

Larvicidal and Anti-inflammatory Activities of *Funtumia Africana* (Benth) Stapf Leaf and Stem.

Fred-Jaiyesimi A. Adediwura¹, Dunkwu Tochukwu¹, Okunroumu Oluwanisola¹

*Corresponding author:

Fred-Jaiyesimi A. Adediwura

¹Department of Pharmacognosy, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu Campus, Ogun State, Nigeria.

Abstract

Funtumia africana (Benth) Stapf, one of the two species of the genus *Funtumia*, is used in folklore medicine for the treatment and management of fever, inflammation and incontinence of urine. In this study, a comparative phytochemical analysis, larvicidal and anti-inflammatory effects of the leaf and stem of *F. africana* using established procedures were carried out. The phytochemical analysis revealed the presence of saponins, flavonoids, anthraquinone and cardiac glycosides. The methanol extracts, petroleum ether, ethyl acetate and chloroform fractions of the leaf and stem exhibited larvicidal and anti-inflammatory effects.

Keywords: *Funtumia africana*, Larvicidal, Antiinflammatory Activities.

Introduction

Funtumia africana (Benth) Stapf, a member of the Apocynaceae family is characterized as a tree of about 30 m in height and usually found in the deciduous and evergreen forests of Guinea-Bissau through West Cameroons to Fernando-Po. In the genus *Funtumia*, there are only two species *F. elastica* (Female) and *F. africana* (Male) [1,2]. The two species of the genus *Funtumia* have similar characteristics and can only be distinguished from each other by rubbing the latex between fingers. The latex of *F. elastica* coagulates into balls when rubbed between fingers while that of *F. africana* does not [3] and the flowers of *F. elastica* are shorter than those of *F. africana* [1].

F. africana commonly referred to as bush rubber, bastard wild rubber or Lagos rubber, Ako ire (Yoruba, SW Nigeria) [2] is used in traditional medicine for the treatment of fire burns [4,5], inflammation [6], incontinence of urine [7], amoebic dysentery [8] and malaria [9].

Previous chemical studies have reported the isolation of alkaloids [10] such as Funtumine and funtumidine, which are used as hypotensive, antipyretic and as a local anaesthetic [10]. This study aims at investigating the larvicidal and anti-inflammatory activities of the extracts and fractions of the leaf and stem bark of *Funtumia africana*.

Methodology

Plant Material

The fresh leaves and stem bark of *F. africana* were collected from Oyo State in September, 2011. The plant was authenticated by Mr

T.K Odewo at the Department of Botany, Faculty of Science, University of Lagos. The herbarium specimen deposited at the herbarium with voucher number LUH 4063.

Extraction

The fresh leaves and stem bark were dried and powdered in an electric miller. The powdered samples were each macerated in 80% methanol for 72hrs. The filtrate were concentrated in a rotary evaporator. The dried methanol extracts were reconstituted in water and successively partitioned with Pet ether, ethyl acetate and chloroform. The fractions were dried under reduced pressure and stored for further studies.

Phytochemical Screening

The phytochemical screening of the leaf and stem bark of *F. africana* was carried out using standard procedures [11,12].

Antiinflammatory Activity

Animals

Wistar albino rats (60-90g) of both sexes were used and assay was performed according to International guiding principles concerning the care and use of laboratory animals. The egg-induced oedema model was used according to the methods of Winter *et al.*, 1962 and Arunachalam *et al.*, 2009 [13,14] with little modifications.

The rats were divided into six groups (n=5)

Group A – Rats received 180mg/kg of methanol extract

Group B – Rats received 180mg/kg of Pet ether fraction

Group C – Rats received 180mg/kg of ethyl acetate fraction

Group D- Rats received 180mg/kg of chloroform fraction

Group E- Rats received 180mg/kg of distilled water

Group F- Rats received 5mg/kg of Diclofenac sodium

The methanol extracts, fractions and standard drugs were administered to the respective groups. Thirty minutes after administration of the extracts/fractions, standard drugs and control, 0.1 ml of the egg albumin were injected into each of the right hind rat paw. The rat paw circumference of the animals were measured at interval of thirty minutes (30 minutes) (0, 30, 60, 90, 120, 150 and 180min) for 3 hrs.

