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# *In-Vivo* Studies on the Analgesic and Anti-inflammatory Potentials of Novel *Xylopia Aethiopica* Formulations.

**Nwafor Arthur[1], Nworah Doris Chinwe[1], Ukpuho Etisang Inyang[1], Aniyeloye Michael Amadi[2]**

*1. Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, PMB 5323 Choba, Port Harcourt, Rivers State, Nigeria*
*2. Department of Pharmacology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, PMB 5323 Choba, Port Harcourt, Rivers State, Nigeria*

## ABSTRACT

The present experimental research work was undertaken to determine the analgesic and anti-inflammatory activities of novel *xylopia aethiopica* formulations based on folklore methods used in Nigeria for the management of chronic severe painful conditions on eggwhite induced paw oedma in both sex of albino wistar rats using the pain models of Randall and Selitto (1957) as adopted by Ugo Basile (Italy). Analgesic and anti-inflammatory activities of the different analgesia models at doses as low as 0.5mg/ kg & 2mg/ kg respectively were evaluated against the standard analgesic drug Piroxicam, at dose of 20mg/ kg. The analgesic and anti-inflammatory activities was dose, and sex dependant: females appearing to endure more pains than males and in the ratio 2:1; suggesting that xylopia aethiopica is efficacious in ameliorating pains .The results from the study demonstrated strong anti- nociceptive than anti-inflammatory activities and as evidenced the bioactive constituents of hydro-alcoholic formulations, particularly, hydro-methanolic, potentiated the effects which resonated with the local use of finished products and practices for medicine purposes. Studies with the pure samples are on the way in order to understand the precise mechanisms of action.

**Key words**: Analgesic & anti-inflammation activity, Piroxicam, *xylopia aethiopica*, hydro-alcoholic formulation, paw oedema, sex.

*Corresponding Author Email: anwafor2000@yahoo.com,
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## INTRODUCTION

The efficacy of plant-based drugs used in traditional medicine has received much attention recently: accordingly 80% of the populations in some Asian and African countries depend on traditional medicine for primary health care; and in many developed countries, 70% to 80% of the population has used some form of alternative or complementary medicine[1.] However, a wide variety of the available orthodox or synthetic analgesics and anti-inflammatory agents and many non-steroidal anti-inflammatory agents are not without adverse side effects such as respiratory depression, addiction and gastrointestinal irritations, and the development of a truly non-toxic, yet potent, broad spectrum anti-inflammatory-non-narcotics from natural substances is required. Hence, there is therefore the necessity for a worldwide trend search for alternative anti-inflammatory drugs or biologically active botanically substances which are co-friendly in approach and interfere with the natural patterns of body functions. Locally, in Nigeria, one of the large numbers of cheap indigenous drugs – xylopia aethiopica has been mentioned to possess potent anti-inflammatory and anti-nociceptive properties. It is traditionally employed alone or in combination along other medicinal plants for the management of chronic severe painful conditions such as post partum tonic or usage as abortifacient and in the control of fertility locally. It has been employed in the management of pain and treatment of boils, skin eruptions and rheumatism [2.]Chemical analysis of novel xylopia aethiopica formulations based on folklore methods of the finished natural products and practices revealed varying and statistically significant differences in the percent bio-active phyto-medicines [3] and phyto-mineral elements [in our unpublished data] and ranked the percent bioactive contents of the novel formulations as : hydro-methanolic > hydro-ethanolic > methanolic > ethanolic = aqueous respectively. Perusal of the literature on scientific evidence from tests done to evaluate anti-nociceptive and anti-inflammatory activities of medicinal uses of xylopia aethiopica based on folklore methods of finished medicine products and practices are limited. Similarly, there is little research regarding information on the analgesic and anti-inflammatory activities of xylopia aethiopica in vivo and in vitro. Hence in the present investigation anti-nociceptive and anti-inflammatory potential of novel xylopia aethiopica formulations based on folklore methods are explored in rodent models based on the method first reported [4] and adopted by Ugo Basile (Italy) which is associated with the involvement of the peripheral mechanisms of inhibiting cyclooxygenases (COXs) with the resultant decrease in prostaglandin (PG) production; and consequently reduction of pain and inflammation.

