IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
February 2021 Vol.:20, Issue:3
© All rights are reserved by Ukwueze S. E et al.

Phytochemical and Comparative Study of the Analgesic and Anti-Inflammatory Activity of *Alafia barteri* (Apocynaceae) against Common NSAIDs Using Albino Wistar Rats

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Ukwueze S. E*[1], Nwuzi P. O[1], Nwoke E. A[1], Ajibo D. N[2].

[1]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Port Harcourt, PMB 5323, Choba, Nigeria. [2]Department of Experimental Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, University of Port Harcourt, PMB 5323, Choba, Nigeria.

Submitted: 03 January 2021
Revised: 23 January 2021
Accepted: 12 February 2021

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: Phytochemical, Analgesic, Anti-inflammatory, *Alafia barteri*, Oedema

ABSTRACT

This study investigated the phytochemical constitution and the analgesic/anti-inflammatory effects of the methanol leaf extracts of *Alafia barteri* (MeAB) using standard procedures. The analgesic/anti-inflammatory effectswere compared with that of common NSAIDs. The preliminary phytochemical screening of the methanol leaf extract of *Alafia barteri* revealed the presence of glycosides, flavonoids, anthraquinones, and saponins, while tannins and alkaloids werefound to be absent. The analgesic and anti-inflammatory activities of the extracts (200 and 400 mg/kg, orally), diclofenac, ibuprofen and aspirin (200 mg/kg. orally) were evaluated using acetic acid-induced writhing test, hot plate-induced pain test and carrageenan oedema tests, respectively. MeAB(200 mg/kg), diclofenac, ibuprofen and aspirin inhibited acetic acid-induced abdominal constriction by 26.56, 71.90, 54.70 and 50%, respectively,while MeAB (400 mg/kg) inhibited constriction by 32.80%. MeAB, diclofenac, ibuprofen and aspirin (200 mg/kg) pre-treatments significantly increased the reaction time by 21.6, 56.0, 40.5 and 21.62%, respectively, while MeAB (400 mg/kg) increased the reaction time by 43.24%, 80 min post-treatment in the hot-plate test. MeAB(200 mg/kg), diclofenac, ibuprofen and aspirin showed significant anti-inflammatory activity with 12.50, 44.40, 33.3 and 33.3% (at 4 h) inhibition of paw oedema respectively, while MeAB (400 mg/kg) showed significant anti-inflammatory activity with 44.4% inhibition of paw oedema at the same time interval. The anti-inflammatory and analgesic activities of the NSAIDs (diclofenac, ibuprofen, and aspirin) used in the study were found to be greater than that of methanol leaf extract of *A.barteri.*

1. INTRODUCTION:

Inflammation is a reaction or response of the body against an aggressive agent. It can be evoked by a wide variety of noxious agents (e.g. infections, antibodies, and physical injuries). It is characterized by; vasodilation, infiltration of fluid and cells into the target tissue, one of the major signs of inflammation is the pain that can be triggered by direct stimulation of nociceptors or by the action of inflammatory receptors.[1] These mediators, for example, cytokines, histamine, serotonin, leukotriene, and prostaglandins, increase the vascular permeability, local vasodilatation and migration of leukocytes to inflamed tissues.[1] The cardinal signs of inflammation are redness, swelling, heat, pain and loss of function. [2] Prostaglandins act as short-lived localized hormones that can be released by any cell of the body during tissue, chemical, or traumatic injury, and can induce fever, inflammation, and pain, once they are present in the intercellular space. Thromboxane, which is also hormone activator, can regulate blood vessel tone, platelet aggregation, and clot formation to increase the inflammatory response.[3, 4] The inflammatory pathway is a complex biochemical pathway that, once stimulated by injury, leads to the production of these and other inflammatory mediators whose initial effect is pain and tissue destruction, followed by healing and recovery. [5, 6] A major component of the inflammatory pathway is called the arachidonic acid pathway because arachidonic acid is immediately released from traumatized cellular membranes. Membrane-based arachidonic acid is transformed into prostaglandins and thromboxane partly through the enzymatic action of cyclooxygenase (COX). [5. 7] There are two types of COX enzymes, COX-1 and COX-2. Both the enzymes act similarly, but selective inhibition (as accomplished by selective COX-2 inhibiting NSAIDs) can make a difference in terms of side effects. Inflammation can be acute [8] or chronic [9] , the former resolving within few hours to few days, while the later lasts for weeks to months, usually greater than six months.

