PHARMACOGNOSTIC AND ANTI-INFLAMMATORY STUDIES OF THE LEAVES OF *ALLOPHYLUS AFRICANUS* BEAUV (SAPINDACEAE)

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By

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FACULTY OF PHARMACEUTICAL SCIENCES

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NIGERIA

AUGUST, 2016

DECLARATION

I declare that the work in this Dissertation entitled "Pharmacognostic and Anti-inflammatory Studies of the Leaves of *Allophylus africanus* Beauv (Sapindaceae)"has been carried out by me in the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this Dissertation was previously presented for another degree or diploma at this or any other institution.

FATIMA SENEIRE IBRAHIM

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Name of Student Signature Date

CERTIFICATION

This Dissertation entitled "PHARMACOGNOSTIC AND ANTI-INFLAMMATORY STUDIES OF THE LEAVES OF *ALLOPHYLUS AFRICANUS* BEAUV (SAPINDACEAE)" by Fatima seneire Ibrahim meets the regulations governing the award of the degree of Master of Science in Pharmacognosy of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This research work is dedicated to all men, women and children out there, who are suffering from any form of pain.

ABSTRACT

This study evaluated some pharmacognostic standards and anti-inflammatory activity of the leaves of *Allophylus africanus* Beauv (Sapindaceae). The plant has a wide distribution in West Africa, with Nigeria (Mambila Plateau, Calabar) inclusive. It has been used in communities for both its medicinal and economic importance. Traditionally, the boiled bark, root and leaves are used in aches, fever and rheumatic pains and economically, the wood is a good source of building materials, and as insect plants. The aim of the study was to determine some pharmacognostic parameters of *A. africanus* leaves and profer scientific basis for its use in the treatment of inflammatory disorders. Methods used included macroscopic, microscopic, chemo microscopic, physical constant, water and alcohol extractive values determination and carrageenan induced paw edema study in Wister rats. Results of the study revealed the presence of anomocytic stomata, polygonal epidermal cells with straight anticlinal walls on the abaxial surface and irregular shaped epidermal cells with wavy anticlinal walls on the adaxial surface. The physical constants evaluated were moisture contents (7.8%), total ash value (7.0%), water soluble ash (1.0%), acid insoluble ash (1.5%), water extractive (16.6%) and ethanol extractive value (9.4%) respectively. Phytochemical screening on the methanolic leaf extract revealed the presence of carbohydrates, steroids and triterpenes, glycosides, tannins, flavonoids and alkaloids. Acute toxicity study showed the plant to have an LD_{50} of 3,807 mg/kg body weight (*p.o*) in rats. The methanolic leaves extract at doses 250 and 1000 mg/kg was found to produce significant anti-inflammatory effect at the 3^{rd}, 4^{th} and 5^{th} hour with the effect being dose dependent at the 4^{th} and 5^{th} hour. Thin layer chromatographic analysis of the fractions of the leaves extract showed the presence of some chemical constituents in the leaves extract specifically flavonoids, steroids and triterpenes. The results provide some

pharmacognostic standards for the plant and scientific basis for the traditional use of the leaves in the treatment of inflammation and inflammatory disorders.

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ACRONYMS

%: Percentage.

ANOVA: Analysis of Variance.

ASA: Acetyl Salicylic Acid.

B.P: British Pharmacopoeia.

CAM: Complementary and Alternative Medicine.

CM: Centimeter.

COX: Cyclooxygenase.

DAMPs: Damage-Associated Molecular Patterns.

DPPH: 1,1 – Diphenyl-2-2-picrylhydrazyl.

FAA: Formalin Acetic Acid.

Fig: Figure

G: Gram

GC – MS: Gas Column Spectrophotometry.

H_2SO_4: Sulphuric acid.

HCC: Hydrochloric acid.

KG: Kilogram.

L: Liter.

LD_{50}: Median lethal dose.

Ml: Mililiter.

MM: Milimeter.

N/S: Normal saline.

NSAIDs: Non-steroidal anti-inflammatory drugs.

PAMPs: Pathogen-associated molecular patterns.

PRRs: Pattern recognition receptors.

R_f: Retention Factor.

SEM: Standard Error of Mean.

TLC: Thin layer chromatography.

UV: Ultraviolet Light.

W/W: Weight per weight.

WHO: World Health Organization.

CHAPTER ONE

1.0 INTRODUCTION

Traditional medicine includes diverse health practices, approaches, knowledge and beliefs incorporating plant, animal and or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness (WHO, 2002). It is the sum total of all knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation whether verbally or in writing (WHO, 2002). It is a cultural gem of various communities around the world and encompasses all kinds of folk medicine, unconventional medicine and indeed any kind of therapeutical method that had been handed down by tradition of a community or ethnic group (Adesina*et al*., 2013). Elements of traditional medicine includes various forms such as herbal medicine, massage, homeopathy, aromatherapy, mind and spirit therapies, reflexology, hydrotherapy, etc. It is approximated that about 75% of the populace by choice solve their health problems consulting traditional healers in Nigeria, (Adeshina*et al*., 2013). In Africa, up to 80% of the population use traditional medicine to help meet their health care needs. Meanwhile, in many developed countries, complementary and alternative medicine (CAM) is becoming more popular. The percentage of the population which has used CAM at least once is 48% in Australia, 70% in Canada, 42% in United States of America, 38% in Belgium and 75% in France (WHO, 2003). Nigerians especially those living in rural communities, don't have access to orthodox medicine and still prefer to solve their health problems consulting traditional healers. This shows the importance of traditional medicine to the majority of Nigerians. Many rural communities have great faith in traditional

medicine, especially the explicable aspect which also recognize their socio-cultural and religious background which orthodox medicine seems to neglect.

Different traditional medicine systems have played an important role in terms of providing information for drug discovery purposes. It is reported that about 122 compounds of defined structure have been obtained from only 94 species of plants that are used globally as drugs and shows that 80% of these have had an ethno medical use similar or related to the present use of the active elements of the plant (Fabricant *et al*., 2001). Artemesinin, atropine, aspirin, camptothecin, codeine, digoxin, morphine and pilocarpine, are a few examples of useful plant drugs (Kumar *et al.,* 2012).

The only true medicines ever used initially are plants (Kadans, 1970). The use of plants as medicinal agents have been over thousands of years with records as far back to Mesopotamia (Newman *et al*., 2003). Biologically active molecules can be obtained from plants and these serve as lead structures for the synthesis of modified derivatives. A number of chemically useful plant drugs have been identified from the lead provided by their ethno medical uses (Mate *et al*., 2008). The chemical constituents that the medicinal plants do have are indicative for their uses which may have the ability to alter specific physiological action on the human body. Flavonoids and other phenolic compounds, terpenes and alkaloids are some examples of the bioactive chemical constituents (Gurib-Fakin, 2006).

1.1Pharmacognosy

Pharmacognosy, derived from the Greek words “Pharmakon” (drug) and “gnosis” (knowledge), is probably the oldest modern science, and generally the study of crude drugs of plants and animal origin (in the form of tinctures, teas, poultices, powders, and other herbal formulations). It incorporates authentication and quality control of

such drugs, based on macroscopic and microscopic examination of crude drugs. Historically, the emphasis of botanical aspect in pharmacognosy started in the early 1900s out of a need to establish the quality of /and adulterants in plant and animal drugs. At the beginning, the Pharmacognosist engaged in his pursuit with the use of his five senses, reinforced at time with the microscope and few chemical reaction. Therefore, up to the beginning of the 20th century, pharmacognosy was more a descriptive subject that involved identification of drugs both in the entire or powdered condition by microscopic and macroscopic studies.

1.2 Standardization and Quality Control of Herbal Medicines

All medicines that we take or use, be it of plant origin or synthetic, is expected to meet basic requirements to be termed as safe and effective (WHO, 2002). Herbal preparation must be used overtime at required dosages to achieve the wanted result but some of these herbs could be toxic associated with unwanted side effects. The quality of the raw material for herbal medicine is often a problem and this also applies to its availability as well.

In vast number of countries, there is no adequate structured process to regulate quality standard and manufacturing. Herbal products are put forward into the market without evaluating them scientifically and embarking on mandatory safety and toxicological studies. In order to produce a quality drug, a well-defined and constant composition of the drug is necessary (Adeshina, 2013). Standardization of herbal medicines is the means of establishing a set of standards or inherent characteristics, definitive quantitative and qualitative values, constant parameters that carry an assurance of quality, safety, efficacy and reproducibility. Set of characteristics possessed by the plant drug can be made by obtaining specific standards via observations and experimentation. Standardization can be said to be a tool in quality

control process (Kunle *et al.*, 2012). Standardization and quality control of herbs also involves evaluation of crude drug covering aspects such as how crude drugs are handled, safety, efficacy and stability assessment of finished product. It serves as a tool for the identification of right variety and search for adulteration, the criteria to judge the identity and purity of a drug and also identification and characterization of crude drug with respect to phytochemical constituent (Upton *et al.*, 2010).

Plant derived drugs form significant proportion of global market and in this respect, internationally recognized guidelines for their quality control and quality assessment is a necessity (Kunle *et al*, 2012).

1.3 Inflammation

Inflammation is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants.

1.3.1 Concept of inflammation

Inflammation is a protective response that involves immune cells, blood vessels, and molecular mediators. It also involves the activation of compliment factors which brings forth edema formation due to extravasations of fluid and proteins and accumulation of leukocytes at the inflammatory site. The purpose of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the inflammatory process, and to initiate tissue repair.

1.3.2 Types of inflammation

Acute Inflammation

Acute inflammation is a short-term process, usually appearing within a few minutes or hours and begins to cease upon the removal of the injurious stimulus. It is

characterized by 5 basic signs: pain, heat, redness, swelling and loss of function. Causative agents include bacteria pathogens and injured tissues. Major cells involved includes neutrophils (primarily), basophils (inflammatory response), eosinophils and mononuclear cells (monocytes, macrophages). Primarymediators are vasoactive amines and eicosanoids. Onset is immediate, with duration of a few days. Resulting outcomes are resolution, abscess formation, chronic inflammation.

Chronic Inflammation

Chronic inflammation is caused by persistent acute inflammation due to non-degradable pathogens, viral infections, persistent foreign bodies or autoimmune reactions.Major cellsinvolved are mononuclear cells (macrophages, monocytes, lymphocytes, plasma cells), fibroblasts. Primary mediators are cytokines, growth factors, hydrolytic enzymes, reactive oxygenspecies. Onset is delayed, with the duration of up to many months or years. Outcome includes tissue destruction, fibrosis and necrosis.

1.3.3 Causes of inflammation

Physical:

- Burns
- Physical injury (penetrating or blunt)
- Frostbite
- Ionizing radiation
- Trauma
- Foreign bodies including splinters, debris and dirt

Biological:

- Infection by pathogens

- Immune reactions
- Stress

Chemical:

- Chemical irritants
- Alcohol
- Toxins

Psychological:

- Embarrassment
- Excitement

1.3.4 Propagation of the acute inflammatory response

A sequence of biochemical events propagates and matures the inflammatory response, involving the immune system, the local vascular system and various cells within the injured tissue. The process of acute inflammation is initiated by resident immune cells already present in the involved tissue, mainly mast cells, macrophages, Kupffer cells, histocytes and dendritic cells. These cells have surface receptors known as pattern recognition receptors (PRRs), that recognize and binds to two subclasses of molecules namely pathogen-associated molecular patterns (PAMPs), which are compounds that are associated with various pathogens, but are distinguishable from host molecules, and damage-associated molecular patterns (DAMPs), which are compounds that are associated with host-related injury and cell damage.

At the inception of a burn, infection or other injuries, one of the PRRs recognize a PAMP or DAMP) and release inflammatory mediators responsible for the clinical signs of inflammation. Vasodilation and its resulting increased blood flow causes the increased heat and redness. Increased permeability of the blood vessels results in an exudation (leakage) of plasma proteins and fluid into the tissue (edema), which manifests itself as swelling. Some of the released mediators such as bradykinin increase the sensitivity to pain (hyperalgesia). The mediator molecules also alter the blood vessels to permit the migration of leukocytes, mainly macrophages and neutrophils, outside of the blood vessels (extravasation) into the tissue. A neurological reflex in response to pain probably results in loss of function. Sustaining the acute inflammatory response requires constant stimulation. Inflammatory mediators are short-lived and are quickly degraded in the tissue. Therefore, once the stimulus has been removed, acute inflammation begins to cease.

1.3.5 Inflammatory disorders

Inflammatory abnormalities are a large group of disorders that bring about enormous array of human diseases. The immune system is often involved with inflammatory disorders, demonstrated in both allergic reactions and some myopathies with many immune system disorders resulting in abnormal inflammation. Non-immune diseases with etiological genesis in inflammatory processes include atherosclerosis, ischemic heart disease and cancer.

