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Original article

Chemical analysis and analgesic activity of methanol extract of *Crinium Jagus* bulb in BALB/c mice

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Abstract: *Crinum jagus*, family- *Amaryllidaceae*, commonly called river lily is an important medicinal plant used in the treatment of cough. It is an anti-venom agent used among the rural people in south-western Nigeria. The research is aimed to investigate the phytochemical constituents and analgesic activity of *Cinium jagus* bulb in BALB/c mice. The bulb was extracted with methanol in a soxhlet extractor apparatus. Phytochemical screening was performed according to standard methods. Alkaloid fraction was obtained using separation by extraction and characterized by infra-red (I.R) analysis. Acute toxicity test was done before analgesic activity was determined in BALB/c albino mice using the hot plate model. The result indicated the presence of some bioactive constituents like alkaloids, terpenoids and saponin. I.R bands were observed at 1064.74 cm^{-1} (C-O) stretch of alcohol; 1415.80 cm^{-1} (C-H) bend of alkyl groups and 1639.55 cm^{-1} (N-H) stretch of amine among others. The extract significantly increased the mean latency time of mice on the hot plate when compared with control with 100 mg/kg at 120 minutes. This study indicates that the *Crinum Jagus* bulb possesses analgesic potential.

Introduction

Plants have been used from antiquity to treat, manage and cure various diseases by man [1]. The potential significance of these plants is due to the medicinal value contained in the bioactive chemical constituents which includes alkaloids, flavonoids, phenolics, terpenes and steroids [2]. Medicinal plants have been used to treat infectious diseases for many years worldwide leading to a growing interest in the development of drugs of plant origin. Nigeria is one of the countries in the world with unique wealth of medicinal plants and vast traditional knowledge of use of herbal medicine for treatment of various diseases. *Crinum jagus* (*C. jagus*, river lily), family *Amanyllidaceae* is an herbaceous plant with large, tunicates bulb producing a pseudo-stem. It's used in the treatment of backache and to increase lactation in animal and human mothers [3]. In Nigeria, the plant is locally called "Ebe-eyen" in Bini, "Albasar kwadi" in Hausa, "Ede chuku" in Ibo and "Ogede odo" in Yoruba.

They are used traditionally as emetics, laxatives, expectorants, antipyretics, among others [4]. Extracts of Crinum species have been reported to possess cytotoxic, antitumor, antiviral, antimicrobial, antimalarial, analgesic, and immune-dilating activities [5]. These activities have been attributed to the presence of alkaloids in these *Crinum* species, like *Crinum pedunculatum*, *C. firmifolium* and *C. latifolium* [4, 6]. More so, scientific investigation have been conducted on the analgesic, anti-inflammatory and anti-pyretic activity of *Crinum* bulb species [7]. Their findings revealed that the methanol, ethanol, and ethyl acetate extracts of *Crinum pedunculatum* possess significant peripheral analgesic, activities. From related literature, more pharmacological activities of *Crinum* jagus bulb have been reported to include anti-tuberculosis and anti-diabetic [8], antihemorrhagic, antioxidant, antibacterial [9], healing and hepatoprotective activity [10]. Phytochemical constituents which are of physiological importance have also been reported recently, among which terpenes, coumarine and host of alkaloids are including hippadine, lycorine, 3-O-demethyltazettine, ambelline, acetylambelline, acetylcaranine, crinanine acetate and crinine [11]. The study was conducted to explore the analgesic activity of the methanol extract of *Crinum jagus* bulb in mice.

Materials and methods

Extraction and treatment of plant sample: The fresh bulbs of *C. jagus* were collected from its natural habitat on March 21, 2018 in Akure, Ondo State, Nigeria. The plant was identified and authenticated by a taxonomist Prof. J. F. Bamidele, with herbarium voucher number (UBHm 0198) deposited in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria.

