CYTOTOXICITY AND ANTI-PROLIFERATIVE STUDIES OF *Crinum Jagus* L. (Amaryllidaceae) BULB EXTRACT

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ABSTRACT

Crinum jagus is a flowering plant, commonly called poison bulb. Traditionally, the bulb extract is used in the treatment of several ailments including cancer. Cancer is a global cause of death characterized by abnormal cell proliferation. This research thus aimed to identify secondary metabolites present in the crude extract of *C. jagus* and evaluate its cytotoxic and antiproliferative activities using bench top assays. Whole *C. jagus* bulb was collected, air-dried under the shade and extracted into distilled methanol. The extract was concentrated *in vacuum* and subjected to; phytochemical analysis, brine shrimp lethality (BSL) assay, *Sorghum bicolor* radical and *Allium cepa* root growth inhibitory assays. Data obtained was analyzed by Graphpad prism version 6.0. The whole bulb on extraction had a percentage yield of 12.15 % $^{w}/_{w}$. The phytochemical content of the extract includes alkaloids, flavonoids, tannins and some glycosides. The extract demonstrated concentration dependent brine shrimp lethality (LC_{50} of 65.62±0.74 µg/mL), *Sorghum bicolor* radical growth inhibition (IC_{50} = 5.36±3.21µg/mL) and significant *Allium cepa* root growth inhibition comparative to cyclophosphamide (a standard anticancer drug). The extract was found to be rich in secondary metabolites which elicited significant cytotoxicity and antiproliferative activities. This is the first report of antiproliferative activity of *C. jagus* bulb extract. Hence, this study justifies the traditional use of the bulb in the treatment of cancer.

Keywords: *Crinum jagus,* Brine Shrimps, *Sorghum bicolor, Allium cepa,* Cytotoxicity, antiproliferative

INTRODUCTION

Cancer is a global cause of death characterized by proliferation of abnormal cells and there are about a hundred thousand new cases reported in Nigeria annually (Ferlay and Bray, 2010), it was estimated in a WHO report that by the year 2020 there will be more than 30 million people living with cancer worldwide (WHO, 2002; Lyerly *et al*., 2010). Hence, there is a need to increase effort toward the search for new anticancer agents.

Since ancient times medicinal plants have been used in the treatment of several diseases (Salawu *et al*., 2017) and over 80% of modern Africans are still relying on plant-based medicines for their primary health care needs (Ekor, 2014). Currently

medicinal plants are used in the development of novel drugs, such as artemisinin, a novel anti-malarial compound isolated from a Chinese medicinal plant *Artemesia annua* (Muangphrom *et al*., 2016). Statistically, more than 75% of all anti-infectives are derived from plants sources (Cragg *et al*., 2005; David *et al*., 2012), and medicinal plants still remain a viable source of novel bioactive compounds (Kumar *et al.*, 1997; Cragg & Newman, 2013).

Crinum jagus is a flowering plant that belongs to the Amaryllidaceae family. It is commonly called poison bulb and widely used traditionally in the treatment of wounds and several other ailments (Udegbunam *et al*., 2015). In southern Nigeria, *C. jagus* is used for the treatment of memory loss and other mental symptoms associated with ageing (Ogunkunle & Olopade, 2011). The methanol extract of *C. jagus* has been reported to have anti-snake venom activities (Ode & Azusu, 2006). Traditionally, the bulb extract of *C. jagus* is used in the treatment of several other ailments including cancer. Meanwhile, the focus of this research is to identify the secondary metabolites present in *C. jagus* crude extract and to screen the extract for cytotoxic and antiproliferative activity using Brine Shrimps Lethality and other *in vitro* antiproliferative bench to assay.

MATERIALS AND METHODS

Plant Collection

Whole bulb of *C. jagus* was obtained from Ipata market in Ilorin, Kwara State. It was identified and authenticated at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Ilorin, Kwara State, Nigeria, a voucher number UILH/001/1022 was issued.

Plant Preparation

The whole bulbs of *Crinum jagus* were washed and cut into smaller bits. It was air-dried under the shade and extracted into distilled methanol. The extract was concentrated using a rotary evaporator (40 - 50°C), weighed, and refrigerated (stored) at 4ºC until needed.

