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## Anti-Snake venom Activities of the leaf extracts of *Asystasia gangetica* (L) and *Newbouldia leavis* (p. Beauv)

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### ABSTRACT

The leaf extracts of two medicinal plants *Asystasia gangetica* (L) and *Newbouldia leavis* (p. Beauv) were assessed for anti-snake venom activity *in vivo* using mice. The result of the phytochemical analysis revealed that the two plants contain flavonoids, glycosides, saponins, tannins and alkaloids. The methanolic extracts of the two plants *A. gangetica* (L) and *N. leavis* (p. Beauv) significantly ( $p < 0.05$ ) neutralized the *Naja melanoleuca* venom-induced lethality activity in the mice. The extracts (flavonoids, tannins and saponins) of the two plants also showed significant ( $p < 0.05$ ) neutralization of the venom-induced lethality activity in mice. The work confirmed that *Asystasia gangetica* and *Newbouldia leavis* possess significant anti-venom activity and can therefore be used in the treatment of snake bites.

**Keywords:** Snake Venom, Medicinal plants, *Naja melanoleuca*, *Newbouldia leavis*, *Asystasia gangetica*

### 1. INTRODUCTION

In many parts of the world, especially in the tropical countries of Africa, South America and South East Asia, snakebite remains a serious medical, social and economic problem. African countries are global diversity center for feared snake families like Vipers, Cobras, etc.

The risk of snakebite is higher in the rural areas, where most people engage in agricultural, pastoral and other outdoor activities. The only medical approach to snakebite is the use of

antisera, which have their own limitations. These include high cost and lack of availability which makes it difficult for the rural dwellers to access. There is also the problem of storage difficulty and short expiry dates which restrict their usage. Snake venom antiserum or AVS is associated with administration problems, the exact dosage is also a present problem. AVS administration is equally associated with hypersensitivity reactions which require further medical research [1, 2]. Many medicinal plants have been used in folk and traditional medicines against snakebites [3]. However till date, such drugs are yet to be made available in the market [4]. Using a combination of rural survey and immunoassay techniques, it is estimated that approximately 23,000 people die each year as a result of snake bite in the West African sub-region (**Figure 1**).



**Fig. 1.** Unbearable pains caused by the bites of venomous snake. Source: [3]

Snake venom is secreted by venomous snakes and is synthesized and stored in the venomous gland. The gland which secretes the zootoxin is a modification of the parotid salivary gland. They are situated on each side of the head below and behind the eye encapsulated in muscular sheath. The glands have large alveoli in which venom is stored before being conveyed by the duct to the tubular fangs, through which it is injected. Snake venom is a complex mixture

of enzymatic and toxic proteins, which include phospholipase A<sub>2</sub>(PLA<sub>2</sub>s), mycotoxins, hemorrhagic metalloproteinases and other proteolytic enzymes, coagulant components, cardiotoxins, cytotoxins, and neurotoxins [5-7].

Snake venom comprises many complex compounds, like proteins, enzymes, neurotoxins, coagulants, anti-coagulants, as well as other substances with cytotoxic effects (**Figure 2**). The venom is water-soluble and has a specific gravity of 1.03 and is acidic in nature [8]. An enzyme known as phosphodiesterase A<sub>2</sub> lyse the cell membrane of erythrocytes which leads to haemolysis. Digestion is made possible by oxidases and proteases. Inorganic cations, like sodium, potassium, magnesium, as well as small amount of zinc, nickel, cobalt and iron are equally found in snake venom. The toxins found in the venom of a snake could either be neurotoxins (which attack the nervous system) and cytotoxins (which attack the cells).



**Fig. 2.** Snake venom  
Source: [3]

Many medicinal plants have been recommended for the treatment of snake bites. *Acalypha indica*, *Hemidermis indica*, *Pluchea indica*, *Guiera senegalensis*, *Pentaclethra maculosa*, *Tamarindus indica*, *Parkia biglobosa* and others are known to inhibit a variety of snake venom toxicity [9-14]. *Asystasia gangetica* belongs to the family *Acanthaceae*. Common names are creeping foxglove (English) Iyeri nti umuagbogho, ikere, Nni-nwaturu (Igbo), and Lobiri (Yoruba). It is widely distributed from tropical Asia to South Africa. In Gold Coast (Ghana), the plant is commonly used as a woman's medicine, a decoction taken internally and also mixed with pepper and administered as an enema during the later months of pregnancy to lighten the pains of childbirth [15]. In Southern Nigeria it is applied to the wound after piercing the lobe of the ear. It is also used in many parts of Nigeria for the management of asthma [16].

