Evaluation Of The Anti-Inflammatory Activity Of Methanol Stem Bark Extract Of *Hymenocardia acida* Tul (Euphorbiaceae)

[*1]Okpo, S. O., [2]Ayinde, B. A., [1]Nuka, P, A., [3]Sode, O. S.

[*1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City 300001, Nigeria.

[2]Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City 300001, Nigeria.

[3]Department of Pharmacology, Therapeutics and Toxicology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Lagos, Nigeria

*Corresponding Author: E-mail: steveokpo@uniben.edu Phone: +234 806 402 8832

ABSTRACT

Hymenocardia acida Tul (Euphorbiaceae), also known as "Heart-fruit" in English, is very popular in African traditional medicine practice. The stem bark of the plant is widely used as an effective remedy for inflammatory conditions and in treatment of chest pain by herbal medicine practitioners. The methanol stem bark extract of *Hymenocardia acida*(100, 200, 500 mg/kg) was evaluated using acute inflammatory models in rodents including: carrageenan-, dextran- and histamine-induced paw edema, carrageenan-induced peritonitis and croton-oil ear edema. The highest dose of the extract, administered one hour before carrageenan showed significant ($p<0.05$) reduction of the oedema response (31.3%) at the 3rd hour and the inhibitory effect lasted till the 6th hour. The extract also caused a dose-dependent suppression of the paw swelling, at 1 hour in the dextran-induced (Control: 70.30±6.90; 100mg/kg: 55.08±9.89; 200mg/kg: 55.34±4.67; 500mg/kg: 48.60±5.87) and histamine-induced (Control: 67.26±6.67; 100mg/kg: 47.59±10.53; 200mg/kg: 47.48±10.30; 500mg/kg: 42.24±8.20) paw oedema models. Administration of *H. acida* (500 mg/kg) and indomethacin (10 mg/kg) significantly ($p<0.05$) decreased the volume of peritoneal exudate formed with the injection of carrageenan (Control: 0.93±0.12; 500mg/kg: 0.24±0.03; Indomethacin: 0.23±0.07), without any significant changes on white blood cell (WBC) count, sodium (Na^+) and potassium (K^+) ion concentrations. All doses of the extract significantly ($p<0.05$) decreased ear oedema induced by croton oil but these were lower than that of dexamethasone. The results of the study showed that methanol stem bark extract of *H. acida* possesses anti-inflammatory effects, as demonstrated by inhibition of oedema induced by carrageenan, dextran and histamine, peritoneal exudate formation by carrageenan, and ear oedema induced by croton oil. These observations provide support for the use of the plant in folk medicine.

Keywords: Paw oedema, inflammation, carrageenan, dextran, *Hymenocardia acida*

INTRODUCTION

Inflammation is an important and integral part of host defense mechanisms, which acts to remove and repair damaged tissue or to neutralize harmful agent. There are three phases of inflammation which include: acute phase which involves transient local vasodilatation and increased capillary permeability; sub-acute phase involving infiltration of leucocytes and other phagocytic cells and chronic phase which results in degeneration of the affected tissue and fibrosis(Kumar *et al*.,2004; Lacerda *et al*., 2009).Some of the most common and disabling human diseases such as rheumatoid arthritis, atherosclerosis, tuberculosis and chronic lung disease and Alzheimer's disease are due to chronic inflammation (Kiecotl-Glaser, 2010). The available anti-inflammatory drugs such as NSAIDs and corticosteroids only provide symptomatic relief and possess many side effects including gastric ulcers, renal failure, and hormonal suppression, among other toxic reactions (Jaiswal and Santakke, 2012). Hence there is a continued search for plant-derived medicines that possess anti-inflammatory activity as a means of treating inflammation with little or no side effects.

