

ORIGINAL RESEARCH article

Chemical analysis and analgesic activity of methanol extract of *Crinum 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 aims 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. The alkaloid fraction was obtained using separation by extraction and characterized by infra-red (I.R) analysis. An acute toxicity test was done before the 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 the control with 100 mg/kg at 120 minutes. This study indicates that the *Crinum Jagus* bulb possesses analgesic potential.

Introduction

Plants have been used since 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 include 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 a unique wealth of medicinal plants and vast traditional knowledge of use of herbal medicine for the treatment of various diseases. *Crinum jagus* (*C. jagus*, river lily), family *Amaryllidaceae* is an herbaceous plant with a large, tunicate 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, and 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 investigations has been conducted on the analgesic, anti-inflammatory and antipyretic 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, coumarin and a host of alkaloids including hippadine, lycorine, 3-O-demethyltazettine, ambelline, acetylabelline, 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 their 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 the 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 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 an insoluble precipitate. The precipitate which should contain a 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 by 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 orally at 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 during 24 hours for any effect or mortality.

Determination of analgesic activity in albino mice: 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 to get familiarized with 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, and 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 to 100) maintained at 55 ± 0.00 °C. 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 were recorded on a Buck IR M500 Spectrophotometer $4000\text{-}350\text{ cm}^{-1}$

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, or 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 compares the mean value of latency time following the administration of *C. jagus* extract in albino mice. The analgesic results revealed a significantly increased 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** show that the dosage of control and extract showed a significant difference in 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)

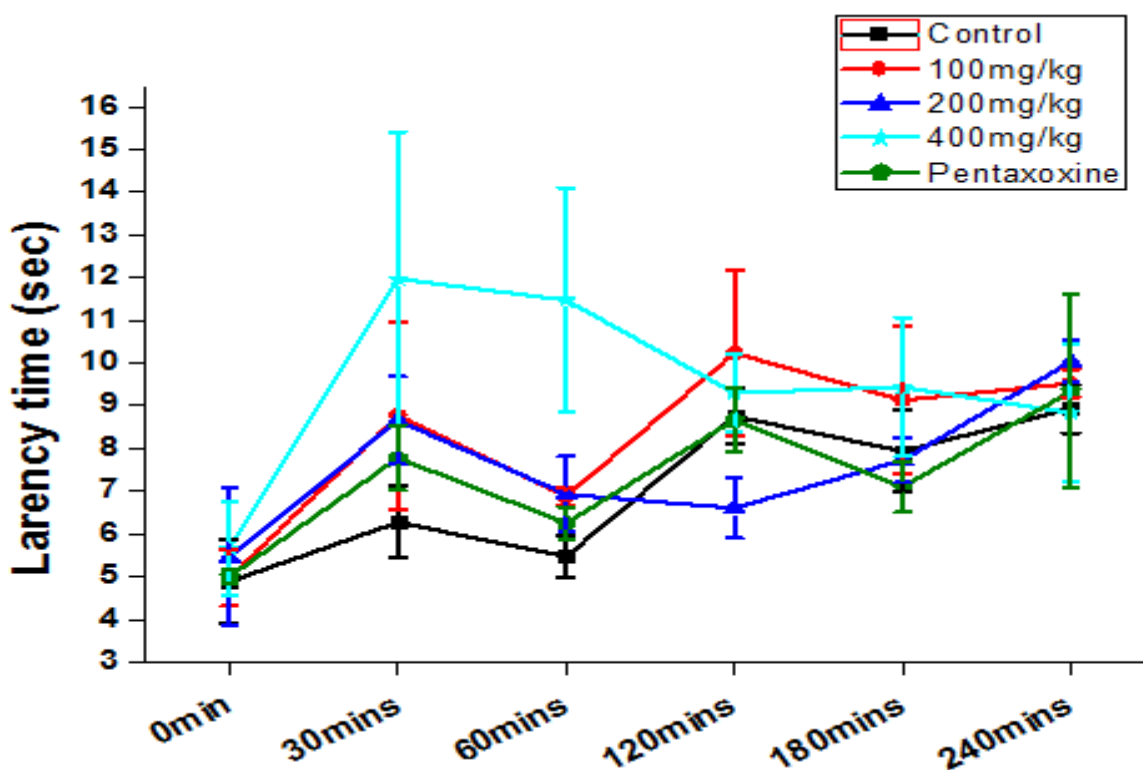


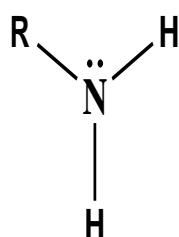
Figure 1: Graphical representation of latency time following the administration of *C. jagus* extract at different time intervals in albino mice

