

VALIDATING TRADITIONAL SNAKE REPELLENT USE THROUGH PHYTOCHEMICAL AND ANTIOXIDANT ANALYSES OF *Andrographis paniculata* (Burm. F.) Nees AND *Petiveria alliacea* Linn.

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ABSTRACT

High incidence of snakebite in the tropical regions necessitates prompt exploration of eco-friendly deterrents, entrenched in traditional knowledge. In this study, Phytochemical composition together with the antioxidant potential of two plant species traditionally utilised as snake repellents in the Southwestern Nigeria (*Andrographis paniculata* and *Petiveria alliacea*) were investigated. Methanolic leaf extracts of the plants were prepared and analysed for total flavonoid and phenolic contents, ferric reducing antioxidant power, DPPH radical scavenging activity, hydroxyl radical inhibition and total antioxidant capacity. The studies showed a high antioxidant potential activity in both species, with *A. paniculata* exhibiting greater flavonoid (499.1 mgQE/g) and phenolic (752.6 mgGAE/g) contents, alongside notable radical scavenging and reducing power. *P. alliacea* demonstrated comparable hydroxyl radical inhibition and stronger DPPH activity (IC₅₀ = 0.4 mg/mL). The recorded high antioxidant in the studied species lends credence to the traditional use of these plants as natural snake repellent and supports their potential integration into sustainable ecofriendly and public health strategies.

Keywords: *Andrographis paniculata*, Antioxidant activity, *Petiveria alliacea*, Snake repellents, Traditional medicine.

INTRODUCTION

Snakes are noxious crawling animal found far and wide in the ecosystem (Ilundu and Lemy, 2018). They are mostly attracted to an environment by other small animals such as geckos, mice and such like, which potentially form part of their foods. One of the challenging public health challenges of tropical countries like Nigeria is snakebite. Its occurrences are common in many rural communities and their venoms being deadly cause morbidity and mortality among farmers, hunters, and children (Maregesi *et al.*, 2013). About 81,000 deaths worldwide occurring to at least 1.8 - 2.7 million people yearly has been attributed to snakebites (Asante-Kwatia *et al.*, 2021, Suhita *et al.*, 2022; Afroz *et al.*, 2024). Mortality estimates in sub-Saharan Africa alone ranges between 7,000 - 32,000 annually with West Africa recording about 5,450 mortality a year (Gutierrez *et al.*, 2017).

Traditional and modern methods for snakebite management present several challenges and synthetic chemical repellents, though effective, are often expensive and environmentally harmful (Wanzala *et al.*, 2014). Plants are known

to provide sustainable and eco-friendly alternatives which have long been in use by tropical communities for protection from snakebite. Notable examples include *Andrographis paniculata* (Burm. F) Nees and *Petiveria alliacea* Linn., known for their natural repellent properties. For example, *A. paniculata* has been extensively reported used as immune booster, blood cleanser and, as treatment for infectious diseases, fevers, colic pain, diarrhea, and viral infections in Ayurvedic and traditional medicine (Saxena *et al.*, 1998; Shalini and Narayanan, 2015).

Andrographis paniculata is domesticated in Nigeria but it has its centre of diversity in India, Southeast Asia, Sri Lanka, China, and America where it is cultivated for its medicinal importance (Latto *et al.*, 2006). The plant is in the Acanthaceae family. It is herbaceous and annual. It thrives in moist and shaded areas, and grows 30 - 110 cm tall. It possesses lanceolate leaves, a fragile, quadrangular stem, and small bilabiate flowers with white and violet markings. Its fruit contains tiny, subquadrate seeds. The genus *Andrographis* consists of a number of species ranging from 19 to 44

species (Tewari *et al.*, 2010; Patidar *et al.*, 2011; Bhattacharya *et al.*, 2012).

Phytochemical analyses of *A. paniculata* have shown the presence in this plant, compounds such as flavonoids, diterpenoids, xanthenes and noriridoides among others with pharmacological properties and anticancer, anti-inflammatory, antimicrobial, antidiarrheal, antimalarial, and hepatoprotective effects (Jarukamjorn and Nemoto, 2008; Hossain *et al.*, 2014). Toxicity investigations have also revealed that *A. paniculata* is generally safe, with no severe adverse effects in animal models and some clinical trials (Akbarsha and Murugaian, 2000; Kamal *et al.*, 2003).

