



Received on 20 October, 2017; received in revised form, 24 January, 2018; accepted, 27 January, 2018; published 01 July, 2018

EVALUATION OF *IN-VITRO* ANTIOXIDANT AND ANTIPLASMODIAL ACTIVITIES OF THE ACETONE EXTRACT OF *CRINUM JAGUS* BULBS (LILIACEAE) LINN.

O. A. Shorinwa ^{*1}, O. O. Ebong ², A. W. Obianime ² and I. M. Siminialayi ²

Department of Experimental Pharmacology and Toxicology ¹, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Rivers State, Nigeria.

Department of Pharmacology ², Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Rivers State, Nigeria.

Keywords:

Crinum jagus,
Antioxidant, Antiplasmodial,
Gas chromatography,
Mass Spectrometry

Correspondence to Author:

Dr. O. A. Shorinwa

Department of Experimental
Pharmacology and Toxicology,
Faculty of Pharmaceutical Sciences,
University of Port Harcourt, Rivers
State, Nigeria.

E-mail: olusayo.shorinwa@uniport.edu.ng

ABSTRACT: This study evaluated the acetone extract of *Crinum jagus* bulbs for its antioxidant, and antiplasmodial properties. The bulbs of *Crinum jagus* are used in traditional medicine for various ailments. The antioxidant activity of the extract was evaluated using DPPH and nitric oxide free radical scavenging assay. The antiplasmodial activity (suppressive, prophylactic and curative activity) was evaluated *in-vivo* against *Plasmodium berghei* at 250 mg/kg and 500 mg/kg through the oral route in Swiss albino mice. Isolation and characterization of the active principles were done using GC-MS techniques. Phytochemical analysis of the extract revealed the presence of flavonoids, tannins, saponins, steroids and alkaloids. The LD₅₀ was found to be greater than 5000 mg/kg. The extract showed a dose-dependent antioxidant activity against the various assays. The extract caused dose-dependent and statistically significant ($P < 0.05$) reduction of parasitemia in the antiplasmodial suppressive and prophylactic study. Bowdensine and demethoxy bowdensine were the active principles found on chemical analysis of the extract, using GC-MS. The results indicate that this extract may possess antioxidant, prophylactic and suppressive antiplasmodial activities, probably attributable to the bowdensine and demethoxy bowdensine constituents.

INTRODUCTION: *Crinum jagus* is commonly referred to as bush onions in Nigeria and is used for the treatment of various diseases such as chronic cough, memory loss and other mental symptoms associated with aging ¹ in traditional medicine. Oxidative stress is a central pathogenic factor for chronic diseases.

Fruits and vegetables have been shown to exert a protective effect against oxidative stress. The primary nutrients thought to provide the protection afforded by fruits and vegetables are the antioxidants.

ROS are major sources of primary catalysts that initiate oxidation *in-vivo* and *in-vitro* and create oxidative stress which results in numerous diseases and disorders ² such as cancer ³, cardiovascular disease ⁴, neural disorders ⁵, Alzheimer's disease, mild cognitive impairment ⁶, Parkinsons disease, alcohol - induced liver disease ⁷, ulcerative colitis, aging ⁸, atherosclerosis ⁹. Oxygen-derived free radicals such as superoxide anions, hydroxyl

<p>QUICK RESPONSE CODE</p> 	<p>DOI: 10.13040/IJPSR.0975-8232.9(7).2990-98</p> <hr/> <p>Article can be accessed online on: www.ijpsr.com</p> <hr/> <p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(7).2990-98</p>
---	--

radicals and hydrogen peroxide are cytotoxic and give rise to tissue injuries¹⁰. Excessive amounts of ROS are harmful because they initiate biomolecular oxidation which leads to cell death and creates oxidative stress. Also, oxidative stress causes inadvertent enzyme activation and oxidative damage to cellular system¹¹.

Malaria affects world-wide, more than 200 million people out of which between 1 to 2 million die every year. There is also an increase in the development of drug resistance which has necessitated a continued search for newer and safer drugs. During a malaria infection, both host and parasite are under oxidative stress. Increased levels of reactive oxygen species (ROS, e.g. superoxide anion and the hydroxyl radical) are produced by activated neutrophils in the host and during degradation of haemoglobin in the parasite¹².

Using an experimental mouse model for malaria,¹³ reported that antioxidant therapy with N-acetylcysteine and desferroxamine, as additives to chloroquine, prevented cognitive impairment, confirming the importance of oxidative stress in cerebral malaria - associated cognitive sequelae. Administration of additive antioxidants may be a successful therapeutic strategy to control long-lasting consequences of cerebral malaria and in other severe systemic inflammatory syndromes with neurological involvement. Identification and characterization of active principles of plants is necessary for the discovery of new drug substances. Chromatography plays a fundamental role as an analytical technique for quality control and standardization of phytotherapeutics¹⁴. Gas chromatography is mainly used in the separation and analysis of multi component mixtures such as essential oils, hydrocarbons and solvents. Mass spectrometry is the most sensitive and selective method for molecular analysis and can yield information on the molecular weight as well as the structure of the molecule. Combining chromatography with mass spectrometry (GC-MS) provides the advantage of both chromatography as a separation method and mass spectrometry as an identification method.

Some pharmacological properties such as anti-diabetic, and toxicological evaluation of *C. jagus* bulbs have been reported^{15, 16}.