Inflammation involves two major mechanisms, vascular and cellular events. [10] The vascular event which appears few minutes after tissue injury or infection is characterized by vasodilation and increased capillary permeability, with the subsequent entry of inflammatory mediators, and production of interstitial oedema, while the cellular events involve the recruitment and the infiltration of the tissues by the inflammatory cells.[11] Examples of inflammatory cells include; neutrophils (e.g. in acute bacterial infection), eosinophils, mast cells, lymphocytes (e.g. in asthma), monocytes, and macrophages. Available therapy is aimed at interfering with the mediators of inflammation. The major mechanism of action of

the NSAIDs is the inhibition of the COX enzyme system; older drugs are not selective and will inhibit both the COX-1 and COX-2 isoform. [12] Although newer agents have been introduced which are selective for the COX-1 isoenzyme, an example of this is CELECOXIB, which is used in the treatment of arthritis. Corticosteroids are mainly used in the management of chronic inflammatory conditions.[13, 14]

All steroidal and NSAIDs, despite their usefulness, cause undesired and serious side effects which in many cases are severe enough to pose the risk of ulcer perforation, upper gastrointestinal bleeding, sometimes leading to death, thus greatly limiting their use in therapy. [15] Therefore, the development of new and more powerful drugs with low toxicity and higher therapeutic value is still needed. Medicinal plants have long been used worldwide in folk medicine as an alternative treatment of inflammatory processes of diverse origins. [16] Some of the medicinal plants that have been investigated for their analgesic, anti-inflammatory or anti-rheumatic activities include: Cannabis (*Cannabis sativa* L.), Pineapple (*Ananas comosus* L.), Green tea (*Camelia sinensis* L.), Mango (*Magnifera indica* L.), Clove (*Syzygiumaromaticum*), Ginger (*Zingiber officinale* R.), Elderberry (*Sambucus nigra*), Eucalyptus (*Eucalyptus globulus*), European Mistletoe (*Viscum album*), Evening primrose (*Oenothera spp*.), Banyan (*Ficus bengalensis*), etc. [17-20][43]

Alafia berteri. Oliv (Apocynaceae), is a vigorous climbing shrub producing stems up to 35metres long, which scramble over the ground or climb up into trees in the forest, the stems can be 3cm in diameter. It is a tropical rain forest plant grown widely but native to the West and Central Africa, stretching from Guinea Bissau to Cameroon, Congo and Nigeria. [21] In Nigeria it is known as ọta nza amongst the Igbos and agbárí ẹtù in Yoruba. In Nigerian traditional medicine, the stem and root decoctions of *Alafia barteri* are used for treating rheumatic pains, toothache, eye infection and sickle cell anaemia. There have also been reports of its use in south-western Nigeria for the treatment of malaria. [22]Ethanol and water extracts of the leaves of *Alafia barteri* have shown antifungal activity against *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Candida albicans, Microsporum audoninii, Trichoderma viride* and *Trichophyton mentagrophytes*. [23, 24] The ethanol extract showed more activity than the water extracts. A preliminary phytochemical report on the stem extracts of *Alafia barteri* showed the presence of reducing sugars, steroids, flavonoids and anthraquinones. [24]Since most drugs indicated in the treatment of inflammation and pains are associated with various adverse effects, hence the need to search for a safer and more effective alternative. This research is therefore aimed at evaluating the phytochemical

constituents, analgesic and anti-inflammatory activities of the methanolic leaf extract of *Alafia barteri.*

2. MATERIALS AND METHODS:

2.1 Plant materials

The plant material was selected based on traditional healers' information. Fresh leaves of *Alafia barteri* together with the stem were collected in September 2019 from Ibadan, Oyo State, Nigeria. The plant material was identified and authenticated by Mr. John, a taxonomist at the Department of Plant Science and Biotechnology, and a herb identification number (UPH/PHARM/PCH012) assigned.