## MATERIALS AND METHODS:

**Plant material:**

Whole dried fruits of *xylopia aethiopica* (Dunal) A.Rich were purchased from Ariaria International market Aba, Abia State, Nigeria and identified and authenticated by Dr. (Mrs.) Essiet a taxonomist in the Department of Botany University of Uyo, Akwa Ibom State, Nigeria where voucher specimens are preserved. The Voucher number is UUH 1819 and is deposited in the herbarium of the Department of Pharmacognosy and National Medical University of Uyo, Akwa Ibom State, Nigeria for further reference.

**Preparation of novel xylopia aethiopica formulations:**

The carpals of the *xylopia aethiopica* were removed from their strands, washed free of sand and sundried for 7 days. Two kilogram (2kg ) dried fruits of *Xylopia aethiopica* were pulverized using an electric grinding machine/blender (model ms-233 China) into fine powdery form [5,6,] The resulting 100 gram powder was macerated in 1000ml of hydro-methanol (1:4, v/v), hydro-ethanol (1:4, v/v), methanol and aqueous medium respectively under room temperature for 20 hours with constant shaking in a shaker (Stuart Scientific Orbital Shaker, UK). The extracts were filtered with Whatman NO.1 filter paper and the resulting filtrates were concentrated to dryness in vacuum rotary evaporator at $40^0$C to obtain 175gram weight each of hydro-methanol, hydro-ethanolic, methanolic and aqueous residues (brownish-black gel). 8.75gm from which fresh solution was reconstituted in 100ml of extracting solvent to give the required low doses of 0.5 and 2mg/kg body weight respectively for the studies which are relatively below the $LD_{50}$ or that which is generally employed for most investigations [7-10]

**Experimental animals:**

One hundred (100) healthy Albino rats of either sex, weighing between 150-200 gm obtained from the animal unit of University of Nsukka, Enugu State was used in this study. They were kept under standard laboratory condition at the animal house facility of the Department of Human Physiology University of Port Harcourt (temperature 28 – $31^0$C, photo period of 12 hours natural light and 12 hours dark cycle, and humidity of 50 – 55%. They were acclimatized for two weeks before the commencement of experiment with free access to solid rat pellets diet (commercial layer mash, product of to feed LTD, Sapele Nigeria), water and feed were provided *ad-libitum* throughout this study. The animals were divided into 4 groups and 5 subgroups containing 5 animals (3 females and 2 males) per sub-group. The experimental protocol has been approved by institutional animal ethical committee. The control groups (Group 1, males and 11,

females) were given piroxicam at the dose of 20mg/kg intraperitoneally which was considered as standard according to the animal's body weight. Groups 111 and 1V which are males and females received 0.5mg/kg of the various extracts respectively, while Group V and V1 which were also both sexes received 2mg/kg of the various novel xylopia aethiopica formulations respectively. Doses were given intraperitoneally with the help of syringe. All animals were treated according to the National Institute of Health Guidelines for the care and the use of laboratory animals Guide to the Care and Use of Experimental Animals, Vol. 1 (2nd ed., 1993) and Vol. 2 (1984)

