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Phytochemical Investigation, Anti-inflammatory and Analgesic Activities of Ethyl Acetate Extract of Pride of Barbados Pod (*Caesalpinia pulcherrima*)

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Abstract

Pride of Barbados is a therapeutic herb which has wide traditional applications in the treatments and management of diverse ailments. Diseases pose great threats to human race. This research was aimed at evaluating the phytochemicals, proximate composition, acute toxicity, anti-inflammatory, and analgesic activities of the pod extract of Pride of Barbados in order to provide a scientific validation for its use as a therapeutic herb. All analyses were carried out using already established methods; the antioxidant potential was examined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, while the formalin-induced inflammation and acetic acid-induced writhing techniques were used to evaluate the anti-inflammatory and analgesic activities, respectively. Phytochemicals detected were alkaloids, tannins, saponins, flavonoids and phenolic compounds. The moisture content, crude fibre, acid insoluble ash, water soluble ash and total ash were 9.02 ± 0.02%, 9.04 ± 0.01%, 2.78 ± 0.02%, 1.20 ± 0.00% and 4.35 ± 0.13%, respectively. The IC_{50} values for the DPPH radical scavenging capacity of the pod extract and ascorbic acid (standard) were 50.05 ± 0.50 and 5.20 ± 0.85 µg/mL, respectively. The oral administration of crude ethyl acetate pod extract of Pride of Barbados to Swiss mice was not toxic even up to a dose of 5,000 mg/kg. The pod extract showed a significant decrease ($p < 0.05$) in the formation of formalin-induced oedema and the number of writhes in the acetic acid-induced writhing test in a concentration (dose) dependent manner. This study confirms the phytomedicinal use of the pod extract of Pride of Barbados with rich pharmacological and antioxidant properties.

Keywords: Pride of Barbados; *Caesalpinia pulcherrima*; Anti-inflammatory; Analgesic; Phytochemicals.

Introduction

Therapeutic or medicinal plants can also be termed as medicinal herbs. They have been known and applied traditionally in folk medicine practices since primordial times. Numerous wholesome products are produced from plants and have remained in use as long-established cures for various human sicknesses for many decades (Lichterman 2004, Iyasele et al. 2022). The pursuit for divine healthiness, long-life and therapies to distress and relieving of pain forced our fore-fathers to explore their immediate surroundings for solutions. This has given birth to the applications of many plants, minerals and animal products with the

development of diversities of therapeutic mediators (Ighodaro and Ogbeide 2020). Nature has made available very rich botanicals and a good amount of miscellaneous kinds of plants are growing desolate in many areas of the world. Hence, there is now an improved attention to traditional medicine and high demands for more drugs sourced from plants. This resurgence of interest in plant-based drugs can be attributed to the recent general conviction that "green medicine" is relatively safe and more reliable compared to the expensive synthetic drugs, several of which are known to exhibit contrary side effects (Falodun et al. 2013). In this regard, there is a necessity to bring data up to date on the effectiveness, uses, properties as well as safety of curative plant products. Ethno botany is well applied in drug discovery in order to search for naturally occurring bioactive compounds. This has resulted in the discovery of hundreds of useful compounds (American Diabetes Association 2007). The medicinal attributes of different plants are as a result of the active phytochemicals found in different parts of the plant, for instance, fighting sources of oxidative stress in the body which is antioxidant property or managing ailments accompanied with pains being analgesic property.

Antioxidants can be sub-grouped into two (either natural or synthetic) based on their origin. Nowadays, it is generally believed that naturally occurring antioxidant compounds are relatively safe and can aid the improvement of nutritional standards of our diets resulting in sound health (Monin and Kadam 2012, Oluwafemi et al. 2015). They help to put an end to free radicals and the oxidation reactions that are caused by them, thus, precluding cell destruction resulting from the oxidation reactions in the human system (Falodun et al. 2013). Plants remain a rich source of natural antioxidants that can avert any oxidative mutilation in the body (Miller et al. 1990).