Table 3: Data of the ANOVA analysis

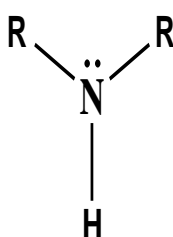
Source of variation	SS	df	MS	F	P value	F crit
Rows	55.19407	5	11.03881	7.34282	0.000473	2.71089
Columns	21.91315	4	5.478288	3.644059	0.021869	2.866081
Error	30.06696	20	1.503348			
Total	107.1742	29				

Discussion

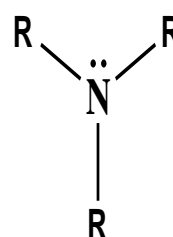
In this study, the presence of alkaloids, flavonoids, phenolics and terpenes was detected in *C. jagus* extract. Alkaloid was also reported in the work of Ying [14], who isolated azettine-type alkaloids which are known for their antimalarial, analgesic, antiviral and anti-proliferative properties. Several 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 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 the 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 the 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 the alkyl group, 1639.55 cm^{-1} (N-H) stretch of amine, 2112.12 cm^{-1} (C \equiv N) stretch of nitrile and 3558.78 cm^{-1} (N-H) stretch of amine. The broad band observed at 3558.78 cm^{-1} does not have 2 or 3 bands (not H-bonded) and hence will likely be a tertiary amine which is present in most alkaloids of heterocyclic origin. The other band at 1639.55 cm^{-1} of N-H stretch suggests that the isolate is rich in alkaloids. 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 in having physiological and medicinal effects 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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Author contribution: OI conceived, designed the study, performed the analysis and drafted the manuscript. AOO contributed to collecting and analysis of data. Both authors have approved the final version of the manuscript and agreed to be accountable for its contents.

Conflict of interest: The authors declare the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical issues: Including plagiarism, informed consent, data fabrication or falsification and double publication or submission were completely observed by the 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.

