examined the venom's cardiotoxicity and the cardio-protection mechanism provided by the tested medicinal plants. Out of the 34 families of plants species with antisnake venom activity, Fabaceae has the highest number of studies reporting its efficacy (five studies), followed by Annonaceae (three studies), Combretaceae (two studies), Sterculiaceae (two studies), and Olacaceae (two studies) as shown in Fig. 4. A summary of the reviewed herbal plants with significant efficacy on venom-induced pathologies is presented in Table 3.

3.7. Secondary outcome

Efficacy in neutralizing neurotoxicity, hematotoxicity, and cytotoxicity were considered secondary outcomes (World Health Organization, 2017). Sixteen studies assessed either one or two of the secondary outcomes with significant effect compared with the control group that received no medicinal plant intervention (Asuzu and Harvey, 2003; Abubakar et al., 2006; Ode and Asuzu, 2006; Nasser et al., 2018; Gabriel et al., 2020), hemolytic effect (Adzu et al., 2005; Adeyemi et al., 2021), histopathologic effect (Fung et al., 2014) and PLA_2 enzyme inhibition (Abubakar et al., 2000; Molander et al., 2014).

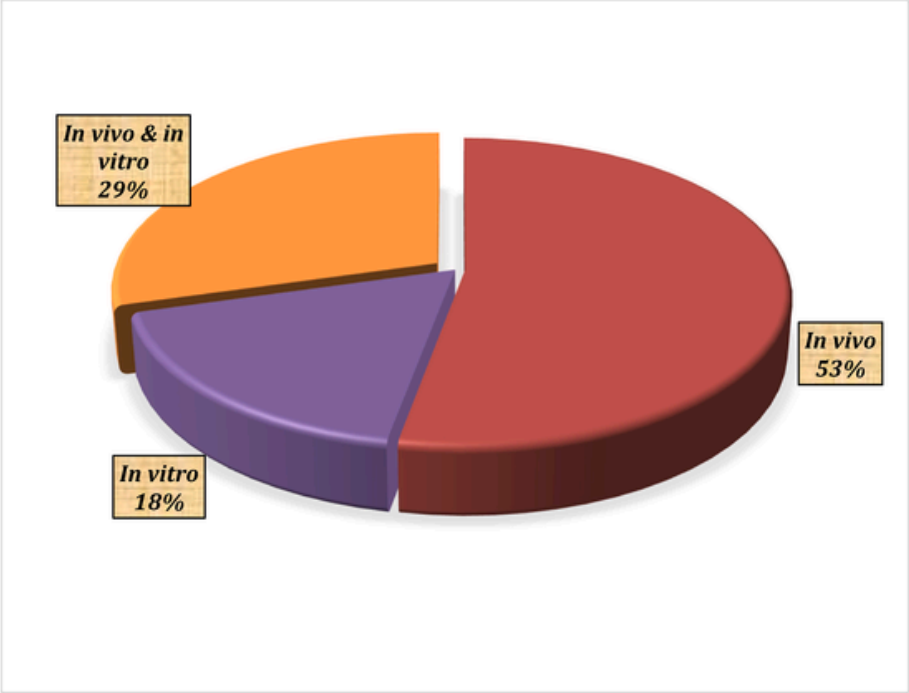

Fig. 3. Types of study models included in the systematic review on the biological activity of plants against snake venom.