**Nociceptive Thresholds:**

The mechanical nociceptive threshold was quantified in the rat paw withdrawal test [4] using an analgesy meter (Model No. 15776, Ugo Basile, Comerio, Italy), based on the Randal and Selitto test[4] which generates a linearly increasing mechanical force. While the inflammation study was carried out using plethysmometer designed to measure inflammatory swelling in rodent model. Small differences in paw volume are detected by a transducer and it has a 0.01ml resolution. The results represented the maximal pressure (expressed in grams) tolerated by the animals. Thus the mechanical nociceptive threshold response utilizes a monosynaptic pathway involving higher centres. Pressure was gradually applied to the right hind paw and paw withdrawal thresholds (PWTs) were assessed as the pressure (grams) required eliciting paw withdrawal. A change in hyperalgesic state was calculated as a percentage of the maximum possible effect (% MPE). On the test day, a baseline measurement was taken 30 min before animals were administered 0.2ml of eggwhite [5] into the planter surface of the right hind limb paw for production of oedema or local swelling. PWTs were determined again 30min after administration of eggwhite to establish that mechanical hyperalgesia had developed. Xylopia aethiopica formulations (0.5 and 2mg $kg^{-1}$) or Piroxicam (20mg $kg^{-1}$) were administered 30 min post-eggwhite and PWTs were taken again 30 and 60 min post eggwhite administration. Reaction time (latency period) was recorded when animals licked their hind paws, or jumped prior to and 30 and 60 min after intra-peritoneal administration of the samples (the reaction of the animals considered as the post – drug reaction time). At each time point, animals were tested with three trials, and the values were averaged.

**Statistical analysis:**

The differences among experimental and control groups were determined using SPSS for window XP software programme (version B.) and one-way ANOVA followed by LSD multiple comparisons. Significant differences between control and experimental groups were assessed. The data were expressed as mean = SEM, n=5, ($P \leq 0.05$) using One way ANOVA followed by

LSD post hoc.

**Analgesic activity in rats**

Both novel xylopia aethiopica formulations at doses as low as 0.5mg/kg and 2mg/kg as well as the reference drug piroxicam caused significant increase ($P<0.001$) in the reaction time. The increase in latency period at different time points significantly differed ($P<0.01$) compared to baseline values within the same drug treated groups. The percentage increase in the reaction time was dose-dependent and differed significantly between both sex of rats ($P<0.001$) receiving different dose levels of the extract. The percentage increase in the reaction time caused by the xylopia aethiopica formulations was detectable and peaked at +60min but thereafter declined after the administration of the extract. Generally, the hydro-methanolic formulations showed significant anti-nociceptive and anti-inflammatory activities compared with others.

## RESULTS AND DISCUSSION

The interplay between the use of safe, effective and quality products and practices, based on local available evidence of finished biological active botanical substances are limited. Our results showed at doses as low as 0.5mg/kg and 2mg/kg respectively, xylopia *aethiopica* dose- and sex dependently *exhibited* potent anti-inflammatory (Figure.1-6 ) and anti-nociceptive (Figure.7-12) activities compared to the reference drug piroxicam as evaluated in eggwhite induced rat paw oedema which highly correlated with its local use in relief of human pain perception.

As shown in Figure 1-6, *xylopia aethiopica* at doses as low as 0.5mg/kg and 2.0mg/kg respectively in the different novel formulations for both sexes, inhibited "mean increase in paw volume" compared with the standard drug, piroxicam, at all the time intervals (+ 30min, +60min). However, xylopia aethiopica anti-nociceptive effects (figure. 7-12) were stronger than that of the anti-inflammatory responses evidenced by significant increase in the reaction time by stimuli in experimental model. The study also showed that xylopia aethiopica effects in ameliorating pain and inflammation was potentiated by hydro-methanolic (1:4 v/v) formulation constituents than other formulations. Preliminary qualitative and quantitative phytochemical [3] in addition to phyto-mineral elements (our unpublished data) studies revealed variability and statistically significant differences in the percent bioactive phyto-medicine and phyto-minerals of novel xylopia aethiopica formulations and showed that hydro-methanolic formulation constituents was rich in phyto-minerals and the following phyto-chemicals alkaloids (66.7%), flavonoids (66.7%), tannin (66.7%) , anthraquinone and combined anthraquinone (44.4 %) and glycosides (100%) compared to other formulation constituents.

