



## Original Article

## *Musanga cecropioides* leaf extract exhibits anti-inflammatory and anti-nociceptive activities in animal models



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## ABSTRACT

Extract obtained from the leaves of *Musanga cecropioides* R. Br. ex Tedlie, Urticaceae, a tree growing in Africa, is used traditionally in the treatment of edema and rheumatism. The anti-inflammatory and anti-nociceptive properties of ethanol extract were studied using the carrageenan, histamine, serotonin and xylene-induced edema tests as well as the formalin, mouse writhing and tail clip tests. Significant dose dependent inhibition was observed in the carrageenan model with peak inhibition at 150 mg/kg (71.43%, 90 min,  $p < 0.001$ ). In the histamine and serotonin models, the extract caused significant inhibition of 83.33% ( $p < 0.05$ ) and 45% ( $p < 0.01$ ) at 120 min respectively. For the xylene model, the extract showed maximum inhibition (59.25%) at 200 mg/kg. Also, *M. cecropioides* produced significant anti-nociceptive activity in the mouse writhing (55.12%,  $p < 0.01$ ), formalin (81.88%,  $p < 0.01$ ) and tail clip (11.78%,  $p < 0.001$ ) tests at 200 mg/kg respectively. The results obtained in this study demonstrated that the ethanolic leaf extract of *M. cecropioides* possesses anti-inflammatory effect possibly mediated via histaminergic and serotonergic inhibition and anti-nociceptive effect mediated via peripheral mechanism with mild central involvement.

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## Introduction

*Musanga cecropioides* R. Br. ex Tedlie, Urticaceae, is a deciduous or evergreen, dioecious medium-sized tree of up to 30 m tall with an umbrella-shaped crown. Traditionally, the leaves are used to prepare a vaginal douche for painful menstruation and also used in the treatment of gonorrhoea and cough (Burkill, 1985; Ayinde et al., 2003). The bark decoction is taken to treat arterial hypertension, constipation, pain during childbirth, cough, diabetes and schizophrenia. The stem sap is used to treat dysmenorrhoea and as galactagogue, while the root sap is used to treat stomach spasms, diarrhea, gonorrhoea, pulmonary complaints, trypanosomiasis, skin diseases, otitis, rheumatism, edema, epilepsy, and to ease childbirth. The root bark is eaten with kolanut to cure cough, and the bark is tied on wounds where it is said to effect a cure (Adejuwon, 2001). The sheath-like stipules are applied as emmenagogue and oxytocic, and to treat stomach complaints, hiccup and wounds (Burkill, 1985).

Pharmacological activities such as the uterotonic, antidiabetic, hypotensive and hypoglycemic properties of the leaf and stem bark as well as the antimicrobial activity of the root sap of *M. cecropioides* have been reported (Kamanyi et al., 1992, 1996; Dongmo et al., 1996; Ayinde et al., 2003, 2006; Adeneye et al., 2006, 2007; Senjobi et al., 2012; Uwah et al., 2013). There is, however, no record of the anti-inflammatory or analgesic activity in literature; hence, the aim of this work is to investigate the anti-inflammatory and analgesic properties of the plant with a view to validating its ethno-medicinal use.

## Materials and methods

## Plant collection and extraction

The leaves of *Musanga cecropioides* R. Br. ex Tedlie, Urticaceae, leaves were collected at Abatadu, Oke-ode, a village about 3 km to Ikere, Osun State, Nigeria (7°30' N 4°30' E), in February, 2013. The plant specimen was identified and authenticated at the Herbarium of the Department of Botany and Microbiology, University of Lagos, Akoka, Lagos, Nigeria where a voucher specimen (LUH 5637) was deposited. The leaves were pulverized and macerated with absolute

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ethanol at room temperature. The extract was filtered and concentrated using the rotary evaporator (Buchi, Switzerland). The yield was 4.58% (w/w).

### Animals

Wistar rats (100–200 g) and Swiss albino mice (18–30 g) of either sex used in this study were purchased from the Animal House of the National Agency for Food and Drug Administration and Control (NAFDAC), Lagos. The animals were maintained under standard laboratory conditions (12 h light/dark cycle at  $22 \pm 2^\circ\text{C}$ ) and fed standard rodent pellets (Livestock Feed PLC, Lagos, Nigeria) and water ad libitum. The protocol was approved by the Experimentation Ethics Committee of the College of Medicine, University of Lagos (CM/COM/08).