The percentage inhibition of oedema was calculated

$$\%PI = \frac{I_0 - I_t}{I_0} \times 100$$

Larvicidal Activity

Larva collection

Larvae of *Culex pipiens* were collected from a stagnant polluted puddle at Aiyeye road, Sagamu, Ogun State, Nigeria.

The collected larvae were authenticated by comparing their features with those reported in the literature. *Culex pipiens* are characterized by slightly longer, constricted antennae and prominent antennal tuft (the tufts on the siphon have multiple hair).

Extraction

The test solutions of the Pet ether, ethyl acetate and chloroform fractions of both the leaf and stembark of *F. africana* were prepared in required well water at doses of 2000µg/ml, 1000µg/ml, 500µg/ml, 250µg/ml, 125µg/ml and 62.5µg/ml.

The larvicidal bioassay was carried out according to the WHO methodology [15]. The extracts and fractions were prepared in well water and transferred into sterile petri dishes. Twenty third and fourth instars larva of *Culex pipiens* were introduced into each of the extracts and fractions.

The mortality rates were recorded after 24 and 48 hr exposure. The larvae were considered dead if the larva did not move after touching with a needle [16,17] or if it showed change in colour or an abnormal position. 1% ethanol was used as reference. The percentage mortality was calculated:

$$PM = \frac{\text{Death count of larvae}}{\text{Initial larvae population}} \times 100$$

Results

Table 1: Phytochemical Screening of leaf and stem bark of *Funtumia africana*

	Saponins	Tannins	Free anthraquinone	Combined anthraquinone	Alkaloids	Flavonoids	Cardiac glycoside
Leaf	+	-	+	+	++	+	+
Stem bark	++	+	+	+	++	+	ND

+ = Present; ++ = Highly present; - = Absent; ND = Not Detected.

Antiinflammatory activities of Extract and Fractions of *F. africana* leaf at 180mg/ml

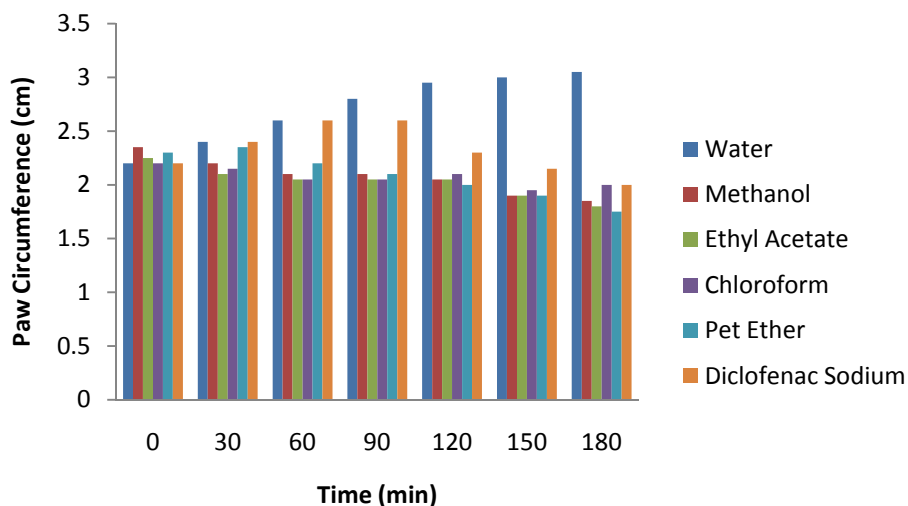


Figure 1: Anti-inflammatory Activities of extract and fraction of *F. africana* Leaf at 180mg/kg

Antiinflammatory Activities of Extract and Fractions of *F. africana* Stembark at 180mg/ml

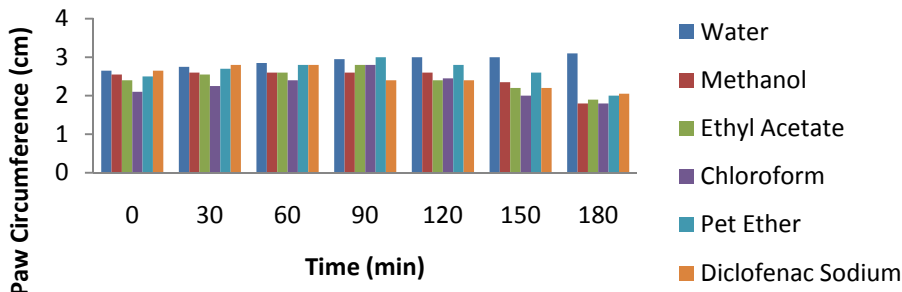


Figure. 2: Anti-inflammatory Activities of extract and fraction of *F. africana* Stem bark at 180mg/kg.