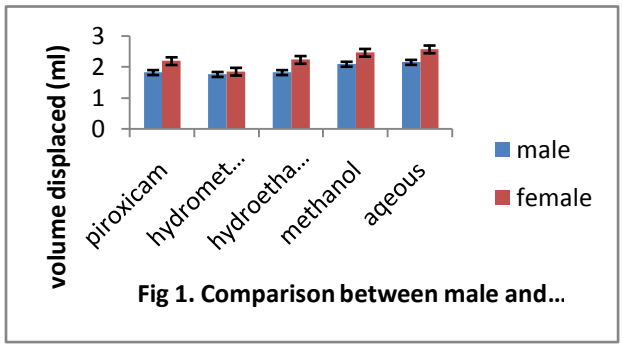


Fig 1. Comparison between male and…

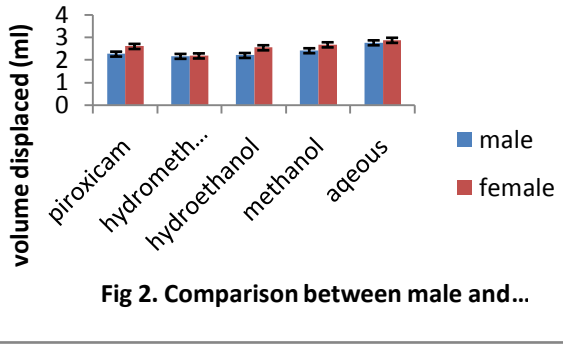


Fig 2. Comparison between male and…

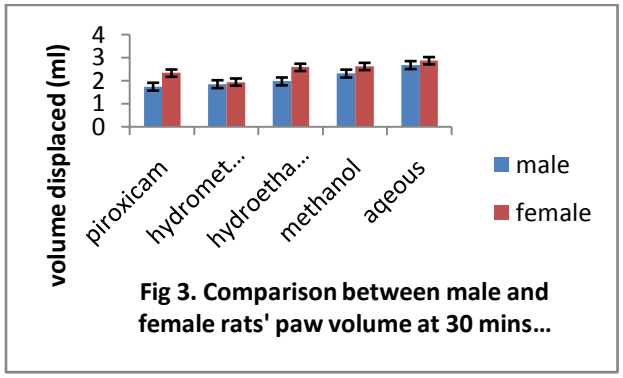


Fig 3. Comparison between male and female rats' paw volume at 30 mins…

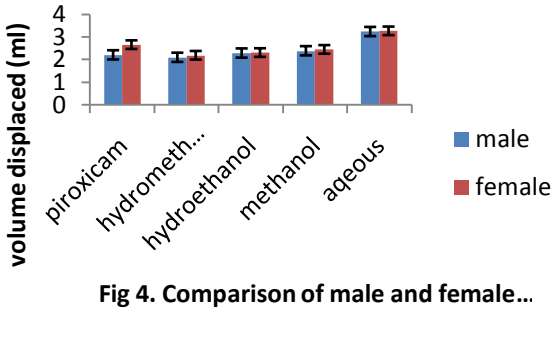


Fig 4. Comparison of male and female…

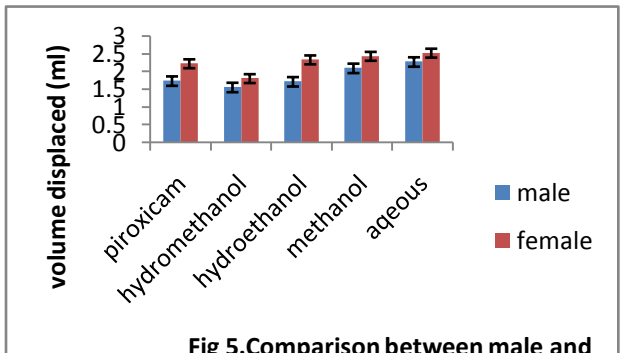


Fig 5.Comparison between male and female rats' paw volume at time 60mins..

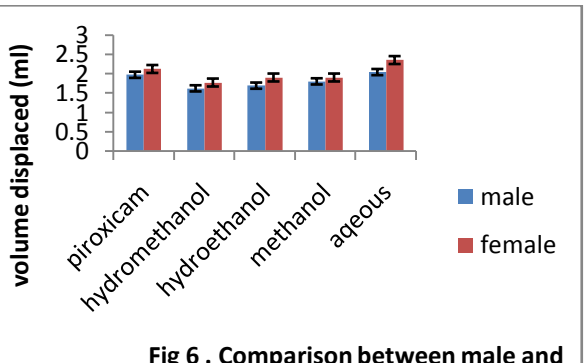


Fig 6 . Comparison between male and female rats' paw volume at time 60 mins.