Hence, this study investigated the *in-vitro* antioxidant activity, *in-vivo* anti-plasmodial activity and chemical composition of *C. jagus* bulbs.

MATERIALS AND METHODS: Plant material and extraction-The bulbs of *Crinum jagus* were collected from Ikono village In Akwa-Ibom State. The plant was identified and authenticated in the herbarium of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Uyo by Prof. Mrs. M. E. Bassey and a voucher specimen has been deposited in the herbarium. The ground plant material was subjected to solvent extraction by maceration and vigorous shaking for 72 hours using 96% v/v acetone. The product was then filtered using a suction pump and Whatman No. 1 filter paper. The filtrate was concentrated to dryness with a Rotary evaporator at 60 °C to give a golden colored crude extract. The crude extract was further dried over a water bath set at 40 °C to give dark brown viscous syrup. The obtained extract was weighed and stored in a refrigerator until needed.

Experimental Protocols:

Phytochemical Screening: Phytochemical screening was carried out on the acetone extract of the *C. jagus* bulbs for the detection of various plant constituents according to the protocol of Harborne¹⁷.

Determination of the Free Radical Scavenging Potential of the Samples:

Total Antioxidant Activity: The antioxidant activity was determined by the conjugated diene method¹⁸. Each extract (0.1 - 20 mg/ml) in water (100 µl) was mixed with 2.0 ml of 10 mM linoleic acid emulsion in 0.2 M sodium phosphate buffer (pH 6.6) in a test tube and kept in the dark, inside a cupboard at 37 °C to accelerate oxidation. After incubation for 15 h, 0.1 ml from each tube was mixed with 7.0 ml of 80% methanol in deionized water and the absorbance of the mixture was measured at 234 nm against a blank in a spectrophotometer. The antioxidant activity was calculated as follows:

$$\text{Antioxidant activity (\%)} = (A_0 - A_1 / A_0) \times 100$$

Where; A_0 is the absorbance of control and A_1 is the absorbance of test. Ascorbic acid was used as positive control¹⁸.

Determination of the Phenolic Content of the Extracts:

Total Phenol Content: The amount of total phenol content was determined by Folin-Ciocateu reagent method¹⁹. The plant extract (0.5 ml) and 0.1 ml of Folin-Ciocateu reagent (0.5 N) were mixed and incubated at room temperature for 15 min. Then 2.5 ml saturated sodium carbonate was added and further incubated for 30 min at room temperature and absorbance measured at 760 nm. Gallic acid was used as positive control. The total phenolic content was expressed in terms of standard equivalent (mgg^{-1} of extracted compound).

Total Flavonoid Content: The amount of total flavonoid content was determined by aluminum chloride method²⁰. The reaction mixture (3.0 ml) comprising of 1.0 ml of extract, 0.5 ml of aluminum chloride (1.2%) and 0.5 ml of potassium acetate (120 mM) was incubated at room temperature for 30 min and absorbance measured at 415 nm. Quercetin²¹ was used as a positive control. The flavonoid content was expressed in terms of standard equivalent (mgg^{-1} of extracted compound).

Free Radical Scavenging Assays: DPPH (1, 1-diphenyl-2-picryl-hydrazyl) is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH in methanol solution in the presence of hydrogen – donating antioxidant due to the formation of the non-radical form DPPH-H²². The antioxidant activity of the plant extracts are examined on the basis of the scavenging effect on the stable DPPH free radical activity. Briefly, in 3 mL of each diluted extract or Vitamin C used as standard, 1 mL of a methanol solution of DPPH 0.1 mM is added. The mixture is kept in the dark at room temperature for 30 min and the absorbance is measured at 517 nm against a blank. The following equation is used to determine the percentage of the radical scavenging activity of each extract.

$$\text{Scavenging effect (\%)} = 100 \times (A_o - A_s) / A_o$$

Where A_o is the absorbance of the blank; A_s is the absorbance of the sample.

Nitric Oxide Radical Scavenging (NO) Assay: Nitric oxide generated from sodium nitroprusside

in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured using the Griess reaction reagent²³. 3.0 ml of 1mM sodium nitroprusside in phosphate buffer was added to 2.0 ml of extract and reference compound in different concentrations (20-100 g/ml). The resulting solutions were then incubated at 25 °C for 60 min. A similar procedure was repeated with methanol as blank, which served as control. To 5.0 ml of the incubated sample, 5.0 ml of Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dihydrochloride in 2% H_3PO_3) was added and absorbance of the chromophore formed was measured at 540 nm. Percent inhibition of the nitrite oxide generated was measured by comparing the absorbance values of control and test preparations. Ascorbic acid was used as the positive control²⁴.

Evaluation of Anti-plasmodial Activity:

Preparation of Animals: Sixty Swiss albino mice of either sex, weighing between 15 - 25 g were used for the study. They were obtained from the animal house of the Nigerian Institute of Medical Research, Yaba, Lagos State. The animals were maintained on commercial feeds and water, *ad libitum*, for the entire duration of the study. The mice were allowed to acclimatize for two weeks before the experiment began. Good hygiene was maintained by constant cleaning and removal of faeces and spilled feeds from the cages daily. All the standard ethical requirements were complied with.

