MECHANISMS OF ANTI-INFLAMMATORY ACTIVITY OF THE LEAF EXTRACT AND FRACTIONS OF *MILLETTIA ABOENSIS*

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ABSTRACT

The acute and chronic anti-inflammatory effect of the crude extracts and fractions of *Millettia aboensis* leaves, a popular Nigerian traditional anti-inflammation remedy and possible mechanism of effect was investigated. Phytochemical analysis of the crude and fraction revealed the presence of anti-inflammatory phyto-compounds – terpenoids, steroids, flavonoids, tannins, saponins and glycosides. No death was recorded from acute toxicity study. The crude and methanol fraction at 300 and 200 mg/kg showed significant ($p < 0.05$) inhibition of egg albumin induced paw edema. Effect of the extract and fraction on topical oedema induced by xylene on mouse ear revealed that methanol fraction at 5 mg/ear had the highest activity with percentage inhibition of 61.90 % compared to the 54.76 % inhibition produced by Indomethacin. Methanol fraction at 300 mg/kg produced a significant ($p < 0.05$) inhibition of formaldehyde induced arthritis with percentage inhibition of 40.93 %. Result of the ulcerogenic effect in rat demonstrated that both the crude and methanol fraction possess ulcerogenic effect though lower than that produced by Indomethacin. Methanol fraction at 400 mg/kg also produced significant ($p < 0.05$) inhibition of leucocytes migration compared with the control. Both extract and methanol fraction produced stabilization effect on the heat-induced and hypotonicity induced red blood cell haemolysis. These results suggest that the ethanol leaf extract of *M. aboensis* possess acute and chronic anti-inflammatory activity that is most active in the methanol fraction. The activity may be as a result of combined inhibition of prostaglandin synthesis, leukocyte migration and membrane stabilization effect.

Keyword: *Millettia aboensis*; Anti-inflammation; Paw edema, Arthritis; Leucocytes migration; Membrane stabilization.

INTRODUCTION

Inflammation is part of the complex biological response to harmful stimuli such as pathogens, damaged cells or irritants¹. While acute inflammation is characterized by rapid onset and short duration, chronic inflammation is of more prolonged duration due to persistent antigen². Sustained or excessive inflammation has been implicated in the pathogenesis of numerous diseases including rheumatoid arthritis and inflammatory bowel disease³. The humoral response to inflammation involves the dilation of arterioles, capillaries and Venules with consequent increase in permeability and blood flow leading to exudation of fluid and proteins; the latter process (Cellular response) involves the migration and subsequent activation of leukocytes. Various mechanisms of anti-inflammatory effects of medicinal plants have been postulated. Some mediate their effects through inhibition of prostaglandin synthesis⁴, leucocytes migration⁵ and membrane stabilization². Traditionally, plants have been used to ameliorate inflammatory conditions. *M. Aboensis* is one of such plants widely used in Eastern Nigeria in the treatment of inflammatory diseases. Its leaves are used for general healing and as laxatives while its root is used for treatment of venereal diseases⁶. It is widely distributed in tropical Africa and found abundant in South East of Nigeria. In the rural communities of Igbo land, Nigeria, its leaf decoction has been widely used for the treatment of inflammatory diseases. Despite the popular use of this leaf, no study has been done on its anti-inflammatory activity.

This study, evaluated the anti-inflammatory activity of the ethanol leaf extract and fractions of *M. aboensis*. Possible mechanism of anti-inflammatory activity of the extract and fraction was also investigated.

MATERIALS AND METHODS

Plant Material

The leaves of *M. aboensis* were collected from Nsukka, Enugu State, Nigeria. The collection and authenticated was done by a taxonomist Mr. Alfred Ozioko of Bioresource Development and Conservation Project, Nsukka, Enugu State, Nigeria. The leaves were cleaned, air-dried and pulverized.

Chemicals

Analytical grade of n-Hexane, Chloroform, Ethyl acetate, Methanol and Ethanol (Sigma Chemicals CO., USA) were used. All laboratory reagents and distilled water were freshly prepared.

Extraction and Fractionation

About 500 g of the pulverized leaves was cold macerated in aqueous ethanol (70 %) for seven days. The resulting solution was filtered and the filtrate concentrated to dryness in vacuo using rotary evaporator at 40°C. The crude ethanol extract was screened for anti-inflammatory activity. A 2 kg portion of the pulverized leaves was macerated at room temperature in 15 L of 70 % ethanol. The ethanol extract was

concentrated in vacuo, adsorbed on silica gel and eluted in succession with n-hexane, Chloroform, Ethyl acetate and Methanol to obtain the n-hexane fraction, Chloroform fraction, Ethyl acetate Fraction and Methanol fraction. All the extracts and fractions were stored in refrigerator between 0-4°C until used.