**Figure 1-6. Comparison of anti-inflammatory potency of novel xylopia aethiopica preparations for both sexes**

This is suggestive probably that the combined effects of both phyto-mineral elements and phyto-chemicals perhaps may be responsible for the potentiating xylopia aethiopica anti-nociceptive anti-inflammatory effects which are in resonate with the local preparations and dispensing of finished products for medicinal purposes; and which needed further investigations. Significant inhibition of paw oedema in the early hours of study by *xylopia aethiopica was* observed which perhaps might be attributable to the inhibition of neurohoromone -serotonin at the crucial points in the brain and /or histamine [12]

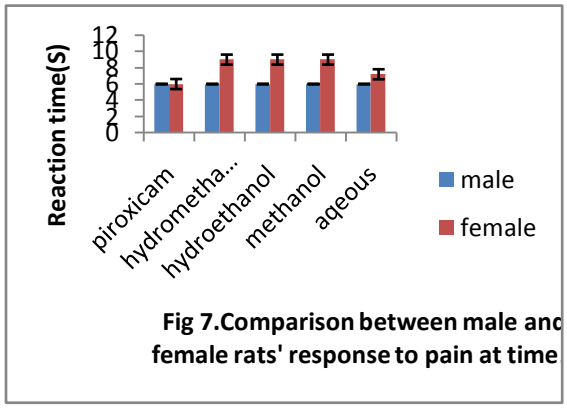


Fig 7.Comparison between male and female rats' response to pain at time

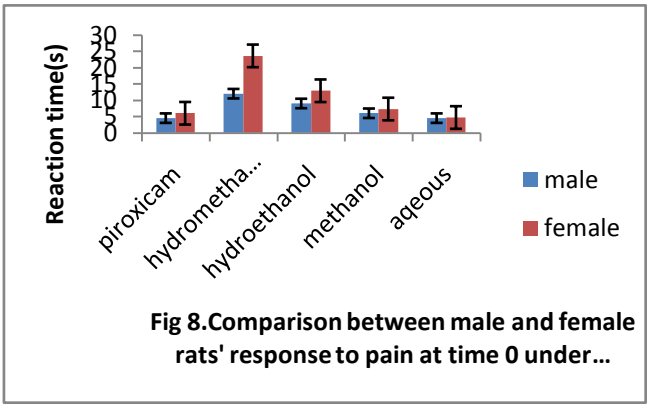


Fig 8.Comparison between male and female rats' response to pain at time 0 under…

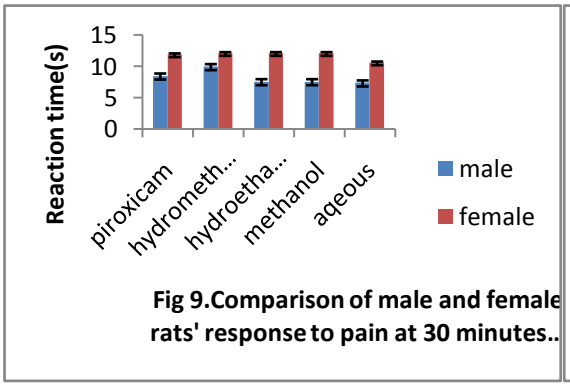


Fig 9.Comparison of male and female rats' response to pain at 30 minutes..

