WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 7, 38-51. Research Article ISSN 2277– 7105

PHTYOCHEMICAL STUDIES AND INVESTIGATION ON THE ANTI-INFLAMMATORY ACTIVITY OF *Detarium microcarpum* GUILL (FABACEAE)

Odoh Uchenna Estella* and Ene Chidiebere

Department of Pharmacognosy and Environmental Medicines, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

Article Received on
27 April 2020,

Revised on 18 May 2020,
Accepted on 08 June 2020,

DOI: 10.20959/wjpr20207-17813

***Corresponding Author**
Prof. Odoh Uchenna Estella
Department of Pharmacognosy and Environmental Medicines, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

ABSTRACT

This work investigates the anti-inflammatory effect of the methanol leaf extract of *Detarium microcarpum* Guill (Fabaceae). The anti-inflammatory activity of methanol leaf extract of *Detarium microcarpum* was determined using the egg albumin and formalin induced paw edema method at doses of 200 mg/kg and 400mg/kg. Piroxicam was used as the positive control at a dose of 50 mg/kg. The acute toxicity was carried out using the standard method of Lorke, and the phytochemical test done with the appropriate laboratory procedure. The phytochemical analysis of the extract showed the presence of saponins, tannins, steroids, terpenoids, glycosides, proteins, carbohydrates, flavonoids, reducing sugar, while fats and oil, alkaloids, and resins were absent. Toxicological studies showed that the extract is safe at a dose 5000 mg/kg. The result show that the methanol leaf extract of *Detarium microcarpum* has significant ($P<0.05$) dose dependent anti-inflammatory activity in both egg albumin and formalin induced inflammation. At the dose of 200 and 400 mg/kg, the extract exhibited 26.5% and 29.4% percentage inhibition at the 5^{th} hour for egg albumin. The percentage inhibition for formalin induced edema was 32.5% for both doses (200 & 400 mg/kg). This study ascertains the use of *Detarium microcarpum* leaves as an anti-inflammatory agent.

KEYWORDS: *Detarium microcarpum*; phytochemical analysis; anti-inflammatory activity, acute toxicity, edema.

INTRODUCTION

Herbal medicine (also called botanical medicine or phytomedicine) refers to using plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Herbalism has a long tradition of use outside of conventional medicine. It is becoming more mainstream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in the treating and preventing disease. Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plants, animals and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singly or in combination to treat or to diagnose and prevent illnesses or maintain well being.[1] Plants had been used for medicinal purposes long before recorded history. Ancient Chinese and Egyptian papyrus writings describe medicinal uses for plants as early as 3,000 BC. Indigenous cultures (such as African and Native American) used herbs in their healing rituals, while others developed traditional medical systems (such as Ayurveda and Traditional Chinese Medicine) in which herbal therapies were used.[2] Researchers found that people in different parts of the world tended to use the same or similar plants for the same purposes. In the early 19th century, when chemical analysis first became available, scientists began to extract and modify the active ingredients from plants. It has been found that the active principles responsible for therapy consists of alkaloids, tannins, glycosides, saponins, gums, resins etc.[3] Later, chemists began making their own version of plant compounds and, over time, the use of herbal medicines declined in favour of orthodox or conventional drugs. Almost one fourth of pharmaceutical drugs are derived from botanicals. Large numbers of plants are constantly being screened for their possible pharmacological value particularly for their anti-inflammatory, hypotensive, hypoglycaemic, amoebicidal, anti-fertility, and anti-Parkinson's properties.[3] Recently, the World Health Organization estimated that 80% of people worldwide rely on herbal medicines for some part of their primary health care.[1] In Germany, about 600 - 700 plant based medicines are available and are prescribed by some 70% of German physicians. In the past 20 years in the United States, public dissatisfaction with the cost of prescription medications, combined with an interest in returning to natural or organic remedies, has led to an increase in herbal medicine use. The majority of herbal medicines have not been scientifically tested. But some have been adequately analyzed, standardized, and submitted to clinical trials.[4] Medicinal plant is any plant which one or more of its organs, contain substances or constituents that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs.[5] Two thousand five hundred years ago, Hippocrates (the father of medical literature), stated as part of his oath: "I

will give no deadly medicine to anyone." Hippocrates used only food and herbs and is best known for the Saying: "Let your food be your medicine and let medicine be your food".[6] "Sickness is caused by the body's inability to digest its environment" The latter is perhaps particularly relevant in the modern world. It has been found that the active principles responsible for therapy consists of alkaloids, tannins, glycosides, saponins, gums, resins etc.[3]

Inflammation is a protective and defense mechanism of the body. It is a complex of sequential changes in the tissue in-response to injury.[7] During inflammatory conditions various pathological changes are taking place, the production of active inflammatory mediators is triggered by microbial products or by host proteins, such as proteins of the complement, kinins, and coagulation systems that are themselves activated by microbes and damaged tissues.

