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Phytochemical Investigation, Proximate Composition, Acute Toxicity, Anti-Inflammatory and Antinociceptive Activities of Extracts of *Caesalpinia Pulcherrima* Linn Flower

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Abstract

The management and treatment of inflammation and pains have continued to gain increasing interest in recent times due to the challenge they pose to health. The study evaluated the phytoconstituents, proximate content, acute toxicity, antioxidant, anti-inflammatory, and antinociceptive properties of Caesalpinia pulcherrima flower extracts using different solvents. The bioactive chemical constituents, acute toxicity, and antioxidant property were investigated via standard methods while the anti-inflammatory and analgesic activities were determined using formalin-induced inflammation and acetic acid-induced writhing methods respectively. Phytochemicals determined were alkaloids, glycosides, saponins, tannins, phenolics, terpenoids, and steroids. The moisture, crude fibre, crude lipid and total ash content determined were 6.20 ± 0.01%, 15.00 ± 0.07%, 1.80 ± 0.03% and 7.00 ± 0.00% respectively. The n-hexane extract possessed the chief % inhibition (86.49 ± 0.30) and for ascorbic acid 88.29 ± 0.00 at 500 µg/mL extract from the antioxidant study. The acetone extract displayed the greatest significant anti-inflammatory potential at both 100 and 200 mg/kg dose at four hours, being the most effective and there exist a momentous reduction at $p < 0.05$ in the writhes' number in a manner depended on dose in the acetone and n-hexane extracts. At 5000 mg/kg, there was 100% mortality when the crude extracts were orally administered to the Swiss mice. This study ratifies that Caesalpinia pulcherrima flower extract is a potential spring of phytomedicine which could be applied for managing of inflammation, pain and oxidative stress-related infections but higher dosages could potentially be lethal.

Keywords: *Caesalpinia pulcherrima*; *phytochemical; analgesic; anti-inflammatory; antioxidant*

Introduction

Plants are known to be the ministry of traditional herbs medicine particularly among the rural occupiers across the globe ever since antiquity till date. Over hundreds different plant species have been used for medicinal purpose, thus, plant products have remained a central area of the primordial traditional systems of medicine which include; Chinese, Ayurvedic and Egyptian (Sarker & Nahar, 2007). Expensive synthetic drugs as well as poor healthcare services in the developing countries have forced a large percentage of the masses into using traditional medicine (Uraku *et al.*, 2015). As a result of the increasing inefficiency of various recent synthetic

drugs with several of them having adverse effects, the World Health Organisation (WHO) supports the application of traditional medicine as far as they are proven to be effective and safe. Copious nutritious and healthy products are produced from plants which may be contained in their leaves, flowers, barks, roots, fruits, seeds or stems (WHO, 1985; Iyasele *et al.*, 2022).

The medicinal attributes of different plants e.g. antioxidant, ant-inflammatory, and analgesic property, are due to the bioactive components present in diverse areas of the herb and as a result, the knowledge of these phytochemicals is very essential to unearth chemotherapeutic agents and can also disclose the new source of economic and pharmacological materials (Monin & Kadam, 2012). The naturally found antioxidant molecules are generally harmless and can help us raise the nutritional value of our foods, which will lead to good health. In the regulation of herbal drugs, the WHO has underscored the necessity and importance of determining proximate and micro-nutrients (Niranjan *et al*., 2008; Oluwafemi *et al.*, 2015).

Inflammation is among the defensive mechanism of the body; it is the manner through which the immune system detects and eradicates destructive and strange stimuli and activates the curative process. It is either chronic or acute (Pahwa *et al.*, 2022). Chronic inflammatory ailments and pain such as cancer, heart diseases and stroke are the major causes of demise in the world today, causing the death of about three out of five individuals; hence, Global scientific research has turned to it as a focal point (Onasanwo *et al.,* 2012; Pahwa *et al.,* 2022). Pain is divided into nociceptive and neuropathic types when used therapeutically (Rajagopal, 2006).