Phytochemical Screening of *C. jagus* Crude Extract

Crinum jagus bulb extract was screened for the presence of secondary phytochemicals using the method described by Sofowora (2008) and Trease and Evans (2009).

Biological assays

Brine shrimp lethality (BSL) assay

The eggs of *Artemia salina* were hatched by incubating the eggs in natural seawater at room temperature for 48 h. The BSL assay was conducted using a method described by McLaughlin (1991). Ten milligrams of *C. jagus* bulb extract was diluted to 1000 µg/mL by dissolving in 2 mL of 0.5% DMSO (dimethyl sulfoxide) in sea water. Serial dilution of the extract was done in 96-well microplates in triplicates. The negative control wells contained 0.5% DMSO in sea water. A 250 µL suspension of nauplii in the extract was added to each well. The plates were incubated at RT (25-33°C) for 24 h. The number of dead nauplii in each well was counted.

Sorghum bicolor Radical Growth Inhibitory Assay

The *Sorghum bicolor* Radical Growth Inhibitory Assay was performed using the method described by Ikpefan and co-worker (2013). *Sorghum bicolor* seeds were purchased from Ipata market in Ilorin, Kwara State. Viable seeds were selected using a simple viability test method. A handful of the seeds were placed in distilled water, viability seeds were selected based on their ability to remain submerged in water. The viable seeds thus collected were washed with 95% ethanol for sterilization for 1 minute and they were finally rinsed with distilled water, dried and stored until needed. Ten milliliters (10 mL) of 39.06, 156.25, 625, 2500 and 10000 µg/mL were prepared by four-fold dilution from an initial twenty milligrams (20,000 µg) of the *C. jagus* extract dissolved in 5% DMSO. The same concentrations as above were prepared for cyclophosphamide (positive control). A volume of 10 mL different concentrations of the extract (39.06 - 10000 µg/mL) was poured into the petri-dish of 6 cm wide containing filter (Whatman No.1) underlay with cotton wool, after which ten of the sterilized seeds were spread on each of the petri-dishes. The petri-dishes were incubated in a dark cupboard at room temperature and the lengths of the radicle emerging from the seeds were measured at 48 and 96 hours. The negative control seeds were treated with 10 mL 5% of DMSO in distilled water. The experiment was repeated in three replicates for all concentrations and controls. The radical lengths were measured to the nearest millimeter.

Allium cepa Root Growth Inhibitory Assay

The *A. cepa* root growth inhibitory assay was performed based on the method described by Saboo and co-worker (2013). Locally available onion bulbs (*Allium cepa* 50 ± 10 g) were also purchased from Ipata market in Ilorin. The bulbs were washed with distilled water and grown in the dark over tap water at ambient temperature until the roots have grown to approximately 2-3 cm length. A volume of 20 mL different concentrations of the extract (39.06, 156.25, 625, 2500 and 10000 µg/mL) was poured into the petri-dish of 6 cm wide and the base of each of three *A. cepa* bulbs were placed on a petri-dishes containing each extract (39.06 - 10000 µg/mL). The same concentrations as above were prepared for cyclophosphamide (positive control), while the negative control bulbs were treated with 10 mL of 5% DMSO in distilled water. The root lengths were measured at 0, 48, 96 hours for each concentration of extract and control was measured. The percentage root growth inhibition after treating with extract/cyclophosphamide at 48 and 96 hours was determined.

Data Analysis

Data obtained was analyzed by Graphpad Prism computer program version 6.0. The concentration with 50% lethality (LC_{50}) in the BSL, 50% growth inhibition (GI_{50}) in *Sorghum bicolor* radical growth inhibitory and 50% growth inhibition (GI_{50}) in *Allium cepa* root growth inhibitory were estimated from a dose-response inhibition curve using a non-linear regression curve data analysis. The results were displayed as mean ± SEM of three replicates. Statistical significance

was evaluated using student's t-test and values with $P < 0.05$ were considered significant.

RESULTS

Table 1: Phytochemical analysis of *C. Jagus* bulb extract

Bioactive constituent	Chemical Test	Extract
Alkaloid	Drangendorff's	++
	Wagner's	++
	Meyer's	+
Flavonoids	Shinoda's test	++
	Lead acetate	++
Tannins	$FeCl_3$	++
Saponins	Frothing	+
	Emulsifying	+
Anthraquinone	Combined	+
	Free	++
Cardiac glycoside	Kella-Killiani	-
	Kedde	+
Steroid/terpenoids	Salkowski	–

KEYS: -, Absence of component; + Trace presence of component; ++ moderate amount of component, +++ Copious amount of component.

