PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF THE STEMBARK EXTRACT OF *ERYTHROPHLEUM AFRICANUM* (Welw. ex Benth) Harms

BY

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FACULTY OF SCIENCE
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

APRIL, 2015

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Shehu Ahmad ALHAJI

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DEPARTMENT OF CHEMISTRY, FACULTY OF SCIENCE
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA.

APRIL, 2015

DECLARATION

I solemnly and honestly declare that the work reported in the dissertation entitle **"Phytochemical and antimicrobial studies of the stembark extracts of *Erythrophleum africanum* plant"** has been carried out by me, in the Department of Chemistry A.B.U Zaria under the supervision of **Professor A.O. Oyewale** and **Professor J.O. Amupitan**. The work has not been accepted in any previous application for a higher degree elsewhere and the information derived from the works of other investigators are acknowledged and referenced accordingly.

April, 2015

Shehu Ahmad Alhaji _____ _____

Signature Date

CERTIFICATION

This dissertation entitled the **"Phytochemical and antimicrobial studies of the stembark extracts of *Erythrophleum africanum* plant"** by Shehu Ahmad Alhaji meets the requirements of the regulations governing the award of a Master of Science Degree in Organic Chemistry of Ahmadu Bello University, Zaria and it is approved for it contribution to knowledge and literary presentation.

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Head Department of Chemistry	Signature	Date

Prof. A. Z. Hassan

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Dean, School of Post Graduate Studies	Signature	Date

DEDICATION

This thesis is dedicated to the glory of almighty Allah and his beloved prophet Muh'd (SAW) and His apostles, My Parent late Alhaji Muh'd Saba and Malama Fatima saba and Ramatu saba, my uncle Aliyu Muh'd Babanna, his wives Hajiya Hawawu and Hajiya Amina, my brother Alhassan K. Saba, Abdullahi Adamu, Mohammed Saba, Mohammed Haruna, Aishat Adamu and late sister Fatimah Saba (Nana) may her soul rest in peace.

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ABSTRACT

The Stembark of the *Erythrophleum africanum* plant (Welw. ex Benth) collected from Niger State, Nigeria was air-dried, pulverized and exhaustively extracted by maceration using ethanol. The phytochemical screening of the extract revealed the presence of secondary metabolites which include cardiac glycosides, flavonoids, terpenoids, saponins, alkaloids and steroids. The antimicrobial sensitivity test showed that the extract was sensitive to these organisms (with zone of inhibition indicated): *S. aureus* (25 mm), *S. faecalis* (27 mm), *E. cloacae* (29 mm), *P. mirabilis* (22 mm) and *C. stellatoidea* (24 mm) while *E. coli*, *P. aeruginosa* and *C. albicans* were resistant. The minimum inhibitory concentration (MIC) results revealed that the extract inhibited the growth of five (5) out of eight (8) tested organisms at 5 mg/ml for *S. aureus, S. faecalis, E. cloacae, P. mirabilis* and *C. stellatoidea*. *P. aeruginosa, E. coli, C. ulcerans* and *C. albicans* are not inhibited even at 20 mg/ml. The minimum bactericidal/fungicidal concentration (MBC/MFC) of the extract was 20 mg/ml against *S. aureus* and *P. mirabilis,* and 10 mg/ml for *S. faecalis, E. cloacae* and *C. stellatoidea,* while *P. aeruginosa, E. coli, C. ulcerans* and *C. albicans* proved to be resistant. The crude ethanol extract was dissolved in distilled water and then partitioned with petroleum ether, ethyl acetate, chloroform and n-butanol respectively. Preparative TLC on the n-butanol fraction developed with ethyl acetate/methanol (7:3 v/v) led to the isolation of the compound coded, N_A. The isolated compound N_A was screened for antimicrobial properties with the zone of inhibition found to be higher than that of crude ethanol extract. For the isolated compound N_A, the zone of inhibition are *S. aureus* (26 mm), *S. faecalis* (29 mm), *E. cloacae* (30 mm), *P. mirabilis* (26 mm), and *C. stellatoidea* (29 mm). The minimum inhibitory concentration of the isolated compound N_A against *S. aureus* and *P. mirabilis* was observed at 5 mg/ml and for *S. faecalis, E. cloacae* and *C. stellatoidea* at 2.5 mg/ml while *P.*

aeruginosa, *E. coli*, *C. ulcerans* and *C. albicans* are not inhibited even at 20 mg/ml. The minimum bactericidal/fungicidal concentration of the isolate N_A against *S. aureus*, *P. mirabilis*, and *C. stellatoidea* was observed at 10 mg/ml while *S. faecalis* and *E. cloacae* was observed at 5 mg/ml, and the organisms showed moderate to very heavy colony growth at 2.5 to 1.25 mg/ml. The isolated compound N_A was subjected to NMR analysis i.e ^{1}HNMR, ^{13}CNMR, DEPT, COSY, NOESY, HMBC and HSQC from which the structure of the isolated compound N_A was elucidated and found to be ipolamiide which was confirmed from spectra data reported in the literature.