Examples of disorders related with inflammation include:

- Rheumatoid arthritis
- Asthma
- Pelvic inflammatory disease
- Hypersensitivities
- Glomerulonephritis

- Acne vulgaris
- Auto inflammatory diseases
- Autoimmune diseases
- Inflammatory bowel diseases
- Chronic prostatitis
- Transplant rejection

1.3.6 Drug treatment

Anti-inflammatory drugs

Drugs used in the management of inflammatory conditions are called anti-inflammatory drugs. They are classified into two categories:

Steroidal anti-inflammatory drugs – these are synthetic derivatives of cortisol, the major endogenous glucocorticoids and are used extensively in the management of inflammatory disorders and for their immunosuppressive actions. They act on the inflammatory cells and the inflammatory mediators. Some examples include cortisol, prednisolone, triamcinolone, dexamethasone and betamethasone.

Non-steroidal anti-inflammatory drugs (NSAIDs) – They alleviate pain by counteracting the cyclooxygenase (COX) enzyme which synthesizes prostaglandins, creating inflammation.

Some common examples are aspirin, ibuprofen, naproxen, diclofenac. The newer specific COX-Inhibitors are not classified together with the traditional NSAIDs even though they presumably share the same mode of action. Long term use of NSAIDs can cause gastric erosions, which become stomach ulcers, gastro-intestinal bleeding, exacerbation of asthma and can cause kidney damage.

1.3.7 Plants as sources of anti-inflammatory agents

Owing to the numerous adverse effects associated with the use of these synthetic drugs, attention is now drawn on exploring anti-inflammatory agents of plant origin which are considered to be equally effective with minimal or no side effects. Plants have contributed to the development of some anti-inflammatory drugs such as *Salix alba* (white willow tree) from which salicin is obtained and it is believed to be less toxic than Aspirin.

Plants secondary metabolites have provided an important source of drugs since time in memorial and now partly of the practical drugs used are obtained from natural sources and many of this herbal constituents are being prescribed widely for the treatment of inflammatory conditions.

Pharmacological value of phenolic compounds have been reported, with reports of some having anti-inflammatory properties. Different types of phenolic compounds such as flavonoids, condensed tannins, have been reported to inhibit some molecular targets of pro-inflammatory mediators in inflammatory responses and inhibit particular enzymes such as COX-2 enzymes (Mona *et al.*, 2014).

Some flavonoids, such as quercetin has been reported to have the ability to block both the cyclooxygenase and lipooxygenase pathways at relatively high concentrations Other metabolites from plants reported to show potential anti-inflammatory activities are Alkaloids, saponins, sterols, terpenoids, coumarins and essential oils (Mona *et al.*, 2014) and these may provide important sources of anti-inflammatory agents.

1.4*Allophylus africanus*

Allophylus africanus is a specie of the genus *Allophylus* of the family Sapindaceae. It is commonly known as African false currant, ebe/ukpe (esan tribe in Edo state), akanro, akaraesu (in Yoruba), akaito (in Igbo) and karki (in Hausa). It is a shrub with flowers that are small and creamy-yellow (Burkill, 1985). The fruit is fleshy, red to black when ripe (Burkill, 1985). *A. Africanus* mostly grows in reverine thickest, open wood land and forest edges, often associated with mounds, at an altitude of 960-1540m. Its flowering time is usually December to March. It is widely distributed throughout tropical Africa, extending to the Eastern Cape South Africa (Burkill, 1985). *A. Africanus* has been reported to be used as not just for medicinal purposes but, also as food and horticulture (Burkill, 1985). In ethnomedicine, the leaves are used for the treatment of various ailments such as arthritis, rheumatism, gout, haemorrhoids, dysentery, veneral diseases and malnutrition (Burkill, 1985), root and twig are used as chewing stick for dental and oral healthcare and diarrhoea treatment. *A. Africanus* is reported to demonstrate biological activities such as anti-bacterial, anti-oxidant (Sofodiya *et al*, 2012) and anti-malarial activity (Oladosu *et al*., 2013). Some chemical components reported from *A. Africanus* leaves are tannins, saponins, flavonoids and carbohydrates (Oladosu et al., 2013).

1.5 Statement of Research Problem

For 99% of the population, chronic inflammation is an affliction of lifestyle which often serves as a precursor for chronic diseases such as arthritis (Gil, 2002). Most medicinal plants used in localities of various parts of Nigeria as well as those used by traditional medicine practitioners in management of inflammation and other ailments are not scientifically validated of which *A. africanus* is included. In the

Northern part of Nigeria, *A. africanus* is used for the management of arthritis which is an inflammatory condition.

1.6 Justification of Research Problem

In various countries across Tropical Africa, *A. allophylus* has been used as herbal remedy for various disease conditions such as arthritis without proper standardization. It becomesvery important to make an effort towards standardization of the plant as crude drug.Non-steroidal anti-inflammatory drugs ($NSAID_S$) are often used in the management of acute and chronic inflammation and these drugs have serious limitations due to their side effects. Therefore, there is the need to intensify research for plant based anti-inflammatory agents that are efficacious with low toxicity profile. *A. africanus* is known to be used in the Northern part of Nigeria for management of arthritis, therefore there is a need to validate its folkloric claim.

1.7 Hypothesis

The leaves of *A. africanus*contain constituents or bioactive compounds with anti-inflammatory activity.

1.8AIM AND OBJECTIVES

The aim of this research work is to determine pharmacognostic features of *A. africanus* leaves and to profer scientific basis for its use in the management of inflammatory disorders by traditional medicine practitioners.

1.8.1 Specific Objectives

1. To evaluate pharmacognostic features of *A. africanus* leavesthat can serve as standards for the identification of the plant

2 To determine the LD_{50} of the crude extract of *A. africanus* in Wistar rats using Lorkes method

3 To evaluate the crude extract of *A. africanus* for anti-inflammatory activity using carrageenan induced paw edema in Wistar rats

4 To evaluate the phytochemical constituents of the crude extract of *A. africanus* and fractions using Thin layer chromatography

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction of Family Sapindaceae

The Sapindaceae plant family is a family of flowering plants. Also known as the soap berry family, as currently grouped by Harrington *et al*., (2005) consist of 142 genera and 1900 species spread among four subfamilies: Dodonaeoideae, Hippocastanoideae, Sapindoideae and Xanthoceroideae. Members belonging to this family (Sapindaceae) occurs in temperate to tropical regions throughout the world. Many of the members contain a milky sap (latex). Also contained in their foliage, seeds or roots are mildly toxic saponins with soap-like qualities.

2.2 Identification and Characteristics of Sapindaceae Family

Plants belonging to the Sapindaceae family ranges from herbaceous vines or lianas to trees and shrubs. They are either monoecious or dioecious plants. Flowers are small and unisexual. Most often, the number of petals are four or five, though some members do not have petals (e.g. Dodonaea), Buerki *et al*., (2009). Fruits are either fleshy or dry and could be berries, drupes, nuts, capsules or schizocarps Buerki *et al*.,(2009). The Sapindaceae richly contain in their seeds toxic cyanolipids. It has been found that these metabolites are characteristics only of the Sapindaceae family and the seed oils are the source of the cyanolipids from which they are extracted, Avato *et al*.,(2005).

2.3 Classification of Sapindaceae Family into Genera

The Sapindaceae family consists of 142 genera as currently circumscribed (Harrington *et al*., 2005 and Buerki *et al*., (2009). According to the recent circumscription of the Sapindaceae family as mentioned above, the members are

spread into 4 subfamilies (Buerki *et al.*, 2009). But the treatment at the generic level remains partially resolved, especially in the Sapindoideae subfamily (Rodriguez *et al*., 2005). Recent alterations have added the synonymization of Distichostemon with Dodonaeae (Harrington *et al.*, 2010). The below is a list of some genera of the Sapindaceae family.

Genus	**Distribution**
Allophylus L.	Pantropical
Paullinia L.	Neotropics and one African species
Aporrhiza Radlk.	Tropical Africa
Bizonula pellegr	Gabon
Blighia pellegr	Gabon
Litchi	Southeastern China to Malaysia
Sapindus .L	Circumtropical
Hirania	Somalia
Smelophyllum	South Africa
Placodiscus	Tropical Africa
Pometia	Indian and pacific islands
Pancovia	West Africa
Mischocarpus	Southeastern Asia to Australia

Hornea	Mauritius
Gongrospermum	Phillippines
Erythrophysopsis	Madagascar
Acer .L	Temperate Northern Hemisphere
Xanthoceras	China
Billia	Mexico to South America
Dodonaea	Pantropical

2.4 Distribution and Description of *Allophylus* Species

As reported by (Piago and Delfino, 2014), there are about 255 species of *Allophylus*, which are distributed worldwide. These include subtropical and tropical regions of Africa, America, Asia, Australia, Indian Archipelago, pacific and Madagascar. The number of reported species of *Allophylus* in China is 11 species. Other reported species of *Allophylus* include *A. edulis* which occurs in Argentina, Bolivia, Uruguay (occurs in the coastal forest) and Brazil.*A. cobbe*is distributed in Bengal Srilanka and peninsula (Leenhouts, 1967) and found in the edge of mangroves, strand forest and semi-deciduous vine forest (Cook *et al., 2008*). Reported found species at Gilbert Island, Kiribati, in the United States are *A. litoralis, A.cobbe, A. laetus* and *A.timoriensis* (Cook *et al*., 2008). Nine (9) species are reported to be found in India. Martin *et al.,* (2009), reported 18 genera of *Allophylus* and 22 genera in West Africa, out of which 13 are spread widely throughout Nigeria (Keay *et al*., 1964).

Allophylus species are trees or shrubs to lianas. Flowers are unisexual which are usually female and male in the same inflorescence, small in size. Sepals are 4 in number with the 2 outer ones larger. Petals are 4, whitish, yellowish or greenish in

colour with each possessing a hairy scale. It has been reported that fruit is indehiscent, having 1-2 subglobose lobes, while seeds possess aril (Liu *et al*, 2015). *Allophylus* species are reported to have 3 foliate leaves (Hyde *et al*., 2005), though there are reports that some West Indian species of *Allophylus* are unifiolate but that all Central American species are trifiolate. They are alternate and pinnately compound.

2.5 Occurrence and Description of *Allophylus* species in Africa

Members of the Sapindaceae family have a wide distribution in West Africa, being spread over 26 genera and 104 species (Onuminya, 2014). They are reported to occur in forms varying from shrubs to trees and climbers with leaves that are either paripinnate (e.g. *Deinbolia*), compound trifiolate (e.g. *Allophylus*) or imparipinate (e.g *paullina*) while fruits are capsules or drupes, berry or trilobed. (Onuminya, 2014).

It is reported that there has been no complete revision of the African species of the genus *Allophylus* (De winter, 1956). However, a number of species have been reported to be found in different counties in Africa. Those found in West Africa includes*: Allophylus ujori cheek*, a small tree or spiny shrub of upper submontane forest in Cameroon and Nigeria, *Allophylus* conraui, a lowland to lower submontane spineless small shrub (Cameroon) (Martin *et al*., 2009), *A. africanus*and *A. spicatus* (Olokemeji forest reserve), *A. bullatus* (Buea mountain), *A. hirtellus* (Bakingili forest reserve), *A. spicatus* (Olokemeji forest reserve), *A. ferrugineus* (Bakingili forest), *A. abyssimicus* (Trinderet forest), *A. cobbe* (Buea mountain), *A. conraui* (Mambila plateau), *A. grandifolius* (Muyuka), *A. macrobotrys* (Limbe Botanic Gardens), *A. megaphyllus* (Ndian falls), *A. nigericus* (Calabar), *A. rubifolius* (Ndian falls), *A. talboti* (Yaounde) and *A. Zeakeri* (Batouri) (Onuminya, *et al*., 2014).

2.5.1 Occurrence and Description of Common *Allophylus* species in Nigeria

No comprehensive report has been made on the occurrence of *Allophylus* species in Nigeria, but a few reported by Onuminya *et al*., include *Allophylus conrani Gilg ex Radlk*. (Mambila Plateau), *A. nigericus Bak*. (Calabar), *Allophylus spicatus Radlk*. (Olokemeji forest reserve), *A. africanusP. Beauv* (Olokemeji forest reserve) and *A. bullatus*.

2.5.2 Description of *A. africanus*

Allophylus africanus is a shrub or small tree with trifoliolate leaves, obovate leaflets, variously hairy, margin dentate, serrate or almost entire. Inflorescences axillary, often branched, spike-like. Flowers are small and creamy-yellow (Burkill, 1985). The fruit is near spherical, fleshy, red to black when ripe (Burkill, 1985).