Phytochemical screenings of the crude extract lead to the identification of alkaloids, flavonoids, saponins and both free and combined anthraquinines (Table 1). However, the extract tested negative for the presence of steroids and terpenes while the presence of cardiac glycoside is not confirmed.

The extract demonstrated a comparable concentration dependent cytotoxicity to cyclophosphamide. At 1000 µg/mL the extract demonstrated 100% lethality similar to cyclophosphamide. Interestingly, the extract displayed higher cytotoxicity compared to cyclophosphamide up to 125µg/mL.

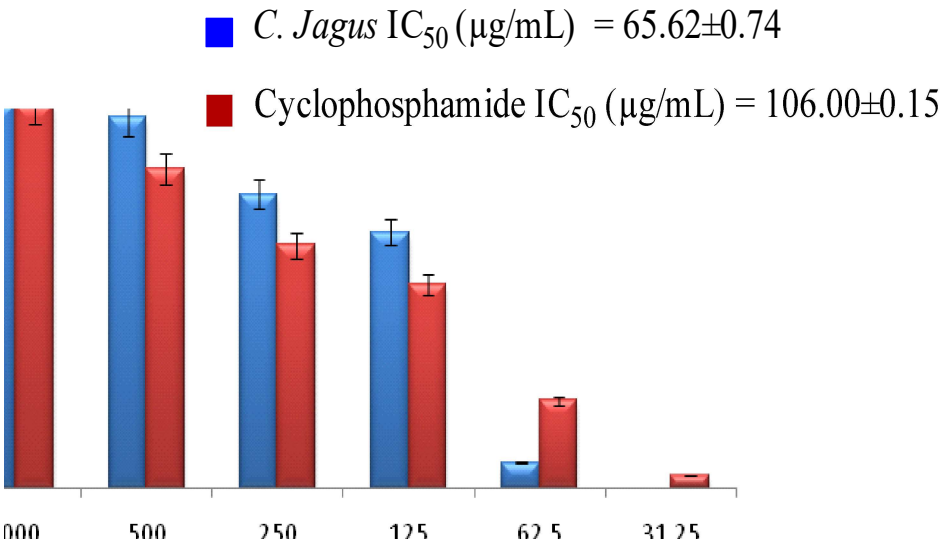


Figure 1: *In vitro* Brine Shrimp Lethality of *C. Jagus* Bulb Extract and cyclophosphamide (CTZ).

