Antiinflammatory, analgesic and antioxidant activities of *Cyathula prostrata* (Linn.) Blume (Amaranthaceae)

Bolanle Ibrahim[a], Abimbola Sowemimo[a,*], Anzel van Rooyen[b], Maryna Van de Venter[b]

[a] Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, College of Medicine Campus, Idi-Araba, Lagos, Nigeria
[b] Department of Biochemistry and Microbiology, PO Box 77000, Nelson Mandela Metropolitan University, Port Elizabeth 6031, South Africa

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ABSTRACT

Ethnopharmacological relevance: Cyathula prostrata (Linn) Blume (Amaranthaceae) is an annual herb widely used traditionally in the treatment of various inflammatory and pain related health disorders in Nigeria. The aim of this study is to evaluate the anti-inflammatory, analgesic and antioxidant activities of the methanolic extract of *Cyathula prostrata* (Linn) Blume.

Materials and methods: The anti-inflammatory (phorbol 12-myristate 13-acetate (PMA)-induced reactive oxygen species (ROS), lipopolysaccharide (LPS) induced nitric oxide production in U937 macrophages, LPS-induced COX-2 expression, carrageenan-induced rat paw oedema, arachidonic acid-induced ear oedema and xylene-induced ear oedema), analgesic (acetic acid-induced writhing and hot plate tests) and antioxidant activities (DPPH [1,1-diphenyl-2-picrylhydrazyl] and lipid peroxidation assays) activities of the plant extract were investigated.

Results: The methanolic extract of *Cyathula prostrata* did not show inhibitory activity in the *in vitro* PMA-induced reactive oxygen species, LPS-induced nitric oxide production and LPS-induced COX-2 expression assays. In the *in vivo* anti-inflammatory assays, the extract (50, 100 and 200 mg/kg) showed a significant ($P < 0.05$) dose-dependent inhibition in the carrageenan, arachidonic acid and xylene-induced tests. *Cyathula prostrata* produced a significant ($P < 0.05$, 0.001) dose-dependent inhibition in the acetic acid and hot plate analgesic tests respectively. The plant extract did not exhibit any antioxidant activity in the DPPH and lipid peroxidation assays.

Conclusion: The results suggest that the methanolic extract of *Cyathula prostrata* possesses anti-inflammatory and analgesic activities and this authenticates the use of the plant in the traditional treatment of ailments associated with inflammation and pain.

1. Introduction

Due to their implication in virtually all human and animal diseases, inflammation and pain have become the focus of global scientific research. Adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs) and opioids have necessitated the search for new drugs with minimal side effects (Dharmasiri et al., 2003; Vittalrao et al., 2011). The current trend of research is the investigation of medicines of plant origin because of their affordability and accessibility with minimal side effects.

Cyathula prostrata (Linn.) Blume (Amaranthaceae) is a straggling to more or less erect annual herb of up to 1 m long and is widely distributed in tropical Africa, Asia, Australia and tropical America. The young foliage is often coloured red with burred and adhesive fruits. The plant is a weed of cultivated land, waste places as well as forest margins. In Ivory Coast, the sap is applied to sores and chancres and used as ear drops for otitis and headache while leafy twigs, inflorescences and seeds pulped into a paste with or without clay are used on sores, burns and fractures. In Nigeria and Cameroon, it is used in the treatment of articular rheumatism and dysentery while in Gabon, it is used in treating eye troubles, wounds and urethral discharges (Burkill, 1995). The phytochemical study, proximate analysis and antibacterial activity of *Cyathula prostrata* have been reported (Oladimeji et al., 2005; Unni et al., 2009). The cytotoxic activity of the ethanolic extract of the plant against cervical and breast cancer have also been reported (Sowemimo et al., 2009, 2011). Ecdysterone, a plant ecdysteroid has been isolated from the plant (Shah and De Souza, 1971).

The aim of this study was to investigate the anti-inflammatory, analgesic and antioxidant activities of the methanolic extract of *Cyathula prostata*.

2. Materials and methods

2.1. Plant material

Cyathula prostata (Linn.) Blume [Amaranthaceae] (whole plant) was collected from the front of the Faculty of Science Building, University of Lagos, Nigeria in April 2011. They were identified by Mr. Daramola and authenticated at the Herbarium of the Department of Botany, Faculty of Science, University of Lagos, where a voucher specimen (LUH 3671) was deposited.

2.2. Extraction procedure

The plant was air-dried for 2 days and further dried in a hot air oven at 40 °C, ground to powder and stored in amber coloured bottles. Five hundred grams of the powdered material was macerated with 80% methanol at room temperature. The extract was filtered and concentrated *in vacuo* at 30 °C using the rotary evaporator (Buchi, Switzerland). The extract obtained was deep brown in colour and the crude yield was 4.4% (w/w). The extract was reconstituted in distilled water to appropriate concentrations before administration to experimental animals.