In recent times, non-steroidal anti-inflammatory drugs (NSAIDs) are being applied to manage pain, some of which were obtained from plants like aspirin. In light of this, herbal drugs derived from plants are used in complementary and alternative medicine (CAM) to treat and manage inflammatory conditions, pain, and other allied illnesses (Ogbeide et al., 2022a; Singh et al., 2008). *Caesalpinia pulcherrima* of the family "Fabaceae" is a plant of interest used under traditional herbal medicine which has become a subject of many research studies. It has variety of English common names which include; dwarf poinciana, pride of Barbados, Mexican bird of paradise and pride of Barbados (Atienza *et al.,* 2009).

Figure 1. Overview of the Leaves with an Orange Red Variant Flower of *C. Pulcherimma*

They are normally grown for their ornamental flowers. Its vernacular names include: *Eko-omode* in Yoruba, *Akasi-ibieka* in Edo, *Konkehl* in Fulani and *Gumgoroci* in Nupe. (Schiebinger, 2004; Iwalewa *et al.,* 2007). Herbal and folkloric medicinal values from various areas of *Caesalpinia pulcherrima* are recognised in several places across the world; the juice from the flower and leaves is known to treat sores and fever respectively while the seeds are known to treat difficulty in breathing, chronic cough and chest pain. It is also known that four grams (4 g) of the root might cause an abortion in the first trimester of pregnancy in human (Schiebinger, 2004; Counter *et al.*, 2006).

According to reports, C. pulcherrima's stem bark and leaves contain varying degrees of antiplasmodial potential (Okoro *et al.*, 2013; Ogu *et al.*, 2012). All parts of *C. pulcherrima* have been confirmed to have anti-inflammatory activities (Khan *et al.*, 2018). Again, Ogbeide and his co-workers established that the pod of *C. pulcherrima* contain anti-inflammatory and analgesic agents (Ogbeide *et al.*, 2022a). The extracts

of the flowers (fresh and dried) have likewise been reported to display powerful antibacterial potential against *E. faecalis, B. cereus, S. aureus, P. aeruginosa, E. coli and K. pneumonia* (Pulipati *et al.*, 2012). Nevertheless, this study is meant to add to the limited scientific documentations of *C. pulcherrima* flower by evaluating the bioactive chemical constituents, acute toxicity, antioxidant, anti-inflammatory and antinociceptive properties of the extract using different solvents.

Methods

Flower Samples Collection

In August 2019, fresh *C. pulcherrima* flowers were obtained from the University of Benin's flower garden in Benin City, Nigeria. Dr. Akinnibosun from the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, identified the plant and flower samples, and a voucher specimen number, UBH-C54, was put down.

Preparation and Extraction of Sample

The flowers were washed with tap water, let to air dry for three weeks, and then processed via the British milling machine (AJT 200 turret mill) to create powder. The pulverised flowers 500 g each were macerated in 750 mL of water, methanol, n-hexane, chloroform and acetone separately with constant shaking and stirring for three days. Utilising Whatman paper no. 1, the extracts obtained from various macerations were filtered, and the filtrates were then concentrated to dryness with the help of a rotary evaporator. The extracts' weight was taken, and it was afterwards put in storage in the fridge until analysis.

Preliminary Phytochemical Screening

Phytochemical screening was done on the methanol, chloroform, n-hexane and aqueous extracts to identify the existence of alkaloids, steroids, saponins, terpenoids, flavonoids, tannins, phenols and glycosides using standard methods as summarised below (Harborne, 1993; Sofowora, 1993; Trease and Evans, 2002; Ogbeide *et al.*, 2020).

Test for Alkaloids (*Picric Acid Test*): 2 mL of 10% picric acid was added to 2 mL of the extract. The formation of a yellow precipitate showed a positive test.