Detarium microcarpum Guill is an African tree belonging to the Fabaceae family (legumes). It is a multipurpose species, with a wide range of uses due to its medicinal properties, edible fruit (eaten raw, cooked or made into flour with many uses of its own) and hardwood used as fuel-wood. Among the Ibo tribe of south eastern Nigeria, the plant known as "Ofo" is believed to be a "religious" tree which grows in God's own compound, symbolizing truth and honestly.[8] It is the most investigated specie of the genus because of its popular use in African traditional medicine. Its many uses make it a valuable and appreciated species to local communities but further research and efforts are needed for its domestication. In terms of growth rate, the shoots of the trunk can reach a height of 1.5 m – 2 m in 1 to 2 years and are much more vigorous than seedlings which on average grow to 0.6 m after 3 years and may reach 1.5 m in 4 years.[9] The plant parts are harvested according to need and availability. Fruits are harvested from March to May and can be kept for 1–3 years in jute bags. Leaves are harvested from April to November and roots and bark are harvested year round, all of which are used fresh or dried for future use. It flowers during the rainy season (July to September/November), but the main flowering period only lasts up to 8 days. It bears fruit from September – January/May and in November; the tree sheds its leaves and produces new leaves in March.[10] *Detarium microcarpum* occurs naturally in the drier regions of West and Central Africa (Benin, Cameroon, Central African Republic, Chad, Gambia, Ghana, Guinea, Guinea Bissau, Côte d'Ivoire, Mali, Niger, Nigeria, Senegal, Sudan and Togo). Unlike the other species of its family, *D. microcarpum* grows in dry savanna, while *Detarium*

senegalense grows in the dry forest, and *Detarium macrocarpum* grows in humid forest. Many different vernacular names exist for this species, including the English, sweet dattock or tallow tree, and the French, dankh or petit détar, as well as Abu-laili (Sudan). Propagation of this species may be vegetative or from seed. It is capable of vegetative propagation by coppice regeneration and suckering from stumps or roots, as well as propagation by rooted cuttings and grafting using scions from mature trees. This species is mainly found on shallow, stony and lateritic soils, often on hills, as well as in regions with an annual rainfall of 600–1000 mm.[11] It is most common in wooded savannahs or savannahs, semi-cleared dry forest areas and fallows, growing in sandy or hard soils with high iron content. In Mali the bark is used to treat measles, nocturia, hypertension, itch and tiredness, while a decoction of the leaves or roots is taken against paralysis, meningitis, tiredness, cramps and difficult delivery.[11] The powdered seeds are applied to skin infections and inflammations, whereas the fruit is eaten to cure meningitis and malaria. Throughout western Africa the genus Detarium is believed to possess medicomagical powers. In African ethnomedicine, they are used in the treatment of diverse diseases, notably, syphilis, dysentery, diarrhea, bronchitis, pneumonia, sore throat, malaria, leprosy and meningitis.[12;13;14;15] In Burkina Faso, the fruit pulp of *Detarium microcarpum* is used to treat skin infection. In Mali the bark is used to treat measles, itching, hypertension, nocturia and tiredness, while the decoction of the leaves or roots is used for paralysis, meningitis, tiredness, cramps and difficult delivery.[11] A preparation of the fruits is taken against dizziness in Niger and Togo. In Senegal a mixture of the leaves of *Detarium microcarpum*, *Sclerocarya birrea* (A.Rich.) Hochst. and *Acacia macrostachya* pounded in milk is considered very efficient for snakebites.[14] In Benin a decoction of the leaves is taken to treat fainting and convulsions. In veterinary medicine the leaves and roots are used to treat diarrhoea in cattle in southern Mali, and in Benin to treat constipation. In Niger cattle are made to inhale the smoke of the leaves to treat fever.[11] The fruit is sweet and commonly eaten fresh, while the pulp is used in the preparation of cakes and couscous. The pulp is used as a substitute for sugar. The seeds and leaves are eaten as a condiment and vegetable. The wood is hard and tough, with a regular grain, and is easy to work. It is used for carpentry, fence poles and joinery. It is durable and long-lasting even under water. The wood is well appreciated as firewood, as it lights quickly even if wet. *Detarium microcarpum* is well integrated in the traditional agro forestry systems of the Sahel, and it can be coppiced well. It is widely in herbal medicine in Nigeria. It has a considerable commercial in food and pharmaceutical industries.[16] The leaves and flowers are used as fodder, and the seeds as pig feed. In southern Mali the leaves are used as roofing material,