2.3. In vitro anti-inflammatory activity

2.3.1. Culture differentiation and treatment of U937 cells
U937 cells (human promonocytic leukaemia cell line) were cultured in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum, penicillin and streptomycin. The cells were differentiated into monocyte–macrophages before treatment by incubating them in the presence of 100 nM 1α,25-dihydroxyvitamin D3 (1,25(OH)$_2$D3) for 24 h. After differentiation, cells were seeded into 48-well plates at 1×10^6 cells/ml and incubated in the presence of the various treatments. Incubation period for each method is indicated in the relevant section below. Treatments included vehicle (0.125% DMSO), 50 and 125 µg/ml extract, 100 or 250 ng/ml lipopolysaccharide (LPS) and 10 nM phorbol 12-myristate 13-acetate (PMA).

2.3.2. Measurement of intracellular ROS level
The membrane permeable 2′,7′-dichlorofluorescin diacetate (DCF-DA) (Molecular Probes, Eugene, OR, USA) was used to assess intracellular reactive oxygen species (ROS) (Liu et al., 2005; Lee et al., 2008). Oxidation of DCF-DA by ROS particularly H$_2$O$_2$, yielded fluorescent 2′,7′-dichlorofluorescin (DCF). Differentiated U937 monocyte–macrophage cells were washed with warm phosphate buffered saline (PBS) and incubated in culture medium containing 2.5 µM DCF-DA for 30 min in the dark. Subsequently, the cells were centrifuged for 5 min at $250 \times g$, washed twice with PBS to remove excess stain and resuspended in fresh medium. The cells were then incubated with the extract and controls for 90 min in the dark. The reaction was terminated through addition of 50 mM EDTA. Cells were analysed using a Beckman Coulter FC500 flow cytometer with a 488 nm laser for excitation and green fluorescence recorded in FL1.

2.3.3. Measurement of nitric oxide production
The differentiated cells were incubated with the test compound and LPS (100 ng/ml) for 24 h. Fifty microliters of the supernatant was removed, transferred to another plate and mixed with 50 µl of Griess reagent to assay the nitrite (Suh et al., 1998). The solution was incubated at room temperature for 30 min in the dark. The nitrite concentration in the culture medium was measured spectrophotometrically on a BioTek® PowerWave XS spectrophotometer at 540 nm as an indicator of nitric oxide (NO) production according to the Griess reaction (Kim et al., 1995).

2.3.4. Measurement of COX-2 expression
Differentiated U937 cells were seeded into 48-well plates and incubated with extract and LPS (250 ng/ml) for 6 h. The cells were lysed with Cytobuster™ Protein Extraction Reagent (Novagen, USA) to obtain the total cell lysate and stored at −80 °C until required. The cell lysates (100 µl) were used to measure the amount of COX-2 using the COX-2 ELISA kit (Calbiochem, USA) according to the manufacturer's instructions. ELISA results were recorded using a BioTek® PowerWave XS spectrophotometer at 450 nm. The results were expressed in ng/mg protein (Shigemasa et al., 2003). Each sample was measured in triplicate.

2.4. Animals

Wistar rats (100–200 g) and Swiss albino mice (20–30 g) of either sex used in this study were obtained from the animal house facility of National Agency for Food and Drug administration and Control (NAFDAC), Lagos. All the animals were maintained under standard laboratory conditions (12 h light/dark cycle at 22 ± 2 °C) and fed with standard rodent pellets (Livestock Feed PLC, Lagos, Nigeria) and water *ad libitum*.

The procedures used in this study were in accordance with the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (NIH, 1985).

2.5. Acute toxicity study

Acute oral toxicity assay was performed on five groups of 6 mice each fasted for 12 h prior to the experiment. The plant extract was administered at doses of 2.0, 2.5, 3.0, 3.5 and 4.0 g/kg (p.o.) and 1.8, 2.0, 2.2, 2.4 and 2.6 g/kg (i.p.). The animals in each group were observed for 2 h after treatment for immediate signs of toxicity. The number of deaths in each group within 24 h was recorded. Animals which survived were further observed for 7 days for signs of delayed toxicity. The log dose-probit analysis method was employed in the LD$_{50}$ estimation (Adeyemi et al., 2009).