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ABBREVIATIONS

TLC	Thin Layer Chromatography
Kg	Kilogram
ml	Milliliter
g	Gram
%	Percentage
mm	Millimeter
v/v	Volume By Volume
mg/ ml	Milligram Per Milliliter
MIC	Minimum Inhibition Concentration
MBC/MFC	Minimum Bactericidal/Fungicidal Concentration
^{1}HNMR	Proton Nuclear Magnetic Resonance
^{13}CNMR	Carbon 13 Nuclear Magnetic Resonance
DEPT	Distortionless Enhancement by Polarization Transfer
H-HCOSY	Proton-proton Correlation Spectroscopy
HMBC	Heteronuclear Multiple Bonds Correlation Spectroscopy
HSQC	Heteronuclear Single Quantum Correlation Spectroscopy
NOESY	Nuclear Overhauser Enhancement Spectroscopy

CHAPTER ONE

1.0 INTRODUCTION

Traditional medicine is an important part of human health care. It is the sum total of knowledge, skill and practice based on the theory, belief and experience used in maintaining health as well as curing diseases using medicinal plants. The early man lived and depended on plant for his food, fuel, shelter and medicare. In the course of doing this, they notice that some plants when consumed gave them some relief from symptoms of discomfort. Some were just for nourishment, some elevated their mood through hallucinations and quite a number make them ill or even killed them (Thomson, 1978).

However, going by this gradual proceeding of trial and error, primitive man was able to sort out flora they could eat, those they could not, those with healing properties and the knowledge concerning their medicinal usage. Hence, varieties of plants have been used medicinally and for this reason the plant kingdom was described as the sleeping giant of drug development by Verpoorte *et al.* (1987), while the forest is generally referred to as "God's Own Pharmacy" (Treben, 1986).

The medicinal plants are those in which one or more of its parts contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Sofowora, 1993). The medicinal property of plants depends on the presence of a variety of chemical substances known as secondary metabolites. These secondary metabolites are constituents synthesized by plants in addition to the primary metabolites, which may be concentrated in different parts of the plants (Evans, 1997).

Some of these compounds include saponins, glycosides, flavonoids, alkaloids, steroids, terpenoids, tannins, volatile oil, organic acids, e.t.c which differs from one plant to another at varying concentrations (Miller, 1973). The phytochemical examination of these plant constituents has been made possible and easier by improved methods of extraction, separation, isolation and characterization (Harbone, 1973).

The application and research for drugs, food and feed supplements obtained from plants have increased over the years (Frankič *et al.,* 2009). Spices, herbs and their constituents are generally recognized to be safe, either because of their traditional use without any documented detrimental effect or because of much toxicological studies (Frankič *et al.,* 2009). Plants being a rich source of secondary biomolecules which exhibit significant pharmacological effects, spices and herbs appeal to many consumers who question the safety of synthetic food additives (Craig, 1999).

Plant extracts and essential oils have been used as alternatives to antibiotics due to their antimicrobial activities and the favorable effect on the animal intestinal system (Al-Kassien, 2009). Spices and herbs can have a great influence on the function and reactivity of the immune system of the farm animals (Craig, 1999). The growth promoting active feed supplements improve stability of feed and auspiciously impact the digestive micro population mostly through inhibition of pathogenic microorganism growth. In consideration of promoted health status of intestinal tract, farm animals are less exposed to the toxins produced by different microorganisms (Frankič *et al.,* 2009).

Windisch *et al.* (2008) reported that spices and herbs have beneficial effect on the stress resistance of the animals and amplify the absorption of essential nutrients. There is also, one other important advantage of using plant extracts of herbs and spices or their essential oils instead of synthetic drugs in feed stocks: synthetic drug residues in animal meat and eggs can cause health problems in people who consume them, especially due to increasing resistance of pathogens present in the human body as a result of prolonged use of synthetic drugs (Barbour *et al.,* 2010).

Despite the tremendous progress in human medicine, infectious diseases caused by bacteria, fungi, virus and parasites are still a major threat to public health. Their impact is particularly large in developing countries due to relative unavailability of medicine and emergence of drug resistance (Zampini *et al.,* 2009). During the last two decades, the resistance of microorganisms to synthetics drugs

as well as the appearance of undesirable side effects of certain antibiotics reported by Okemo *et al.* (2003), has led to the search for new biologically active compounds from natural sources as new antimicrobial agents with the view to discover new chemical compounds, which could overcome the above disadvantages (Meenakshi *et al.,* 2001; Bouamama *et al.,* 2006).