*Corresponding Author: E-mail: steveokpo@uniben.edu Phone: +234 806 402 8832

Hymenocardia acida (Euphorbiaceae) is very popular in traditional (folk) medicine. Commonly known as "Heart-fruit" in English (Schmelzer, 2008), *ikalaga* by the Igbo of Southeast Nigeria**,** and *Orunpa* by the Yoruba of South West Nigeria (Ibrahim *et al*., 2007), it occurs in the savanna and deciduous woodland, and mainly on sandy, loamy or clayey soils (Keay, 1989; Adjanohoun *et al*, 1991; Schmelzer, 2008).

All parts of the plant have been found useful as remedies for various ailments (Abu et al., 2011). Decoction or infusion of leaves and other parts of this plant alone or mixed with other plant species are used for chest complaints, abdominal and menstrual pains and as poultices on abscesses and tumours (Burkill, 1994). The stem bark is widely used in traditional medicine as an anti-inflammatory agent (Abubakar *et al*., 2007), for bone setting by traditional bone healers and in the treatment of chest pains (Igoli and Gray, 2008). The stem bark and leaves have also been used for the treatment of haemorrhoids or prolapsed recta (Schmelzer, 2008; Ibrahim *et al*., 2010). Other reported ethnomedical uses include antifungal and antimycobacterial, anti-HIV and anti-inflammatory activity (Muanza *et al*., 1995); anti-sickling (Mpiana et al., 2009); antiplasmodial (Senecheau *et al*., 2003); antimicrobial (Mann et al., 2008), anti-ulcer (Ukwe 1997); antidiarrhoeal (Tona *et al*., 1999).

The anti-inflammatory activity of the aqueous extract of the stem bark of *H. acida* had previously been investigated (Sackeyfio, 1988). The aqueous leaf extract had also been shown to possess significant anti-inflammatory and anti-nociceptive activities (Sofidiya *et al*., 2010), hence providing a rationale for the use of the plant in folk medicine. The present study investigates the use of the methanol stem bark extract in inflammatory conditions, using various acute models of inflammation.

MATERIALS AND METHODS

Plant collection

The fresh stem barks of *Hymenocardia acida* were collected in June 2014 at Iwo Town in Osun State Nigeria. The plant was identified by Prof. B. A. Ayinde of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. Authentication of the plant was done by Dr. O. S. Sashanya at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria where a herbarium specimen (FHI 110465) was deposited for future reference.

Preparation of Extracts

The fresh barks were air dried and blended to coarse mass using a mechanical blender. A quantity (750g) of the dried powdered stem bark was successively extracted with 1.5L of methanol, for 4 hours, using a sohxlet apparatus followed by concentration with a rotary evaporator maintained at 40°C. The resulting dried extract gave a yield of 15.03 g (2% w/w). A stock solution (200mg/ml) of the extract was prepared from which other concentrations were made as required.

Animals

Albino mice (22.0±1.5g) and Sprague-Dawley rats (180.0±16.0g) of both sexes were used for this study. The animals were obtained from the Animal House of University of Ibadan, Ibadan, Nigeria. They were allowed to acclimatize for two weeks under standard laboratory conditions prior to the commencement of the experiment. The animals were fed with pelleted chow and water *ad libitum* and handled according to standard protocols for the use of laboratory animals (National Institute of health USA; Public health service policy on humane care and use of laboratory animals, 2002).

Drugs and chemicals

Acetone, Carrageenan, Croton oil, Dextran, Egg albumin, Histamine and Indomethacin were all purchased from Sigma-Aldrich Laborchenikalien, GmbH Germany. Chlorpheniramine (Pharmanova, Accra); Cyproheptadine (PT KALBE FARMA, Indonesia); Dexamethasone (Jiangsu Pharm. Inc., China); Heparin (GlaxoSmithKline, England). Other reagents used were of the analytical grade. Stock solutions of drugs and reagents were prepared, appropriately, prior to the experiment.