Petiveria alliacea also known as Guinea hen weed is a perennial shrub or herb (up to 1 meter tall). It is native to tropical America and now naturalized in parts of Africa like Nigeria. *P. alliacea* thrives in disturbed areas and near settlements. It has simple alternate leaves with a garlic-like scent when crushed. Its roots also emit a strong sulfuric odor (Benevides *et al.*, 2001). The plant is recognised for analgesic, antipyretic, sedative, anti-inflammatory, hypoglycaemic, antitumor, antifungal and antibiotic properties (Lopes-Martins *et al.*, 2002; Santander *et al.*, 2012). *Petiveria. alliacea* is a member in the family Phytolaccaceae and thrives in tropical forests and disturbed habitats, where its pungent odour serves as a natural deterrent to herbivores and even snakes. Its phytochemical analyses have revealed that it contains secondary metabolites such as

saponins, alkaloids, steroids, tannins, coumarins, triterpenes and lipids alongside other metabolites which are responsible for the various bioactivity properties (Kubec *et al.*, 2002; Duarte and Lopes, 2005). More importantly, studies have reported *P. alliacea*'s antiophidic activity and its potential as a natural snake repellent (Trevisan *et al.*, 2021).

However, despite the traditional claims, scientific validation of *A. paniculata* and *P. alliacea* as snake repellents remains limited. There is a need for controlled laboratory studies to confirm their effectiveness and as well identify the specific bioactive chemical compounds responsible for the repellent attribute. Hence this research aimed at bridging this gap through laboratory analyses of the antioxidant and phytochemicals in *A. paniculata* and *P. alliacea* related to their efficacy. Some antioxidant compounds in plants such as phenolics, flavonoids possess strong odors, bitter taste, or irritant properties that deter snakes by causing sensory discomfort or avoidance behaviour.

MATERIALS AND METHODS

Plant Materials

The study investigated two plant species namely; *Andrographis paniculata* (Burm. F) Nees (Figure 1) and *Petiveria alliacea* Linn., (Figure 2). The samples were collected in the South-Western part of Nigeria at various locations. The GPS coordinates and locations of plants are as shown in Table 1.



Figure 1: *Andrographis paniculata* (Burm. F) Nees



Figure 2: *Petiveria alliacea* Linn.

Table 1: GPS Coordinates and Locations of the Studied Plants

Plant Species	Locations	GPS Coordinates
<i>Andrographis paniculata</i>	1. Ilesha, Osun State	7°37'40"N, 4°44'29"E
	2. Akungba Akoko, Ondo State.	7°28'11"N, 5°44'10"E.
	3. Ibadan, Oyo State	
	4. Obafemi Awolowo University	7°22'36"N, 3°56'23"E.
	5. Igbogbo, Ikorodu, Lagos State.	7°31'14"N, 4°31'49"E.
	6. Staff Quarters, OAU	6°35'60"N, 3°30'0"E 7°31'14.76"N, 4°31'49.13"E
<i>Petiveria alliacea</i>	1. Botany Department, OAU, Ile Ife.	7°31'8"N 4°31'35"E
	2. First Bank Lecture theatre OAU, Ile-Ife	
	3. Staff Quarters, OAU	7°31'14"N, 4°31'23"E
	4. Ilesha, Osun State	7°31'14.76"N, 4°31'49.13"E 7°37'40"N, 4°44'29"E

Preparation of plant extracts

Dried and pulverized leaves of *Andrographis paniculata* (51.99 g) and *Petiveria alliacea* (115.73 g) were soaked separately in 1 L of 70% methanol. After 48 hours of macerating, the extracts were filtered using cheesecloth, then air-dried in Petri dishes, until a constant weight was achieved. The percentage yields of the obtained crude extracts were determined using the formula:

$$\text{Percentage yield} = \frac{\text{Weight of concentrated extract}}{\text{Total amount of extract}} \times 100$$

The obtained crude extracts were stored in sealed jars for further laboratory analyses.