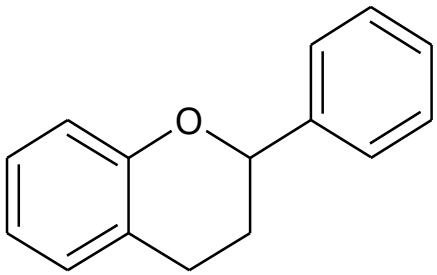
The development of resistance to current antibiotics by disease causing microbes has also reinforced research for discovery of new ones. Current trends in drug development process are focused on natural sources, especially of plant origin due to some proven correlation between the folkloric medicinal uses of some of these plants to biological activity. Therefore, the use of plant materials to prevent and treat infectious diseases successfully over the years has continued to attract the attention of scientist's worldwide (Osawa *et al.,* 1990; Begum *et al.,* 2002; Sophon *et al.,* 2002).

In some parts of the world, the administration of decoction of plants is still in practice (Chopra, 1958; Said, 1969). Traditionally, this treasure of knowledge has been passed on orally from generation to generation without any written documentation (Perumal and Ignacimuthu, 2000). The importance of herbs in the management of human ailments cannot be overemphasized. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Furthermore, the active components of herbal plants have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components (Shariff, 2001). In the past century, remarkable progress in the discovery of antimicrobial drugs has been made and thus several numbers of synthetic drugs have also been synthesized. These new synthetic antimicrobial drugs play a vital role in treatment of various types of microbial infections and reducing the number of fatalities that are associated with infections caused by microbes. However, despite this remarkable progress, multiple **drug resistance** pathogenic

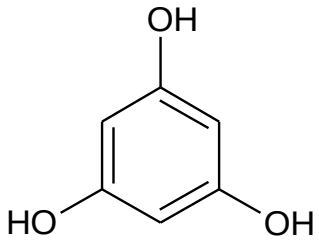
microorganisms have emerged due to indiscriminate use of the commercially available antimicrobial drugs in human (Cowan, 1999; Newman *et al*., 2000; Poole, 2005). Thus, the emergence of previously uncommon infections is considered as a serious medical problem by World Health Organization, governments, the public health and scientific communities as well as health care providers at large. In fact, approximately 25 % of prescribed medicines in industrialized countries are obtained directly or indirectly from plant sources (Newman *et al*., 2000). In addition, according to an estimate of the World Health Organization, approximately 70 to 80 % of the world populations presently use plants for medicinal purposes (Wills *et al*., 2000).

It is also worthy of note that in **developing countries** where medicines are quite expensive, investigation on antimicrobial activities from **medicinal plant**s may still be needed. Nonetheless, plants used in folk medicine in most developing are still understudied, particularly in clinical microbiology (Kirby, 1996; Wills *et al*., 2000; Pavrez *et al*., 2005; Zakaria *et al*., 2007). Recently, several numbers of plant species have been investigated for their potential **antimicrobial activity** using various extraction methods (percolation, tincture, soxlet, maceration e.t.c) with water, methanol, ethanol, n-hexane, chloroform, butanol and acetone. These extracts have been assessed for their **antimicrobial activity** against several numbers of bacteria isolates by broth dilution (Bacteria are inoculated in liquid growth media), microtiter plate and/or disc diffusion methods(Bacteria are inoculated in agar media) (Scalbert *et al*., 2005; Zakaria *et al*., 2007; Lawrence *et al*., 2009). For example, Sweet Basil, stem bark of *Distemonanthus benthamianus* Baill, *Muntingia calabura* (L.) and *Dicranopteris linearis* (L.) and *E. suaveolens, E guineense, E. ivorense,* have been shown to exhibit **antibacterial activity** against some selected bacterial strains (Zakaria *et al*., 2007; Aiyegoro *et al*., 2007; Moghaddam *et al*., 2009). These studies demonstrated that plant extracts derived from these plant species could be a potential source of antibacterial agents for the treatment of normal infection caused by some bacteria such as *H. pylori* strains, *S. aureus* strains, *E. coli* and others. Additionally, several mechanisms of action have been

suggested with regards to the chemical compounds which might be present in these plant extracts, particularly, flavonoids (**1**) (rutin and quercetin), tannins (**2**) and others (Aiyegoro *et al*., 2008).