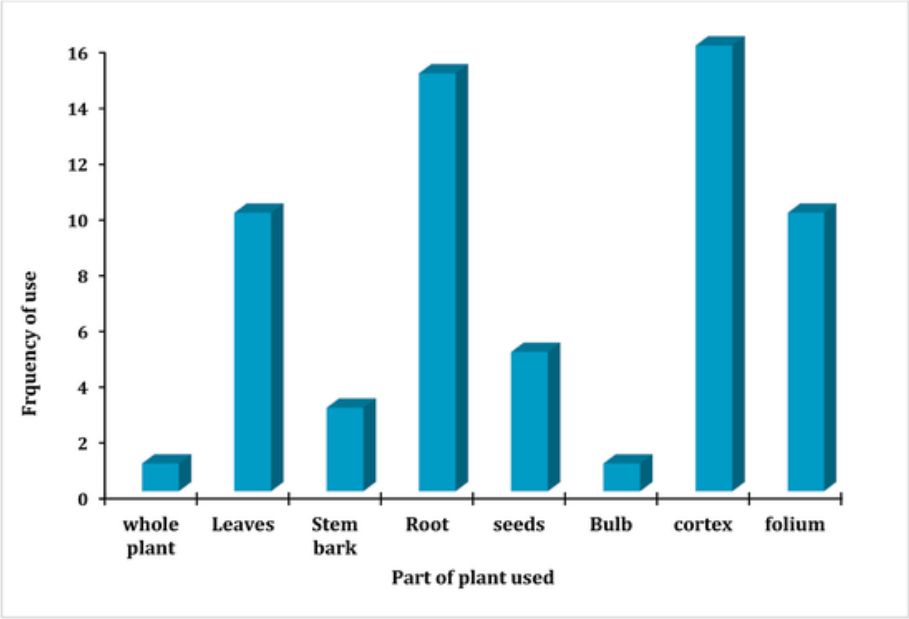

Fig. 4. Part of the medicinal plants used in the included studies.

3.8. Summary of important characteristics and key findings from the reviewed studies

A total of seventeen studies reported the screening of 240 medicinal plants for biological activity against the venom of 15 different snake species as shown in Table 3. Furthermore, the extracted parts of plants used for antisnake venom screening in the included studies comprise leaves, stem bark, roots, seeds, bulb, cortex, or folium, except for the survey conducted by Nasser et al. (2018), which used the whole plant in extracting the test sample. Cortex was used 16 times, followed by roots (15 times), leaves, and folium (10 times). Seeds and stem bark were used only five and three times, respectively. We observed three medicinal plants with the highest percentage of protection (50–100%) from venom-induced lethality among laboratory animal groups, ranging from 3 to 6 animals (Abubakar et al., 2000; Adzu et al., 2005; Ode et al., 2006) as shown in Fig. 4.

3.9. Plant families with efficacy in the included studies

Thirty-four plant families were used in the included studies, and the highest number of plant species are from Fabaceae (12), followed Malvaceae (5), Annonaceae (4) and Anacardiaceae (3), as shown in Fig. 5.

Table 3
Summary of important characteristics and key findings from the included studies.