Preparation of Inoculum of Chloroquine Sensitive Strain of *Plasmodium berghei* NK 65 strain was obtained from the laboratory of Nigerian Institute of Medical Research, Yaba by serial blood passage from mouse to mouse was used for the study. Donor mouse with a rising parasitaemia of 20 - 30% confirmed by thin and thick blood film microscopy was used. Blood (0.2 ml) was collected in a heparinized tube through retro-orbital puncture in the donor mouse using heparinized capillary tubes. The blood was diluted with 5 mL of phosphate buffer solution (PBS) pH 7.2, so that each 0.2 ml contained approximately 1×10^7 infected red cells^{25, 26}. Each animal was inoculated with about 10 million parasites per kilogram body weight intraperitoneally, which is expected to produce a steadily rising infection in mice.

Preparation of Drugs:

Chloroquine: Fifty milligram of powdered chloroquine sulphate was dissolved in 20 mL of distilled water so that 1 ml will contain 2.5 mg of chloroquine sulphate. Dosage administered to the animals in the standard drug group was 25 mg/kg. Hence, the 0.2 ml of solution administered contained 0.5 mg of chloroquine sulphate.

Suppressive Test: A total of 20 mice were used for this study. On the first day (D0), a standard inoculum of (1×10^7) *Plasmodium berghei* infected erythrocytes was injected intraperitoneally into each rat.

The animals were divided into four groups of five mice each. Doses of 250 mg/kg and 500 mg/kg of the extract were administered orally to groups A and B with the aid of an oral cannula. Chloroquine sulphate, 25 mg/kg/day was given as positive control to animals in group C and 0.2 ml of distilled water was administered to group D as negative control. All were given daily for 4 consecutive days starting 2 hours after infection, i.e., from day 0 (D0) to day 3 (D3), receiving a total of 4 oral doses. Thin and thick smears of blood films were obtained from the peripheral blood by snipping the tail of each mouse on day 4 after infection. The smears were placed on microscopic slides while the thin films were fixed with methanol, air dried and then stained along with the thick films with 3% Giemsa and then microscopically examined at 100-x magnification. The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected controls with those of treated mice²⁷.

Prophylactic Test: Four groups of 5 mice per group were used for the study. Two groups were administered with 250 and 500 mg/kg/day doses of the extract orally. The other two groups were administered with 25 mg/kg of chloroquine sulphate and 0.2 ml of distilled water orally. The animals were dosed for four consecutive days (D0-D3). On the fifth day (D4), the mice were inoculated with *P. berghei* infected red blood cells. Seventy-two hours later, thin and thick blood smears were prepared from the tail of each mouse so as to determine the parasitaemia level. The parasitaemia levels of the treated groups were

compared to both the positive and negative control group²⁷.

Curative Test: On the first day (D0), standard inoculums of (1×10^7) *Plasmodium berghei* infected erythrocytes were injected intraperitoneally. Seventy two hours later, the mice were randomly divided into four groups of five mice each. Two groups received 250 mg/kg and 500 mg/kg doses of the extract for 5 days. The remaining two groups received chloroquine sulphate (25 mg/kg/day) for 3 days and 0.2 ml of distilled water for 5 days. Two thick blood smears were prepared from the tail of each mouse for 10 days, to monitor the parasitaemia level. The parasitaemia levels of the treated groups were compared to the negative and positive control groups²⁸.

Isolation and Characterization of Constituents:

The acetone extract (12.5 g) was dissolved in 50% methanol and was partitioned with three times equal volume of dichloromethane. The constituents of the dried dichloromethane fraction were then separated using column chromatography. The dry packing technique was used. The column was packed with 25 g silica gel (mesh size 70 - 230). The column was eluted with the following mobile phase gradient in increasing order of polarity: n-hexane : dichloromethane (4:0; 300 mL, 3:1; 300 mL, 2:2; 300 mL, 1:3; 300 mL and 0:4; 300 mL) to dichloromethane: methanol (3:1; 300 mL and 2:2; 300 mL). Seven major fractions were collected, CJ-1 (hexane: dichloromethane 4:0), CJ-2 (hexane: dichloromethane 3:1), CJ-3 (hexane: dichloromethane 2:2), CJ-3 (hexane: dichloromethane 1:3), CJ-4 (hexane: dichloromethane 1:3), CJ-5 (hexane: dichloromethane 0:4), CJ-6 (dichloromethane: methanol 1:3) and CJ-7 (dichloromethane: methanol 2:2). Based on thin layer chromatography analysis of the column fractions CJ-1-CJ-5 were pooled together as A2D and subjected to Gas Chromatography - Mass Spectroscopic (GC- MS) analysis to characterize the constituents.

Gas Chromatography - Mass Spectroscopic Analysis Pooled Column Fraction Coded A2D:

This was done according to the method²⁹. The pooled column fraction coded A2D (underivatized) was dissolved in chloroform and the chemical constituents were characterized using an Agilent

gas chromatograph Model 6890, coupled to a mass spectrometer model, equipped with a DB DB-1MS capillary column (30 m long \times 320 μ m nominal diameter). The oven temperature was programmed from 120 °C (5 min) to 250 °C at 3 °C/min, 5 min hold. Helium was used as carrier gas at 1.0 ml/min. Sample injection was done in split mode (50:1). The injector and detector temperatures were set at 250 and 280 °C, respectively. The MS working in electron impact mode at 70 eV; electron multiplier, 1600 V; and ion source temperature, 180 °C. Mass spectra data were acquired in the scan mode in m/z range 50 - 550. The compounds assayed in A2D were identified and characterized by comparing their retention times with those of reference compounds in the library and by comparison of their mass spectra with those of reference substances from the library with a quality factor >80 used as criterion for acceptance^{30,31}.