Flavonoid (1)



Tannin (2) phloroglucinol

Furthermore, previous as well as recent investigators reported that phenols, polyphenol and flavonoids are natural antioxidant plant products that have been found in various concentrations in most **medicinal plant**s as well as other plant kingdom (Rice-Evans *et al*., 1995; Middleton *et al*., 2000; Scalbert *et al*., 2005; Abdel-Hameed, 2009). They also indicated that these compounds have been shown to possess various therapeutic values such as antibacterial, anti-mutagenic, anti-carcinogenic and anti-inflammatory, anti-aging activities by reducing the oxidative damages or stresses that are commonly induced by **free radical** species. Furthermore, the growing interest in the substitution of synthetic

antioxidants with natural ones has encouraged research on plant sources and screening of raw materials for identifying new ones. Plant-derived antioxidants such as ascorbic acid, β-carotene, α-tocopherol, **phenolic acids** and flavonoids, among others, are becoming increasingly known as a primary dietary factors for humans (Ferguson, 2001; Martin and Appel, 2010). The interest in these compounds is largely due to the growing evidence of their potential health benefits, particularly for their role in reduced risk for heart diseases, cancer and age-related diseases as well as protection against cellular damage caused by **reactive oxygen species** (free-radicals) (Ferguson, 2001; Abdel-Hameed, 2009; Martin and Appel, 2010).

Reduced susceptibility to antibacterial drugs is continuously increasing which is attributed to indiscriminate use of broad-spectrum antibacterials and immunosuppressive agents (Dean and Burchard, 1996). This situation provided the impetus to search for new antimicrobial substances from various sources like medicinal plants reported by Cordell (2000) and screening of these plants may result in the discovery of new effective compounds and can raise the spectrum of untreatable bacterial infections and adds urgency to the search for new infection fighting strategies (Tomoko and Takashi, 2002). There is a need to search for new infection-fighting strategies to control microbial infections. Several studies have been carried out on various medicinal plants screening for their antimicrobial activity (Parekh and Chanda, 2006; Firas and Hassan, 2008). For the past two decades, antibacterial properties of various plants and plant parts like root, stem, leaves, seeds and flowers have been well documented for some of the medicinal plants (Nandagopal and Kumari, 2007).

The acceptance of traditional medicine using medicinal plants as an alternative form of health care and the development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial herbal extracts (Bisignano *et al*., 2000; Hammer *et al*., 1999). Plants containing flavonoids, terpenoids, steroids, phenolic compounds and alkaloids have been reported to

have antimicrobial activity (Hostettmann *et al*., 1977). The medicinal plant plays an important role in the healthcare system of the third world population (Akerele *et al.*, 1991). Africa, particularly Nigeria is rich with indigenous plants which are used in herbal medicine to cure diseases and to heal injuries.

Investigators have implicated alkaloids to be directly involved in eliciting diverse biochemical and pharmaceutical characteristics on biological systems (Clarke, 1970). In the Savannah regions of Nigeria, the cattle herders always prevent their animals from grazing along the routes where these species are known to grow (Nwude and Chineme, 1981). Other investigators have reported cases of accidental human poisoning after the use of the bark of *Erythrophleum* species for traditional uses (Nwude, 1981).

1.2 Aim and Objectives of the research

1.2.1 Aim of the research

To isolate and characterize the chemical components of the stembark extract of *E. africanum* and investigate its antimicrobial activities as well as ethnomedicinal/cultural claims for its use as medicinal plant.

1.2.2 Objectives of the research

The objectives of the study are:

1. Collection, identification, drying, grinding of plant material and extraction of powdered plant with ethanol to exhaustion.
2. Partitioning of crude ethanol extract with petroleum-ether, ethyl acetate, chloroform and n-butanol.
3. Phytochemical screening of the plant extract.
4. Antimicrobial studies of the plant extract

5. Isolation and structural elucidation of the isolated compound(s) using spectral techniques e.g ^{1}HNMR, ^{13}CNMR, DEPT, COSY, NOESY, HMBC and HSQC.

1.3 Statement of the Problem

Many active compounds are known which are use in the treatment of different types of diseases, though only few were identify by the scientists, thus there is need to search for more active compounds which can be use to cure disease.

1.4 Justification of the Research

The uses of *Erythrophleum africanum* as claimed by traditional healers in the treatment of various ailments has necessitated the need for a scientific study to ascertain its antimicrobial properties and the active phytochemical components responsible for curing the said disease conditions.

1.5 Scope and Limitation of the Research

The scope of the study is phytochemical and antimicrobial screening, isolation and structural elucidation of active compounds of *E. africanum*. To investigate if any isolated compound could account for the use of the plant as claimed by traditional healers, the isolated compound must be tested for antimicrobial activities against suspected microbes that can causes the said disease condition.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Taxonomical Description of the Plant *Erythrophleum africanum* (Welw. ex Benth) Harms

Name: *Erythrophleum africanum* (Welw. ex Benth) Harms

Kingdom: Plantae

Class: Magnoliopsida

Order: Fabales

Family: Leguminosae-Caesalpinioidae

Subfamily: Caesalpinioidae

Phylum: Magnoliophyta

Genus: *africanum*

Species: *Erythrophleum*

Scientific name author: (Welw. ex Benth) Harms

English: Ordeal tree or African black wood, Sasswood

Hausa: *Samberu, Gwaska*

2.1.1 Origin and Geographical Distribution:

Erythrophleum africanum occurs in much of tropical Africa, from Senegal east to Sudan and South throughout Central Africa, Kenya, Tanzania and South Africa to Transvaal (Neuwinger, 2000) and Nigeria (Burkill, 1995).

