Exploring the Antivenom Potentials of *Crinum Jagus* Bulbs in the Treatment of Snake Bite from Girei, Adamawa State, Nigeria

[*]Anthony Amakor and Mohammed Hassan Shagal

Department of Chemistry, Modibbo Adama University Yola, Adamawa State, Nigeria

[*]Corresponding Author

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ABSTRACT

Crinum jagus is a plant barely used in the treatment of snake bite in Nigeria and other African countries. This study focuses on evaluating the efficacy of the bulb of the plant against venom from Carpet viper snake. The plant samples were obtained, dried, blended while successive extraction was carried out using methanol. The crude methanolic extract and two distinct fractions obtained from column chromatography were used. Crystallized venom of Carpet viper was administered at varying concentrations. Forty mice were allocated into ten equal groups of four each. Mice in groups 4, 2 and 1 were given 150, 300 and 450 mg/kg of fraction 1 of *Crinum jagus* bulb respectively. Group 6, 5 and 7 were given same dose of fraction 2 obtained from *Crinum jagus* bulb. Group 7, 8 and 9 were given the crude methanolic extract at 150, 300 and 450 mg/kg, while group 10 served as the control. Apart from the Carpet viper venom that was administered intraperitonially, all others were administered orally. The Mice were observed over 24 hours before readings were taken. All the animals in the control group succumbed to envenomation within 7 to 15 minutes. In contrast, mice treated with both fractions and the crude methanolic extract exhibited significant survival rates, with survival rates increasing with higher dosages. Specifically, two mice from group 5 (F2 = 300 mg/kg), one from group 4 (F1 = 150 mg/kg), and one from group 3 (Cr = 300 mg/kg) survived the venom. The average weights of the mice across the groups varied, but no significant weight loss or adverse reactions were observed in the treated groups. These findings suggest that *Crinum jagus* bulb extracts possess potential antivenom properties against Carpet viper venom.

Keywords: Antivenom, chromatography, *Crinum jagus*, methanolic extract, snake

INTRODUCTION

Snakebites remain a significant public health concern, particularly in regions where venomous snakes coexist with human populations [10]. This has caused high morbidity and mortality [11]. The World Health Organization estimates that approximately 5.4 million snakebite incidents occur annually, resulting in over 100,000 deaths and severe complications [2]. Timely administration of effective antivenom is crucial in reducing morbidity and mortality associated with snake envenomation [6]. However, the availability, cost, and limited efficacy of conventional antivenoms necessitate the exploration of alternative sources for snakebite treatment [7]. Research has shown that antimicrobials derived from plants are safer than their synthesized ones [3]. *Crinum jagus* is a medicinal plant indigenous to various parts of Africa. Traditionally, different parts of the plant, including the bulb and root, have been used for their therapeutic properties, including wound healing and antimicrobial effects [8]. Previous studies have reported the presence of various bioactive compounds in *Crinum jagus*, suggesting its potential for the treatment of snakebite envenomation [13]. The classes of bioactive antivenom compounds include flavonoids, phenolic compounds, steroids, terpenes and alkaloids [14].

This present study aims to investigate the antivenom immunoglobulin activities of *Crinum jagus* bulb methanolic extracts on venom-induced animals. The objective is to assess the potential antivenom properties of the extracts, contributing to the ongoing efforts in developing safer and more accessible treatments for snakebite envenomation.

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Crinum jagus, a plant found in Nigeria and other African countries has long been utilized in traditional medicine [12]. Despite its historic significance, a comprehensive investigation into the antivenom potentials and pharmacological applications of the bulb remains limited [9]. This study would bridge this gap by accessing the bulb for its antivenom activity.

MATERIALS AND METHODS

Sample Collection and Preparation

Crinum jagus plants were collected from Girei Local Government Area of Adamawa state, Nigeria. The samples were collected in September 2023. The bulbs of the *Crinum jagus* plant were washed using distilled water, out-most skin peeled off, while the bulb was chopped into small portions to ensure uniformity during drying. It was allowed to dry at room temperature. The dried sample was ground into fine powder, and preserved in sample containers for further analysis. Extractions were carried out using Soxhlet extraction method, according to Saim *et al*., 1997[1]. The extracts were concentrated to dryness using a rotary evaporator. The crude extract was kept in an airtight container at room temperature till further use.

Snake Venom

The snake venom from *Echis ocellatus* was sourced from the Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University Zaria, Nigeria.