2.2 Animals used

Albino Wistar rats and albino mice of either sex were obtained from the animal house in the Department of Pharmacology, University of Port Harcourt. The animals were kept in polyacrylic cages with five rats or six mice per cage and maintained under standard housing conditions (room temperature 28°C and humidity 60–65%) with a 12-hr light and dark cycle. Food, in the form of dry pellets, and water was available *ad libitum*; however, they were fasted 12 h before and until completion of the experiments.[44]Experiments were performed according to International Ethical Standards and the protocol was approved by the Research Ethics Committee of Central Drug Research Institute and CPCSEA (Committee for Control and Supervision of Experiments on Animals). The experimental procedures adopted in this study were in accordance with the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research. [25]

2.3 Drugs/ Chemicals and Materials.

Chemicals and drugs used in this work include acetic acid, carrageenan, methanol, dimethylsulphoxide (DMSO), diclofenac tablet, ibuprofen tablet, aspirin tablet and normal saline. Materials used are BUCHI Rotavapor (Newcastle DE), vacuum pump, Magnetic stirrer with hot plate, thermometer, plethysmometer.

2.4 Extraction

The leaves were air-dried at room temperature, for one week. The air-dried leaves (623 g) were pulverized into coarse powder using a blender. The powdered leaves were extracted five

times, on each occasion with 9.6L of methanol at room temperature for 48 h (with occasional shaking). The combined methanol extracts were filtered and the filtrates were concentrated to dryness under reduced pressure in BUCHI Rotavapor (New Castle, DE) at 40^{o}C. They were further dried with a vacuum pump to remove moisture, giving 74 g (11.87% yield) of *Alafia barteri*. The extracts were dissolved in 0.5% v/v dimethylsulfoxide in normal saline and were administered to the animal by oral gavage.

2.5 Acute Oral Toxicity Test-Fixed Dose Procedure

The acute oral toxicity test was conducted using the fixed-dose procedure according to OECD test guidelines on acute oral toxicity (TG 42) as adopted on 17 December 2001. Fifteen female mice (25–30 g) were randomly selected from a group of 40 mice. Based on the recommendations of several expert meetings in 1999, testing in one sex (usually females) is generally considered sufficient for acute toxicity testing. [26] The selected mice were divided into three groups of five mice each. Each group was kept separately in polyacrylic cages, feed but the water was withheld for 4 hr before the test. A stepwise procedure using fixed doses of 300, 2000, and 4000 mg/kg/body weight per extract with 3 days in-between each dose. Each animal was observed for the first 10 min after treatment then every 30 min for the next 6 hr for any behavioral or acute toxicity symptoms. The mice were further observed for up to 14 days following treatment for any signs of toxicity and mortality.

2.6 Phytochemical screening

The phytochemical screening of the methanolic leaf extract of *Alafia barteri* leaves was done using the standard procedure by Sofowora [27]to detect the presence of steroids, alkaloids, tannins, glycosides, reducing sugars, flavonoids, and saponins.

2.7 Evaluation of Analgesic Activity

2.7.1 Hot Plate Test

The hot-plate was used to measure response latencies according to the method described by Eddy and Leimbach, [28] with minor modifications. Mice of either sex were screened for a response to thermally induced pain by placing them on (Columbus Analgesiometer, Columbus, OH), a hot plate maintained at 55^{o}C. The time between the placement of the mouse on the hot plate and shaking or licking of the paws or jumping was recorded as the reaction latency. Mice with baseline latencies higher than 45 s were excluded from the study.

Animals that reacted to the thermally induced pain in less than 10 s were divided into 6 groups, with 5 animals per group (n=5) followed by administration of different concentrations of drugs and extract: group 1: 0.5% v/v dimethylsulfoxide in normal saline (10 ml/kg, p.o.), group 2-3: *Alafia barteri* (200 and 400 mg/kg, p.o), group 4: diclofenac (200 mg/kg, p.o.), group 5: ibuprofen (200 mg/kg,p.o.), groups 6: aspirin (200 mg/kg, p.o.). The reaction latency was recorded at 0, 20, 40, 60, and 80 min post-drug treatment. The prolongation of the latency times comparing the values before and after the administration of the extract- or vehicle-treated control was considered analgesic response. [29]

$$\%\textbf{(MPE)}=\frac{(test-baseline)}{(cutoff-baseline)}\times 100$$

Where the test is the latency to respond after treatment; the baseline is the latency to respond prior to treatment; and cut-off (10 s), is the preset time at which the test will be ended in the absence of a response.