Determination of Total Phenolic Content

The extracts total phenolic content (TPC) was determined following the Folin-Ciocalteu colorimetric method (Singleton *et al.*, 1999). A standard gallic acid solution (0.1 g/mL) was prepared, and varying volumes (0.2 - 1 mL) were diluted to 1 mL. Each test tube received 10% Folin-Ciocalteu reagent (0.2 mL), vortexed for 5 minutes, followed by 7% Na₂CO₃ (1 mL) and this was made up 2.5 mL with distilled water and then incubated at room temperature for 90 minutes. The absorbance was read at 750 nm. Samples were run in triplicate. The TPC of the extracts was expressed as mg gallic acid equivalents (GAE) per gram dry weight (mg/g) using the formula;

$$c = \frac{c \times v}{m}$$

Where:

(C) = Total phenolic content (mgGAE/g dry extract)

(c) = Gallic acid concentration from calibration curve (mg/mL)

(v) = Volume of extract (mL)

(m) = Weight of crude extract (g)

Determination of Total Flavonoid Content

Total flavonoid content was determined using the aluminium chloride colorimetric method (Zhilen *et al.*, 1999). Standard quercetin solutions (0.2 - 1.0) mg/mL were prepared. To each assay, the extract or the standard (0.1 mL) was added distilled water (0.4 mL), followed by the addition of 5% sodium nitrite (0.1 mL). This was left for 5 minutes after which 10% aluminium chloride (0.1 mL) and sodium nitrite

(0.2 mL) were added, and the volume was adjusted to 2.5 mL with distilled water. Samples were run in triplicate, and absorbance was taken at 510 nm against a blank. The flavonoid content was expressed as milligram quercetin equivalents (mgQE/g) using the formula:

$$X = \frac{q \times v}{w}$$

Where:

(X) = Total flavonoid content (mgQE/g)

(q) = Quercetin concentration from calibration curve (mg/mL)

(v) = Volume of extract (mL)

(w) = Weight of crude extract (g)

DPPH Radical Scavenging Activity

The plant extracts antioxidant activities were evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method (Brand-Williams *et al.*, 1995). Different concentrations of the extracts and standard (ascorbic acid) ranging from 0.1 to 0.5 mg/mL were mixed with 1 mL of 0.3 mM DPPH in methanol. These were incubated in the dark for 30 minutes after which the absorbance was read at 517 nm. The percent inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \frac{\text{Abs(blank)} - \text{Abs(Sample)}}{\text{Abs(blank)}} \times 100$$

Where;

Abs(blank) = Absorbance of the control

Abs(Sample) = Absorbance of test compound

The IC₅₀ value (concentration required to inhibit 50% of DPPH radicals) was determined from the plot of percentage inhibition against extract concentration.

Evaluation of Total Antioxidant Capacity

Total antioxidant capacity of the extracts was determined following the phosphomolybdenum method (Prieto *et al.*, 1999). To 0.3 mL of extract (5 mg/mL) or standard (ascorbic acid, 1 mg/mL), 3 mL reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) was added. The tubes were capped, incubated at 95 °C for 90 minutes and cool at room temperature. The absorbance was read at 695 nm against a blank containing distilled

water in place of extract. Antioxidant activity was expressed as ascorbic acid equivalents.

Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity was determined based on the metal-chelating ability of antioxidants under Fenton-like reaction conditions, following the method of Chang and But (1987). A reaction mixture containing phosphate buffer (1.2 mL) at pH 7.4 and extract (200 μ L) was combined with 200 μ L each of 10 mM $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 10 mM EDTA and 10 mM 2-deoxyribose. Thereafter, 200 μ L of 10 mM H_2O_2 was added. The mixture was incubated at 37 °C for 3 hours after which 1 mL each of 1% thiobarbituric acid (TBA) and 2% trichloroacetic acid (TCA) were added. This was heated in a water bath for 10 minutes and allowed to cool to room temperature before the absorbance was read at 532 nm. The percentage scavenging activity was calculated using the formula;

$$\% = \frac{\text{Abs}(\text{blank}) - \text{Abs}(\text{Sample})}{\text{Abs}(\text{blank})} \times 100$$

Where:

Abs(blank) = Absorbance of the control reaction

Abs(Sample) = Absorbance of test compound
Sample concentration providing 50% Scavenging activity (IC_{50}) was calculated from the graph of percentage scavenging activity against concentration.

