

## EVALUATION OF ANTIHAEMORRHAGIC AND ANTIOXIDANT POTENTIALS OF *Crinum jagus* BULB

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**ABSTRACT:** *Crinum jagus* plant belongs to Amaryllidaceae. The plant is distributed worldwide in the tropics and subtropics. The extract was prepared by cold maceration in 80% methanol at 37°C with intermittent shaking for 48 h. A yield of 12.6% w/w dry extract was obtained. *C. jagus* extract at various concentrations (2.5, 5.0 and 10 µg/1.5 µl) completely inhibited the haemorrhagic activity of *Echis ocellatus* venom (4.2 µg/1.5 µl). Phytochemical analysis revealed the presence of alkaloids, tannins, reducing sugars, sterols and terpenes in the crude extract. *C. jagus* extract possessed a significantly high antioxidant activity, an effect that was more pronounced when compared with vitamin C at increased concentrations (50-400 µg/ml). The bulb extract of *C. jagus* could therefore be used as adjunct therapy to handle myriads of health challenges.

**Keywords:** Envenomation; *Crinum jagus*; Antioxidant activity; *Echis ocellatus*; Antihemorrhagic, DPPH.

### INTRODUCTION

Snakebites have constituted a particularly alarming public health problem in most developing countries. In Nigeria, the rural communities of the Northern states are the worst affected. The state governments continued to expend appreciable part of the annual budgets on importation of polyvalent antivenins (PVA). Unfortunately, PVA are not only expensive, some cause anaphylaxis in patients (Grant *et al.*, 2000; Gutierrez *et al.*, 1980; Ferreira *et al.*, 1992). PVA are sometimes, unavailable due to storage difficulties as a result of epileptic power supply in our country. The need for the development of specific or polyvalent antivenin from naturally occurring substances becomes paramount.

It is however, on record that herbal plants were employed by traditionalists to effectively manage various cases of envenomation in different places in our country (Akunyili and Akubue, 1987). Many snake venoms, especially those from viperine and crotaline snakes cause local and systemic bleeding (Theakston and Reid, 1983). Bleeding is basically the consequence of damage to blood vessel walls by venom components. The capability of a plant extract to neutralize or inhibit the haemorrhagic activity of snake venoms underscores its efficacy as an antidote and therefore needs to be measured (WHO, 1981). Antihemorrhagic and antioxidant tests are *in vitro* models; hence the problems of cost, pain and ethical considerations in the use of animals for experimentation are eliminated (Russell and Burch, 1959).

## MATERIALS AND METHODS

### Solutions, reagents, Drugs and chemicals

Freshly prepared solutions and analytical grade chemicals were used in all the experiments. Freeze dried *E. ocellatus* venom (Liverpool School of Tropical Medicine, UK), DPPH, Ascorbic acid (Sigma Aldrich, Germany), spectrophotometer (Spectrolab, USA) and a locally fabricated incubator were used.

### Fertile eggs

Day-old fertile eggs were obtained from a hatchery, John Gloree in Nsukka, Enugu State, Nigeria.

### Preparation of plant material

Fresh bulbs of *C. jagus* plant were collected in March, 2010 from farm locations in Ochimode village, Oju Local Government Area of Benue State, Nigeria. The plant was duly identified by Mr Ozioko, a botanist with the University of Nigeria, Nsukka. The plant bulbs were cut into small pieces with kitchen knife, dried under mild sunlight and pulverized into powder with a laboratory mill. 500 g of the powder was exhaustively extracted with 2 L of 80% methanol. The extraction was by cold maceration at 37°C with intermittent shaking for 48 h. The extract was concentrated by vacuum rotary evaporation and stored in a refrigerator at 4°C. The concentration of the extract was determined and the percentage yield calculated.