2.6. In vivo antiinflammatory activity

2.6.1. Carrageenan-induced paw oedema
The method of Henriques et al. (1987) was used to determine the anti-inflammatory activity of the extract. Five groups of 6 rats each were treated with *Cyathula prostrata* (50, 100 and 200 mg/kg, p.o.), indomethacin (10 mg/kg p.o.) and distilled water (10 ml/kg, p.o.). One hour after the administration of the various agents, oedema was induced by injection of carrageenan (0.1 ml, 1%, w/v in saline) into the subplantar tissue of the right hind paw. The linear paw circumference was measured using the cotton thread method (Bamgbose and Noamesi, 1981). Measurements were made immediately before injection of the phlogistic agents and at 30 min interval for 3 h.

$$\%\text{Inhibition} = \frac{\text{increase in paw oedema, control} - \text{increase in paw oedema, treated}}{\text{increase in paw oedema, control}} \times 100$$

2.6.2. Arachidonic acid-induced right ear oedema

Mice were divided into five groups of 6 animals each. Thirty minutes after oral treatment of mice with distilled water (10 ml/kg), acetylsalicyclic acid (1 mg/kg) and extract (50–200 mg/kg), oedema was induced by applying 0.03 ml of arachidonic acid to the inner surface of the right ear. The left ear was considered as control. Thirty minutes after the application of arachidonic acid, the mice were killed under ether anaesthesia and both ears were removed and weighed (Young et al., 1984)

$$\%\text{Inhibition} = \frac{\text{difference in weight of ear, control} - \text{difference in weight of ear, treated}}{\text{difference in weight of ear, control}} \times 100$$

2.6.3. Xylene-induced ear oedema

Mice were divided into five groups of 6 animals each. Thirty minutes after oral treatment of mice with distilled water (10 ml/kg), dexamethasone (1 mg/kg) and extract (50–200 mg/kg), oedema was induced by applying 0.03 ml of xylene to the inner surface of the right ear. The left ear was considered as control. Fifteen minutes after the application of xylene, the mice were killed under ether anaesthesia and both ears were removed and weighed (Nunez Guillen et al., 1997).

$$\%\text{Inhibition} = \frac{\text{difference in weight of ear, control} - \text{difference in weight of ear, treated}}{\text{difference in weight of ear, control}} \times 100$$

2.7. Analgesic activity

2.7.1. Hot plate test

The hot plate test was performed at a fixed temperature of $55 \pm 0.5 ^\circ$C. Five groups of six mice each which were fasted overnight were used in this experiment. Pre-treatment reaction for each mouse was determined after which treatment was done as follows: distilled water (10 ml/kg, p.o.), morphine (10 mg/kg, s.c.) and *Cyathula prostrata* (50, 100 and 200 mg/kg). Sixty minutes after oral administration, the reaction time was recorded. Post-treatment cut-off time of 15 s was used (Omisore et al., 2004).

$$\%\text{Inhibition} = \frac{\text{post-treatment latency} - \text{pre-treatment latency}}{\text{cut-off time} - \text{pre-treatment latency}} \times 100$$

2.7.2. Acetic acid-induced abdominal writhing test

The method of Koster et al. (1959) was used for this test. The mice were divided into five groups of six mice each and fasted overnight. The animals were treated with acetylsalicyclic acid (10 mg/kg, p.o.), distilled water (10 ml/kg, p.o.) and *Cyathula prostrata* (50, 100 and 200 mg/kg, p.o.). The mice were treated with acetic acid (0.6%, v/v in saline, 10 ml/kg, i.p.) 1 h after the above treatment was carried out. The number of writhes was counted for 30 min.

$$\%\text{Inhibition} = \frac{\text{number of writhes (control)} - \text{number of writhes (test)}}{\text{number of writhes (control)}} \times 100$$

2.8. Antioxidant activity

2.8.1. Determination of antioxidant activity by DPPH radical scavenging ability

The effect of the extract on DPPH radical was estimated using the method of Liyana-Pathirana and Shahidi (2005). A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with five different concentrations of the extract ranging from 20 to 500 μg/ml. The reaction mixture was shaken thoroughly and left on the bench at room temperature for 30 min for complete reaction. The absorbance was measured at 517 nm using a spectrophotometer (T80 spectrometer, PG Instrument Ltd.).

Quercetin was used as standard. All determinations were done in triplicate.

The ability of the sample to scavenge radical was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)}$$
$$= \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

2.8.2. Determination of antioxidant activity by lipid peroxidation (LPO) assay

An *in vitro* model using the liver homogenate was used for the induction of lipid peroxidation mediated by $FeSO_4$ as pro-oxidant and assessed by the method of Ohkawa et al. (1979). The reaction mixture contained 1 ml of liver homogenate, 0.1 ml of potassium dihydrogen sulphate buffer (10 mM), 0.1 ml of $FeSO_4 \cdot 6H_2O$ (25 μM), 0.1 ml ascorbic acid (100 μM) and 1 ml of various concentrations of the extract (20–500 μg/ml). Distilled water (0.7 ml) was added to make up to 3 ml. Incubation was carried out at 37 °C for 1 h. After the incubation period, the reaction mixture was treated with 1 ml thiobarturic acid in HCl (0.8%) and 1 ml trichloroacetic acid (20%, pH 3.5). The solution was boiled in a water bath at 95–100 °C for 30 min. The absorbance was measured at 532 nm. The assay was performed in triplicate. Quercetin was used as standard. The percentage inhibition of lipid peroxidation was calculated using the following equation:

$$\%\text{Inhibition of lipid peroxidation} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100$$

where A_{control} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample extract.