and as organic fertilizer. The heated roots are sweet scented and are used as a perfume by Dinka women in Sudan, and as a mosquito-repellent in Chad. The fragrance of other parts of the tree is useful as well. If the bark is damaged, a sticky, fragrant gum is secreted that is used to deter mosquitoes. Heated roots produce a sweet scent that is used as a perfume by women in Sudan, and as a mosquito repellent in Chad. In African ethno-medicine, the plant and the closely related species *Detarium senegalense* are used in the treatment of syphilis, dysentery and bronchititis. Nutritionally, the seed which is used as a traditional soup thickener contain lipids, carbohydrates, proteins, crude fibre and the essential elements. The ethanol extract of the bark showed antimicrobial action against *Pseudomonas aeruginosa, Citrobacter freundii, Klebsiella pneumonia, Staphylococcus aureus, Streptococcus pyogenes* and *Listeria monocytogenes.*(Abreu *et al*, 1998). The extract showed moderate antitumor activity against breast cancer cells.[13] The flavanes present in a methanol extract of *Detarium microcarpum* showed strong inhibitory effects on HIV-1 or HIV-2 infection. A bark extract showed significant molluscicidal activity against *Lymnaea natalensis.* They are prepared as infusions or decoctions to treat rheumatism, venereal diseases, urogenital infections, hemorrhoids, intestinal worms and diarrhea. The fruit is rich in vitamin C (3.2 mg), with 4.8 g protein and 64.5 g of sugar per 100 g. It was found to have the highest total phenolic, flavonoid and antioxidant values among fourteen wild edible fruits from Burkina Faso. The fruit pulp has been found to have high proportions of carbohydrate (40-42.0%) and protein (29.1-30.9%) The seeds yield 7.5% oil with the predominant fatty acid being linoleic acid.[9] Its many uses make it a valuable and appreciated species to local communities but further research and efforts are needed for its domestication. Recently modern day pharmacy practice employs a systematic screening technique to establish the actual constituents responsible for exerting a given action, isolate them and to determine the quality and purity. Hence, this work investigates the phytochemical properties and anti-inflammatory activity of *Detarium microcarpum* in order to lend scientific support to its use in traditional medicine as a folk remedy for inflammatory diseases.

Taxonomic profile of *Detarium microcarpum*

Kingdom - Plantae
Division - Magnoliophyta
Class - Magnoliopsida
Order - Fabales
Family - Fabaceae

Subfamily - Caesalpinioideae
Genius - *Detarium*
Species - *microcarpum* Guill.
Common names - sweet detar, tallow tree (English) petit détar (French), *Ofo* (Ibo)

Figure 1: Leaves of *Detarium microcarpum*.

MATERIAL AND METHODS

Collection and preparation of leaves of *Detarium microcarpum*

Fresh leaves of *Detarium microcarpum* were collected during the month of May, 2013 at Nsukka, Enugu state, Nigeria. It was identified by Mr. A.O. Ozioko of International Centre for Ethno medicine and Drug and Development (InterCEDD) Nsukka, Enugu State. The leaves were cleaned, dried, and reduced to a powder form using the hammer mill.

Animals

Wistar rats (70 g – 165 g) were purchased from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were kept in standard laboratory conditions and fed with rodent commercial diet and water. Approval for the use of animal subjects was secured from the Animal Research Ethics Committee, University of Nigeria, Nsukka. The animals were handled according to International protocol for handling laboratory animals as documented in the European Community guideline.