Test for Steroids (*Salkwoski Test*): To 2 mL of the extract, 2 mL of concentrated sulphuric acid and 2 mL of chloroform were added. 'Reddish brown' ring formation indicated a positive test.

Test for Saponins (Frothing Test): 10 mL of distilled water was added to 2 mL of the extract and vigorously shaken for a minute and observed for persistent foam.

Test for Terpenoids (Salkowski Test): 5 mL of the extract were combined with 2 mL of chloroform and 3 mL of sulphuric acid. A ‘reddish brown’ ring at the interface showed a positive result.

Test for Flavonoids (Lead Acetate Test): To 2 mL of the extract, a few drops of lead acetate solution were added. The formation of a milky precipitate showed a positive test.

Test for Tannins (Ferric Chloride Test): To 2 mL of the extract, 10% ferric chloride was added. The formation of a greenish grey colour indicated a positive test.

Test for Phenolics (Ferric Chloride Test): A drop of freshly made 10% ferric chloride was added after 5 mL of 90% ethanol and 2 mL of the extract were combined. A darkish blue colour that formed indicated a positive outcome.

Test for Glycosides (*Keller Kiliani Test*): 2 mL of the extract were combined with 1 mL of glacial acetic acid, which was then cooled; two to three drops of ferric chloride were then added. To this solution, 2 mL of concentrated sulphuric acid was cautiously added to the walls of the test tube. A'reddish-brown' coloured ring that

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appeared at the intersection of the two layers indicated the presence of glycosides.

Proximate Analysis

Standard techniques were used to examine quantitative characteristics like moisture content, crude fiber, crude lipid, and total ash (Arlington, 1984).

Antioxidant Activity (DPPH Radical Scavenging Assay)

Using a method adopted from Jain et al. (2008), the crude acetone and n-hexane extracts of the *C. pulcherrima* flower were evaluated for their capacity to scavenge DPPH free radicals. Ascorbic acid was used as a reference standard. The DPPH radical scavenging capacity was estimated using equation 1.

$$\% \text{ DPPH scavenging capacity} = \frac{A_0 - A_1}{A_0} \times 100 \quad (1)$$

Where: A_0 = absorbance of DPPH radical in methanol; A_1 = absorbance of DPPH radical + test sample in methanol.

The fifty percent inhibitory concentrations (IC_{50} values) were calculated using linear regression analysis as described by Sebaugh (2011) and used to indicate antioxidant capacity.

Experimental Animal Handling

In the acute toxicity, anti-inflammatory and analgesic tests, about 84 healthy Swiss mice (male and female) weighing 19–25 g were used. These animals were gotten from the University of Benin's Department of Biochemistry's animal breeding facility. The animals were housed in the Animal House division (Department of Animal and Environmental Biology) of the University of Benin. All procedures of the experiment were approved by the Ethical Review Board of Faculty of Life Sciences, University of Benin (LS19107). The animals had a two-week acclimatisation period and were kept in conventional environmental conditions, including a 12-hour day/night cycle, water *ad libitum* and were fed with standard grower mash diets. The National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals was followed when handling them.

Evaluation of Acute Toxicity

The method used was adapted from Igbe *et al.* (2010). The mice were divided into four distinct sets (groups) containing three mice each marked I-IV. Groups II-IV (test groups) was administered 100, 1000, 5000 mg/kg of the crude extract suspended in gum acacia respectively via oro-gastric syringe, whereas group I (control group) was administered ten percent (10%) gum acacia solution orally. This was done using acetone and n-hexane extracts separately. Within twenty-four hours, the mice were examined for usual toxicity and death symptoms; the mice that stayed alive after twenty-four hours were further examined for any indications of deferred toxicity for another fourteen days. Using equation 2, the LD_{50} (median lethal dose) was calculated.

$$LD_{50} = \sqrt{LD_0 \times LD_{100}} \quad (2)$$

Where: LD_0 = maximum lethal dose without death; LD_{100} = minimum lethal dose with death.

