

Phytochemical and *in vitro* antsnake venom activity of the methanol leaf and stem bark extracts of *Leptadenia hastata* (Asclepiadaceae) against *Naja nigricollis* venom

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ABSTRACT

Snake envenomation is a major cause of death and morbidity in many developing countries. *Leptadenia hastata* (Pers.) Decne (Asclepiadaceae) has been reportedly used in traditional medicine as an antivenom, antiulcer, antidiabetic, analgesic, antibacterial, antiviral agent and cardiovascular disorders. This research design is to investigate the phytochemical analysis and phospholipase A₂ enzyme inhibition potential of *L. hastata* leaf and stem bark extracts using standard procedures. Preliminary phytochemical screening revealed the presence of key constituents such as carbohydrates, tannins, flavonoids, alkaloids, triterpenes, steroids, saponins, and diterpenes. The methanol leaf and stem extracts were able to inhibit the hydrolytic action of phospholipase A₂ enzyme in a concentration-dependent manner. The research findings lay credence to the folkloric claim of the leaf and stem of *L. hastata* as an anti-snake venom.

Keywords: *Leptadenia hastata*, Phytochemical, *Naja nigricollis*, Phospholipase A₂
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INTRODUCTION

Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries due to the unmatched availability of chemical diversity.^[1] It has been estimated that approximately over half of the pharmaceuticals in clinical use today are derived from natural products.^[2] Some natural product-derived drugs that are a hallmark of modern pharmaceutical care include quinine, theophylline, penicillin G, morphine, paclitaxel, digoxin, vincristine, doxorubicin, cyclosporine, and vitamin A among many other examples.^[2] For centuries, natural substances, particularly plants, have been used to control and treat diseases and this has culminated in the discovery of the majority of modern pharmaceutical agents.^[3]

Leptadenia hastata (Pers.) Decne a member of the Asclepiadaceae family are mostly herbs and shrubs with white sap comprising about 250 genera and 2,000 species, many of which are lianous and some cactus-like succulents with reduced leaves.^[4] *L. hastata* is referred locally in Nigeria as "yahdiya" in Hausa and as "hagalhadja" in Arabic. The plant leaves are used traditionally in the management of various ailments such as onchocerciasis,^[5] scabies,^[6] hypertension, catarrh, skin diseases, wound healing, snakebite, and prostate complaint.^[7]

Snakebite is a major cause of morbidity and mortality in Nigeria, especially in rural areas, where large numbers of envenoming and deaths are yearly reported. Venom from snake bite is largely composed of many complex compounds such as proteins, enzymes, neurotoxins, coagulants, anticoagulants, and other substances with cytotoxic effects. The venom is water-soluble, has a specific gravity of 1.03, and is acidic in nature.^[8] Phospholipase A₂ enzyme (PLA₂E) is known to stimulate the phospholipid membrane releasing arachidonic acid causing inflammation and pain at the bite site.^[9] Snake venom is hemotoxic, mainly affecting the circulatory system and muscular system, causing excessive scarring, hemorrhagic, coagulant defects, hypovolemic shock, pyrogen reaction, and serum sickness.^[10,11] The immediate administration of antsnake venoms is the only current treatment for snake bite

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victims which is accompanied by a number of side effects coupled with its unavailability and problem of storage conditions especially in the hot humid climate of Northern Nigeria thereby restricting its usage. The side effects of ASVs are largely due to the action of high concentrations of non-immunoglobulin proteins present in commercially available hyperimmune antivenom.^[12,13] Over the years, many attempts have been made for the development of snake venom antagonists, especially from plants sources;^[14] hence, this study explores the phospholipase A₂ inhibition potential of *L. hastata* leaf and stem bark against *Naja nigricollis* venom [Figure 1].

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The leaves and stem of *L. hastata* were collected in July 2019 from Dange town, Dange Shuni local government area of Sokoto State, Nigeria. They were authenticated at the herbarium unit, Department of Pharmacognosy and Ethnomedicine, Usmanu

Danfodiyo University Sokoto, Nigeria, where herbarium specimen were deposited and voucher number PCG/UDUS/894 was issued. The samples were shade dried at room temperature for 10 days, reduced to a fine powder using mortar and pestle, labeled and stored in an airtight glass container until ready for use.

Extraction of Plant Materials

The powdered leaves (84.23 g) and stem (64.42 g) of *L. hastata* were cold macerated using absolute methanol for 48 h. The extracts were concentrated using a rotary evaporator at 40°C at reduced pressure. The residues referred to as the methanol leaf and stem extracts were obtained and coded MEL and MES, respectively.

Preliminary Phytochemical Screening

Chemical tests were carried out on the MEL and MES extracts to identify the presence of various phytoconstituents such as alkaloids, flavonoids, and tannins, among others using standard procedures.^[15-18]

Snake Venom Sample

The venom sample of *Naja nigricollis* with an LD₅₀ value of 5.75 mg/kg was obtained from Dr. Amina Yusuf Jega of the Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto. It was preserved in a desiccator at 8°C until ready for use.

