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Original article

Studies on the mechanisms of anti-inflammatory activity of the extracts and fractions of *Alchornea floribunda* leaves

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Abstract

Objective: *Alchornea floribunda* leaves are widely used in ethnomedicinal management of inflammatory disorders. The present work is aimed at investigating this folkloric use. **Methods:** The anti-inflammatory effect of the leaf extracts and fractions was investigated in experimental animal models of acute and chronic inflammation. The possible mechanisms by which the two most active fractions, hexane (HE) and ethyl acetate (EF) exert their effects were also investigated. **Results:** The crude extract (200 mg/kg) showed moderate inhibition of egg albumen-induced edema in rats (% edema inhibition = 54.69) at 4 h. HE and EF showed very high activity (% edema inhibition of 81.25 and 67.19 respectively at 200 mg/kg) at 4h as compared to the control. Both fractions ameliorated arthritis induced by formaldehyde in rats. At 400 mg/kg, HE evoked a significant irritation of gastric mucosa in rats. EF (200 mg/kg, p.o.) significantly inhibited leucocytes (% inhibition = 36.79) migration *in vivo*, but could not stabilize heat and hypotonicity-induced lysis of human erythrocyte at 200 and 400 μg/mL *in vitro*. Phytochemical investigation revealed the presence of terpenoids and steroids in HE and flavonoids, tannins and saponins in EF. **Conclusion:** These results suggest that the leaves of *Alchornea floribunda* possess anti-inflammatory activity in acute and chronic inflammation. The activity may derive from a combination of inhibition of prostaglandin synthesis and leucocytes migration. The phytochemical constituents detected in HE and EF may account for the anti-inflammatory activity.

Keywords: *Alchornea floribunda*; Anti-inflammatory; Arthritis; Leucocytes migration; Membrane stabilization; Plant extracts

INTRODUCTION

Alchornea floribunda (Müll. Arg.) and *Alchornea cordifolia* (Schumach. and Thonn.) (Euphorbiaceae) are found growing luxuriantly along the coastal regions of West Africa. The two plant species normally referred to as 'Iporuru' are widely used in ethnomedicine for the management of a variety of inflammatory disorders. *Alchornea floribunda* is used traditionally as a local remedy for arthritis and muscle pain[1]. The pain-relieving properties appear in topical treatments; crushed leaves are rubbed on painful joints and are beaten into a paste to apply to painful stingray wounds[2]. The leaves are used to increase female fertility and also highly regarded as a remedy for impotence[2]. It is also used as aphrodisiacs and for reducing sugar in the blood and urine of diabetics[2]. *Alchornea cordifolia* leaves are used as topical anti-inflammatory agent in chancre, yaws[3], wounds, cicatrisation, ulcers[4], caries, toothache and gum inflammation[5] and conjunctivitis[3].

The ethnomedicinal uses of these plant species are

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as a result of their anti-inflammatory and antimicrobial properties among others. The anti-inflammatory properties of *Alchornea cordifolia* have been studied and documented[6-9]. There are also documented studies on the antimicrobial properties of *Alchornea cordifolia* and *Alchornea floribunda*[10, 11]. Hitherto, there is no scientific study confirming the anti-inflammatory properties of *Alchornea floribunda*, even though this specie is more widely used in ethnomedicine as anti-inflammatory agent[1, 2]. In this study, we investigated the anti-inflammatory properties of *Alchornea floribunda* leaves in experimental animal models of acute and chronic inflammation. We also investigated the possible mechanisms by which the active constituents elicit their anti-inflammatory activity.

MATERIALS AND METHODS

Plant materials

The leaves of *Alchornea floribunda* were collected in August 2005 from Oba Town Nsukka, Enugu State, Nigeria. The plant material was authenticated by a taxonomist Mr. Alfred Ozioko of Bioresources Development and Conservation Project, Nsukka, Enugu State, Nigeria. The plant material was cleaned, air-dried for 10 days and pulverized. A voucher specimen was deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Nigeria.