### Antihaemorrhagic test

The test was conducted following a standard method (Dunn and Boone, 1976; Sells *et al*, 1997). Day-old fertile eggs were disinfected with serviette soaked in methanol before incubation till day 4 in a humid incubator at 38°C. The eggs were cracked on day 4 into cling film hammocks following a standard method (Sells *et al*, 1997) and incubated further till day 6. Discs of 2 mm diameter cut with a hand punch from filter paper (Whatman no. 2) were separately impregnated with a standard haemorrhagic dose (SHD) of *E. ocellatus* venom (4.2 µg/1.5 µl) only. Various concentrations (2.5, 5.0 and 10.0 µg/1.5 µl) of *C. jagus* extract and the same volume (4.2 µg/1.5 µl) of *E. ocellatus* venom were mixed individually and applied to different paper discs. Each of the discs was placed on the yolk sac membrane over a major bilateral vein and left for 3 h for haemorrhagic corona to form. The coronas were measured with a transparent metre rule. Control experiments were performed with the buffered saline solution used to prepare the extract and venom solutions. Readings were taken in duplicates and recorded. The minimum concentration required to abolish haemorrhage was recorded as the minimum effective neutralizing dose (MEND).

### Phytochemical tests

Preliminary phytochemical tests were carried out on *Crinum jagus* extract (CJE) using the method of Trease and Evans (1989). Equal volume of distilled water in a separate test tube served as the control for each of the tests.

### Test for alkaloids

To a test tube containing 2 ml (100 mg/ml) of CJE each time, 3 drops of either Dragendorff's, Mayer's or Wagner's reagent were added, shaken and the mixture observed for colour change or presence of precipitate.

**Test for flavonoids****Ammonia test**

To 2 ml of the extract in a test tube was added 3 drops of ammonia solution. The content was mixed and then observed for colour change or presence of precipitate.

**Test for tannins**

To a test tube containing 2 ml of the extract (100 mg/ml) was added 3 drops of ferric chloride ( $\text{FeCl}_3$ ) solution. The mixture was observed for colour change or presence of precipitate.

**Test for saponins****Emulsifying test**

To a test tube containing 2 ml of the extract (100 mg/ml) was added 3 drops of olive oil and the content shaken vigorously, properly mixed by inverting the tubes several times for formation of frothing foam.

**Test for sugar**

Freshly prepared Fehling's solutions A and B were added to 1 ml of the extract (100 mg/ml) in a test tube and then boiled in water bath for 5 min and observed. Presence of brick-red precipitate indicates presence of a reducing sugar.

**Test for carbohydrates (reduction test)**

To 1 ml of the extract (100 mg/ml) was added 2 drops of 1 % iodine solution, and then observed for blue black colouration.

**Molisch's test**

This reaction is a general test for the presence of carbohydrate and other organic compounds that could form furfuraldehyde (furfural) or hydroxymethyl furfuraldehyde (hydroxyl-methylfurfural) in the presence of concentrated tetraoxosulphate (IV) acid ( $\text{H}_2\text{SO}_4$ ). In the Molisch's test, the basic principle is one in which  $\text{H}_2\text{SO}_4$  hydrolyses glycosidic bonds to give the monosaccharides, which are then dehydrated to furfural and its derivatives.

For the test, two drops of  $\alpha$ -naphthol solution was added to 2 ml of the extract and mixed thoroughly. Then 1 ml quantity of concentrated  $\text{H}_2\text{SO}_4$  was carefully poured down the side of the tube and observed.

**Test for sterols and terpenes**

*C. jagus* extract (5 ml) was evaporated to dryness in a beaker. The residue was dissolved in 1 ml of acetic anhydride and 1 ml of chloroform. The solution was transferred to a dry test tube and 2 ml of concentrated  $\text{H}_2\text{SO}_4$  was added. Formation of a brownish or violet ring at the zone of contact with the supernatant indicates presence of sterols and terpenes.

**Test for polyuronides**

To a test tube containing 2 ml of absolute ethanol, 1 ml of *C. jagus* extract (100 mg/ml) was added drop-wise and then observed for formation of violet or blue precipitate.