Inflammation can be viewed as the composite genetic reaction of vascular tissues to destructive stimuli like irritants or pathogens, through a protecting effort by the organism to get rid of the damaging stimuli and also commence the restorative development for the tissue. Inflammation has become the focus of international scientific exploration due to its impacts on both animal and human illnesses (Onasanwo et al. 2012).

In therapeutic applications, pain is classified into nociceptive and neuropathic (Rajagopal 2006). In order to subdue pain, non-steroidal anti-inflammatory drugs (NSAIDs) are prescribed all over the world (Boursinos et al. 2009, Sparkes et al. 2010). However, prolonged applications of these NSAIDs can only offer asymptomatic relief, but one of their grave consequences is toxicity to the gastrointestinal linings, kidney and liver (Shah et al. 2006). Based on this, herbal medications sourced from plants are being used in corresponding and substitute medicines (CSM) for the cure and management of inflammations, pains and other related diseases (Singh et al. 2008). It is important to note that several synthetic analgesics, anti-pyretic and anti-malarial drugs like morphine, aspirin, atrophine, artemisinin, and chloroquine were all obtained from plants (Gupta et al. 2006).

Among the plants that have been explored for medicinal value is Pride of Barbados (*Caesalpinia pulcherrima*). It is a leguminous flowering plant belonging to the family 'Fabaceae'. It is grown and nurtured as a decorative flower in humid gardens. Its common names are dwarf poinciana, poinciana, red bird of paradise, flospavonis, Mexican bird of paradise, flamboyant-de-jardin and peacock flower (Schiebinger 2004). It is generally known to be *Nwayi ibem* or *Nwoke ibem* by Igbos, *Eko-omode* by Yorubas and *Waken bature* by the Hausas. Different parts of the plant are globally known to possess great healing properties which include treatment of fever, jaundice, malaria and gastrointestinal disorders. It is also well-known to have antioxidant, anti-inflammatory and analgesic properties as well as prevention of chronic rheumatic malady (Iwalewa et al. 2007, Jayasri et al. 2009). There are reports on different parts of the plant, for instance, the antiplasmodial potentials of the stem bark and leaves of *C.*

pulcherrima have been reported to display modest and significant antiplasmodial activities, respectively against *Plasmodium berghei* (Ogu et al. 2012, Okoro et al. 2013). Many compounds have also been isolated from different areas of the plant, including pulcherrin A, pulcherrin B, pulcherrimin A, pulcherrimin B, pulcherrimin C and many other cassane-type diterpenoids (Ogbeide et al. 2018). Similarly, the anti-inflammatory potentials of the methanol, acetone and aqueous extracts of all parts of *C. pulcherrima*, including fractions of its fresh pods have been documented (Khan et al. 2018). Again, Kumbhare and Sivakumar (2011) postulated that *C. pulcherrima* pod extracts are capable of inhibiting inflammatory reactions as well as pain where the petroleum ether and methanolic extracts were found to possess a prominent anti-inflammatory activity compared to the chloroform extract. Nevertheless, this study stands to update the various works and information on the scientific investigation and assessment of Pride of Barbados pod for the cure of pain and various ailments as established by folklore. Hence, the purpose of this research work was to evaluate the phytochemicals, proximate, antioxidant, acute toxicity, anti-inflammatory and analgesic activities of the pod of Pride of Barbados using ethyl acetate extract.

Materials and Methods

Collection of plant samples

The fresh pods of *C. pulcherrima* were procured in August 2019 in the University of Benin Teaching Hospital (UBTH), Benin City, Nigeria. They were identified and authenticated by Dr. Akinnibosun of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria, and a voucher specimen number (UBH-C354) has been deposited.

Sample preparation and extraction

The pods were sanitized and air-dried for three weeks and thereafter they were pulverized using the British milling machine. The pulverized pods (500 g) were macerated in 750 mL of ethyl acetate with continuous stirring and shaking for 72 hours. The extract was filtered by means of Whatman grade 1 qualitative filter paper and the filtrate was concentrated to dryness in the vacuum at 40 °C using a rotary evaporator. The weight of the extract was taken and then stored in the fridge-freezer at 4 °C for analysis.

