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Research Article

Pharmacognostic Screening and Anti-inflammatory Investigation of the Methanol extract of stem bark of *Blighia unijugata* Baker (Sapindaceae)

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ABSTRACT

Introduction: *Blighia unijugata* is a potent medicinal plant extensively employed in traditional herbal remedies for the treatment of various diseases such as fever, fertility, inflammation, hypertension, migraine and treatment of infections due to microorganisms.

Aim: This work, investigates the pharmacognostic screening and anti-inflammatory activities of the methanol extract of *B. unijugata* stem bark.

Method: Collection, drying, pulverization, and methanol extraction of the stem bark were done accordingly. The screening of phytochemical constituents and Pharmacognostic numerical data were carried out. The chromatographic analysis was carried out using TLC. The acute toxicity was determined using Lorke's method. Methanol extract was investigated for anti-inflammatory effect in albino rats using egg-induced hind paw oedema at doses of 200, 400 and 600mg/kg body weight respectively.

Result: The macroscopical investigation showed the stem bark outer layer is greyish and, the inner layer is pale reddish brown, disagreeable odour, bitter, Microscopical screening revealed the presence of starch grains, trichomes and sclerenchyma cells. Chemomicroscopic result revealed the presence of lignin, starch, calcium oxalate, cellulose, stone cells. Qualitative phytochemical screening of the powdered bark showed the presence of saponins, steroids, tannins, and resins. Numerical data: moisture content/ weight loss on drying gave value of 7.7%, percentage yield of 6.7%, alcoholic soluble extractive 5.6%, water soluble extractive 6.3%, total ash of 7.1%, acid insoluble ash 0.57% and water soluble ash 4.56%. The chromatographic screening results were close compared with the standard drug. Toxicity test established the lethal dose of greater than 5000mg/kg. There was a significant inhibition of the edema $p > 0.05$, the presence of various bioactive constituents may have contributed to the anti-inflammatory properties of the plant extract.

Conclusion: The result of this study confirms that the barks of *B. unijugata* have anti-inflammatory effect and justifies the use as traditional treatment of inflammation and pain.

Keywords: *Blighia unijugata*, Inflammation, Inflammatory agents, Anti inflammatory

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INTRODUCTION

Herbal medicine includes herbs, herbal materials, herbal preparation and finished herbal products that contain active ingredients of plants' parts, or other plant materials or combinations (Saad *et al.*, 2011)¹. Herbal medicine has been used to treat, alleviate virtually every possible medical condition.

Blighia is a genus of four species of flowering plants in the soapberry family. *Blighia unijugata* belongs to Sapindaceae, native to tropical Africa from Guinea east to Kenya. The species are evergreen trees growing to 15–20 m tall, with pinnate leaves. The flowers are produced in small panicles, unisexual, regular, 5-merous, whitish or yellowish, sweet-

scented. The fruit is an oval capsule 4–8 cm long containing three seeds, each surrounded by an edible fleshy yellow aril, and a thick, leathery orange or red skin; the fruit apart from the aril is poisonous. (Ilesanmi *et al.* 2006)². *Blighia unijugata* is a potent medicinal plant used in Herbal medicine for the treatment of rheumatism, kidney pain and stiffness, and is reputed to have oxytocic action in childbirth. Bark pulp is applied as an enema, a bark decoction is taken to treat fever, and as purgative (Ayodele *et al.*, 2008)³. The common names for *Blighia unijugata* include; Africa-Driehoektolletjies, Kefgna-komy, English-TriangleTops, Oromugna-Adakebo, tucho, Shona-musadima and Isi zulu-umdlaguva. The leaves are eaten as a vegetable in Nigeria. Various parts of the tree are considered to have