### Acute toxicity

Acute oral toxicity assay was performed on three groups of six mice each fasted for 12 h prior to the experiment. The plant extract was administered at doses of 1, 2 and 3 g/kg (*p.o.*). The animals were observed for immediate signs of toxicity and mortality for 24 h and further observed for seven days for signs of delayed toxicity.

### Pharmacological studies

#### *In vivo anti-inflammatory activity*

**Carrageenan-induced paw edema.** Increase in the rat hind paw linear circumference induced by plantar injection of the phlogistic agent was used as the measure of acute inflammation (Henriques et al., 1987). The rats ( $n=6$ ) were administered the plant extract (50, 100, 150 and 200 mg/kg, *p.o.*) while the control rats received indomethacin (10 mg/kg *p.o.*) and distilled water (10 ml/kg, *p.o.*) respectively. One hour after treatment, 0.1 ml of carrageenan (1%, w/v in water) was administered into the sub-plantar tissue of the right hind paw. The linear paw circumference was measured immediately before injection of the phlogistic agents and at 30 min interval for 3 h using the cotton thread method (Bamgbose and Naomesi, 1981).

Anti-inflammatory activity is determined by analyzing the reduction in edema size and calculating % inhibition of edema. A mean reduction in edema when compared with control and an increase % inhibition in the treated groups is an indication of anti-inflammatory activity.

**Serotonin and histamine induced rat paw edema.** In order to elucidate the mechanism of action of *M. cecropioides*, selected anti-inflammatory mediators (histamine and serotonin) were used. The dose which gave maximum inhibition in the carrageenan assay for was used for the tests.

Adult rats (100–200 g) fasted overnight were divided into three groups of six animals each. Distilled water, 10 ml/kg was administered to group one (control), group two received 10 mg/kg indomethacin and group three, 150 mg/kg *M. cecropioides*. All treatments were done orally. One hour post treatment, edema was induced by injection of 0.1 ml serotonin or histamine ( $10^{-3}$  mg/ml) into the sub-plantar tissue of the right hind paw. The linear circumferences of the paws were measured using cotton thread method. Measurements were made at 0 min and thereafter at an interval of 30 min for 3 h. The mean of the paw size were computed and percentage inhibitions were calculated (Agbaje and Fageyinbo, 2011).

**Xylene-induced ear edema.** Adult mice (18–30 g) fasted overnight were divided into six groups of six animals each and were treated as follows: *M. cecropioides* (50, 100, 150 and 200 mg/kg, *p.o.*), dexamethasone (1.0 mg/kg *p.o.*) and distilled water (10 ml/kg *p.o.*). Ear

edema 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 anesthesia and both ears were removed and weighed. Increase in weight caused by the irritant was measured by subtracting the weight of the untreated left ear section from that of the treated right ear sections (Núñez Guillén et al., 1997).

#### *Anti-nociceptive activity*

**Mouse writhing test.** Mice used for this experiment were divided into five groups of six animals each. Group I was given distilled water (10 ml/kg, *p.o.*); Group II received the standard drug acetylsalicylic acid (100 mg/kg, *p.o.*) while the remaining Groups III, IV and V were given the plant extract (50 mg/kg, 100 mg/kg and 200 mg/kg, *p.o.*) respectively. Sixty minutes after treatment, acetic acid (0.6% v/v in saline, 10 ml/kg *i.p.*) was administered. The number of writhes (characterized by contraction of the abdominal musculature and extension of the hind limbs) was counted for 30 min (Singh and Majumdar, 1995; Mbagwu and Anene, 2007).

**Formalin test.** In this study, the animals ( $n=6$ ) were arranged into five groups which received distilled water (10 ml/kg), extract (50, 100 and 200 mg/kg) and morphine (10 mg/kg *s.c.*) respectively. For the induction of pain, formalin (20  $\mu\text{l}$  of 1% solution) was injected into sub-plantar tissue of the right hind paw of each mouse 60 min after administration for the oral route and 30 min for the subcutaneous route. The nociceptive response was considered as the time spent in licking and biting of the injected paw. The responses of the mice were observed for 5 min (first phase) and 15–30 min (second phase) post formalin injection (Shibata et al., 1989; Vianna et al., 1998).