Antiinflammatory studies

Carrageenan-induced paw oedema

Rats were randomly allotted to different treatment groups of six animals per group. Group 1 (control) was pretreated orally with distilled water (5ml/kg) while groups 2, 3 and 4 were given 100, 200 and 500 mg/kg of the methanol extract, respectively. Indomethacin (10mg/kg) was used as the standard drug. Oedema was induced in rats by injection of freshly prepared carrageenan (0.1 ml, 1% in normal saline) into the sub plantar region of the right hind paw (Winter *et al*., 1962). Measurements of paw diameter were made, using a digital vernier caliper (Marry et al, 1998), immediately before and after injection of carrageenan at hourly intervals for six hours. The paw swelling at each time was calculated as the difference between the paw diameter at time t (D_t) and that at zero hour (D_o).

Histamine-induced paw oedema

Rats were divided into groups of at least five animals per group. Groups 1, 2 and 3 were pretreated orally with the aqueous extracts (100, 200 and 500 mg/kg) respectively, while the standard group (group 4) received chlorpheniramine (10mg/kg). Distilled water (5ml/kg) was used as the control. Oedema was induced one hour after administration of the extract and standard drug by injection of histamine (0.1 ml, 1% w/v in normal saline) into the sub-plantar tissue of the right hind paw (Singh and Pandey, 1996). The paw diameter was measured using a digital vernier calipers. Measurements were made before injection of histamine (i.e basal reading) and at 0.5, 1, 2 and 3 hours post injection of histamine.

Dextran-induced paw oedema

Rats were randomly selected into different treatment groups of six animals per group. Control group was given distilled water (5ml/kg, oral). Groups 2, 3 and 4 animals were pretreated orally with the methanol extract at doses of (100, 200 and 500 mg/kg) respectively, while the standard group received cyproheptadine (10mg/kg). Paw swelling was induced in the rats by injection of freshly prepared dextran (0.1 ml, 1% w/v in normal saline) into the sub-plantar aponeurosis of the right hind paw (Nishida *et* al., 1979). Measurement of paw diameter was made before and at one hour intervals after injection of dextran, for 3 hours, using a digital vernier calipers. Oedema was monitored as the percentage increase in paw thickness in the dextran injected paw.

Croton oil-induced ear oedema

Mice were selected into six groups of six animals each. Groups 1, 2 and 3 received 100, 200 and 500 mg/kg of the methanol stem bark extract of *Hymenocardia acida*, respectively. Group 4 was given dexamethasone (2mg/kg) while group 5 served as the control and was given distilled water (5 ml/kg). All administrations were via the oral route. Thirty minutes after drug administrations, inflammation was induced by application of 20µL croton oil (1% *v/v* in acetone solution) to the inner surface of the right ear while the left served as the control (Tubaro *et al*., 1985). Four hours later, the animals were sacrificed by cervical dislocation, and the left and right ears were cut off. The difference between the weights of the two ears was recorded as the result of the oedema induced by the croton oil.

Carrageenan-induced peritoneal exudate formation

Rats were divided into 5 groups of, at least, six animals each. Groups 1, 2 and 3 received 100, 200 and 500 mg/kg of the methanol stem bark extract of *Hymenocardia acida*, respectively. Group 4 was given indomethacin (10mg/kg) while group 5 served as the control and was given distilled water (5 ml/kg). Pre-treatment of animals was via the oral route, one hour before intraperitoneal injection of carrageenan (0.15ml, 1% w/v in normal saline) (Ribeiro *et al*., 1991). The animals were sacrificed three hours later and the peritoneal cavity washed with 2 ml phosphate buffered saline (containing 5 iu/ml heparin and 3% egg albumin). The exudate volume (minus the wash fluid, 2 ml) was measured, using a measuring cylinder. Total leucocyte counts were performed using Neubauer WBC counting chamber. The potassium and sodium contents of the wash fluid were determined using a flame photometer (FP 640).The percentage inhibition of leukocyte migration was calculated as: 100 × (1-T/C) where, T represents the WBC counts of the treated groups and C represents the WBC counts of the control groups (Umapathy et al., 2010).

Statistical analysis

The values were expressed in Mean ± SEM animals in each group. All groups were analysed for one way ANOVA by Dunnette's test using Graph Pad Prism for Windows version 6.0 (Graph Pad Software, San Diego, CA, USA). The groups with $p<0.05$ were considered significant.