Phytochemical screening

The secondary metabolites contained in the concentrated crude extract were qualitatively screened. The phytochemicals were assessed using established methods (Harborne 1993, Sofowora 1993, Trease and Evans 2002). The presence of alkaloids, steroids, tannins, saponins, glycosides, terpenoids, flavonoids and phenolic compounds were investigated.

Proximate analysis

Quantitative parameters such as moisture content, crude fibre, acid insoluble ash, water soluble ash and total ash were analyzed using standard methods (Arlington 1984).

Antioxidant activity assessment

The scavenging potential of the crude ethyl acetate pod extract of *C. pulcherrima* on DPPH free radical was estimated by applying the technique designed by Jain et al. (2008). The reaction of DPPH with an antioxidant species is shown in Scheme 1. The capacity to scavenge DPPH radical was estimated using the equation below.

DPPH radical scavenging ability (in percent)

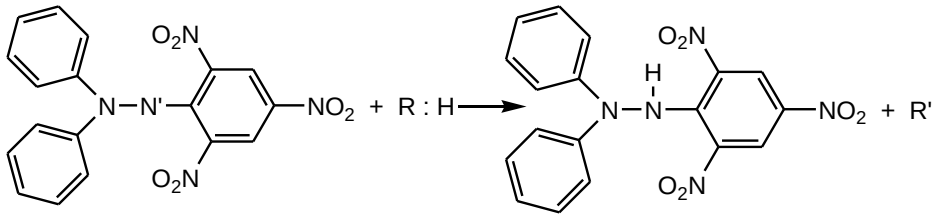
$$= \frac{A0-A1}{A0} \times 100$$

Where:

A_0 = DPPH radical absorbance in methanol;

A_1 = DPPH radical absorbance + sample extract/standard in methanol.

The IC_{50} value, otherwise known as the 50% inhibitory concentration was determined by an exponential equation to match data into the concentration-response.



DPPH radical DPPH reduced form

Scheme 1: The reaction of DPPH with an antioxidant species (R: H).

Experimental animals

Forty-five (45) healthy Swiss mice (male and female) weighing between 19 and 25 g were utilized for the acute toxicity, anti-inflammatory, and analgesic studies. These animals were acquired from the animal breeding unit in the Department of Biochemistry, University of Benin. The mice were kept in the Animal House section of the Department of Animal and Environmental Biology, University of Benin and were acclimatized for two weeks. They were maintained under the standard environmental settings: 12 hours-day/night cycle and were fed with typical mash grower feeds and water *ad libitum*. They were handled in accordance with the standard protocols guiding handling of experimental animal(s) throughout the experimental period (Takem et al. 2014).

Experimental design

The obtained forty-five (45) Swiss mice of either sex weighing between 19 and 25 g were distributed arbitrarily into five different groups of three animals each. The mice were grouped for the evaluation of both anti-inflammatory plus analgesic activities as follows:

Group I (Normal control): received 0.5 mL distilled water;

Group II (Positive control): administered with100 mg/kg aspirin (standard drug);

Group III: administered with 50 mg/kg of ethyl acetate pod extract;

Group IV: administered with 100 mg/kg of ethyl acetate pod extract;

Group V: administered with 200 mg/kg of ethyl acetate pod extract.

While for acute toxicity determination, graded doses of 1000, 1600, 2900 and 5,000 mg/kg of pod extract were administered to groups II-V, respectively.

Evaluation of acute toxicity

The acute toxicity determination was conducted by applying the new approach to practical acute toxicity testing according to Igbe et al. (2010) as revised by Ogbeide et al. (2018). The median lethal dose (LD_{50}) value was estimated by the application of the equation below.

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

Where: LD_0 = Maximum dose without death;

LD_{100} = minimum dose with death.