Chemicals

Analytical grades of hexane, chloroform, ethyl acetate, and methanol (BDH) were used. Other solvents used are dimethylsulfoxide (DMSO) and Tween 80 (BDH). All laboratory reagents were freshly prepared and freshly distilled water was used when required.

Extraction and Fractionation

About 100 g of the pulverized leaves was extracted for 48 h by cold maceration in aqueous methanol (90 %). The crude methanolic extract (CME, 4.98 g) was screened for anti-inflammatory activity. A 1 - kg portion of the leaves was macerated at room temperature (25℃ ±1℃) in 5 L of n-hexane for 48 h. The hexane extract was concentrated *in vacuo* to yield HE (7.18 g). The marc was air-dried for about 1 h and extracted for 48 h in 5 L of absolute methanol at room temperature. The methanolic extract (ME) was concentrated *in vacuo*. ME (25 g) was adsorbed on silica gel and eluted in succession with chloroform, ethyl acetate and methanol to obtain the chloroform fraction (CF, 3.6 g), ethyl acetate fraction (EF, 18.22 g) and methanol fraction (MF, 3.31 g). All the extracts and fractions were stored in the refrigerator between 0 - 4 ℃ until used.

Phytochemical tests

The phytochemical tests on the extracts and fractions were carried out using standard procedures[12].

Pharmacological tests

Animals

Wister rats 120 ± 20 g and Albino mice 20 ± 5 g obtained from the laboratory animal facilities of the Faculty of Veterinary Medicine, University of Nigeria were used for the experiments. The animals were housed under standard conditions (25℃ ±1 ℃ and 12 h light/dark cycle). They were fed with standard rodent pellets (Vital Feed, Nigeria) and had unrestricted access to clean drinking water. All animal experiments were in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals[13] (Pub. No. 85-23, revised 1985).

Acute toxicity tests

The LD_{50} of the crude methaolic extract was determined according to the Lorke's method[14].

Egg albumen induced paw edema in rats

The test was carried out as previously reported[6]. The animals ($n=5$, per group) were fasted for 5 h and deprived of water only during the experiment. They were given intraperitoneal (i. p.) injection of the extracts and fractions solubilized in 10 % Tween 80 at doses of 100 and 200 mg/kg. Control animals received 0.4 mL of 10 % Tween 80 or 100 mg/kg aspirin. All the substances were administered i. p. 30 min before the subplanta injection of the phlogistic agent (0.1 mL of fresh undiluted egg albumen) in the rats. Paw volumes were measured by water displacement method at 0, 1, 2, 3 and 4 h after induction of edema. The anti-inflammatory effect was calculated at each time of observation as percent inhibition of edema[15] in the animals treated with the

substances under test in comparison with the vehicle treated animals. The percent inhibition of edema was calculated using the formula

$$\% \ Inhibition = \frac{(v_0 - v_t)}{v_0} \times 100$$

Vt is the volume of edema at corresponding time and Vo the volume of edema of vehicle treated rats at the same time.

Arthritis induced by formaldehyde in rats

The method of Seyle[16] was used. Adult Wister rats ($n = 4$, per group) received 100 or 200 mg/kg of HE and 100 mg/kg of EF administered i. p. on day one. One hour later, arthritis was induced by subplantar injection of 0. 1 mL of 2. 5 % formaldehyde solution and repeated on day 3. Arthritis was assessed by measuring the rats´ paw volume by water displacement method before the induction of arthritis and once daily for 10 days, starting from day 1, after induction of arthritis. Drug administration was continued once daily for the first five days and once every other day for the next five days. Control animals received either i. p. administration of diclofenac sodium (50 mg/kg) or equivalent volume of the vehicle (10 % Tween 80). The global edematous response was quantified as the area under curve (AUC) of the time course of the arthritic event. The AUC was calculated using the trapezoid rule. The level of inhibition of arthritis was calculated using the following relation:

$$\% \ Inhibition = \frac{(AUCc - AUCt)}{AUCc} \times 100$$

AUCc = AUC of the control animal groups; AUCt = AUC of treated group.