2.9. Determination of total phenolic content

The total phenolic content (TPC) of the extract was determined by Folin and Ciocalteu (1927) method. In the preparation of the calibration curve five dilutions of standard solution of gallic acid (0.005–1.0 mg/ml) were prepared. In a 96-well-plate, 4 μl of extract (250 μg/ml) or standard solution was added to distilled water (16 μl) and Folin–Ciocalteu reagent (100 μl). Immediately after, 80 μl of 7.5% sodium bicarbonate was added to the mixture to make up to a total volume of 200 μl in each well. The solution was incubated for 90 min at 30 °C after which the absorbance was read at 765 nm using a BioTek® PowerWave XS spectrophotometer. All determinations were performed in quadruplicate. The results were expressed as mg gallic acid equivalent (GAE) per gram of dry mass.

2.10. Determination of total flavonoid content

The total flavonoid content (TFC) of the extract was determined using the aluminium chloride colorimetric assay (Kumar et al., 2008). In the preparation of the calibration curve five dilutions
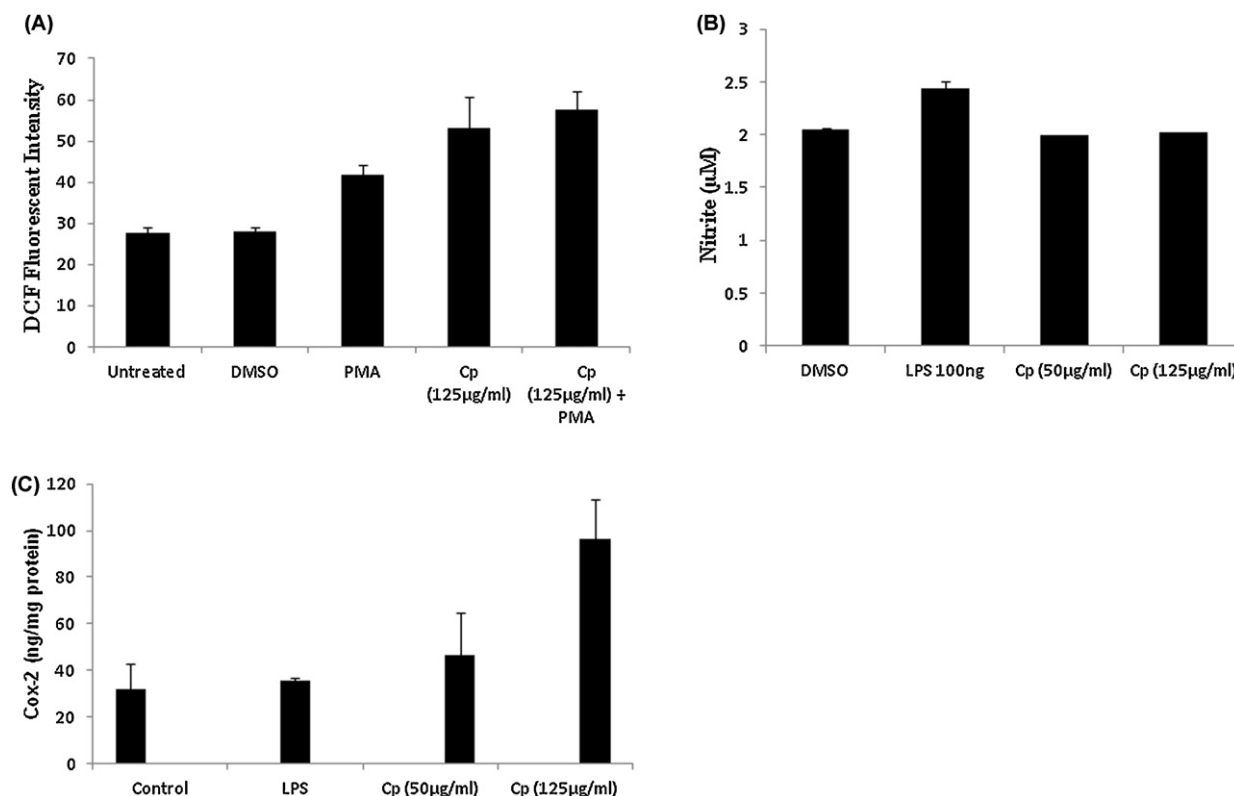
Fig. 1. Effect of *C. prostrata* on ROS (A) NO production (B) and COX-2 protein expression (C).

of standard solution of rutin (0.005–1.0 mg/ml) were prepared. In a 96-well-plate, 4 μl of extract (250 μg/ml) or standard solution (dissolved in 16 μl of distilled water) was added to 6 μl of 15% $NaNO_2$ and 80 μl of distilled water. After 6 min, 6 μl of 10% $AlCl_3$ was added. After another 6 min, 80 μl of 4% NaOH was added and made up to a total volume of 200 μl with 8 μl of distilled water. The solution was mixed well and allowed to stand for 15 min after which absorbance was read at 510 nm on a BioTek® PowerWave XS spectrophotometer. All determinations were performed in quadruplicate. Total flavonoids content was expressed as mg rutin equivalent (RE) per gram weight of sample.