2.7.2 Acetic Acid-Induced WrithingTest

The test was performed as described by Koster *et al*. [30] Mice (20–30 g, n=5 per group) were pre-treated with 0.5% v/v dimethylsulfoxide in normal saline (10 ml/kg, p.o.), MeAB, (200 and 400 mg/kg, p.o.), diclofenac (100 mg/kg, p.o.), ibuprofen (200 mg/kg) and aspirin (200 mg / kg), 60 min before intraperitoneal injection of acetic acid (0.6% v/v, 0.1 ml/10 g body weight). The number of abdominal writhes (contraction of the abdominal muscle together with a stretching of the hind limbs) was cumulatively counted every 5 min for 20 min. The antinociceptive activity was expressed as percentage inhibition of abdominal writhes. [31]

$$\textbf{Inhibition}(\%)\ \frac{W_{Control}-W_{test}}{W_{control}}\times 100$$

Where,$W_{control}$=(no of writhing in control)

W_{test}=(no of writhing in test).

2.8 Evaluation of Anti-inflammatory Activity

2.8.1 Carrageenan Induced Paw Oedema

This test was carried out using carrageenan as a phlogistic agent to induce paw oedema in the right hind limb of rat, which serves as a model of acute inflammation. [32] Albino rats (170–300 g) were randomly divided into groups of six animals each, and were used after 12-h fast but allowed free access to water except during the experiment. A 0.5%v/v dimethylsulfoxide in normal saline (10ml/kg,p.o.), MeAB (200 or 400 mg/kg/body weight, p.o.), diclofenac (200 mg/kg, p.o.), ibuprofen (200 mg /kg. p.o) and aspirin (200 mg/kg p.o), were administered 1 h before subcutaneous injection of 100 µl of carrageenan (1% w/v in 0.9% normal saline) into the right hind paw. The needle is inserted to a depth of approximately 1 mm into the callus to deliver an accurate and uniform amount of carrageenan into the sub plantar site. Paw volume was measured employing a volume displacement method using a plethysmometer (Ugo-Basile, Varese, Italy) before the injection of carrageenan and thereafter at 2, 3, and 4 h. Oedema was expressed as the change in paw volume (mm) after carrageenan injection relative to the pre-injection value for each animal. Percentage inhibitions of oedema were calculated. [29] [33]

$$\textbf{Inhibition (\%)} = \frac{PIC - PITD}{PIC} \times 1OO$$

PIC=Paw inflammation of control

PITD=Paw inflammation of treatment drug

2.9 Statistical Analysis

The various data obtained from the experiments were analysed using the two-way analysis of variance (ANOVA) method.

3. RESULTS AND DISCUSSION:

RESULTS:

3.1 Acute Oral Toxicity Test

Oral administration of methanol extract of *Alafia barteri* (MeAB), up to 4 g/kg neither induced mortality nor toxic behaviours.

3.2 Phytochemical Screening

Table No. 1: Phytochemical screening of methanol extract of *Alafia barteri*.

PHYTOCHEMICALS	PRESENCE (+) ABSENCE (-)
Alkaloid	--
Saponin	++
Tannin	--
Anthraquinone	++
Glycoside	++
Reducing Sugars	++
Flavonoids	++

Table No. 2: Effect of *A. barteri*, Diclofenac, Ibuprofen, and Aspirin on Latency period.

Treatment	Doses (mg/kg)	Latency period (s)				
		0 min	20 min	40 min	60 min	80 min
Vehicle	10	8±0.2	8±0.4	7±0.9	9±0.5	8±0.2
Alafia barteri	200	14±07	18±0.6 (27.0%)	17±0.5 (26.9%)	20±0.7 (30.6%)	19±0.2 (21.6%)
	400	19±0.3	20±0.6 (32.4%)	21±0.2 (36.8%)	18±0.3 (25.0%)	24±0.6 (43.2%)
Diclofenac	200	18±0.7	22±0.2 (27.0%)	26±0.3 (50.0%)	30±0.7 (58.0%)	29±0.4 (56.0%)
Ibuprofen	200	15±0.2	12±0.5 (20.0%)	14±0.5 (18.0%)	15±0.3 (16.0%)	23±0.6 (40.5%)
Aspirin	200	17±0.6	19±0.2 (21.6%)	17±0.2 (26.3%)	18±0.3 (25.0%)	20±0.3 (32.4%)

*Values in parenthesis represent percentage maximum possible effect (%MPE).