RESULTS

Carrageenan – induced oedema

Administration of carrageenan into the hind paw of rats caused a peak swelling at the 3rd hour. The methanol stem bark extract of *Hymenocardia acida*, administered one hour before carrageenan caused dose-dependent suppressions of the peak oedema response at 3 hours (Table 1). The greatest and significant ($p<0.05$) inhibition (31.30%) was seen at the highest dose (500 mg/kg) of the extract but this was significantly ($p<0.01$) less than that produced by the standard anti-inflammatory drug-indomethacin (64.93%).

Dextran-induced paw oedema

Pretreatment of rats with the methanol extract (500mg/kg) caused a significant suppression of the paw swelling one hour after sub-plantar injection of dextran into the right hind paw of rats. The effects of the extract, at the different doses did not differ significantly from each other but were significantly ($p<0.01$) lower than that obtained with cyproheptadine (Table 2).

Table 1: Effect of methanol extract of *H. acida* stem bark on carrageenan-induced oedema

Treatment	Dose (mg/kg)	Time (Hours)						Inhibition (%) #
		1	2	3	4	5	6	
Control	10	33.02±2.87	42.34±2.54	44.22±6.96	38.22±3.17	31.37±1.96	25.94±4.70	-
H. acida	100	29.89±3.75	39.10±3.32	37.21±5.41	33.51±9.82[a]	32.70±10.95	21.03±3.16	15.85
	200	27.37±3.35	37.64±2.90[a]	33.96±3.45[a]	31.46±2.15[a]	20.23±3.16*[a]	12.29±2.81*	23.20
	500	21.01±6.54*	34.58±6.13[a]	30.38±5.39*	28.75±7.50*[a]	22.34±8.44*[a]	14.71±7.24*	31.30
Indomethacin	10	18.56±4.41*	16.84±3.74**	15.51±2.96**	15.88±3.52**	9.25.00±2.47**	6.78±2.11**	64.93

Values are mean ± SEM. *p<0.05, **p<0.001significantly different from control; [a]p<0.01, significantly different from indomethacin, One-way ANOVA (n= 6 rats per group). [#]Percentage inhibition: oedema at 3hours.

Table 2: Effect of methanol extract of *H.acida* stem bark on dextran-induced oedema

Treatment	Dose (mg/kg)	Time (Hours)				Inhibition (%)[#]
		0.5	1	2	3	
Control	10	69.55±4.54	70.30±6.90	61.75±6.31	46.22±7.22	-
H. acida	100	43.19±9.23*	55.08±9.89[a]	63.53±10.68[a]	41.48±10.99	21.65
	200	40.17±8.93*	55.34±4.67[a]	58.51±6.50[a]	40.04±7.19[a]	21.28
	500	46.61±4.97*	48.60±5.87*[a]	48.12±6.37*[a]	30.63±6.03*	30.87
Cyproheptadine.	10	29.31±4.03**	26.49±3.10**	19.61±3.99**	17.09±3.31**	61.74

Values are mean ± SEM. *p<0.05, **p<0.01significantly different from control; [a]p<0.01, significantly different from cyproheptadine, One-way ANOVA (n= 6 rats per group).[#]Percentage inhibition: oedema at 1hour.

Table 3: Effect of methanol extract of *H.acida* stem bark on histamine-induced oedema

Treatment	Dose (mg/kg)	Time (Hours)				Inhibition (%)[#]
		0.5	1	2	3	
Control	10	66.02±5.39	67.26±6.67	47.11±4.72	38.67±3.29	-
H. acida	100	42.79±9.23	47.59±10.53	30.17±4.93	17.99±2.81*	29.24
	200	47.40±9.30	47.48±10.37	23.50±6.54*	16.41±4.12*	29.40
	500	44.97±10.27	42.24±8.20*	22.11±6.13*	16.93±4.21*	37.20
Chlorpheniramine	10	33.25±3.45	31.48±6.91*	19.64±3.44*	7.95±3.60**	53.20

Values are mean ± SEM. *p<0.05, **p<0.01 significantly different from control; One-way ANOVA (n= 6 rats per group). [#]Percentage inhibition: oedema at 1hour.