Experimental Animals

Forty albino mice weighing 20 – 36 g were used for this study. They were procured from the National Veterinary Research Institute, NVRI, Vom, Plateau state, Nigeria. Animals were kept at the Micro biology laboratory, in neat cages at room temperature, and allowed to acclimatize for 12 days under standard conditions before the study was carried out. Mice were fed with standard pelleted feed and distilled water were provided *ad libitum*

RESULTS AND DISCUSSION

Percentage Yield

The percentage yield of the extract is shown in table 1 using the expression below:

$$\%\ yield = \frac{Weight\ of\ Extract}{Weight\ of\ sample\ before\ extraction} \times 100$$

Table 1: Percentage yield of *Crinum jagus* bulb extract

Weight of sample (g)	Weight of Extract (g)	% Yield
440	98	22.27

Column Chromatography

The solvents used for the fractionation of the crude extract were n-hexane, ethyl acetate and methanol in the ratio 40:20:40. This ratio showed a better Rf value using a thin layer chromatographic plate (TLC). This mixture ensured that compounds in the samples were separated based on differences in their Polarities. Fractions obtained in beakers were subjected to TLC and those with similar Rf values were pooled together. A total of two distinct fractions were obtained, namely Fraction 1 (F1) and Fraction 2 (F2).

Preliminary Antimicrobial Studies

Antimicrobials in this study were carried out using clinical strains of *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*) and *Salmonella typhi (S. typhi)* as shown in table 2.

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Table 2: Antimicrobial activities, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Crinum jagus* methanolic bulb fractions against *E. coli*, *S. aureus* and *S. typhi*

Fraction	Test Organisms	200 µg/ml	100µg/ml	50µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	MIC µg/ml	MBC µg/ml
F1	***E. coli***	-	-	-	-	-	+	12.5	100
F1	***S. aureus***	-	-	-	+	+	+	50	> 200
F1	***S. typhi***	+	+	+	+	+	+	> 200	> 200
F2	***E. coli***	-	-	-	+	+	+	50	> 200
F2	***S. aureus***	-	-	-	+	+	+	50	> 200
F2	***S. typhi***	+	+	+	+	+	+	200	200

Key: F1 = Fraction 1; F2 = Fraction 2; + = Growth, – = No growth

In vitro Antimicrobial Assay of Fractions

Graded concentrations (6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml and 200 µg/ml) of the sample were prepared. 2ml of each concentration was added to about 18mls of nutrient agar at 50^0C. These were mixed together and poured into the sterile plates.

Exactly 0.2 ml of 1:100 dilution of the culture of each bacterium were used to seed sterile. The plates were allowed to set. After this, the organisms were streaked on the plates at different concentrations in order to determine the minimum concentration that would inhibit the growth of the test organisms. All plates used were incubated at 40^0C for 24 hours. After 18 hours, the plates were observed for the growth of the microorganisms after the incubation period.

Table 2 outlines the minimum inhibitory concentration and minimum bactericidal concentration of the bulb methanolic extract of *Crinum jagus* against *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi*. Two fractions, F1 and F2, were evaluated for their antibacterial activity.

The highest MIC obtained against *Escherichia*. *coli* for fraction one was 12.5µg/ml while the highest obtained against *Staphylococcus aureus* and *Salmonella typhi were 50 and* > 200 respectively. The least for F1 fraction was 100 µg/ml against *E. Coli* and >200 for *Staphylococcus aureus* and *Salmonella typhi respectively.*

For the second fraction obtained from the column (F2), The highest MIC obtained against *Escherichia Coli* and *Staphylococcus aureus* were both 50µg/ml, while that of *Salmonella species* was *200* µg/ml. The least for F2 fraction was > 200 for *E. Coli* and *Staphylococcus aureus* while *Salmonella species* was at 200 µg/ml.

For F1, the highest MIC was 12.5 µg/ml against *Escherichia. coli,* while for *Staphylococcus aureus* and *Salmonella typhi*., it was 50 µg/ml and > 200 µg/ml, respectively. The lowest MIC recorded for F1 was 100 µg/ml against *Escherichia*. coli, > 200 µg/ml for both *Staphylococcus aureus* and *Salmonella typhi*. The activities of this fractions are consistent with the result from [4], who found notable antibacterial effects of different solvent extracts of *Crinum jagus* bulb against common pathogens, including *Escherichia. coli, Staphylococcus aureus*, and *Salmonella typhi*., exhibiting significant inhibitory activity.