Ferric Reducing Antioxidant Power Assay

The determination of ferric reducing antioxidant power (FRAP) of the extracts was carried out following the method of Iris and Strain (1999). The working FRAP reagent comprising acetate buffer (300 mM) at pH 3.6, tripyridyltriazine (10 mM), and ferric chloride (20 mM) in a ratio 10:1:1 was prepared. To 2 mL of this reagent, 20-100 μ L of extract (5 mg/mL) or standard ascorbic acid solution (1 mg/mL) was added. Samples were prepared in triplicates and absorbance was read at 593 nm using a reagent blank containing distilled water. The antioxidant capacity was expressed as ascorbic acid equivalent concentration, defined as the concentration of antioxidant that produces a ferric reducing ability equivalent to that of the ascorbic acid standard.

Statistical Analysis

Data were expressed as percentages. Replicate values ($n=3$) were expressed means $\pm$ standard errors.

RESULTS

Extracts Yield

Table 2 below shows the percentage extract yields of *Andrographis paniculata* extract and *Petiveria alliacea* extract. The result showed that *A. paniculata* had higher extract percentage yield than *P. alliacea*.

Table 2: Yield of the methanolic extract of *Andrographis paniculata* and *Petiveria alliacea*

Methanolic Extract	Percentage yield (%)
<i>Andrographis paniculata</i>	53.97
<i>Petiveria alliacea</i>	30.75

Total Phenolic Content

Total Phenolic Content in *Andrographis paniculata* and *Petiveria alliacea* extracts (Table 3) showed that *A. paniculata* exhibited a higher total phenolic content compared to *P. alliacea*.

Table 3: Total Phenolic Content (TPC) of *Andrographis paniculata* and *Petiveria alliacea* extracts

Extracts	Total Phenolic Content (mgGAE/g)
<i>Andrographis paniculata</i>	752.6 $\pm$ 128.6
<i>Petiveria alliacea</i>	710.4 $\pm$ 15.2

Values are mean $\pm$ standard error; where $n = 3$

Total Flavonoid Content

Table 4 presents the results of the analyses of total flavonoid content in *A. paniculata* and *P.*

alliacea extracts expressed as quercetin equivalent (mgQE/g). *A. paniculata* showed a higher total flavonoid content than *P. alliacea*.

Table 4: Total Flavonoid Contents of *Andrographis paniculata* and *Petiveria alliacea* extracts

Extract	Total Flavonoid Content (mgQE/g)
<i>Andrographis paniculata</i>	499.10 ± 84.60
<i>Petiveria alliacea</i>	398.30 ± 15.90

Values are mean ± standard error; where n = 3

Total Antioxidant Content

The results of the analyses of *A. paniculata* and *P. alliacea* extracts for total antioxidant capacity expressed as mg ascorbic acid equivalent

(mgAAE/g) are shown in Table 5. The total antioxidant capacity was higher in *A. paniculata* extract than that of *P. alliacea*.

Table 5: Total Antioxidant Content of *Andrographis paniculata* and *Petiveria alliacea* extracts

Extract	Total Antioxidant Content (mgAAE/g)
<i>Andrographis paniculata</i>	103.90 ± 4.70
<i>Petiveria alliacea</i>	87.30 ± 8.60

Values are mean ± standard error; where n = 3

Ferric Reducing Antioxidant Potential

Table 6 below shows the Ferric reducing antioxidant potential of *A. paniculata* and *P. alliacea* methanolic extracts expressed as

Ascorbic acid equivalent (mgAAE/g). *A. paniculata* displayed a stronger ferric reducing power than *Petiveria alliacea*.

Table 6: Ferric Reducing Antioxidant Potential of *Andrographis paniculata* and *Petiveria alliacea* extract

Extract	Ferric Reducing Antioxidant Potential (mgAAE/g)
<i>Andrographis paniculata</i>	49.19 ± 0.16
<i>Petiveria alliacea</i>	26.94 ± 0.16

Values are mean ± standard error; where n = 3

Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activities of *A. paniculata* and *P. alliacea* extracts are presented in Figure 3. Both *A. paniculata* and *P. alliacea*

exhibited identical hydroxyl radical scavenging activities, with an IC₅₀ value of 0.075.