Ulcerogenic effects in rats

The method of Cashin et al[17] was used. Adult Wister rats (120 ± 20) were fasted for 24 h. After the fasting period, 400 mg/kg of HE and EF were administered orally to two animal groups ($n = 3$, per group). Control animals received either indomethacin 40 mg/kg or equivalent volume of the vehicle (10 % Tween 80). 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 glass (×10). The ulcer index was determined as previously described[18].

In vivo leucocytes migration tests

The effects of HE and EF on *in vivo* leucocytes migration induced by inflammatory stimulus was investigated using the method of Ribeiro et al.[19]. One hour after oral administration of HE or EF (100 and 200 mg/kg) each rat in the group ($n = 4$) received intraperitoneal injections of 1 mL of 3 % Agar suspension in normal saline. Four hour later the rats were sacrificed and the peritoneum washed with 5 mL of 5 % solution of EDTA in Phosphate Buffer Saline (PBS). The peritoneal fluid was recovered and the total and differential leucocytes counts (TLC and DLC) were performed on the perfusates.

Membrane stabilization effects

a. Preparation of erythrocyte suspension: Fresh whole human blood (5 mL) was collected and transferred to EDTA centrifuge tube. The tube was centrifuged at 2 000 rpm for 5 min, 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), NaH_2PO_4 (1. 6 g) and Na_2HPO_4 (7. 6 g).

b. Heat - induced hemolysis: The isotonic buffer solution (5 mL) each containing 200 and 400 μg/mL of EF were put in four sets (per concentration) of centrifuge tubes. Control tubes contain 5 mL of vehicle or 5 mL of 100 μg/mL prednisolone. Erythrocyte suspension (0. 005 mL) was added to each tube and gently mixed. A pair of the tubes was incubated at 54 ℃ for 20 min in a regulated water bath. The other pair was maintained at 0 - 4 ℃ in a freezer for 20 min. At the end of the incubation, the reaction mixture was centrifuged at 1 000 rpm for 3 min and the absorbance (OD) of the supernatant measured spectrophotometrically at 540 using 2102 PC Spectrophotometer (UNICO®, USA). The percent inhibition of hemolysis[20] was calculated using the relation

$$Inhibition \ of \ haemolysis(\%) = \left[1 - \frac{OD2 - OD1}{OD3 - OD1}\right] \times 100$$

OD1 = absorbance of test sample unheated, OD2 = absorbance of test sample heated and OD3 = absorbance of control sample heated.

c. Hypotonicity - induced hemolysis: The hypotonic solution (distilled water, 5 mL) containing 200 and 400 μg/mL of EF were put in two pairs (per con-

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centration) of centrifuge tubes. Control tubes contain 5 mL of the vehicle or 100 μg/mL predniolone. Erythrocyte suspension (0.005 mL) was added to each tube and after gentle mixing, the mixtures were incubated for 1 h at room temperature (30 ℃). At the end of the incubation, the reaction mixture was centrifuged at 1 000 rpm for 3 min and the absorbance (OD) of the supernatant measured spectrophotometrically at 540 using 2102 PC Spectrophotometer (UNICO®, USA). The percent inhibition of hemolysis[20] was calculated using the relation

$$Inhibition\ of\ haemolysis(\%) = [1-\frac{OD2-OD1}{OD3-OD1}] \times 100$$

OD1 = absorbance of test sample in isotonic solution, OD2 = absorbance of test sample in hypotonic solution and OD3 = absorbance of control sample in hypotonic solution.

Statistical Analysis

Results obtained were analyzed by SPSS version 10 using student´s *t*-tests and expressed as mean ± SEM. Differences between means were considered significant at $P < 0.05$.

RESULTS

Extraction, phytochemical and acute toxicity tests

The extracts, fractions, their yields and phytochemical constituents are shown in Table 1. Acute toxicity studies indicate that the crude methanolic extract is safe up to 567.7 mg/kg i.p.

Table 1 Extracts/fractions and their phytochemical constituents.