Table 4: Effect of methanol extract of *H. acida* stem bark on carrageenan-induced exudate formation.

Treatment	Dose (mg/kg)	Exudate volume (ml)	Exudate inhibition (%)	WBC count (10^3/µl)	Na^+ (mM/L)	K^+ (mM/L)
Control	10	0.93±0.17	-	4.09±0.93	136.42±4.88	4.02±0.09
H. acida	100	0.48±0.07	48.38	3.86±0.79	149.93±13.36	3.93±0.12
	200	0.48±0.14	48.38	2.73±0.32	163.33±1.96	3.68±0.11
	500	0.24±0.03*	74.19	2.70±0.36	180.27±1.04	3.97±0.16
Indomethacin	10	0.23±0.07*	75.27	2.71±0.39	172.50±4.70	3.82±0.20

Values are mean ± SEM. *p<0.05, significantly different from control; One-way ANOVA (n= 6 rats per group).

Histamine-induced paw oedema

Histamine produced a peak oedema response one hour after injection and gradually faded out (Table 3). The methanol extract of *Hymenocardia acida* at various doses reduced the oedema produced, but only the highest dose showed a significant (*p<0.05) reduction (42.24±8.20) relative to the control (67.26±6.67).

Carrageenan- induced exudate formation

Table 4 shows the effect of oral administration of the extract on peritoneal exudate formation. Only the highest dose (500mg/kg) of the extract showed a significant (p<0.05) reduction in exudate volume, an effect similar to that of indomethacin. However no significant alterations were produced by the extract, at any dose level, on white blood cell (WBC) count as well as sodium ion (Na^+) and potassium ion (K^+) concentrations.

Croton oil – induced ear oedema

The effect of the extract on croton oil-induced mouse ear oedema is summarized on Table 5.

All doses of the extract caused significant ($p<0.05$) decreases in the ear oedema, which were however, lower than that of the positive control – dexamethasone.

Table 5: Effect of methanol extract of *H.*acida stem bark on croton oil-induced ear oedema

Treatment	Dose (mg/kg)	Change in ear weight (mg)	Inhibition (%)
Control	10	30.33±1.61	-
H. acida	100	20.60±5.49*	32.08
	200	17.20±3.30*	43.29
	500	17.40±4.97*	42.63
Dexamethasone	2	10.60±3.31**	65.05

Values are mean ± SEM. *p<0.05, **p<0.001, significantly different from control; (n= 6 rats per group).

DISCUSSION

Acute models of inflammation in rodents were employed in investigating the possible anti-inflammatory activity of the methanol stem bark extract of *Hymenocardia acida*. The results obtained showed dose dependent anti-inflammatory effects on the various models used.

The carrageenan-induced oedema in rat is a useful model to detect the action of anti-inflammatory agents (Di Rosa *et al*., 1971) and consists of three distinct phases. The first phase is mediated by histamine and serotonin released from mast cells, the second phase is provided by kinins and the third phase is mediated by prostaglandins, particularly cyclooxygenase products, including prostacyclins and thromboxanes (Nishida *et al* 1979; Silva et al., 2005). Suppression of carrageenan-induced inflammation after the third hour correlates reasonably with therapeutic doses of most clinically effective anti-inflammatory agents (Mequanint *et al*., 2011).

The highest dose of the extract showed significant effect at both the early and later stages of the carrageenan-induced paw oedema, with more prominent effects on the later stages, mediated by prostaglandins. This is similar to the results obtained by Sofidiya *et al*. (2010) using the aqueous leaf extract of *Hymenocardia acida*, which showed significant inhibitory effects between the third and sixth hour of oedema response.

Dextran-induced oedema results from liberation of histamine and serotonin from mast cells (Aziz *et al*., 2011). The highest dose of extract showed a significant activity on the oedema produced by dextran. This effect is a further proof that the extract may inhibit the cellular release of histamine or serotonin. Additionally, the significant reduction of paw oedema observed in the histamine-induced model suggests that the extract, apart from interfering with mediator (histamine) release from the mast cells, may antagonize the effect(s) of already released histamine at the receptor sites.