Antinociceptive, anti-inflammatory properties⁴ In Côte d'Ivoire leaf pulp is administered as an embrocation, serve as rejuvenate and relaxant. In Congo the leaves are used in vapour baths for the treatment of fever in children, and as tonic. Fruits have been used in Nigeria for the treatment of nausea and vomiting In the Central African Republic seed oil is used as medicinal ointments and the leaf decoction is used in treatment of vertigo . Macerated twigs, leaves, flowers and fruits are used as fish poison. In Sierra Leone the seeds are edible. In South Africa the pleasantly scented flowers are soaked in water to make a fragrant cosmetic perfumes and lotion. *Blighia unijugata* are commonly planted in villages as shade trees (Bekele, 2007)⁵. In Kenya and Ethiopia, the trees are often left after land clearing or sometimes planted to provide top storey shade in coffee plantations. The flowers provide nectar and pollen for honey bees. A leaf decoction is used to treat vertigo. The seed oil is used in preparation of medicinal ointments. Ethanol extracts of the roots, bark and leaves exhibit antibacterial activity (Ayodele *et al.*, 2008) ³. Other uses of *Blighia unijugata* are for light flooring, interior trim, joinery, furniture, mine props, toys, novelties, agricultural implements, musical instruments, boxes, crates, turnery and carving. It is suitable for ship building, preparation of veneer, plywood and pulpwood. It is also used as firewood and for charcoal production (Chi kamai *et al.*, 2010).⁶

Inflammation is a complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells or irritants (Ferrero *et al.*, 2007) ⁷. It is also defined as the reaction of the living body's microcirculation to injury or stressful stimuli. Inflammation is a generic response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen (Abbas, 2009).⁸ When there is a swelling, redness and hurts in a wound, it is a sign of inflammation. Inflammation is the body's immune system's response to stimulus. This can be bacteria colonizing a wound or a splinter piercing through the skin, for instance. Inflammation happens when the immune system combats something that may turn out to be harmful. In some diseases, like arthritis, the body's defence system- triggers an inflammatory response when there are no foreign invaders to fight off. In these diseases, called autoimmune diseases, the body's normally protective immune system causes damage to its own tissues. The body responds as if normal tissues are infected or somehow abnormal. Inflammation is a protective response which naturally involves immune cells, blood vessels, and molecular mediators. The natural purpose of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult, and to initiate tissue repair (Guyton and Hall, 2011).⁹ When inflammation occurs, chemicals from the body's white blood cells are released into the blood or affected tissues to protect the body from foreign substances. The release of chemicals increases the blood flow to the area of injury or infection, and these results in redness and warmth. Some of the chemicals cause a leak of fluid into the tissues, resulting in swelling. It is the protective process that stimulates the nerves and cause pain (Parakrama *et al.*, 2005).¹⁰ Increase in number of cells and inflammatory substances within the affected joint, cause irritation, swelling of the joint lining and eventually wearing down of cartilage (Porth *et al.*, 2007) ¹¹. Inflammation can be classified as either acute or chronic. Acute inflammation is a short-term process, usually appearing within a few minute or hours and begins to cease upon the removal of the injurious stimulus (Cortan *et al.*, 1998).¹² It is characterized by the five cardinal signs (Parakrama *et al.*, 2005).¹⁰ Acute inflammation starts rapidly (rapid onset) and quickly becomes severe. Signs and

symptoms may remain only for a few days, but in some cases may persist for a few weeks. Chronic inflammation means long-term inflammation, which can last for several months and even years. It can result from failure to eliminate whatever the cause of an acute inflammation.

Previous works on *Blighia unijugata*

Ayodele, et al 2008³. Nutritional elements, antibacterial activity and cytotoxicity of the leaf, root and stem bark of *Blighia unijugata* Baker (Sapindaceae). Medicinal and Aromatic Plant Science and Biotechnology 2(2): 137-140.

Ilesanmi, O.O., 2006 ². Comparative studies of the morphology and foliar anatomy of *Blighia sapida* K.D.Koenig and *Blighia unijugata* Baker (Sapindaceae) in Nigeria. BSc Botany degree thesis, Department of Botany, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. 54 pp.