Statistical Analysis: Statistical analysis of data was done using Microsoft Excel (Microsoft Office Professional Plus, 2013). Student's t-test was done to determine the significant differences between the means of control groups and the treated groups. P-values <0.05 were considered to be statistically significant³².

RESULTS AND DISCUSSION:

Phytochemical Screening: The phytochemical screening of the plant extract revealed that alkaloids, flavonoids, steroids, carbohydrates and reducing sugar were most abundant, while tannins were present in moderate quantities. Saponins were only mildly (+) present while anthraquinones and phlobatannins were absent **Table 1**.

TABLE 1: PHYTOCHEMICAL CONSTITUENTS OF THE ACETONE EXTRACT OF CRINUM JAGUS BULBS

Test	Observation
Alkaloid	+++
Tannins	++
Steroids	+++
Flavonoids	+++
Anthraquinones	-
Saponins	+
Carbohydrates	+++
Reducing sugar	+++
Phlobatannins	-

Key: - Absent, + slightly present, ++ moderately present, +++ highly present

Acute Toxicity Study: The LD₅₀ of the acetone extract of *Crinum jagus* bulbs is greater than 5,000

mg/kg since no death was recorded in the two phases of the study.

Antioxidant study:

In - vitro Antioxidant Activity of the Acetone Extract of *Crinum jagus* Bulbs: The plant extract was found to have a tannin acid content of 194.1 ± 5.33 mg/g AAE, flavonoids (220 ± 0.19 mg/g QAE), tannin (259.6 ± 0.58 mg/g TAE) and phenol content of 136.9 ± 0.29 mg/g GAE.

1, 1, -diphenyl- 2- picryl- hydrazyl Radical Scavenging Assay (DPPH) Activity: The acetone extract of *Crinum jagus* showed a comparable dose related inhibitory activity against DPPH at the various concentrations when compared to that of ascorbic acid. The extract at the concentration of 25 ug/ml had an inhibitory activity of 19.7% while it demonstrated 66.4% inhibitory activity at a concentration of 100 ug/ml **Fig. 1**.

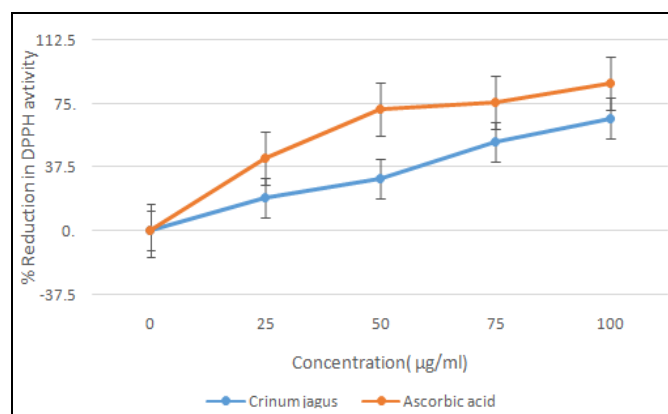


FIG. 1: PLOT OF INHIBITORY ACTIVITY OF CRINUM JAGUS AND ASCORBIC ACID AGAINST DPPH

The acetone extract of *Crinum jagus* showed a comparable dose related inhibitory activity against DPPH at the various concentrations when compared to that of ascorbic acid. The extract at the concentration of 25 ug/ml had an inhibitory activity of 19.7% while it demonstrated 66.4% inhibitory activity at a concentration of 100ug/ml **Fig. 1**.

Evaluation of Inhibitory Activity of *Crinum jagus* Extract against Nitric Oxide: The figure represents the bar chart values of the concentrations of *Crinum jagus* and ascorbic acid that produced 50% reduction in nitric oxide generation. *Crinum jagus* at a concentration of 55 ug/ml was able to bring about 50% inhibition which ascorbic acid produced at 35 ug/ml

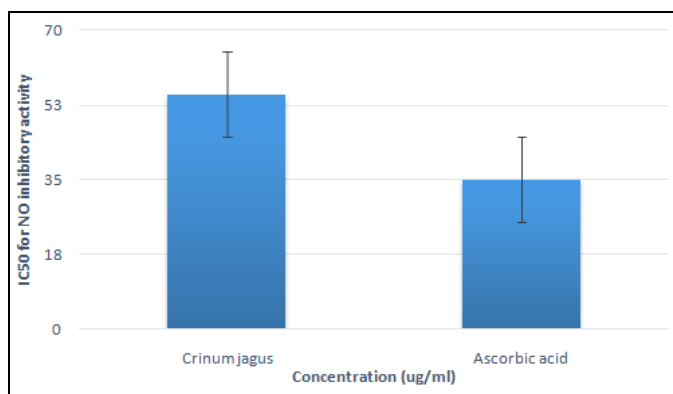


FIG. 2: PLOT OF IC₅₀ OF *CRINUM JAGUS* AND ASCORBIC ACID USING NITRIC OXIDE

Antiplasmodial Activity Study:

Evaluation of Antiplasmodial Suppressive Activity of the Acetone Extract of *C. jagus* Bulbs: The acetone extract of *Crinum jagus* bulbs produced 43.02% of parasitaemia suppression at a dose of 250 mg/kg, while it exhibited 61.42% chemo suppression of parasitaemia level when compared against control and chloroquine which were associated with 0% and 100% reduction in parasite load respectively. The chemo suppressive antiplasmodial activity of the plant extract at 500 mg/kg was significant (P<0.05) when compared against the control. Meanwhile the extract demonstrated statistically significant (P < 0.05) suppressive activity against parasitaemia at the two doses when compared with chloroquine. The chemo suppressive activity of the extract was dose-dependent **Fig. 3**.

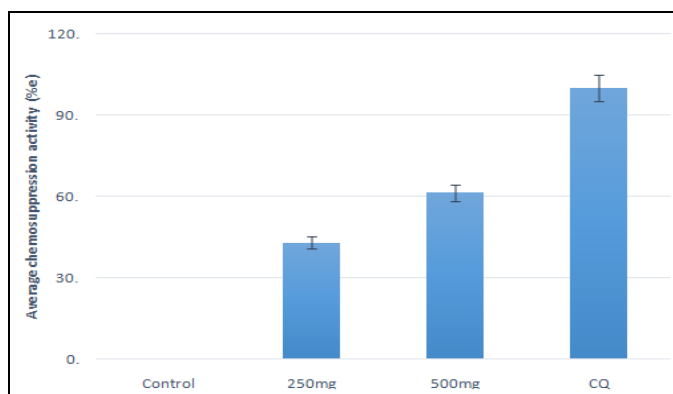


FIG. 3: CHEMOSUPPRESSION ACTIVITY OF *CRINUM JAGUS* ON *PLASMODIUM BERGHEI* IN RATS

The bar chart of percentage average chemosuppression activity of the plant extract at the two doses when compared to control and chloroquine. The *Crinum jagus* extract activity increased with increased dose even though its suppressive activity is lower than that of chloroquine.

Evaluation of Antiplasmodial Chemoprophylactic Activity of the Acetone Extract of *C. jagus* Bulbs: The plant extract exhibited chemoprophylactic activity of 52.5% at 250 mg/kg and 70.08% at the dose of 500 mg/kg. The chemoprophylactic effect of the extract was significantly higher than that of negative control but significantly less than chloroquine as shown in **Fig. 4**.

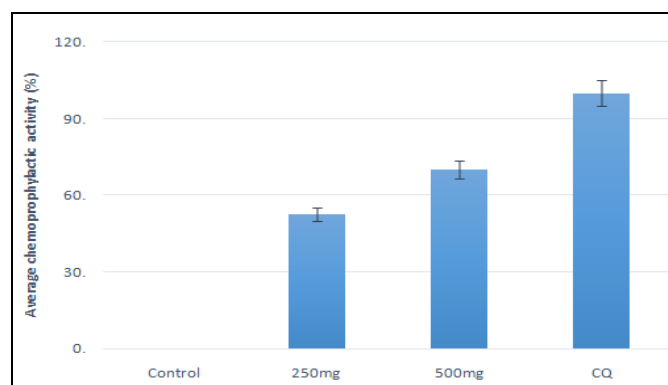


FIG. 4: CHEMOPROPHYLACTIC ACTIVITY OF *CRINUM JAGUS* ON *PLASMODIUM BERGHEI* IN RATS. Graphical representation of average chemoprophylactic activity of the plant extract, chloroquine and distilled water. The plant extract caused a dose-dependent prophylactic activity.

Curative Activity Study:

Evaluation of Antiplasmodial Curative Activity of the Acetone Extract of *C. jagus* Bulbs: The acetone extract of *C. jagus* showed no curative activity against *P. berghei* parasites at the two doses tested. Chloroquine was able to clear the parasites on day 2, however parasites showed up on day 8 and day 9 again.

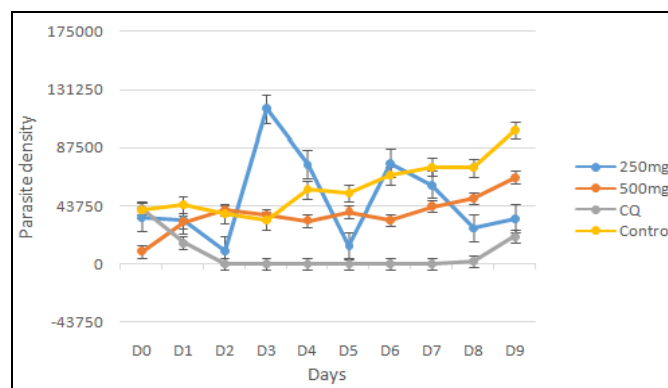


FIG. 5: GRAPHICAL REPRESENTATION OF THE PARASITE DENSITY OF *CRINUM JAGUS* EXTRACT, CHLOROQUINE AND DISTILLED WATER DURING THE STUDY PERIOD. The result showed that the plant extract does not possess curative activity when compared to chloroquine.