Percentage mortality of Extract and fractions of leaf of *F. africana* after 24hr Exposure

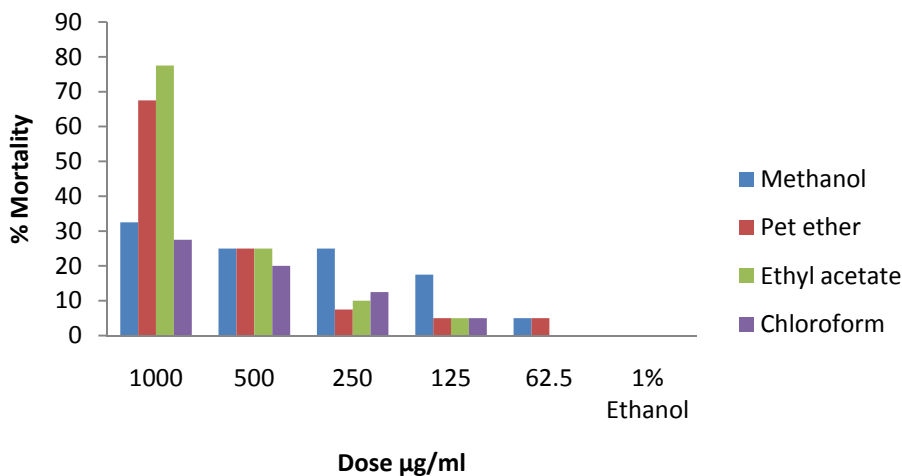


Figure. 3: % Mortality of Extract and fractions of *F. africana* after 24 hr Exposure.



% Mortality of Extract and Fractions of *Funtumia africana* Leaf After 48hrs Exposure

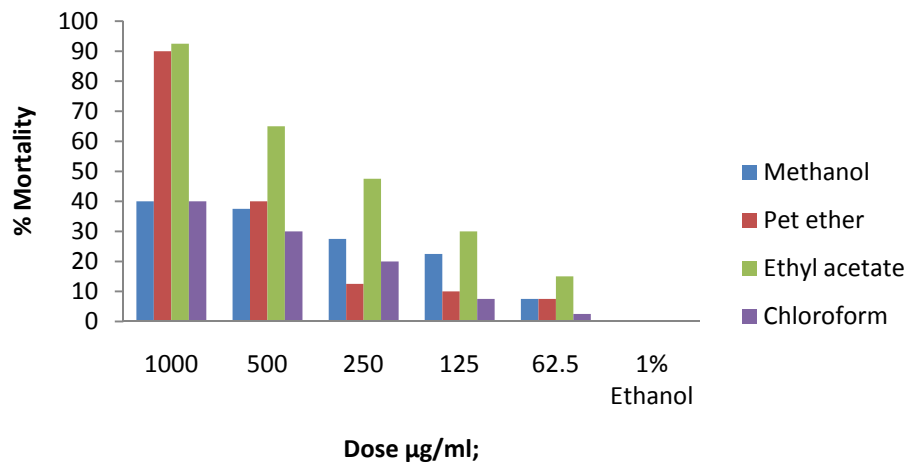


Figure 4: % Mortality of Extract and Fractions of *F. africana* leaf after 48 hr Exposure.

% Mortality of Extract and Fractions of *F. africana* stem bark after 24 hrs Exposure

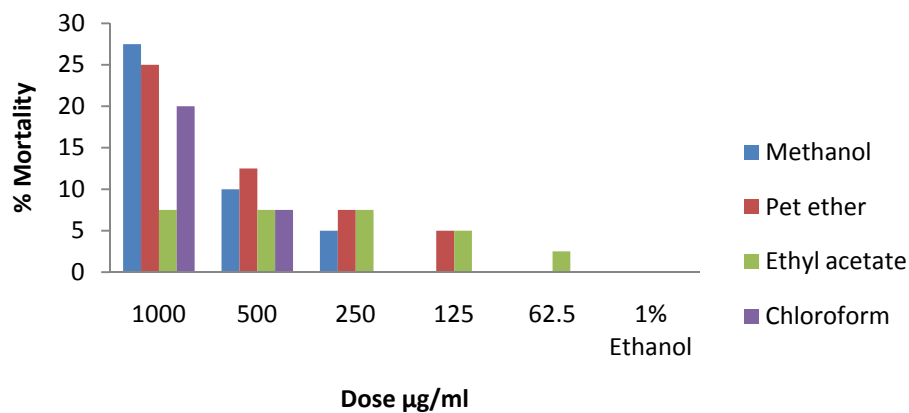


Figure 5: % Mortality of Extract and Fractions of *F. africana* stem bark after 24 hrs Exposure.