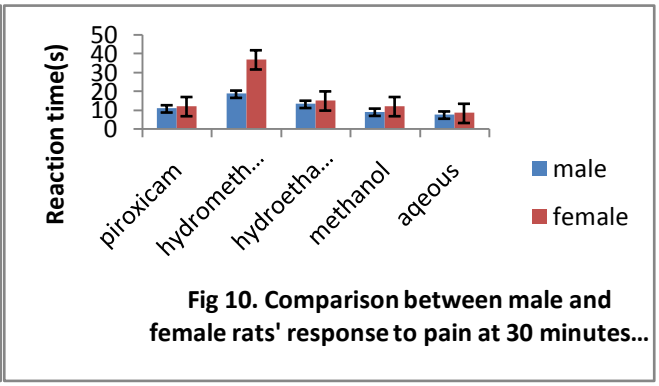


Fig 10. Comparison between male and female rats' response to pain at 30 minutes…

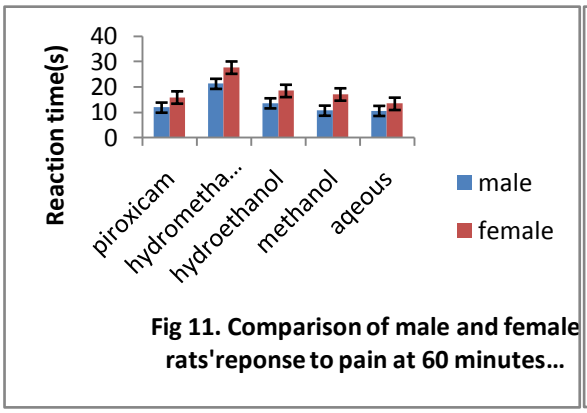


Fig 11. Comparison of male and female rats'reponse to pain at 60 minutes…

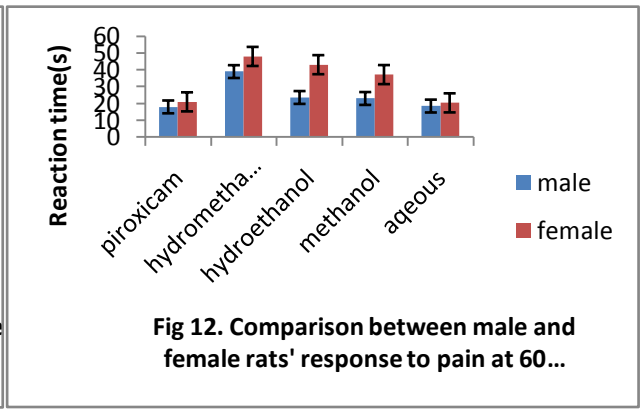


Fig 12. Comparison between male and female rats' response to pain at 60…

**Figure.7-12. Comparison of the relationship between xylopia aethiopica formulations and reaction time for the both sexes**

It is plausible that the significant pain reduction of xylopia aethiopica, might be due to the presence of anti-nociceptive and anti-inflammatory principles acting within the prostaglandin pathways -the inhibition of the enzyme cyclooxygenase and subsequent inhibition of prostaglandin synthesis mechanisms associated with many non-steroidal anti-inflammatory agents (NSAIDS)[13,14] The increase in prostaglandin levels within the peritoneal cavity has been implicated in enhancement of the inflammatory pains by increasing capillary permeability[15] Flavonoids "nature's biological response modifiers" have been reported to play a role in differential anti-nociceptive and anti-inflammatory activities primarily by targeting

prostaglandins [16].There are also reports on the role of tannins in anti-nociceptive activity[17] Besides, alkaloids are well known for their ability to inhibit pain perception [18] Our results therefore are suggestive that xylopia aethiopica extract may be acting via mechanisms similar to non-steroidal anti-inflammatory/ anti-nociceptive drugs.

## CONCLUSION:

In conclusion our results showed that *xylopia aethiopica* fruit possesses anti-nociceptive and anti-inflammatory properties and its effects were potentiated by hydro-methanolic formulation constituents compared to others. Studies to identify the active components, specifically, in hydro-methanolic formulations, are needed for further pharmacological and toxicological characterization, such as the research of the mechanisms involved in the central and peripheral analgesic effect.