Extracts/fractions	Yield (% w/w)	Phytochemical constituents
CME	4.98[a]	Terpene, steroids, flavonoid, tannins, saponnins, alkaloids, glycosides
HE	0.718[b]	Terpenes, steroids
CF	14.4[c]	Terpenes, flavonoids
EF	72.88[c]	Flavonoid, tannins, saponins
MF	13.23[c]	Tannins, alkaloids, saponnins, glycosides

CME = Crude methanolic extract, HE = hexane extract, CF = Chloroform fraction, EF = Ethyl acetate fraction, MF = Methanol fraction.

[a] Yield calculated from 100 g of powdered leaves

[b] Yield calculated from 1 kg of powdered leaves

[c] Yield calculated from 25 g of methanol extract.

Egg albumen-induced edema in rats

The result of acute inflammatory study using egg albumen-induced edema in rats as a model is shown in Table 2. Fractions HE and EF exhibited higher anti-inflammatory activity than CME and fractions CF and MF. All the fractions, however, at 200 mg/kg exhibited anti-inflammatory activity higher than a standard anti-inflammatory drug aspirin (100 mg/kg). The activity of HE is dose - dependent. As shown in Table 2, edema volume peaked at 1 h and progressively decreased up to 4 h.

Ulcerogenic effects in rats

Evaluation of the ulcerogenic effects of the fractions on rat stomach showed that HE at 400 mg/kg (p.o.) significantly evoked irritation of gastric mucosa. EF at 400 mg/kg (p.o.) showed only very mild irritation, which is not different from that caused by the vehicle (Table 3). The gastric irritation caused by HE was, however, statistically lower than that caused by indomethacin (40 mg/kg, p.o.).

In vivo leucocytes migration in rats

The results as shown in Table 4 indicate that EF significantly inhibited the migration of leucocytes to the site of Agar - induced inflammatory stimulus. HE only showed a mild inhibition of *in vivo* leucocytes migration at 200 mg/kg.

Membrane stabilization effects

The result of the effect of EF on the stability of human erythrocytes *in vitro* is shown in Table 5. EF did not stabilize both heat - induced and hypotonicity - induced hemolysis of human erythrocyte in vitro. Prednisolone (100 μg/mL) inhibited heat - induced hemolysis, but did not have any effect on the hypotonicity - induced hemolysis.

Formaldehyde induced arthritis in rats

The result is shown in Table 6. Both HE and EF at 100 mg/kg (i. p.) inhibited the global edematous response to formaldehyde - induced arthritis. The inhibitions are comparable to that of standard anti-inflammatory drug, diclofenac sodium (50 mg/kg, i. p.)

Table 2 Effect of the extracts and fractions on egg albumen-induced acute paw edema in rats.

Treatment	Dose mg/kg	Mean edema (mL, mean ± SEM)			
		1 h	2 h	3 h	4 h
CME	200	0.65 ±0.05(26.14)	0.54 ±0.06(40.00)	0.44 ±0.04(40.54)	0.29 ±0.02[b](54.69)
HE	100	0.57 ±0.06(35.23)	0.49 ±0.06(45.56)	0.33 ±0.07[a](40.54)	0.26 ±0.06[a](59.38)
HE	200	0.47 ±0.06(46.60)	0.37 ±0.06[b](58.89)	0.24 ±0.08[a](67.57)	0.12 ±0.04[b](81.25)
CF	200	0.56 ±0.09(36.36)	0.46 ±0.09(48.89)	0.30 ±0.11[a](59.46)	0.23 ±0.08[a](64.06)
EF	100	0.38 ±0.07(56.82)	0.31 ±0.07[b](65.56)	0.2 ±0.05[a](72.97)	0.19 ±0.06[a](70.31)
EF	200	0.44 ±0.06(50.00)	0.35 ±0.02(61.11)	0.28 ±0.03[a](62.16)	0.21 ±0.03[b](67.19)
MF	200	0.63 ±0.01(28.40)	0.52 ±0.01(42.22)	0.46 ±0.03[a](37.84)	0.25 ±0.03[b](60.9)
Aspirin	100	0.78 ±0.10(11.36)	0.55 ±0.12(38.89)	0.40 ±0.12(45.95)	0.30 ±0.10[a](53.13)
10 % Tween 80	0.4 mL	0.88 ±0.04	0.90 ±0.06	0.74 ±0.09	0.64 ±0.08

[a]$P < 0.05$, [b]$P < 0.01$, $n = 5$, values significantly different from control (vehicle treated animals).
Values in parentheses represent percent inhibition of edema.