Extraction of plant material

The powdered leaves were extracted by cold maceration with 2.5 l of methanol with intermittent shaking for 48 h and then filtered. The filtrate was then evaporated to dryness using a rotary vacuum evaporator. The dried extract was kept for further analysis.

Phytochemical analysis

The extract was subjected to preliminary phytochemical analysis to test for the presence of alkaloids, saponins, glycosides, resins, proteins, carbohydrates, flavonoids, steroids, terpenoids, and fats and oils.[17]

Preparation of sample solution

The dried extract was used to determine the concentration in mg/ml of extract by dissolving 26.59 g of the dried extract in 98 ml of distilled water and 2 ml of 10 % Dimethylsulfoxide.

PHARMACOLOGICAL STUDY

Acute Toxicity Test

The acute toxicity tests (LD_{50}) were performed using various methods of Lorke.[18] Essentially the method involves an initial dose range determination stage in which twelve mice were used. The aqueous extracts of *Detarium microcarpum* were given at a dose of 10, 100, and 1000 mg/kg by intraperitoneal route. The animals were observed after 24 hours. No death occurred in the animals after 24 hours. A second stage was carried out, doses of 1600, 2900 and 5000 mg/kg were given to the mice and observed after 24 hours.

Anti-inflammatory evaluation

Two models were used to test for the activity of the aqueous extract of *Detarium microcarpum* against acute inflammation.

Egg albumin-induced inflammation

Twelve animals divided into four groups of three animals each were used for the experiment. They were fasted for about 8 hours before the experiment and deprived of water only during the experiment. The deprivation of water was to ensure uniform hydration and to minimize variability in edematous response. The animals were given oral administration of *Detarium microcarpum* at doses of 200 mg/kg and 400 mg/kg. The negative control animals were treated with 5 ml/kg of dimethylsulfoxide. The positive control group received 50 mg/kg piroxicam. All the substances except piroxicam were administered 30 minutes before the

administration of the inflammatory agent (0.1 ml of fresh egg albumin) in the sub plantar region of the right hind paw after oral administration of the extract. Piroxicam (positive control) was administered one hour before induction of inflammation. The paw volume measurements were taken at 0 hour, 30 minutes, 1, 2, 3, 4, and 5 hours after egg albumin induction. Edema was assayed in terms of volume of water displaced by the treated paw using a plethysmometer.[19;20]

Formalin-induced inflammation

The procedure according to Singh *et al*.,[21] was used to evaluate the effect of the extract on acute inflammation induced by 2.5 % $^{v}/_{v}$ formaldehyde. Twelve animals divided into four groups of three animals each were also used. A 0.1 ml of 2.5 % $^{v}/_{v}$ formaldehyde was injected into the sub plantar region of the left hind paw of the rat on the day of the experiment. The edema volume was taken at 0 hour, 30 minutes, 1, 2, 3, 4, and 5 hours each. The negative control animals were treated with 5 ml/kg of dimethylsulfoxide and the positive control animals received 50 mg/kg piroxicam. All drugs were administered orally. Edema was accessed in terms of volume of water displaced by the treated paw using same plethysmomemeter.

Statistical Analysis

The data were statistically analysed by analysis of variance (ANOVA) at (*) $P < 0.05$ significant level.

RESULTS

Percentage yield

The percentage yield is 6.4 w/w of plant material.

Result of phytochemical test

The result of the preliminary phytochemical analysis is represented in the Table 1. It shows the presence of the phytochemicals tested that are responsible for the pharmacological property conferred on the plant.

Table 1: Results of the phytochemical analysis of methanol leaf extract of *Detarium microcarpum*.

Test	Inference
Carbohydrates	+
Reducing Sugars	++
Alkaloids	+
Flavonoids	++
Glycosides	+
Saponins	++
Tannins	++
Resins	+
Proteins	+
Fats and oils	+
Steroids	+
Terpenoids	+

Key: - = absence of metabolite, + = slightly present, ++ = moderately present.

ACUTE TOXICITY RESULT

The result of the acute toxicity test indicates no toxicity of the plant at 5000 mg/kg (Table 2).

Table 2: Results of the acute toxicity study of methanol leaf extract of *Detarium microcarpum*.