% Mortality of Extract and Fractions of *F. africana* Stembark after 48 hrs Exposure

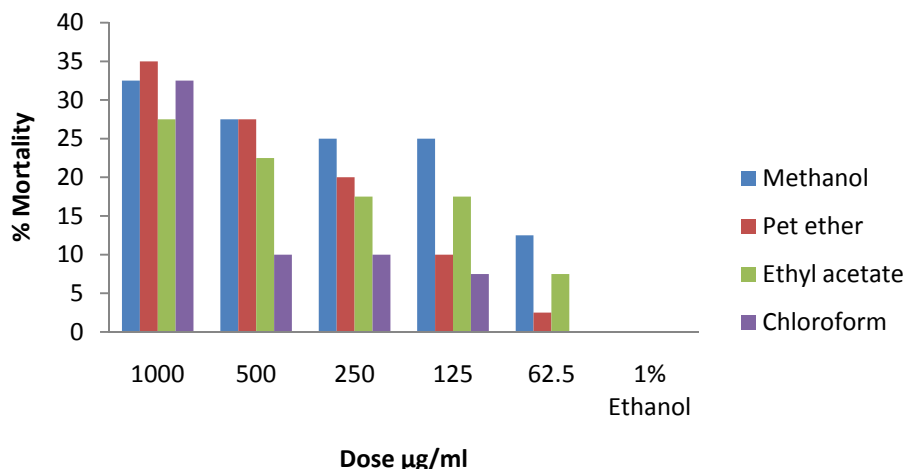


Figure 6: % Mortality of Extract and Fractions of *F. africana* stem bark after 48 hrs Exposure.

Discussion

F. africana (Benth) Stapf a member of the Apocynaceae family is used in the treatment of several ailments including inflammation and malaria. Phlogistic agent (egg albumin model) used in this study to induce inflammation and exhibit inflammation in three phases. The early phase mediated by histamine within the first two hours, intermediate phase caused by bradykinin and the late phase by synthesizing of prostanoid [18,19]. In previous studies on the acetone crude extract of *F. africana* leaf and the hexane and chloroform fractions of the acetone crude extract exhibited moderate activity against the COX-1 and COX-2 assays.[20] The previous study and this study have been able to establish that the compounds responsible for anti-inflammatory activity are moderately polar in nature.

The phytochemical screening of the leaf and stem bark of *F. africana* revealed the presence of Saponins, Anthraquinone, flavonoids, cardiac glycosides in both the leaf and stem bark. However tannins were found present in the stem bark but absent in the leaves. Table 1. These secondary metabolites in *F. africana* are similar to those present in *F. elastica* as reported by Adekunle and Ikumapayi (2006) [21]. This therefore suggests that the two species of the genus *Funtumia* are rich sources of flavonoids, saponins, anthraquinone and steroids.

In the anti-inflammatory study, there were similarities in the activities of the methanol extract, ethyl acetate, chloroform and pet ether fractions of the leaf with the highest percentage inhibition of 39.3%, 40.9%, 34.4% and 42.6% respectively at 180 mins. Fig 1.

The extract and fractions were able to reduce inflammation at the early, intermediate and later phases.

In the case of the stem bark, the effect of the extract and fractions on inflammation were not as much as those of the leaf between 0 and 150 mins, while at 180 mins, the methanol extract, ethyl acetate, chloroform, pet ether fractions of the stem bark and Diclofenac Sodium exhibited a percentage decrease in inflammation of 41.9%, 38.7%, 41.9%, 35.5% and 33.9% respectively. Fig. 2. The effect of the methanol extract, ethyl acetate, chloroform fractions of the leaf at 30, 60, 90, 120 and 150 minutes were more pronounced than the extract and fractions of the stem bark at the same time interval.