2.11. Statistical analysis

All values were expressed as means ± SEM. Data were analysed by one way ANOVA followed by Dunnett's multiple comparison, Tukey's multiple comparison and Student's *t*-tests. Results were considered statistically significant at $P < 0.05$.

3. Results

3.1. Effect of Cyathula prostrata on PMA-induced intracellular reactive oxygenase species

3.1.1. (ROS) accumulation in U937 cells

The intracellular reactive oxygen accumulation as reflected by the DCF fluorescence triggered by PMA was measured in this assay. The concentration of *Cyathula prostrata* (125 μg/ml) used in this assay increased the generation of intracellular reactive oxygen species induced by PMA (Fig. 1A). The results suggest that *Cyathula prostrata* is not a reactive oxygenase inhibitor.

3.2. Effect of Cyathula prostrata on LPS-induced NO production

The ethanolic extract of *Cyathula prostrata* was checked for its inhibitory effect on nitric oxide production from macrophages (U-937 cells) induced by LPS. Two different concentrations of the crude extract were used (Fig. 1B). The extract did not show inhibitory activity on nitric oxide production in the induced cells. This suggests that the ethanolic extract of *Cyathula prostrata* is not an inhibitor of the NO related inflammation pathway.

3.3. Effect of Cyathula prostrata on LPS-induced COX-2 expression

The influence of *Cyathula prostrata* on LPS-induced COX-2 protein expression was determined. At 50 and 125 μg/ml, the crude extract caused an increase in the COX-2 protein expression (Fig. 1C) and this result suggests that extract does not inhibit the COX-2 protein expression in the inflammation pathway.

3.4. Acute toxicity

No death was observed for the oral route of administration at all doses. However 83% mortality (2.0 and 2.2 g/kg) and 100% mortality (2.4 and 2.6 g/kg) were recorded for the intraperitoneal route and the LD_{50} was estimated to be 1.9 g/kg. The physical signs of toxicity included excitation, paw licking, increased respiratory rate, decreased motor activity, gasping and coma which was followed by death.

3.5. Carrageenan-induced rat paw oedema

The ethanolic extract of *Cyathula prostrata* administered 1 h before the injection of carrageenan caused a significant ($P < 0.01$–0.05) and dose dependent inhibition of increase in paw

oedema from 30 to 150 min. The peak inhibitory effect of the extract was recorded with a dose of 50 mg/kg (66.67%) at 150 min and 10 mg/kg indomethacin (76.92%) at 150 min. The inhibition elicited by the extract at 150 min (66.67%) was comparable to that of indomethacin at 120 min (Table 1).

3.6. Arachidonic acid-induced ear edema

Cyathula prostrata significantly ($P < 0.05$) reduced the ear oedema induced by arachidonic acid and peaked at the dose of 100 mg/kg (62.44%). The level of inhibition observed at this dose was not significantly different from that produced by 10 mg/kg acetylsalicyclic acid (65.38%) (Table 2).

3.7. Xylene-induced ear edema

A significant ($P < 0.05$) dose dependent inhibition of oedema development was produced by *Cyathula prostrata* and peaked at the highest dose of 200 mg/kg (26.26%). The percentage inhibition produced by dexamethasone (1 mg/kg; p.o.) was however higher than that of the extract at 200 mg/kg (Table 3).

3.8. Analgesic activity

3.8.1. Hot plate test
Cyathula prostrata showed a significant ($P < 0.001$) dose dependent effect with maximum inhibition (44.28%) at the highest dose of 200 mg/kg. Morphine showed a significant protective effect (62.26% inhibition, $P < 0.001$) at a dose of 10 mg/kg (Table 4).

3.8.2. Acetic acid writhing reflex
The effect of *Cyathula prostrata* on the writhing response in mice is shown in Table 5. The plant extract significantly ($P < 0.01$) inhibited the acetic acid induced writhing response in a dose dependent manner with a maximum inhibitory response (50.16%) at the dose of 200 mg/kg. The inhibition elicited by the extract at 200 mg/kg was however lower than that observed for acetylsalicyclic acid at a dose of 10 mg/kg (62.58%).

3.9. Antioxidant activity

The DPPH radical scavenging and lipid peroxidation activities of the extract are shown in Fig. 2. These results suggest that *Cyathula prostrata* had no antioxidant activity in the DPPH assay and a low activity (<35%) in the lipid peroxidation assay.

