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Original Article

Analgesic, Anti-inflammatory, and Anti-pyretic Activities of *Crinum pedunculatum* R.Br. Bulb Extracts

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ABSTRACT

Background: *Crinum pedunculatum* R.Br. bulbs are used for the topical management of inflammation by traditional healers in the southern region of Ghana. **Objectives:** This study aims to assess the analgesic, anti-inflammatory, and anti-pyretic activities of different solvent extracts of *Crinum pedunculatum*. **Methods:** The analgesic, anti-inflammatory, and anti-pyretic activities of the bulb extracts of *Crinum pedunculatum* were determined in rats at doses of 100, 200, and 400 mg/kg. The acetic acid induced writhing test was used to determine the analgesic activity, carrageenan was employed to determine the anti-inflammatory activity, and Brewer's yeast-induced pyrexia was studied to evaluate the extract's anti-pyretic activity. **Results:** All solvent extracts of *Crinum pedunculatum* significantly decreased ($P < 0.001$) the frequency of writhing in rats at all doses with 400 mg/kg of the ethanolic extract showing a 98% inhibition comparable to that obtained with diclofenac sodium at 94%. These extracts also caused the inhibition of the increase in paw diameter induced by the administration of carrageenan with 400 mg/kg of the ethyl acetate extract of *Crinum pedunculatum* causing a 97% inhibition of paw oedema. All doses (100, 200, and 400 mg/kg) of the methanol extract caused a significant decrease ($P < 0.0001$) in the temperature of rats induced via the administration of yeast with ethanol and ethyl acetate extracts also showed a significant reduction ($P < 0.001$) in rectal temperature. **Conclusion:** These results obtained indicate that the methanolic, ethanolic, and ethyl acetate extracts of *Crinum pedunculatum* R.Br. possess analgesic, anti-inflammatory, and antipyretic activities.
Key words: Analgesic, Anti-inflammatory, Anti-pyretic, *Crinum pedunculatum*, Rats.

INTRODUCTION

Several disease conditions are routinely present with pain and pyrexia. Nonsteroidal anti-inflammatory drugs (NSAIDS) are frequently prescribed to manage these conditions, but gastrointestinal bleeding, perforation, exacerbation of gastric ulcers and cardiac irregularities are some of the side effects associated with their use.[1] Natural products and their derivatives are principal sources for the management of several diseases worldwide.[2] The scientific investigation of plants used as analgesics, anti-inflammatory, and antipyretic agents in traditional medicine is a strategy that has yielded and will continue to yield promising prospects. *Crinum* species have a substantial medicinal reputation as potent traditional remedies with their use extending to present times in Africa, tropical Asia, and South America.[3,4] They are used traditionally as emetics, laxatives, expectorants, antipyretics, among others. Extracts of *Crinum* species have been reported to possess cytotoxic, antitumor, antiviral, antimicrobial, antimalarial, analgesic, and immunomodulating activities. These activities have been attributed to the presence of alkaloids in these *Crinum* species.[5–9]

Crinum pedunculatum, also known as swamp lily, belongs to the family Amaryllidaceae. Plants of the *Crinum* species have been reported to contain phytoconstituents such as coumarins, catechic tannins, triterpenes, anthocyanidins, polyphenols among others.[10] The bulbs of *Crinum pedunculatum* plant are used by traditional healers of the southern region of Ghana for the management of inflammation, pain, and fever. However, there are no scientific studies carried out to validate these activities and to the best of our knowledge no pharmacologic or biologic evaluations of any activities concerning this plant have been reported. Consequently, this study was carried out to evaluate the analgesic, anti-inflammatory, and anti-pyretic activities of the methanolic, ethanolic, and ethyl acetate extracts of the bulbs of *Crinum pedunculatum*.