Formalin-Induced Paw Oedema in Mice (Evaluation of Anti-Inflammatory Activity)

The effect of acetone and n-hexane extracts of *C. pulcherrima* flower on formalin-induced inflammation in rat paw was studied using a modified Afolabi et al. (2017) approach. The mice were randomly divided into five groups of three mice each (n = 3). Group I served as the "negative control" and received just distilled water (DW); groups II, III, and IV each received 50, 100, and 200 mg/kg of the floral extracts respectively; while group V served as the "positive control" and received 100 mg/kg of aspirin. Prior to the experiment, the animals were fasted for 12 hours, and the drugs were orally given.

Each animal received 0.1 mL of a 10% formalin suspension by hypodermic injection thirty (30) minutes after receiving the extracts orally. Oedema (localised

inflammation) in situ materialised as a result. Following formalin administration, the paw oedema volume was quantified hourly for four hours using a vernier calliper. T The average % increase in paw volume over the course of the experiment was calculated and compared to the control group. % inhibition was evaluated using equation 3.
% Inhibition of paw oedema is:

$$1-\frac{Vt}{Vc}\times 100 \qquad (3)$$

Where Vt and Vc denote average paw volume of the control and treated animals respectively.

Acetic Acid-Induced Writhing in Mice (Evaluation of Analgesic Activity)

The peripheral analgesic activity of the acetone and n-hexane extracts of *C. pulcherrima* flower was assessed by the acetic acid-induced writhing inhibition technique adapted from Akuodor *et al.* (2011). The Swiss mice which were prescreened before being used for this experimentation were divided into five distinct groups. The doses administered orally to the animals were 50, 100, and 200 mg/kg body weight. Writhing inhibition in mice by the extracts was related to the writhing inhibition by a reference drug (aspirin 20 mg/kg) which was administered orally. 0.1 mL/10 g acetic acid (0.7%) was given intraperitoneally (i.p.) to generate the sensation of pain. After the acetic acid inoculation, the number of writhes was sharply assessed for 10 minutes. When compared to the control group, there was a decrease in writhing; this was interpreted as a sign of analgesic activity. Equation 4 was used to estimate the index of analgesia and evaluate it as a percentage inhibition of writhing.

$$\%\ \text{Writhing inhibition} = \frac{C-D}{C}\times 100 \qquad (4)$$

Where, C = average no. of writhes for the control group of animals; D = average no. of writhes for treated animals.

Statistical Analysis

Windows SPSS version 20.0 for was used to analyse the data and standard errors of the mean (SEM) were used to express the results as mean. They were then subjected to 1-way analysis of variance (ANOVA) to determine whether there were any statistically significant differences between the groups, followed by the Bonferroni t-test (student-Newman-Keulsposthoc test), which was used to compare the means and identify any significant differences between the various groups. $P < 0.05$ was taken to be significant.

Results and Discussion

Phytochemical Screening

The phytochemical investigation of the n-hexane, aqueous, methanol and chloroform crude extracts of *C. pulcherrima* flower indicated the existence of alkaloids, glycosides, steroids, saponins, phenolics, tannins and terpenoids (Table 1). Meanwhile, the phytochemicals detected greatly depended on the type of extracting solvent used and this is attributable to the polarity difference. Polarity refers to a molecule's relative ability to engage in interactions (Oluwafemi et al., 2015). The presence of these secondary metabolites in the extracts confirms its uniqueness as essential medicinal plant. The alkaloids are a group of bioactive compounds that are well known for their analgesic potentials and they serve as precursors of various drugs (Zohra *et al.*, 2012).