Dose mg/kg	Stage I	Stage II
10	No death	-
100	No death	-
1000	No death	-
1600	-	No death
2900	-	No death
5000	-	No death

PHARMACOLOGICAL EVALUATION

Results of egg albumin induced inflammation

The results of the effect of the extract on the egg albumin-induced inflammation and the percentage inhibition are shown in Tables 3 and 4.

Table 3: Effects of leaf extract of *Detarium microcarpum* on egg albumin induced inflammation.

Treatment Groups	Mean Edema Volume (ml) at different hours						
	0h	0.5h	1h	2h	3h	4h	5h
Negative control	0.800±0.000	1.567±0.167	1.533±0.088	1.433±0.133^{+}	1.467±0.067^{+}	1.300±0.116^{+}	1.133±0.145^{+}
200 mg/kg extract	0.800±0.000	1.467±0.033	1.400±0.058	1.267±0.033	1.167±0.033*$^{+}$	1.000±0.000*	0.833±0.033*
400 mg/kg extract	0.933±0.120	1.733±0.120	1.567±0.088	1.367±0.033	1.200±0.000*$^{+}$	1.000±0.058*	0.800±0.100*
Positive control 50 mg/kg	0.700±0.058	1.600±0.058	1.400±0.116	1.133±0.067*	0.933±0.088*	0.833±0.033*	0.733±0.033*

Values are Mean ± SEM, n=3. *Values are significantly different from the negative control group at ($p < 0.05$),$^{+}$ Values are significantly different from the positive control group at ($p < 0.05$), Negative control: 5 ml/kg of Dimethylsulfoxide, Positive control: 50 mg/kg of Piroxicam.

Table 4: Percentage inhibition for egg albumin inflammation.

Treatment (mg/kg)	30min (%)	1h (%)	2h (%)	3h (%)	4h (%)	5h (%)
DME 200	6.4	8.7	11.6	20.5	23.1	26.5
DME 400	-10.6	-2.2	4.6	18.2	23.1	29.4
Piroxicam 50	-2.1	8.7	20.9	36.4	35.9	35.3

DME – *Detarium microcarpum* extract

Results of Formalin induced inflammation

The results obtained from the effect of the extract on the formalin-induced inflammation and the percentage inhibition is as shown in Tables 5 and 6.

Table 5: Mean edema effect of leaf extract of *Detarium microcarpum* on formalin induced inflammation.

Treatment Group	Mean Edema Volume (ml) at different hours						
	0h	0.5h	1h	2h	3h	4h	5h
Negative control	0.567±0.033	1.167±0.067	1.300±0.100	1.433±0.033^{+}	1.400±0.000^{+}	1.333±0.033^{+}	1.433±0.033^{+}
200 mg/kg extract	0.600±0.100	1.100±0.100	1.200±0.058	1.167±0.033*	1.067±0.088*	1.033±0.067*	0.967±0.033*
400 mg/kg extract	0.700±0.100	1.067±0.067	1.267±0.067	1.233±0.067*	1.200±0.000*	1.033±0.033*	0.967±0.033*
Positive control 50 mg/kg	0.567±0.033	1.033±0.067	1.167±0.088	1.133±0.067*	1.033±0.033*	1.000±0.058*	0.900±0.058*

Values are Mean ± SEM, n=3. *Values are significantly different from the negative control group at ($p < 0.05$), $^{+}$ Values are significantly different from the positive control group at ($p < 0.05$), Negative control: 5 ml/kg of Dimethylsulfoxide, Positive control: 50 mg/kg of Piroxicam.

Table 6: Percentage Inhibition for Formalin inflammation.

Treatment (mg/kg)	30min (%)	1h (%)	2h (%)	3h (%)	4h (%)	5h (%)
DME 200	5.7	7.7	18.6	23.8	16.9	32.5
DME 400	8.6	2.5	14.0	14.3	16.9	32.5
Piroxicam 50	11.5	10.2	20.9	26.2	25.0	37.2

DME – *Detarium microcarpum* extract

DISCUSSION

The result of the preliminary phytochemical analysis of the methanol leaf extract of *D.microcarpum* revealed the presence of alkaloids, saponins, tannins, proteins, flavonoids, reducing sugars, carbohydrates, steroids and terpenoids These compounds contribute to the various pharmacological activities of *D. microcarpum.* One or a combination of these phytoconstituents may be responsible for the observed antinociceptive and anti-inflammatory activities observed in this study. Saponins and related phytosterols, tannins, and some glycosides have been reported to have antinociceptive and/or anti-inflammatory activities.[22;23]