Study	Snake venom used	Medicinal plant tested	Family	Part of the plant used	Efficacy report
Abubakar et al. (2000)	*Echis carinatus* and *Naja nigricollis*	*Guiera senegalensis* J.F. Gmel.	Combretaceae	Leaf	The extract alleviated the venom-induced lethality and neurological symptoms
Asuzu and Harvey, 2003	*Naja nigricollis*, and *Echis ocellatus*	*Parkia biglobosa* (Jacq.) G. Don.	Mimosaceae	Stem bark	The extract protected the venom-induced lethality, neurotoxic, myotoxic, cytotoxic and haematoxic effects.
(Adzu et al., 2005)	*Naja nigricollis*	*Annona senegalensis* Pers.	Annonaceae	Root bark	The extracts provided up to 83% protection form death and inhibited venom-induced hyperthermia caused by *N. nigricollis* venom
Abubakar et al. (2006)	*Naja nigricollis*	*Indigofera pulchra* Willd. *Aristolochia albida* Duch. *Guiera senegalense* J.F. Gmel. *Sterculia setigera* K. Schum.	Fabaceae Aristolochiaceae Combretaceae Sterculiaceae	Ariel part Rhizome Leaf and bark	The plant *I. pulchra* and *A. albida* produced in mice 33.3% and 44.4% protection from death respectively. It also inhibited PLA_2 activities and hemolytic effect of *N. nigricollis* venom
Ode and Asuzu, 2006	*Echis ocellatus, Bitis arietans* and *Naja nigricollis*	*Crinum jagus* J. Thomps. Dandy.	Amaryllidaceae	Bulb	The bulb extract protected the mice from death, myonecrosis and haemorrhage induced by the venoms
Fung et al. (2009)	*Naja sputatrix*	*Mucuna pruriens* (L.) DC.	Fabaceae	Seed	*M. pruriens* seed extract protected mice against the snake venom induced-histopathological changes on the rat brain, liver and blood vessels.
Tan et al. (2009)	*Ophiophagus hannah, Naja sputatrix, Bungarus candidus, Notechis scutatus, Pseudechis australis, Trimeresurus purpureomaculatus, Naja nigricollis, Bothrops asper, Agkistrodon piscivorus, Vipera russelli russelli*	*Mucuna pruriens* (L.) DC.	Fabaceae	Seed	The seeds moderately neutralized the lethal effect of *Calloselasma rhodostoma* venom.
Fung et al. (2011)	*Naja sputatrix*	*Mucuna pruriens* (L.) DC.	Fabaceae	Seed	The seed extract prevented venom cardio-respiratory and neuromuscular effect by neutralizing the cobra venom cardiotoxins and neurotoxins
Omale et al., 2013	*Naja katiensis*	*Olax viridis* Oliv. *Syzygium guineense* (Willd.) DC.	Olacaceae Myrtaceae	Leaf	The extracts could be used to treat venom-induced-edema and lethality in rats
Fung et al. (2014)	*Naja sputatrix*	*Mucuna pruriens* (L.) DC.	Fabaceae	Seed	The seed extract elicited cardio-respiratory protection by up regulation of five immunomodulatory genes that maintained heart homeostasis
Molander et al., 2014	*Naja nigricollisBitis arietans*	44 plants with activities were screened (list on appendix 1)	34 plant families with the (list on appendix 1*)	Leaf, root, cortex, stem bark and folium	There was more than 90% inhibition of enzyme activity (PLA_2 Hyaluronidase, and Proteases) by 40 of the tested extracts from 226 medicinal plants
Molander et al., 2015	*Naja nigricollis* and *Bitis arietans*	44 plants with activities were screened (list on appendix 1*)	34 plant families with the (list on appendix 1*)	Leaf, root, cortex, stem bark and folium	The extracts did not exhibit any topical effect in inhibiting enzyme activities and prevention of tissue damage
Nasser et al. (2018)	*Cerastes cerastes*	*Alkannaorientalis* (L.) Boiss.	Boraginaceae	Whole plant	*The* extract has activity against *Cerastes cerastes* venom in rats
Fasuba et al., 2019	*Bitis arietans*	*Euphorbia continifolia* L.	Euphorbiaceae	Leaf	Pre-treatment with the extract increased the mean survival time
Abubakar et al. (2020)	*Naja nigricollis*	*Commiphora Africana* (A. Rich.)	Burseraceae	Leaf and stem bark	The extracts of *C. Africana* inhibit the phospholipase A_2 activity from *N. nigricollis* crude venom
Gabriel et al. (2020)	*Naja nigricollis*	*Uvaria chamae* P. Beauv	Annonaceae	Leaf	The extract showed activity against the venom induced lethality and toxicity signs
Adeyemi et al. (2021)	*Echis ocellatus*	*Moringa oleifera* Lam.	Moringaceae	Leaf	The extract exerted anti haemorrhagic effect

3.10. Origin of the plant species preclinically screened in the included studies

In total, there are 43 plant species screened in the included studies in which most are from Mali (44%), followed by Nigeria (42%), Congo DR (9%), South Africa (3%), and Egypt (2%) as shown in Fig. 6.

4. Discussion

4.1. Evidence of efficiency

In this systematic review, we identified seventeen studies that met the inclusion criteria; these include three *in vitro* models, nine *in vivo* models, and five studies that combined both *in vivo* and *in vitro* models. At the end of the review, we found evidence for the preclinical efficacy of some (54 species) African medicinal plants against snakebite envenoming from plant families, including Fabaceae, Malvaceae, and Annonaceae. This evidence is more qualitative in studies that combined *in vivo* and *in vitro* models involving the pre-incubation of venom and plant extracts before intervention. The finding also indicates that contact of venom and ASV is an important step in neutralizing snake venoms by ASV, as reported for IgG-based antisnake venom (World Health Organization, 2017).