Masette, 2006.¹³ Utilization of *Blighia unijugata* by grey-cheeked mangabeys (*Lophocebus albigena*) in Lwamunda forest reserve, Uganda. In: Biodiversity, conservation and community development. Biodiversity and Medicinal Plant

Oderinde, et al 2009¹⁴. Evaluation of the mineral nutrients, characterization and some possible uses of *Blighia unijugata* Bak. seed and seed oil. Electronic Journal of Environmental, Agricultural and Food Chemistry 8(2): 120-129.

Fowden, et al 1972¹⁵ . Characterized new amino acids from *Blighia unijugata*. Phytochemistry 11: 1105-1110.

Effects of Ethanol Stem Bark of *Blighia unijugata* (Sapindaceae) on Monosodium Glutamate-induced Uterine Leiomyoma in Sprague-Dawley Rats was carried out by George-Asumeng et al 2013 ¹⁶.

MATERIALS AND METHODS

The stem bark of *Blighia unijugata* used for this study was collected in Madonna garden from of Madonna University Elele, Rivers state on January 24th, 2016. The plant was identified and authenticated by Mr. A.O Ozioko of International Centre for Ethno Medicine and Drug Development (InterCEDD) with the number 'InterCEDD 16012' for its botanical identity.

The stem bark were dried at room temperature and pulverized; to provide a large surface area for extraction (Adesanwo *et al.*, 2007).¹⁷ Then it was stored in a closed container.

Chemomicroscopic Analysis of the powdered drug

The tests for the different cell wall components of the plants using powdered stem barks material were carried out based on procedure outlined by Evans (1993).¹⁸

Phytochemical Analysis

The phytochemical tests were carried out to detect constituents of the powdered drug. The procedures are in accordance with the method used by Undie, (1987) and in Pharmacognosy Practical Schedule (Iwu, 1982;¹⁹ Ayodele, 2005)²⁰.

Determination of Analytical standard

The analytical standards were determined using the method of Odoh *et al.*, (2011).²¹

Determination of moisture content/water loss on drying

Powdered drug of *Blighia unijugata* stem bark (2g) was accurately weighed into 10 crucibles and placed in an oven at 105°C for 5 hours, cooled in a desiccator and weighed. The

procedures were repeated until there was a constant weight. The average percentage weight loss in relation to air dried powdered drug was determined for 5 replicates.

% Moisture content=

$$\frac{\text{Wt of sample in crucible (w2)} - \text{constant wt of sample in crucible (w3)} \times 100}{\text{Wt of sample in crucible (w2)} - \text{wt of crucible (w1)}}$$

Determination of water soluble extractive value

Distilled water was mixed with 1g of the powdered sample and macerated for 24 hours at room temperature. The mixture was rapidly filtered into a clean dry beaker. 2ml of the extract was transferred into a crucible and dried in the oven at 105°C. The weight of the crucible and sample were recorded before and after drying and their difference obtained and the percentage extractive value was calculated with reference to the air-dried powdered drug.

$$\% \text{ Extractive value} = \frac{W_3 - W_1 \times 100}{W_2 - W_1}$$

W₁ = Weight of flask

W₂ = Weight of the powder + flask

W₃ = Weight of the dried extract + Flask

Determination of alcohol soluble extractive value

One gram of dry sample of *Blighia unijugata* stem bark was macerated with 50ml ethanol (absolute-sigma adrich) for 24 hours at room temperature. The mixture rapidly filtered into a clean dried beaker. 1ml of the filtrate was evaporated to dryness on water bath using an evaporating dish of known weigh. It was further dried at 105°C. The dish was weighed after cooling in a desiccators. The weight of the alcohol extractive value was obtained by subtracting the weight of the dish from the weight of the dish containing the dry alcohol extractive. The alcohol-soluble extractives were calculated with reference to the air dried powdered drug.