### Evaluation of antioxidant capacity of *C. jagus* Extract with DPPH (1, 1- diphenyl-2-picrylhydrazyl radical) photometric assay

The free radical scavenging activity of the extract was analysed by the DPPH assay following a standard method (Mensor *et al*, 2001) using a spectrophotometer. CJE (2 ml) at varying concentrations ranging from 10-400 µg/ml each was mixed with 1 ml of 0.5 mM DPPH (in methanol) in a cuvette. The absorbance at 517 nm was taken after 30 minutes of incubation in the dark at room temperature. The experiment was done in triplicate. The percentage antioxidant activity was calculated as follows:

% Antioxidant Activity [AA] =  $100 - \left[ \frac{(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}) \times 100}{\text{Abs}_{\text{control}}} \right]$ . 1 ml of methanol plus 2 ml of the extract was used as the blank while 1 ml of the 0.5 mM DPPH solution plus 2 ml of methanol was used as the negative control. Ascorbic acid (vitamin C) was used as reference standard.

Data were collected as mean antioxidant activity for the various concentrations (10, 50, 100, 200 and 400 µg/ml) and analysed using one-way ANOVA followed by Duncan New Multiple Range post-hoc test, p-values < 0.05 were considered statistically significant.

## RESULTS

### Plant extract

*Crinum jagus* extract (CJE) was yellowish in colour with a sweet aromatic odour. The total solid recovered from the extract was 12.6% (w/w).

### Antihaemorrhagic test

Standard Haemorrhagic Dose of *E. ocellatus* venom (4.2 µg/1.5 µl) produced a distinct haemorrhagic lesion, 2 mm in diameter (Plate 1). A mixture of *E. ocellatus* venom (4.2 µg/1.5 µl) and various concentrations (2.5, 5.0 and 10.0 µg/1.5 µl) of *C. jagus* bulb extract did not produce any haemorrhagic spot (Plate 2).

### Phytochemical test

Phytochemical analysis revealed the presence of alkaloids, tannins, reducing sugars (glucose or fructose), sterols and terpenes but no flavonoid, starch, saponin or mucilage in the extract of *C. jagus* bulb.

### Antioxidant test with DPPH

The crude extract of *C. jagus* at the same concentrations (50-400 µg/ml), exhibited appreciable antioxidant capacity when compared with ascorbic acid. At 50 µg/ml, CJE had percentage antioxidant activity of  $81.47 \pm 0.3$  while that of ascorbic acid was  $74.1 \pm 0.1$  and at 400 µg/ml, the extract produced AA of  $84.43 \pm 4.2$  percent but that of ascorbic acid at this concentration was  $79.21 \pm 1.6$  (Table 2, Figure. 1).

## DISCUSSION

*C. jagus* extract at 2.5, 5.0 and 10.0 µg/1.5 µl completely blocked the haemorrhagic activity of *E. ocellatus* venom (Plate 1). The antihemorrhagic activity of the extract could be an important therapeutic potential for handling envenomation from viperid snakes. Most bites from viperid snakes can cause serious and uncontrolled bleeding from gums, eyes, scars of old wounds, skin, orifices etc. (Rosenfeld, 1971). The haemorrhagins are major constituents of venoms from viperid and crotalid snakes, causing loss of red blood cells from blood vessels in poisoned victims (Gutierrez *et al*, 2000). Neutralization of systemic effects of venoms is vehemently rational for successful snakebite therapy.



Plate 1: Control, *Echis ocellatus* venom (4.2 µg/ml) without extract. Forceps point to the spot of haemorrhagic lesions.

