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ORIGINAL ARTICLE

Acute Toxicity Study on the Bulb of *Crinum ornatum* (Ait Bury)

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ABSTRACT
The acute toxicity of the Bulb of Crinum Ornatum was studied using white albino rats of the winster strain. Phytochemical screening of the ethanol extract of Crinum ornatum bulb revealed the probable presence of alkaloids, tannins, saponins, terpenes and flavonoids. The total phenolic antioxidant content of the extract was evaluated by the Folin ciocalteau method. The extract revealed a total phenol antioxidant content of 271.5 expressed in mg/g GAE. Acute toxicity study of the extract upon oral administration revealed adverse effects at the lowest dose of 200mg/kg body weight with median lethal dose LD_{50} calculated to be 440 mg/kg body weight using the Karber arithmetic method. In conclusion, the plant should be used with caution.
KEY words: Toxicity, acute, rats

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INTRODUCTION

The plant kingdom is a rich source of herbal remedies to both man and animal. Plant extracts have been utilized for many purposes and their uses in natural therapies and alternative medicine have been profound and revolutionary [1]. The increasing interest in the use of naturally occurring substances for the preservation of food is reported by Kadri et al., [2]. Plants and their components have been known to exhibit biological activities, especially antimicrobial and antioxidant, and have also been the subject of study, particularly by the chemical, pharmaceutical, and food industries. The reason that antioxidants are important to human physical well being comes from the fact that reactive oxygen is a potentially toxic element since it can be transformed by metabolic activity into more reactive forms such as superoxide, hydrogen peroxide, singlet oxygen and hydroxyl radicals, collectively known as active oxygen [2]. These molecules are formed in living cells by various metabolic pathways. Free radicals can also cause lipid peroxidation in foods that leads to their deterioration. Antioxidants play important roles in preventing the diseases induced by reactive oxygen species (ROS) which result in oxidative damage to DNA, proteins and other macromolecules. Therefore, research in the determination of natural sources of antioxidants and the antioxidant potential of plants is important. The initiation of many diseases is associated with oxidative stress. Oxidative stress arises from the generation of free radicals in cells leading to cell damage. Oxidative stress plays an important role in the etiology of many pathological conditions like diabetes, atherosclerosis, malaria and cancers. Antioxidants are naturally occurring polyphenols that have the ability to fight and scavenge free radicals. The antioxidant property of plants is due to the presence of active phytochemicals like vitamins, flavonoids, terpenoids, carotenoids, coumarins, curcumins, lignin, saponin and plant sterol [3].

Crinum ornatum (Ait) Bury of the Amaryllidaceae family is a medicinal plant commonly found in tropical Africa which is native to sub Saharan Africa. It is known as 'albasar kwaadi' in Hausa; 'gaadal' in Kanuri; 'isumeri' in Yoruba and gaadal fabru' in Fulfulde. The bulb of some species of Crinum is interesting because they are being used all over the world to treat disorders like urinary tract infection, coughs and cold, renal and hepatic conditions, sores, sexually transmitted diseases and backaches as well as increase lactation in animals and humans. Previous work done on crinum species reported anti bacterial and antifungal activities, antitumour and immune stimulating activities and anti- malarial,anti viral and analgesic [4]; anti insecticidal activities [5] and anticonvulsant activity [6]. Traditionally in areas of Borno and Adamawa states of Nigeria where this study was conducted herbalists claim that the bulb in very small amount increases lactation and helps in relieving breast inflammation and other breast related illnesses. However, there is no scientific validation of such claims. The assessment of plant extracts for

potential toxicity is considered an important step in evaluating their suitability for commercial applications [7]. Therefore, in accordance with the resolutions of the 31st World Health Organization assembly requesting a complete inventory of the safety and standardization of medicinal plants, this study was undertaken with the aim to identify the phytochemical composition of ethanol extract of *Crinum ornatum* bulb from Maiduguri and to evaluate its total phenolic antioxidant content and provide information on the toxic or safety limits of the crude extract.

MATERIALS AND METHODS

Plant Material

Fresh whole plant *Crinum ornatum* were collected from a herb seller at Monday market Maiduguri. These were identified and authenticated by the chief taxonomist, Department of Botany, University of Maiduguri, Nigeria. Voucher specimen was deposited in the herbarium. The bulbs were excised and the outer part of the bulb peeled off. The bulbs were washed and thinly sliced before shade drying. The dried plant material was milled to powder and stored in airtight bottle until needed.

Extraction of Plant Material

100g of the powdered plant material was extracted by macerating in 0.5L absolute ethanol (97%) purity) for 48 H. This was filtered using Whatmann no 1 filter paper. The filterate was concentrated in- vacuo using a rotary evaporator before finally evaporating to complete dryness. The dry extract was then kept refrigerated in airtight bottle.

Phytochemical Screening

The Phytochemical components; alkaloids, tannins, saponins, flavonoids and terpenes were analyzed using the methods described by Sofowora, [8].

Total Phenol Antioxidant

The total phenolic antioxidant content of the ethanol extract of *Crinum ornatum* bulb was estimated by the Folin Ciocalteau method as described by Kajala et al., [9]. The extract was treated with 5ml Folin Ciocalteau (Sigma Aldrich) and 4ml sodium carbonate (7% w/v). The solution was mixed and allowed to stand for 30 minutes in the dark at room temperature. The absorbance was measured by UV-spectrophotometer at 760nm. The phenol content was expressed as gallic acid equivalent (GAE) in mg/g dry weight of extract.