The anti-inflammatory activity of the standard drug Diclofenac sodium (5mg/ml) was not as effective between 30, 60 and 90 mins compared to the effect of the leaf extract and fractions.

The anti-inflammatory activity exhibited by pet ether, ethyl acetate and chloroform fractions of *F. africana* indicates that the compounds responsible for this effect have varying polarity in nature.

The results therefore indicate the possible ability of the extract and fractions of *F. africana* to inhibit bradykinin, the synthesis of prostanoid, generation of excessive nitric oxide and cytokinase.

The presence of flavonoids as indicated in the phytochemical analysis, could also be responsible for the anti-inflammation effects. This is because previous studies have reported the importance of flavonoids in the inhibition of prostaglandin synthesis [22,23,24].

In the larvicidal studies, the crude extracts and fractions of *F. africana* exhibited varying degree of larvicidal activities at different concentrations by exhibiting a dose-dependent larvicidal activities.

The ethyl acetate and pet ether fractions of the leaf exhibited the most pronounced mortality of 77.5% and 67.5% at 1000µg/ml over 24 hr exposure as well as 92.5% and 90% over 48 hr exposure. The least activity was observed in the chloroform fraction over 24 and 48 hr exposure (27.5% and 40% respectively) at 1000µg/ml. Fig. 3 and 4.

The larvicidal activities exhibited by the extract and fractions of the stem bark were low. The pet ether fraction of the stem bark of *F. africana* exhibited a 35% mortality at 1000µg/ml over 48 hrs exposure. Fig.6.

The Pet. Ether and ethyl acetate fractions of the leaf of *F. africana* exhibited a dose-dependent effects. There were however similarities in the larvicidal effects of the two fractions after 24 hr and 48hr exposure. The ethyl acetate fraction at 1000, 500, 250, 125 and 62.5 µg/ml doses exhibited 92.5%, 65%, 47.5%, 30% and 15% mortality rate while the petroleum ether fraction exhibited 90%, 40%, 12.5%, 10% and 7.5% mortality rate respectively after 48hr exposure at 1000µg/ml, 500µg/ml, 250µg/ml, 125µ/ml, 6.5µg/ml..

The larvicidal effect of the leaf of *F. africana* was more pronounced than that exerted by the stem bark with the ethyl acetate fraction of the leaf exerting the most pronounced larvicidal effects.

This study therefore suggests that compounds mainly responsible for the larvicidal activity in the leaf of *F. africana* are mainly non-

polar and moderately polar. This is similar to previous reports on the activities of non-polar and moderately polar compounds exhibiting larvicidal activity against the mosquito larvae. Furthermore, the study shows diversity in the nature of compounds exhibiting these larvicidal activities in the leaf.

The larvicidal activities by the extract and fractions of the leaf and stem bark of *F. africana* might be due to independent or synergistic effects of the compounds by inhibiting the growth of larvae, reduced fertility or fecundity [25].

The ethyl acetate and chloroform fractions of the leaf exhibited the highest percentage decrease of inflammation at 60 min and 90 mins respectively. Though the activities of the methanol, ethyl acetate and chloroform fractions were comparable and more effective than the reference drug Diclofenac sodium.

The chloroform fraction of the stem bark of *F. africana* was the most effective of all the fractions of the stem bark.

Though both the stem and leaf of *F. africana* possesses anthraquinone, flavonoids, saponins, alkaloids and cardiac glycosides, the pronounced anti-inflammatory activities exhibited by the leaf might be due to the synergistic effect of these secondary metabolites. This study therefore has been able to justify the use of *F. africana* as part of traditional recipes for the treatment of inflammation and malaria as previously reported by other studies.