4.2. Preclinical systematic reviews

A preclinical systematic review is an important tool, especially for biomedical scientists searching for ASV from medicinal plants. System-

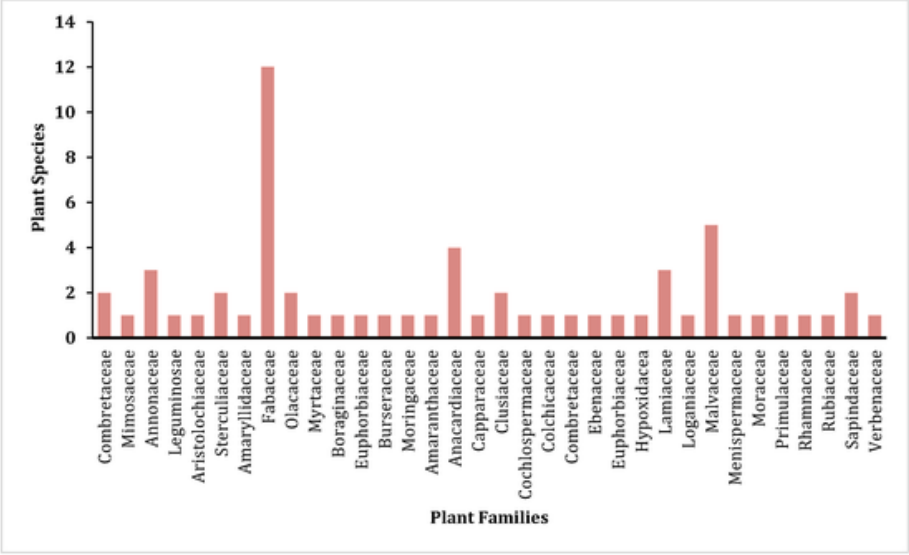

Fig. 5. Plant families used in the included studies that showed efficacy against snake venom-induced pathologies.

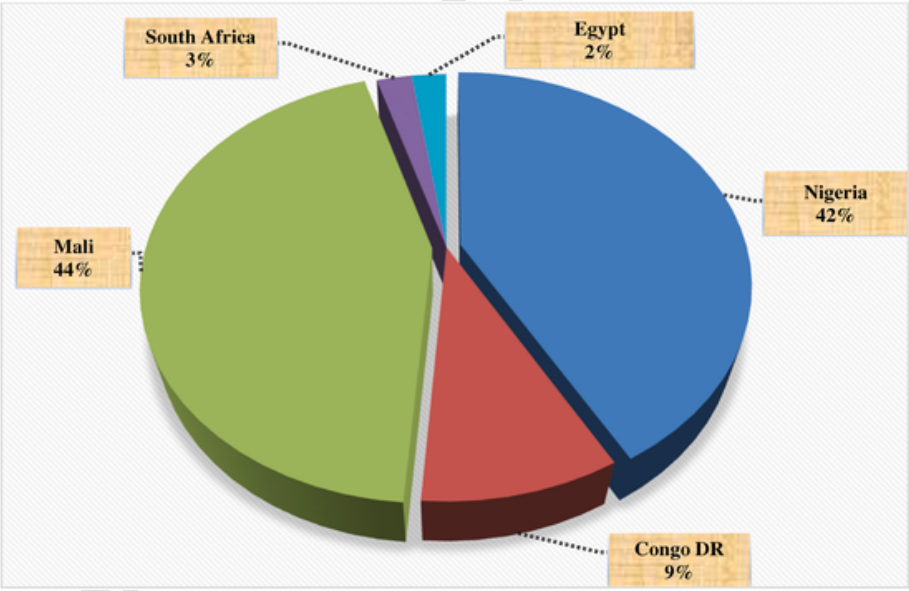

Fig. 6. Origin of the medicinal plants preclinically screened for pharmacological activity against snake venom-induced envenoming?.

atic reviews are more common in clinical trials, and it is not a common practice in preclinical studies despite their importance to researchers in the design, conducting, and analysis of laboratory experiments that rely on animal and non-animal models. It also helps strengthen the ethical concept of the 3Rs in animal experiments (Van Luijk et al., 2014). Systematic reviews on the preclinical efficacy of medicinal plants used in treating SBE are scarcely reported in the literature. Perhaps, this study is the first and could pave the way for further studies in this exciting area, especially in a snakebite-endemic region like sub-Saharan Africa. Most SR on SBE focused on the efficacy of conventional ASVs (Schaeffer et al., 2012; Das et al., 2015; Maduwage et al., 2015; Potet et al., 2019) and other supportive care (Avau et al., 2016) in the management of the SBE. Even though the mainstay of treatment of SBE is the administration of animal-derived ASVs, the challenges associated with accessing this treatment, particularly for resource-limited regions, necessitate SBE victims to seek alternative and complementary remedies from traditional healers. This SR analyzed data of medicinal plants utilized by traditional snake charmers to treat snakebites. An SR of this nature can provide valuable data to inform health system policy decisions, guidelines, and practice in managing SBE to meet the WHO target.