Four hundred and twenty grams of the powdered bulb were extracted with methanol in a soxhlet apparatus for eight hours. The crude extract was dried with Na_2SO_4 and then concentrated in a rotary evaporator (model, RE, 200) to obtain a syrupy consistency (97 g, yield: 23.10%)

Sourcing of animals: Twenty (20) Swiss balb/c albino mice were obtained from the Pharmacology animal house Faculty of Pharmacy, University of Benin, Benin City, Nigeria. The animals were kept in clean cages and allowed to acclimatize for two weeks before experiment. They were maintained on standard animal pellets and water *ad libitum* while permission and approval for animal studies were obtained from the Institutional Ethical Review Committee of the Faculty of Life Sciences, University of Benin, Benin City, Nigeria with the ethics reference project number LS191185.

Phytochemical screening of methanol extract: Phytochemical screening was done to find the presence of the active chemical constituents such as alkaloids, glycosides, steroids, flavonoids, saponins, terpenoids, phenolics, tannins and eugenols by using the standard procedures [7].

Isolation of alkaloid fraction: Twenty six grams of dried methanol extract of *C. jagus* was dissolved in 50 ml of diethyl ether and treated with 20 mL, three times, each of 2M HCl. The aqueous layer (lower layer) which contains the soluble organic salt was separated and treated with 60 ml of sodium carbonate (Na_2CO_3) to release the soluble bases as insoluble precipitate. The precipitate which should contain basic fraction was then re-extracted with ether and dried for IR analysis.

Mice: Balb/c mice weighing 20 - 33 g were used. They were housed in netted metal cages under standard conditions of light and temperature and were maintained on a standard diet and water ad libitum. They were acclimatized for 14 days and were treated in accordance with guidelines for animal care approved by the Institutional Ethical Review Committee of the Faculty of Life Sciences, University of Benin with reference number LS191185.

Evaluation of acute toxicity: The acute toxicity was performed with the methanol extract of *C. jagus* bulb according to guidelines prescribed by the Organisation for Economic Cooperation and Development [12] A group of mice (n = 6) were injected with extract orallyat a dose of 500, 1000 and 2000 mg/kg. The doses were increased as the mice survived at the smaller doses. Distilled water was used as a control and the mice were observed carefully during 24 hour for any effect or mortality.

Determination of analgesic activity in albino mice: Albino albino mice were divided into five groups of four animals each after acclimatization. This acclimatization involved placing the mice on the hot plate analgesia meter prior to administration to allow them get familiarized to the hot plate environment. After the initial screening, distilled water which served as control was given orally to group A at a dose of 0.05 ml, the extract at 100, 200 and 400 mg/kg was given orally to groups B, C and D, respectively. Group E received the standard drug, pentazoxine at 10 mg/kg subcutaneously [13].

The animals were dropped gently on the hot plate analgesia meter (Ugo Basile hot/cold plate -35 100) maintained at 55 ± 0.00 ℃. This was done after the oral administration of water, extract and pentazoxine at 30, 60, 120,180 and 240 min, respectively. The time in seconds for the mouse to either jump or lick its paws was taken as the reaction time. This was recorded carefully [13]

Infra-Red (IR) analysis: The IR spectra of the alkaloid isolate was recorded on a Buck IR M500 Spectrophotometer 4000-350 cm^{-1}

Statistical analysis: A two-way ANOVA was done to compare the effect of dosage extract and controls on the analgesic activity using EXCEL 2013. $P < 0.05$.

Results

The findings of phytochemical screening of the methanol extract are shown in **Table 1**. Several components were found in this extract such as glycoside, saponin, tannins, flavonoids and alkaloids, however, no phenolics, steroid were detected in this extract. Furthermore, the chemical analysis of the extract revealed different functional groups (alcohol, amine, amide and alkyl groups) with different peaks, appearance and band by IR absorption bands (**Table 2**).