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Table 1. Phytochemical Components of Flower Extract from *C. Pulcherrima*

Phytochemicals	Aqueous extract	Chloroform extract	Methanol extract	n-Hexane extract
Alkaloids	+	-	+	+
Tannins	+	-	+	-
Saponins	+	+	+	+
Phenolics	+	-	+	-
Terpenoids	+	-	+	-
Glycosides	+	+	+	+
Steroids	-	+	-	+
Flavonoids	-	-	-	-

(+) indicates present and (-) indicates absent

Saponins and steroids contain anti-inflammatory agents and exhibit erythrocyte haemolysis. Among others, glycosides contain hepatoprotective agents while tannins and phenols are known for their antidiarrhoeal, anthelmintic and antimicrobial potentials; more also, saponins and terpenoids are good antioxidants (Wojtunik *et al.,* 2014; Oghogho *et al.*, 2022). The presence of terpenoids in the flower of C. pulcherrima suggests that the extract could be used to prepare anti-viral and anti-tumor medications and therapies because terpenes are known to have cytotoxic characteristics (Oghogho *et al.*, 2022). Virtually all of these bioactive compounds have been demonstrated to be present in the stem bark, leaf, pod and aerial parts of the herb (Ogbeide *et al.*, 2018; Savasankari *et al.,* 2010; Ogbeide *et al.*, 2022a; Sharma and Rajani, 2011).

Proximate Composition

The proximate analysis results are presented in Table 2. The moisture content was found to be 6.20 ± 0.01% w/w. A crude drug has a better chance of inhibiting the activities of fungi, bacteria and yeast at lower moisture content during storage. The moisture content helps us to evaluate the vulnerability of a crude plant sample to microbial and hydrolytic degradation (Ogbeide *et al.*, 2022b). The value obtained is within the permissible range (6-8%) for a crude drug (AOAC, 1984). This implies that the phytochemicals present in the crude drug sample might not be susceptible or vulnerable to microbial and hydrolytic degradation. The mean total ash obtained from the analysis was 7.00 ± 0.0% w/w. The total ash is an amount of the residue (non-volatile inorganic matters) left after ashing, thus, indicating impurities such as silicate, carbonate and oxalate (Ogbeide *et al.*, 2022b). The total ash value obtained from the flower of *C. pulcherrima* is higher than the value 4.35 ± 0.13% w/w obtained for the pod and less than the value 8.67 ± 1.63% w/w obtained for the leaves from previous studies (Ogbeide *et al.*, 2022a; Ogbeide and Falodun, 2016).

Table 2. Proximate analysis of powdered *C. Pulcherrima* Flower

Parameters	Mean ± SEM
Moisture content	06.20 ± 0.01
Crude lipid	01.80 ± 0.03
Crude fibre	15.00 ± 0.07
Total ash	07.00 ± 0.00

SEM represents standard error of the mean

Total ash is one of the significant parameters generally used to assess the quality of the functional properties of crude drugs and food which also indicates the degree of the original food's mineral contents (Unuigbe *et al.*, 2021). Hence, *C. pulcherrima* flower could serve as a significant source of natural minerals, because it is relatively rich in minerals. Similarly, the crude fibre content obtained was 15.00 ± 0.07% w/w which is above 9.606 ± 0.045% obtained from the whole

seed and the value obtained (12.33 ± 0.12%) from the stem bark as documented by Ilori *et al.* (2015) and Ogbeide *et al.* (2020) respectively. Meanwhile, fibre is a well-known substance that reduces cholesterol level in the body, thereby, aiding human health (Magu *et al.*, 2018). Lipid are important in the diet; the crude lipid obtained (1.80 ± 0.07% w/w) is less than those contained in the seed (5.89 ± 0.24% w/w) as well as in the stem bark (5.45 ± 0.12% w/w) (Aremu *et al.*, 2012; Ogbeide *et al.*, 2020).

Antioxidant Activity

The scavenging consequence for ascorbic acid and crude extracts is greatly influenced by dose. Thus, at 500 µg/mL being the highest concentration considered, the % inhibition of the n-hexane crude extract (86.49 ± 0.30%) found is slightly higher than the value 85.59 ± 0.20% obtained for the acetone crude extract which are all close to the value 88.29 ± 0.00% obtained for the standard (Figure 2 and Figure 3).