2.1.2 Traditional Uses:

In Ghana, a mouth wash for relieving toothache is made from the back of *Erythrophleum africanum*, in Zimbabwe an infusion of the bark is drunk to treat stomach-ache or dysmenorrhaea, the bark steeped in water is applied externally and internally to cure cardiac diseases and epilepsy. In Namibia the powdered root bark mixed with urine is applied to skin to treat leprosy. Hot water extracts from the pounded root is drunk to induce vomiting in case of poisoning and as a cure for insanity. Inhaling the smoke of burning leaves is said to relieve pain. A paste of root bark is applied to skin to cure scabies. The bark has been used as an ordeal poison in Tanzania, Malawi and Zimbabwe (Burkill, 1995; Jonathan *et al.,* 2000).

The timber of *Erythrophleum africanum* and several other *Erythrophleum* species is marked under the trade name "*Missanda*" it is used for furniture, heavy and light construction, posts, poles and tool handles. The wood is used as firewood and to make good-quality charcoal, used in iron working. Cutting is used to establish living fences. The gum from the bark is used to make basket water proof and to fix arrow heads and axe handle. In Zambia the folliage is reportedly used as a fodder but source from several other countries report that it is toxic and that cattle are kept away from it (Tulikki, 2003).

2.1.3 **Botany**:

Erythrophleum africanum is a tropical plant commonly found in arid land. In Nigeria, the stem bark is dark brown, rough and cracked or slash reddish and gritty (Mabberley, 1997). It is a small tree up 15 m tall bole strength and cylindrical, up to 120 cm in diameter, crown spreading, fairly dense, young parts have variable hairy, bipinnately compound with 3-4 pairs of opposite pinnae, stipulate minute; petotiole 3, 5 cm long rachis 3-15 cm long; leaves alternate, 8-17 per pinna, elliptical or ovate, up to 6 cm x 3.5 cm, base symmetrical, apex obtuse to rounded. Inflorescence an auxiliary or terminal penicles consisting of spike-like raceme up to 10 cm long, often smoothly hairy, flower bisexual, regular, 5-merous, white to yellowish green; pedical 1 mm long, hairy, calyx c.2.5 mm long, tube about as long as lobes. Petals narrowly obovate, up to 4 mm x 1 mm; stamens 10, free, up to 8 mm long; ovary superior, long woolly hairy, 1-called stigma broadly peltate, fruit a flat straight, dehiscent pod, elliptical in outling 5-19 cm x 2-4.5 cm base rounded, apex rounded ortopering, thick leathery, pendulous, 3-4 seeded. Seeds ovoid, compressed, C.12 mm x 10 mm x 4 mm (Arbonnier, 2004).

The genus *Erythrophleum* comprises of about 10 species, 4 or 5 of which occur in continental Africa, 1 in Madagascar, 3 in eastern Asia, and 1 in Australia. The genus is one of the few Caesalpiniacae reported to contain alkaloids. The area of distribution of *Erythrophleum africanum* and *Erythrophleum suaveolens* largely overlap and the two species share many uses and properties and therefore confusion is quite likely. The results of earlier pharmacological work are blurred by doubtful identifications. The 2 species differ in ecology, a number of morphological characteristics and the alkaloid profile of the bark. Modulation and vesicular *arbuscular mycorrhizae* were observed in the roots of *Erythrophleum africanum*. The *rhizobium* involved in nodulation probably belongs to the genus *Bradyrhizobum* (Brummitt *et al.,* 2007).

2.1.4 **Fruit**:

The young fruit are green and are about 5-10 cm long (Mabberley, 1997).

2.1.5 Ecology:

Erythrophleum africanum is common in deciduous woodland, and is absent from riparian wood lands and dry savanna of the Sahel. It occurs at 600-1400 m altitude, and resists bush fires (Neuwinger, 2000).

2.1.6 Management (morphology):

Erythrophleum africanum can be grown from seeds, but wildings are used as well. Coppicing and pollarding are recommended management practices for *Erythrophleum africanum* in Zambia but coppicing often gives better results (Neuwinger, 2000).

2.1.7 Genetics resources and breeding:

Erythrophleum africanum is widespread and not commercially exploited on a large scale. There is no reason to assume it will become threatened in the near future (Neuwinger, 2000).

2.1.8 Pest species:

The *Gonimbrasia richelmanni* which is called the waymer in English is the pest species that attack the plant.