Evaluation of anti-inflammatory activity

Formalin-induced paw oedema in mice

The influence of ethyl acetate pod extract of *C. pulcherrima* on formalin-induced inflammation in mice paw was investigated by applying the technique illustrated by Winter et al. (1962) and Agbaje et al. (2008) except that single dose of 0.1 mL aqueous suspension of formalin (6% w/v) was used to induce inflammation after thirty (30) minutes of all treatments. The inflammation inhibition (%) was evaluated using the equation below.

$$\% \text{ inhibition} = \frac{D0-Dt}{D0} \times 100$$

Where: D_0 = average value of the inflammation or hind paw oedema of the control group of mice at a particular time;

D_t = average inflammation of the treated group (extracts or reference aspirin) of mice at a given time.

Evaluation of analgesic activity

Acetic acid-induced writhing in mice

Writhing was induced in the mice as described by Akuodor et al. (2011) and Koech et al. (2017) except that 1% acetic acid

was used for the intraperitoneal (IP) injection via an oral gastric syringe to induce pain sensation. Decrease in the writhing amount when compared with that of the control group was taken as a reflection for analgesia. Analgesia index was evaluated as percentage writhing inhibition and estimated from the equation given below.

$$\% \text{ Writhing inhibition} = \frac{C-D}{C} \times 100$$

Where, C = average writhing number for the control group of mice; D = average writhing number for the drug and extract treated mice.

Statistical analysis

The results of the analyses are given as mean ± standard error of mean (SEM). The significant difference was evaluated by applying Newman-Keuls Multiple Comparison Test ANOVA. Values with $p \leq 0.05$ were taken to be significant.

Results and Discussion

Phytochemical screening

Phytochemicals have continued to be major sources of drugs for pharmaceuticals due to their various medicinal potentials. The qualitative phytochemical screening of ethyl acetate pod extract of *C. pulcherrima* revealed the presence of alkaloids, tannins, saponins, flavonoids and phenolics as shown in Table 1. These findings are in agreement with those contained in the extract from aerial parts of the plant as reported by Sharma and Rajani (2011) as well as those contained in the stem bark as reported by Ogbeide et al. (2018). The presence of all these phytochemicals makes this pod a promising therapeutic agent that could be used in the management of diseases.

Alkaloids as a group of bioactive compounds have been known to possess many analgesic properties. They are precursors of various drugs and also serve as anti-hypertensive agents. Tannins possess the capacity to subdue any bacterial cell propagation by hindering certain vital enzymes of bacterial metabolism such as the proteolytic enzymes (Zohra et al. 2012). In the treatment of illnesses like hyperglycemia and hypercholesterolemia, saponins are of great importance. This group of phytochemicals (saponins) also serves as antioxidant and anti-inflammatory agents (Oyinlade 2014).

Table 1: Phytochemical constituents of *C. pulcherrima* pod extract

Phytochemicals	Result
Alkaloids	+
Steroids	-
Tannins	+
Saponins	+
Glycosides	-
Terpenoids	-
Flavonoids	+
Phenolics	+

(+) = Present and (-) = Absent.

Flavonoids and phenolic compounds are powerful phytochemicals that have health promoting potentials as a result of their antioxidant activities. Flavonoids as well as some polyphenolic compounds possess great nutritional values due to the fact that they have the ability to scavenge free radicals by working against oxidative cell mutilation (Oluwafemi et al. 2015). Again, these various phytochemicals have also been confirmed to be present in the extracts from the leaves of the plant as put forward by Savasankari et al. (2010).

Proximate composition

The results obtained for the proximate analysis of *C. pulcherrima* pod extract are shown in Table 2. The moisture content was 9.02 ± 0.02% w/w. The lower the moisture content of drugs, the better it could inhibit the growth of yeast, bacteria and fungi during storage (Pandey et al. 2012, Shehu et al. 2021). It has been recommended, and is generally accepted that the moisture content of crude drugs should not exceed 14% (Shehu et al. 2021). Therefore, the fine particles (powder) of this pod material can be stored for a long time with less possibility of any biological degradation or microbial attacks that can denature its bioactive compounds.