There is a clear indication that *Detarium microcarpum* leaf extract has promising potential as an anti-inflammatory agent. This is consistent with the findings that phytochemicals such as saponins, tannins, flavonoids, terpenoids, and steroids have anti-inflammatory effects.[24]

Result of the anti-inflammatory test showed that the extract suppressed the acute inflammation of the rat paw with 200 and 400 mg/kg. In the egg albumin induced inflammation, there was an initial increase in mean volume of inflammation at 30 minutes, then gradual decrease from one hour to the fifth hour. The fifth hour having the lowest mean volume of inflammation explains the fact that *Detarium microcarpum* leaf extract has anti-inflammatory effect. The result shows that at 200 mg/kg and 400 mg/kg, there were significant differences from the negative control group at ($P < 0.05$) as from the third hour to the fifth hour. The positive control group has its significant difference from the negative control group at ($p < 0.05$) as from two hours to five hours. Unlike in formalin induced inflammation, in the egg albumin induced inflammation both the positive control and the

extract were able to effect a return to basal paw size at the end of the fifth hour. Egg albumin-induced edema results from the release of histamine and serotonin.[25]

The inhibition by the extract of the early acute phase of edema produced by egg albumin-induced progressive increase in edema suggests that the extract may suppress both the early and later phases of the acute inflammatory response. In acute inflammation there is liberation or release of various chemical mediators known as proinflammatory mediators, such as histamine, 5-HT, kinins and prostanoids mediating acute inflammation-induced phlogistic agents.[26;27;28;29;30], likely also involved in egg albumin-induced edema. It is therefore not unlikely that the extract may exhibit its action through inhibition of these proinflammatory mediators.

This same effect was also seen in the formalin induced inflammation result where there was a decrease in inflammation from the second hour down to the fifth hour. The mean volume of inflammation of the negative control (dimethylsulfoxide) fluctuates at different time interval, with increase at some time and decrease in some. The reduction from second to fifth hour could be explained by the fact that anti-inflammatory agents cause inhibition of arachidonic acid synthesis as the extract administered followed same pattern as the positive control (piroxicam) in decreasing the inflammation. In the above table, at 200 mg/kg, 400 mg/kg, and the positive control, there was significant difference from the negative control group at ($P < 0.05$) as from the second hour down to the fifth hour.

CONCLUSION

From the results obtained it is obvious that the leaf extract of *Detarium microcarpum* has significant anti-inflammatory activity. The effectiveness of this extract can be furthered with more research studies to characterize the structure of the active constituents and determine the mechanism of anti-inflammatory effects.

REFERENCES

1. WHO. Resolution – Promotion and Development of Training and Research inTraditional Medicine, WHO Document No. WHA, 2003; 40–49.
2. Izzo AA, Ernst E. Department of Experimental Pharmacology, University of Naples Federico II; Interaction between Herbal medicine and Prescribed drug, 2009.
3. Evans WC. Textbook of Pharmacognosy, 15th edition, Harcourt Publishers Limited, 2002; 125: 318.