References

1. Gelaw H, Adene L, Tariku Y, Hailu A (2012) Isolation of crotepoxide from berries of *Croton macrostachyus* and evaluation of its anti-leishmanial activity. *Journal of Pharmacognosy and Phytochemistry*. 1 (4): 15-24. Corpus ID: 55172535.
2. Yuan H, Ma Q, Ye L, Piao G (2016) The traditional medicine and modern medicine from natural products. *Molecules*. 21 (5): 559-577. doi: 10.3390/molecules21050559
3. Aigbokhan EI (2014) Annotated checklist of vascular plants of Southern Nigeria- a quick reference guide to the vascular plants of southern Nigeria: a systematic approach. Uniben Press, Benin City. 165. doi: 10.13140/RG.2.1.1604.0808
4. Aziz A, Raju GS, Das A, Ahmed J, Moghal MMR (2014) Evaluation of in vitro anthelmintic activity, total phenolic content and cytotoxic activity of *Crinum latifolium* L (family: Amaryllidaceae). *Advanced Pharmaceutical Bulletin*. 4 (1): 15-19. doi: 10.5681/apb.2014.003
5. Presley CC, Krai P, Dalal S, Su Q, Cassera M, Goetz M (2016) New potentially bioactive alkaloids from *Crinum erubescens*. *Bioorganic and Medicinal Chemistry*. 24 (21): 5418-5422. doi: 10.1016/j.bmc.2016.08.058
6. Presley CC, Du Y, Dalal S, Merino EF, Butler JH, Rakotonandrasana S, Rasamison VE, Cassera MB, Kingston DGI (2017) Isolation, structure elucidation, and synthesis of anti-plasmodial quinolones from *Crinum firmifolium*. *Bioorganic and Medicinal Chemistry*. 25 (15): 4203-4211. doi: 10.1016/j.bmc.2017.06.017
7. Doe P, Danquah CA, Ohemeng KA, Opare AE, Sharif A, Akua-Abora D, Akuoko AK, Kpabitey A, Quarshie E, Asante OO, Amponsah EK, Mariyana, Mutwaliku M, Nuro-Brefo C, Ofori M (2021) Analgesic, anti-inflammatory, and anti-pyretic activities of *Crinum pedunculatum* R. Br. Bulb extracts. *Pharmacognosy Research*. 14 (1): 24-29. doi: 10.5530/pres.14.1.5
8. Kouadio ATG, Kabran GRM, Mamyrbekova-Bekro JA, Lebrun A, Virieux D, Pirat J-L, Bekro Y-A (2021) Alkaloids isolated from *Crinum jagus* L. bulb (Amaryllidaceae) from Côte d'Ivoire. *Journal of Pharmacognosy and Phytochemistry*. 10 (2): 36-39. doi: 10.22271/phyto.2021.v10.i2a.13694
9. Udegbuman SO, Kene RO, Anika MS, Udegbuman IR, Nnaji TO, Anyanw UM (2015) Evaluation of wound healing potential of methanolic *Crinum jagus* bulbs extract. *Journal of Intercultural Ethnopharmacology*. 4 (3): 194-201. doi: 10.5455/jice.20150405064050
10. Olusayo A, Shorinwa L, Omatayo EO, Obianime WA, Siminialayi MI (2016) Evaluation of potential anti-diabetic activity of acetone extract of *Crinum jagus* bulbs in Albino rats. *Indo American Journal of Pharmaceutical Research*. 6 (2): 4581-4586. doi: 10.1044/1980-iajpr.160233
11. Kouadio ATG, Kabran GRM, Mamyrbekova-Bekro JA, Virieux D, Pirat JL, Bekro YA (2020) Total alkaloids and in vitro antioxidant activity of *Crinum jagus* L. (Amaryllidaceae) organs from Côte d'Ivoire. *International Journal of Green and Herbal Chemistry*. 4: 451-453. doi: 10.24214/IJGHC/HC/9/4/45163
12. Organization for Economic Cooperation and Development (OECD) (2002) Test number 423: Acute oral toxicity. Acute toxic class method. OECD guideline for testing of chemicals. 1-14. ISSN: 20745788. doi: 10.1787/20745788
13. Eddy NB, Leimbach D (1953) Synthetic analgesic II. Dithienylbutenyl and dithienylbutylamine. *Journal of Pharmacology and Experimental Therapeutics*. 107 (3): 385-393. PMID: 13035677.



14. Ying G (2015) Research on the alkaloids of amaryllidaceae plants: Genera Lycoris and Hippeastrum. Thesis. Publisher: Universitat de Barcelona. November 5, 2015. 195. <http://hdl.handle.net/2445/67769>
15. Fan SH, Ali NA, Basri DF (2014) Evaluation of analgesic activity of the methanol extract from the Galls of *Quercus infectoria* (Olivier) in rats. *Evidence-Based Complementary and Alternative Medicine*. 2014: 976764. doi: 10.1155/2014/976764
16. Doughari JH (2012) Phytochemicals: extraction methods, basic structures and modes of action as potential chemotherapeutic agents. *Phytochemicals - A Global perspective of their role in Nutrition and health*. In Tech. 1-35. doi: 10.5772/26052. ISBN: 978-953-51-0296-0.
17. Karak P (2019) Biological activities of flavonoids: an overview. *International Journal of Pharmaceutical Sciences and Research*. 10 (4): 1567-1574. doi: 10.13040/IJPSR.0975-8232.10(4).1567-74
18. Wigdor S, Wilcox GL (1987) Central and systemic morphine-induced antinociception in mice: contribution of descending serotonergic and noradrenergic pathways. *The Journal of Pharmacology and Experimental Therapeutics*. 242 (1): 90-95. PMID: 3612540.