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PHYTOCHEMICAL ANALYSIS OF CRUDE EXTRACTS FROM *ANNONA SENEGALENSIS (L)* AND ITS ANTI-SNAKE VENOM POTENTIAL ON ALBINO RATS

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Abstract
This work seeks to determine the phytochemical analysis of crude extracts from Annona senegalensis (L) and its anti-snake venom potential on albino rats, the plant part (stem) was collected using cutlass, air-dried, pulverized into powder using mortar and pestle,the powdered sample was soaked using cold maceration method. Two black-necked spitting cobra were captured and their venom milked by holding the snake at the head region using a conical flask covered with a black polythene and the snake brought closer to the flask such that any bite on the covered flask allows for the collection of the venom. Phytochemical screening of petroleum ether and ethanol crude extracts of A. senegalensis revealed the presence of alkaloids, flavonoids, saponins, glycosides and tannins. The lethal dose of snake venom capable of killing 50% of the rats was observed to be 0.194mg/ml among other concentrations (0.240mg/ml, 0.068mg/ml and 0.036mg/ml) administered. The effective dosages of petroleum ether and ethanol extracts of A. senegalensis needed for survival of venom injected rats are 75mg/ml and 100mg/ml respectively. This study showed that A. senegalensis possesses bioactive ingredients that have the potential to be used in managing black neck spitting cobra snakebites.

Keywords: *Phytochemical analysis, Crude extracts, Annona senegalensis, Anti-snake venom.*

Introduction

Traditional medicine is an ancient art of medical practice that many rural populations of developing countries rely on for their health care due to economic accessibility and the belief that natural products are more acceptable to the body and have fewer side effects than synthetic drugs (Aida *et al*., 2001). Despite the fact that traditional medical practices are limited by lack of precise diagnosis, standardization, hygiene, ethics in dosaging and sometimes exaggerated claims which cannot be proved scientifically; traditional medicine still enjoys a wide acceptability among the citizens of these nations. Most bioactive substances are discovered as a result of scientific investigations of the plants used in traditional medicine (Ahmad and Beg, 2001). Many natural products isolated from plants serve as template or lead molecules for the design and generation of new drugs. The potential of plants as sources of new drugs is still largely unexplored. Among all species of plants in the world, very few have been investigated phytochemically and their fraction subjected to biological or pharmacological screening (Aida *et al*., 2001). Chemical compounds in plants mediate their effects on the human body through processes similar to those used in conventional drugs thus, there is no wide variation between herbal and conventional medicines, however, they both have the same potential to cause harmful side effects (Mesfin *et al*., 2009). *Annona senegalensis (A. senegalensis)* makes up one of the important plants used in herbal medicine.

A. senegalensis takes the form of either a shrub or small tree, growing between 2-6m tall. Occasionally it may grow as tall as 11m. It has a bark of smooth or coarse texture that can be gray-brown. It is atraditional food plant in Africa. The fruit of *A. senegalensis* have the potential to improve food nutrition, boost food security, foster rural development and support sustainable land care. It has green to blue-green leaves which are alternate, simple oblong to ovate or elliptic from 18.5cm long by 2.5-11.5cm wide with nearly hairless upper sides. It is native of many places including east, northeast, west, western central and southern Africa (Bawaskar, 2004). In Africa, traditional treatments made from plants play an important role in the health of a million people thus, its significance as one of the surest means to achieve total health care coverage worldwide. The indigenous systems of medicine use medicinal plants for the treatment of snake bites (Techacondo, *et al*., 2011).

Over the years, there have been several casualties of black neck spitting cobra *(Naja nigricollis)* attacks which has raised concerns. Despite the efforts put forward by scientists in remediating black neck spitting cobra attacks using synthetic drugs and other plants that include *Perkia biglobosa* (Asuzu and Harvey, 2003), treatments are still expensive thus the need for an alternatively cheaper and easier method. Therefore, this work is aimed exploring the phytochemical analysis of crude extracts from *Annona senegalensis (L)* and its anti-snake venom potential on albino rats as a model to that of human treatment.

Materials and Methods

Sampling, test animals, source of snake venom and cold maceration of stem bark

The bark of the stem of *A. senegalensis* was collected using a cutlass from Tsiga, Bakori local government area, Katsina State, Nigeria. Plants were collected at flowering stage in the evening of August, 2017. It was identified by Mr. Iliya Muhammad and authenticated by Prof. Sanusi Muhammad using a standard chart in the Department of Biological Sciences, Federal University Dutsin-Ma, Katsina State, Nigeria. Collected plant sample was air-dried at room temperature before cold maceration.