The extract caused a reduction in the peritoneal exudate volume without significantly affecting the number of leucocytes in the exudate. The main characteristics of acute inflammation are the exudation of fluid and plasma proteins (oedema) and the emigration of leucocytes, predominantly neutrophils; a process dependent on the release of chemotactic mediators by resident cells (Sonza *et al*., 1988; Furst and Muster, 2001). The decrease in the WBC count by the methanol stem bark extract of *H. acida* suggests that it inhibited migration of WBCs to the site of inflammation. Inhibition of cell migration is associated with anti-inflammatory effect (Okoli *et al*., 2008).

During the inflammatory process, leucocytes are known to release lysosomal enzymes resulting in cell membrane damage and inflammation (Chou, 1997). Membrane proteins are largely responsible for the physical properties of the cell membrane and are implicated in regulation of the volume and water content of cells by controlling the movement of sodium ions (Na^+) and potassium ions (K^+) (Rowman, 1996). Inflammatory exudate has a low Na^+ and high K^+ content (Guarino, 1966; Cividalli and Nathan, 1974). An anti-inflammatory agent will therefore increase the level of Na^+ while reducing the K^+ content. The effect of the extract in elevating the Na^+ content coupled with its ability to reduce the peritoneal exudate volume, WBC count and K^+ content though not significantly, suggest anti-inflammatory property.

Dermatitis induced by croton oil represents a model of acute inflammatory response. Topical application of croton oil provokes an intense dermatitis, characterized by vasodilatation, oedema, leukocyte migration and local liberation of the inflammatory mediators such as histamine, serotonin, bradykinin and prostaglandins (Tubaro *et al*., 1985, Inoue *et al*., 1986). Inhibition of croton oil effect is an indication of anti-inflammatory activity (Veras *et al*., 2013). The pronounced effect observed with *H. acida* at all doses used may be related to its ability to inhibit the formation of

inflammatory mediators, especially arachidonic acid and its metabolites, prostaglandins and leukotrienes.

The results of this study have provided evidence to support the use of methanol stem bark extract of *Hymenocardia acida* as an anti-inflammatory agent in traditional medicine. These effects might be partially or wholly due to possible inhibition or interference with the formation, release or receptor activity of some inflammatory mediators, especially prostaglandins and histamine. Thus the present results indicate the efficacy of *H. acida* as an efficient therapeutic agent in acute anti-inflammatory conditions.

REFERENCES

Abu AH, Uchendu CN (2011). Phytochemical and Medicinal activities of*Hymenocardiaacida*Tul (Euphorbiaceae). *J Med Plant Res* 5(8): 1280-1283.

Abubakar MS, Musa AM, Ahmed A, Hussaini IM(2007).Phytochemical and medicinal activities of *Hymenocardiaacida*Tul (Euphorbiaceae): *J Ethnopharmacol*111: 625-629.

Adjanohoun E, Ahyi MRA, AkeAssi L, Dramane K, .Elewude JA, Fadoju SO, Gbile ZO, Goudote E, Johnson CLA, Keita A, Morakinyo O,

Ojewole JAO, Olatunji AO, Sofowora EA (1991): Traditional medicine and pharmacopoeia: contribution to ethnobotancal and floristic studies in Western Nigeria. Publication of the OAU/STRC., Pp 140-141.

Aziz TA, Marouf BH, Mohammed N, Mahmood A, Hussain SA (2011). Anti-inflammatory activity of benfotiamine in adjuvant-induced arthritis and cotton pellet-induced granuloma models of inflammation in rats.*Glo J Pharmacol* 2: 86-91.

Burkill HM (1994). Useful plants of West Tropical Africa, Vol. 2, Families E-I, Royal Botanical Gardens, Kew,pp. 85.

Chou CT (1997). The anti-inflammatory effect of *Tripterygiumwilfordii* Hook F on adjuvant-induced paw edema in rats and inflammatory mediators release. *Phytother Res* 11: 152-154.