Table 2: Proximate composition of the pod extract of *C. pulcherrima*

Parameters	Values (% w/w)
Moisture content	9.02 ± 0.02
Crude fibre	9.04 ± 0.01
Acid insoluble ash	2.78 ± 0.02
Water soluble ash	1.20 ± 0.00
Total ash	4.35 ± 0.13

Values are mean ± standard error of the mean of triplicate analysis.

The crude fibre content of the pod extract obtained was 9.04 ± 0.01% w/w which is slightly above the value 7.92% obtained from the leaf protein concentrate of the leaves (Nwokoro et al. 2022). Similarly, the fibre content obtained is very close to the value 9.606 ± 0.045% obtained from the whole seed as reported by Ilori et al. (2015) and less than the value 12.33 ± 0.12% obtained from the stem bark as put forward by Ogbeide et al. (2020). Fibre is important in the body as it aids human health and it is a recognized substance that decreases cholesterol level in the body (Magu et al. 2018). The mean acid insoluble ash obtained was 2.78 ± 0.02% w/w. This is used to estimate the amount of silica present, especially sandy matter which is the indication of contamination with earthy materials. Water soluble ash value is a reflection of water soluble content of ash of crude drug. The mean water soluble ash obtained was 1.20 ± 0.00% w/w; it is a measure of the amount of inorganic compounds present in the crude drug. Similarly, the value of 4.35 ± 0.13% w/w was obtained as the mean total ash. Total ash is a measure of the residue left after ashing and it is made up of non-volatile inorganic constituents, which indicates the impurities like carbonate, oxalate and silicate (Kokate 1994, Prakash et al. 2019). Hence, these ash parameters such as acid insoluble ash, water soluble ash and total ash values are very significant parameters to check the purity, authenticity as well as the quality of crude drug, especially in powdered form (Shehu et al. 2021). The ash values obtained for the pod extract are less than those obtained for the root bark extract as put forward by Ogbeide et al. (2016) and also less than those obtained for the leaves extract as documented by Ogbeide and Falodun (2016). Therefore, relatively less amount of these three parameters indicate low impurities and also suggests that the pod extract could be better for drug action and effects (Bhargava et al. 2013).

Antioxidant estimation

The discolouration of DPPH radical has remained the most commonly applied approach in assessing the antioxidant potential of various extracts from herbs (Unuigbe et al. 2014). DPPH free radical scavenging action of *C. pulcherrima* pod extract showed an appreciable and dose-dependent increase in scavenging effect for the standard (ascorbic acid) and crude extract (Table 3).

Table 3: DPPH-scavenging potential of crude pod extract of *C. pulcherrima*

Concentration (µg/mL)	Ascorbic acid (% inhibition)	Crude extract (% inhibition)
100	46.93 ± 1.30	42.28 ± 0.09
200	48.04 ± 0.32	65.08 ± 0.28
300	79.54 ± 0.50	81.98 ± 0.25
400	86.75 ± 0.42	87.38 ± 0.55
500	95.76 ± 0.62	88.29 ± 1.28

Values are mean ± standard error of the mean of triplicate analysis.

At the highest concentration (500 µg/mL), the percentage inhibition of the crude pod extract obtained was 88.29 ± 1.28%, while the percentage inhibition of ascorbic acid (standard) obtained was 95.76 ± 0.62% (Table 3). The IC_{50} value is the concentration which will inhibit 50 percent of the original DPPH radical. The lower the concentration of inhibition at IC_{50} (50%) value, the greater the strength in scavenging free radicals (Oluwafemi et al. 2015).

The pod extract displayed a moderate antioxidant activity with an IC_{50} value of 50.05 ± 0.50 µg/mL (Table 4). This was significantly less active ($p < 0.05$) than the reference, ascorbic acid (5.20 µg/mL). Thus, the mild antioxidant property displayed by the extract may be attributed to the phytochemicals detected in *C. pulcherrima* pod (Oyinlade 2014, Oluwafemi et al. 2015).