2.1.9 Prospects:

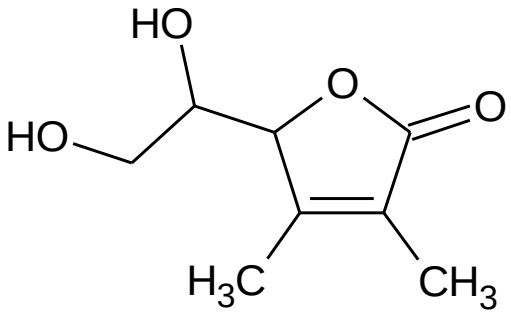
The huge variety in alkaloid contents such as diterpenoids and amide norcassaide alkaloids of *Erythrophleum africanum* bark warrants caution in medicinal use because of their toxicity effect reported by Neuwinger (2000). The local population in Nigeria use the leaf extract for poisoning wild

animals (rats, dogs and other predators) around their farm land. In northern Nigeria (Nwude, 1981), severally herbal claims of animal's death after feeding on *E. africanum* were reported (Hassan *et al.,* 2014). The leaves of the plants were among those used in national parks in East Africa to protect big animals (Caro, 2003). *E. africanum* (African black wood), is an endangered African medicinal plant.

2.2 Chemical Constituents and Antimicrobial Studies on the Phylum, Family and Species of *E. africanum*

2.2.1 Chemical Constituents and Antimicrobial Studies on the Phylum (*Magnoliophyta*)

The pharmacognostic and traditional properties of *Cissus quadrancularis* Linn extract was studied. The extracts showed antioxidant properties and healing of fractures, due to its high vitamin C (**3**) content while the compound isolated was very effective against organisms (*E. cloacae* and *P. mirabilis*) that caused osteoporosis, cough, hemorrhoids (Justin, 2011).



Vitamin C (3)

In investigation of fresh flower, dried leaves, stem and bark extract of *Nytanthes arbor-tritis* Linn, all the extracts tested positive for numerous phytochemical compounds which are responsible for

activities such as antispasmodic, antihelmintic, cytotoxic, anti-inflammatory, hepatoprotective, immuno-stimulant, antidiabetics and antibacterials (Harleen *et al.,* 2011). In the evaluation of the antibacterial activity of flavonoids, extracted from *Fiscus benghalensis* extract, the flavonoids gave excellent antibacterial activity against several pathogenic bacteria i.e gram positive and gram negative bacteria (Manimozhi *et al.,* 2012).

Pharmacognostic and phytochemical properties of *Ficus carica Linn* indicated that the extracts exhibited high pharmacognostic activity against the tested organisms due to the presence of numerous bioactive components (Justin and Baby, 2011). The phytochemical screening, quantitative estimation of total phenolic, flavonoids and antimicrobial activity of *Trachyspermum ammi*, only methanol extract showed positive test for numerous phytochemicals, indicated methanol as best solvent for extraction of phytochemical. Methanol and other extracts showed high antimicrobial activities against microbes and fungi due to the presence of phytochemical constituents (Katasini *et al.,* 2011).

The phytochemical, antimicrobial, and anti-diarrhoea properties of *Spaeranthus amaranthoides* were studied and the extracts showed the antibacterial activity of the entire tested organism which might be due to the presence of the potent phytochemical in the extracts (Latha and Reddy, 2009). The assessment of potentials of *Asparagus racemosus* (wild), *Butea frondosa* (Robx), *Carica papaya* Linn, *Annona squamoza* leaves and bark extracts as antiulcer agents in Wistar rats studied by Gadekar *et al.* (2010), the extracts tested positive for phytochemicals and the antiulcer activities exhibited by extracts was due to the presence of flavonoids in these plants.

The antimicrobial and anticancer activities of the aqueous extract of *Geranium incanum, Artemisia afra* and *Artemisia absinthium* was investigated by Kumar *et al.* (2006), and the aqueous extract showed the presence of various bioactive components which showed high antimicrobial activity against gram positive bacterial compared to gram negative bacteria. Though there is no literature report

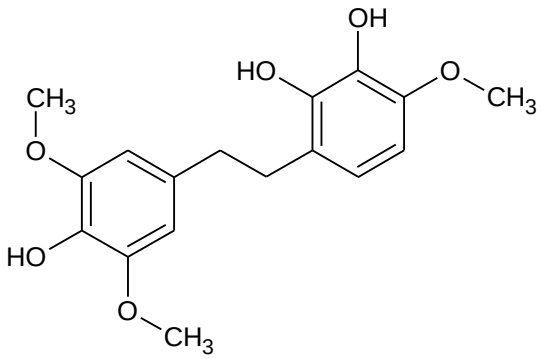
on the use of these plants traditionally as anticancer agents, various extracts of these plants showed anticancer activity in the study reported by Ryno (2009).