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MATERIALS AND METHODS

Plant collection and identification

Bulbs of *Crinum pedunculatum* were collected from Oframatin, Kwahu-Asakrakra in the Eastern region of Ghana (Latitude: 6.62942 N 6° 37'45.9048". Longitude:-0.68647 W 0° 41'11.30253"). They were identified and authenticated by Mr Clifford Asare, an herbalist at the Herbal Medicine Department, Faculty of Pharmacy and Pharmaceutical Sciences (FPPS), Kwame Nkrumah University of Science and Technology, Ghana. A sample was kept at the herbarium (voucher specimen number CP/01/19) of Central University, Ghana, where part of the research was carried out.

Plant preparation and extraction

The dried bulbs were ground into coarse powder and extracted with 99.8% ethanol, 99.8% methanol, and ethyl acetate using cold maceration method. The preparation was shaken intermittently for 7 days, after which filtration through a No. 1 Whatman filter paper was carried out. A rotary evaporator was used to evaporate the solvents, and the dried extracts were stored in separate air-tight containers and refrigerated at 4°C for use.

Experimental animals

Wistar albino rats weighing 93-110 g obtained from the University of Ghana animal house were used in this study. They were allowed to acclimatize for 14 days. Animals were kept in plastic cages and fed with a standard pellet diet and granted unrestrained access to clean water. All experimental protocols and handling of animals were carried out in compliance with the Institute for Laboratory Animal Research[11] and were authorized by the Institutional Review Board on Animal Experimentation, Kwame Nkrumah University of Science and Technology with the ethics reference number FPPS/PCOL/010/2019.

Phytochemical screening

Preliminary phytochemical analysis was conducted on the methanolic, ethanolic, and ethyl acetate extracts of *Crinum pedunculatum* using standard methods described by Trease and Evans.[12] Qualitative screening was carried out for tannins, phlobatannins, flavonoids, saponins, cardiac glycosides, and alkaloids.

Acute toxicity test

Acute toxicity test was performed on the methanol, ethanol, and ethyl acetate extracts of *Crinum pedunculatum* following the guidelines stated by The Organization for Economic and Co-operative Development.[13]

Analgesic activity

Acetic acid induced writhing test

The method described by Koster *et al.*[14] with some adjustment was employed to determine the analgesic effect of the crude extracts in rats. Experimental animals were weighed and distributed into 5 groups of 5 animals each. Group 1 served as the negative control and were administered normal saline 10 ml/kg, group 2 received diclofenac 75 mg/kg (standard drug), while groups 3, 4, and 5 received *Crinum pedunculatum* extracts at 100, 200 and 400 mg/kg respectively. All administrations were done orally. Thirty minutes after pretreatment, each animal received 1% acetic acid (10 ml/kg) intraperitoneally. Frequency of abdominal writhes were counted for 15 min commencing 5 min following acetic acid administration. Percentage of analgesic activity was determined using the following formula:

$$\text{Percentage inhibition of writing} = \left[\frac{W_{\text{control}} - W_{\text{treated}}}{W_{\text{control}}} \times 100\right]$$

Where W = number or frequency of writhes

Anti-inflammatory activity

Carrageenan-induced rat paw oedema

Anti-inflammatory activity was carried out using Wistar rats according to the method reported by Winter *et al.*[16] Group 1 served as the negative control and were administered normal saline 10 ml/kg, group 2 (positive control) received diclofenac 75mg/kg (standard drug), and groups 3, 4 and 5 were administered 100, 200 and 400 mg/kg of *Crinum pedunculatum* extract respectively. One hour following pretreatment, inflammation was induced by the administration of 0.1ml carrageenan (1%w/v) in 0.9% normal saline to the right hind paw of each animal by sub-plantar injection. Diameter of the injected paw was measured every hour for 6 hr using digital callipers. Percentage reduction in diameter of the treated group was calculated as follows:[17]

$$\text{Percentage reduction in paw diameter} = \left[\frac{(Ct - Co)\ \text{control} - (Ct - Co)\ \text{treated}}{(Ct - Co)\ \text{control}} \times 100\right]$$

Where C_t = paw diameter at time t; C_0 = paw diameter before carrageenan injection.