Table No. 3: Effect of methanol leaf extracts of *A. barteri*, Diclofenac, Ibuprofen, Aspirin against acetic acid-induced mouse writhing test.

Treatment	Doses (mg/kg)	Onset of writhing	No of writhing	% inhibition
Vehicle	10	12±2.24	64±3.42	
Alafia barteri	200	20±1.76	47±2.81	26.56%
	400	21±1.69	43±2.23	32.80%
Diclofenac	200	47±2.35	18±3.31	71.90%
Ibuprofen	200	28±2.21	29±2.41	54.70%
Aspirin	200	24±1.87	32±1.98	50.00%

Table No. 4: Effect of *A. barteri*, Diclofenac, Ibuprofen and Aspirin, against carrageenan - induced paw edema in albino mice.

Treatment	Dose (mg/kg)	Change in paw volume (mm)			
		1 h	2 h	3 h	4 h
Vehicle	10	0.5±0.1	0.7±0.1	0.9±0.1	0.9±0.1
Alafia barteri	200	0.5±0.1(0.0%)	0.6±0.1(14.4%)	0.6±0.0 (33.3%)	0.8±0.1 (12.5%)
	400	0.5±0.1 (0.00%)	0.5±0.2 (28.6%)	0.4±0.2 (55.5%)	0.5±0.2 (44.4%)
Diclofenac	200	0.3±0.0 (40.0%)	0.5±0.1 (28.5%)	0.4±0.1 (55.5%)	0.5±0.1 (44.4%)
Ibuprofen	200	0.4±0.0 (20.0%)	0.5±0.1 (28.6%)	0.5±0.1 (44.4%)	0.6±0.2 (33.3%)
Aspirin	200	0.4±0.1 (20.0%)	0.4±0.2 (20.0%)	0.5±0.1 (44.4%)	0.6±0.1 (33.3%)

***** Values in parenthesis represent percentage inhibition (%).

4. DISCUSSION:

The preliminary phytochemical screening of the methanol leaf extract of *Alafia barteri* revealed the presence of glycosides, flavonoids, anthraquinones, and saponins (Table 1). However, tannins and alkaloids were not found in the extract.

The data illustrated in Table 2 above shows the time course of the various treatments against the hot plate test. The hot-plate test is used to detect centrally-acting analgesics. [34] The extent of the extract's central analgesic effects was also determined using same method. The hot-plate test produces two kinds of behavioral response, paw licking and jumping. Both are considered to be supra-spinally integrated responses. [35, 36] A good analgesic agent should be able to significantly increase the pain threshold, that is, the time taken for an untreated animal (control animal) to jump out of the beaker. In this test, the animals were treated with the agents and evaluated at different time intervals to allow for absorption prior to the commencement of the procedure. Analgesic agents produce higher activity at a higher time interval.[34] In the hot plate test, oral treatment of methanol extract of *Alafia barteri* (200 and 400 mg/kg) was able to cause a significant increase in the latency period, 19±0.2 and 24±0.6 s, respectively ($p<0.05$), after 80 minutes of pretreatment compared to the control. Diclofenac had the highest maximum possible effect (56.0%), and significantly ($p<0.05$) increased the latency period by 29±0.4s, after 80 minutes of pretreatment (Table 2). Oral pretreatment with ibuprofen and aspirin also produced a significant increase in latency period of 23±0.6 and 20±0.3s respectively.

Table 3 showed the percentage inhibition produced by the various treatments against the acetic acid-induced writhing test. This test is often used to distinguish between central and peripheral analgesic actions. [34, 37] Acetic acid stimulates the vanilloid receptor (VR1) and bradykinin B2 receptor in the pathway comprising sensory afferent C-fibers. [38] Acetic acid induces peripheral stimuli by activation of the arachidonic acid metabolites; this sensitizes and activates peripheral nociceptors or sensitization of the nerve fibers involved in the pain transmission pathway. [39] Agents that significantly reduce writhing in the animals are said to act peripherally. Oral treatment with diclofenac (200mg/kg, p.o) significantly reduced the number of writhing by 18±3.31, followed by ibuprofen (200 mg/kg, p.o), which recorded a value of 29±2.41 ($p<0.001$). Oral administration of MeAB (200 or 400 mg/kg) significantly ($p<0.01$; $p<0.001$) reduced the mean number of writhes from 64.0 ± 3.42 in control to 47± 2.81 and 43.40 ± 2.23 (26.56% and 32.80% inhibition) respectively. Aspirin (200 mg/kg, p.o)

also resulted in a decrease in the number of writhes by 32±1.98 (%inhibition of 50.0%). This shows that diclofenac has the highest activity compared to the other treatments.