Determination of Ash values

Total ash: A 4g of powdered sample was placed in weighed silica. Sample was placed on even layers and ignited by gradually increasing the heat to a temperature of 500-600°C until it was white indicating the absence of carbon. The material was cooled in a desiccator and weighed. Content of total ash was calculated in mg/g of air dried material.

$$\% \text{ Ash value} = \frac{W_3 - W_1 \times 100}{W_2 - W_1}$$

W₁ = Weight of the silica crucible

W₂ = Weight of the silica crucible + powder

W₃ = Weight of the ash + silica crucible

Acid insoluble ash

To the above crucible and sample, add 25ml of HCL and cover with watch glass and boiled gently for 5mins, the watch glass was rinsed with 5ml of hot water and liquid added into the crucible. The insoluble matter was collected on an ash less filter paper and washed with hot water until neutral. The insoluble matter left on the filter paper was transferred to the original crucible, dried on hot plate and ignited to constant weight. The residue was cooled for 30mins and then weighed without delay.

Water soluble ash

Ash is dissolved with distilled water and heated to nearly boiling point and the resulting solution is filtered. The amount of soluble ash is determined by drying the filtrate.

Preparation of extract or Extraction

Four hundred and fifty grams of *Blighia unijugata* powdered was macerated with 1250ml of methanol for 72 h filtered with a muslin cloth and finally filtered with filter paper. The extract was dried using a water bath and the percentage yield calculated.

Chromatographic analysis

Thin layer plate of 20/10 cm coated with silica gel was used. The plate was first activated by heating in the laboratory oven at a temperature of 110 for 5 minutes a 2 mg of the extract which was dissolved in 4 ml of methanol. A starting line was drawn 2 cm from the base, a drop of the methanol extract and a drop of solution of the standard drug (diclofenac sodium) were introduced at the starting line 5cm apart using a capillary tube and then the plate was allowed to dry. It was put into the chromatographic tank containing a concentrated solvent system of methanol and chloroform in ratio of 1:5, the plate was brought out when the capillary movement of the solvent has reached near the top end of the plate. The solvent front was marked and the plate was then dried and viewed. Then the distance moved by the different spots, were measured, the values obtained were used to determine the R_f values. The R_f for each spot was calculated using the formula

$$R_f = \frac{\text{Distance moved by the spot}}{\text{Distance moved by the solvent}}$$

Hrf values (%) = (R_f × 100)

Pharmacological evaluation

Acute toxicity

A total of 12 animals were used for the study. The Dietrich Lorke (1981)²² procedure of LD₅₀ determination was employed for the acute toxicity test using oral route of drug administration and the animals were monitored for 24 h. In the first stage, the rats were grouped into 3 groups of 3 rats. Groups 1, 2 and 3 received 100, 400 and 1000 mg/kg of extract of *Blighia unijugata* stem bark. In the second stage, the rats were also grouped into 3 groups of 3 rats. Groups 1, 2 and 3 received 1500, 2500 and 5000 mg/kg of extract of *Blighia unijugata* stem bark.

Anti inflammatory Tests

The test was carried out using a phlogistic agent- induce rat hind paw oedema as a model of acute inflammation (winter *et al.*, 1963)²³. The phlogistic agent employed in this study was Egg albumen. The albino rats (150-214g) were used after a 12 hour fasting. Animals were deprived of water only during the experiment. Inflammation of the hind paw was induced by injection of Egg albumen into the sub plantar surface of the right hind paw of the rat. Paw diameter were measured immediately before the administration of the phlogistic agent and 5 hours thereafter. The routine drug testing holds at end of 5 hours after administration of the phlogistics agent (winter *et al.*, 1992)²³. Thus (inflammation) was assessed as the difference between zero time paw diameter and that 5 hours after administration of phlogistic agent (Hess and Milonig, 1972).²⁴ The extracts were administered orally 1 hour before inducing inflammation. Control rat received equivalent amount of distilled water and the reference group administered diclofenac sodium injection 75mg/ml. Average oedema (Ct- Co) where Ct is paw size on inflammation, Co is paw size on inhibition and percent inhibition (Co/Ct x100) were calculated for each dose (Oriowo, 1982).²⁵

RESULTS

Macroscopical examination

Table 1: Macroscopical examination of *B. unijugata* stem bark

Features	Appearance (Outer layer)	Appearance (Inner layer)
Colour	Grayish slightly greenish beneath	Pale reddish brown
Odour	Odourless	Disagreeable
Taste	Tasteless	Bitter
Texture	Smooth	Smooth
Markings	Lenticular horizontal	Furrowed
Shape	Curved	Curved