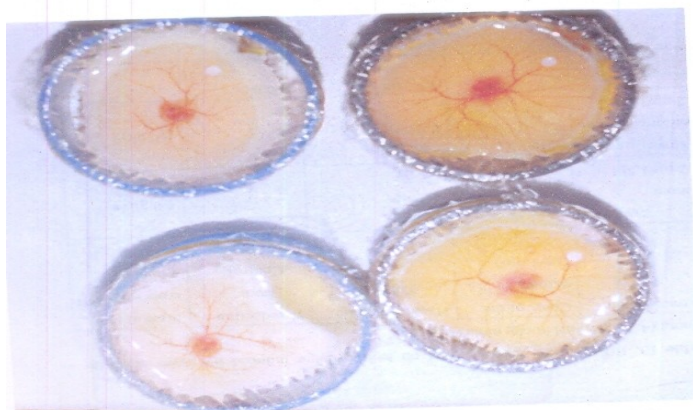


Plate 2: *E. ocellatus* venom (4.2 µg/1.5µl) + *C. jagus* extract (2.5 µg/1.5µl, 5.0 µg/1.5µl and 10.0 µg/1.5µl).

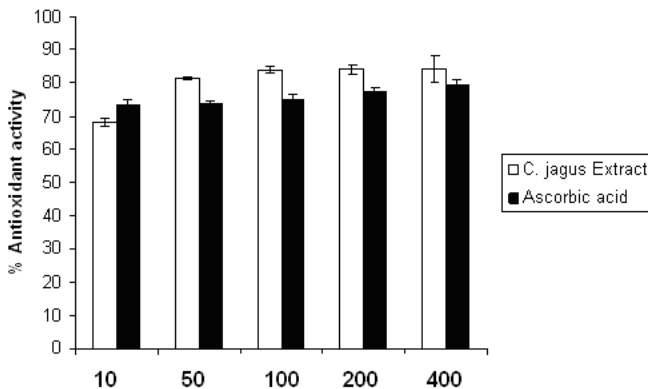


Figure. 1. The Antioxidant activity of *C. jagus* Extract (CJE) and Ascorbic acid determined with DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging method. Values are mean ± SE. \*P<0.05 show significant difference in the same group.

CJE exhibited a high profile of antioxidant activity, a dose dependent effect which became more pronounced when compared with vitamin C at increased concentrations (50-400 µg/ml). Reactive oxygen species (ROS) are oxygen-centered molecules which include the non-radicals, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), hypochlorous acid (HOCl), hydroxyl anion (HO) and single oxygen (O<sub>2</sub>); and the radicals, superoxide anion (O<sub>2</sub><sup>-</sup>), hydroxyl radical (HO<sup>•</sup>), and nitric oxide (NO<sup>•</sup>) (Al-Omar *et al*, 2004). Potentially harmful reactive oxygen species are produced as a consequence of normal aerobic metabolism. Snakebites are most often accompanied by signs of inflammation and local tissue damage. Neutrophils and macrophages are induced to produce superoxide radical anion (O<sub>2</sub><sup>-</sup>) which belongs to a group of reactive oxygen species and this reacts with cellular lipids leading to the formation of lipid peroxides and the observed necrosis.

The high antioxidant property of the extract could be a reason behind its popular folkloric use as anecdotal therapy for snakebites (Ode and Asuzu, 2006). Oxidative stress-induced tissue damage with ROS is implicated as a cause and consequence of a variety of disorders (Trouillas *et al*, 2003). Many of such diseases and disorders include ageing, allergies, Alzheimer's disease, angina, arthritis, asthma, arterosclerosis, bleeding gums, cancer, cataracts, liver cirrhosis, diabetes type II, dry skin, fatigue, heart attacks, haemorrhoids, hypertension, kidney damage, leukemia, liver damage, male sexual inadequacy, memory loss, menstrual disorders, migraine, multiple sclerosis, night blindness, Parkinson's disease, phlebitis, prostate problems, psoriasis, retinopathy, varicose veins etc.

Antioxidants consist of vitamins, polyphenols, flavonoids, minerals and endogenous enzymes such as superoxide dismutase, catalase and glutathione peroxidase that have the capability to neutralize unstable molecules called free radicals (Réka and Varga, 2002). Vitamin A (retinol), vitamin C (ascorbic acid), vitamin E (tocopherol) and selenium are valuable antioxidants. Antioxidants are among the first link between chemical reactions and biological activity. *C. jagus* bulb extract contains alkaloids, tannins, reducing sugars (glucose or fructose), sterols and terpenes.