Cividalli G, Nathan DN (1974). Sodium and potassium concentration and transmembrane fluxes in leukocytes. Blood 43: 861-869.

Di Rosa M, Giround JP, Willoughy DA (1971). Studies of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. *J Pathol* 104:15-29

Furst DE, Munster T (2001). Non-steroidal anti-inflammatory drugs, disease-modifying anti-rheumatic drugs, non-opioid analgesics and drugs used in gout. In Katzung BG. (Ed.): Basic and Clinical Pharmacology, 8th ed. Lange Medical Books/McGraw-Hill Companies, Inc, pp 596-97.

Guarino AM (1966). Toxicology of muconomycins A and B: Inflammatory properties of two new antibiotics; electrolyte and protein characterization of inflammatory exudate anti-production of a model state of ascites in male albino rats. Open Access Dissertations. Paper 152.

Ibrahim JA, Muazzam I, Jegede IA, Kunle OF (2010). Medicinal plants and animals sold by the "YanShimfidas" of Sabo Wuse in Niger State, Nigeria. *Afr J Pharm Pharmacol* 4(6): 386-394.

Ibrahim H, Sani FS, Danladi BH, Ahmadu AA (2007). Phytochemical and antisickling studies of the leaves of *Hymenocardia acida* Tul (Euphorbiaceae).*Pak J Biol Sci* 10(5): 788-791.

Igoli JO, Gray LA (2008). Phytochemical and Medicinal activities of *Hymenocardi aacida* Tul (Euphorbiaceae): *A Review Int J Phy Sci* 3(6): 156-158.

Inoue H, Saito K, KoshiharaY, Muroto S (1986).Inhibitory effect of glycyrrhetinic acid derivatives on lipoxygenase and prostaglandin synthetase. *Chem Pharm Bull* 34: 897-901.

Jaiswal SR, Santakke SD (2012). Experimental evaluation of analgesic and anti-inflammatory activity of simvastatin and atorvastation. *Indian J Pharmacol* 44(4): 475-479.

Keay RWJ. (1989). Trees of Nigeria. Clarendon Press Oxford, pp 179-181.

Kiecotl-Glaser JK, Gouin J, Hantsoo L(2010). Close relationship, inflammation, and health. *J Neurol Bio Rev* 35(1): 33-38.

Kumar V, Abbas AK, Fausto N (2004). Robbins and Cotran Pathologic Basis of Disease 7th Edition WB Saunders Company, Philadelphia, pp.48, 56-80.

Lacerda RB, de Lima CKF, da Silva LL, Romeiro NC, Miranda AL, Barreiro EJ (2009). Discovery of novel analgesic and anti-inflammatory 3-arylamine-imidazo [1, 2-a] pyridine symbiotic prototypes. *JBMC* 17: 79-84.

Mann A, Amupitan JO, Oyewale AO, Okogun JI, Ibrahim K, Oladosu P, Lawson L, Olajide J, Nnamdi A (2008). Antimicrobial activity of *Hymenocardiaacida*. *Afr J Biotech* 7:1630-1636.

Marry RL, Geetha T, Varalakshmi P (1998). Effect of *Vernoniacinerea* less flower extract in adjuvant-induced arthritis. *Gen Pharmac* 31:601–606.

Mequanint W, Makonnen E, Urga K (2011).*In vivo* anti-inflammatory activities of leaf extracts of *Ocimum lamiifolium* in mice model. *J Ethnopharmacol* 134:32-36.

Mpiana PT, Mudogo V, Kabangu YF, Tshibangu DST, Ngbolua KN, Atibu EK, Mangwala KP, Mbala MB, Makelele LK, Bokota MT (2009). Antisickling activity and thermostability of anthocyanins extracts from a Congolese plant, *Hymenocardia acida* Tul. *Int J Pharmacol* 5(1): 65-70.