**Haffner's tail clip test.** Mice used in this experiment were pre-screened by placing a metal artery clip 1 in. from the base of the tail and animals which did not respond to the clip placement by turning or biting at the clip within 10 s were discarded. Eligible mice were divided into five groups of five animals each. The pre-treatment reaction time of all mice to clip was determined after which the animals were treated as follows: Group 1: distilled water (10 ml/kg), Groups 2, 3 and 4 were treated with the extract at 50 mg/kg, 100 mg/kg and 200 mg/kg respectively, Group 5: Morphine 10 mg/kg *s.c.* The presence or absence of anti-nociceptive activity was determined 60 min after drug administration for oral administration and 30 min for subcutaneous administration (Adeyemi et al., 2004). A post-treatment cut-off time of 30 s was used (Adeyemi et al., 2004; Agbaje and Adeneye, 2008).

#### *Quantitative analysis*

##### *Determination of total phenol content*

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method (Wolfe et al., 2003). Gallic acid was used as a standard with a concentration range of 0.01–0.05 mg/ml prepared in methanol (Folin and Ciocalteu, 1927). The extract (0.5 ml) (0.1 mg/ml) together with the gallic acid was mixed with 2.5 ml Folin-Ciocalteu reagent (previously diluted with distilled water 1:10, v/v) and 2 ml (75 g/l) of sodium carbonate. The mixtures were vortexed for 15 s and allowed to stand for 30 min at room temperature before the absorbance was measured at 765 nm using a spectrophotometer. All determinations were performed in triplicates. The total phenolic content was expressed as mg gallic acid equivalent (GAE) per gram of sample.

##### *Determination of total flavonoid content*

Total flavonoid was estimated using the method of Miliauskas et al. (2004). A 2%  $\text{AlCl}_3$  in ethanol (2 ml) was added to 2 ml of

the sample. To obtain the calibration curve, a concentration range of 0.01–0.05 mg/ml was used for quercetin. The absorbance was measured at 420 nm after 60 min at room temperature. The total flavonoid content was calculated as milligram quercetin equivalent (QE) per gram of sample.

#### Determination of mineral content

Powdered leaf sample (1 g) was digested using 10 ml of 1 M HNO<sub>3</sub>. The solution was then filtered and made up to 50 ml with distilled water. Concentrations of sodium, potassium, zinc, copper, calcium, magnesium, iron and manganese were determined by atomic absorption spectrophotometry (Analyst 200, Perkin Elmer, Waltham, MA, USA).

#### Statistical analysis

All values were expressed as means ± SEM. Results were analyzed by one-way ANOVA followed by Dunnett's multiple comparison or by two-way ANOVA followed by Bonferroni test using Graph Pad Prism 6. Results were considered statistically significant at  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$ .

## Results

#### Acute toxicity test

The ethanol leaf extract of *M. cecropioides* was found safe at the doses tested (1, 2, 3 g/kg). During the seven days assessment time, the test animals were found normal with no visible signs of delayed toxicity.

#### Anti-inflammatory activity

##### Carrageenan-induced rat paw edema test

The leaf extract of *M. cecropioides* (50, 100, 150 and 200 mg/kg) produced a significant ( $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$ ) inhibition of edema relative to control (10 ml/kg distilled water) in a dose independent manner. The highest inhibition of edema was obtained with 150 mg/kg dose (71.43%) of the extract at 90 min (Table 1). The extract compared effectively with standard drug indomethacin (10 mg/kg) used in this study, which produced a peak inhibition of edema (68.47%) at 30 min.

#### Inflammatory mediators

##### Serotonin-induced and histamine-induced rat paw edema

In the serotonin model, the extract of *M. cecropioides* at a dose of 150 mg/kg gave a mild inhibitory effect (45.0%) at 120 min while the standard produced a more pronounced inhibitory (64.52%) effect at 150 min (Fig. 1A). For the histamine model, at the same dose of extract, there was a peak inhibitory effect (83.33%) relative to the control (distilled water 10 ml/kg) observed at 120 min. The extract was more effective than the standard (indomethacin 10 mg/kg) which also produced a peak inhibitory effect (57.14%) at the same time with the extract (Fig. 1B).