Table 4: IC_{50} values of pod extract of *C. pulcherrima* and standard (ascorbic acid)

Sample	IC_{50} (µg/ml)
Ascorbic acid	5.20 ± 0.85
Crude extract	50.05 ± 0.50

Acute toxicity study

The oral administration of crude pod extract of *C. pulcherrima* at graded doses of 1000, 1600, 2900 and 5000 mg/kg body weight showed no indication of acute toxicity (Table 5), because there was no record of mortality of the animals up to 72 hours of cautious watching from the least to the maximum concentration (dose). Thus, there was no any indication of toxicity and variation in the pattern of behaviour and physiological responses detected such as raised tails, salivation or paw licking.

According to Hodge and Sterner (2005), an experimental drug which is orally administered can be classified into six categories based on the LD_{50}. If the LD_{50} is equal to or less than 1 mg/kg, it is regarded as 'extremely toxic' and when the LD_{50} is between 1 and 50 mg/kg, then it is said to be 'highly toxic'. Similarly, when the LD_{50} is between 50 and 500 mg/kg, it is termed to be 'moderately toxic' and when the LD_{50} is between 500 and 5000 mg/kg, it is only 'slightly toxic'. Furthermore, when the LD_{50} is between 5000 and 15000 mg/kg, it is practically or basically 'non-toxic' and finally, an experimental drug is declared relatively 'harmless' at LD_{50} equal to or greater than 15,000 mg/kg (Hodge and Sterner 2005). Hence, *C. pulcherrima* pod extract is practically non-toxic up to a dose of 5000 mg/kg.

Table 5: Oral acute toxicity results of the crude pod extract of *C. pulcherrima*in mice

Group	Doses (mg/kg)	Number of lethality	Percentage mortality
Control	DW	0/3	0
Pod extract	1000	0/3	0
Pod extract	1600	0/3	0
Pod extract	2900	0/3	0
Pod extract	5000	0/3	0

Anti-inflammatory and analgesic studies

Formalin-induced inflammation is one of the experimental techniques used for determining the anti-inflammatory effectiveness of compounds or natural products (Singh et al. 2016). It displays some level of reproducibility. From the formalin-induced paw licking experiment, the maximum dose of 200 mg/kg (at $p < 0.05$) significantly decreased the licking time compared with that of the control within a specified time interval. The oedema inhibition increased with the dosage. Thus, the inhibitory action of the pod extract is dose-dependent (Table 6).

The inhibition at the longest time (3 hours) is comparable to aspirin (reference drug); as there is no significant difference (at $p < 0.05$) between aspirin and the pod extract at graded doses of 100 and 200 mg/kg except at 50 mg/kg graded dose. Furthermore, there is no significant difference (at $p < 0.05$) between 100 and 200 mg/kg pod extract, meanwhile there exist significant differences (at $p < 0.05$) between 50 mg/kg pod extract and 100, 200 mg/kg pod extract from the % inhibition at 3 hours. Therefore, it can be inferred that this pod extract provided defense to counter the activities of chemo-irritants and inflammatory agents and also the activation of chemoreceptors (Onasanwo et al. 2012).

Table 6: Anti-inflammatory effect of the pod extract of *C. pulcherrima* and aspirin on formalin-induced oedema in mice

		Paw oedema volume (mm) and % inhibition		
Groups	Doses (mg/kg)	1 hour	2 hours	3 hours
Control	DW	2.05 ± 0.33	1.38 ± 0.23	1.06 ± 0.29
Aspirin	100	0.54 ± 0.03 (73.66)*	0.17 ± 0.02 (87.68)*	0.12 ± 0.05 (88.68)*
Pod extract	50	1.21 ± 0.09 (40.98)	0.84 ± 0.20 (39.13)	0.30 ± 0.19 (71.70)*
Pod extract	100	0.84 ± 0.07 (59.02)*	0.62 ± 0.15 (55.07)*	0.20 ± 0.17 (81.13)*
Pod extract	200	0.71 ± 0.14 (65.37)*	0.51 ± 0.33 (63.04)*	0.19 ± 0.06 (82.08)*

Values are mean ± SD (n= 3 mice); *Significantly different from control ($p < 0.05$).