Anti-pyretic Activity

Anti-pyretic activity was determined in rats using Brewer's yeast following standard procedures.[18,19] The basal rectal temperature of each animal was taken using a clinical digital thermometer, after which pyrexia was induced by the subcutaneous injection of 20% w/v Brewer's yeast suspension in normal saline at 10 ml/kg of rat. Increase in rectal temperature of each animal was recorded 18 hr after yeast injection and animals that showed a rise in temperature of ≥ 1F (0.6°C) were chosen for the study. Animals were subsequently distributed into five groups of 5 animals with group 1 receiving 10 ml/kg normal saline, group 2 receiving 125 mg/kg paracetamol, groups 3, 4, and 5 receiving 100, 200 and 400 mg/kg of *Crinum pedunculatum* extract respectively. Rectal temperature of each rat was recorded for the first 6 hr and at 12 and 24 hr to confirm activity of the extract.

Statistical analysis

All results are expressed as mean ± standard error of the mean (SEM). Results were analysed statistically using GraphPad prism version 8 software and $P < 0.05$ was regarded as statistically significant.

RESULTS

Phytochemical analysis of *Crinum pedunculatum* extracts indicated the presence of saponins, alkaloids, and phlobatannins among others (Table 1).

Acute toxicity test

Acute toxicity experiments carried out with all *Crinum pedunculatum* extracts showed that the limit dose of 2000 mg/kg did not result in mortality and any visible toxic manifestations such as changes in skin, fur, eyes, respiration, tremors, convulsions, salivation, diarrhoea, sleep and lethargy.

Acetic acid induced writhing test

All doses of the ethanolic, methanolic, and ethyl acetate extracts of *Crinum pedunculatum* (100, 200, and 400 mg/kg) exhibited significant decrease in the number of writhes induced by acetic acid relative to the

Table 1: Phytochemical analysis of *Crinum pedunculatum* extracts.

Crinum pedunculatum extracts			
Phytochemical Constituent	Methanol extract	Ethanol extract	Ethyl acetate extract
Saponins	+	+	+
Alkaloids	+	+	+
Phlobatannins	+	-	+
Phenols	-	+	-
Flavonoids	-	+	-
Tannins	-	+	-
Cardiac glycosides	-	+	-
Steroids	-	-	+
Reducing sugar	+	+	-
Oil	+	+	-
Carbohydrates	-	-	+

+ = present; - = absent

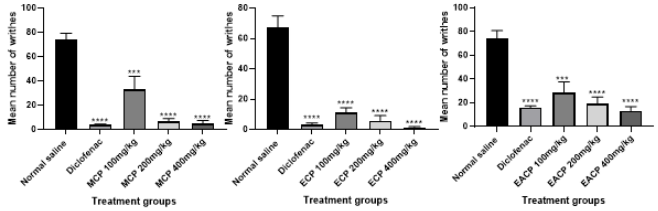

Figure 1: The effect of methanol (MCP), ethanol (ECP), and ethyl acetate (EACP) extracts of *Crinum pedunculatum* on acetic acid induced writhing. ***P< 0.001, ****P< 0.0001 relative to the control (One-way ANOVA followed by Dunnett's multiple comparison test).

Table 2: The percentage inhibition of different extracts of *Crinum pedunculatum*.

Treatment groups	Dose (mg/kg)	Percentage inhibition of writhes (%)
Normal saline	10	0
Diclofenac sodium	75	93.75
Methanolic extract	100	30.1
	200	86.11
	400	90.28
Ethanolic extract	100	84.44
	200	91.94
	400	98.06
Ethyl acetate extract	100	60.28
	200	73.61
	400	81.39

control ($F_{4, 20}$ = 29.50, P< 0.0001), ($F_{4, 20}$= 44.73, P< 0.0001), ($F_{4, 20}$ = 17.79, P< 0.0001) respectively. The most significant (P< 0.0001) reduction was observed with the methanolic, ethanol and ethyl acetate extracts at doses 200 mg/kg and 400 mg/kg (Figure 1). The percentage inhibition of writhing of the ethanolic extract at 100 and 200 mg/kg are 84.44% and 91.94%, respectively, with the 400 mg/kg dose showing the highest inhibition at 98.06% (Table 2).