#### Xylene-induced ear edema

Animals pre-treated with ethanolic leaf extract of *M. cecropioides* (50, 100, 150 and 200 mg/kg) produced a significant ( $p < 0.05$ ) inhibition of edema (Table 2). The effect produced was dose dependent and compared effectively with the standard drug dexamethasone (1 mg/kg) used in the study.

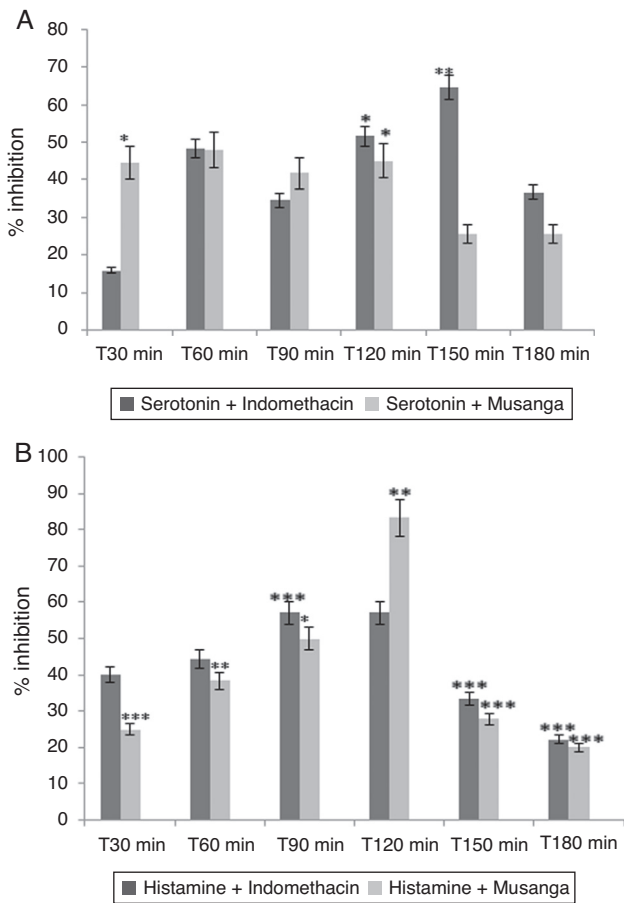
**Table 1**  
Effect of *Musanga cecropioides* on carrageenan induced edema.

Treatment	Dose (mg/kg)	Increase in paw circumference							
		T <sub>0</sub>	T <sub>30</sub>	T <sub>60</sub>	T <sub>90</sub>	T <sub>120</sub>	T <sub>150</sub>	T <sub>180</sub>	
<i>M. cecropioides</i>	50	2.05 ± 0.022	2.27 ± 0.033 (62.86)***	2.45 ± 0.062 (47.83)***	2.70 ± 0.045 (36.07)***	2.82 ± 0.048 (32.35)***	2.95 ± 0.072 (28.94)***	2.72 ± 0.031 (35.48)***	
<i>M. cecropioides</i>	100	1.97 ± 0.021	2.08 ± 0.040 (71.07)***	2.23 ± 0.049 (51.81)***	2.27 ± 0.049 (48.86)***	2.32 ± 0.048 (45.03)***	2.20 ± 0.062 (55.13)***	2.62 ± 0.031 (30.60)***	
<i>M. cecropioides</i>	150	1.78 ± 0.031	1.87 ± 0.021 (52.63)***	1.83 ± 0.021 (66.67)***	1.70 ± 0.037 (71.43)***	1.67 ± 0.022 (52.63)***	1.87 ± 0.021 (52.63)***	1.90 ± 0.00 (45.45)***	
<i>M. cecropioides</i>	200	1.87 ± 0.033	2.08 ± 0.031 (46.28)***	2.10 ± 0.058 (44.44)***	2.08 ± 0.031 (46.28)***	2.07 ± 0.033 (48.28)***	2.20 ± 0.026 (35.90)***	2.60 ± 0.026 (20.29)***	
Indomethacin	10	1.93 ± 0.042	2.05 ± 0.022 (68.47)***	2.17 ± 0.021 (52.05)***	2.17 ± 0.056 (52.05)***	2.15 ± 0.022 (53.90)***	2.17 ± 0.021 (52.05)***	2.58 ± 0.031 (28.04)***	
Distilled water	10 ml/kg	1.68 ± 0.031	2.43 ± 0.045 (-)	2.55 ± 0.084 (-)	2.78 ± 0.048 (-)	2.85 ± 0.102 (-)	2.92 ± 0.122 (-)	3.17 ± 0.191 (-)	

Values in parenthesis indicate percentage inhibition of edema development.