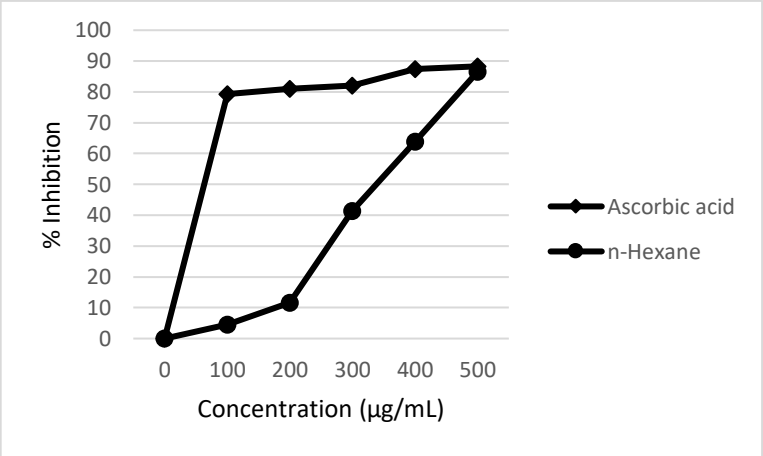

Figure 2. DPPH Scavenging Capacity of n-Hexane Extract of *C. Pulcherrima* Flower

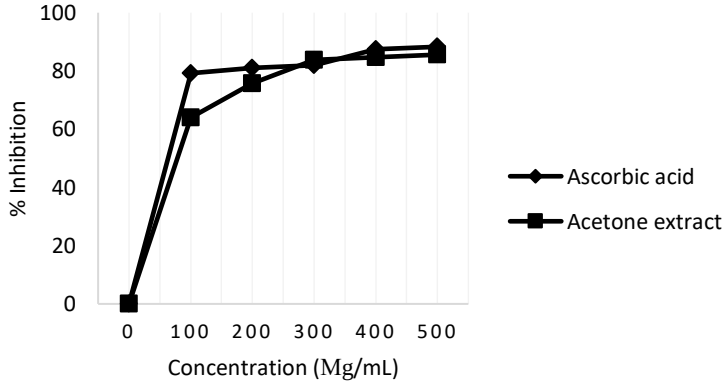

Figure 3. DPPH Scavenging Activity of Acetone Extract of *C. Pulcherrima* Flower

Furthermore, the IC_{50} values for the n-hexane and acetone extracts obtained were 25.27 ± 0.05 and 37.54 ± 1.00 µg/mL respectively while that obtained for the standard was 23.07 ± 0.01 µg/mL (Table 3).

Table 3: IC_{50} Values of *C. Pulcherrima* Flower Extracts and Ascorbic Acid

Samples	IC_{50} value (µg/mL)
Ascorbic acid	23.07 ± 0.01
Acetone extract	37.54 ± 1.00
n-Hexane extract	25.27 ± 0.05

The percentage of DPPH scavenging activity was plotted against the extract concentration, and from the plot, the plant extract concentration that can generate 50% inhibition (IC_{50}) was extrapolated. According to Oluwafemi et al. (2015), the IC_{50}-value is the concentration that will inhibit fifty percent of the original DPPH radical. The lower the IC_{50}-value, the more effective the compound is at scavenging free radicals. Thus, the extracts manifested considerably high antioxidant property with the highest being displayed by the n-hexane extract which is close to that of the standard. This antioxidant property demonstrated by the extracts is attributable to the phytochemicals previously detected.

Acute Toxicity Study

The same result was obtained for both the acetone and n-hexane extracts from the acute toxicity study (Table 4 & 5). It was observed that 100, 1600 and 2900 mg/kg body weight produced 0% mortality, signifying that there were no substantial deviations identified in the wellness of the mice apart from drowsiness which was dose dependent. Meanwhile, 5000 mg/kg body weight produced 100% mortality which implies that all the animals died at this dose level.