Table 1: Extraction yield of the plant extracts

Part used	Quantity macerated (g)	Volume of methanol used (mL)	Weight of obtained extract (g)	% yield
Leaves	84.23	750	16.10	19.12
Stem	64.20	750	21.05	32.80

Table 2: Preliminary phytochemical screening of *L. hastata* MEL and MES extracts

Phytoconstituents	Test	Observation	Inference (MEL)	Inference (MES)
Carbohydrates	Molisch	Red precipitate	+	+
	Fehling's	Red precipitate	+	+
Tannins	Ferric chloride	Precipitate	+	+
Flavonoids	Alkaline test	Orange color	+	+
Alkaloids	Wagner's	Cream-colored precipitate	+	+
	Mayer's	Reddish-brown absent	+	+
Triterpenes/Steroids	Salkowski's	Yellow color	-	+
Saponin	Frothing	Persisting froth	+	+
Diterpenes	Copper acetate	Emerald green color	+	+

+: Presence, -: Absence of phytochemical, MEL: Methanol leaf extract, MES: Methanol stem extract

Table 3: Phospholipase A₂ activity of MEL and MES extracts of *Leptadenia hastata*

Samples	Conc. (mg/mL)	ΔT	ΔpH	Fatty acid released (μmol)	Enzyme activity (μmol/FA/min)	Enzyme activity (%)	Enzyme inhibition (%)
MES	0.6	15	0.41	54.53	3.64	49	51
MES	0.3	16	0.46	61.18	3.82	50	50
MES	0.1	17	0.50	66.50	3.91	52	48
MEL	0.6	18	0.48	63.84	3.54	47	53
MEL	0.3	19	0.53	70.49	3.71	49	51
MEL	0.1	20	0.59	78.47	3.92	52	48
VENOM	0.1	15	0.85	113.05	7.53	100	0
VENOM	0.3	16	0.92	122.36	7.64	100	0
VENOM	0.5	17	0.98	130.34	7.66	100	0
ASV	-	-	-	-	0.93	27.1	72.1

MEL: Methanol leaf extract, MES: Methanol stem extract, ASV: Standard antivenom, ΔT: Change in time, ΔpH: Change in pH

Phospholipase A₂ Assay

Acidimetric assay for PLA₂, as described by Tan and Tan,^[19] was adopted. Constant volumes of a substrate comprising calcium chloride (18 mM), sodium deoxycholate (8.1 mM), and egg yolk were mixed and stirred for 10 min to produce a homogeneous egg yolk suspension. Using 1 M sodium hydroxide solution, the pH of the suspension was adjusted to 8.0. 0.1 mL of the snake venom (0.1- 0.5 mg/mL) was added each to 15 mL to initiate the process of hydrolysis. About 0.9% normal saline was used as the negative control. The pH of the suspension was observed after 2 min using a pH meter. While a 1.0 unit pH decline corresponds to 133 μmol fatty acids released in the egg yolk mixture, the enzymatic activity of PLA₂ was recorded in micromoles of fatty acids released/minute.

To test, the anti-snake venom potential of *L. hastata*. 0.1 mg of the snake venom was pre-incubated with 0.1-0.6 mg/mL concentration of the extracts to neutralize the hydrolytic action of PLA₂. The protection offered by the extracts that were calculated and represented in terms of percentage using the following expressions:

$$\text{Enzyme activity} = \frac{\mu\text{mol of fatty acid released}}{\text{Time taken in minute}} \quad (1)$$

$$\% \text{ Enzyme activity} = \frac{\text{Enzyme activity of the test sample}}{\text{Enzyme activity of the control}} \times 100 \quad (2)$$

$$\% \text{ Enzyme inhibition} = 100 - \text{Enzyme activity} \quad (3)$$

RESULTS AND DISCUSSION

Percentage Yield

The result of the extraction yield of the leaves and stem bark of *L. hastata* with respect to the quantity macerated is presented in Table 1.

Phytochemical Screening

Preliminary phytochemical screening of the MEL and MES of *L. hastata* showed the presence of important secondary metabolites (Table 2).

Polyphenols were the major secondary metabolites found in the extracts which could be attributed to the antsnake venom activity of the plant. Thus, studies have shown that polyphenols possess protein – binding and enzyme inhibiting properties, which could invariably inhibit snake venom PLA₂ activities, an enzyme presents in cobra venom.^[20]

Phospholipase A₂ Assay

The result of the phospholipase A₂ inhibition of the snake venom when challenged with the MEL and MES extracts of *L. hastata* is presented in Table 3.

PLA₂ assay is an important assay mainly used to test for the anti-snake venom of plant extracts. In this assay, the enzyme was hydrolysed and free fatty acids were released in the presence of sodium deoxycholate. *N. nigricollis* venom was found to liberate fatty acids, which was measured in terms of a decrease in pH of egg yolk suspension. MES inhibited the hydrolytic action of PLA₂ enzyme in a concentration-dependent manner with 48, 50, and 51% inhibition at 0.1, 0.3, and 0.6 mg/mL, while MEL had an inhibition of 48, 51, and 53% at 0.1, 0.3, and 0.6 mg/mL. Comparably, MEL had better antsnake venom effect than MES at the highest concentration. These results significantly blocked many of the toxic effects of *N. nigricollis* venom *in vitro*. Which could possibly be due to the extracts acting through a mechanistic intervention rather than a direct physical interaction with the venom *in vitro* as this is similar to the mode of action of many polyphenolic compounds found in plant extracts.^[21]

This result is in agreement with the report of Ode and Asuzu^[22] who used different venom concentrations different from that reported with the results following the same trend, although snake venom neurotoxin content varies both qualitative and quantitative from region to region within the same species.^[23,24] Further, research is currently being advanced in the fractionation assay of the *L. hastata* extract to identify the component(s) responsible for the inhibitory effect reported.

CONCLUSION

The present findings validated the ethnomedicinal claim of the use of *L. hastata* as an antsnake venom, particularly *N. nigricollis* venom. The plant extracts were significantly effective in neutralizing the main toxic and enzymatic effects of *N. nigricollis* venom. The antivenom properties of both plant extracts were potent enough to neutralize the lethality and various pharmacological activities of *N. nigricollis* venom. This activity might be corroborated with the diverse phytoconstituents revealed to be present in the plant materials. The plant could serve as therapy for patients with snakebite envenomation.

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