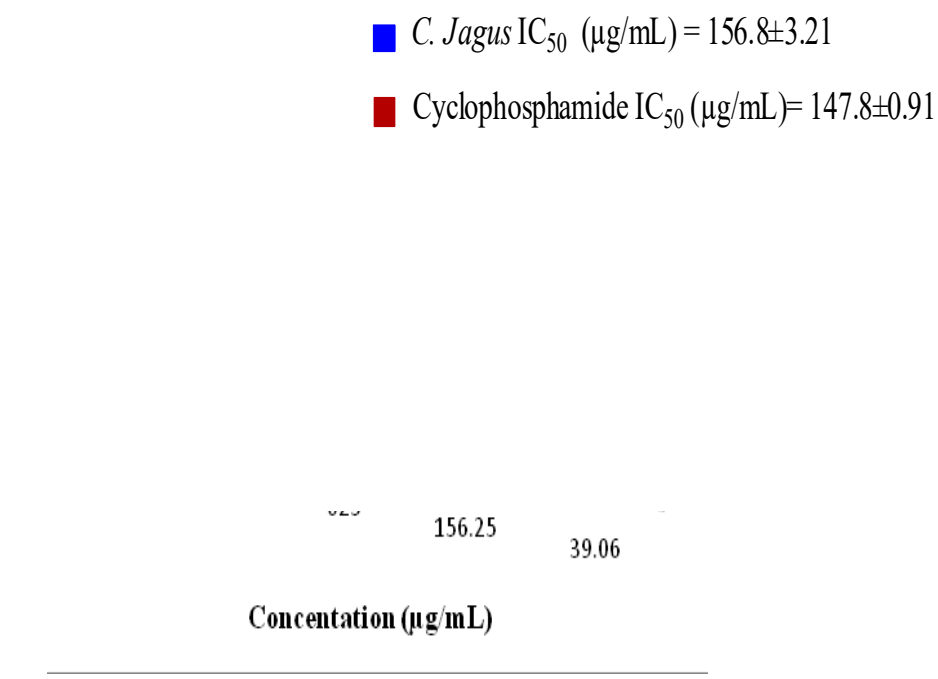


Figure 2: Percentage Inhibition and IC_{50} of *Sorghum bicolor* Radical Growth by extract and control

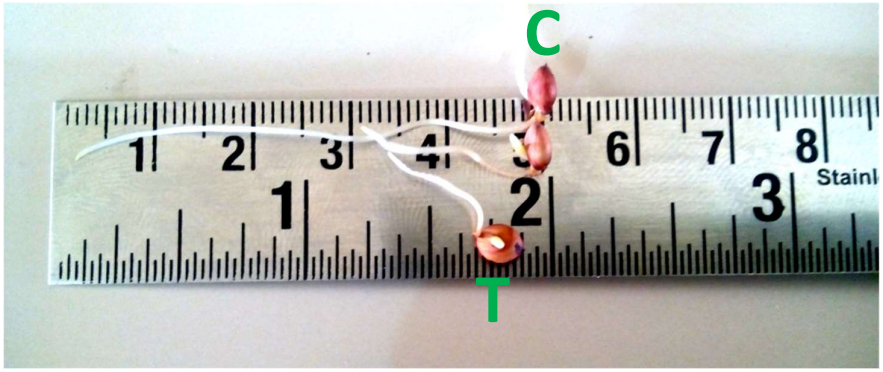
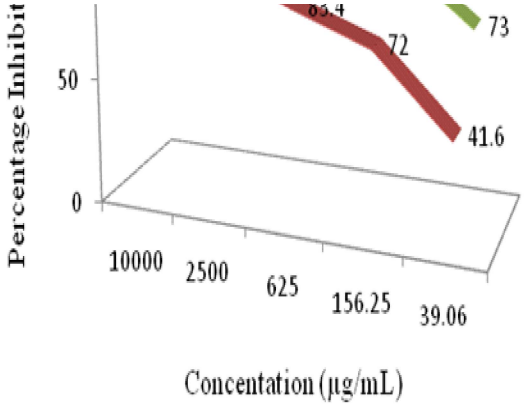


Figure 3: Measure of *Sorghum bicolor* Radical Length of Treated (T) and Negative Control (C) Group.

The extract exerted a significant concentration dependent *Sorghum bicolor* radical growth inhibition at 10000, 2500 and 625 µg/mL and inhibition of 100, 97.9 and 93.5 % were observed. There was a sharp decrease in radical growth inhibition (64.1 %) at 156.25 µg/mL from 93.5% to 625 µg/mL. The extract (IC_{50} = 156.8 ± 3.21) showed similar and comparable radical growth inhibition to cyclophosphamide (IC_{50} = 147.8 ± 0.91).



C.jagus (µg/mL) = 206.2±2.07

Cyclophosphamide (µg/mL) = 139.4±2.47

Figure 4: Percentage Inhibition and IC_{50} of *A. cepa* Radical Growth by extract and control.

Figure 5: Treated and control *A. cepa* bulb root length.

In the *A. cepa* root growth inhibition assay, the extract showed a concentration dependent root growth inhibition. At 10000, 2500 and 625 µg/mL the extract showed 100 %. The extract however has an estimated IC_{50} of 206.2 ± 2.07 at shown in table showed similar and comparable radical growth inhibition to cyclophosphamide (IC_{50} = 139.4 ± 2.47).

DISCUSSION

The lack of scientific evidence and continuous use of *Crinum jagus* bulb in the management of cancer by trado-medical practitioners informed the need to evaluate the cytotoxicity and antiproliferative activities. On extraction the dried bulb (200 g) yielded 24.3 g (12.15 % $^{w}/_{w}$) of dark brown extract with distinct aromatic smell. The methanol extract of *C. jagus* had been reported to be dark brown with distinct aromatic smell (Udegbunam *et al*., 2015).