Phytochemical screenings

Table 2: Results of phytochemical screening of methanol extract of *B. unijugata* stem bark

Constituents	Results
Alkaloids	-
Glycosides	+
Tanins	+
-Ferric chloride test	+
-Lead acetate test	+
Resins	+
Fats and oils	-
Saponins	
-Frothing test	+
-Emulsion test	+
Carbohydrates	-
Flavonoids	+
Terpenoids	+
Steroids	+
Proteins	-

KEY: + = present - = absent

Determination of Analytical Standards

Table 3: Analytical standards of *Picralima nitida*

Parameter	Concentration (%)
Moisture content	6.1
Total ash value	7.5
Acid insoluble ash	0.7
Water soluble ash	4.0
Sulphated ash	2.5
Alcohol soluble extractive	18.7
Water Soluble extractive	10.3

Results of Thin Layer Chromatography

Table 4: Result of Thin Layer Chromatographic Analysis

Distance moved (CM)	Solvent Front (CM)	R _f Values	HR _f Values (%)
Extract	10		
7.7		0.77	77
7.9		0.79	79
8.0		0.8	80
8.3		0.83	83
8.5		0.85	85
8.7		0.87	87
Standard drug	10	0.85	85
8.5			

Anti-inflammatory effect of *Blighia unijugata* methanol stems extract and Diclofenac Sodium injection (standard drug)

Table 5: Results of Anti-inflammatory effect of *Blighia unijugata* methanol stem extract and Diclofenac Sodium injection (standard drug) within a period of 5 h

Treatment	Dose	0(h)	1(h)	2(h)	3(h)	4(h)	5(h)
Normal control (distilled water)	1ml	2.60±0.20	5.63±0.71	5.60±0.66	5.53±0.68	5.40±0.66	5.30±0.62
Standard drug (diclofenac)	75mg/kg	2.60±0.46	5.97±0.38	5.33±0.56	4.93±0.51	4.23±0.25	3.37±0.40
Extract	200 mg/kg	2.67±0.15	5.80±0.27	5.40±0.36	4.97±0.42	4.50±0.53	3.93±0.38
	400 mg/kg	2.97±0.12	5.70±0.27	5.37±0.40	4.47±0.15	3.77±0.06	3.10±0.10
	600 mg/kg	2.67±0.06	5.73±0.55	5.17±0.75	4.60±0.95	3.90±1.95	2.87±0.12

Value are mean ± SDM, n=3, p>0.05

Table 6: Potency comparison between inflammatory effect of *B. unijugata* and Diclofenac sodium (standard drug).

Treatment	0 (h)	1 (h)	2(h)	3 (h)	4(h)	5(h)
Normal Control (Distilled water)	2.06	5.63	5.60	5.53	5.40	5.30
Standard drug (Diclofenac sodium 75mg/kg)	2.60	5.97	5.33	4.93	4.23	3.37
Extract 200 mg/kg	2.67	5.80	5.40	4.97	4.50	3.93
Extract 400 mg/kg	2.77	5.70	5.37	4.47	3.77	3.10
Extract 600mg/kg	2.67	5.73	5.17	4.60	3.90	2.87

Value are mean ± SDM, n=3, p>0.05

Table 7: Percentage inhibition.

Treatment	Dose (mg/kg)	Control Diameter (CM)	Mean diameter (cm)	Inhibition %
Standard drug (diclofenac sodium)	75	4.9	4.4	11
Extract	200	4.9	4.5	9
	400	4.9	4.2	17
	600	4.9	4.2	17

DISCUSSION AND CONCLUSION

In this study, stem of *Blighia unijugata* were subjected to pharmacognostic studies such as macroscopic, microscopic, and phytochemical screening. The extract contains Saponins which exert antimicrobial, anti-inflammatory, antiulcerogenic and spasmolytic properties, also used as emulsifying, demulcent and mild expectorant agents (Evans, 1984)²⁶.