Characterization of Constituents:

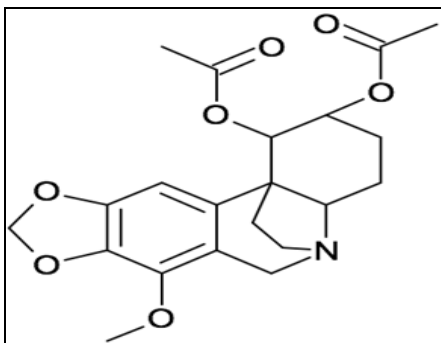


FIG. 6: STRUCTURE OF BOWDENSINE IDENTIFIED IN THE ACETONE EXTRACT OF *CRINUM JAGUS* BULBS (S2A). THE MOLECULAR FORMULA IS $C_{21}H_{25}NO_7$

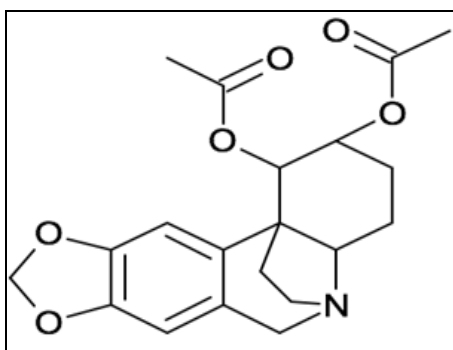


FIG. 7: STRUCTURE OF DEMETHOXYBOWDENSINE IDENTIFIED IN THE ACETONE EXTRACT OF *CRINUM JAGUS* BULBS (S2B). THE MOLECULAR FORMULA IS $C_{20}H_{23}NO_6$

DISCUSSION: *Allium flavum* L. (Yellow onion) was investigated by ³³ for its anti-oxidant activity, total phenolic content and concentrations of flavonoids present in its extracts. They reported that the antioxidant activity ranged from 64.34 to 243.34 $\mu\text{g/ml}$, total phenolic content ranged between 42.29 and 80.92 mg GA/g and concentration of flavonoids in various extracts of *A. flavum* varied from 64.07 to 95.71 mg RU/g. Comparing this report with the result of the present study, it would appear that the acetone extract of *Crinum jagus* seems to possess a high content of phenolic constituents and flavonoids. The 1, 1-Diphenyl- 2-picryl-hydrazyl radical scavenging (DPPH) test provides information on the reactivity of the test compounds with a stable free radical. DPPH gives a strong absorption band at 517 nm in the visible region. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract. *Crinum jagus* was able to reduce the absorbance to 50% at a concentration of 70 $\mu\text{g/ml}$ when compared

to ascorbic acid IC_{50} of 31.7 $\mu\text{g/ml}$. This showed that the acetone extract is less potent than ascorbic acid against DPPH.

Under aerobic conditions nitric oxide reacts with oxygen to produce stable products (nitrate and nitrite). This leads to reduction of nitrite concentration in the assay media ³⁴. An IC_{50} value is the concentration of the sample required to scavenge 50% of the free radicals present in the system. The extract had an IC_{50} of 55 $\mu\text{g/ml}$ when compared to the standard (ascorbic acid) value of 35 $\mu\text{g/ml}$. A lower IC_{50} value corresponds to a larger scavenging activity ³⁵. This showed that the extract is less potent even though it is able to inhibit the generated nitrite oxide. The acetone extract of *Crinum jagus* seems to exert its antioxidant activity more by acting as an electron donor which can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants ³⁶, observed a high correlation between phenolic compositions and antioxidant activities of legume extracts studied.

The presence of flavonoids, phenols and tannins in this plant may likely be responsible for the free radical scavenging effects observed in this plant. Flavonoids and tannins are phenolic compounds and plant phenolic are a major group of compounds that act as primary antioxidants or free radical scavenger ³⁷. This corresponds to the report of ³⁸ which stated that the extracts of *P. guajava*, *M. indica*, *C. papaya*, *V. amygdalina* which are used for malaria therapy in South-western Nigeria exhibited potent antioxidant activity. The acetone extract of *Crinum jagus* showed a dose-dependent reduction of parasitaemia at the different doses used. The extract at the doses of 250 mg/kg and 500 mg/kg showed 43.02% and 61.42% suppression of parasitaemia in the suppressive activity test as shown in **Fig. 4**. This chemosuppressive activity the extract was dose dependent even though it was significantly ($p < 0.05$) lower than that of chloroquine.

The extract also exhibited a chemoprophylactic activity of 52.5% and 70.08% at the doses of 250 mg/kg and 500 mg/kg, respectively, when compared with chloroquine. However, the extract does not seem to possess curative activity as the

parasite density was higher than that of chloroquine throughout the study period. The parasite density for chloroquine was zero from the third day till the seventh day, but parasites suddenly appeared in the blood smear on day 8 and day 9. This is quite unusual and may be due to the fact that the NK65 parasites might have undergone mutation over the years or it could be due to recrudescence which usually happens after 7 days in humans. Chloroquine phosphate has been used as the standard antimalarial in this study for curative, suppressive and prophylactic antiplasmodial assessment because of its established activity against *P. berghei*³⁹. *P. berghei* a rodent malaria parasite, though not able to infect man and other primates, has been used because of its sensitivity to chloroquine⁴⁰.