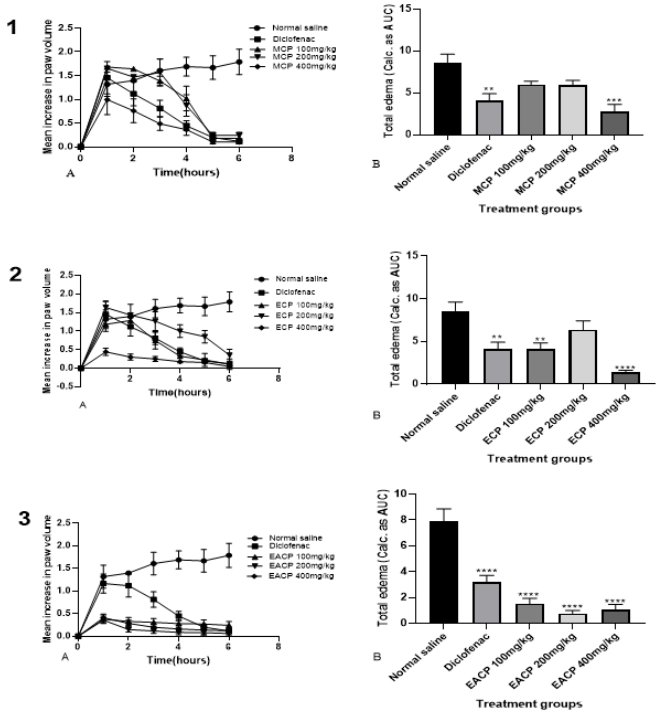

Figure 2: The effect of MCP-methanol, ECP-ethanol, and EACP-ethyl acetate extracts of *Crinum pedunculatum* on mean increase in paw diameter (A) and total oedema (B) (calculated as AUC) in carrageenan-induced paw inflammation. **P< 0.01, ***P< 0.001, ****P< 0.0001 relative to the control (One-way ANOVA followed by Dunnett's multiple comparison test).

Carrageenan-induced inflammation in rats

Oral administration of methanol, ethanol, and ethyl acetate extracts of *Crinum pedunculatum* showed anti-inflammatory activity by significantly decreasing paw oedema induced by carrageenan (Figure 2). The protection from inflammation exhibited by the ethanol extract was non-dose dependent with 100 mg/kg of the ethanol extract showing better protection than 200 mg/kg and 400 mg/kg showing the highest protection. Although all doses of the ethyl acetate extract showed significant inhibition of inflammation, a ceiling effect was observed at 200 mg/kg. The methanol extract at 400 mg/kg showed a significant reduction (P< 0.0001) in paw diameter from the first to the sixth hour compared to the negative (normal saline) and positive control (diclofenac).

Brewer's yeast-induced pyrexia

Rectal temperature was recorded for each animal every hour for 6 hr after the administration of different solvent extracts of *Crinum pedunculatum* as well as the standard drug paracetamol. All doses of the methanol and ethyl acetate extracts showed significant reduction of rectal temperature (P< 0.0001 and P< 0.001), which was not dose-dependent. The ethanol extract at the dose of 200 and 400 mg/kg caused significant reduction in temperature (P< 0.0001 and P< 0.001), but a ceiling effect was observed at 200 mg/kg (Figure 3).