4.3. Study quality and risk of bias

Study quality and risk of bias assessment have been an important debate in evaluating preclinical studies because researchers tend to use divergent *in vivo* and *in vitro* techniques in preclinical testing, especially in rare and neglected tropical diseases. Despite the availability of tools for assessing the quality and risk of bias for studies included in preclinical systematic reviews, there are still inconsistencies in many findings (Pussegoda et al., 2017; Sheth et al., 2022). These inconsistencies have made quality or risk of bias assessments challenging because some investigators include different models in one study. Hence, we adopted and slightly modified an *in vivo* quality assessment tool (CAMERADES) and risk of bias for animal studies (SYCLE'S) to accommodate studies that use *in vitro* models or a combination of both (Klimisch et al., 1997; Macleod et al., 2004; Hooijmans et al., 2014). We consider the studies

included in our review to be of acceptable quality for preclinical systematic reviews because all the studies scored above 10 points, out of which ten studies scored 12–14 points on a scale of 15 points (Table 1). Equally, the risk of bias assessment is considered acceptable because thirteen studies were found to have a low risk of bias. In comparison, the remaining four studies have a medium risk of discrimination based on the 12-item tool.

4.4. Ethical considerations

The most common animal specimen for preclinical evaluation of antisnake venom is the use of laboratory animals, including mice, rats, and rabbits (World Health Organization, 2017). However, using laboratory animals for venom research is still an ethical controversy among researchers and animal rights activists, leading to many recommendations, including the need to establish *in vitro* efficacy before exposing the animals to *in vivo* models. The *in vivo* studies in our SR reported the use of either mice or rats in evaluating medicinal plants, while the *in vitro* studies used egg yolk, blood samples, and pig muscle cell lines. Ten of the included studies reported efficacy in terms of statistical significance ($p \leq 0.05$) on specific pathologies induced by SBE compared with negative control groups, including neurotoxicity, hematotoxicity, cytotoxicity, and effect on cholesterol profile (Tan et al., 2009; Fung et al., 2011;Omale et al., 2013; Molander et al., 2015 Nasser et al., 2018; Abubakar et al., 2020; Adeyemi et al., 2021). In ethnopharmacology, the family of any plant of interest is paramount to drug discovery. Similar to other reports, we found that Fabaceae (20%) is the most explored for preclinical efficacy against snake venom-induced pathologies followed by Malvaceae (8%) and Annonaceae (6%) (Félix-Silva et al., 2017; Omara et al., 2020; Gbolade and AuthorAnonymous, 2020).

4.5. Methodological strategies for antisnake venom screening

Implementing the 3Rs (Replacement, Reduction, and Refinement) in using animals for laboratory experiments has been one of the critical focuses of experts in toxinological research (Sells, 2003; World Health Organization, 2017). Using *in vitro* techniques as alternative methods or at least a combination of both has been the point of advocacy by experts in antivenomics. These techniques include Enzyme-Linked Immunosorbent Assay (ELISA), western blotting, organ-bath experiments, chromatographic techniques, and the use of invertebrates as substitutes for rodents (Gutiérrez et al., 2009; Calvete et al., 2014; Gutiérrez et al., 2017). Only three studies (18%) were conducted using *in-vitro* models. In comparison, the rest of the studies either conducted *in vivo* studies (53%) or a combination of both models (29%). Although significant pharmacological activity was observed in our systematic review, we believe that the methodological design can be improved by initially designing and conducting *in vitro* studies before exposing the animals to uncomfortable *in vivo* experiments in antisnake venom studies. This will maintain the ethical consideration in the concept of 3Rs as duly recommended by World Health Organization (2017). It is also important to note that *in vitro* techniques will provide insight into the cellular mechanism of antisnake venom activity. Nevertheless, researchers should consider improving the design of the classical *in vivo* antisnake venom screening methods that involve pre-mixing venom and plant extract before administration into the experimental animal. We observed that some studies combined the pre-treatment of animals with plant extracts before the administration of venom while some pre-mixed venom and extract before the administration; we observed better efficacy reports in pre-mixing venom and extract with zero-to insignificant efficacy reports when animals were pre-treated with extract before administration of venom.