Both male and female Swiss albino rats (8-12kg), were purchased from the Department of veterinary medicine, Ahmadu Bello University (ABU) Zaria, Kaduna State, Nigeria and were kept at the animal house (Biological garden) to

acclimatize for one week in August. The rats were fed with standard animal feed until the experiment.

Two black neck spitting cobras (*Naja nigricollis*) were captured in July, (2017), identified using a standard chart, kept, and fed at the herpeterium of the faculty of veterinary medicine, ABU Zaria. Venom was collected using the snake milking method described by Theakston *et al*., (2003). The snakes were held at the neck region with thumb and index finger using a snake handler. Conical flask top covered with black polythene was brought close to the mouth of the snake in such a way that any bite on the polythene allows dropping of the venom into the conical flask. The venom was crystallized using silica gel and was stored at -4^{0}C until required. This was considered as the crude venom.

Powdered stem bark (200g) was extracted serially using two solvents of different polarity in three days. The solvents used were petroleum ether and ethanol. Each extract was evaporated to dryness in a fume chamber and stored until required (Aida *et al*., 2001).

Phytochemical screening of stem back extracts

Phytochemical screening using petroleum ether and ethanol extract of the plant were examined for the presence of alkaloids, flavonoids, saponins, tannins, glycosides and volatile oil as reported by Asuzu and Harvey., (2003). To test for tannins, flavonoids and saponins, 3 drops of ferric chloride ($FeCl_3$) solution, ammonium solution and olive oil were added to individual test tubes containing 2ml of the extract (100mg/ml). The mixtures for tannins and flavonoids were observed for colour change or presence of precipitates while that of saponins was checked for formation of frothing foam after vigorous shaking and inversion of tubes. In determining the presence of sugar and carbohydrate, freshly prepared fehling's solution A and B were added to 1ml of the extract (100mg/ml) in a test tube then boiled in water bath for 5 mins. Observation was made for presence of brick-red precipitate. In testing for carbohydrate, 2 drops of iodine solution (1%) was added to 1 ml of the extract (100mg/ml) then observed for blue black coloration.

In determining the presence of sterols and terpenes, *Senegalensis* extract (5ml) was evaporated to dryness in a beaker. The residue was dissolved in 1ml of acetic anhydride and 1 ml of chloroform. The solution was transferred to a dry test tube and 2 ml of tetraoxosulphate was added. Observation was checked for formation of a brownish or violet ring at the zone of contact with the supernatant indicates presence of sterols and terpenes.

Preparation of stock venom of *Naja nigricollis*

The snake venom (25mg) was dissolved in 10ml of clinical normal saline solution from which 4.35ml was measured out into 10ml flask and used to prepare 1.00mg/ml stock solution. From the stock solution, 0.240mg/ml, 0.194mg/ml, 0.068mg/ml and 0.036mg/ml solutions were prepared for the study.

Determination of lethal (LD50) and effective dose (ED50) of snake venom

The venom solutions prepared at various concentrations (0.240mg/ml, 0.194mg/ml, 0.068mg/ml and 0.036mg/ml) were administered intraperitoneally to four groups of six rats each using insulin syringe and needle in the evening. The rats were kept in separate wooden cages and were observed for deaths. The LD50 was determined using the method described by Theakston *et al*., (2003). Control rats were injected with normal saline solution only.

From the stock of plant extracts obtained, 100mg/ml, 75mg/ml, 50mg/ml and 25mg/ml were prepared in normal saline solution. To determine ED50, each solution of these concentration was injected intraperitoneally into the four groups of rats that had been previously administered with the venom after five (5) minutes. Controlled rats were treated with crude 0.194mg/ml of venom. The rats were kept in separate wooden cages for observation within five hours (Theakston *et al.,*2003).

Results

Phytochemical and pharmacological screening of plant extract

The result of phytochemical screening is presented in table 1. Alkaloids, flavonoids, tannins, saponins and glycosides were detected however, volatile oils (Terpenes) were not seen.

LD50 Testing.