*Newbouldia leavis* belongs to the family *Bignoniaceae*. It is known as 'Aduruku' in Hausa, 'Ogirisi' in Igbo, and 'Akoko' in Yoruba languages [17]. *Newbouldia leavis* is a native of tropical Africa and grows from Guinea savannah to dense forests. In Nigeria, the bark is

chewed and swallowed for stomach pains, diarrhea, and toothache [18]. The plant has been found to be effective in the treatment of elephantiasis, dysentery, rheumatic swellings, syphilis, constipation, pile earache, sore feet, chest pain, epilepsy, and convulsion in children [19]. It was against this backdrop that the interest arose to evaluate the anti-snake venom activities of the leaf extracts of *Asystasia gangetica* and *Newbouldia leavis*.

## **2. MATERIALS AND METHODS**

### **2. 1. Venom sample**

The lyophilized snake venom (*Naja melanoleuca*) was obtained from SIGMA-ALDRICH INC., 3050 spruce street, St. Louis no 63103 USA and was preserved in a freezer for further use. 100 mg of the lyophilized venom was dissolved in 5 ml of 0.9% saline (20 mg/ml stock venom solution) and centrifuged at 2000 rpm for 10 min. The supernatant was used as venom and kept at 4 °C for further use.

### **2. 2. Collection of plant materials**

Fresh leaves of *A. gangetica* and *N. leavis* were collected from the premises of Nnamdi Azikiwe University, Awka, Anambra state, Nigeria. The plant was identified and authenticated by Prof. J. C. Okafor, a consultant plant taxonomist of No. 7 Dona Drive, Independence Layout, Enugu, Nigeria. The fresh leaves were dried in a shade, blended with a laboratory milling machine into powdered form and stored in tight containers until required for further use.

### **2. 3. Preparation of methanolic extracts**

The powdered plant materials, *A. gangetica* (100 g) and *N. leavis* (100 g) were soaked in 800 ml and 1200 ml of methanol, respectively, for 48 hours. These were sieved using a mesh and filtered using the No. 1 filter paper to obtain a clear filtrate. A semi-solid extract was obtained using BUCHI rotary evaporator (Rotavapor R-210/R-215) at 40 °C and kept in a desiccator at room temperature. The plant extracts were dissolved in saline and centrifuged at 2000 rpm for 10 minutes at room temperature. The supernatant was used for further investigation and kept at 4 °C. The plants extracts were expressed in terms of dry weight.

### **2. 4. Determination of phytochemical constituents**

The freshly prepared extracts were subjected to a standard phytochemical analysis for different constituents, such as tannins, alkaloids, flavonoids, glycosides, saponins, etc., as described in [20].

### **2. 5. Test animals**

Albino mice (18-20 g) of both sexes were used for the studies of acute toxicity of the extracts and venom, and in the experiments and venom neutralization. All the animals were conditioned in standard cages. They were kept in a 12/12-h light- dark cycle. Food pellets and water were available *ad libitum*. Each experimental group was matched with parallel control group treated with saline solution (0.9%). Experiments were carried out at laboratory temperature (30-35 °C).

## 2. 6. Acute Toxicity (LD<sub>50</sub>) of the extracts

A total of 18 mice were used. 3 mice each were injected intraperitoneally (ip) with graded doses (0.4 mg/kg, 0.5 mg/kg, 0.6 mg/kg, and 0.7 mg/kg) of the venom. The doses were given according to the body weight. Mortality was recorded within 24 hours [21].

## 2. 7. Neutralization of lethal effects of venom (crude)

*In vivo* neutralization test, as described by [22], was followed with modification. Mice were injected with crude extract preparation (1000 mg/kg) ip 30 min prior to the administration of lethal dose of 0.7 mg/kg body weight. The deaths were recorded for seven days after admixture injection of venom.

## 2. 8. Preparation of plants polyphenols

Extraction of tannins, saponins, and flavonoids were carried out according to the methods described in [23-25].

## 2. 9. Neutralization of the lethal effects of venom (polyphenols)

*In vivo* neutralization test, as described by [22], was followed with a modification. Mice (n=5) were injected ip 30 min prior to the administration of lethal doses of 0.7 mg/kg body weight. The deaths were recorded for 7 days after admixture injection of venom.

## 2. 10. Statistical analysis

Test of significant differences were carried out using Chi-square ( $\chi^2$ ) method. A P-value of <0.05 was considered significant.

## 3. RESULTS

The phytochemical screening of the crude methanolic leaf extracts of *A. gangetica* and *N. leavis* revealed the presence of flavonoids, glycosides, saponins, tannins, and alkaloids (Table 1).

The venom of *Naja melanoleuca* was highly lethal to mice with a lethal dose of 0.7 mg/kg body weight of mouse by ip route. The acute toxicity studies carried out using albino mice showed the two plants *A. gangetica* and *N. leavis* to be non-toxic up to 5000 mg/kg body weight through intraperitoneal route. The intraperitoneal administration of the crude extracts of the two plants, *A. gangetica* and *N. leavis* (1000 mg/kg) 30 min before the injection of the venom (0.7 mg/kg) gave 60% and 80% protection, respectively (Table 2).