Furthermore, the included studies conducted LD_{50} determination mainly to determine the exposure dose used in the *in vivo* studies. However, preclinical safety studies of the medicinal plants in the included studies have not been thoroughly reported. Since some medicinal plant concoctions have been used even clinically (Bhaumik et al., 2020), it is therefore very important to conduct some safety studies of medicinal plants with pharmacological activity against the venom-induced pathologies.

4.6. Public health/clinical implications of the study findings

The public health impact of snakebite envenoming cannot be overemphasized; the incidence of SBE is more common among young people who are often engaged in economic activities, such as farmers and herders in rural areas of Africa and Asia. It has been reported that SBE has caused the loss of 6–8 billion DALYs, comparable to prostate and cervical cancer. This impact could be one of the reasons why SBE victims in rural areas resort to readily available medicinal plants for treatment. The clinical impact of SBE and the paucity of antisnake venom have even led to the clinical use of medicinal plants, as reported by Bhaumik et al. (2020).

4.7. Limitations

Our SR, reviewed only medicinal plants originating from Africa. However, there could be other effective medicinal plants against snake venom in other regions of the world. Another limitation is the inclusion of studies reported only between the years 2000–2021. Single study identified, characterized, isolated, and screened the biological actions of the bioactive compounds present in various medicinal plants reported.

4.8. Future directions

As Trim et al. (2020) reported earlier, we also observed that the research for antisnake venom from the traditional plant is somewhat limited to preliminary preclinical techniques centred on immunoassays which might not be suitable for other conventional pharmaceutical drug discovery approaches such as molecule-receptor interactions. Therefore, there is a need to explore a robust approach to pharmaceutical drug discovery for SBE, just like other diseases. The introduction of antivenomics has provided an important avenue for exploring clear and more precise methods in the preclinical screening of medicinal plants for biological activity against snake venom-induced pathologies. We observed that most of the included studies used crude extracts of plant parts during screening, while others used fractionated extracts (Nasser et al., 2018; Abubakar et al., 2020; Gabriel et al., 2020). Although plants contain many bioactive compounds, only one out of the seventeen studies used Gas Chromatography coupled with a Mass Spectrometer (GC/SM) to separate and identify the active constituents in the extract. Hence, there is a need for further experiments on bioassay-guided fractionation, identification, isolation, and characterization of different medicinal plants' constituents. In contrast, Fung et al. (2014) used the Polymerase Chain Reaction technique (PCR) to determine the mechanism of cardio-protection provided by plant extract. For all that, there is a need to explore the omics techniques in venomics and antivenomics for a comprehensive preclinical efficacy evaluation, particularly the evaluation of medicinal plants against venom-induced pathologies, including ELISA, Blotting techniques, High-performance chromatography, Mass spectrometry, and cell line-based analysis.

5. Conclusion

There are African medicinal plants with clinical efficacy against venom-induced lethality and secondary pathologies, including hematoxicity and cytotoxicity. The most effective plant families are Fabaceae, Malvaceae, and Annonaceae. However, there is a need for robust antivenomics that will implement the ethical concept of the 3Rs

and explore other pharmaceutical approaches in drug discovery including isolation and identification of active components of effective medicinal plants.

Author statement

We declared that this work was conducted by the authors named in this article, and all liabilities relating to the article's content were borne by them.

Auwal A. Bala, Basheer Chedi, Sani Malami, Mustapha Mohammed, and Umar Sharif Abdussalam conceived the original idea. All other authors equally contributed to the review work and manuscript writing, editing, and proofreading.

Funding

No funding was received for this study.