Result obtained from the lethal dose (LD) assay following observations of the rats for effects of the cobra venom is shown in table 2. Hundred percent (100%) survival was observed in the samples that were injected with normal saline. In all the rats injected with the different concentration of the venom, the same symptoms (swollen body, weakness and bleeding) were observed, however, the minimum dose of venom that killed 50% of the rats was 0.194mg/ml. This concentration was taken as the LD50.

ED50 Testing.

Hundred percent (100%) mortality was recorded in the samples injected with crude venom (table 3 and table 4). The effective dosages of petroleum ether and ethanol extracts of *Annona senegalensis* needed for good survival of venom injected rats are 75mg/ml and 100mg/ml.

Table 1: Phytochemical screening of petroleum ether and ethanol extracts

Extracts	Petroleum ether	Ethanol
Saponins	+	+
Tannins	+	-
Alkaloid	+	+
Flavonoids	+	+
Glycosides	_	+
Terpenes	_	_

Keys:
(+): Detected
(-): Not detected

Table 2: LD50 of *Naja nigricollis* venom injected in albino rats

Treatments	Number of rats tested (Experimental)	Number of rats tested (Control)(mg/ml)	Venom Conc. (Experimental)	Mortality (%) (Control)	Mortality (%)
1.	6	6	0.240	6 (100)	0 (0.00)
2.	6	6	0.194	6 (100)	0 (0.00)
3.	6	6	0.068	2 (33.33)	0 (0.00)
4.	6	6	0.036	0 (0.00)	0 (0.00)

Table 3: The effective dose (ED50) of the petroleum ether extracts needed for the survival of 50% of the rats

Treatments	Number of rats tested (Experimental)	Number of rats tested (Control)	Conc.of extract (mg/ml)	Mortality (%) (Experimental)	Mortality (%) (Control)
1.	6	6	25	6 (100)	6 (100)
2.	6	6	50	6 (100)	6 (100)
3.	6	6	75	2 (33.33)	6 (100)
4.	6	6	100	0 (0.00)	6 (100)

Table 4: The effective dose (ED50) of the ethanol extracts needed for the survival of 50% of the rats.

Treatments	Number of rats tested (Experimental)	Number of rats tested (Control)	Conc. of extract (mg/ml)	Mortality (%) (Experimental)	Mortality (%) (Control)
1.	6	6	25	6 (100)	6 (100)
2.	6	6	50	6 (100)	6 (100)
3.	6	6	75	2 (33.33)	6 (100)
4.	6	6	100	0 (0.00)	6 (100)

Discussion

A. senegalensis contain phytochemicals that include alkaloids, flavonoids and saponins which is in line with the work of Swarna and Ravidhran, (2013) and Arrais-Silva *et al*., (2014) that realized the same phytochemicals in the stem and leaves while studying some medicinal plants. These critical phytochemicals especially flavonoid could be responsible for retraction of hemorrhage induced by the snake venom as described by Emmanuel *et al.,* (2015) who reported 75% survival having performed similar research using the leaf extract of *A. senegalensis* on mice treated with *Echis ocellatus* venom.Bernard *et al.,* (2015) also reported alkaloids, flavonoids and saponins to have the highest mean concentrations in the leaves of some medicinal plants which agrees with results obtained in table 1. This study also observed the presence of these metabolites in the extracts of the two solvents (petroleum ether and ethanol) used for the crude extraction of the plant used in this study. The absence of tannins in ethanol extract (table 1) contradicts the findings of Ijaiya *et al.,* (2014) who recorded high concentration of tannins extracted from leaves and roots of the same plant using similar solvent. This suggests a very low concentration or absence of this metabolite in the stem back of *A. senegalensis* as with other parts of the plants. The presence and absence of tannins and glycoside respectively in petroleum ether extracts (table 1) disagrees with the results obtained by Yisa *et al.,* (2010) who recorded the absence and presence of the two constituents having used the same solvent for extraction. These variations could arise from difference in the plant parts sampled. Yisa *et al.,* (2010) sampled seed plant of *A. senegalensis* while results from table 1 was restricted to the stem back. This suggests that phytochemical constituents vary quantitatively in various plant parts.