Based on the toxicity standard classification proposed by Hodge and Sterner (2005), which established that any compound having an oral intake LD_{50} between 500 and 5000 mg/kg should be considered as virtually "slightly toxic", therefore, the flower extract of *C. pulcherrima* could be said to be slightly toxic. This finding is comparable with that obtained by Patel *et al.* (2010) using graphical method.

Table 4. Acute Toxicity Outcomes of the Acetone Crude Extract of *C. Pulcherrima* Flower in Mice

Groups	Doses (mg/kg)	Amount of mortality	Percentage mortality
Control	DW	0/3	0
Flower extract	100	0/3	0
Flower extract	1600	0/3	0
Flower extract	2900	0/3	0
Flower extract	5000	3/3	100

Table 5. Acute Toxicity Outcomes of the n-Hexane Crude Extract of *C. Pulcherrima* Flower in Mice

Groups	Doses (mg/kg)	Number of mortality	Percentage mortality
Control	DW	0/3	0
Flower extract	100	0/3	0
Flower extract	1600	0/3	0
Flower extract	2900	0/3	0
Flower extract	5000	3/3	100

Studies on Anti-Inflammatory and Anti-nociceptive properties

Formalin-induced inflammation is recognised to have some degree of repeatability, making it an acceptable experimental approach used to ascertain the anti-inflammatory characteristics of natural products or compounds (Singh et al., 2016). Rat paw oedema, or swelling of the paw, materialized as a result of the inflammation caused by formalin (Tables 6 and 7). The extracts and the reference drug (100 mg/kg aspirin) were both observed to

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significantly ($p < 0.05$) shorten the licking duration compared to the control within a predetermined time period at doses of 50, 100, and 200 mg/kg body weight. This is suggestive of the dose-dependent manner of several formulations of herbs in managing diverse ailments because the extracts' unequivocal ability to reduce inflammation is dose-dependent (Ogbeide et al., 2022a). The highest percentage inhibition of inflammation at the peak dose (200 mg/kg) for the acetone extract obtained was 26.08 and that obtained for the n-hexane extract was 25.65 while that obtained for the reference drug (100 mg/kg aspirin) was 27.16 after four (4) hours being, the longest time. Thus, the extracts displayed anti-inflammatory potential compared to the reference drug; meanwhile, the acetone extract is more effective. Therefore, it can be deduced that the extracts might have provided defense which paralyzed the activities of inflammatory agents and chemo-irritants (Ogbeide *et al.*, 2022a).

Table 6. Effect of *C. Pulcherrima* Flower Acetone Extract on Formalin-Induced Inflammation in Rat Paw

		Volume of paw oedema (mm) and inhibition rate			
Groups	Doses (mg/kg)	1hrs	2hrs	3hrs	4hrs
Control	DW	4.61 ± 0.33	4.67 ± 0.23	4.69 ± 0.29	4.64 ± 0.24
Aspirin	100	3.55 ± 0.03 (22.99)	3.71 ± 0.19 (20.56)	3.68 ± 0.18 (21.54)	3.38 ± 0.09 (27.16)
Flower extract	50	4.15 ± 0.07 (09.98)	4.15 ± 0.11 (11.13)	4.15 ± 0.21 (11.51)	3.60 ± 0.03 (22.41)
Flower extract	100	4.11 ± 0.02 (10.85)	3.93 ± 0.02 (15.85)	4.06 ± 0.17 (15.52)	3.43 ± 0.02 (26.08)
Flower extract	200	3.64 ± 0.09 (21.04)	3.53 ± 0.22 (24.41)	3.73 ± 0.18 (20.47)	3.43 ± 0.11 (26.08)

Values signify mean ± standard error of mean; n= 3 mice; $P < 0.05$

Table 7. Effect of n-Hexane Extract of *C. Pulcherrima* Flower on Formalin Induced Inflammation in Rat Paw