3.10. Total phenolic and flavonoid content

The total phenolic and the flavonoid contents of *Cyathula prostrata* were 16.95 ± 3.32 mg GAE/g dry weight and 117.69 ± 4.56 mg RE/g dry weight respectively.

4. Discussion

Inflammatory reactions can be triggered off by exogenous or endogenous aggressions which are characterised by vascular and cellular events. These reactions in turn induce the production of ROS which is known to play an important role in the expression of cyclooxygenase (COX) which augments the production of inflammatory mediators such as leukotrienes and prostaglandins (Hakim, 1993; Pepicelli et al., 2002). Nitric oxide also plays an important role in acute and chronic inflammatory processes (Saha et al., 2004).

In our study of the *in vitro* anti-inflammatory activity of *Cyathula prostrata*, the inhibitory effect of the extract on nitric oxide production in U937 cells as well as the inhibition of the generation of ROS induced by PMA and COX-2 expression were investigated in order

Table 1
Effect of extract of *Cyathula prostrata* on carrageenan-induced rat paw oedema.

Treatment	Dose (mg/kg)	Increase in paw circumference (cm)						
		T_0	$T_{30\,min}$	$T_{60\,min}$	$T_{90\,min}$	$T_{120\,min}$	$T_{150\,min}$	$T_{180\,min}$
Cyathula prostrata	50	2.00 ± 0.04	2.30 ± 0.04 (25.20)	2.28 ± 0.04 (26.32)	2.25 ± 0.09 (28.57)	2.15 ± 0.09 (40.0)	$2.05 \pm 0.04^{**}$ (66.67)	2.30 ± 0.12 (25.0)
Cyathula prostrata	100	2.00 ± 0.03	2.28 ± 0.05 (26.32)	2.22 ± 0.05 (31.25)	2.18 ± 0.04 (35.71)	$2.08 \pm 0.02^{**}$ (53.65)	$2.05 \pm 0.03^{*}$ (58.82)	2.33 ± 0.10 (23.26)
Cyathula prostrata	200	2.02 ± 0.03	2.28 ± 0.05 (31.57)	2.25 ± 0.08 (34.29)	2.23 ± 0.08 (36.36)	2.22 ± 0.07 (38.71)	2.18 ± 0.12 (42.86)	2.83 ± 0.13 (31.56)
Indomethacin	10	1.95 ± 0.03	2.27 ± 0.02 (27.0)	2.20 ± 0.07 (33.3)	$2.12 \pm 0.07^{*}$ (45.46)	$2.05 \pm 0.06^{**}$ (66.67)***	$2.03 \pm 0.06^{*}$ (76.92)	2.22 ± 0.06 (31.25)
Distilled water	10 ml/kg	1.90 ± 0.06	2.22 ± 0.02 (−)	2.33 ± 0.05 (−)	2.33 ± 0.07 (−)	2.32 ± 0.05 (−)	2.27 ± 0.06 (−)	2.30 ± 0.05 (−)

Values are expressed as mean ± SEM ($n = 6$). Figures in parenthesis indicate percentage inhibition of oedema development.
* $P < 0.05$.
** $P < 0.01$ vs. control (One way ANOVA followed by Student's t test).
*** $P < 0.05$ vs. control (one way ANOVA followed by Dunnett's multiple comparison test).
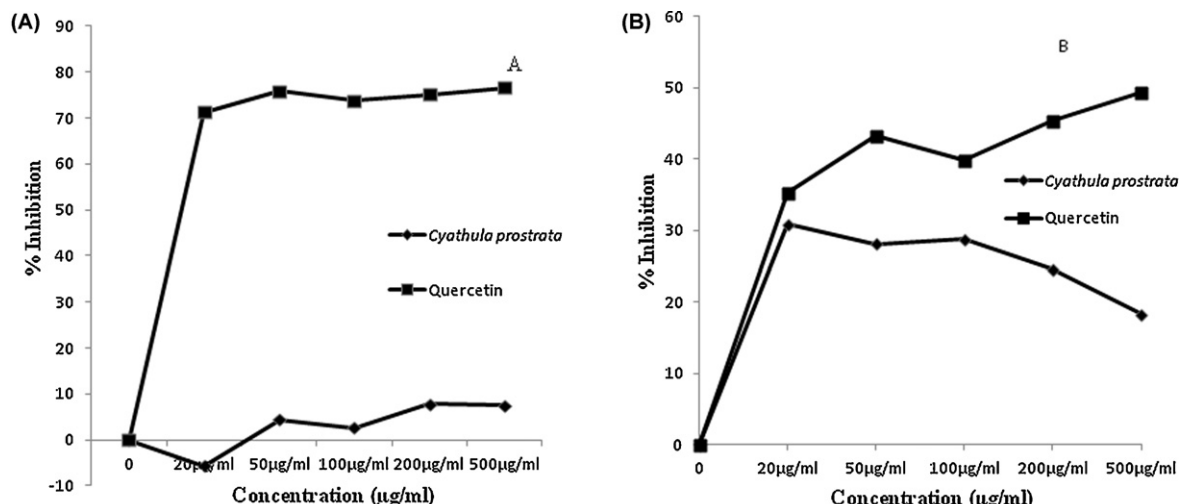
Fig. 2. Result of antioxidant activity of *Cyathula prostrata*, (A) DPPH and (B) lipid peroxidation.