Tannins in *Blighia unijugata* enable it to exert astringent and haemolytic properties. There are two types to tannins; hydrolysable, which are esterified with glucose that is really hydrolyzed to yield sugar and phenol nuclei or link to carbohydrates or proteins (Evans, 1993)²⁷. All these constituents enables *Blighia unijugata* to exert anti-inflammatory activity, aid wound healing, also in proper blood circulation that is needed in suppression of inflammation.

Chemomicroscopic and chromatographic evaluation, pharmacognostic numerical values such as ash value, water extractive value, alcohol extractive value and moisture content of the plant part were carried out to confirm the identity of the plant. The result of the moisture content 7.70 % fell within the normal range of moisture content which is 10 % (African pharmacopoeia, 1986). This result indicates the ability of the powdered drug to withstand microbial contamination to a very maximal level, percentage yield of 6.7, and average value of 5.60 % alcoholic extractive, 6.30 % of water soluble extractive, and total ash of 7.10 %, 0.57 % of acid insoluble ash and 4.56 % of water soluble ash. The chromatographic analysis was carried out on chromatographic plates and the results revealed the comparison between the extract and the standard drug. The extract contains some compounds and yielded some spot, but the standard drug contains a pure substance and yielded one spot. Acute toxicity test established the lethal dose of greater than 5000 mg/kg from which the safe doses were arbitrarily determined.

The holistic action of the steroids, saponins, tannins of the stem bark may have accounted for the observed anti-inflammatory properties of the plant. Chemomicroscopical features reveal the presence of lignin, trichomes, cellulose, and stone cells.

The anti-inflammatory investigated of *B. unijugata* stem bark extract on albino rats using fresh egg albumen induced pedal (paw) edema, significantly inhibited the fresh egg albumen induced acute inflammation. Also, the extract exhibited anti-inflammatory properties. From the anti-inflammatory test 200mg/ml, 400mg/ml and 600mg/ml of methanol extract of *Blighia unijugata* produced a significant ($P < 0.05$) dose dependent inhibition of the inflammation. The result obtained confirmed that edema formation was inhibited in all treated animal except the control animals. The percentage inhibition was shown to be highest with the extract dose of 600 mg/kg. Also the percentage inhibition establishes the fact that *B. unijugata* extract 600 mg/kg exhibited high anti-inflammatory activity comparable with the standard drug (diclofenac sodium). Investigation of anti-inflammatory properties have focused on suppression of primary inflammatory end points such as platelet aggregation (Gwenewegen *et al.*, 1990)²⁸ and egg albumen induced mouse (Schinella *et al.*, 1998)²⁹ and rat paw edema (Jain *et al.*, 1999)³⁰.

This study have evaluated the methanol extract of *blighia unijugata* stem bark inhibitory effect on inflammation mediators including activities and expression of cyclooxygenase (COX) (Sumner *et al.*, 1992)³¹ generation of

the prostaglandins (O'Neil *et al.*, 1987)³²; Pugh *et al.*, 1988)³³ and leukotrienes (LT) (Sumner *et al.*, 1992)³¹ and expression of pro inflammatory cytokines (Hwang *et al.*, 1996)³⁴; The plant had a significant reduction ($p > 0.05$) in anti-inflammatory response of all the treated groups. *B. unijugata* showed a great promise as an anti-inflammatory agent. The result of this study conducted confirmed that the methanol extract of *B. unijugata* bark do possess anti-inflammatory activity. Egg albumen induced paw size edema is a valuable test used in predicting the value of anti-inflammatory agents acting by inhibiting the mediators of acute inflammation (Mossa *et al.*, 1995)³⁵.

CONCLUSION

It has been shown from the above investigation that methanol stem bark extract of *Blighia unijugata* has anti-inflammatory effect since they inhibited egg albumin-induced inflammation. The qualitative phytochemical tests carried out on the methanol stem bark extract of *B. unijugata* revealed it contains tannins, saponins, steroids, which may explain the role of this plant in ethnomedical uses.