Ethical statement

The systematic review does not require ethical clearance.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgments

The authors would like to thank and appreciate all members of the Pharmacy Scholars Initiative (PSI) for their support and recommendations. We acknowledge and appreciate Prof. Elda Sanchez, Dr. Jacob Galan, Dr. Montamas Suntravat, and Dr. Emelyn Salazar of the National Natural Toxin Research Centre, Texas A&M University-Kingsville, USA, for providing training and support. We also appreciate Prof. Habib G. Abdulrazaq of the Nigerian Snakebite Research and Intervention Centre (NSRIC) for their recommendations and training. We appreciate the Universiti Sains Malaysia, Malaysia, and the University of Edinburg, UK, for providing institutional access to research databases.

Appendix 1. List of medicinal plants with more than 90% efficacy in the study conducted by Molander et al. (2014) and Molander et al. (2015).

S/N	Medicinal plant tested	Family	Part of the plant used
1.	*Pupalia lappacea Juss*	Amaranthaceae	Herba
2.	*Lannea acida A Rich*	Anacardiaceae	Cortex
3.	*Sclerocarya birrea Hochst.*	Anacardiaceae	Cortex
4.	*Spondias mombin* L.	Anacardiaceae	Radix
5.	*Spondias mombin* L.	Anacardiaceae	Cortex
6.	*Annona senegalensis pers.*	Annonaceae	cortex
7.	*Capparis tomentosa Lam.*	capparaceae	Radix
8.	*Psorospermum corymbiferum Hochsr.*	clusiaceae	Radix
9.	*Psorospermum corymbiferum Hochsr.*	clusiaceae	Cortex
10.	*Cochlospermum tinctorium Perr.*	Cochlospermaceae	Radix
11.	*Gloriosa superb* L.	Colchicaceae	Radix
12.	*Combretum mole R. Br. Ex G. Don*	Combretaceae	Folium
13.	*Guiera senegalensis J.F. Gmel*	Combretaceae	Radix
14.	*Diospyros mespiliformis Hochst*	Ebenaceae	Cortex
15.	*Alchornea laxiflora* Pax & K. Hoffin	Euphorbiacea	Cortex
16.	*Securinega virosa* (Roxb.) Baill.	Euphorbiacea	Radix
17.	*Bauhinia thonningii* Schumach.	Fabaceae	Cortex
18.	*Bauhinia thonningii* Schumach.	Fabaceae	Radix
19.	*Burkea africana* Hook.	Fabaceae	Cortex
20.	*Dichrostachy cinerea* (L.) Wight &Arn.	Fabaceae	Folium
21.	*Parkia biglobosa* Benth	Fabaceae	Cortex
22.	*Swartzia madagascariensis* Desv.	Fabaceae	Folium
23.	*Tamarindus indica* L.	Fabaceae	Folium
24.	*Tamarindus indica* L.	Fabaceae	Cortex
25.	*Curculigo recurvata* W.T.Aiton	Hypoxidaceae	Folium
26.	Haumaniastrum sp.	Lamiaceae	Herba
27.	*Teucrium krausii* Codd	Lamiaceae	Radix
28.	*Teucrium krausii* Codd	Lamiaceae	Herba
29.	*Strychnos innocua* Delile	Loganiceae	Folium
30.	*Dombeya quinqueseta* (Delile) Excell	Malvaceae	Cortex
31.	*Grewia mollis* Juss.	Malvaceae	Folium
32.	*Grewia mollis* Juss.	Malvaceae	Radix
33.	*Grewia mollis* Juss.	Malvaceae	Cortex
34.	*Watheria indica* L.	Malvaceae	Radix
35.	*Cissampelos mucronata* A. Rich	Minispermaceae	Herba
36.	*Ficus platyphylla* Delile	Moraceae	Folium
37.	*Xirnenia americana* L.	Olacaceae	Folium
38.	*Maesa lanceolata* Voigt.	Primulaceae	Cortex
39.	*Ziziphus mucronata* Wild	Rhamnaceae	Radix
40.	*Pentanisia prunelloides* (Klorzsch) Walp.	Rubiaceae	Radix
41.	*Paulinnia pinnata* L.	Sapinadaceae	Folium

S/N	Medicinal plant tested	Family	Part of the plant used
42.	*Paulinnia pinnata* L.	Sapinadaceae	Radix
43.	*Sterculia setigera* Delile.	Sterculiacea	Cortex
44.	*Lantana trifolia* L.	Verbenacea	Cortex

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