DISCUSSION

Efforts made to develop new, efficacious and relatively safe agents for the management of inflammation are still necessary today to find an alternative to the use of NSAIDs. Natural product drug discovery remains

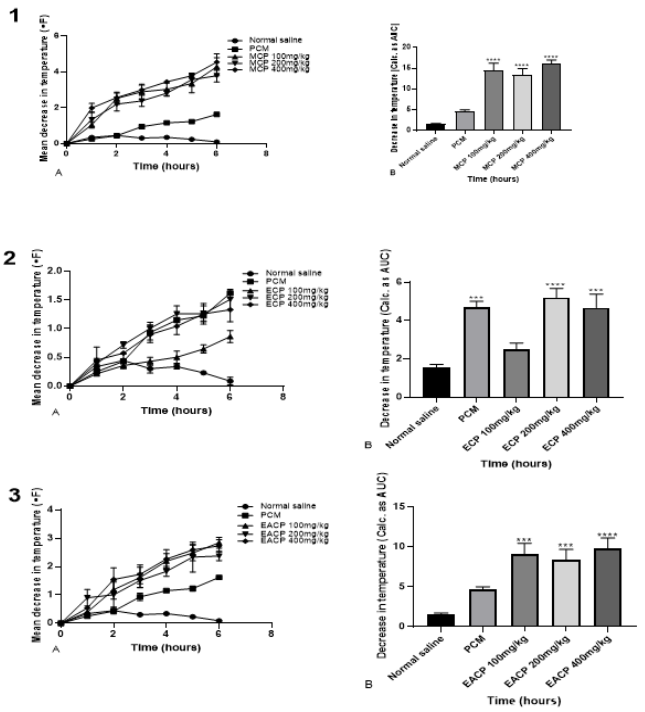

Figure 3: The effect of MCP-methanol (1), ECP-ethanol (2), and EACP-ethyl acetate (3) extracts of *Crinum pedunculatum* on the change in rectal temperature (A) and total decrease in temperature (B) (calculated as AUC) on brewer's yeast-induced pyrexia. ***P< 0.001, ****P<0.0001 relative to the control (One way ANOVA followed by Dunnett's multiple comparison test).

a major contributor to the development of novel therapeutic agents.[20] This study was carried out to evaluate the analgesic, anti-inflammatory, and antipyretic activities of the bulbs of *Crinum pedunculatum*, family Amaryllidaceae. Acute toxicity experiments showed no mortality all the extracts at 2000 mg/kg. Analgesic activity was determined by intraperitoneal injection of acetic acid, which results in contortions of the animal's abdominal muscle (writhing) along with the stretching of the hind limbs, and these reactions are considered to be caused by local peritoneal receptors.[21] In the acetic acid-induced model, several cytokines are released like interleukin 1β, tumour necrosis factor α, and chemokines which act together to induce writhing.[22,23] Furthermore, several studies have shown elevated levels of prostaglandins, mainly PGF2α, PGE2, PGI2 in the peritoneal fluids of animals administered with acetic acid[24,25] and NSAIDs have been established to alleviate pain by inhibiting the synthesis of prostaglandins along with several inflammatory mediators by inhibiting these cyclooxygenase enzymes.[26] Therefore, any substance that causes a reduction in the frequency of abdominal writhes induced by acetic acid can be postulated to possess analgesic effect. The methanol, ethanol, and ethyl acetate extracts of *Crinum pedunculatum* significantly decreased the frequency of abdominal writhes in a dose-dependent form (Figure 1). It can be postulated that the analgesic activity observed by the extracts could be due to the inhibition of the pathway involved in the synthesis of prostaglandins as well as the local inhibition of peritoneal inflammation. Carrageenan-induced hind paw oedema was employed to investigate the anti-inflammatory activity of *Crinum pedunculatum*. Oedema induced by carrageenan is regarded as the initial phase of the process of inflammation and is characterized by fluid and cell exudation.[27] The development of oedema in the hind paws of the animal after carrageenan administration is caused initially by the release of mediators like histamine and serotonin and subsequently by prostaglandins which further facilitates the oedema.[27-29] This study showed that the standard drug diclofenac caused the inhibition of paw oedema from the 3rd to the final hour, which is consistent with its mechanism of action. Diclofenac acts by inhibiting cyclooxygenase-1 and 2 enzymes, thereby inhibiting the synthesis of prostaglandins released during the late phase after carrageenan administration.[30,31] All doses of the ethyl acetate extracts showed a significant decrease in paw diameter from the first hour after carrageenan administration; 200 and 400 mg/kg of the methanol extract as well as 100 and 400 mg/kg of the ethanol extract also showed significantly lower paw diameter compared to the negative control. (Figure 2). The highest and most significant inhibition of the increase in paw oedema caused by carrageenan was observed at the fourth hour for all solvent extracts of *Crinum pedunculatum* (Figure 2). It can be postulated that the ethanol, methanol, and ethyl acetate extracts of *Crinum pedunculatum* inhibit fluid exudation, as well as several mediators of inflammation such as serotonin and histamine that contribute to the acute inflammatory process. Further studies are required to determine the specific inflammatory mediators inhibited by these extracts.