		Paw oedema volume (mm) and % inhibition			
Groups	Doses (mg/kg)	1hrs	2hrs	3hrs	4hrs
Control	DW	4.61 ± 0.33	4.67 ± 0.23	4.69 ± 0.29	4.64 ± 0.24
Aspirin	100	3.55 ± 0.03 (22.99)	3.71 ± 0.19 (20.56)	3.68 ± 0.18 (21.54)	3.38 ± 0.09 (27.16)
Flower extract	50	4.60 ± 0.23 (00.22)	4.15 ± 0.11 (11.13)	4.13 ± 0.39 (11.94)	3.57 ± 0.29 (23.06)
Flower extract	100	4.13 ± 0.38 (10.41)	3.96 ± 0.29 (15.20)	3.80 ± 0.12 (18.98)	3.50 ± 0.23 (24.57)
Flower extract	200	4.01 ± 0.45 (13.02)	3.67 ± 0.15 (21.41)	3.54 ± 0.45 (24.52)	3.45 ± 0.41 (25.65)

Values signify mean ± standard error of mean; n= 3 mice; $P < 0.05$

Acetic acid-induced writhing test finds application in evaluating the peripherally acting analgesics. Similarly, the acetic acid-induced writhing experiment showed that the extracts significantly reduced the writhing response (Tables 8 and 9). The maximum analgesic influence for the acetone flower extract manifested at a dose of 50 mg/kg with % inhibition of pain obtained as 53.85 while the maximum analgesic influence for the n-hexane flower extract displayed at a dosage of 100 mg/kg with a percentage inhibition of pain obtained as76.92, being the most effective

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compared to the acetone extract and even the reference drug (aspirin). Thus, these findings are consistent with the results obtained for the methanol extract of the flower as put forward by Patel and co-workers (Patel *et al.*, 2010)

Table 8. Acetic acid-induced peripheral pain in mice: effects of aspirin and acetone extract of *C. pulcherrima* flower

Groups	Doses (mg/kg)	Writhing number per 10 minutes	% inhibition of pain
Control (DW)	0.5 mL	13.00 ± 0.58	0
Aspirin	100	05.33 ± 0.88**	59.00
Flower extract	50	06.00 ± 0.00**	53.85
Flower extract	100	06.67 ± 0.33**	48.69
Flower extract	200	09.00 ± 2.65**	30.77

Values signify mean ± SEM; n= 3 mice; **Significantly different compared with control ($P < 0.05$)

Table 9. Acetic acid-induced peripheral pain in mice: effects of aspirin and n-Hexane Extract of *C. pulcherrima* flower

Groups	Doses (mg/kg)	Writhing number per 10 minutes	% inhibition of pain
Control (DW)	0.5 mL	13.00 ± 0.58	0
Aspirin	100	05.33 ± 0.88**	59.00
Flower extract	50	10.00 ± 2.00**	23.08
Flower extract	100	03.00 ± 1.16**	76.92
Flower extract	200	07.00 ± 3.51**	46.15

Values signify mean ± SEM; **Significantly different compared with control ($P < 0.05$); n= 3 mice

The anti-nociceptive and anti-inflammatory potentials of *C. pulcherrima* flower are attributable to the existence of alkaloids, terpenoids, and saponins earlier detected (Zohra *et al.*, 2012; Ogbeide *et al.*, 2022b).

Conclusion

In this study, it is unequivocal that the acetone and n-hexane extracts of *C. pulcherrima* flower are rich sources of antioxidants, anti-inflammatory and analgesic agents thus, confirming its bioactivities as claimed by other authors and folk medicine practitioners. Meanwhile, it could be somewhat toxic at a higher dose; hence, great cautiousness must be taken when being administered for medicinal purposes. Nevertheless, it is recommended to further isolate, purify and characterise the bioactive components contained in the flower.

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