REFERENCES

1. Saad B and Saad O. Greco-Arab and Islamic herbal medicine Traditional system, ethics, safety, efficacy and regulation issues. John Wiley and Sons 2011; p.80. ISBN 9780470474211.
2. Ilesanmi O.O., Comparative studies of the morphology and foliar anatomy of *Blighia sapida* K.D.Koenig and *Blighia unijugata* Baker (Sapindaceae) in Nigeria. BSc Botany degree thesis, Department of Botany, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. 2006; 54 pp.
3. Ayodele R.O., Ajayi I.A. & Adewuyi A., Nutritional elements, antibacterial activity and cytotoxicity of the leaf, root and stem bark of *Blighia unijugata* Baker (Sapindaceae). Medicinal and Aromatic Plant Science and Biotechnology 2008; 2(2):137-140.
4. Ojewole J.A.O, Antinociceptive, anti-inflammatory of *Blighia unijugata* bark aqueous extract, *Journal of Ethnopharmacology*; 2005; 99:13-19.
5. Bekele TA. Useful trees and shrubs for Ethiopia: Identification, Propagation and management for it agroclimatic Zones Technical manual No 6. RELMA in ICRAF project, Nainbi, Kenya. 2007; SS2 pp.
6. Chikamai B.N, Githiani jik, Gachathi F.N. and Njenya M.G, under dated 2010 commercial timber resources of Kenya. Kenya forestry research constitute (Ker Ri), Nainbi, Kenya. 2010; 164pp.
7. Ferrero- Miliani L, Nielsen OH, Andersen Ps, Giradin SE, Nielsen, Andersen Ciradin "chronic inflammation"importance of nod2 and NALP3 in interleukin- 1 bta generation *clin exp Immunol*. 2007; 147(2)
8. Abbas AB, Lichtman A.H. "ch.2 innate Immunity" in saunders (Elsevier). Basic immunology. Function and disorders of the immune system 2009 ; (3rd ed).
9. Guyton and Hall: Textbook of Medical Physiology, 2011; 13th edition.
10. Chandrasoma P, Taylor O "part a general pathology, section, the host response to injury chapter 3 the acute inflammatory response subsection cardinal clinical signs"concise pathology (3rd edition (computed tilled) New York, NY :MC Graw Hill. 2005.
11. Porth, Carol, Essentials of Pathophysiology; Concepts of altered health states. Hagers town, MD Lippincott Williams and Wilkins 2007; p.20.
12. Cortan, Kumar, Collins, Robbins pathologic basis of disease. Philadelphia. Wis saunders company, 1998.
13. Masette. Utilization of *Blighia unijugata* by grey-cheeked mangabeys (*Lophocebus albigena*) in Lwamunda forest reserve, Uganda. In: Biodiversity, conservation and community development. Biodiversity and Medicinal Plant, 2006.
14. Oderinde et al. Evaluation of the mineral nutrients, characterization and some possible uses of *Blighia unijugata*