Table 1: Phytochemical screening of methanol extract of *C. jagus bulb*

S/N	Phytochemical	Methanol extract
1	Glycoside	+
2	Saponin	+
3	Phenolics	-
4	Tannins	+
5	Eugenol	+
6	Steroid	-
7	Terpenoids	+
8	Alkaloids	+
9	Flavonoids	+

Key: **-** = absent, + = present

Figure 1 shows compares the mean value of latency time following the administration of *C. jagus* extract in albino mice. The analgesic results revealed significantly increased the mean latency time of mice on the hot plate, when compared with the control at 100 mg per kg and 200 mg per kg at 120 minutes and 30 minutes, respectively, and 400 mg per kg at 30 minutes and 60 minutes. This effect was, however, more significant at 100 mg per kg and 400 mg per kg. At all the doses, the extract significantly

increased mean latency time in mice when compared to pentazoxine except at 200 mg per kg at 120 minutes. Furthermore, the results from **Table 3** shows that dosage of control and extract showed significant difference on the analgesic activity of mice.

Table 2: Infra-Red absorption bands of functional groups detected in alkaloid fraction of *C. Jagus*

S/N	Peak (cm^{-1})	Appearance	Band	Functional group
1.	1064.74	Short	C-O stretch	Alcohol
2.	1415.80	Strong, short	C-H bend	Alkyl group
3.	1639.55	Strong	N-H stretch	Amine, amide
4.	2112.12	Short	(C ≡ N) stretch	Nitrile
5.	3558.78	Broad	N-H stretch	Amine (RNH_2)

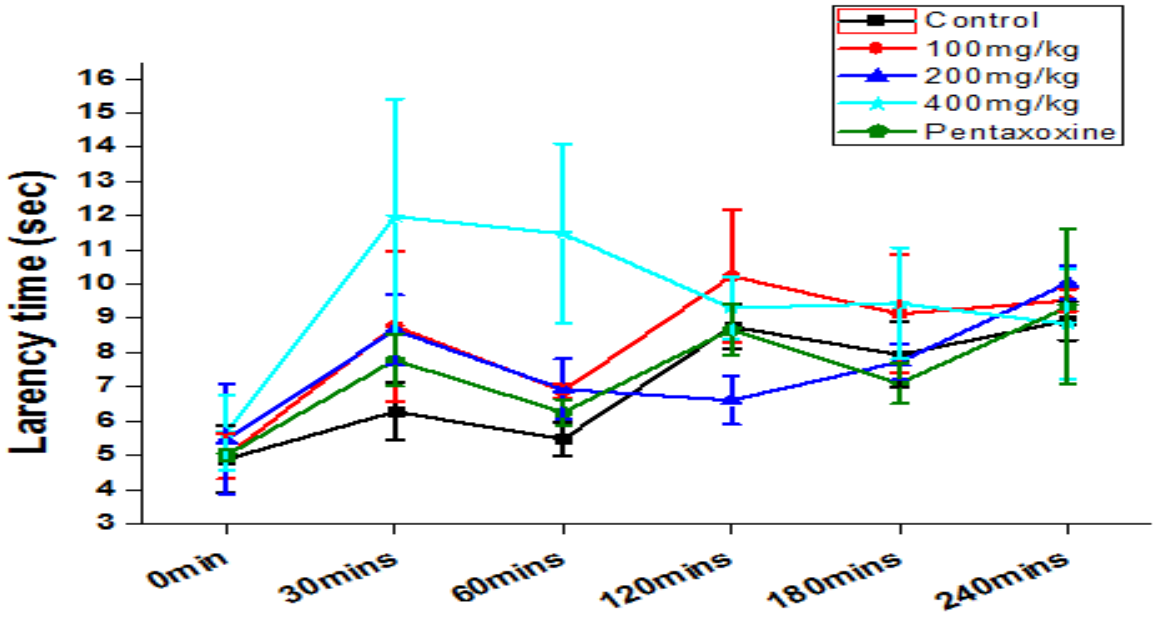


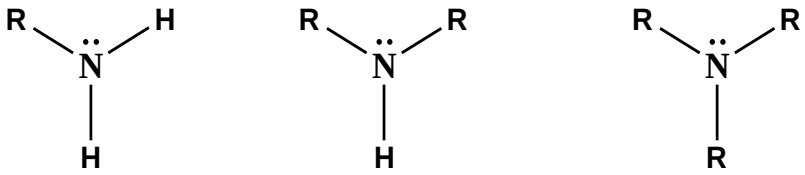
Figure 1: Graphical representation of latency time following the administration of *C. jagus* extract at different time intervals in albino mice