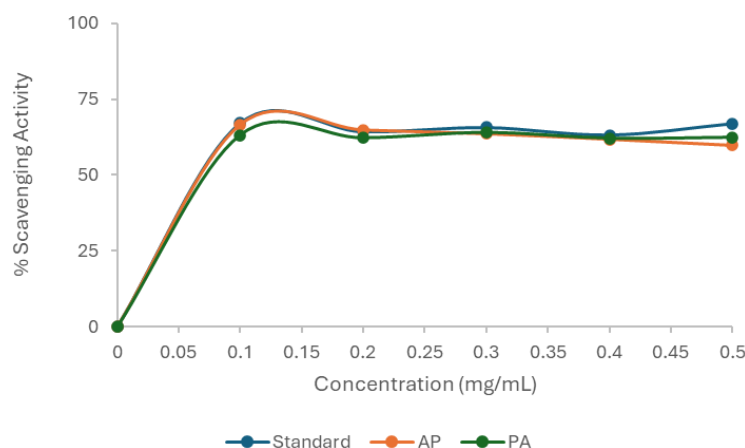


Figure 3: Hydroxyl Radical Scavenging Activity of Methanolic Extracts of *Andrographis paniculata* and *Petiveria alliacea*

DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Scavenging Activity

Figure 4: presents the DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity of *A. paniculata* and *P. alliacea* extracts. The DPPH

assay revealed that *P. alliacea* exhibited stronger free radical scavenging activity with a lower IC₅₀ value of 0.4 mg/mL compared to *A. paniculata* (0.5 mg/mL).

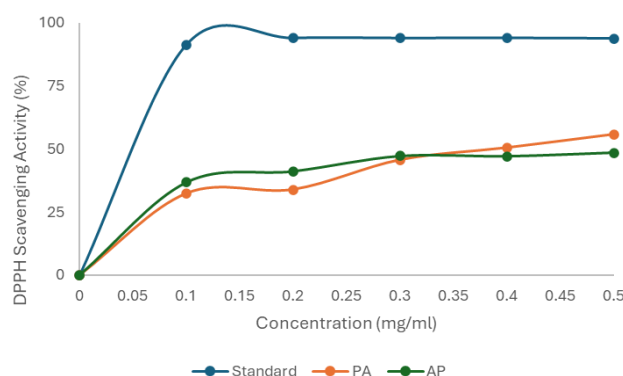


Figure 4 : DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Scavenging Activity of *Andrographis Paniculata* and *Petiveria Alliacea* Extracts

DISCUSSION

The use of plant-based snake repellents to deter snakes has been reported capable of significantly influencing public health policies, especially in regions with high incidences of snakebites (Ilundu and Lemy, 2018). These plant-based snake repellents offer eco-friendly alternatives to chemicals, thereby reducing environmental harm and supporting public health. Their integration into sustainable agriculture can also enhance ecosystem health

and lessen pesticide use. Several authors have reported the use of various plant species across different geographical regions (Ghana, Senegal, Nigeria) for their antidotal effects against snake envenomation and as natural snake repellents (Hassan *et al.*, 2020; Ahmed *et al.*, 2021; Asante-Kwatia *et al.*, 2021).

The use of *Andrographis paniculata* as snake repellent was reported by Shalini and Narayanan (2015) in their work. According to

their findings, the repellent property was linked to the presence of bitter lactones such as kalmegh and andrographolide in the leaves. The analysed antioxidants in this study as well as other secondary metabolites present have also been linked to the characteristic odour of the plant contributing to its snake repellent potential (Asante-Kwatia *et al.*, 2021). Other studies have also shown methanolic extracts of the plant parts demonstrate strong antioxidant activity across multiple oxidative tests (Akowuah *et al.*, 2008; Tewari *et al.*, 2010; Kurzawa *et al.*, 2015).