Table 3 Ulcerogenic effect of the fractions.

Test compound	Dose (mg/kg)	Ulcer index (mean ± SEM)
HE	400	5.40 ± 0.12[b]
EF	400	1.33 ± 0.03
Indomethacin	40	12.10 ± 0.23[c]
10 % Tween 80	0.4 mL	0.93 ± 0.09

[b]$P < 0.01$, [c]$P < 0.001$, $n = 3$, values significantly higher than the irritation caused by the vehicle.

Table 4 Membrane stabilization effect of EF.

Test compound	Dose(μg/mL)	Percent inhibition of haemolysis (%)	
		Heat - induced	*Hypotonicity - induced*
EF	200	-	-
EF	400	-	-
Prednisolone	100	30.0	-

The negative sign (-) indicates no inhibition of haemolysis.

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Table 5 Effect of the extracts on in vivo leucocytes migration.

Fraction	Dose (mg/kg)	TLC (cells. mm^{-3})	Inhibition of TLC (%)	DLC		
				N	M	L
HE	100	4 250 ± 606	-	11.50 ± 4.33	7.00 ± 0.57[a]	81.50 ± 4.90
HE	200	2 312 ± 757	12.75	12.30 ± 2.53	1.00 ± 0.57[a]	86.3 ± 1.8
EF	100	1 850 ± 259	30.19	8.50 ± 2.53	5.70 ± 0.63[a]	84.3 ± 4.8
EF	200	1 675 ± 72[a]	36.79	4.50 ± 1.50	15.00 ± 5.06	80.5 ± 5.9
Control	-	2 650 ± 259	-	5.00 ± 1.87	21.70 ± 4.33	75.8 ± 5.4

TLC = Total leucocytes count; DLC = Differential leucocytes count; N = Neutrophils;
M = Monocytes; L = Lymphocytes
[a] $P < 0.05$, $n = 4$, values significantly lower than the control (vehicle treated animals)

Table 6 Effect of HE and EF on formaldehyde - induced arthritis in rats

Test compound Inhibition	Dose (mg/kg)	AUC (mL. day, mean ± SEM)	%
HE	100	2.81 ± 0.38	37.1
HE	200	2.58 ± 0.46	42.3
EF	100	3.19 ± 0.62	28.6
Diclofenac sodium	50	2.38 ± 0.31	46.7
10 % Tween 80	0.4 mL	4.47 ± 0.43	-

AUC = Area under curve of time course of the arthritic event.

DISCUSSION

Alchornea floribunda (Iporuru) leaves have been widely used in ethnomecine for the management of a variety of inflammatory disease states (Duke et al 2002). The crude methanol extract (CME) showed a moderate inhibition of acute edema induced by sub-plantar injection of egg albumen in rats. The result of the acute toxicity study indicates that the extract is relatively safe thus giving credence to the claimed ethnomedicinal use.

Bioassay-guided fractionation of the crude methanol extract gave rise to two most active fractions HE and EF. The fractions were found to inhibit the early phase of acute inflammation (acute vascular response), which is associated with the release of some inflammatory mediators like histamine, serotonin, bradykinin and prostanoids[21-23]. It is possible that the fractions may have either inhibited the release, or antagonize the actions of these inflammatory mediators.