Table 3: Data of the ANOVA analysis

Source of variation	*SS*	*df*	*MS*	*F*	*P value*	*F crit*
Rows	55.19407	5	11.03881	7.34282	0.000473	2.71089
Columns	21.91315	4	5.478288	3.644059	0.021869	2.866081
Error	30.06696	20	1.503348			
Total	**107.1742**	**29**				

Discussion

In this study, the presence of alkaloid, flavonoid, phenolics and terpenes were detected in *C. jagus* extract. Alkaloid was also reported in the work of Ying [14], who isolated azettine-type alkaloids which are known for their antimalarial, analgesic, antiviral and anti-proliferative properties. Several studies have indicated the analgesic activity of phytochemical constituents like flavonoids, saponins and alkaloids [15 - 17]. The acute test conducted on the three doses of the extracts showed that the highest dose of 2000 mg/kg did not cause toxic manifestations like changes in the skin, eye hair (fur), respiration, salivation, sleep and mortality. The mean value of latency time following the administration of *C. jagus* extract in albino mice was significantly increased the mean latency time of mice on the hot plate, when compared with the control at 100 and 200 mg/kg at 120 and 30 min, respectively, 400 mg/kg at 30 and 60 min. This effect was, however, more significant at 100 and 400 mg/kg. At all the doses, the extract significantly increased mean latency time in mice when compared to pentazoxine. Therefore, since the effect of the extract on the mean reaction time of mice on the hot plate was not dose dependent, the findings suggested that the extract possesses a centrally acting analgesic activity rather than peripheral [18] due to prolongation of time responses following the administration of the extract as observed for the 100 and 400 mg/kg doses which increased the pain threshold of the mice at 120 min and 30 min, respectively. However the analgesic studies of Doe and others [7] indicated a dose dependent activity for methanol extract of *Crinum pedunculatum.* This effect may thus be explained on the basis of the action of some receptors in the central nervous system, which when stimulated have the intrinsic potential to reduce the effective components of pain [7]. The I.R. spectrum of the alkaloid fraction and the wave numbers of functional groups were detected. The functional groups detected from the I.R. bands were observed at 1064.74 cm^{-1} (C-O) stretch of alcohol, 1415.80 cm^{-1} (C-H) band of alkyl group, 1639.55cm^{-1} (N-H) stretch of amine, 2112.12cm^{-1} (C ≡ N) stretch of nitrile and 3558.78cm^{-1} (N-H) stretch of amine. The broad band observed at 3558.78cm^{-1} does not have 2 or 3 band (not H-bonded) and hence will likely be a tertiary amine which is present in most alkaloids of heterocyclic origin. The other band at 1639.55cm^{-1} of N-H stretch suggests that the isolate is rich in alkaloid. This supports the work by Kouadio et al. [11] which revealed a total of fifteen alkaloids in *C. jagus* bulb.



Primary amine **Secondary amine** **Tertiary amine**

Conclusion: The extract of *Crinum jagus* contains bioactive constituents like alkaloids, flavonoids, tannins, terpenes, saponin, eugenol and glycosides which have been implicated to have physiological and medicinal effect in humans. The analgesic findings suggest that the *Crinum jagus* may be a potential analgesic agent when properly screened and characterized.

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Ethical issues: Including plagiarism, informed consent, data fabrication or falsification and double publication or submission have completely been observed by authors.
Data availability statement: The raw data that support the findings of this article are available from the corresponding author upon reasonable request.
Author declarations: The authors confirm that all relevant ethical guidelines have been followed and any necessary IRB and/or ethics committee approvals have been obtained.

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