Similarly, F2 displayed a maximum MIC of 50 µg/ml against both *E. coli* and *Staphylococcus aureus*, whereas for *Salmonella typhi*., it was 200 µg/ml. The lowest MIC observed for F2 was > 200 µg/ml against *E. coli* and *Staphylococcus aureus*, while for *Salmonella typhi*., it was 200 µg/ml. This result is synonymous with the findings by [5], where there was a significant inhibitory activity of *Crinum jagus* extract against clinical isolates of bacteria, including *E. coli*, *Staphylococcus aureus*, and *Salmonella typhi*. Their study supported the broad-spectrum antibacterial potential of *Crinum jagus* bulb extract.

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These findings suggest that both fractions (F1 and F2) exhibit varying degrees of antibacterial activity against the tested organisms, with F1 showing slightly higher potency against *E. coli*, and F2 demonstrating consistent MIC values across different bacteria. Further analysis and characterization of these fractions could provide insights into their mechanisms of action and potential applications in antibacterial therapy. This result is in agreement with that of Akintola *et al.,* (2017) which said that all fractions obtained from the extract of *Crinum jagus* inhibited the growth of test organisms used.

In-vivo Activity of *Crinum Jagus* Extract and Its Fractions on Venom Induced Mice

Table 3 shows the groups, dose per kg administered and the weight (in grams) of the mice used for this study.

Table 3: Groups, dose per kg administered and the weight (in grams) of the mice

	G4 (F1 = 150mg/kg)	**G2 (F1 = 300mg/kg)**	**G1 (F1 = 450mg/kg)**	**G6 (F2 = 150mg/kg)**	**G5 (F2 = 300mg/kg)**	**G7 (F2 = 450mg/kg)**	**G8 (Cr = 150mg/kg)**	**G3 (Cr = 300mg/kg)**	**G9 (Cr = 450mg/kg)**	**G10 (Neg)**
Weight (g)	15.11	26.89	32.45	17.65	* 22.69	34.8	18.41	25.62	26.3	19.05
	18.94	18.97	34.02	15.04	* 27.45	29.37	16.47	* 24.06	24.32	16.94
	19.48	20.89	28.79	16.53	23.19	28.21	16.44	24.06	27.48	21.26
	* 20.26	21.64	30.16	18.04	20.18	28.48	19.73	21.98	22.39	15.78
AVG. Wgt.	**18.4475**	**22.0975**	**31.355**	**16.815**	**23.3775**	**30.215**	**17.7625**	**23.93**	**25.1225**	**18.2575**

Key: G = groups, F1 = fraction 1, F2 = fraction 2, Cr = Crude extract * = mice that survived after 24 hours.

Based on the results of the statistical analysis, several conclusions can be drawn regarding the effectiveness of different fractions and controls in preventing the death of mice:

Comparison between Group 4 (F1 = 150 mg/kg) and Group 5 (F2 = 300 mg/kg) showed that there was no statistically significant difference in mean weights between the two groups ($t_{(3)} = -2.402$, $p \approx 0.054$), indicating that the application of F1 at 150 mg/kg and F2 at 300 mg/kg did not significantly affect the life of the mice.

However, when comparing Group 5 (F2 = 300 mg/kg) and Group 7 (F2 = 450 mg/kg), a statistically significant difference was observed ($t_{(3)} = -3.425$, $p \approx 0.022$), suggesting that the application of F2 at a higher concentration of 450 mg/kg resulted in a significant improvement in preserving the life of the mice compared to F2 at 300 mg/kg.

In contrast, comparison between Group 8 (Cr = 150 mg/kg) and Group 10 (Negative control) indicated no significant difference in mean weights ($t_{(3)} = -0.526$, $p \approx 0.625$), suggesting that the application of the crude extract at 150 mg/kg did not have a significant impact on preventing mouse death, after coming in contact with the venom of carpet viper compared to the negative control group.

The results suggest that the effectiveness of the fractions varied depending on the concentration applied. Specifically, while F2 at a higher concentration (450 mg/kg) showed promise in preserving the life of mice, F1 at 150 mg/kg and Crude extract at 150 mg/kg did not demonstrate significant effects compared to their respective controls. These findings provide valuable insights into the potential applications of these fractions in mitigating the death of mice when they are bitten by carpet viper, contributing to the understanding of the use of *Crinum*

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jagus in treatment of snake bite in animals.

CONCLUSION

The results showed that the fractions obtained from the extract of *Crinum jagus* bulb exhibits significant pharmacological potential as evidenced by its diverse antimicrobial activities on some selected microorganisms and antivenom activity on mice. These findings underscore the importance of *Crinum jagus* bulb as a valuable source of natural bioactive compounds with promising medicinal properties.

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