Table 4 shows the effect of *A. barteri,* diclofenac, ibuprofen and aspirin on the carrageenan induced paw edema test. Carrageenan-induced inflammation consists of three phases: early phase involves the production of histamine, serotonin, nitric oxide, and bradykinin, i.e., 1h post phlogistic injection; second phase (at 2 h) mediated by kinins, leukotrienes, platelet-activating factor, and possibly cyclooxygenase products; and a third phase (3–24 h; late phase) primarily from the formation of pro-inflammatory prostanoids and nitric oxide (synthesized by the inducible nitric oxide synthase isoform), cytokines, neutrophil infiltration and the production of neutrophils derived free radicals, such as hydrogen peroxide, superoxide and OH^- radicals. [40-43] From this study *A. barteri* (200 and 400 mg/kg, p.o.), diclofenac (200 mg/kg), ibuprofen (200 mg/kg) and aspirin (200 mg/kg) significantly attenuated the development of edema in the middle phase (at 2 h) and more pronouncedly in the late phase (at 3 h) of carrageenan-induced inflammation (Table 4). This suggests that the methanol extract of *Alafia barteri* acts by inhibiting the release and/or actions of kinins, pro-inflammatory prostanoids, and inducible nitric oxide synthase isoform.[40, 43] However the anti-inflammatory activity produced by MeAB 400 mg/kg was similar to diclofenac at the 3 h, suggesting that methanol extract of *Alafia barteri*(at 400 mg/kg) can be used as an alternative for diclofenac (400 mg/kg) in the management of inflammation. The effect produced by ibuprofen was similar but higher than that produced by MeAB 200 mg/kg (Table 4). The peak effect produced by aspirin at 200 mg/kg is slightly higher (44.4%) than the effect produced by MeAB at 200 mg/kg (33.3%).

CONCLUSION:

The present study has demonstrated the anti-inflammatory and analgesic activity of methanol leaf extract of *Alafia barteri* in albino wistar rats. The study also indicated that methanol leaf extracts of *Alafia barter*i have potent analgesic and anti-inflammatory activity in a dose-dependent manner. However, the anti-inflammatory and analgesic activity of commercially available NSAIDs used (diclofenac, ibuprofen, and aspirin) had higher activity than the extracts at similar doses. This study also indicated that methanol leaf extract of *Alafia barteri* showed the presence of saponins, glycosides, reducing sugars, anthraquinones and flavonoids, but didnot contain tannins and alkaloids.

ACKNOWLEDGEMENT:

We specially appreciate the Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical sciences, University of Port Harcourt.

CONFLICT OF INTEREST:

None

REFERENCES:

1. Brenner, P. &Krakauer T. (2003). Regulation of inflammation: A review of recent advances in anti-inflammatory strategies. *Current Medicinal Chemistry of Anti-Inflammatory and Anti-Allergy Agents* 2:274–83.
2. Purnima A, Koti BC, Thippeswamy A, Jaji M, Swamy A, Kurhe Y. Anti-inflammatory, analgesic and antipyretic activities of *Mimusop selengi* linn. *Indian Journal of Pharmaceutical Science 2010;*72:480–485.
3. Nelson A, Lau B, Ide N, Rong Y. Pycnogenol inhibits macrophage oxidative burst, lipoprotein oxidation, and hydroxyl radical-induced DNA damage. *Drug Development and Industrial Pharmacy 1998;* 24:139–44
4. Rehman Q,Sack K. When to try COX-2-specific inhibitors: Safer than standard NSAIDS in some situations. *Postgraduate Medical Journal 1999;* 106:95–106.
5. Fitzgerald G. Coxibs and cardiovascular disease. *New England Journal of Medicine 2004;* 351:1709–1711.
6. Harris W, Von Schacky C. The Omega-3 Index: A new risk factor for death from coronary heart disease? *Preventive Medicine* 2004; 39:212–220.
7. Hostanska K, Daum G, Saller R. Cytostatic and apoptosis inducing activity of boswellic acids toward malignant cell lines in vitro. *Anticancer Research*2002; 22:2853–286
8. Serhan C, Dalli J, Colas R, Winkler J,Chiang N. Protectins and maresins:New pro-resolving families of mediators in acute inflammation and resolution bioactive metabolome. *Biochim. Biophys. Acta (BBA) Mol. Cell Biol. Lipids 2015;* 1851:397–413.
9. Isailovic N, Daigo K, Mantovani A,Selmi C. Interleukin-17 and innate immunity in infections and chronic inflammation. *Journal of Autoimmunity* 2015; 60:1–11.
10. Nguyen T. Systems Biology Approaches to Corticosteroid Pharmacogenomics and Systemic Inflammation (Doctoral dissertation, Rutgers University-Graduate School-New Brunswick, 2012)
11. Porter S. Tidy's Physiotherapy. Amsterdam: Elsevier Health Sciences, 2013.
12. Vane J. Differential inhibition of cyclooxygenase isoforms: An explanation of the action of NSAIDs. *Journal of Clinical Rheumatology*1998; 4:S3–10.
13. Watanabe S,Bruera E. Corticosteroids as adjuvant analgesics. *Journal of Pain Symptom Management* 1991; 9(7):442–5.
14. Chandrasekharan N, Dai H, Roos K, Evanson N, Tomsik J, Elton T, Simmons DL. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic drugs: Coloning, structure and expression. Proct. Natl. Acad. Sci. USA. 2002; 99. 3926-3931
15. Bjamason I, Hayllar J, MacPherson A,Russell A.Side effects of nonsteroidal anti-inflammatory drugs on the small and large intestine in humans. *Gastroenterology 1993*; 104 (6): 1832-1847.
16. Farnsworth N.Screening plants for new medicines, In: E.O. Wilson (Ed.) Biodiversity: Part II. Washington: National Academy Press 1998, pp. 83-97
17. Anilkumar M. Ethnomedicinal plants as anti-inflammatory and analgesic agents. In: Debprasad C (Ed.), Ethnomedicine: A source of complementary therapeutics. *Indian Research Signpost 2010,* pp 267-293
18. Inoue K, Motonaga A, Dainaka J, Nishimura T, Hashii H, Yamate K, Ueda F, Kimura K. Effect of etodolac on prostaglandin E2 biosynthesis, active oxygen generation and bradykinin formation. *Prostaglandins Leukot Essent Fatty Acids*. 1994; 51(6): 457-62.