The morphology, phytochemical and pharmacological aspects of *Erythrina variegate* were studies by Kumar *et al. (*2010). The morphological studies revealed *E. variegates* to be a thorny deciduous tree, growing to 60 feet tall. The methanol extract tested positive for phytochemicals (flavonoids, saponnins, alkaloids, tannins and glycosides) and alkaloids was reported to be responsible for its antiinflammatory and analgesic activities while isoflavonoid isolated from the plant were reported to have antibacterial and antihelminthic effects on the test organisms (Kumar *et al.,* 2010).

2.2.2 Chemical Constituents and Antimicrobial Studies on the Family (*Leguminosae*)

The phytochemical and antibacterial activities of the extracts of some medicinal plants in Malaysia Peninsular showed the presence of some secondary metabolites which were active against gram positive bacteria but inactive against gram negative bacteria and the antifungal activity was correlated to the antioxidant activity, where flavonoids could scavenge the free radical produced by fungal (Yik *et al.,* 2011).

Eloff *et al*. (1998) reported the isolation of an antibacterial agent stilbene from *Combretum woodii* leaves through partitioning using acetone and chloroform, the chloroform fraction was observed to have the highest total activity against the microbes. The chloroform fraction was chromatographed by open column chromatography and only one compound was isolated and identified by nuclear magnetic resonance (NMR) and mass spectroscopy (MS) techniques as stilbene 2^1,3^1,4-trihydroxyl-3,5,4^1-trimethoxybibenzyl (combretastatin B5 (**4**)).



Stilbene 2^1,3^1, 4-trihydroxyl-3, 5, 4^1-trimethoxybibenzyl(combretastatin B5 (4))

The antibacterial and anticandidal activities of *Tylosema esculantum* (Marama) extracts and seed coat were investigated and the extracts showed high activities against multiple species of bacteria and candida at concentrations comparable to that of some conventional drugs (Walter *et al.,* 2011).

The extract of *Psoralea corylifolia L*. *(leguminosae)* is one of the extracts of twelve species of Indian medicinal plants studied by Jigna and Chanda (2007) which revealed the presence of phytochemical constituents that are active on gram positive and not on gram negative bacteria, although Parekh and Chanda (2006) demonstrated that gram positive bacterial are more susceptible toward plant extracts as compared to gram negative bacteria and the differences were attributed to the fact that the cell wall in gram positive bacteria is single layered, while the gram negative cell wall is multilayered structure (Yao *et al.,* 1995).

The phytochemical and antibacterial activity of the extracts of *Distemonanthus benthamianus* was investigated by Cowan (1999) against some oral pathogens. The extracts showed the presence of phytochemicals that are responsible for the observed antibacterial effects on the isolate (selected pathogen).

Donatus and Uchegbu (2009) investigated the phytochemical constituents and antibacterial activity of stem bark extracts of *Detarium senegalensis* Gmeline (*leguminosae)* which showed positive

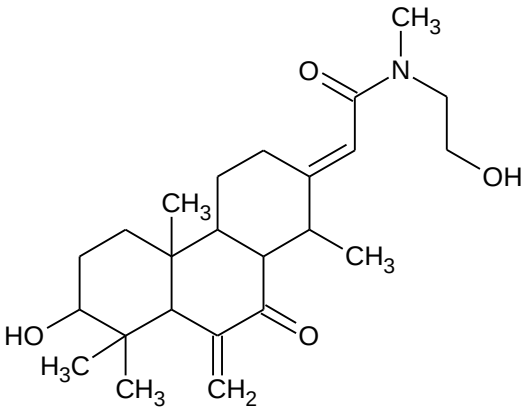
test for some phytochemicals that are responsible for the observed zone of inhibition of some micro-organism and this was the reason behind the use of stem bark of *Detarium senegalensis* as a preservative agent in palm wine by natives in south eastern Nigeria and as a stimulant.

2.2.3 Chemical Constituents and Antimicrobial Studies on the Species *(Erythrophleum)*

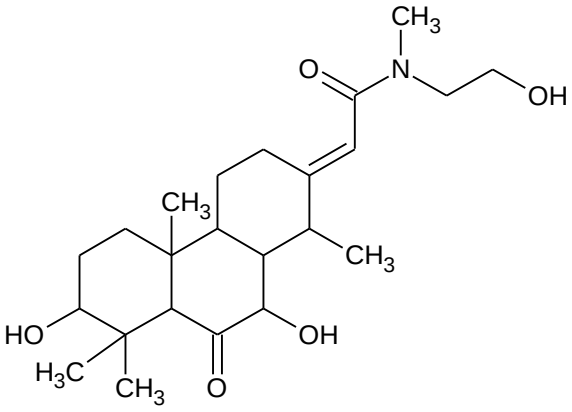
The study of macromorphological and micromorphological results for seed characteristic of 12 species representing 7 genera using both light and scanning electron microscope (SEM) to mark out the limits of diversity in the characters of both the shape, areole, micropyle, hilum and lens (Lock, 1990). Adeoye and Oyedapo (2004) studied the phytochemical and toxicity effect of the stem bark extract of *Erythrophleum guineense* which showed the presence of phytochemical constituents which could be attributed to toxic effect of the plant extracts.