The *in vivo* antiplasmodial activities of the extract show that the acetone extract of *Crinum jagus* contains biologically active substances that are relevant in the treatment of malaria. The observed antiplasmodial activity of the acetone extract of *Crinum jagus* may be likely due to the presence of phytochemical constituents such as alkaloids, phenolic compounds and flavonoids. Acetone solvent was used in this study because it has been reported as a very good extraction solvent for the polyphenol group of plant phytochemical compounds. However, the principal constituents of the acetone extract of *Crinum jagus* bulbs on characterization were found to be Bowdensine and demethoxy bowdensine which are alkaloids, even though the plant extract exhibited potent antioxidant activity which confirms the presence of phenolic substances, flavonoids and tannins, as observed in the phytochemical screening. This seems to suggest that the acetone extract of *Crinum jagus* bulbs has a higher content of alkaloids than the polyphenols.

CONCLUSION: This study revealed that the acetone extract of *Crinum jagus* bulbs possesses antioxidant activity, antiplasmodial suppressive and prophylactic activity. The characterization of the constituents of the acetone extract of *Crinum jagus* bulbs has shown the presence of biologically active principles which may be most likely responsible for the plant's use in traditional medicine. These phyto-components will be useful in differentiating this specie from other species. The principal

constituents of this plant may be evaluated for pharmacological activities.

ACKNOWLEDGEMENT: The authors hereby acknowledge the staff of the Department of Biochemistry, Nigerian Institute of Medical Research, Lagos, Nigeria for their assistance during this study.

CONFLICT OF INTEREST: The authors hereby declare no conflict of interest.

REFERENCES:

- Houghton PJ, Agbedahunsi JM and Adegbulugbe A: Choline esterase inhibitory properties of alkaloids from two Nigerian *Crinum* species. *Science Direct* 2004; 65(21): 2893-2896.
- Rackova L, Oblozinsky M, Kostalova D, Kettmann V and Bezakova L: Free radical scavenging activity and lipoxygenase inhibition of *Mahonia aquifolium* extract and isoquinoline alkaloids. *Journal of Inflammation* 2007; 4: 15.
- Kinnula VL and Crapo JD: Superoxide dismutases in malignant cells and human tumours. *Free Radical Biology and Medicine* 2004; 36(6): 718-744.
- Singh U and Jialal I: Oxidative stress and atherosclerosis. *Pathophysiology* 2006; 13(3): 129-142.
- Sas K, Robotka H, Toldi J and Vecsei L: Mitochondrial, metabolic disturbances, oxidative stress and kynurenine system, with focus on neurodegenerative disorders. *Journal of the Neurological Sciences* 2007; 257(1-2): 221-239.
- Guidi I, Galimberti D, Lonati S, Novembrino C, Bamonti F, Tiritico M, Fenoglio C, Venturelli E, Baron P and Bresolin N: Oxidative imbalance in patients with mild cognitive impairment and Alzheimer's disease. *Neurobiology of Aging* 2006; 27(2): 262-269.
- Arteel GE: Oxidants and antioxidants in alcohol induced liver disease. *Gastroenterology* 2003; 124(3): 778-790
- Hyun DH, Hernandez JO, Mattson MP and De Cabo R: The plasma membrane redox system in aging. *Ageing Research Review* 2006; 5(2): 209-220
- Upston JM, Kritharides L and Stocker R: The role of Vitamin E in atherosclerosis. *Progress in Lipid Research* 2004; 42(5): 405-422.
- Jainu M, Shyamala Devi CS: *In vitro* and *in vivo* evaluation of free radical scavenging potential of *Cissus quadrangularis*. *African Journal of Biomedical Research* 2005; 8: 95-99.
- Wiseman H and Halliwell B: Damage to DNA by reactive oxygen and nitrogen species: Role of inflammatory disease and progression to cancer. *Biochemistry Journal* 1996; 313(1): 17-29.
- Postma NS, Mommers EC, Eling WM and Zuidema J: Oxidative stress in malaria; implications for prevention and therapy. *Pharmaceutical World Sciences* 1996; 18 (4): 121-129.
- Reis PA, Comim CM, Hermani F, Silva B and Barichello T *et al.*: Cognitive dysfunction is sustained after rescue therapy in experimental cerebral malaria, and is reduced by additive antioxidant therapy. *PLOS Pathogen* 2010; 6(6): e1000963.
- Andrew M: Role of advances in chromatographic techniques in phytochemistry. *Phytochemistry* 2007; 68: 2785-2797.