- Bak. seed and seed oil. *Electronic Journal of Environmental, Agricultural and Food Chemistry* 2009; 8(2):120–129.
15. Fowden et al. Characterized new amino acids from *Blighia unijugata*. *Phytochemistry* 1972; 11:1105–1110.
 16. Effects of Ethanol Stem Bark of *Blighia unijugata*(Sapindaceae) on Monosodium Glutamate-induced Uterine Leiomyoma in Sprague-Dawley Rats was carried out by George-Asumeng et al 2013.
 17. Adesanwo J.K., Raji Y, Olaleye SB, Onasanwo SA, Fadare OO, Ige OO, Adesanya AA, Antiulcer Activity of Methanolic Extract of *Bryophyllum pinnatum* in Rats, 2007.
 18. Evans WC. *Trease and Evans Textbook on pharmacognosy*, Fifteenth edition, WB Saunders 1993; 125-134.
 19. *Iwu M, Igboko O. J. Nat. Prod. , 1982; 45 (5):650–651.*
 20. Ayodele A. E. "The Medicinally Important Leafy Vegetables of Southwestern Nigeria," *Ethnobotanical Leaflets*: 2005; 1:16. Available at: <https://opensiuc.lib.siu.edu/ebl/vol2005/iss1/16>
 21. Odoh U.E, Inya-agma S.I. and Ugwuoke C. E. C., Ezugwu C.O, Ezejiofor M. And Ezea S. C. Determination of Pharmacognostic Standards of Medicinal Plants. Paschal Communications, Enugu State, 2011; pp. 32 -45
 22. Lorke D. A new Approach to practical Acute Toxicity Testing. *Arch Toxicol* 1992; 53(4):275-289.
 23. Winter E.A, Risley E. A and G.V Nuss, Carrageenan-induced oedema in hind paw of rats as an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. Med.* 1992; 111:544-547.
 24. Hess S.M., Milonig R.C. Assay for anti-inflammatory drugs. In: Lepow, I.H., Ward P.A (eds), *Inflammation, Mechanisms and Control*. Academic Press, New York, 1972; pp. 1-22.
 25. Oriowo M.A. Anti-inflammatory activity of piperonyl -4-acrylic isobutyl amide, an extractive from *Zanthoxylum zanthoxyloides*. *Plants Medica* 1982; 44: 54 - 56.
 26. Evans JG, Stonehenge the environment in the Late Neolithic and early Bronze Age and a Beaker Burial Wiltshire *Archaeological and Natural History Magazine* 1984; 78:7-30
 27. Evans JG, The influence of Human communities on the English Chalkland from the Mesolithic to the Iron Age: the moluscan evidence. In F.M Chambers (ed), *climate change and human impact on the landscape*, London Chapman & Hall. 1993; 147-56.
 28. Groenewegen HJ, Berendse HW, Wolters JG, Lohman AH. The anatomical relationship of the prefrontal cortex with the striatopallidal system, the thalamus and the amygdala: evidence for a parallel organization. *Prog Brain Res.* 1990; 85:95–116. discussion 116–118. [PubMed] [Google Scholar]
 29. Guillermo R. Schinella Rosa-María Giner María Del Carmen Recio Perla Mordujovich De Buschiazzo José-luis Ríos Salvador MÁñez 12 April 2011, Anti-inflammatory Effects of South American *Tanacetum vulgare* *Journal of Pharmacy and Pharmacology* <https://doi.org/10.1111/j.2042-7158.1998.tb06924.x>Citations: 70
 30. Jain NK, Kulkarni SK: Antinociceptive and anti-inflammatory effect of *tanacetum parthenium* L.extract in mice and rat. *J ethnopharmacol.* 1999; 68:251-259, 10.1016/S0378-8741(99)00115-4. ArticlePubMedGoogle Scholar.
 31. Sumner MJ, Cannon TR, Mundin JW, White DG, Watts IS, Endothelin ET_A and ET_B receptors mediate vascular smooth muscle contraction: *British Journal of Pharmacy.* 1992. <https://doi.org/10.1111/j.14765381.1992.tb14537.x>Citation s:188:
 32. O'Neil R.J., and Wiedenmann R.N. Adaptations of arthropod predators to agricultural systems. *Fla. Ent.* 1987; 70:41–48. Google Scholar
 33. Pugh WJ, Sambo K, October: Prostaglandin synthetase inhibitors in feverfew: *Journal of Pharmacy and Pharmacology*, 1988. <https://doi.org/10.1111/j.20427158.1988.tb07010.x>Citation s: 45
 34. Hwang S, Benjamin LE, Oh B, Rothstein JL, Ackerman SL, Beddington RS, Solter D, Knowles BB, Genetic mapping and embryonic expression of a novel, maternally transcribed gene *Mem3. Mamm Genome* 1996; 7(8):586-90
 35. Mossa J.S, Rafallah S, Galal AM, AL- Yahya M, Pharmacological studies of *Rhus retinorrhoea*. *International Journal of Pharmacognosy.* 1995; Pp. 242-246.