Phytochemical Test

The phytochemical analysis of the leaves extract and fractions were carried out using standard methods^{7,8}.

Animals

Swiss albino rats (130 + 20 g) and mice (20 – 27 g) were employed for the study. The animals were obtained from the animal house of the Department of Pharmacology and Toxicology, Nnamdi Azikiwe University, Awka. The animals were housed in standard laboratory conditions and fed with rodent feed (Guinea feed Nigeria Ltd) and had free access to food and water *ad libitum*. The animals were fasted overnight with free access to water prior to experiments. All animal experiments were conducted in compliance with the laid down procedures by the relevant ethical committee on laboratory animal use.

Acute toxicity study

Thirty Swiss albino mice and rats each, divided into six groups of five animals per group was used for this study. After six hours fasting for mice and overnight fasting for rats, groups 1-5 received oral administration of 100, 1000, 2000, 4000, 5000 mg/kg doses of the extract respectively. Group 6, served as the control and received oral administration of 10 ml/kg normal saline. The animals were observed for obvious toxic symptoms and mortality for 24 hours post administration of the extract. The median lethal dose of the extract (LD₅₀) was estimated using probity analysis⁹.

Egg albumin induced paw edema in rats

The test was carried out as described by Osadebe and Okoye¹⁰. The animals (n = 5 per group) were fasted for 5 hours and deprived of water only during the experiment. They were given intra peritoneal (i.p) injection of the crude extract and fractions solubilised in 10 % Tween 80 at doses of 100, 200, and 300 mg/kg. Control animals received 0.4 ml of 10 % Tween 80 or 100 mg/kg aspirin (i.p); all substances were administered 30 minutes before the sub planta injection of the phlogistic agent (0.1 ml of fresh undiluted egg albumin). Paw volumes were measured by water displacement method at 0, 1, 2, 3, and 4 hours after induction of edema. The anti-inflammatory effect was calculated at each time of observation as percentage inhibition of edema in the animals treated with the substances under test in comparison with the vehicle treated animals. The percentage inhibition of edema was calculated as described by Perez¹¹ using the formula

$$\% \text{ inhibition} = \frac{V_0 - V_t}{V_0} \times 100$$

V_0 = the volume of edema of the control (vehicle treated) group at time t;
 V_t = the volume of edema at corresponding time of the treated rats

Topical Oedema Induced by Xylene in the Mouse Ear

The effect of the methanol extract on acute topical inflammation was evaluated by a modification of the method of Atta and Alkohafi¹². Adult Swiss albino mice (20–28 g) of either sex were divided into four groups of 5 animals each. The treatment groups received crude extract and methanol

fraction (5 mg/ear) applied on the anterior surface of the right ear. Topical inflammation was instantly induced on the posterior surface of the same ear by application of xylene (0.05 ml). Control animals received either the vehicle (normal saline) or indomethacin (5 mg/ear). Two hours after induction of inflammation, mice were killed by overdose of chloroform anesthesia and both ears removed. Circular sections (7 mm diameter) of both the right (treated) and left (untreated) ears were punched out using a cork borer, and weighed. Edema was quantified as the weight difference between the two earplugs. The anti-inflammatory activity was evaluated as percent edema reduction/inhibition in the treated animals relative to control animals¹³ using the relation:

$$\text{Percentage edema reduction (\%)} = [100 (R_t - L_t / R_c - L_c)]$$

Where R_t = mean weight of right ear plug of treated animals;

L_t = mean weight of left ear plug of treated animals;

R_c = mean weight of right ear plug of control animals;

L_c = mean weight of left earplug of control animals.