15. Shorinwa OA, Ebong OO, Obianime AW and Siminialayi IM: Acetone extract of *Crinum jagus* bulbs (Liliaceae): An acute and sub chronic toxicological evaluation in albino rats. International Research Journal of Pharmacy 2014; 5(7): 560-564. <http://dx.doi.org/10.7897/2230-8407.0507114>.
16. Shorinwa OA, Ebong OO, Obianime AW and Siminialayi IM: Evaluation of potential anti-diabetic activity of acetone extract of *Crinum jagus* bulbs in albino rats. Indo American Journal of Pharmaceutical Research 2016; 6(2): 4581-4586
17. Harborne JB: Phytochemical methods: A guide to modern techniques of plant analysis. 3rd Ed. London: Chapman and Hall 1998.
18. Lingnert H, Vallentin K and Eriksson CE: Measurement of antioxidative effect in model system. Journal of Food Protection and Preservation 1979; 3: 87-103
19. McDonald S, Prenzler PD, Autolovich M and Robards K: Phenolic content and antioxidant activity of olive extracts. Food Chemistry 2001; 73: 73-84.
20. Chang C, Yang M, Wen H and Chern J: Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis 2002; 10: 178-182.
21. Ordonez AA, Gomez JG, Vattuone MA and Isla MI: Antioxidant activities of *Sechium edule* swart extracts. Food Chemistry 2006; 97: 452-458.
22. Pieme C, Kumar S, Dongmo M, Moukette B, Boyoum F, Ngogang J and Saxena A: Antiproliferative activity and induction of apoptosis by *Annona muricata* (Annonaceae) extract on human cancer cells. BMC Complementary and Alternative Medicine 2014; 14(1): 514-516.
23. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS and Tannenbaum SR: Analysis of nitrate, nitrite and (¹⁵N) nitrate in biological fluids. Analytical Biochemistry 1982; 126(1): 131-138.
24. Jayaprakasha GK, Jaganmohan Rao L and Sakariah KK: Antioxidant activities of flavidin in different *in vitro* model systems. Bioorganic and Medicinal Chemistry 2004; 12(19): 5141-5146.
25. Peters W: Drug resistance in *Plasmodium berghei* Vincke and Lips (1948). 1. Chloroquine resistance. Experimental Parasitology 1965; 17(1): 80-89.
26. Fidock DA, Rosenthal PJ, Croft SL, Bruna R and Nwaka S: Antimalarial drug discovery: efficacy models for compound screening. Nature Reviews Drug Discovery 2004; 3(6): 509-520.
27. Knight DJ and Peters W: The antimalarial activity of N-benzoyloxydihydrotriazines. I. The activity of clociguanil (BRL 50216) against rodent malaria, and studies on its mode of action. Annals of Tropical Medicine and Parasitology 1980; 74(4): 393-404.
28. Chandel S and Bagai U: Antiplasmodial activity of *Ajuga bracteosa* against *Plasmodium berghei* infected BALB/c mice. Indian Journal of Medical Research 2010; 13(3): 440-444.
29. Kreh M, Matusch R and Wittie L: Capillary gas chromatography-mass spectrometry of Amaryllidaceae alkaloids. Phytochemistry 1995; 3(38): 773-776
30. Mallard WG and Reed J: Automated Mass Spectral deconvolution and Identification System –User guide. A publication of the United States. Department of Commerce Technology Administration, NIST Standard Reference Data Program Gaithersburg 1997. MD 20899. 58pp In:chemdata.nist.gov/mass-spc/amdis/docs/amdis.pdf.
31. Swigar AA and Silverstein RM: Monoterpenes, Infrared, Mass, NMR Spectra and Tram NTN, Mitova M, Bankova V, Handjieva N, Popov SS: GC-MS of *Crinum latifolium* L. Alkaloids. Zeitschrift fur Naturforschung 2002; 57c: 239-242.
32. Hasan I, Nasir UM, Forhad K, Humayera KH and Moktesur RS: Alpha amylase enzyme inhibitory and anti-inflammatory effect of *Lawsonia inermis*. Pakistan Journal of Biological Sciences 2013; 16(23): 1796-1800.
33. Curcic MG, Stankovic MS, Radojevic ID, Stefanovic OD, Comic LR, Topuzovic MD, Djacic DS and Markovic SD: Biological effects, total phenolic content and flavonoid concentrations of fragrant yellow onion (*Allium flavum* L.). Medicinal Chemistry 2012; 8(1): 46-51.
34. Sakat SS, Juvekar AR and Gambhire MN: *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. International Journal of Pharmacy and Pharmaceutical Sciences 2010; 2(1): 146-155.
35. Tung YT, Wub HJ, Hsieh C, Chen PS and Chang ST: Free radical-scavenging phytochemicals of hot water extracts of *Acacia confuse* leaves detected by an on-line screening method. Food Chemistry 2009; 115: 1019-1024.
36. Xu BJ and Chang SK: A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. Journal of Food Sciences 2007; 72(2): S159-166.
37. Polterait O: Antioxidants and free-radical scavengers of natural origin. Current Organic Chemistry 1997; 1: 415-440.
38. Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC and Atangbayila TO: Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in South-western Nigeria. Tropical Journal of Pharmaceutical Research 2008; 7(3): 1019-1024.
39. Ajaiyeoba E, Falade M, Ogbale O, Okpako L and Akinboye D: *In vivo* antimalarial and cytotoxic properties of *Annona senegalensis* extract. African Journal of Traditional, Complementary and Alternative Medicine 2006; 3: 137-141.
40. Dapper DV, Aziagba BN and Ebong OO: Antiplasmodial effect of the aqueous extract of *Phyllanthus amarus* Schumacher and Thonn against *Plasmodium berghei* in Swiss Albino mice. Nigerian Journal of Physiology 2007; 22(1-2): 19-25.

How to cite this article:

Shorinwa OA, Ebong OO, Obianime AW and Siminialayi IM: Evaluation of *in-vitro* antioxidant and antiplasmodial activities of the acetone extract of *Crinum jagus* bulbs (liliaceae) linn. Int J Pharm Sci & Res 2018; 9(7): 2990-98. doi: 10.13040/IJPSR.0975-8232.9(7).2990-

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)