## CONCLUSION

This study demonstrated that *C. jagus* extract has proven antihaemorrhagic and antioxidant activities which could complement other protective and therapeutic mechanisms in the extract. The findings support earlier report (Ode and Asuzu, 2006) that the methanolic extract of the bulb of *C. jagus* plant possessed anti-snake venom activities. However, further work is needed to fractionate CJE, isolate and elucidate the active principle responsible for its antihaemorrhagic and antioxidant activities.



## REFERENCES

- Akunyili, D. and Akubue, P.I. (1987). Antisnake venom properties of the stem bark juice of *Schumanniohyton manicum*. *Fitoterapia* 58, 47-49.
- Al-Omar, M. A., Beedham, C. and Alsarra, I. A. (2004). Pathological roles of reactive oxygen species and their defence mechanisms. *Saudi pharmac. J.* 12 (1), 1-18.
- Dunn, B.E; Boone, M.A. (1976). Growth of the chick embryo *in vitro*. *Poult. Sc.* 55, 1067-1071.
- Ferreira, M.L; Moura-da-Silva, A.M; Franca, F.O.S; Cardoso, J.L; and Mota, I. (1992). Toxic activities of venoms from nine Bothrops species and their correlation with lethality and necrosis. *Toxicon* 30, 1603-1608.
- Grant, G.A; Ownby, C. and Peel, R. (2000). Comparison of the neutralizing abilities of three antivenom preparations. XIIIth World congress of the International society on toxinology, Paris. p. 228.
- Gutierrez, J.M; Chaves, F. and Bolano, R. (1980). Estudio comparativo de venenos de ejemplares recién nacidos y adultos de *Bothrops asper*. *Rev de Biologica Tropicana* 28, 341-351.
- Mensor, L. L., Fábio, S. M., Gilda, G. L., Alexandre, S. R., Tereza, C. D., Cintia, S. C. and Suzana, G. L. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother. Res.* 15, 127-130.
- Ode, J.O. and Asuzu, I.U. (2006). The anti-snake venom activities of the methanolic extract of the bulb of *Crinum jagus* (Amaryllidaceae). *Toxicon* 48, 331-342.
- Réka, S. and Varga, I. S. (2002). Total antioxidant power in some species of Labiatae (Adaptation of FRAP method). Proceedings of the 7<sup>th</sup> Hungarian Congress on Plant Physiology 46 (3-4), 125-127.
- Rosenfeld, G. (1971). Symptomatology, pathology and treatment of snakebites in *South American Journal of veterinary science* 2, 345-403.
- Trouillas, P., Calliste, C. A., Allias, D. P., Simon, A., Marfak, A., Delage, C. and Durous, J. I. (2003). Antioxidant, anti-inflammatory and antiproliferative properties of sixteen water plant extracts used in the Limousine countryside as herbal teas. *Food Chem.* 80, 399-407.
- Russel, W. and Burch, R.I. (1959). The principles of humane Experimental technique. Muthuen, London. pp. 5-10.
- Sells, P.G; Richards, A.M; Liang, G.D. and Theakston, R.D. (1997). The use of hen's eggs as an alternative to the conventional *in vivo* rodent assay for antidotes to haemorrhagic venoms. *Toxicon* 36, 1413-1421.
- Theakston, R.D.G. and Reid, H.A. (1983). Development of simple standard assay procedures for the characterization of snake venoms. *Bulletin of the World Health Organization* 61, 949-956.
- Trease, E. C. and Evans, W. C. (1989). Pharmacognosy 12<sup>th</sup> ed. Bailliere and Tindall, London. pp. 687-689.
- World Health Organisation (1981). Progress in the characterization of venoms and Standardization of antivenoms. Offset publication, No.58. World Health organization (WHO), Geneva.