Experimental Animals

Thirty mice weighing (25 - 35 g) of either sex were grouped into five cages. The animals were maintained under standard conditions of temperature (25 ± 2°C) and light, (approximately 12/12 h light-dark cycle), fed on standard commercial pelleted marsh (Vital Feeds Ltd. Jos, Nigeria) and water *ad libitum.* All experiments performed on the laboratory animals in this study followed approved Standard Operation Procedures.

Acute Toxicity Study

The lethal median dose (LD_{50}) of the ethanol extract of *Crinum ornatum* bulb was calculated by the Karber arithmetic method of Aliu and Nwude, [10]. Animals were administered graded doses of ethanol extract of the plant. The maximum dose of the extract that did not produce any death and the minimum dose that caused 100% death were identified through an oral administration of the extract. Signs of toxicity viz; hyper excitability, weakness, dilated pupil, depression, convulsion, irritability or death was observed in the animals from the time of administration for up to a period of 72H.

$LD_{50} = LD_{100}$ – sum of (Dose difference X Mean Death) / Number of animals per group

RESULTS AND DISCUSSION

The results of Phytochemical screening of the ethanol extract of *Crinum ornatum* bulb is presented on Table 1. The extract contained alkaloids, tannins, saponins, flavonoids and terpenes but anthraquinones were not detected. The presence of many active phytocomponents in plants has been documented over the years. Flavonoids are reported to possess pharmacological activity as free radical scavengers (Cotelle et al., 1996). Nwanorh, 2011 reported the presence of hydroquinone and benzoquinone, and absence of quinoline and anthraquinone derivatives in *Crinum ornatum.*

The total phenol antioxidant of the extract was determined to be 271.5 mg/g expressed in gallic acid equivalent. Antioxidant properties of the plant have been reported by Oloyede et al., [11]. They reported the presence of free radical scavenging activities of the plant being due to the presence of crinamine, lycorine, hamyne and haemanthamine alkaloids. The results of phytochemistry showed that the bulb in this study contained alkaloids. Qader et al., [7] reported similar total phenol content (207 ± 0.011mg/g) in the ethanol extract of *P.minus*. Ling et al., 2010, reported that ethanol extracts of plants show high total

phenol contents than aqueous extracts probably due to the polarity of the solvent of extraction. A total phenol content of 121.9 ± 3.1 mg/g GAE in *Torilis leptophylla* was reported by Saeed et al., [12]. Natural antioxidants protect dietary lipid from oxidation and may also provide health benefits associated with preventing damages due to biological degeneration [13]. Antioxidants are used in many industrial processes as food preservative, in cosmetics and the prevention of degradation of rubber and gasoline [14]. These agents help protect the skin from sun exposure and decrease skin roughness, wrinkles depth, UV- induced skin cancer and skin swelling from sunlight [14]. Women in Eastern Nigeria have been reported to use the paste of the plant to tone their skin [15]. This could probably be attributed to the antioxidant content of the plant.

The acute toxicity of the ethanol extract of *Crinum ornatum* bulb is presented on Table 2. The extract revealed an LD_{50} value of 440 mg/kg body weight on oral administration of extract. Clinical signs of toxicity viz; weight loss, hypoactivity and weakness were observed at a dose of 400 mg/kg of the extract. The extract was 100% lethal at a dose of 1600 mg/kg body weight of the extract. The Chemical Toxicity Data Base [16] reported that the lowest published lethal dose on *Crinum ornatum* bulb on intraperitoneal administration is 500 mg/kg body weight. Ghosal et al., 1985 reported acute toxicity of *Crinum ornatum* bulb intraperitoneally in albino rats with a value >200mg/kg ip. Patricia et al., 2011 reported that the secondary metabolites in the plant are most times toxic. The cytotoxicity of the essential oil of *Crinum ornatum* Ait Bury. was studied by Oloyede et al., [11]. Their findings revealed LC_{50} value of 1.70 µg/ml using brine shrimps assay.

Table 1 Phytochemical screening

COMPONENT	RESULT
Alkaloid	+++
Tannins	++
Saponins	+
Flavonoids	++
Terpenes	+++
Anthraquinones	–

+++ high
++ moderate
+ present
– negative

Table 2 Acute toxicity

Dose mg/kg bw	No of Deaths	Dose Difference	Mean of Deaths
Control	-	-	-
200	0	200	0
400	0	200	0
800	3	400	1.5
1600	5	800	4
2000	5	400	5

$LD_{50} = LD_{100}$ – sum (Dose difference X Mean Death) / Number of animals per group
$LD_{50} = 1600 – (5800/5)$
LD_{50} = 440 mg/kg body weight

CONCLUSION

Crinum ornatum Ait. (Bury) is a plant with much potential in cosmetic, pharmaceutical and food industries. This work has shown that the plant is rich in active phytocomponents, has antioxidant potentials and is toxic and lethal at dose of 400 mg/kg body weight orally. Therefore its use in folkloric treatment needs caution. However, its use for industrial purposes needs to be fully explored.

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