References

- [1]. Burkill HM. The useful plants of the West Tropical Africa. Royal Botanic Garden, Kew, 1997
- [2]. Keay RWJ, Onochie CFA, Stanfield DF. Nigerian Trees. Nigeria National Press Ltd. Lagos, 1964: 495.
- [3]. Burkill HM. The Useful plants of West Tropical Africa, Royal Botanic Garden, Kew, 1985: 318 – 388.
- [4]. Cooper GP and Record SJ. The evergreen Forest of Liberia. Bull Yale Sch For., 153pp
- [5]. Wagner H, Seegert K, Sonnenbichler, H, Ilyas M. Steroids alkaloids of *Funtumia africana*. Planta Medica, 1987; 53(5): 444 – 9.
- [6]. Barros D, Amaral OB, Izquierdo I, Geracitano L, Do Carmo Bassols Rajeira M, Henriques AT, Ramirez MR. Behavioural and genoprotective effects of *Vaccinium* berries intake in mice. Pharmacol Biochem Behav., 2006; 84(2): 229 -34.
- [7]. Ainslie JR (1937). A list of plant used in Native Medicines in Nigeria. Imperial Forestry Institute, Oxford Paper 7, 1937; 30.
- [8]. Haerdi F. Native Medicinal Plants of Ulanga District of Tangayika (East Africa) Dissertation, Verlag Fur Recht Und Gesellschaft, AG Basel.1964
- [9]. Odugbemi TO, Odunayo R, Akinsulire IE. Medicinal plants useful for malarial therapy in Okeigbo. African Journal of Traditional Complementary and Alternative Medicine, 2007; 4(2): 191 – 198.
- [10]. Bouquet A and Debray, M. Plantae Medicinalis de Cote d'Ivoire. Document Orstrum, 1974; 32:63
- [11]. Harborne JB. Phytochemical Methods. 2nd ed., Chapman and Hall, Ltd, London, 1991; 228.
- [12]. Evan, WC. Trease and Evans Pharmacognosy 15th ed.. Harcourt Publisher Limited, London, 2002; 585.
- [13]. Winter C, Risley E, Nuss G. Carrageenan- induced edema in hind paw of rats as an assay for anti-inflammatory drug. Proc Soc Exp Biol Med., 1962, 111: 207 – 210.
- [14]. Anosike CA and Obida O. Anti-inflammatory and antiulcerogenic effects of ethanolic extracts of Coconut on experimental rats. African J Food Agric Nutr Dev., 2010, 10(10):4286 – 4360.
- [15]. WHO. Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides, 1981; WHO/VBC/81.807

- [16]. Abdul RA, Geetha G, Venkatesan P and Kannappan G. Larvicidal activity of some Euphorbiaceae plant extracts against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitol. Res., 2008; 102: 867 – 873.
- [17]. Das NG, Goswani D and Rabha B. Preliminary evaluation of mosquito larvicidal efficacy plant extracts. J Vector Borne Dis., 2007; 44: 145 – 148.
- [18]. Amos S, Chindo B, Edmond I, Akah P, Wambebe C, Gamaniel K. Antiinflammatory and anti-nociceptive effects of *Ficus platyphylla* in rats and mice. *J. Herbs, Spices and Med Pl.*, 2002, 9:47 – 53.
- [19]. Pérez Guerrero C, Herera MD, Ortiz R, Alvarez e Sotomayo M, Fernández MA. A pharmacological study of *Cecropia obtusifolia* Bertol aqueous extract. J Ethnopharmacol. 2001; 76(3); 279 – 284.
- [20]. Ramadwa TE. Isolation and characterization of antimicrobial compounds from *Funtumia Africana* (Apocyanaceae) leaf extracts, M.Sc dissertation, University of Pretoria, Pretoria. 2010
- [21]. Adekunle AA and Ikumapayi A. Antifungal property and phytochemical screening of the crude extracts of *Funtumia elastica* and *Mallotus oppositifolius*. West Indian Med J, 2006; 55(4): 219.
- [22]. Clavin M, Redko F, Aceredo C, Martino V, Gorzalgang S. In vivo anti-inflammatory activity and flavonoid identification of medicinal *Eupatorium* species. Phamacog J. 2013; 5(2): 91 – 93.
- [23]. Wang BS, Huang GJ, Lu YH, Chang LW. Anti-inflammatory effects of an aqueous extract of Welsh onion green leaves in mice. Food Chem. 2013; 138: 751 -756.
- [24]. Mensah AY, Donkor PO and Fleischer TC. Anti-inflammatory and antioxidant activities of the leaves of *Wissadula amplissima* var *Rostrata*. Afr. J Tradtl Complement Altern Med 2011; 8(2): 185 – 195.
- [25]. Maniafu BM, Lwande Wilber, Isaiah O. Ndiega, Cornelius C. Wanjala, Teresa Ayuko Ayenga. Larvicidal activity of extracts from three *Plumbago* spp against *Anopheles gambiae* Mem Inst Oswaldo Cruz, Rio de Janeiro 2009; 104 (6): 813 – 817.