Phytochemical investigation of the two most active fractions (HE and EF) revealed the presence of terpenoids (volatile oils, triterpenes and steroids) in HE, and flavonoids, tannins and saponins in EF. These phytochemical constituents have been shown in several other studies to exhibit anti-inflammatory effects[24-27]. Various mechanisms of anti-inflammatory effects have been postulated for some of these phytochemical constituents[20, 27, 28]. We therefore investigated the mechanisms by which the constituents of these most active fractions elicit their anti-inflammatory effect.

Irritation or ulceration of gastric mucosa by anti-inflammatory drugs usually indicates that inhibition of prostaglandins is involved in their mechanism of action[29]. The observed gastric irritant effect of HE may be as a result of high concentration of steroids found on phytochemical analysis. Steroids are known to induce the synthesis of a family of proteins (lipocortin or macrocortin) that inhibits the activity of phospholipase A2, an enzyme involved in the pathway leading to production of prostaglandins[30]. This gastric irritant effect may constitute a major drawback to the systemic use of HE in management of chronic arthritis. EF which lacked this activity may

be more suited for this indication. HE will, however, be more effective topically, since the lipophylic constituents will easily permeate the lipoidal layers of skin. The later phase of acute inflammatory response (acute cellular response) involves the migration of neutrophils to the site of inflammatory stimulus[31-33]. We investigated the effect of the active fractions on in vivo leucocytes migration. Leucocytes usually migrate to the site of inflammation in response to chemotactic stimulus[34]. This plays a pivotal role in the pathogenesis of inflammatory disorders of both acute and chronic types. During phagocytosis, the activated leucocytes release superoxide radicals and other cytoplasmic contents at the site of inflammation; this can further cause tissue damage and inflammation[35, 36]. Inhibiting the migration of leucocytes to the site of inflammation may be an important mechanism of action of the anti-inflammatory constituents in EF. Apart from inhibiting the migration of leucocytes, EF may also prevent the release of cytoplasmic pro-inflammatory mediators from these leucocytes by virtue of membrane stabilization. Besides, the inflammatory response is usually associated with release of inflammatory mediators following the degranulation of mast cells[37]. Consequently, we investigated the membrane stabilization effect of EF. It was, however, observed that EF did not stabilize both heat - induced and hypotonicity - induced hemolysis of human erythrocyte in vitro (Table 5). It is not very clear if the anti-inflammatory constituents present in EF altogether lacked this activity. Flavonoids have been shown in previous studies to possess membrane stabilization effect[38, 39]. It is possible that this effect was antagonized by hemolytic action of saponins[40, 41] also present in EF. The membrane stabilization effect of HE was not determined due to insolubility of this fraction in both distilled water and the isotonic buffer solution.

We also investigated the effect of the active fractions on the proliferative phase of inflammation. Formaldehyde is a potent edematous agent and produces inflammation through the release of several inflammatory mediators including prostaglandins[42]. The ability of these fractions to inhibit the global edematous response induced by formaldehyde suggests that they contain chemical agents which can be very useful in the management of chronic arthritis. EF will be of particular interest in elderly patients since it lacked gastric irritant effect, which is pronounced in HE.

In conclusion, the results of this study provide a rationale for the ethnomedicinal uses of the leaves of *Alchornea floribunda* in the management of both acute and chronic inflammatory disorders. The exact mechanisms of action of the anti-inflammatory constituents are not yet clear. However, it is likely that the constituents of HE exact inhibitory effect on prostaglandin synthesis while that of EF exact anti-inflammatory effect possibly by a combination of inhibition of leucocytes migration and membrane stabilization. Isolation and structure elucidation of these active constituents are currently going on in our laboratory.

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REFERENCES

1 **Duke JA**, Mary JB, Judi D. Handbook of Medicinal Herbs. Boca Raton: CRC Press Inc, 2002.

2 **Schwontkowski D**. Herbs of the Amazon, Traditional and Common uses. Utah: Science Student Brain Trust Publishing, 1993.

3 **Neuwinger HD**. African traditional medicine: A dictionary of plant use and application. Germany: Medpharm, Stuttgart, 2000.

4 **Bouquet A**, Debray M. Plantes médicinales de la Côte d´Ivoire. Travaux et documents de lO. R. S. T. O. M., Paris: O. R. S. T. O. M, 1974.