Arthritis Induced by Formaldehyde in Rats

The method of Seyle¹⁴ was used. Adult swiss albino rats (n = 5 per group) received 100, 200 and 300 mg/kg of crude and methanol fraction of *M. aboensis* i.p on day one. One hour later, arthritis was induced by sub-planter injection of 0.1 ml of 2.5 % formaldehyde solution and repeated every 5 days. Inflammation was assessed by measuring the rats paw volume by water displacement method before the induction of paw edema and once daily for 14 days. Control animals received either i.p administration of diclofenac sodium (50 mg/kg) or equivalent volume of the vehicle (10 % Tween 80). The overall edematous response was quantified as the area under curve (AUC) of the time course of the inflammatory event. The AUC was calculated using the trapezoid rule. The level of inhibition of inflammation was calculated using the following relation

$$\% \text{ inhibition} = \frac{AUC_c - AUC_t}{AUC_c} \times 100$$

AUC_c = AUC of the control group; AUC_t = AUC of treated groups

Ulcerogenic Effect in Rats

The method of Cashin *et al.*¹⁵ was used. Adult Albino rats of both sexes (150-180 g) were fasted for 24 hours. After the fasting period, 200 mg/kg and 400 mg/kg of crude and methanol fraction were administered orally to treatment groups (n = 5, per group). Control animals received either indomethacin 40 mg/kg or equivalent volume of vehicle (10 ml/kg normal saline). Three hours after drug administration, animals were sacrificed, the stomach was removed and cut along the larger curvature and opened to expose the mucosal surface. The mucosa was washed with normal saline and observed with magnifying lens (10 x). The ulcer index was determined according to the method described by Main and Whittle¹⁶.

In vivo Leucocytes Migration Test

The effect of the extract and methanol fraction on cell migration *in vivo* was evaluated in albino rats (150–200 g) of either sex using the method described by Ribeiro *et al.*¹⁷. One hour after oral administration of crude extract and methanol fraction (200 or 400 mg/kg), animals received intra peritoneal injection of 1 ml of 3 %, w/v agar suspension in normal saline. Four hours later, the animals were killed and the

peritoneal cavities washed with 5 ml of phosphate buffer saline containing 0.5 ml of 10 % EDTA. The peritoneal fluid was recovered and the total and differential leucocytes counts (TLC and DLC) were performed on the perfusates.

Membrane Stabilization Effect

The method described by Shinde *et al.*,¹⁸ was used with little modification. Fresh whole human blood (5 ml) was collected and transferred to EDTA centrifuge tube. The tube was centrifuged at 2000 rpm for 5 minutes and washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as a 40 % v/v suspension with isotonic buffer solution (pH, 7.4). The composition of the buffer solution (g/l) was NaCl (4.4 g), NaH₂PO₄ (1.6 g) and Na₂HPO₄ (7.6).

Heat-induced Haemolysis

The isotonic buffer solution (5 ml) each containing 200 and 400 µg/mL of crude and methanol fraction was put in four set (per concentration) of centrifuge tubes. Control tubes contain vehicle while 5 ml of 100 µg/ml Diclofenac sodium was used as the positive control. Erythrocyte suspension (0.005 ml) was added to each tube and gently mixed. A pair of the Tubes was incubated at 54°C for 20 minutes in a regulated water bath. The other pair was maintained at 0-4°C in a freezer for 20 minutes. At the end of the incubation, the reaction mixture was centrifuged at 1000 rpm for 3 minutes and the absorbance of the supernatant measured spectrophotometrically at 540 nm. The percentage haemolysis was calculated as follows

$$\text{Inhibition of haemolysis (\%)} = [1 - (\text{OD}_2 - \text{OD}_1 / \text{OD}_3 - \text{OD}_1)] \times 100$$

OD₁ = absorbance of test sample unheated; OD₂ = absorbance of test sample heated; OD₃ = absorbance of control sample heated

Hypotonicity-induced Haemolysis

The hypotonic solution (distilled water, 5 mL) containing 200 and 400 µg/mL of crude and methanol fraction was put in two pairs (per concentration) of centrifuge tube. Control tubes contain 5 mL of the vehicle or 100 µg/mL of diclofenac sodium. Erythrocyte suspension (0.005 ml) was added to each tube and after gentle mixing, the mixture were incubated for 1 h at room temperature (30°C). At the end of the incubation, the reaction mixture was centrifuged at 1000 rpm for 3 minutes and the absorbance of the supernatant measured at 540 nm using spectrophotometer. The percentage inhibition of haemolysis was calculated thus

$$\text{Inhibition of Haemolysis (\%)} = [1 - (\text{OD}_2 - \text{OD}_1 / \text{OD}_3 - \text{OD}_1)] \times 100$$

OD₁ = absorbance of test sample in isotonic solution;
OD₂ = absorbance of test sample in hypotonic solution;
OD₃ = absorbance of control sample in hypotonic solution

Statistical Analysis

The results were analysed using SPSS version 15 and presented as mean + SEM. Significance between control and extract treated group were determined using students t-test and one way ANOVA. Differences between mean were considered significant at P < 0.05.