Values are expressed as mean ± SEM (n = 6).

\*\*\*  $p < 0.001$  vs. control (one-way ANOVA, Dunnett's multiple comparisons).



**Fig. 1.** The effects of *Musanga cecropioides* (MC) and indomethacin on rat hind paw edema induced by (A) serotonin and (B) histamine. Data represented as mean  $\pm$  SEM ( $n = 6$ ). \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$  compared to the control (one-way ANOVA followed by Dunnett's multiple comparison).

**Table 2**

Effect of *Musanga cecropioides* on xylene induced ear edema.

Treatment	Dose (mg/kg)	Increase in ear weight (mg)	% Inhibition
<i>M. cecropioides</i>	50	12.83 $\pm$ 0.003	34.63
<i>M. cecropioides</i>	100	13.00 $\pm$ 0.004	33.79
<i>M. cecropioides</i>	150	11.00 $\pm$ 0.006	43.96
<i>M. cecropioides</i>	200	8.00 $\pm$ 0.002 <sup>a</sup>	59.25
Dexamethasone	1	4.80 $\pm$ 0.002	75.55
Distilled water	10 ml/kg	19.63 $\pm$ 0.004	–

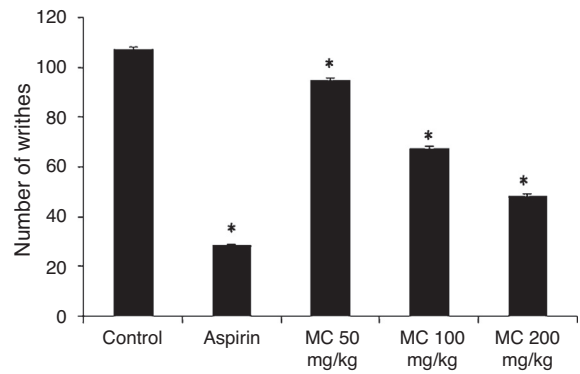
Values are expressed as mean  $\pm$  SEM ( $n = 6$ ).

<sup>a</sup>  $p < 0.05$  vs. control (one-way ANOVA, Dunnett's multiple comparison test).

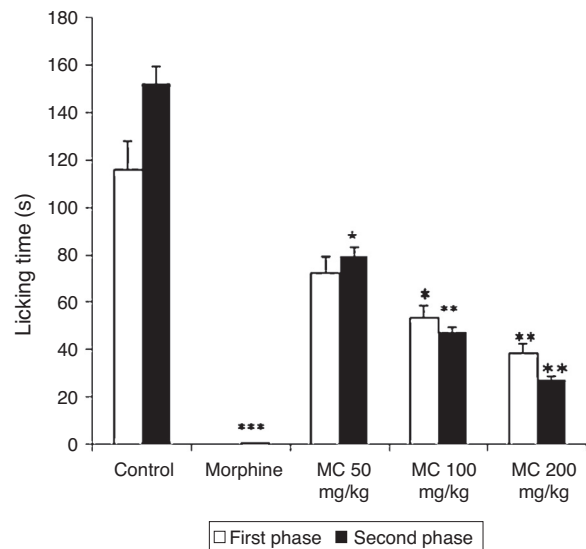
### Anti-nociceptive activity

#### Mouse writhing test

Writhing reflex was produced by intraperitoneal injection of acetic acid in control animals with  $107.3 \pm 14$  number of writhes counted in 30 min. The plant extract (50, 100, 200 mg/kg) produced a significant ( $p < 0.01$ ) reduction in the number of writhes in a dose dependent manner. The peak inhibitory effect (55.12%) was produced at the dose of 200 mg/kg. The standard drug (Aspirin, 100 mg/kg) also produced a significant (73.30%,  $p > 0.01$ ) reduction in the number of writhes with a greater inhibition compared to the extract (Fig. 2).



**Fig. 2.** Effect of *Musanga cecropioides* (MC) on mouse writhing test in mice. Values are expressed as mean  $\pm$  SEM ( $n = 6$ ). \* $p < 0.01$  compared to the control (one-way ANOVA followed by Dunnett's multiple comparison).