## REFERENCES

1. National Policy on Traditional Medicine and Regulation of Herbal Medicines. Report of a WHO Global Survey, update May 28, 2012.
2. Iwu M. Handbook of African Medicinal Plants CRC Press, Boca Raton FL; 2003.
3. Nworah DC, Nwafor A, and Bekinbo MT. Comparative Characterization of Phytomedicinal Constituents of Xylopia Aethiopica. Am J PharmTech Res 2012; 2(2): 1-7.
4. Randall LO, Selitto JJ. A Method for Measurement of Analgesic Activity on Inflamed Tissue. Arch Int Pharmacodyn Ther 1957;111( 4): 409-419.
5. Nwafor A, Kalio ID. Physiological effects of *xylopia aethiopica* on pregnancy in albino rats. J Med Pharm Sci 2006;2(1): 1-4
6. Nwafor A, Egberike EF and Nworah DC. Assessment of contraceptive effect of aqueous fruit extract of *xylopia aethiopica* in female wistar rats. Afri J Med Sci 2011;4(2): 93-96.
7. Abolaji OA, Adebayo AH, Odesanmi OS. Nutritional qualities of three medicinal plant parts (*xylopia aethiopica*, Blighia sapida and Parinari polyandra) commonly used by pregnant women in the western part of Nigeria. Pakistan J Nutri 2007;6(6): 665 -668
8. Nnodim J, Emejulu A, Amachi A, Nwosu NEC. Influence of *Xylopia aethiopica* fruits on some hematological and Biochemical Profile. Al Ameen J Med Sci 2011;4(2): 191-196
9. Edeoga HO, Okwu DE, Mbaoble BO. Phytochemical constituent of some Nigerian Medicinal plants. Afri J Biotecol 2005; 4(7): 685-688

10. Ogbonnia S, Adekunle AA, Bosa MK, and Enwuru VN. Evaluation of acute and sub acute toxicity of Alstonia congensis Engler back and *xylopia aethiopica*(Dunal) A. Rich (Annonaceae) fruits mixtures used in treatments of diabetes. Afri J Biotechnol 2008;7(6) : 701-705
11. Winder, CV, Wax, J, Burr, V, Been, M. & Rosiere, CE. A study of pharmacological influences on ultraviolet erythema in guinea-pigs. Arch Int Pharmacodyn 1958; 116: 261-292.
12. Hirasawa N, Watanabe M, Mue S, Tsurufuji S, Ohuchi K. Downward regulation of neutrophil infiltration by endogenous histamine without affecting vascular permeability responses in air pouch type carrageenan inflammation in rats. J Inflammation 1991; 15: 117–126.
13. Seibert K, Zhang Y, Leahy K et al. Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. Proc Natl Acad Sci 1994; 91: 12013–12017.
14. Panthong A, Norkaew P, Kanjanapothi D, Taesotikul T, Anantachoke N, Reutrakul V. Anti-inflammatory, analgesic and antipyretic activities of the extract of gamboge from *Garcinia hanburyi* hook f. J Ethnopharmacol 2007;111: 335-340.
15. Zakaria ZA, Abdul Gani ZDF. Antinociceptive, anti-inflammatory, and antipyretic properties of an aqueous extract of Dicranopteris linearis leaves in experimental animal models. J Natural Medicines. 2008; 62: 179-187.
16. Rajnarayana K, Reddy, MS, Chaluvadi MR, Krishna DR. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. Indian J Pharmacol 2001; 33: 2-16
17. Vanu MR, Palanivelu S, Panchanatham S. Immunomodulatory and anti-inflammatory effects of *Semecarpus anacardium* Linn. Nut milk extract in experimental inflammatory conditions. Biological and Pharma Bulletin. 2006;29: 693-700. DOI:10.1248/bpb.29.693.
18. Uche FI, Aprioku JS. The phytochemical constituents, analgesic and anti-inflammatory effects of methanol extract of *Jatropha curcas* leaves in mice and Wister albino rats. J Applied Sci Environmental Management. 2008; 12(4): 99–102.
19. Guide to the Care and Use of Experimental Animals, Vol. 1 (2nd ed., 1993) and Vol. 2 (1984)