4. Ernst E, Pittler MH, Wider B. The Desktop Guide to Complementary and Alternative Medicine, 2nd ed. Edinburgh: Elsevier Mosby, 2006.
5. WHO. Resolution – Drug and Management: Medicinal Plants, WHO Document No. WHA, 1978; 31.33.
6. Geoffrey Lloyd. Hippocratic writing, 1978.
7. Guyton, A.C. Textbook of medicinal physiology, W.B. Saunda Co. Philadelphia 6th edition, 1981; 65–73.
8. Ejizu CI. Ofo: Igbo ritual symbols. Fourth Dimension publishers, Enugu, Nigeria, 1986; 190.
9. Abdalbasit, Adam Mariod, Mohamed Elwathig S. Mirghani, Ahmad Bustamam Abdul and Siddig Ibrahim Abdelwahab. "*Detarium microcarpum* Guill and Perr fruit proximate chemical analysis and sensory characteristics of concentrated juice and jam," African Journal of Biotechnology, 2009; 8(17): 4217-4221.
10. Kouyate AM, Van Damme P. *Detarium microcarpum* Guill & Perr. In: Schmetrer GH, Gurib Fakim A Edn. Prota (11)1: Medicinal plants/plantesmeduinales I (CD-Rom) PROTA, Wageningen, Netherlands, 2006.
11. Kouyate AM. Aspects ethnobotaniqueset etudea de la variability morphologique biochemique et phenologuque de *Detarium microcarpum*, Guill & PCN. AU. Mali. Ph.D Thesis, Faculty of Agriculture and Applied Biological Sciences, University of Kent Belgium, 2005.
12. Dalziel JM. The useful plants of West tropical Africa. Gown Agents for Overseas Colonies London, 1937.
13. Abreu PM, Rosa VS, Araujo EM, Canda AB, Kayser O, Bindseil KU, Siems K, Seeman A. Phytochemical analysis and antimicrobial evaluation of *Detarium microcarpum* bark extracts. Pharmaceutical and Pharmacological letters, 1998; 8(3): 107-109.
14. Keay RWJ, Phil D, Biol TT. (1998). Trees of Nigeria. Oxford University Press, London, 1998; 204-207.
15. Burkill HM. The useful plants of West tropical Africa. Royal Botanical Gardens Kew London, 1995; 3: 101.
16. Wang Q, Ellis PR, Ross-Murphy SB, Feid PR, Ross Murphy SB, Rcid JS9. A new poly-sacch- arides from a traditional Nigerian plant food *Detarium senegalense* Gmelin. Carbohydrate Research, 1996; 284(2): 229-239.
17. Trease GE and Evans WC. (1989). Textbook of Pharmacognosy, Edited by Evans, W. C. 13th Edition, ELBS, Great Britian, 1989; 19-672.

18. Lorke D. A New Approach to Practical Acute Toxicity Testing. Arch. Toxicol., 1983; 54: 275 -287.
19. Winter CA, 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: 544-547.
20. Odoh UE, Ezugwu CO, Onwugbenu NC and Osadebe PO (2010). Evaluation of the Phytochemical constituents, anti-inflammatory and analgesic properties of the ethanol extract of *Acanthus montanus* (Acanthaceae) leaf. Nigerian Journal of Pharmaceutical Research, 2010; 8(1): 122–128.
21. Singh S, Bani S, Singh GB, *et al.*. Anti-inflammatory activity of Lupeol. Fitoterapia, 1997; 68: 9–16.
22. Raaman N: Categories of phytochemicals. In: Phytochemical Techniques. New India Publishing Agency, New Delhi, India, 2006; 197–274.
23. Larkins N, Wynn S: Pharmacognosy: Phytomedicines and their mechanisms. Vet Clin North Am Small Anim Pract, 2004; 34: 291–327.
24. Manach C, Scalbert A, Morand C, Remesy C, Jimenez L. Polyphenols: Food sources and bioavailability. Am J Clin Nutrition, 2004; 79(5): 727-747.
25. Adeyemi OO, Okpo SO, Okpaka O: The Anti-nociceptive effect of the methanolic extract of *Acanthus montanus*. J Ethnopharmacol., 2004; 90: 45–48.
26. Dumas J, Bourdon U, Remade-Volon G, Adam A. Kinins and peritoneal exudates induced by carragenin and zymosan. J. Pharmacol, 1990; 101: 418-422.
27. Raychaudhuri A, Colombo C, Pastor G, Wong M, Jeng AY. Effect of capsacin on carrageenan-induced inflammation in rat pleurisy and exudates substance P level. Agent Actions, 1991; 34: 251-253.
28. Utsunomiya I, Nagai S, Ohinishi S. Sequential appearance of IL-1 and IL-6 activities in rat carrageenan-induced pleurisy. J. Immunol, 1991; 147: 1803-1809.
29. Ialenti A, Ianaro A, Moncada S, Di Roossa M. Modulation of acute inflammation by endogenous nitric oxide. Euro. J. Pharmacol, 1992; 211: 177-182.
30. Dumas J, Bourdon, Remade-Volon G. Influence of a long acting bradykinin antagonist HOE B140, on acute inflammatory reactions in the rat. Eur. J. Pharmacol, 1992; 211: 81-86.