Table 2
Effect of *Cyathula prostrata* on arachidonic acid-induced ear oedema in mice.

Treatment	Dose (mg/kg)	Increase in ear weight (mg)	% Inhibition
Cyathula prostrata	50	27.00 ± 0.004	30.34
Cyathula prostrata	100	14.70 ± 0.005[*]	62.44
Cyathula prostrata	200	34.00 ± 0.004	13.93
Acetylsalicyclic acid	10	13.50 ± 0.090[*]	65.38
Distilled water	10 (ml/kg)	39.00 ± 0.007	–

Values are expressed as mean ± SEM ($n = 6$).
[*] $P < 0.05$ *vs.* control (one way ANOVA followed by Dunnett's multiple comparison test).

Table 3
Effect of *Cyathula prostrata* on xylene-induced ear oedema in mice.

Treatment	Dose (mg/kg)	Increase in ear weight (mg)	% Inhibition
Cyathula prostrata	50	8.30 ± 0.002	16.67
Cyathula prostrata	100	8.20 ± 0.002[*]	17.17
Cyathula prostrata	200	7.30 ± 0.002[*]	26.26
Dexamethasone	1	5.00 ± 0.003	49.5
Distilled water	10 (ml/kg)	9.80 ± 0.003	–

Values are expressed as mean ± SEM ($n = 6$).
[*] $P < 0.05$ *vs.* control (one way ANOVA followed by Dunnett's multiple comparison test).

to determine the mechanism of action *in vitro*. Our results indicate that the *Cyathula prostrata* did not inhibit inflammation through any of these pathways.

The carrageenan, arachidonic acid and xylene-induced oedema tests were used to investigate the *in vivo* anti-inflammatory activity of *Cyathula prostrata*. Carrageenan paw oedema is a test used largely to study anti-inflammatory drugs as it involves several media-

Table 5
Effect of *Cyathula prostrata* on acetic acid- induced writhing reflex in mice.

Treatment	Dose (mg/kg)	Number of writhes	%Inhibition
Cyathula prostrata	50	63.83 ± 8.71[*]	39.02
Cyathula prostrata	100	62.00 ± 8.74[*]	40.77
Cyathula prostrata	200	52.17 ± 5.52[*]	50.16
Acetylsalicyclic acid	10	39.17 ± 5.33[*]	62.58
Distilled water	10 (ml/kg)	104.67 ± 9.27	–

Values are expressed as mean ± SEM ($n = 6$).
[*] $P < 0.01$ *vs.* control (one way ANOVA followed by Tukey's multiple comparison test).

tors (Mazzanti and Braghiroli, 1994). The development of oedema induced by carrageenan is a three phase event; the early phase (the first 90 min) involves the release of histamine and serotonin; the second phase (90–150 min) is mediated by kinin and the third phase (after 180 min) is mediated by prostaglandin (Di Rosa et al., 1971). The results from this study suggest that the extract possibly acts by inhibiting the release and/or action of histamine, serotonin and kinin since the extract showed a significant inhibitory activity at the middle phase (150 min) of the oedema development. Forestieri et al. (1996) reported anti-inflammatory activity of the decoction and 95% ethanolic extract of *Cyathula prostrata* at 500 mg/kg after 3 h subplantar injection of carrageenan, however in this study, the highest anti-inflammatory activity was observed at 50 mg/kg at 150 min.

Arachidonic acid induced ear inflammation has been reported to be sensitive in detecting the anti-inflammatory activity of lipoxygenase and cyclooxygenase inhibitors (DiMartino et al., 1987; Khan et al., 2011). In this study, the extract showed a significant inhibitory effect (62.44%) on the mice ear oedema at 100 mg/kg and this suggests that the plant extract acts as a lipoxygenase inhibitor since no inhibition was observed in the expression of COX-2 in

Table 4
Effect of *Cyathula prostrata* on hot plate-induced pain in mice.