5 **Kerharo J**, Adams JG. Pharmacopée, Sénégalaise traditionalle. Paris: Vigot Frère, 1974.

6 **Osadebe PO**, Okoye FBC. Anti-inflammatory effects of crude methanolic extrats and fractions of Alchornea cordifolia leaves. *J Ethnopharmacol.* 2003; 89: 19 - 24.

7 **Osadebe PO**, Ebi GC, Okoye FBC. Anti-inflammatory effects of triterpenoids from Alchornea ordifolia leaves. *Rec Progr Med Plants.* 2008; 22: 571 - 577.

8 **Mavar-Manga H**, Brkic D, Marie DEP, Quetin-Leclercq J. In vivo anti-inflammatory activity of Alchornea cordifolia (Schumach. & Thonn.) Müll. Arg. (Euphorbiaceae). *J Ethnopharmacol.* 2004; 92: 209 - 214.

9 **Mavar-Manga H**, Haddad M, Pieters L, Baccelli C, Penge A, Quetin-Leclercq J. Anti-inflammatory compounds from leaves and root bark of Alchornea cordifolia (Schumach. & Thonn.) Müll. Arg. *J Ethnopharmacol.* 2008; 115: 25 - 29.

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10 **Ebi GC**. Antimicrobial activities of Alchornea cordifolia. *Fitoterapia*. 2001; 72: 69 - 72.

11 **Okoye FBC**, Ebi GC. Preliminary antimicrobial and phytochemical investigation of the extracts and column fractions of Alchornea floribunda leaves. *J Pharm Allied Sci*. 2007; 4(1): 395 - 402.

12 **Harbourne JB**. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysi. 3rd ed. London: Chapman and Hall, 1998.

13 National Institute of Health Guide for Care and Use of Laboratory Animals[12] (Pub. No. 85-23, revised 1985)

14 **Lorke D**. A new approach to practical acute toxicity testing. *Arch Toxicol*. 1983; 53: 275 - 289.

15 **Perez GRM**. Anti-inflammatory activity of Ambrosia artemisaefolia and Rheo spathacea. *Phytomedicine*. 1996; 3 (2): 163 - 167.

16 **Seyle H**. Further studies concerning the participation of adrenal cortex in the pathogenesis of arthritis. *BMJ*. 1949; 2: 1129 - 1135.

17 **Cashin CH**, DawsonW, Kitchen EA. The pharmacology of benoxaprofen (2,4-chlorophenyl-methyl-5-benzoxazole acetic acid) LRC. L3694, a new compound with anti-inflammatory activity apparently unrelated to inhibition of prostaglandin synthetase. *J Pharm Pharmacol*. 1979; 29: 330 - 336.

18 **Main IHM**, Whittle NB (Jnr). Investigation of vasodilator and antisecretory role of prostaglandin in the rat mucosa by use of NSAIDs. *Br J Pharmacol*. 1975; 53: 217 - 224.

19 **Ribeiro RA**, Flores CA, Cunha FQ, Ferreira SH. IL-8 causes in vivo neutrophil migration by a cell dependent mechanism. *Immunology*. 1991; 73: 472 - 477.

20 **Shinde UA**, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Saraf VO. Membrane stabilizing activity-a possible mechanism of action for the anti-inflammatory activity of Cedrus deodara wood oil. *Fitoterapia*. 1999; 70: 251 - 257.

21 **Damas J**, Bourdon V, Remade-Volon G, Adam A. Kinins and peritoneal exudates induced by carrageenan and zymosan. *Br J Pharmacol*. 1990; 211: 81 - 86.

22 **Ialenti A**, Ianaro A, Moncada S, Di Rosa M. Modulation of acute inflammation by endogenous nitric oxide. *Eur J Pharmacol*. 1992; 211: 177 - 182.

23 **White M**. Mediators of inflammation and inflammatory process. *J Allergy Clin Immunol*. 1999; 103: 5378 - 5381.