Plate I: ***Allophylus africanus*** **Leaves**



Plate II: *Allophylus africanus* growing in Samaru village, Zaria

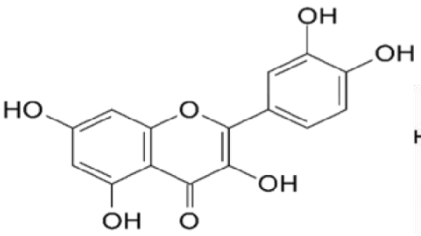
2.6 Chemical Constituents of *Allophylus* Species

The oil of seeds of *A. natalensis* and *A. dregeanus* have been reported to both contain type I cyano-lipids, 1-cyano-2-hydroxymethyl prop-2-en-1-ol diesters and also small amounts of type III cyanolipids, 1-cyano-2-hydroxymethyl prop-1-en-3-ol diesters (Avato *et al.,* 2005). *Allophylus serratus* is reported to contain plant constituents like apigenin -4-0-β-0-glucoside, pinitol, quercetin, luteolin-7-o-β-D glucopyranoside, rutin (Kumar *et al*., 2010), phenacetamide and β-sitosterol (Hegnauer, 1961). L-Quebrachitol is reported found in *A. edulis* (Diaz *et al.,* 2008). Reported in preliminary chemical characterization of the aqueous extract of *A. cominia* leaves are protein concentration, fatty acids and carbohydrates (Rodriguez *et al*., 2005). Reported Gas Column-Mass Spectrophotometry (GC-MS) analysis of *A. serratus Kurz* showed the plant to be rich in phenolics, flavonoids, tanning substances, saponins. Various fatty acid were also identified (Priya *et al*., 2012). Predominance of tannins, saponins, flavonoids and carbohydrates in leaves and other parts of *A. africanus* have been reported (Oladosu *et al*., 2013). It has been reported that 32 compounds representing 88% of the essential oil from leaves of *A. africanus* were characterized and 35 compounds from oil of flowers of *A. africanus* were identified (Oladosu *et al*., 2014). In addition, isolation and identification of four newcompounds, allotaraxerolide,alloeudesmenol, hanocokinoside and alloaminoacetaldehyde together with 2 compounds known, pinitol and stigmastane-3_{β}, 4_{β}-diol were reported in the whole plant of *A. africanus* (Oladosu *et al*., 2015).

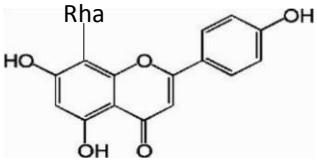
Reported isolated compounds from whole plant of *A. africanus* are allotaraxerolide, hanocokinoside, alloeudesmenol, alloaminoacetaldehyde, stigmastane-3_{β}, 4_{β}-diol and pinitol. The essential oil from leaves of the plant is reported to be dominated by 2- (1, 1dimethylethoxy)-ethanol, caryophyllene oxide, trimethylbenzene, 1-Ethyl-

1,4dimethylbenzene and hexahydrofarnesyl acetone (Oladosu *et al*., 2014). Phytochemcial studies reportedly revealed a predominance of carbohydrates, flavonoids, saponins and tannins (Oladosu *et al*., 2013).

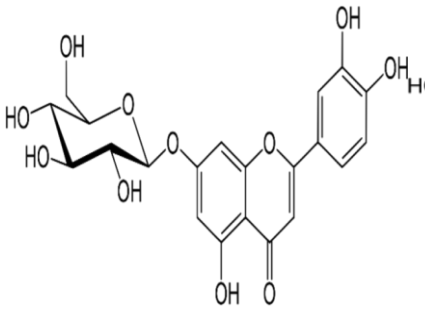
2.6.1 Chemical structures of some compounds isolated from *Allophylus* species



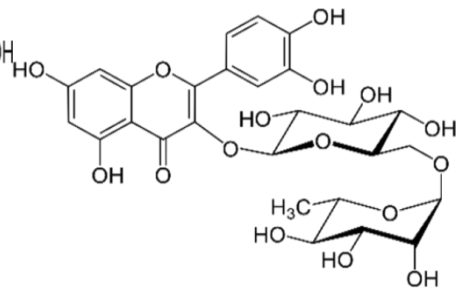
Pinitol



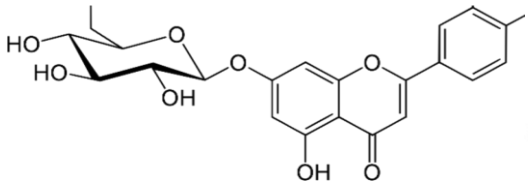
Apigenin-8-c-β-rhamnopyranoside



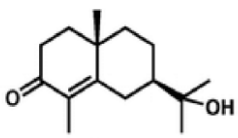
Luteolin-7-o-β-D-glucopyranoside



Rutin



Apigenin-4-o-β-D-glucoside



carrisone

Figure 2.1: Some chemical compounds isolated from various *Allophylus* species

Sources: (Dominguez *et al*., 1975; Ulubelen *et al*., 1971; Ghanta *et al*., 2007; David*et al*., 2004).

2.7 Biological Activities of *Allophylus* Species

The ethanolic extact of *Allophylus serratus Kurz* has been assayed for antiviral activity and was found to show antiviral activity against Ranikhet disease virus. It also showed gross effect on hypothermia and the central nervous system. (Priya *et al*., 2012). Antimalarial activity of *A. africanus* extract has been reported following suppression of parasitaemia in infected mice by 92.82% - 97.81% on day 7 post-infection against 96.81% for chloroquine (Oladosu *et al.,* 2013).

2.7.1 Antimicrobial Activity

Aqueous and ethanolic extracts of young and mature leaves of *Allophylus cobbe* and *Allophylusserratus* were evaluated for antibacterial activity and were found to both show good activity against *bacillus subtilis* and *staph. aureus* as compared to cefotaxime (Chavan *et al*., 2013). As reported by (Sofidiya *et al*., 2012), the methanolic extract of *A. africanus* was found to show strong antibacterial activity against gram-positive bacteria. Also reported is the antimicrobial activity of ethanolic and aqueous extracts of *A. edulis* against *Staph. aureus*. (Tirloni *et al.,* 2015).

2.7.2 Antidiabetic Activity

Hypoglycemic effect of aqueous extract of *A. cominia* was evaluated in type 2 diabetes model induced by streptozocin treatment in neonatal rats and was found to significantly decrease blood glucose values as compared to untreated diabetic group without the extract. This therefore show potential antidiabetic property of this plant. (Sanchez *et al*., 2014)

2.7.3 Anti-inflammatory and analgesic activity

Studies by Jain *et al* (2014), showed that the ethanolic extract of *A. cobbe* had significant analgesic and anti-inflammatory activity in laboratory mice and rats using hot plate, acetic acid induced writhing and carrageenan induced paw edema method.

2.7.4 Anti-Ulcer Activity

As reported by Jyothi *et al* (2012), the leaves of *A. serratus* was found to contain phenacetamide and beta-sitosterol which are responsible for its anti-ulcer activity. Hepato protective property of this plant has also been reported (Jyothi *et al*., 2012).

2.7.5 Anti-oxidant activity

The methanolic leaf extract of *A. africanus* is reported to show very good DPPH (1,1-Diphenyl-2-picrylhydrazyl) scavenging activity with % inhibition values >70% at an effective test concentration of 50 µg/ml, indicating its anti-oxidant activity (Sofidiya *et al*., 2012). Also, the ethanolic extract of leaves of *A. edulis* was reported to have prevented the lipid peroxidation in human erythrocytes and also to have inhibited oxidative hemolysis, indicating its antioxidant activity (Tirloni *et al*., 2015). Ethanol leaf extract of *A. cobbe* has also been reported for scavenging activity which was comparable to the standard ascorbic acid (Jain *et al*., 2014).

2.8 Ethnomedicinal and Economic Importance of *Allophylus africanus*

Various ethno botanical and economic uses have been attributed to the plant in Nigerian and West African folk medicine. The boiled bark, root and leaves are used in aches, fever and rheumatic pains (Sofidiya *et al.*, 2007). Twig and root are chewed for teeth cleaning, tooth ache and diarrhea in Edo State, NigeriaIdu *et al*., 2009). The decoction of leaves and powder of roots are used as appetite stimulant. Other medicinal uses of *A. africanus* in Senegal includes use of leaves in arthritis,

eye treatment, as febrifuges, in malnutrition, gout, veneral diseases and insanity. Leaf and root are used in dysentery, menstrual cycle disorders, dropsy and edema. The root is used as lactation stimulant, abortifacients and ecbolics, while the fruit is used in heart conditions, haemorrhoids and eaten as food.

In Agro-horticulture, the plant is used as bee or honey plants and insect plants. Economically, the branches of the plant are used as building materials. Forestry, farming, hunting products and fishing apparatus are obtained from the wood. Leaf and wood are used as fuel and lighting. Twig and root are also used for household, domestic and personal items (Burkill, 1985).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Equipment, Solvents, Reagents

3.1.1Chemicals/Solvents

n-Hexane (JHD, AR, Lobal Chem.

India)Ethyl acetate (Qualikems)

Methanol (Merck-Germany)

n-Butanol (Lobal Chem,

India)

3.1.2 Reagents

Xylene

Ferric chloride

Sulphuric acid (Sigma-Aldrich, St.Lous, MO, USA)

Antimony trichloride

p-anisaldehyde (Sigma-Aldrich, St.Lous,

MO, USA) Libermann-Bucchard reagent

Chloral hydrate

Sodium Hypochloride

Phloroglucinol

Aluminum Chloride

Acetic acid

Glycerol

Formalin Acetic acid Alcohol (FAA)

Hydrochloric acid

Fast green

Methylene blue

Safranin

Paraffin wax (BDH Laboratory Chemicals Division, POOLE England)

Dragendorff

Mayer's reagent

Wagner reagent

Sudan Red Solution

Zinc chloride Solution

3.1.3Equipment and materials

Photographic camera

Compound Microscope (Fisher Scientific, UK)

Stage Micrometer

Ocular Lens

Mechanical shaker (Stuart Scientific Flask Shaker, Great Britain)

Water bath (HHS, MC Donald Scientific International)

UV lamp

Ashless filter paper

Laboratory glass wares (Beakers, Measuring cylinder, conical flask, Funnel)

Weighing balance

TLC tanks

Microtome

TLC silica gel 60 F_{254} pre-coated plates (Merck-Germany)

Metallic cages and feeding bottles for mice

Carrageenan powder

Aspirin

Venier caliper

Disposable syringes 1ml, 5ml and 10ml

Normal saline

Acacia

3.2 Plant Collection, Identification and Preparation

Fresh, matured plant material comprising leaves, stem, fruits and flowers were collected in February, 2014, from Samaru village, Zaria, Kaduna State, Nigeria. The plant was identified as *A.africanus* with voucher number 1,540 at the herbarium unit, Department of Biological Sciences, Ahmadu Bello University, Zaria Nigeria by taxonomist Mallam M. Muhammad. The plant leaves were cleaned, air dried under shade and powdered to a suitable size and stored in appropriate container for further use.

3.3 Extraction of the leaves of *A. africanus*

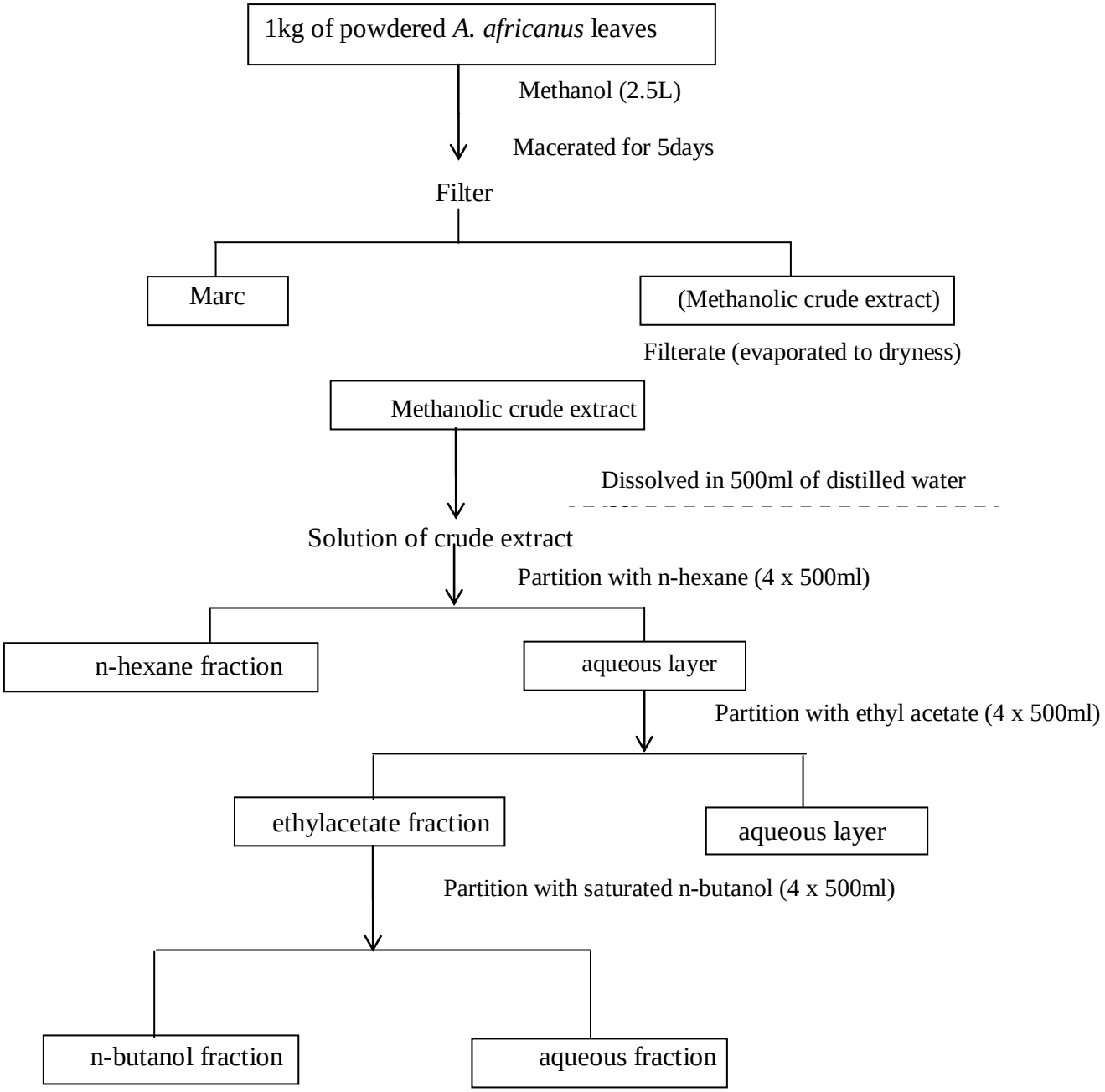


Figure 3.1: Extraction of Plant leaves with Methanol and fractionation with n-hexane,ethyl acetate and n-butanol