Treatment	Dose (mg/kg)	Pre-treatment reaction latency (s)	Post-treatment reaction latency (s)	Percentage inhibition
Cyathula prostrata	50	1.50 ± 0.02	4.35 ± 0.16[*]	21.11
Cyathula prostrata	100	1.20 ± 0.06	6.38 ± 0.22[*]	37.54
Cyathula prostrata	200	1.20 ± 0.06	7.21 ± 0.31[*]	44.28
Morphine	10	2.52 ± 0.07	10.29 ± 0.18[*]	62.26
Distilled water	10 (ml/kg)	1.73 ± 0.20	1.73 ± 0.2	–

Values are expressed as mean ± SEM ($n = 6$).
[*] $P < 0.001$ *vs.* pre- treatment reaction latency (Student's *t* test).

the *in vitro* assay. The xylene-induced ear oedema is used in the screening of anti-acute inflammatory activity (Wang et al., 2011) and in the evaluation of anti-inflammatory steroids (Zanini et al., 1992). The inhibition produced by 200 mg/kg of the extract (26.26%) was lower than that produced by 1 mg/kg dexamethasone (49.5%). The effect of the extract in this model suggests that *Cyathula prostrata* may not be effective in anti-acute inflammatory disorder but rather in acute inflammatory disorder. It also suggests that the extract may contain non-steroidal anti-inflammatory agents since this model has been reported to be less sensitive to non-steroidal anti-inflammatory agents (Zanini et al., 1992).

The analgesic activity of *Cyathula prostrata* in this study was investigated using the hot plate and mouse writhing tests. The hot plate test is useful for the evaluation of centrally acting analgesics which are known to elevate the pain threshold of mice towards heat (Hiruma-Lima et al., 2000). It also indicates narcotic involvement with opioid receptor (Turner, 1965). The low percentage inhibition (<50%) shown by the plant extract in this assay suggests that plant extract is not a centrally acting analgesic. The work of Forestieri et al. (1996) reported no activity for the ethanolic extract of *Cyathula prostrata* at 500 mg/kg. The writhing model is a sensitive method for screening peripheral analgesic efficacy agents and it is more sensitive to non-steroidal analgesics (Collier et al., 1963). The analgesic effect of acetic acid is due to the liberation and increased level of several mediators such as histamine and serotonin which act by stimulation of peripheral nociceptive neurons (Cui et al., 2010). The results from this study suggest that the extract mediates its analgesic activity peripherally and this could be attributed to the histamine and serotonin mediators. In the work of Forestieri et al. (1996) 45% inhibition in this model at 500 mg/kg was reported, however, in this study, 50% inhibition was observed at 200 mg/kg. The observed differences between our work and the results reported by Forestieri et al. (1996) may however be due to the differences in the mode of extraction as there was no application of heat in the extraction of the plant in this study.

Phytochemicals are known to have a complex nature hence the antioxidant activities of plant extracts cannot be evaluated by a single method. In this study, the antioxidant activity of *Cyathula prostrata* was evaluated using the DPPH and lipid peroxidation assay methods. DPPH is a simple, rapid and reliable method commonly used in the assessment of radical scavenging activities of plant extracts *in vitro* (Munasinghe et al., 2001). The lipid peroxidation assay gives information on the scavenging of free radicals induced by oxidative stress which can initiate and propagate the lipid peroxidation cascade (Nuutila et al., 2003). *Cyathula prostrata* did not exhibit scavenging capacity against the DPPH radical. It also showed a very low activity (<30%) in the lipid peroxidation assay. These results indicate that *Cyathula prostrata* has no antioxidant potential.

The poor antioxidant activity of the plant extract may also explain the inability of the plant to act as a ROS inhibitor since ROS (generated from many redox processes) are known to be major free radicals in the human body and antioxidants have the ability to scavenge free radicals and thus prevent intracellular oxidative damage (Valko et al., 2007).

Flavonoids are known to exhibit a wide range of pharmacological properties such as antiviral, antimicrobial, antioxidant, anti-inflammatory and analgesic effects (Pelzer et al., 1998; Clavin et al., 2007; Sanchez de Medina et al., 2009; Mosquera et al., 2011). They have been reported to inhibit the cyclooxygenase and 5-lipooxygenase pathways (Williams et al., 1995; Li et al., 2011). They have also been reported to have antinociceptive activity in acetic acid and p-benzoquinone-induced writing tests (Picq et al., 1991; Toker et al., 2004). The flavonoids present in this plant may be responsible for anti-inflammatory and analgesic activities observed

in *Cyathula prostrata* however the involvement of other secondary metabolites present in the plant cannot be ruled out.

5. Conclusions

The methanolic extract of *Cyathula prostrata* was found to possess anti-inflammatory activity mediated by the inhibition of the lipoxygenase pathway and by inhibiting the release and/or action of histamine, serotonin and kinin. The extract also showed peripheral analgesic activity but no antioxidant activity. The flavonoid content may also play a role in the observed anti-inflammatory and analgesic activities. The results obtained in this work justify the use of *Cyathula prostrata* in the treatment of inflammatory disorders in Nigeria.

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