**Fig. 3.** Effect of *Musanga cecropioides* (MC) on formalin induced reflex in mice. Data represented as mean  $\pm$  SEM ( $n = 6$ ). \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$  compared to the control (two-way ANOVA followed by Bonferroni multiple comparison).

#### Formalin test

Injection of formalin into the sub-plantar tissue of the right hind paw of control mice in the first phase produced nociceptive response of biting and licking of the paw with duration of  $116 \pm 26.98$  s. The plant extract (50, 100, 200 mg/kg) produced a statistically significant ( $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$ ) dose dependent inhibition of nociceptive reaction with peak effect produced (66.81%) at a dose of 200 mg/kg. Morphine produced a greater inhibitory response (100%). In the second phase, the duration of the nociceptive reaction in the control group was  $151.8 \pm 41.3$ . The extract, in a dose dependent manner, significantly inhibited nociceptive reaction with peak effect (81.88%) at 200 mg/kg. In this phase, the effect produced by the extract compared effectively with morphine (96.4%) (Fig. 3).

#### Haffner's tail clip test

Application of the metal artery clip unto the tail of animals in the control group elicited reactions toward clip removal with the post-treatment latency being  $3.18 \pm 0.86$  s,  $5.04 \pm 0.63$  s and  $1.17 \pm 1.11$  s measured at 60, 90, and 120 min, respectively, with a pre-treatment latency of  $3.17 \pm 0.52$  s. The ethanolic leaf extract of *M. cecropioides* (200 mg/kg) produced a significant ( $p < 0.01$ ) increase in reaction latency time with peak effect (17.02% inhibition) at 90 min

**Table 3**  
The effects of *Musanga cecropioides* on tail clip reflex in mice.

Treatment	Dose (mg/kg)	Pre-treatment latency period	Post treatment latency period		
			60 min	90 min	120 min
<i>M. cecropioides</i>	50	3.061 ± 0.84	6.14 ± 0.64 (5.418)	8.74 ± 2.02 (9.97)	6.25 ± 0.79 (5.56)
<i>M. cecropioides</i>	100	3.26 ± 0.65	8.77 ± 1.51 (9.71)	10.26 ± 0.78 (12.35)	7.49 ± 1.03 (7.45)
<i>M. cecropioides</i>	200	2.94 ± 0.36	10.84 ± 1.60 (13.86)*	12.65 ± 2.27 (17.02)*	9.66 ± 0.89 (11.78)*
Morphine	10	1.95 ± 0.48	48.41 ± 8.48 (80.03)****	45.75 ± 9.88 (75.45)****	49.22 ± 9.05 (81.43)****
Distilled water	10 ml/kg	3.17 ± 0.52	3.18 ± 0.86	5.04 ± 0.63	1.17 ± 1.11

Values are expressed as mean ± SEM (n = 6). Figures in parenthesis represent percentage inhibition.

\* p < 0.01 vs. control group (two-way ANOVA followed by Bonferroni multiple comparison test).

\*\*\*\* p < 0.001 vs. control group (two-way ANOVA followed by Bonferroni multiple comparison test).

**Table 4**  
Mineral composition of *Musanga cecropioides* leaves (mg/g of dry matter).

S/n	Element	Concentration (mg/g)
1	Sodium (Na)	1.08
2	Potassium (K)	0.85
3	Zinc (Zn)	0.31
4	Copper (Cu)	0.0075
5	Calcium (Ca)	1.47
6	Magnesium (Mg)	1.06
7	Iron (Fe)	0.94
8	Manganese (Mn)	0.35

post-treatment. This effect was mild compared to the effect produced by morphine (81.43% inhibition) at 120 min (Table 3).

#### Quantitative analysis

The total phenolic and flavonoid contents of the extract were 385.46 ± 7.44 (GAE/g of dried extract mg/g) and 255.86 ± 12.53 (QE mg/g) respectively.

#### Mineral composition

The mineral composition of the leaves of *M. cecropioides* is presented in Table 4. Of all four macro-elements (Na, K, Ca, Mg) investigated, calcium occurred in the largest amount and for the micro-elements (Zn, Cu, Mn, Fe), iron occurred in the largest amount.

#### Discussion

The use of plants as medicines predates written human history. Plants are able to synthesize a wide variety of chemical compounds which have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases.