Acetic acid-induced writhing test is a widely applied model of instinctual pain and it is a highly sensitive test for analgesics. It mainly comprises histamine mediators, histaminic peritoneal receptors, cholinergic and acetylcholine. It finds applications in assessing the marginally acting analgesics (Onasanwo et al. 2012). Similarly, from the acetic acid-induced writhing experiment, results obtained reveal that the extracts considerably lowered the writhing response, as intraperitoneal inoculation of the acid formed abdominal writhing through the activation of chemo-sensitive nociceptors. However, the pod extract acted as a defense to the animals, thereby, displaying analgesic effect. From Table 7, it can be seen that the least dose of 50 mg/kg produced a little analgesic influence when compared to the reference drug (aspirin). However, at 100 and 200 mg/kg, the pod extract was very effective compared to aspirin. The maximum analgesic effect of the pod extract was manifested at a dose of 200 mg/kg with a reduction of the amount of writhes by 84.53%. Whereas 100 mg/kg dose reduced the amount of writhes by 82.46% and 50 mg/kg reduced the amount of writhes by 54.52% showing an inverse dose-dependent pattern (Table 7). This dose dependent pattern of inhibiting acetic acid-induced writhing exhibited by the pod extract is a reflection of the outlying effect. Hence, it is evocative of the dose related mode of general herbal formulations in the management of pain and diseases relating to pain (Onasanwo et al. 2012, Olajide et al. 2000).

Table 7: Effects of pod extract of *C. pulcherrima* and aspirin on acetic acid induced-writhing test in mice

Group	Dose (mg/kg)	Number of writhing per 10 minutes	Inhibition of pain (%)
Distilled water (control)	0	32.33 ± 3.84	0
Aspirin	100	6.00 ± 1.73**	81.44
Pod extract	50	14.67 ± 7.36**	54.62
Pod extract	100	5.67 ± 0.33**	82.46
Pod extract	200	5.00 ± 2.52**	84.53

Values are indicated as mean ± SD (n = 3 mice); **Significantly different from control ($p < 0.05$).

Acetic acid writhing inhibition indicates that the pod extract could have crucial repercussion and depressant influence on the nervous system. This is because depressants of the central nervous system are well-known to prevent or decrease the amount of writhes in the acetic acid pain experiments (Hasan et al. 2009, Stevenson et al. 2009). The presence of alkaloids, and flavonoids in *C. pulcherrima* pod could be attributed for its anti-nociceptive property because these phytoconstituents have been shown to possess analgesic as well as anti-

inflammatory activities (Fernanda et al. 2004, Onasanwo and Elegbe 2006).

Conclusion

This research has clearly illustrated that the ethyl acetate extract of *C. pulcherrima* pod is relatively non-toxic. The ethyl acetate extract contains certain phytochemicals which could be responsible for its moderate antioxidant activity, dose-dependent anti-inflammatory activity as well as its analgesic activity which supports these bioactivities from methanol, petroleum ether, chloroform and acetone extracts as claimed by other authors. Therefore, to the best of our knowledge, this is the first report of the anti-inflammatory and analgesic activities of ethyl acetate extract of Pride of Barbados pod. Hence, these results further demonstrate that *C. pulcherrima* pod could serve as a potential source from which therapeutic drugs and specific bioactive products could be developed. However, it is necessary for these bioactive compounds present in the extract to be isolated, purified and characterized. In essence, this study has updated the works formerly reported on Pride of Barbados pod and other parts of the plant by different researchers.

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Conflict of Interest: The authors declare no conflict of interest.

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