The intraperitoneal (ip) administration of the polyphenol extracts (1000 mg/kg) 30 min before the injection of the venom (0.7 mg/kg) gave 60%, 80%, and 60% protection, respectively (Table 3). Also, the intraperitoneal (ip) administration of the polyphenol extract (flavonoids, tannins, and saponins) of *N. leavis* plant (1000 mg/kg) 30 min before the injection of the venom (0.7 mg/kg) gave 60%, 80%, and 80% protection, respectively (Table 4).

**Table 1.** Phytochemical constituents of methanolic extracts of *A. gangetica* and *N. leavis*.

Phytochemical constituents	Abundant/ level	Abundance/level
flavonoids	++	++
glycosides	+++	+++
saponins	++	+++
tannins	+++	+++
alkanoids	+++	+
terpenoids	–	–
steroids	–	–

**Table 2.** Lethal action neutralized of *Naja melanoleuca* venom by methanolic crude extract of *A. gangetica* and *N. leavis*

Groups n=5	Treatment	Dose (mg/kg); l.p	No of animal protected	0/0 protection
i	Control (venom)	0.7	0/5	0
ii	<i>A.gangetica</i>	1000	3/5	60
iii	<i>N.leavis</i>	1000	4/5	80

**Table 3.** Lethal action neutralization of *Naja melanoleuca* venom by *A. gangetica* polyphenols

Group n=5	Treatment	Dose (mg/kg); l.p	No of animals protected	0/0 protected
i	Control (venom)	0.7	0/5	0
ii	flavonoid	1000	3/5	60
iii	tannins	1000	4/5	80
iv	saponin	1000	3/5	60

P < 0.05 as compared with the control.

**Table 4.** Lethal action neutralization of *Naja melonoleuca* venom by *N. leavis* polyphenols.

Group n=5	Treatment	Dose (mg/kg); i.p	No of animals Protected	0/0 Protected
i	Control (venom)	0.7	0/5	0
ii	flavonoid	1000	3/5	60
iii	tannins	1000	4/5	80
iv	saponins	1000	4/5	80

P < 0.05 as compared with the control.

#### 4. DISCUSSION

The result of phytochemical screening of the two plants, *A. gangetica* and *N. leavis* revealed the presence of polyphenol (tannins, saponin, and flavonoids). This suggests that *A. gangetica* and *N. leavis* leaf extracts have anti-snake venom activities since polyphenols possess protein-binding and enzyme inhibiting properties which also inhibit snake venom phospholipase A2 (PLA2) activities, an enzyme present in cobra venom [26]. Phospholipase A2 (PLA2) is almost invariably the most toxic component of the venom and responsible for a wide range of pharmacological effects, including neurotoxicity, cardiotoxicity, hemolytic and damage to biological membrane [8]. The acute toxicity signs noted in the mice when injected with the venom were lethargy, refusal to eat, salivation, respiratory problems, stiffening of neck, convulsion, and death within 2 hours interval. This result tallies with the work of [27] that elapid (cobra) are mainly neurotoxin, causing muscular and respiratory paralysis, convulsion and drowsiness which may lead to coma. The acute toxicity studies carried out using mice showed *A. gangetica* and *N. leavis* to be non-toxic up to a dose of 5000 mg/kg body weight as no death of animal was recorded. Acute toxicity values, greater than 5000 mg/kg body weight, were of no practical interest [21].

*A. gangetica* and *N. leavis* can then be said to be non-toxic and can therefore be safely used for food and medication. This result is in line with the acute toxicity results of the work of [28].

The methanolic leaves extracts of the *A. gangetica* and *N. leavis* produced a significant (P < 0.05) anti-snake venom protection when compared with the control. The significant anti-snake venom protection is indicated by the survival of the greater number of the test animals when compared with the control. The polyphenol (flavonoid, tannin, and saponin) of *A. gangetica* and *N. leavis* showed significant (P < 0.05) anti-snake venom protection when compared with the control.

*A. gangetica* and *N. leavis* showed no significant difference (P > 0.05) in their neutralization effect on the venom. They have the same neutralization potential and can both be used as substitute in the treatment of snake bite. *N. leavis* ability to protect greater number of animals could be as a result of its more active saponin constituent.

## 5. CONCLUSION

The result of this study showed that *A. gantica* and *N. leavis* leaves possess snake venom inhibiting activity. This confirms the claim of traditional medicine practitioners and other users that extract from the leaves of *A. gangetica* and *N. leavis* is used in the treatment of snake bite.

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