This study investigated the anti-inflammatory and anti-nociceptive activities of *M. cecropioides*. Carrageenan, xylene, serotonin and histamine tests were used to screen the anti-inflammatory activity while mouse writhing, formalin and tail clip tests were used to evaluate the anti-nociceptive activity.

The paw edema induced by carrageenan involves several chemical mediators such as histamine, serotonin, bradykinin, and prostaglandins (Vinegar et al., 1987). *M. cecropioides* extract significantly (p < 0.001) inhibited the edema size. The effect produced by the extract compared effectively with the standard drug (indomethacin) with a peak inhibitory effect at 150 mg/kg observed at 90 min. This result suggests that the extract of *M. cecropioides* is effective in the early phase of inflammation which is primarily due to the release of histamine and serotonin.

For the inflammatory mediators, the plant extract (150 mg/kg) showed a significant (p < 0.05) inhibition of serotonin compared with the control. The peak inhibition was obtained at 120 min post induction. On the other hand, the same dose of the extract

(150 mg/kg) showed a significant (p < 0.05) inhibition of histamine when compared with the control (10 ml/kg distilled water). The peak inhibition was obtained at 120 min. These data indicate that the plant extract is likely to interfere with histamine release at the initial phase of inflammation. Thus, it can be speculated that the inhibition of edema by the extract at the early phase of inflammation-induced by carrageenan may be mediated possibly through the inhibition of histamine. However, the difference in the time of peak inhibition observed in the carrageenan and histamine/serotonin models maybe due to interference of other mediators such as bradykinin, and prostaglandins which are present when carrageenan is used in edema induction.

The xylene-induced ear edema test is useful for the evaluation of anti-inflammatory steroids. The swelling induced by xylene can cause an acute inflammatory response which may lead to severe vasodilation and plasma extravasations. This model is linked with phospholipase A2 which is involved in the pathophysiology of inflammation due to xylene (Zaninir et al., 1992; Akindele and Adeyemi, 2007). In this study, dexamethasone, a steroidal anti-inflammatory agent, produced significant reduction in the ear edema. The extract of *M. cecropioides* also significantly (p < 0.05) inhibited edema induced by xylene and this suggests a steroidal anti-inflammatory activity of the plant possibly mediated by the inhibition of phospholipase A2.

Intraperitoneal injection of acetic acid elicits a response characterized by a wave of abdominal musculature contraction followed by extension of the hind limb (writhing). This response is used to establish the peripheral and non-steroidal action of an analgesic drug and is thought to involve local peritoneal receptors at the surface of the cell lining the peritoneal cavity (Bentley et al., 1983; Berkenkopf and Weichmann, 1988; Nùñez Guillén et al., 1997; Zakaria et al., 2008). The agent reducing the number of writhing will render anti-nociceptive effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition (Duarte et al., 1988; Ferdous et al., 2008). Peripherally acting drugs such as aspirin have been reported to exhibit anti-nociceptive activity in the writhing test only (Zakaria et al., 2008). *M. cecropioides* extract produced a significant reduction in number of writhes in this study and this suggests a peripheral mechanism of action involving direct action on nociceptors, direct inhibition of prostaglandin action or indirect inhibition of prostaglandin synthesis by inhibition of cyclo-oxygenase (COX) activity (Franzotti et al., 2000).

Biphasic nociceptive response is produced by subcutaneous injection of 1% formalin into the mice right hind paw. The first transient phase which is short lived, involves direct effect of formalin on sensory C-fibers while the second prolonged phase is associated with the development of the injury induced spinal sensation which is responsible for facilitated pain processing. This is a central sensitization of the dorsal horn neuron, a process which occurs during inflammatory pain (Rezayat et al., 1999; Ashok et al., 2006; Da Rocha et al., 2011). Changes in the dorsal horn of the spinal cord are said to be initiated by C-fiber barrage during the first phase (Tjølsen

et al., 1992). Centrally acting analgesic drugs such as morphine inhibit both phases while peripherally acting drugs inhibit only the latter phase (Santos et al., 1994; Chen et al., 1995; Agbaje and Adeneye, 2008). Studies have shown the involvement of serotonin, histamine, substance P, excitatory amino acid and prostaglandin in the late phase of formalin test with bradykinin affecting both phases (Tjølsen et al., 1992; Doak and Sawynok, 1997; Rezayat et al., 1999). *M. cecropioides* inhibited both the early and the late phase of formalin induced pain but greater inhibition was observed in the late phase which compared favorably with the standard drug (morphine) used in the study. Thus, suggesting more peripheral action than central effect.