The extracts were also evaluated for antipyretic activity using Brewer's yeast model which is associated with fever through an inflammatory reaction[32] caused by the synthesis of pro-inflammatory cytokines like interleukin-1β and interleukin-6, interferon-α, tumour necrosis factor α and prostaglandins E2 and I2. These mediators are responsible for causing an increase in body temperature through their action on the brain.[33,34] Antipyretic agents like paracetamol, used as a standard drug in this study, exert their effects by decreasing prostaglandin synthesis through the inhibition of cyclooxygenase enzymes as well as by activating anti-inflammatory signals at the site of tissue damage.[35] The methanol, ethanol, and ethyl acetate extracts all showed a significant reduction in temperature induced by the administration of yeast (Figure 3), which could be as a result of the inhibition of pro-inflammatory cytokines.

Phytochemical analysis was carried out on all extracts of *Crinum pedunculatum* used in this study. Flavonoids, alkaloids, tannins, and saponins were found to be present in these extracts (Table 1) and several studies have described the analgesic, antipyretic, and anti-inflammatory activities of these constituents.[36-39] It can therefore be postulated that the analgesic, anti-inflammatory, and anti-pyretic activities observed by the methanol, ethanol, and ethyl acetate extracts of *Crinum pedunculatum* may be due to the presence of these phytochemical constituents.

CONCLUSION

This study showed that the methanol, ethanol, and ethyl acetate extracts of the bulbs of *Crinum pedunculatum* R.Br. possess significant peripheral analgesic, anti-inflammatory and antipyretic activities justifying their use by traditional healers in the southern regions of Ghana. Further studies are ongoing to isolate and characterize compounds that are responsible for these observed activities to offer new leads for the development of agents with analgesic, anti-inflammatory, and antipyretic effects.

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Author Contributions

PD and CAD: Conceptualization, Methodology, Literature search; PD, AEO, AS, DAA and AKA: Experimental Studies and data analysis for the methanolic extract of *Crinum pedunculatum*, PD, AK, EQ and OOA: Experimental studies and data analysis involving the ethyl acetate extract of *Crinum pedunculatum*. PD, EKA, MM and CNB: Experimental studies and data analysis involving the ethanol extract of *Crinum pedunculatum*. PD, CAD and KAO: Data analysis, Statistical analysis Manuscript preparation, editing and reviewing. CAD and KAO: Supervision. Guarantor: Peace Doe. All authors have read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest

ABBREVIATIONS

NSAIDS: Nonsteroidal anti-inflammatory drugs; **OECD:** Organization for Economic and Co-operative Development; **IP:** Intraperitoneal Administration; **PGE2:** Prostaglandin E2; **PGI2:** Prostaglandin I2; **PGF2α:** Prostaglandin F2 α.

SUMMARY

Natural plants still provide important source of new compounds and potential drugs. Since these plants are employed by traditional healers in Ghana, it is imperative to provide scientific basis for their use and this has informed this study. All solvent extracts of *Crinum pedunculatum* investigated in this study possess analgesic, anti-inflammatory and anti-pyretic activities justifying their use.

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