Results of LD50 reported 0.194mg/ml as the minimum dose capable of killing 50% of the rats (table 2). This is in contrast with the findings of Thomas., (2012) who observed a lethal dose of 1.15mg/kg in related experiment however, the size, location and age of the snake can influence the observed variation. ED50 for both petroleum ether (table 3) and ethanol (table 4) extracts showed 100% survival of venom treated rats injected with 100mg/ml of each crude extract which is in accordance with the findings of Emmanuel *et al.,* (2015) who recorded 75% survival in similar experiment using leaf extracts as stated earlier however, 25% significant difference suggests that the stem back extract could possess a stronger anti-venom activity as with that of the leaf extracts. Higher percentage survival of rats treated with stem extract compared to that of leaves could imply a higher concentration of anti-snake venom significant phytochemicals thus, a confirmation of quantitative variation in phytochemical metabolites of the various parts of the plant.

CONCLUSION

This research is placed at laying the basis for developing antivenom therapy for *black neck spitting cobra* attacks. Although, establishing our findings to the number of hours/days in which the therapy will be effective after snake bite is yet to be carried out phytochemical screening of *A. senegalensis* indicates the presence secondary metabolites that include alkaloids, flavonoids, tannins, saponins and glycosides. The minimum dose of venom capable of killing 50% of the rats is 0.194mg/ml. The effective dosages of petroleum ether and ethanol extracts of *A. Senegalensis* needed for survival of venom injected rats are 75mg/ml and 100mg/ml respectively, therefore, *A. senegalensis* possesses bioactive ingredients that have the potential to be used in managing *black neck spitting cobra* snakebites.

References

Aida, P., Rosa, V. Blamea, F., Tomas, A. and Salvador, C. (2001) Paraguan Plants used in Traditional Medicine. *Journal of Ethnopharmacology*. 16:93-98.

Ahmad, I. and Beg, A. Z. (2001). Phytochemical and antimicrobial studies on forty Indian Medicinal Plants Against Multi-drug Resistant Human Pathogens. *Journal of Ethnopharmacology* 74:87-91.

Arrais-Silva, W.W., Nunes, P.S.G., Carvalho, J.D., Brune, M.W., Arrais-Lima, C. andBatalini, C. (2014). Preliminary phytochemical and antileishmanial studies of the ethanolic extracts of Pterodon pudescens. *Rev. bras. Plantas med*. 16: 561-565.

Asuzu, I. U. and Harvey, A. L. (2003). The anti-snake venom activities of *Perkia biglobosa* (*Fabaceae*) stem bark extract. *Journal of toxicology*. 42:763-767.

Bawaskar, H. S. (2004). Snake venoms and antivenom critical supply issues. *Journal of the association of physicians of India*. 52:11-13.

Bernard, O.E., Chimezie, E., Pat, N.O. and Sampson, C. (2015). Phytochemical Evaluation of Leaves of some Medicinal Plants from Parts of Niger Delta.*IOSR Journal of Pharmacy and Biological Sciences.*10: 26 – 31.

Emmanuel, A., Ebinbin, A. and Amlabu, W. (2014). Detoxification of Echis ocellatus venom-induced toxicity by Annona senegalensis Pers. *Journal of Complementary and Integrative Medicine*, 11: 93-97.

Ijaiya, I.S., Arzika, S. and Abdulkadir, M. (2014). Extraction and Phytochemical Screening of the Root and Leave of Annona Senegalesis(Wild Custad Apple).*Academic Journal of Interdisciplinary Studies.*3: 9 – 15.

Mesfin, F., Demissew, S. and Teklehymenot, I. (2009). Ethonobotanical study of world medicinal plants. *Journal of ethnobiological studies* 9:28-32.

Swarna, J. and Ravidhran, R. (2013). Pharmacognostical and phytochemical evaluation of *Talinum triangulare* (Jacq.) wild. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2: 249.

Techacondo, T., Karou, S., Batawila, K., Anani, K., Gbeassor, M. and Comlansouza, C. (2011). Herbal remedies and their adverse effects in traditional medicine. *African journal of Traditional Compliment Alternative Medicine*.8: 460-463.

Theakston, R. D., Warrel, D. A., and Giriffiths, E. (2003). A report of a World Health Organisation and control of anti-venoms. *Journal of Toxicology*. 41:541-557.

Thomas, S. (2012). LD50 for various snakes. Australian venom and Toxin database. www.seanthomas.com. Accessed 7th, November, 2017.

Yisa, J., Egila, J.N. and Darlinton, A.O. (2010). Chemical composition of Annona senegalensis from Nupe land, Nigeria.*African Journal of Biotechnology*. 9: 4106-4109.