To further confirm the involvement of the central mechanism in the anti-nociceptive effect of *M. cecropioides*, the tail clip test was used. In this model, increase in the pain reaction time (latency period) indicates the level of anti-nociception induced by the drug or extract. The extract of *M. cecropioides* produced a significant ( $p < 0.01$ ) dose dependent increase in pain threshold in mice. This effect was however mild when compared with the standard drug (morphine). Maximum inhibition of the extract was observed at 90 min while that of the standard was seen at 120 min. This further confirmed that the plant extract has very mild central activity.

This is the first report of the anti-inflammatory and antinociceptive effects of *M. cecropioides* in literature. However, the anti-inflammatory effect of pomolic acid isolated from *Cecropia pachystachya*, a member of the family Cecropiaceae has been reported (Schinella et al., 2008).

Phenolic compounds are secondary metabolites found in plants. *In vitro* and animal studies have shown that polyphenolic compounds such as flavonoids and phenolic acids can exert multiple activities such as anti-inflammatory and analgesic effects (Larrosa et al., 2010). Flavonoids have been reported to effect anti-nociceptive activity by targeting prostaglandins (Rao et al., 1998; Rajannarayana et al., 2001). The results from this study suggest that the extract of *M. cecropioides* is rich in polyphenols and these may be responsible for the observed biological activities.

The results from the mineral content determination by atomic absorption spectrophotometry revealed the presence of magnesium, manganese, copper, zinc, sodium, potassium, calcium and iron. The role of magnesium in inflammation has been reported. Magnesium deficiency in rats is said to lead to the activation of macrophages and elevation of plasma concentration of interleukin (IL) 6, which is a known mediator of the acute phase response (Maier et al., 1997, 2005). Manganese is reported as a remedy for strains, sprains and inflammation as it has the ability to increase the level or activity of superoxide dismutase (SOD) thereby increasing antioxidant activity (Murray, 1996). Magnesium and manganese block calcium channels, thereby preventing calcium uptake at the presynaptic terminal and subsequent release of synaptic transmitter hence inhibiting nociception (Abdel-Azim, 2001; Giniatullin et al., 2003). Copper supplementation has been reported to have anti-inflammatory effect and this is related to its ability to form complexes that serve as selective antioxidants (DiSilvestro and Marten, 1990). Deficiency of zinc has been reported in diseases associated with chronic inflammation and oxidative stress such as rheumatoid arthritis, diabetes, and cancers (Prasad, 2009). It has also been reported to increase concentration of inflammatory cytokines and oxidative stress and induce apoptosis and endothelial cell dysfunction (Brown et al., 2002; Prasad, 2008; Overbeck et al., 2008). Reports suggest that calcium decreases tumor-promoting pro-inflammatory markers in the plasma of sporadic colorectal adenoma patients. C-reactive protein (CRP), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-6 (IL-6), specific pro-inflammatory markers are reported to be elevated in patients with inflammatory bowel disease (Kim et al., 2008; Groblewska et al., 2008). Calcium is said to bind to bile acids and free fatty acids,

precipitating them from solution in the colon thereby reducing oxidative stress and inflammation in the colon (Newmark and Lipkin, 1992). It is also reported to activate the calcium sensing receptor involved in cell-cycle events and differentiation promoting cell-cell and cell-matrix adhesion (Lamprecht and Lipkin, 2001).

The presence of these elements in the extract of *M. cecropioides* may contribute to anti-inflammatory and anti-nociceptive effects observed.

Conclusively, the extract of *M. cecropioides* has shown anti-inflammatory action mediated possibly through the inhibition of histamine and anti-nociceptive action mediated through peripheral mechanism with a mild central involvement. This study justifies the traditional use of the plant in the treatment of inflammation and painful conditions.

#### Authors' contributions

Plant sample collection, identification and the laboratory work were carried out by EO and OA. FMS and IM contributed to the biological studies and analysis of the data. SA designed the study, supervised the work and wrote the manuscript. All the authors have read the final manuscript and approved submission.

#### Conflicts of interest

The authors declare no conflicts of interest.

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