The plant material (1kg) was extracted with 2.5 liter of methanol (80%w/v) using maceration method for 5days with occasional shaking after which it was filtered and rinsed repeatedly with methanol. The filtrate was collected and then evaporated to dryness using a Rotary vapour and the percentage yield was calculated using the following formula:

$$\text{Percentage yield of extract} = \frac{weight\ of\ total\ extract}{weight\ of\ powdered\ material} \times 100(\%\frac{w}{w})$$

3.4 Fractionation of the crude methanolic extract of *A. africanus* leaves

The methanolic extract (65 g) was dissolved in 500ml of water and placed in a separating funnel. This was partitioned successively based on increasing polarity of solvents which include n-hexane (2L), ethyl acetate (2L) and n-butanol (2L) saturated with water. The fractions obtained were dried over a water bath to obtain dry residues.

3.5 Evaluation of the Pharmacognostic Features of *A. africanus* Leaves

Pharmacognostic studies was carried out on the leaves of *A. africanus* in order to determine characteristic features and parameters that can serve as pharmacopoeial standards. This was done by examining the macroscopic, microscopic, chemomicroscopic features and physicochemical evaluation.

3.5.1 Macroscopic Study of *A. africanus* leaves

Organoleptic evaluation of the leaf of *A. africanus* was carried out using some parameters such as colour, odour, taste and surface characteristics using standard procedure (WHO, 2002., Brain and Turner, 1975).

a. **Colour:** The colour of the leaf sample was determined under daylight and the result was recorded.

b. **Odour:** A small portion of the leaf sample was crushed between the index finger and thumb and between palms of the hands using gentle pressure and also the strength of the odour was determined as weak or as strong (rancid).

c. **Taste;** A small portion of the leaf sample was tasted using the tongue and the taste determined and recorded.

d.Surface texture:

The surface texture was described as smooth or rough or gritty, leathery, papery or fleshy by touching the surface of the leaves and the determined character was noted and recorded.

e. **Surface appearance:** The surface appearance was described as whole or broken by examining the surface which was noted and recorded.

f. **Size:** A graduated ruler in millimeters/centimeters was used for the measurement of length and width of the leaf and the result was recorded.

3.5.2 Microscopic Study of *A. africanus* leaves

a) **Surface preparations**

Anatomical sections of the sample of the leaf were examined under a microscope and description of the features was made by using the terms according to Dutta (2003) and Ahlam and Bouran (2011).

Proceedure

- **Fixation**: The fresh leaves of *A.africanus* was picked off directly from the tree and dipped immediately into the fixative, FAA (90% Ethanol, 10% formalin and Glacial acetic acid at the ratio 90:5:5 and was allowed to stand for 24 hours.

- **Dehydration**: The sample above was transferred into 30%, 50%, 70%,

  95% and 100% ethanol. This was carried out in each graded alcohol for

  2 hours each.

- **Clearing**: The sample was transferred into Ethanol: chloroform (75:25), Ethanol: chloroform (50:50), chloroform: Ethanol (75:25), and 100% chloroform. This was carried out after every 2 hours each.

- **Infiltration and Embedding**: Chips of paraffin wax was added slowly into the leaf sample and this was left to stand for 24hours before transferring into the oven at 60^0C. After melting, the paraffin containing the sample was transferred into the embedding box and allowed to solidify. This was then trimmed and mounted on the microtome to get the transverse or cross section of the leaf. The transverse section was then transferred on to slides.

- **Staining**: The above transverse section of sample was dewaxed in xylene, by changing twice for 5 minutes each. This was then hydrated in 95%, 70%, 50% and 30% ethanol for 2 minutes each. The transverse section was then

transferred into safranin and left to stand for 30minutes before washing with water. It was then transferred into 0.5% HCl in 70% ethanol shortly before dipping into fast green for 2 minutes and then washed with water.

The transverse section was then further dehydrated in 30%, 50%, 70%, 95% and 100% ethanol for 2 minutes each and cleared in xylene for another 2 minutes. Gum (Balsam) was sprayed along the transverse section and cover slip was placed. This was then observed under the microscope and appropriate images were taken and documented.

b). Micrometric evaluation

This involves measurements of dimensions (length and width) of the various diagnostic microscopic characters of the plant; it was carried out by using a binocular microscope with the aid of graticles (Kokate, 2003).

3.5.3 Quantitative microscopy of *A. africanus* leaves

c) Setting up the Camera Lucida

This was done in such a way that the light from the object passes directly to the observer's eye through an opening in the left hand prism. At the same time, light from the drawing paper and the pencil were reflected by the right hand prism, so that the pencil appears superimposed on the object, which was also traced. The illumination of both the object and the paper was suitably adjusted and the paper was tilted at the correct angle to avoid distortion, as the position of the drawing board to which the paper was pinned was correctly determined. Thereafter, the stage micrometer was placed on the microscope stage and its divisions were traced (Brain and Turner, 1975).

d) Calibration of Eyepiece Micrometer

This was performed as described in WHO manual (2011). The ocular micrometer was placed in the eyepiece of the microscope. The stage micrometer was placed on the stage of the microscope and was focused on the scale divisions. The eyepiece was turned in order to place the scale in a parallel position. The other point where two division lines were exactly superimpose was noted on the scale. The number of divisions on the ocular micrometer and the corresponding length on the stage micrometer scale were counted in order to determine the length that is equivalent to one division on the ocular micrometer scale. This serves as the calibration factor.

3.5.3.1 Determination of Parameters

Sections of the fresh leaf sample was cleared in chloral hydrate solution in a test tube using water bath. The cleared sample was mounted using glycerol on a slide with cover slip and viewed under the microscope (Evans, 2009). The parameters stomata number, stomata index, vein- islet and veinlet termination number were determined as described by Brain and Turner (1975) and Evans (2009).

(i) Determination of Stomata Number

Sample of the cleared preparation was viewed under the microscope, and the number of stomata was counted in 10 field of view. The average stomata number per mm^2 of leaf epidermis was calculated and result recorded.

(ii) Determination of Stomata Index

Number of stomata and epidermal cells were counted in 10 fields of view and the stomata index was calculated as percentage.

$$\text{Stomata Index} = \frac{S}{S+E} \times 100$$

Where S = stomata number

E = Number of epidermal cells

(iii) Determination of Vein-islets Number

The vein-islets number was determined by counting the number of vein-islets per mm^2 of the leaf surface midway between midrib and margin

(iv) Determination of Veinlet Termination Number

The veinlet termination number was determined by counting the number of vein terminations per mm^2 of leaf surface midway between midrib and margin

3.5.4 Physicochemical Evaluation of Powdered Leaves

3.5.4.1 Determination of Moisture Content (loss on drying) Method

3g of powdered leaf sample in a crucible was heated in an oven at 105^0C until a constant weight was obtained and recorded. The loss in weight was determined by subtracting the weight of the dish and the sample after heating from the weight of dish and content before heating. The percentage loss in weight was calculated. The determinations were conducted and the average of the values obtained was taken as the moisture content of the drugs (WHO, 2011, Evans, 2009).

3.5.4.2 Determination of Total Ash of *A. africanus* Leaves

2g of the ground air dried powdered leaves was weighed in a platinum crucible. It was ignited by gradual increase in incinerating heat, until it was white. It was cooled in a desiccator and weighed. The ash was then heated again and weighed. The heating and weighing continued until a constant weight was obtained. The contents of the total ash in mg per dried powdered sample were calculated and result was recorded. (WHO, 2011). The total ash value was calculated in percentage as:

$$\text{Total Ash Value (\%)} = \frac{Weight\ of\ Residual\ Ash}{Weight\ of\ Sample} \times 100$$

3.5.4.3 Determination of Acid Insoluble Ash of *A. africanus* Leaves

To the crucible containing the total ash, 25ml of Hydrochloric acid was added and covered with a watch glass and boiled gently for 5minutes. The insoluble matter was collected on an Ash less filter paper and washed with hot water until the filtrate was neutral. This was then transferred back to the crucible and ignited to a constant weight. The residue was allowed to cool in a desiccator for 30 minutes. It was then weighed without delay. The contents of the acid insoluble ash in mg per g of air dried powdered were calculated and recorded (WHO, 2011).

The acid insoluble ash was calculated in percentage as:

$$\text{Acid Insoluble Ash (\%)} = \frac{Weight\ of\ Residual\ Ash}{Initial\ Weight\ of\ Sample} \times 100$$

3.5.4.4 Determination of Water Soluble Ash of *A. africanus* Leaves

To the crucible containing the total ash, 25ml of water was added and boiled for 5minutes. The insoluble matter was collected in a sintered glass crucible. It was then

washed with hot water and ignited in a crucible for 15minutes. The weight of the residue in mg was subtracted from the weight of the total ash. The content of water soluble ash in mg per g of air dried powdered sample was calculated and recorded (WHO, 2011).

The water Insoluble ash was determined in percentage as:

Water soluble ash (%) = $\frac{Weight\ of\ Initial\ ash - Weight\ of\ Residual\ Ash}{Initial\ Weight\ of\ Sample} \times 100$

3.5.4.5 Determination of Water Soluble Extractive Value

5g of powdered drug sample was macerated in 100ml of water in a closed conical flask for 24 hours with frequent shaking for the first 24hours with frequent shaking for the first 6hours using a mechanical shaker and was later allowed to stand for 18hours. It was filtered and 25ml of which was evaporated to dryness at 105^0C to constant weight (WHO, 2011).

The water soluble extractive value was determined as:

Water Soluble Extractive Value = $\frac{Weight\ of\ Extract\ \ in\ 25ml\ X\ 4}{Original\ \ Weight\ of\ Powder}\ X\ 100$

3.5.4.6 Determination of Ethanol Soluble Extractive Value

5g of powdered drug sample was macerated in 100ml of ethanol in a closed conical flask for 24 hours with frequent shaking for the first 6hours using a mechanical shaker and allowed to stand for 18hours. It was filtered and 25ml of the filtrate was measured into an evaporating dish which was evaporated to dryness at 105^0C to constant weight (WHO, 2011). The Ethanol soluble extractive value was calculated (in percentage) with reference to the initial weight of the extract as:

Ethanol soluble Extractive Value (%) = $\frac{Weight\ of\ Extract\ \ in\ 25ml\ X\ 4}{Original\ \ Weight\ of\ Powder}\ X\ 100$

3.5.5 Chemomicroscopical Study of *A. africanus* Leaves

The anatomical transverse section of the powdered leaves was treated with the required reagents on clean slides and reactions observed under the microscope for the presence of cell inclusions and cell wall materials such as carbohydrates, calcium oxalate crystals, starch, cellulose, mucilage, tannins, etc. (WHO, 2011).

Method:

A known quantity of the powdered leaves sample of *A. africanus* was placed in a test tube and a small volume of chloral hydrate solution was added to the sample in the test tube and placed in a hot water bath for about 30minutes. The sample was then thoroughly washed with fresh water and used for the chemomicroscopical examination with glycerol used as the mountant. The procedure was done as described in WHO guidelines (2011).

Test for Cellulose: About 2 drops of zinc chloride was added to the cleared sample on a slide, and this was allowed to stand for few minutes. One drop of sulphuric acid was added, cover- slip applied and observed under the microscope. The appearance of bluish colour indicates the presence of cellulose in the cell walls of epidermal cells.

Suberized cell walls: Two drops of Sudan red was added to the cleared sample on a slide, cover slip was applied and gently heated over hot water bath for 2 minutes. The slide was then observed under the microscope for red colouration which indicates the presence of suberin.