The presence of alkaloids, flavonoids, tannins and some glycosides such as saponins free and combine anthracene derivatives were observed following the phytochemical investigation of *C. jugus* bulb extract as shown in table 1. The extract however appeared to be devoid of unsaturated steroids and lactone rings as observed from Kella-Killiani's and

Salkowski's test. The absences of steroids also suggest that the saponins presence in the extract is of the triterpenoid origin. Triterpenoid saponins are more widely distributed in nature than steroidal saponins (Podolak *et al*., 2010). The presence of alkaloids and saponins in the extract is suggestive of anticancer activity. Several alkaloids have been reported to have potent antiproliferative and antitumor activity (Wink *et al*., 2015). Saponins are known possess a wide range of pharmacological properties including cytotoxic activity. Saponins are believed to be toxic because they readily complex with red blood cells membrane cholesterol, thereby leading to spontaneous pore formation and cell permeabilization leading to haemolysis (Gauthier *et al*., 2009).

The *C. jagus* bulbs extract demonstrated significant concentration dependent brine shrimp lethality compared to the negative control (0.5% DMSO in sea water). Based on Padmaja *et al*., (2002) classification of toxicity in brine shrimp bioassay the extract was said to strongly toxic (LC_{50} of 65.62±0.74 µg/mL) compared to moderately toxic cyclophosphamide with a LC_{50} of 106.00±0.15 µg/mL. The brine shrimp lethality assay is most time employed in preliminary screening of extracts/compounds for cytotoxicity because of the significant correlation between BSL other antitumor assays (Ajaiyeoba *et al*., 2016). Padmaja and co-workers classified the lethality of extracts on brine shrimps as follows; LC_{50} > 1000 µg/ml was considered to be non-toxic, LC_{50} (500 to 1000 µg/ml) was considered to be weakly toxic, LC_{50} (100 to 500 µg/ml) was considered to be moderately toxic and LC_{50} (0 to 100 µg/ml) was considered to be strongly toxic. This is the first report of the brine shrimp leathlity assay of *C. jagus* bulb extract and the report of this assay support the assertion that the bulb is poisonous.

Sorghum bicolor radicle growth inhibitory assay is a bench-top assay. It is predictive of antiproliferative activity. The meristematic cells of *S. bicolor* seeds are highly totipotent and readily proliferate with 24 h when exposed to favourable conditions. The radicle is the first part of a seedling to emerge from the seed during the process of germination and the extent of proliferation is reflected in the increase in the length of the radicles produced in 96 hrs in the control seeds (Ikpefan & Ayinde, 2013). The extract of *C. jagus* displayed a time and concentration dependant radicle growth inhibition. The extract (IC_{50} = 156.8±3.21µg/mL) had similar antiproliferative to a cyclophosphamide (IC_{50} = 147.8±0.91µg/mL) a standard anticancer drug used clinically for its antiproliferative activity.

The extract was observed to display time and concentration dependant *A. cepa* root growth inhibition. The extract (IC_{50} = 206.2 ± 2.07) displayed root growth inhibition comparative to Cyclophosphamide (IC_{50} = 139.4 ± 2.47). This is the first antiproliferative study reported about *C. jagus* bulb extract, unlike other members of the *Crinum* genus such as *C. delagoense* and *Crinum ornatum* have been used traditionally in the management of cancer. Recent scientific reports have justified their traditional use (Nair et al., 1998; Oloyede *et al*., 2010; Refaat *et al*., 2013). Nair and co-worker reported several anticancer compounds such as lycorine, crinamine and 6-hydroxycrinamine isolated from *C. delagoense* bulbs to justify the traditional uses of the plant in the cure of human cancer (Nair *et al*., 1998).

CONCLUSION

Crinum jagus bulbs extract contain alkaloids, flavonoids, tannins and some glycosides. The extract demonstrated a strong toxicity on brine shrimps and displayed time and concentration dependent growth inhibition on *Sorghum bicolor* radicle and *Allium cepa* root. However, this is the first time the antiproliferative activity of *C. jagus* bulb crude extract is reported in literature. Thus, our study justifies the use of crude extract of *Crinum jagus* bulb in the traditional treatment of cancer.

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