RESULTS

Extraction and Phytochemical Analysis

The phytochemical constituents of the extract, fractions and their yields are shown in Table 1. The leaf extract contains abundant of different phytochemicals. Phenolic compounds were found to concentrate in the methanol fraction.

Table 1: Extract / fractions and their photochemical constituents

Extracts / Fractions	Yield (% w/w)	Phytochemical constituents
Crude ethanol Ext	2.84 ^a	Flavonoids, saponins, tannins, terpenoids, glycosides, steroid, fats and oil, carbohydrates and tannins
n-Hexane F.	41.40 ^b	Fats and oil, resins
Chloroform F.	9.75 ^b	Steroids, terpenoids, resins
Ethyl acetate F.	6.32 ^b	Flavonoids, tannins and resins
Methanol F.	40.88 ^b	Flavonoids, saponins, tannins, terpenoids and glycosides

a Yield calculated from 2 kg of powdered leaves; b Yield calculated from 86g of ethanol extract

Table 2: Effects of the extract and fractions on egg albumin induced acute paw edema in rats

Treatment	Dose (mg/kg)	Mean edma (ml, mean ± SEM)			
		1h	2h	3h	4h
n-Hexane F.	300	0.79 ± 0.08	0.66 ± 0.04	0.59 ± 0.07	0.50 ± 0.05
	200	0.87 ± 0.07	0.74 ± 0.05	0.69 ± 0.04	0.60 ± 0.06
	100	0.92 ± 0.08	0.81 ± 0.06	0.72 ± 0.05	0.64 ± 0.06
Chloroform F	300	0.73 ± 0.09	0.64 ± 0.10	0.55 ± 0.08	0.44 ± 0.10
	200	0.76 ± 0.06	0.69 ± 0.05	0.60 ± 0.08	0.47 ± 0.07
	100	0.80 ± 0.07	0.72 ± 0.04	0.64 ± 0.08	0.52 ± 0.06
Ethyl acetate F	300	0.71 ± 0.04	0.60 ± 0.02	0.52 ± 0.05	0.43 ± 0.05
	200	0.77 ± 0.06	0.68 ± 0.04	0.55 ± 0.07	0.46 ± 0.05
	100	0.82 ± 0.07	0.71 ± 0.06	0.60 ± 0.04	0.49 ± 0.06
Methanol F.	300	0.62 ± 0.10	0.50 ± 0.07*	0.35 ± 0.12*	0.20 ± 0.10*
	200	0.68 ± 0.08	0.56 ± 0.06*	0.40 ± 0.06*	0.29 ± 0.04*
	100	0.74 ± 0.09	0.62 ± 0.06	0.47 ± 0.07*	0.37 ± 0.05*
Crude Ext.	300	0.68 ± 0.03	0.54 ± 0.05	0.46 ± 0.05*	0.33 ± 0.04*
	200	0.74 ± 0.06	0.58 ± 0.06	0.51 ± 0.04	0.40 ± 0.05*
	100	0.79 ± 0.05	0.68 ± 0.03	0.58 ± 0.03	0.44 ± 0.02
Aspirin	100	0.84 ± 0.08	0.52 ± 0.10	0.40 ± 0.10*	0.32 ± 0.12*
10 % Tween 80	0.4ml	0.98 ± 0.05	0.89 ± 0.03	0.81 ± 0.04	0.72 ± 0.04

Dose of extract in mg/kg i.p, n/gp = 5. *P < 0.05 compared with control

Table 3: Percentage inhibition of egg albumin induced paw oedema

Treatment	Dose (mg/kg)	Percentage inhibition (%)			
		1h	2h	3h	4h
n-Hexane F.	300	19.39	25.84	27.16	30.56
	200	11.22	16.85	14.81	16.67
	100	6.12	8.99	11.11	11.11
Chloroform F.	300	25.51	28.09	32.10	38.89
	200	22.45	22.47	25.93	34.72
	100	18.37	19.10	20.99	27.78
Ethyl acetate F.	300	27.55	32.58	35.80	40.28
	200	21.43	23.60	32.10	36.11
	100	16.33	20.22	25.93	31.94
Methanol F.	300	36.73	43.82	66.67	72.22
	200	30.61	37.08	50.62	59.72
	100	24.49	30.34	41.98	48.61
Crude Ext.	300	30.61	39.33	43.21	54.17
	200	24.49	34.83	37.04	44.44
	100	19.39	23.60	28.40	38.89
Aspirin	100	14.29	41.57	50.62	55.59