Lignified cell walls: Few drops of phloroglucinol was added to the cleared sample and allowed to stand until almost dry. 1 drop of sulphuric acid was added and cover slip applied. This was observed under the microscope. Appearance of red colouration on the anatomical section indicates the presence of lignin.

Gums and Mucilage: Small amount of cleared powdered leaves sample and a drop of Ruthenium red was added. Appearance of pink colouration indicates the presence of gums andmucilage.

Calcium oxalate: To the cleared sample, few drops of Acetic acid was added, cover-slip applied and this was observed under the microscope. Few drops of hydrochloric acid was then added and observed. The presence of shining structures indicates the presence of crystals.

Tannin: To the cleared sample, a drop of Ferric chloride was added to the cleared sample and cover slip was applied and this was observed under the microscope. The appearance of greenish black coloration on the anatomical section of the leaves indicates presence of tannin.

Starch: Small amount of cleared powdered leaves sample was placed on a slide and a drop of N/50 Iodine was added. The appearance of bluish black colouration in some parenchyma cells of the leaf indicates the presence of starch.

Fats and Oils: Small amount of cleared powdered leaves sample placed on a slide and a drop of Sudan IV reagent was added and covered with a slip. Presence of pinkish colour indicates the presence of oils.

3.5.6 Phytochemical Screening of *A. africanus* Leaves

The methanolic extract of the leaves was subjected to phytochemical screening in order to identify the phytochemical constituents of the plant using the method described by (Evans, 2002;Sofowora 2008).

Methods:

3.5.6.1 Test for carbohydrates

Molisch's test: The extract (10 mg) was boiled in 10 ml of distilled water on a water bath for 3 min. The mixture was filtered while hot. A few drops of Molisch's reagent was added to 2 ml of the cooled filtrate and shaken, and then a small quantity of concentrated sulphuric acid was added and allowed to form a lower layer. The formation of a purple ring at the interface indicated the presence of carbohydrates.

3.5.6.2 Test for tannins

Ferric chloride test: The extract (0.5 g) was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Lead sub-acetate test: To 0.5g of the extract, two drops of lead sub-acetate solution was added and appearance of whitish-yellow precipitate indicated the presence of tannins.

3.5.6.3 Test for saponins

Frothing test: About 0.5 g of the extract was dissolved in 10 ml of water and shaken vigorously in a test tube for 30 seconds and allowed to stand for 30 minutes. The occurrence of frothing column or honey comb-like of at least 1 cm in height and persisting for at least an hour indicates the presence of saponins (Sofowora, 2008).

Haemolysis test: 2 ml of sodium chloride (1.8% solution in distilled water) were added to two test tubes A and B. 2 ml of distilled water were added to test tube A, 2 ml of the extract was added to test tube B. 5 drops of blood were added to each tube and the tubes were inverted gently to mix the contents. Haemolysis in tube B containing the extracts but not in tube A (i.e. control), indicate the presence of saponins in the extract (Brain and Turner, 1975).

3.5.6.4 Test for steroids and triterpenes

Liebermann-Burcchard test: About 1 ml of acetic anhydride was added to 0.5 g of the extract. Two drops of Conc. sulphuric acid was then added gently by the side of the test tube to the solution above and at the junction of the two liquids, a formation of reddish brown or violet brown ring, with the upper layer being bluish green or violet indicates the presence of sterols and triterpenes (Evans, 2002).

Salkowski test: About 2 ml of chloroform and two drops of sulphuric acid were carefully added to about 0.5 g of the extract from the side of the test tube to form a lower layer. A reddish brown coloration at the interface indicates the presence of steroidal ring (Sofowora, 2008).

3.5.6.5 Test for flavonoids

Sodium hydroxide test: few drops of sodium hydroxide was added to 5ml of the extract and the reaction was observed and recorded.

Shinoda test: About 0.5 g of the extract was dissolved in 2 ml of 50% methanol. Two drops of magnesium fillings and 3 drops of hydrochloric acid were added. Appearance of pink coloration indicates the presence of flavonoids (Evans, 2002).

3.5.6.6 Test for alkaloids

The extract was dissolved in 2% HCl. The mixture was filtered and the filtrate was divided into 3 equal portions. One portion was treated with few drops of Dragendorff's reagent, one portion was treated with equal amount of Mayer's reagent and the other portion was treated with an equal amount of Wagner's reagent. Appearance of orange, cream and reddish brown precipitates respectively indicated the presence of alkaloid.

3.5.6.7 Test for glycosides

Keller-Kiliani's test for Cardiac glycosides

To 10 mg of the extract in a test tube, 5 ml of water and 2 ml of glacial acetic acid containing one drop of 10% ferric chloride solution were added. The contents were thoroughly mixed and filtered. To 2 ml of the filtrate, 1 ml of concentrated sulfuric acid was added down the side of the test tube to form a layer underneath. A brown ring at the interface indicates the presence of a deoxysugar, characteristic of cardenolides, indicative of cardiac glycosides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout the layer (Evans, 2009)

3.5.6.8 Test for Anthracenes

Borntrager's test: About 0.5 g of the extract was added to 10 ml of benzene and shaken. This was then filtered and 5 ml of 10% ammonia solution was added to the Filtrate; stirred and the reaction was observed**.** The presence of pink or cherish red color indicates the presence of anthracenes (Evans, 2002).

3. 6.0 Acute Toxicity Study (LD_{50}) of Methanolic Extract of *A. africanus* Leaves

The acute toxicity study was carried out using mice of both sexes according to Lorke (1983) as described below:

- Stock solution of 300mg/ml of the methanolic extract was prepared by dissolving 1.5g in 5ml of distilled water. Acacia was added to enhance the solubility of the extract.

- Serial dilution of 10mg/ml, 100mg/ml and 1000mg/ml of the extract was prepared.
- In the first phase, the weighed animals were grouped into 3 groups with 3 mice each and the volume to be administered for each group of mice was then calculated using the formula:

$$\frac{Dose \times weight\ of\ mice\ in\ kg}{stock\ solution}$$

- The extract was then administered to the animals based on the volume of the extract calculated from above via oral route and observed for signs and symptoms of toxicity for 24 hours.

- The second phase was proceeded with the stated doses according to the method i.e. 1200mg/kg, 1600mg/kg, 2500mg/kg and 5000mg/kg. This involved 4 groups of one animal each. The stock concentration was prepared 500mg/ml and dissolved in 3mls of distilled water.

  The volume to be administered to each animal based on their weight was calculated and administered via oral route. Signs and symptoms of toxicity

was observed for 24 hours. The number of death which occurred was recorded.

- The Acute toxicity (LD_{50}) was calculated as the geometric mean of the lowest lethal dose that caused death and the highest non-lethal dose that did not cause death.

$$LD_{50} = \sqrt{\boldsymbol{minimum\ lethal\ dose \times maximum\ non-lethal\ dose}}$$

3.7.0 Evaluation of the Anti-inflammatory Activity of the Methanolic Extract of *A. africanus Leaves*

Effect of Methanolic Extract of *A. africanus* leaves on Carrageenan Induced Paw Edemain Rats

Thirty adult rats of both sexes of weight ranges (85-105g) were weighed and their weights recorded. They were then divided into 5 groups of 6 animals each. Stock solution of the extract, Aspirin and carrageenan were prepared. The zero reading of the paw sizes of the rats were taken using a venier caliper and recorded. To group I of the animals, 1ml/kg of normal saline was administered to each animal. To group II, Aspirin (positive control) at dose of 300 mg/kg was administered.

To groups III, IV and V, the extract at doses 250, 500, and 1000mg/kg was administered respectively. After an hour, 0.1ml of sterile saline suspension of 1% of carrageenan was injected into the sub planter surface of the left hind paw of all animals in each group. Paw size was measured using venier caliper at time 0, 1, 2, 3, 4 and 5 hours after carrageenan administration (Winter *et al.*, 1963).

3.8.0 To Evaluate the Phytochemical Constituents of the Extract using Fractions of *A. africanus* Leaves by Thin Layer Chromatography

The fractions were subjected `to thin layer chromatography and the retardation factor (R_f) value of each spot was calculated. The various spots were identified using detecting reagents. The solvent system and adsorbent used were as follows, (Trease and Evans, 2002).

1.**Adsorbent**: Precoated Silica gel G plate (Merck), thickness 0.25mm activated at 105℃ for 1hr

2.Solvent system:

(i) Hexane: Ethyl acetate (7:3)

(ii) Chloroform: Ethyl acetate: formic acid (2:2:1)

(iii) Butanol: Acetic acid: Water (8:1:1)

3.Detection techniques

(i) Daylight

(ii) Ultraviolet (280nm)

(iii) Spraying reagents

4.Spraying reagents

(i) P-anisaldehyde

(ii) Ferric chloride (for phenolic compounds)

(iii) Aluminum chloride (for flavonoids)

(iv) Antimony III Chloride (for terpenes and steroids)

3.9 Statistical analysis

Results were expressed as ± standard error of mean (SEM). The data was statistically analyzed using the one-way ANOVA to determine whether results in a particular test group were significantly different from those in the control groups. Results were statistically significant when p value is less than 0.05 ($p < 0.05$).

CHAPTER FOUR

4.0 RESULTS

4.1 Extraction of Plant Material with Methanol

1kg of the plant material was macerated for 5 days using 80%w/v methanol and evaporated to dryness to yield a residue (71 g) and the percentage yield was calculated using the formula: % yield value of methanol extract from plant *A.africanus*

$$= \frac{weight\ of\ total\ extract}{weight\ of powdered\ material} \times 100\% w/w$$

4.1.1 Fractionation of Methanolic Extract

The methanolic extract was dissolved in 500ml of distilled water and was partitioned with n-hexane (4x500ml), ethyl acetate (4x500ml) and n-butanol (4x500ml) successively and evaporated to dryness to yield residues respectively. A gummy residue was also obtained. Their percentage yields were also calculated. Result obtained is summarized in table 4.1

Table 4.1: Mass and Percentage Yield of the Methanol Extract and Fractions

S/N	Extract	Fraction	Mass (g)	% Yield (w/w)
1	Methanol	----------	71.00	7.10
2	----------	Hexane	1.41	0.41
3	----------	Ethyl acetate	5.37	0.54
4	----------	Butanol	9.30	0.93
5	----------	Gummy residue	32.40	3.24

4.2 Pharmacognostic Standards of A. *africanus* Leaves

4.2.1 Macroscopic features of the Leaf of *A. africanus*

The leaves of *A. africanus* was dark green in colour with fruity smell and papery in texture while the surfaces are whole. They are ovate in shape, with acuminate apex and a symmetrical base and the margin of the lamina is dentate. Attachment of leaf is petiolate. The features are summarized as shown in table 2.

Table 4.2: Macroscopic Evaluation of the leaf of *A. africanus*

Morphological characters	Observation
Colour	Green
Shape	Ovate
Size (length)	11.73cm- 10.2cm -8.67cm
Margin (width)	6.56cm- 5.7cm -4.8cm
Apex	Acuminate
Base	Symmetrical
Attachment of leaf	Petiolate
Surface appearance	Whole
Margin of lamina	Dentate
Venation	Pinnate

(—) RepresentsMean Value of 5 counts

4.2.2 Organoleptic Features of Leaves of *A. africanus*

The Organoleptic examination of the leaves of *A. africanus* gave the following sensory characteristics: Green colour, aromatic smell, bland taste and papery surface as summarized in table 4.3.

Table 4.3: Organoleptic Evaluation of Leaves of *A. africanus*

Sensory Characters	Observation
Colour	Green
Odour	Aromatic
Taste	Bland
Texture	Papery

4.2.3 Microscopic Features of *A. africanus* Leaves

Microscopic examination of the lower surface of *A. africanus* leaves revealed the presence of epidermal cells that are polygonal in shape with anticlinal walls that are straight. Also present are characteristics structures such as stoma which consists of a pore surrounded by two guard cells and the arrangement of the subsidiary cells around the stomata is anomocytic. There is also the presence of few trichomes which are covering in nature. Upper surface revealed the presence of epidermal cells that are irregularly shaped with wavy anticlinal walls and covering trichome. Stomata were not observed. The summary of results is given in table 4.4.