In this study, the extract of *A. paniculata* showed considerable antioxidant activity. The total phenolic content of 752 mgGAE/g indicated a high amount of phenolic compounds in the extract, capable of neutralizing free radicals. The total flavonoid content was equally high (499.1 mgQE/g), reflecting the presence of secondary metabolites with antioxidative potents (Chukwu *et al.*, 2024). The total antioxidant capacity (103.9 mg AAE/g) together with the FRAP value (49.19 mgAAE/g) recorded, indicated the potential of *A. paniculata* to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. Additionally, the hydroxyl radical scavenging activity indicated an IC_{50} of 0.075 mg/mL, revealing its potential against highly reactive oxygen species while the IC_{50} of 0.5 mg/mL recorded in the DPPH radical scavenging assay of *A. paniculata*, demonstrated a moderate hydrogen-donating activity.

Although bitter lactones such as andrographolide were not directly measured in this study, previous studies have documented their presence in *A. paniculata*, together with neoandrographolide, andrographolide, and 14-deoxy-11,12-didehydroandrographolide, and have been linked to antioxidant and therapeutic properties (Murthy and Dalawai, 2021; Isibor *et al.*, 2025). They are also known for their anti-inflammatory, antimicrobial, and antioxidant functions and contribute to the plants' characteristic bitterness. Bitter lactones have been reported as a key phytochemical contributing to the overall antioxidant properties of a plant (Laila *et al.*, 2020). Thus,

the antioxidant potency recorded in this study could be a reflection of a synergistic contribution from both polyphenols and these diterpene lactones. Generally, these results lend biochemical credence to the use of *A. paniculata* traditionally as a natural snake deterrent, with potential applications in both the protective and therapeutic contexts.

The FRAP result was slightly lower, however, the phenolic concentration as well as the flavonoid content recorded in this study for *A. paniculata* exceeded those earlier reported by Adetuyi *et al.* (2018) and Omiyale and Karigidi (2023). The high values recorded may be attributed to optimized extraction conditions. The total antioxidant capacity recorded in this

study confirmed the plant's strong oxidative defence, surpassing earlier findings by Omiyale and Karigidi (2023). The DPPH assay measuring hydrogen atom transfer capacity of the extract, showed a 0.5 mg/mL IC_{50} , supporting earlier findings and highlighting room for optimization compared to certain solvent fractions (Abdul Rahman *et al.*, 2017).

The phytochemical analyses in this work also revealed the antioxidant capacity in *Petiveria alliacea* revealing the total phenolic content of 710 mgGAE/g, total flavonoid content (398.3 mgQE/g) and the total antioxidant capacity reaching 87.32 mg AAE/g. The hydroxyl radical scavenging assay recorded for *P. alliacea* was similar to that of *A. paniculata* (IC_{50} = 0.075 mg/mL), but higher in DPPH assay than that of *A. paniculata* with a lower IC_{50} of 0.4 mg/mL. Previous studies have also reported that *P. alliacea* is rich in sulphur-based compounds (such as dibenzyltrisulphide and thiosulfinates) of biological activity and distinct pungent odours, and which may work together with phenolic antioxidants to enhance repellent and therapeutic actions of the plant (Olomieja *et al.*, 2021). The dual mechanisms of combining sulphur-based volatiles with antioxidant metabolites, sustains the case for *P. alliacea* as an effective, eco-friendly plant repellent. The results agreed with previous reports confirming the phytochemicals richness

of *P. alliacea* (De Sousa *et al.*, 1990; Benevides *et al.*, 2001).

CONCLUSION

This study revealed the presence of high antioxidant contents in *Andrographis paniculata* and *Petiveria alliacea*, thereby buttressing the significance use of the plants as ophidian repellents in community health safety, and provided a baseline antioxidant profiles that future researchers can expand upon. Some antioxidant compounds in plants such as phenolics, flavonoids, possess strong odors, bitter taste, or irritant properties that stimulate snakes' chemosensory organ and act as deterrents by causing sensory discomfort or avoidance behavior. However, Further in-vivo assays with snake's venom as well as valuating the plants long-term sustainability and comparing their performance with chemical repellents would help validate their use and support their integration into eco-friendly public health strategies for snakebite prevention.

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AUTHOR'S CONTRIBUTIONS

AAO: Collection of plant materials, data collection and curation, drafting the manuscript. **IMO:** Formal analysis, writing, reviewing and editing. **AKF:** Methodology, visualization, formal analysis, supervision. **FAE:** Conceptualization, collection of plant materials, formal analysis, supervision, review and editing.

POTENTIAL CONFLICT OF INTEREST

None

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