19. Srivastava K,Mustafa T. Ginger (Zingiber officinale) in rheumatism of musculoskeletal disorders. *Med Hypotheses* 1992; 39(4): 342-348
20. Toker G, Küpeli E, Memisoğlu M, Yesilada E. Flavonoids with antinociceptive and antiinflammatory activities from the leaves of Tilia argentea (silver linden). *Journal of Ethnopharmacolog 2004;* 95: 393-397.
21. Irvine R. Woody Plants of Ghana with Special References to their uses. London: Oxford University Press, 1961.
22. Olowokudejo J, Kadiri A,Travail V.An ethnobotanical survey of herbal markets and medicinal plants in Lagos State of Nigeria. *EthnobotanyLeaflets*, Vol.12 2008, pp. 851-856
23. Adekunle A, Okoli S. Antifungal activity of the crude extracts of *Alafia barteri* Oliv. (Apocynaceae) and Chasmanthera dependens (Hochst). Menispermaceae. *Hamdard Medicine* 2002; 45:52-56
24. Hamid A,AiyelaagbeO. Preliminary phytochemical, antibacterial and antifungal properties of *Alafia barteri* stem grown in Nigeria. *European Journal of Medicinal Plants*2011; 1:26–32
25. NIH. Guide for the Care and Use of Laboratory Animals. 8th Ed. Washington DC 2011, 11–133
26. OECD (The Organization of Economic Co-operation Development). The OECD Guideline for Testing of Chemical: 420 Acute Oral Toxicity. Paris: OECD, 2001; 1–14
27. Sofowora A. Medicinal Plants and Traditional Medicines in Africa, 6th ed. New York: Chicester John Willey and Sons, 1982.
28. Eddy N,Leimbach D. Synthetic analgesics. II. Dithienylbutenyl and dithienylbutilamines. *Journal of Pharmacology and ExperimentalTherapeutics* 1953; 107:385–93
29. Ishola IO, Agbaje OE, Narender T, Adeyemi OO, Shukla R. Bioactivity guided isolation of analgesic and anti-inflammatory constituents of *Cnestis ferruginea* Vahl ex DC (Connaraceae) root. *J Ethnopharmacol*. 2012; 142(2): 383-9.
30. Koster R, Anderson M, De-Beer E. Acetic acid for analgesic screening. Fed Proc 1959; 18:412–18.
31. Ishola I, Akindele J, Adeyemi O. Analgesic and antiinflammatory effect of methanol root extract of *Cnestis ferruginea* Vahl DC. *Journal of Ethnopharmacology*2011; 135:55–62.
32. Winter C, Risley E, Nuss G. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proc Soc Exp Biol Med*1962; 111:544–7
33. Adeyemi O, Yemitan O, Afolabi L. Inhibition of chemically induced inflammation and pain by orally and topically administered leaf extract of *Manihot esculenta* Crantz in rodents. *Journal of Ethnopharmacology*, 2008; 119:6–11.
34. Nunez G, Emim J, Souccar C, Lapa A. Analgesic and anti-inflammatory activities of the aqueous extract of *Plantago major* L. *International Journal Pharmacognosy*1997; 35:99–104
35. Chapman CR, Casey KL, Dubner R, Foley KM, Gracely RH, Reading AE. Pain measurement: an overview. *Pain*. 1985; 22(1): 1-31.
36. Chavan M, Kolhe D, Wakte P, Shinde D. Analgesic and anti-inflammatory activity of Kaur-16-en-19-oic acid from *Annona reticulata* L. Bark. *Phytotherapy Research*2012; 26:273–6
37. Umukoro S, Ashorobi R. Further studies on the antinociceptive action of aqueous seed extract of *Aframomum melegueta.Journal of Ethnopharmacology*2007; 109:501–4.
38. Ikeda Y, Ueno A, Naraba H, Oh-ishi S. Involvement of vanilloid receptor VRl and prostanoids in the acid-induced writhing responses of mice. *Life Sciences*2001 ; 69:2911–19
39. Jia Q, Su W, Peng W, Li P, Wang Y. Anti-diarrhoea and analgesic activities of the methanol extract and its fractions of *Jasminium amplexicaule* Buch.-Ham. (Oleaceae). *Journal of Ethnopharmacology*2008; 119: 299–304.
40. Bilici D, Akpinar E, Kiziltunc A. Protective effect of melatonin in carrageenan-induced acute local inflammation. *Pharmacology Research*2002; 46: 133–9
41. Di Rosa M, Giroud J, Willoughby D. Studies on the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. *Journal of Pathology*1971; 104:15–29
42. Handy C, Moore K. A comparison of the effects of L-NAME, 7-NI and L-NIL on carrageenan-induced hindpaw edema and NOS activity. *British Journal of Pharmacology*1998; 123:1083–8.
43. Salvemini D, Wang ZQ, Wyatt PS, Bourdon DM, Marino MH, Manning PT, Currie MG. Nitric oxide: a key mediator in the early and late phase of carrageenan-induced rat paw inflammation. *Br J Pharmacol*. 1996; 118(4): 829-38.

44. Ukwueze S, Aghanya A, Mgbahurike A, Shorinwa O.An Evaluation of the Analgesic and Anti-Inflammatory Activities of the Solvent Fractions of *Aspilia Africana* (Pers.)*World Journal of Pharmacy and Pharmaceutical Sciences*2013;2(6):4177-4189.
45. Shorinwa O, Ubele C, Ukwueze S.Evaluation of the analgesic and anti-inflammatory activities of ethanol extract of the root of Mimosa Pigra Linn (fabaceae) in albino rats *International Journal of Pharmacy and Pharmaceutical Sciences* 2015; 7(7): 376-379.