Table 4.4: Microscopic Features of the Upper and Lower Epidermis of A. *africanus* Leaf

Characters	Observation	
	Upper Epidermis	Lower Epidermis
Epidermal cells	Irregular in shape (28.70 µm X 18.30 µm)	Polygonal in shape (24.30 µm X 16.74 µm)
Anticlinal walls	Wavy	Straight
Stomata	Absent	Anomocytic (18.36 µm X 13.50 µm)
Trichome	Unicellular covering trichomes (36.31 µm x 12.52 µm)	Unicellular covering trichomes (68.39 µm x 14.31 µm) and Unicellular glandular trichomes (33.21µm x 18.10 µm)



Plate III: Micrograph of the upper epidermal layer of *A.africanus* leaf (Mag. x 400). Showing anatomical features, Ec- epidermal cell, Tr- trichome

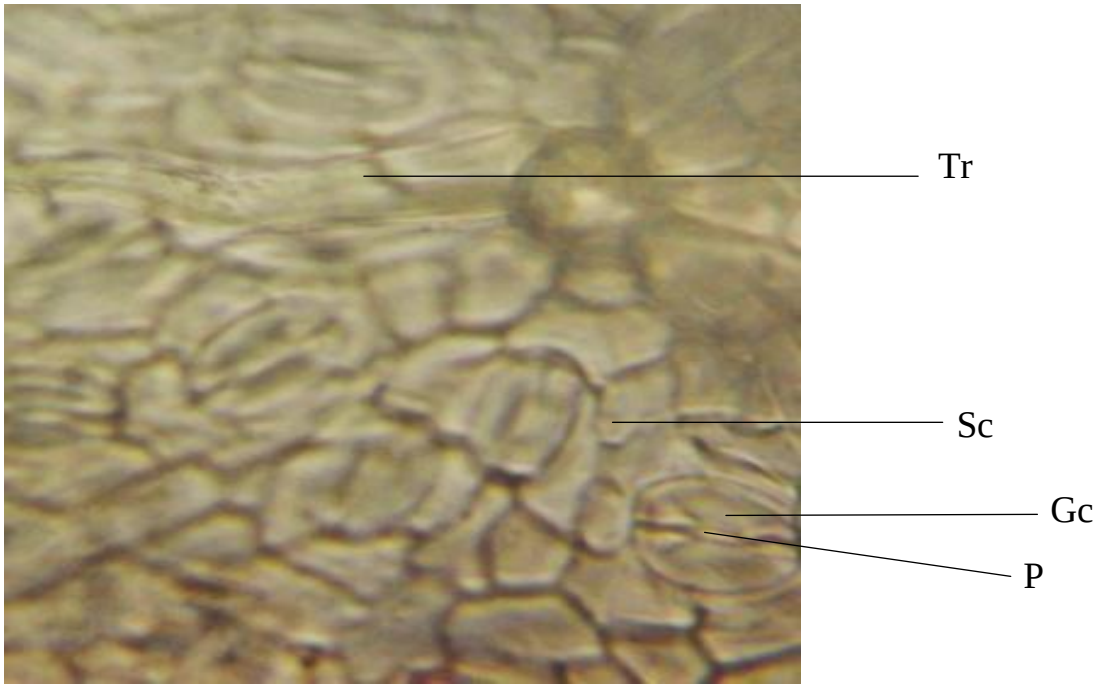


Plate IV: Micrograh of the Lower Epidermal Layer of *A.africanus* Leaf (Mag. x400).
Showing anatomical Features; Tr- trichome, Gc- guardcell, P- pore, - Sc-subsidiary cell

4.2.4 Transverse Section (TS) of *A. africanus* Leaf

The transverse section through the midrib of the leaf revealed a large conspicuous, concentric vascular bundle at the midrib region. Xylem and phloem are arranged in a ring form with the xylem ring occurring towards the center and is surrounded by phloem ring. A sheath of collenchyma appears above the phloem ring.

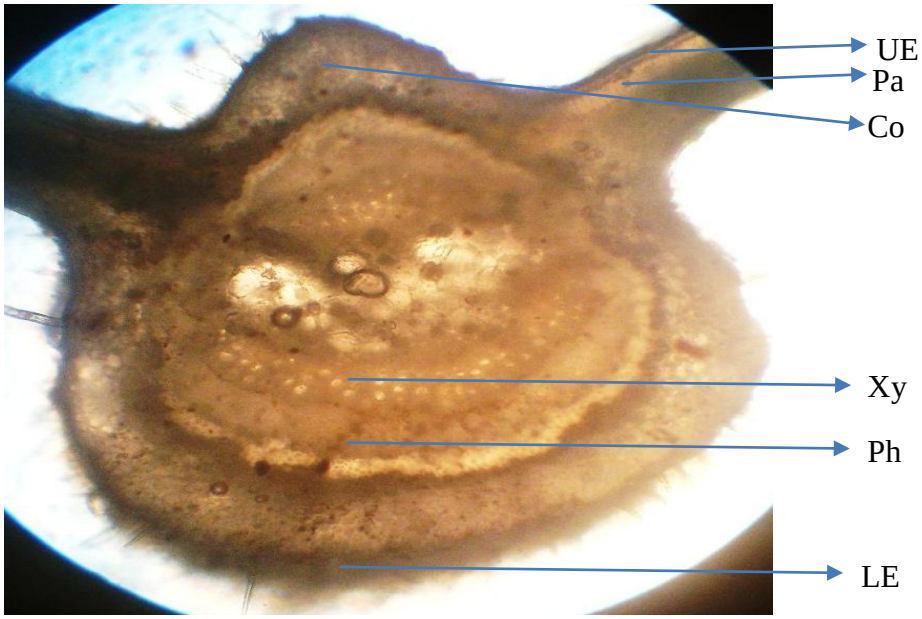


Plate V: Micrograph showing the Transverse section through the midrib of *A.africanus* Leaf (Mag x400); Co- collenchyma, Ph- phloem, Xy- xylem, Pa- Pallisade, UE-Upper epidermis, LE- lower epidermis

4.2.5 Quantitative Microscopic Features of *A.africanus* Leaf

Stomata number (32), stomata index (15), vein islet (15) and vein termination number on the average were determined and recorded. Result is summarized in table 4.5

Table 4.5: Quantitative Microscopic Features of *A .africanus* Leaf

Evaluative Parameter	Values*
Stomata number	27.2 -32 - 36.8
Stomata index	12.5 -15 -17.25
Veinlet termination number	33.15 - 39 - 44.85
Vein islet number	13.26 -15 - 17.94

*****Mean Value of 5 counts

4.2.6 Chemomicroscopic Examination of *A.africanus* powdered Leaves

Chemomicroscopic examination of powdered *A. africanus* leaves indicated the presence of starch, calcium oxalate crystals (13.5µm X 9.72µm), fats and oils, tannins, cellulose cell wall, suberin, gums and mucilage and lignin. Table 4.6

Table 4.6: Chemomicroscopic Features of *A. africanus* Powdered Leaves

Constituents	Reagents	Colour observed	Inference
Starch	N/50 Iodine	Blueblack colouration on grains within the chloroplast	Starch present
Calcium oxalate	80% H_2SO_4	Bright colour in the collenchyma cells with Prism like grey crystals	Calcium oxalate Crystals (Prism-type)
Fats and oils	Sudan (IV) solution	Red patches observed	Fats and oil present
Tannin	5% ferric chloride	Greenish black colouration in some parenchyma cells	Tannin present
Suberin	Sudan red	Orange colour on walls of epidermal cells	Suberin present
Gums and mucilage	Ruthenium red + few drops of lead acetate solution	Pink colouration observed	Gum and mucilage
Lignin	Phloroglucinol in 90% Ethanol + HCl	Red pink colour on the walls of lignified collenchymas cells	Lignin present

4.2.7 Physicochemical Constants of Powdered *A. africanus* Leaves

Quantitative extractive values and physical constants of *A.Africanus* leaves were determined. These include moisture content on drying, ash value, acid insoluble and water soluble values, water and alcohol extractives. The result obtained is(Table 4.7)

Table 4.7: Physicochemical constant values of *A. africanus* leaves

Parameter	Values (% w/w) ± SEM
Moisture content	7.78 ± 0.003
Total ash value	7.0 ± 0.007
Acid insoluble ash	1.5 ± 0.003
Water soluble ash	1.0 ± 0.003
Ethanol extractive	9.4 ± 0.127
Water extractive	16.6 ± 0.133

Average values of three determinations

4.3PhytochemicalScreening of *A.africanus* Methanolic leaf extract

The phytochemical screening of the extract revealed the presence of carbohydrates, steroids and triterpenes, glycosides, tannins, flavonoids and alkaloids.

Table 4.8: Result of Phytochemical Screening of Methanolic Extract of *A. africanus* Leaves

Constituents/Test	Observation	Inference
Carbohydrates		
Molisch's test	formation of a purple ring at the Interface	+
Tannins		
Ferric chloride	appearance of blue black Colouration	+
Lead subacetate	whitish-yellow precipitate	+
Saponins		
Frothing test	absence of frothing	-
Heamolysis test	no observation	-
Steroids and Triterpenes		
Lieberman-Burchard test	reddish brown to violet ring	+
Salkowski test	reddish brown colouration	+
Flavonoids		
Shinoda test	red/pink colouration	+
Cardiac glycosides		
Keller Kiliani test	reddish brown colouration	presence of deoxy sugar, characteristic of cardenolides (Cardiac glycoside)
Anthracenes	absence of pink to violet colour	-
Alkaloids		
Dragendorff's test	orange precipitate	+
Mayer test	Whitish precipitate	+
Wagner test	brown precipitate	+

+ = present, - = absent

4.4 Thin layer Chromatographic Analysis of the Fractions from the Methanol Extract of *A. africanus* Leaves

Thin layer chromatographic analysis was carried out on the fractions obtained from the crude extract in order to identify some of the phytochemical constituents present based on the colour obtained after spraying with reagents. The fractions included hexane and ethyl acetate. The solvent systems used are Hexane: Ethylacetate, (7:3), Chloroform: Ethylacetate: Formic acid (2:2:1) and Butanol: Acetic: Water (8:1:1) . The solvent system Hexane: Ethylacetate (7:3) was found to give the best separation. The colour of the individual spots were noted and their R_f values were calculated respectively.

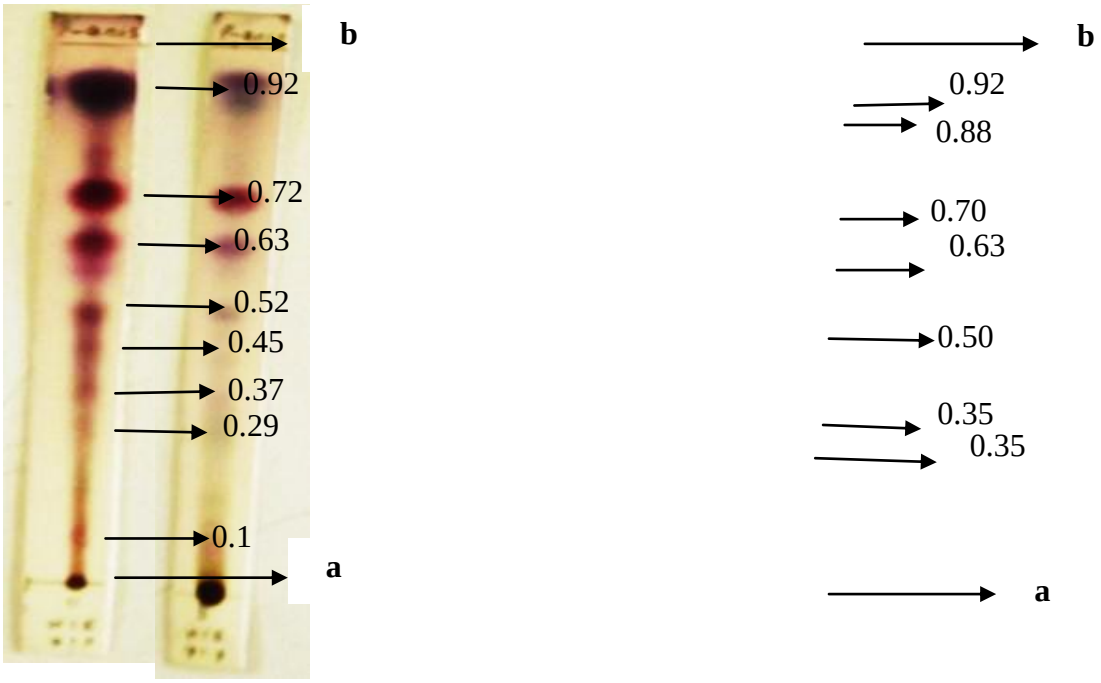


Plate VI: Chromatogram of *A. africanus* fractions after sprayed with p-anisaldehyde

A: hexane B: ethyl acetate(a- origin, b- solvent front)

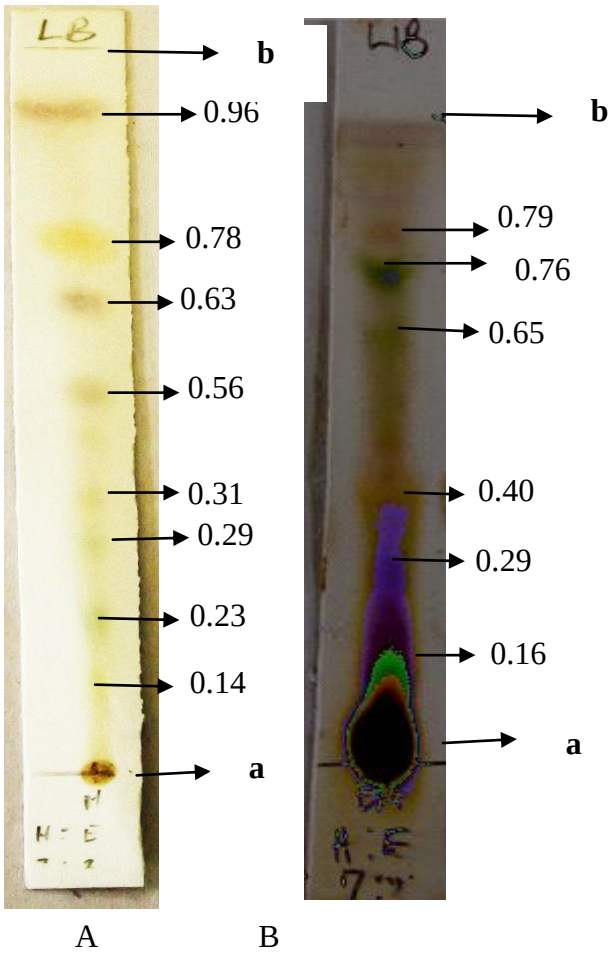


Plate VII: Chromatogram ofA. *africanus* fractions after sprayed with Liebermann-Buchard reagent