Muanza DN, Euler KL, Williams L,Newman DS (1995).Anti-epileptic medicinal plants used in traditional medicine to treat epilepsy. Clinical and genetic aspects of epilepsy. *Int J Pharmacol* 33(2): 98-106.

Nishida S, Kagawa K,Tomizawa S (1979). Dextran-induced paw oedema and 5-hydroxytryptamine release. *Biochem Pharmacol* 28: 3149-3150.

Okoli CO, Akah PA, Onuoha NJ, Okoye TC, Nwoye AC, Nworu CS (2008). *Acanthus montanus:* An experimental evaluation of the antimicrobial, anti-inflammatory and immunological properties of a traditional remedy for furuncles. *BMC Complement Altern Med* 8: 27 doi: 10.1186/1472-6882-8-27.

Ribeiro RA, Frores CA, Cunha FQ, Ferreira SH (1991). IL-8 causes *in vivo* neutrophil migration by cell R-dependent mechanism. *Immunology* 73: 473-477.

Rowman RM (1996). The blood and bone marrow. In MacSween RMN, Whaley K (eds)Muir's Textbook of Pathology 13th edition. Thomas Press Ltd.India, pp 585-644.

Sackeyfio AC (1988). Inhibition of adjuvant arthritis in the rat and pinnal inflammation in the mouse by an extract of *Hymenocardia acida*. Phytother Res 2(1): 42-45.

Schmelzer GH. *Hymenocardia acida* Tul. InSchmelzer GH, Gurib-Fakim A (eds). Prota 11(1): Medicinal plants/ plantesmedicinalis 1. [CD-Rom]. 2008, PROTA, Waganingen, Netherlands.

Senecheau C, Weniger B, Ouattara M, Tra-Bi F, Kamenan A, Lobstein A. (2003).*In vitro*antiplasmodial activity and cytotoxicity of ethnobotanically selected Ivorian plants. J *Ethnopharmacol* 87: 221-225.

Silva GN, Martins FR, Matheus ME (2005). Investigation of anti-inflammatory and anti-nociceptive activities of *Lantana trifolia*. *J Ethnopharmacol* 100: 254-259.

Singh RK, Pandey BL (1996). Anti-inflammatory activity of seed extracts of *Pongamiapinnata*in rat. *Indian J PhysiolPharmacol* 40(4): 355-358.

Sofidiya MO, Odukoya OA, Adedapo AA, Mbagwu, HOC, Afolayan AJ, Familoni OB (2010). Investigation of the anti-inflammatory and antinociceptive activities of *Hymenocardia acida* Tul. (Hymenocardiaceae) *Afr J Biotech* 9(49):8454-8459

Sonza GEP, Cunha EA, Melo R, Ferreira SH (1988). Neutrophil migration induced by inflammatory stimuli is reduced by macrophage depletion. *Agents Actions* 24: 377-380.

Tona L, Kambu K, Masia K, Cimanga R, Aspers S, Rebruyne T, Piatens L, Totten J (1999). Biological screening of traditional preparations from some medicinal plants used as antidiarrhoeal in Kinshasha, Congo. *Phytomed* 6(1): 59-66.

Tubaro A, Dri P, Delbello G, Zilli C, Della LR (1985). Croton oil ear test revisited. *Agents Actions* 17: 347-349.

Ukwe CV (1997). Antiulcer activity of aqueous stem bark extract of *Hymenocardia acida* Tul (Euphorbiaceae). *Int J Pharmacog* 35: 354-357.

Umapathy E, NdebiaEJ, Meeme A, Adam B, Menziwa P, Nkeh-Chungag BN, Iputo JE (2010). An experimental evaluation of *Albucasetosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. *J Med Plants Res* 4(9): 789-795.

Veras HN, Araruna MK, Costa JG, Coutinho HD, Kerntopf MR, Botelho MA, Menezes IR (2013).Topical antiinflammatory activity of essential oil of *Lippia sidoides* Cham: Possible mechanism of action. *Phytother Res* 2(27): 179-185.

Winter CA, Risley EA, Nuss GW (1962). Carrageenan induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proc Soc Exp Biol Med* 111: 544-547.