24 **Lee SI**, Son KH. Chang HW, Do JC, Jung KY, Kang SS, et al. Anti-inflammatory effect of naturally occurring flavones and flavonol glygosides. *Arch Pharm Res*. 1993;16: 25 - 28.

25 **Recio MC**, Giner RN, Manez S, Talens A, Cubells L, Gueho J, et al. Anti-inflammatory activity of flavonoids glycosides from Erythrospermum monticolum depending on single or repeated TPA administration. *Planta Medica*. 1995; 61: 502 - 504.

26 **Viana GSB**, Bandeira MAM, Moura LC, Souza-Filho MVP, Matos FJA, Ribeiro RA. Analgesic and Antiinflammatory Effects of the Tannin Fraction from Myracrodruon urundeuva Fr. *All Phytother Res*. 1997; 11: 118 - 122.

27 **Mills S**, Bone K. Principles and Practice of Phytotherapy - Modern Herbal Medicine. New York: Churchill Livingstone, 2000.

28 **Umukoro S**, Ashorobi RB. Evaluation of anti-inflammatory and membrane stabilizing propertie of aqueous leaf extract of Momordica charantia in rats. *Afr J Biomed Res*. 2006; 9: 119 - 124.

29 **Vane JR**. Inhibition of prostaglandins synthesis as a mechanism of action for aspirin-like drugs. *Nature*. 1971; 231: 232 - 235.

30 **DiRosa M**, Calignano A, Carnuccio R, Ialenti A, Sautebin I. Multiple control of inflammation by glucocorticoids. *Agents Actions*. 1985;17: 284 - 289.

31 **Insel PA**. Analgesic-antipyretics and anti-inflammatory agents; drugs employed in the treatment of rheumatoid arthritis and gout. In: Goodman LS, Gilman A, eds. The Pharmacological Basis of Therapeutics. 8th ed. New York: Pergamon Press, 1990:638 - 681.

32 **Cotran RS**, Kumar V, Collins T. Robbin´s Pathological Basis of Disease. 6th ed. Philadelphia: WB Saunders Co, 1999.

33 **Guyton AC**. Textbook of Medical Physiology. 11th Edition. India: Elsevier, 2006.

34 **Wagner JC**, Roth AR. Neutrophil migration mechanisms, with an emphasis on the pulmonary vasculature 1. *Pharmacol Rev Online*. 2000;52: 349 - 374.

35 **Weissmann G**, Smolen JE, Korchak HM. Release of inflammatory mediators from stimulated neutrophils. *New Engl J Med*. 1980; 303: 24 - 27.

36 **Perez HD**, Weismann G. Lysozymes as mediators of inflammation. In: Keller W. Textbook of Rheumatology. 1st edition. Philadelphia: W. B. Saunder, 1981:179 - 194.

37 **Lagunoff D**, Martin TW, Read G. Agents that release histamine from mast cells. *Annu Rev Pharmacol Toxicol*. 1983; 23: 331 - 351.

38 **Chaika LA**, IaI Khadzhai. Membrane stabilizing effect of medicinal substances used for the treatment of chronic venous insufficiency. *Farmakol Toksikol*. 1977; 40: 306 - 309.

39 **Arora A**, Byrem TM, NairMG, Strasburg GM. Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids. *Arch Biochem Biophys*. 2000; 373: 102 - 109.

40 **Thron CD**. Hemolysis by holothurin A, digitonin, and quillaia saponins: estimates of the required cellular lysine uptake and free lysine concentrations. *J Pharmacol Exp Ther*. 1964;145: 194 - 202.

41 **Zhang J**, Meng Z, Zhang M, Ma D, Xu S, Kodama H. Effect of six steroidal saponins isolated from Anemarrhenae rhizoma on platelet aggregation and hemolysis in human blood. *Clin Chim Acta*. 1999;289: 79 - 88.

42 **Tjolsen A**, Berge O, Hunskaar S, Rosland JH, Hole K. The formalin test: An evaluation of the method. *Pain*. 1992;51: 5 - 14.