A: hexaneB: ethyl acetate(a- origin, b- solvent front)

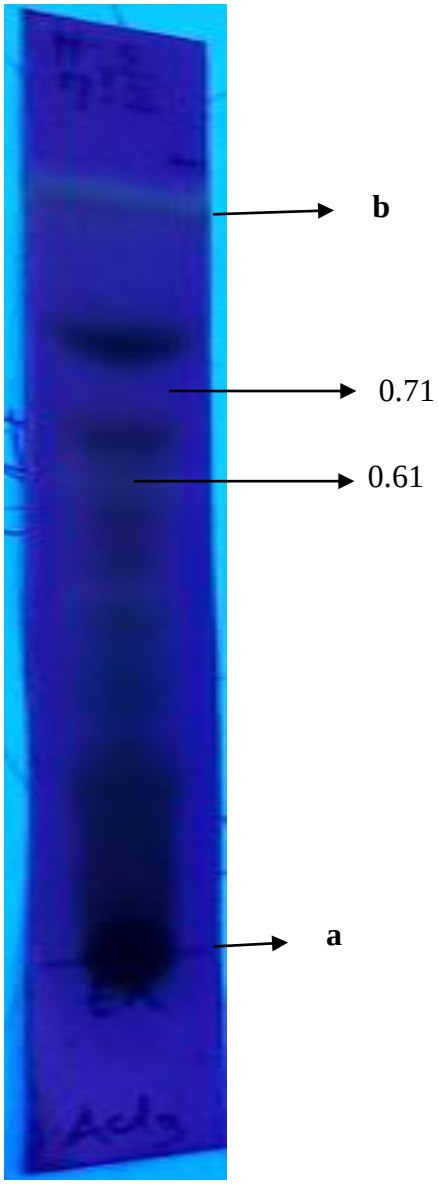


Plate VIII: Chromatogram of *A. africanus*ethylacetate fraction after sprayed with aluminium chloride and passed under UV light (a- origin b- solvent front)

Table 4.9:Summary of the Results of Chromatographic Analysis for the Fractions of the Methanolic extract of *A. africanus* leaves

Extract fraction	Spray reagent	Colour after spray	Number of spots	Retention factor (R_f)
Ethyl acetate	P-anisaldehyde	Green	10	0.05
		Purple		0.09
		Dark green		0.32
		Purple		0.35
		Grey		0.44
		Purple		0.52
		Purple		0.66
		Violet		0.74
		Blue		0.91
Hexane	P-anisaldehyde	Brown	10	0.1
		Green		0.21
		Pale purple		0.32
		Pale purple		0.45
		Dark purple		0.52
		Pink		0.61
		Purple		0.65
		Violet		0.75
		Purple		0.81
		Purple		0.96
Ethyl acetate	Liebermann	Pale green	6	0.21
	Bucchard	Dark green		0.31
		Violet		0.52
		Orange		0.64
		Orange brown		0.76
		Grey		0.93
Hexane	Liebermann	Yellowish green	9	0.13
	Bucchard	Green		0.21
	Reagent	Pale green		0.38
		Brown		0.37
		Orange		0.45
		Grey		0.64
		Violet		0.7
		Orange brown		0.75
		Grey		0.93

4.5 Determination of Median Lethal Dose of Methanolic Extract of *A. africanus* Leaves

The median lethal dose (LD_{50}) of the Methanolic extract of *A. africanus* was determined using Lorkes method via oral route and was calculated to be 3,807.89 mg/kg. The result is summarized as shown in Table 4.10.

Table 4.10: Median Lethal Dose (LD_{50}) of Methanolic Extract of *A. africanus* Leaves

DOSE (mg/kg)	RESULTS
First Phase	
10	0/3
100	0/3
1000	0/3
Second Phase	
1200	0/1
1600	0/1
2900	0/1
5000	1/1

* $LD_{50}= \sqrt{2900} \times 5000 = 3{,}307.89 mg/kg$

4.6 Evaluation of Anti-inflammatory Activity of Methanolic Extract

In the normal saline treated rats, sub-planter injection of 1% carrageenan suspension produced a local edema reaching its maximum at 3hours. The methanolic extract at doses 250mg and 1000mg/kg was able to significantly produce anti-inflammatory effect(at the 3rd, 4th and 5th hour). At the 4th and 5th hour, the effect was dose dependent. The extract at dose 1000mg/kg showed higher inhibition of induced edema compared to standard ASA at the 5th hour.

Table 4.11: Effect of the Methanol extract of *A. africanus* on Carrageenan Induced Paw Edema in Rats

Treatment	Dose	Mean paw edema in Diameters ± SEM (cm)				
	mg/kg	1 hr	2hr	3hr	4hr	5hr
Normal saline	1ml	1.76±0.26	2.29±0.15	2.40 ± 0.37	1.71±0.35	1.11± 0.17
ASA	300	0.89±0.18*	1.36±0.23*	0.94±0.06**	0.75±0.11*	0.58±0.12*
Extract	250	1.46 ± 0.19	1.65 ± 0.17	1.39 ± 0.19*	1.26 ± 0.16	0.58 ± 0.12
Extract	500	1.46 ± 0.16	1.99 ± 0.24	1.68 ± 0.22	0.95 ± 0.23	0.60 ± 0.18
Extract	1000	1.22 ± 0.15	1.81 ± 0.24	1.40 ± 0.25*	0.64±0.24*	0.19±0.13**

* $P < 0.05$, compared with Normal saline group

**$P < 0.01$ compared with Normal saline group

(One way ANOVA followed by Tukey's post hoc multiple comparison test) Data expressed as Mean ± SEM

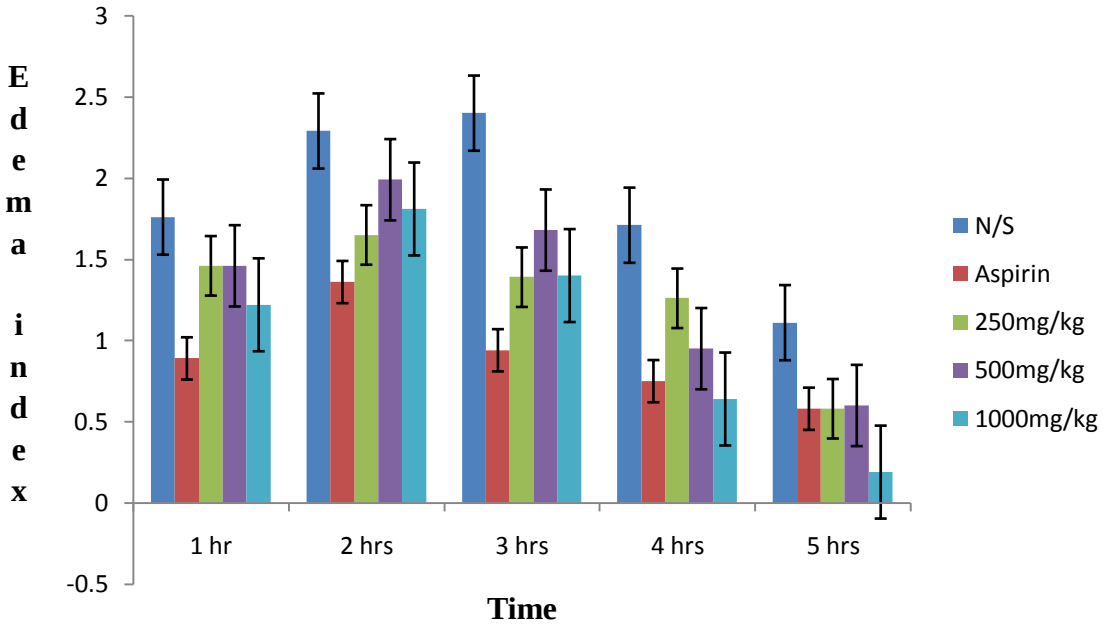


Figure 4.1: Effect of the Methanol Extract of *A. africanus* on Carrageenan induced Paw edema in Rats

CHAPTER FIVE

5.0 DISCUSSION

Macroscopy of the leaf of *Allophylus africanus* is characterized by dark green colour with fruity smell and papery texture while the surfaces are whole. They are ovate in shape with acuminate apex and a symmetrical base and the margin of the lamina is dentate. Attachment of the leaf was observed to be petiolate.

Microscopy of the leaf of *A. africanus* is characterized by epidermal cells in the abaxial and adaxial surfaces that differ in shape. The abaxial surface has epidermal cells that are polygonal in shape with straight anticlinal walls while those in the adaxial surface are irregular in shape with wavy anticlinal walls. Nature of epidermal cells can serve as important diagnostic feature because it greatly varies between species (Brain and Turner, 1975). The leaf was observed to have anomocytic stomata, which is present on the abaxial surface only (hypostomatic).

Anomocytic stomata has been reported in *Dodonaea viscosa*, a member of the Sapindaceae family (Venkatesh *et al.,* 2008). The type of stomata present can be used as diagnostic character in identification of plant drugs.

Transverse section of *A. africanus* leaf through the midrib had revealed that the leaves of the plant are dorsiventral in nature. Dorsiventral nature of the leaves is a characteristic feature of dicotyledonous plant (Dutta 2003) and therefore serve as distinguishing and diagnostic anatomical feature for the leaf. The vascular bundle tissues are cojoint, concentric and of hadrocentric type with the phloem tissue surrounding the xylem tissue entirely (plate III). Anatomical features or the internal structures of plant drugs are very useful in assuming the morphological groups of powdered and entire crude drugs and offer important diagnostic characters for the identification of both entire and powdered crude drugs and detection of adulterants

in them (Ghani, 1990). Determination of quantitative physical constants is of great value in the identification of crude drugs. Stomata number, stomata index, veinlet termination number and vein islets number are constants determined and this is the first of such report on the leaves of the plant. The stomata number for the lower surface of the leaf was found to be (27.2-32-36.8). The limits of the numbers have been shown to vary quite widely according to environmental conditions in which the plant was grown and are wide (Evans,2009). It has been stated by Evans (2009) that early investigation by Timmerman showed that the use of stomata numbers to distinguish between closely allied species are useless, but in certain situations where stomata occurs on the two surfaces, the ratio between the numbers may be of diagnostic importance. The stomata index for the lower surface of the leaf was estimated to be (12.5-15-17.5). The stomata index is a more useful parameter in identification as it is less subjected to variations with external conditions (Brain and turner, 1975).

The vein islet and veinlet termination number was estimated to be (13.36-15-17.94) and (33.20-39-4.9) respectively. The vein islet and veinlet termination number may appear to vary according to the preliminary treatment that the leaf has received (Evans, 2009). Chemomicroscopical features are unique to a particular plant and are used in standardization. The chemomicroscopical studies of the powdered leaves of *A. africanus* showed that the plant leaves contained starch, calcium oxalate crystals, fats and oils, tannins, cellulose, suberin, gums and mucilage and lignin (Table 6). Physico-chemical parameters such as moisture content, total ash content, water soluble ash content, acid insoluble ash content and extractable matter content serve an important role in standardization and quality control by means of purity, stability

and phytochemical composition of plant drugs (Gami and Parabia, 2010). The moisture content in the *A. africanus* powdered leaves was found to be 7.8%. The general requirement of moisture content in crude drug is that, it should not be greater than 14% (B.P., 1990) and the value observed in this research work was within the accepted range. Determination of the moisture content helps prevent degradation of drug during storage. The lower the value, the less likelihood of degradation of drug and suggests better stability of product. Moisture is considered an adulterant because of its added weight as well as the fact that excess of it promotes mold and bacterial growth (WHO, 2002). Ash values are used to determine purity and quality of crude drug. It indicates the presence of various impurities such as carbonate, oxalate and silicate. The water soluble ash (1.0%) contains mainly silica, particularly in sand and it indicates contamination with earthy material. (Gami and Parabia, 2010).

The acid insoluble ash value obtained in this study was 1.5%. The total Ash value (7.0%) represents both the physiological and non-physiological ash from the plant. The non-physiological ash is an indication of inorganic residue after the plant drug is incinerated. Total ash value is a reliable aid for detecting adulteration in drugs (WHO, 2002).