Dose of extract in mg/kg i.p, n/gp = 5

Table 4: Effects of the extract and methanol fraction on xylene induced topical ear edema

Treatment	Dose (mg/ear)	Edema (mg)	Inhibition (%)
Crude Ext	5	0.21 ± 0.11	50.00
Methanol F	5	0.16 ± 0.09	61.90
Indomethacin	5	0.19 ± 0.15	54.76
Control	-	0.42 ± 0.28	-

n/gp = 5

Table 5: Effect of the extract and methanol fraction on formaldehyde induced arthritis in rats

Treatment	Dose	AUC (ml, day, mean ± SEM)	% inhibition
Crude Ext	300	4.29 ± 0.48	24.20
	200	4.37 ± 0.53	22.24
	100	4.70 ± 0.62	16.37
Methanol F.	300	3.32 ± 0.40*	40.93
	200	3.66 ± 0.39	34.88
	100	3.86 ± 0.52	31.32
Diclofenac Sodium	50	2.84 ± 0.34*	49.47
% Tween 80	0.4ml	5.62 ± 0.28	-

Dose of extract in mg/kg i.p, n/gp = 5. *p < 0.05 compared with control; AUC = Area under curve of time course of the inflammatory event

Table 6: Ulcerogenic effect of the extract and methanol fraction in rats

Treatment	Dose (mg/kg)	Ulcer index (mean ± SEM)
Crude Ext	400	3.60 ± 0.14 ^a
	200	2.47 ± 0.10 ^b
Methanol F.	400	3.80 ± 0.09 ^a
	200	2.75 ± 0.12 ^b
Indomethacin	40	5.10 ± 0.26 ^a
Control	10 mg/ml N.saline	0.55 ± 0.03

a p < 0.01 b p < 0.05 n/gp = 5

Table 7: Effect of the extract and methanol fraction on *in vivo* leukocyte migration

Treatment	Dose (mg/kg)	TLC (mm ³)	% inhibition of TLC	DLC		
				N	M	L
Crude	400	2587	24.49	16.16	14.53	69.31
	200	3082	10.04	18.71	13.92	67.36
Methanol F	400	2266*	33.86	15.79	12.78	71.42
	200	2894	15.53	16.46	14.37	69.17
Indomethacin	100	1565*	54.32	12.94	10.45	76.61
Control	-	3426	-	20.85	18.46	60.69

*p < 0.05 n/gp = 5 values significantly lower than control; TLC = Total leukocyte count DLC = Differential leukocyte count; N = Neutrophils
M = Monocytes L = Lymphocytes

Table 8: Membrane stabilization effects of the crude extract and methanol fraction

Test compound	Dose (mg/ml)	Percent inhibition of haemolysis (%)	
		Heat-induced	Hypotonicity-induced
Crude Ext.	400	17.35	31.09
	200	12.66	20.80
Methanol F.	400	25.20	35.72
	200	16.38	27.22
Diclofenac Na	100	45.54	66.28

n = 5

Acute Toxicity

No death was recorded in both species (mice and rats) used however, reduced physical activity was noticed in the groups of mice that received 4000 and 5000 mg/kg of the extract.

Egg Albumin-induced Edema in Rats

The results of acute inflammatory study using egg-albumin-induced edema in rats as a model are shown in Tables 2 and 3. Methanol fraction and crude extract produced better activity than n-hexane, chloroform and ethylacetate fractions. At 300 mg/kg the inhibition produced by methanol fraction was greater than that of standard (Aspirin). There was a progressive decrease in paw edema from 1 h to 4 h.

Xylene Induced Topical Ear Edema

The extract and methanol fraction produced inhibition of topical induced ear edema as shown in Table 4. The percentage inhibition produced by methanol fraction at 5 mg/ear is greater than that produced by positive control (Indomethacin at same concentration)

Formaldehyde – induced Arthritis in Rats

Only methanol fraction at 300 mg/kg produced significant inhibition ($p < 0.05$) of oedematous response to formaldehyde-induced arthritis (Table 5) compared to that of the standard Diclofenac sodium.