Extractive values determination in crude drug are useful because it gives an idea about the nature of the chemical constituents present. It is useful in evaluation of the chemical constituents present in the crude drug and also in estimation of specific constituents soluble in a particular solvent (Subha *et al*., 2014). This study showed that water had high extractive value of 16.6%w/w compared to ethanol which had extractive value of (9.4%w/w).

Preliminary phytochemical screening provides a brief idea about the qualitative nature of active phytochemical constituents present in plant extract. The result of the preliminary phytochemical screening of the methanolic extract indicated the presence of carbohydrates, steroids, triterpenes, glycosides, tannins, flavonoids and alkaloids. This result conforms with the findings of (Oladosu, 2013) who detected the presence of tannins, saponins, flavonoids and carbohydrates in *A. africanus*plant. Plants have different kinds of secondary metabolites such as flavonoids, tannins, saponins and these have been found to have anti-inflammatory activities both in vivo and in vitro (Mona *et al*., 2014). Anti-inflammatory activity of two triterpene saponins from *Quercus imbircaria* have been reported (Mona *et al*., 2014). Flavonoids have been reported to have variety of biological effects in numerous mammalian cell systems such as antimicrobial, antiviral, antineoplastic, anti-inflammatory, antioxidant, antihypertensive and antiplatelet activities (Mona *et al*. 2014).

Chromatographic analysis was conducted on the fractions (hexane and ethyl acetate) obtained from the crude extract on Thin layer chromatographic plates to identify the phytochemical constituents present and to affirm the earlier findings of the phytochemical screening. Thin layer chromatographic technique which is used for the analysis of natural and synthetic substances was used in this study to separate the factions of the extract into their various components which are mixtures of varying polarity using various solvent systems. The Thin layer chromatographic plates developed were visualized with different detecting reagents and some viewed under UV light in order to detect the presence of fluorescent compounds.

The n-hexane andethyl acetate fractions developed in hexane: ethyl acetate (7:3) gave coloured spots with p-anisaldehyde. Spots of various colours grey, green and violet were revealed. These colours obtained could be indicative of the presence of terpenoids (Harborne, 1973). Phenols, terpenes and steroids turn violet, blue, grey or green colors with p-anisaldehyde (Darmstadt, 1974). These colours were observed in the fractions. Liebermann Bucchard reagent yielded orange, orange brown and violet colored spots in hexane and ethyl acetate fractions respectively. These colour of spots may indicate the presence of triterpernoid/steroids.Spots which could indicate the presence of flavonoids were detected in the ethyl acetate fraction. This was observed as a yellow coloured fluorescence which was obtained under UV after spraying plate with aluminum chloride, which was used for the detection of flavonoids.

The median lethal dose LD_{50} of methanolic extract of *A. africanus* was found to be 3,807.89mg/kg. This suggests that the extract is slightly toxic (Lorke, 1983). LD_{50} which is the index of acute toxicity, is a useful index in evaluating safety margin but not to be regarded as a biological constant as differing results are obtained on repetitions or when determinations are carried out in different laboratories (Lorke, 1983). The methanolic leaf extract of *A. africanus* was able to significantly ($p<0.05$) produce anti-inflammatory effect at the peak of carrageenan induced edema but having activity less than that of the standard anti-inflammatory agent Aspirin in comparism. The extract at doses 250mg and 1000 mg/kg was able to significantly produce anti-inflammatory effect at the 3rd, 4th and 5th hour with the effect being dose dependent at the 4th and 5th hour. The extract however showed higher inhibition significantly ($p<0.01$) of induced edema compared to the standard Aspirin at the 4th and 5th hour. According to Musa *et al.,* (2010), inflammation induced by carrageenan

is thought to be biphasic. The early phase is mediated by histamine, serotonin and prostaglandin increased synthesis in the damaged surrounding tissues. The late phase is sustained by prostaglandins produced by tissue macrophages and prostaglandins released and mediated by polymorphonuclear cells, leukotriene and bradykinins.
The extract produced an inhibitory effect on carrageenan induced inflammation over a period of 4 hours which is similar to the effect of most non-steroidal anti-inflammatory drugs.

Anti-inflammatory effects of secondary metabolites like flavonoids, tannins, saponins, alkaloids have been reported (Mona *et al*., 2014), and some of these have been found present in the leaf extract of *A. africanus*. Hence, the anti-inflammatory effect produced by the extract of *A. africanus*plant may be attributed individually or collectively to constituents in the extract.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMENDATIONS

In summary, the leaf of *A. africanus* appeared to be ovate in shape with acuminate apex, symmetrical base and dentate margin with characteristic aromatic smell and papery texture. Important diagnostic features observed included anomocytic stomata on the lower epidermal surface, polygonal epidermal cells with straight anticlinal walls on the abaxial surface, irregular shaped epidermal cells with wavy anticlinal walls and unicellular covering trichomes on the adaxial surface.

Transverse section of the leaf through the midrib revealed vascular bundle tissues that are conjoint, concentric and of hadrocentric type.

The physico-chemical parameters of the plant leaves was determined as moisture content (7.8% w/w), total ash (7.0% w/w), water soluble ash (1.0% w/w), acid insoluble ash (1.5% w/w), water extractive value (16.6% w/w) and ethanol extractive value as (9.4%w/w).

Chemomicroscopical investigation revealed the presence of starch, calcium oxalate crystals, fats and oils, tannins, suberin, gums and mucilage and lignin. Phytochemical investigation revealed the presence of carbohydrates, steroids, triterpenes, glycosides, tannins, flavonoids and alkaloids.

Thin layer chromatographic studies revealed spots of various colours indicating different phytocompounds. Presence of UV active compound was also observed.
The median lethal dose (LD_{50}) of the methanolic leaf extract was determined to be 3,807.89 mg/kg.

The methanolic leaf extract at doses 250 and 1000mg/kg produced significant anti-inflammatory effect at the 3rd, 4th and 5th hour with the effect being dose dependent at the 4th and 5th hour.

6.1 Conclusion

Some pharmacognostic standards for the leaves of *A. africanus* have been determined in this study. The morphological and anatomical studies of the leaf will provide a means to identify the plant. The information obtained from the preliminary phytochemical screening will be useful in finding out the genuity of the drug. Ash values, extractive values can be used as reliable aid for detecting adulteration and authentication of the crude drug. The above studies can provide referential information for identification of the plant drug. Also, the results of the study showed that the methanolic extract of *A. africanus* leaves has anti-inflammatory activity which explained the basis of its use in traditional medicine in the management of inflammation and related inflammatory disorders. The significant anti-inflammatory activity of the methanolic extract observed might be associated to the secondary metabolites triterpenes /steroids and phenolic compounds present in the plant and this may proffer scientific basis for its use.

6.2 Recommendations

Not much research has been done on *A. africanus* which is used in various folkloric medicines in Nigeria and Africa. It is therefore recommended that more research should be channeled towards identifying and isolation of these active principles that are responsible for the anti-inflammatory activity which could possibly be exploited for pharmaceutical use.

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APPENDIX

APPENDIX I

Results of moisture content

Powdered *A. africanus* leaves

a. Weight of evaporating dish (W_1) = 51.58g

   Initial weight of dish + drug (W_2) = 54.58g

   Initial weight of drug (W_3) = 3g

   Final weight of evaporating dish + drug (W_4) =54.3g

   Loss in weight (W) = $W_2 - W_4 = 54.58 - 54.34g = 0.24g$

b. $W_1 = 47.98g$

   $W_2 = 50.98g$

   W_3= 3g

   $W_4 = 50.75g$

   $= 50.98g - 50.75g = 0.23g$

   Average loss in weight = $\frac{0.24+0.23+0.23}{3}$ = 0.233 ± 0.003

   % moisture content = $\frac{0.2333}{3} \; x \; \frac{100}{1} = 7.78\% \pm 0.003$

   Result of Ash value

   Powdered *A. africanus* leaves

a. Weight of empty dish (W_1) = 39.23g

   Weight of dish + powdered drug (W_2) = 41.23g

   Initial weight of powdered drug (W_3) = 2.00g

   Final weight of dish + drug (W_4) = 39.38g

Loss in weight (W) = $W_4 - W_1 = 39.38 - 39.23\text{g} = 0.15\text{g}$

b. W_1 = 39.24g W_2 = 41.24g

$W_3 = 2.00g$

W_4 = 39.37g

W = 0.13g

c.W_1 = 39.23g

$W_2 = 41.23g$

W_3 = 2.00g

$W_4 = 39.38g$

W = 0.15g

$$Average\ total\ ash = \frac{0.15 + 0.13 + 0.13}{3} = 0.137 \pm 0.007$$

$$\%\ Total\ ash = \frac{0.14}{2}\ x\ \frac{100}{1} = 7\% \pm 0.007$$

Result of Alcohol extractive values

Powdered *A.africanus*leaves

a. weight of empty crucible (W_1) = 64.74g

Initial weight of crucible + extract (W_2) = 64.96g

Initial weight of powdered drug (W_3) = 5.00g

Weight of residue (W) = $W_2 - W_1$ = 64.96g – 64.74g = 0.22g

20ml → 0.12g

100ml → $\frac{100\ x\ 0.12}{20}$ 1.1g

b. $W_1 = 64.83$

$W_2 = 64.95$

$W_3 = 5.00g$

W = 0.12g

20ml → 0.12g

100ml → $\frac{100\ x\ 0.12}{20} = 0.6g$

c. $W_1 = 64.84g$

$W_2 = 64.95g$

W = 0.11g

20ml → 0.11g

100ml → $\frac{100\ x\ 0.11}{20} = 0.6g$

Average alcohol extractive value = $\frac{0.22+0.6+0.6}{3} = 0.473 \pm 0.127$

% alcohol soluble extractive value = $\frac{0.47}{5}\ x\ \frac{100}{1} = 9.4\% \pm 0.127$

Result of water-soluble extractive value

Powdered A.*africanus* leaves

a. Weight of empty crucible (W_1) = 49.16g

Initial weight of dish + extract (W_2) = 49.38g

Initial weight of powdered drug (W_3) = 5.00g

Weight of residue (W) = $W_2 - W_1$ = 49.38 – 49.16g = 0.22g

0.22g in 20ml

100ml → $\frac{100\ x\ 0.22}{20} = 1.1g$

b. $W_1 = 49.26g$

W_2 = 49.40g

$W_3 = 5.00g$

W 0.14g

0.4g in 200ml

100ml → $\frac{100\ x\ 0.14}{20} = 0.7g$

c. $W_1 = 49.26g$

$W_2 = 49.40g$

$W_3 = 0.14g$

0.14g in 20ml

100ml → $\frac{100\ x\ 0.14}{20} = o.7g$

Average water soluble extractive value = $\frac{1.1+0.7+0.7}{3}$ $0.833g \pm 0.133$

% water soluble extractive value = $\frac{0.83}{5}\ x\ \frac{100}{1} = 16.6\% \pm 0.133$

Results of Acid insoluble ash

Powdered *A. africanus* leaves

a. Weight of dish (W_1) = 39.23g

Initial weight of dish + residue (W_2) = 39.26g

Initial weight of powdered drug (W_3) = 2.00g

Weight of residue (W) = W_2 - W_1 = 39.26 – 39.23 = 0.03g

b. $W_1 = 39.24g$

$W_2 = 39.26g$

$W_3 = 2.00g$

W = 0.02g

c. $W_1 = 39.23g$

$W_2 = 39.26g$

$W_3 = 2.00g$

W = 0.03g

Average acid-insoluble ash = $\frac{0.03+0.02+0.03}{3}$ 0.027 ± 0.003

% acid-insoluble ash = $\frac{0.03}{2} \; x \; \frac{100}{1} = 1.5\% \pm 0.003$

Results of water soluble ash

Powdered *A. africanus* leaves

a. Weight of dish (W_1) = 39.24g

Initial weight of dish + residue (W_2) $= 39.25g$

Initial weight of powdered drug (W_3) = 2.00g

Weight of residue (W) = $W_2 - W_1 = 39.25 - 39.24 = 0.01g$

b. $W_1 = 39.23g$

$W_2 = 39.25g$

$W_3 = 2.00g$

W = 0.02g

c. $W_1 = 39.23g$

$W_2 = 39.25g$

$W_3 = 2.00g$

W = 0.02g

Average water soluble ash = $\frac{0.01+0.02+0.02}{3}$ 0.017 ± 0.003

% water soluble ash = $\frac{0.02}{2} \; x \; \frac{100}{1} = 1\% \pm 0.003$

APPENDIX II

Percentage inhibition of Methanolic extract of *A. africanus* leaves on carrageenan induced paw edema in rats

Treatment	Dose (mg/ml)	% inhibition at various time				
		1hr	2hr	3hr	4hr	5hr
N/Saline	1ml/kg					
Extract	250	17.05%	27.90%	42.08%	26.32%	47.74%
Extract	500	17.05%	13.10%	30%	44.40%	45.95%
Extract	1000	30.68%	20.96%	41.67%	62.57%	82.90%
Aspirin	300	49.43%	40.60%	60.83%	56.14%	47.74%