Ulcerogenic Effect in Rats

The ulcerogenic effects of the extract and methanol fraction are as shown in Table 6. The ulcer index of both extract and fraction were all below that produced by Indomethacin.

In vivo Leucocytes Migration Test

The result of *In vivo* leucocytes migration revealed that Methanol fraction at 400 mg/kg showed significant ($p < 0.05$) inhibition of leukocyte migration just as Indomethacin (Table 7). The crude extract showed mild inhibition at 400 mg/kg.

Membrane Stabilization Effect

The effect of the extract and methanol fraction on the stability of human erythrocytes *in vitro* is shown in Table 8. Both the extract and fraction showed stabilization effect on both heat-induced and hypotonicity-induced red blood cell haemolysis.

DISCUSSION

Phytochemical analysis of the crude and methanol fraction revealed the presence of phyto- compounds that have been documented to have anti-inflammatory activities. Saponins, steroids, flavonoids, terpenoids, tannins and glycosides have all been shown to exhibit good anti-inflammatory activities^{5,19-21}. The acute toxicity studies showed that the ethanol leaf extract of *M. aboensis* has high safety profile when given orally. The extract and methanol fraction may have inhibited the release or actions of the various chemical mediators such as histamine, 5 HT, Kinins and Prostanoids known to mediate acute inflammation induced by phlogistic agents²²⁻²⁶. The inhibition of acute inflammation by the crude and methanol fraction became significant at the 3rd and 4th hours as Aspirin, suggesting that the extract and fraction may have inhibited the second phase of acute inflammation induced by egg albumin which is essentially mediated by prostaglandins²⁷. Irritation or ulceration of the gastric mucosa by anti-inflammatory drugs usually indicates that prostaglandin synthesis inhibition may be involved in their

mechanism of action²⁸. Following the findings from the acute inflammation and possible inhibition of phase 2 acute inflammation mediated by prostaglandins, we therefore investigated the effect of the extract on the rat gastric mucosa. Just like Indomethacin, the extract and methanol fraction exhibited irritation of gastric mucosa which points to possible inhibition of prostaglandin synthesis. The possible inhibition of prostaglandin synthesis may be traced to the high content of flavonoids in both the crude and methanol fraction. Flavonoids are known to inhibit the enzyme Cyclooxygenase and Lipoxygenase^{29,30} which play important roles as inflammatory mediators. The gastric irritant effect of the extract and fraction may constitute a major drawback to the systemic use of the extract and methanol fraction in the management of chronic inflammatory disorders. However, the extract and methanol fraction exhibited a good anti-inflammatory activity on topical oedema induced by xylene in the mouse ear. The lipophilicity of the crude and methanol fraction may have enhanced its penetration through the skin barrier to exert its effects. Hence, the extract and methanol fraction may be better used topically. Formaldehyde is a potent edematous agent and produces inflammation through the release of several inflammatory mediators including prostaglandins³¹. Only methanol fraction at 300 mg/kg showed significant ($p < 0.05$) inhibition of the global edematous response induced by formaldehyde suggesting that it contains potential agent which may be useful in the management of chronic arthritis. Leucocytes migrate to site of inflammation in response to chemotactic stimulus³² and play an important role in the pathogenesis of acute and chronic inflammatory disorders. During phagocytosis, the activated leucocytes release superoxide radicals and other cytoplasmic content at the site of inflammation; this can further cause tissue damage and inflammation^{33,34}. Methanol fraction at 400 mg/kg showed a significant inhibition of leucocytes migration. In addition to impeding the migration of leukocytes to site of inflammation which certainly suppress the inflammation response, the extract and methanol fraction may also prevent the release of cytoplasmic pro-inflammatory mediators from these leukocytes by virtue of membrane stabilization effect of the extract and fraction. The erythrocyte membrane is analogous to the lysosomal membrane³⁵ and its stabilization implies that the extract may as well stabilize lysosomal membrane. Compounds with membrane stabilizing property are well known for their ability to interfere with the early phase of inflammatory response, namely the prevention of the release of phospholipases that trigger the formation of inflammatory mediators³⁶. In conclusion, the results of this study justify the folkloric use of the leaves of *M. aboensis* in the treatment of inflammatory disorders. The anti-inflammatory activity may be as a result of inhibition of prostaglandin synthesis, leucocyte migration and membrane stabilization. Isolation and characterization of the active constituent of the leaf extract and methanol fraction is on going to elucidate their specific mechanisms of action.

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