The antiinflammatory and analgesic properties of the stem bark extracts of *Erythrophleum suaveolens* studied by Dongmo *et al.* (2001) showed the presence of certain secondary metabolites which were responsible for potent anti-inflammatory and analgesic properties of the plant. The antibacterial potentials of the stem-bark extracts of *E. suaveolens* was investigated against selected bacteria and the extracts showed the presence of some phytochemical which were responsible for cell disruption as the proposed mechanism of action (Aiyegero *et al.,* 2007).

The antimicrobial activities of stem bark extract of *E. suaveolens* was studied and yielded a known amide norcassaide (**6**) and a new diterpenoid alkaloid (norerythrosauvelide (**7**)) whose structure was established by ^{1}H and ^{13}C NMR spectra and the compound showed potent antimicrobial activities against some bacteria (*S. aureus, C. ulcerans and E. coli*) and yeasts (*S. cerevisiae*) (N'gouno *et al.,* 2005).



Amide norcossaide (6)



Norerythrosuavelide (7)

Clinical studies on *Erythrophleum guineense* stem bark extract used in the preparation of local soaps showed the effectiveness of extract to cure skin infections caused by fungi was reported by Oladele *et al.* (2012) and Burkill (1995), while alkaloids were implicated for antimicrobial activity by other workers (Manfouo *et al.,* 2005 and N'gounou *et al.,* 2005). Adedotun *et al.* (2003) reported that the seed oil has been used as a broad spectrum antifungal agent against dermatophytes.

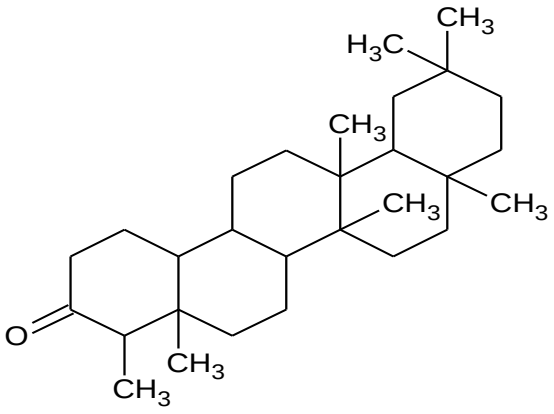
The chemical constituents of the extract of *Caesalpinia paraguariensis* bark was studied by Girma *et al.* (2003) and the compounds isolated from the active fraction showed moderate antimicrobial activity against few organisms. The analysis of the result using Tukey-studentized test, revealed that the

heartwood extract of either *E. suaveolens* or *Milicia excelsa* when applied on perishable sapwood was effective in suppressing the attack of either *Lenzites trabea* or *Poria versicolor* due to the phytochemicals present (Ngane *et al.,* 2003).

The ability of powders and slurries from ten plant species to protect stored grain from attack of *Sitophilus oryzae* and *Prostephanus truncatus* was investigated and the lower concentration of slurries of *Erythrophleum suaveolens, Solanum nigrum* and *Ricinus communis* gave close to, or 100 % protection to the grains compared to the powder, thus significant reduction of damage and increased insects mortality, suggest plant products act as antifeedants (Niber, 1994).

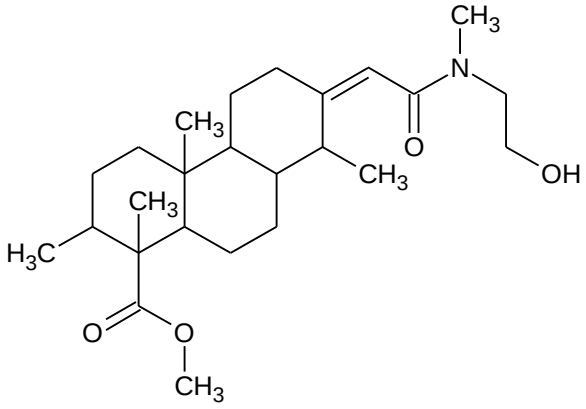
Antwi-Boasiako and Damoah (2010) demonstrated the biocidal effectiveness of water extracts from higher plants such as *Erythrophleum suaveolens* and *Azadirachta indica* (A. Juss) against several microbes and insects. Two alkaloids were isolated from the seeds of *Erythrophleum lasianthum* and their structures were assigned by spectroscopic methods and both alkaloids proved to be very active in inhibiting the Na+/K(+)-ATPase isolated from bovine cardiac sarcolemmal vesicles (Verotta *et al.,*1995).

A phytochemical investigation of the leaves of *Erythrophleum fordii* Oliv has led to the isolation of 3 new diterpenoids and 27 known compounds and selected compounds from this plant were examined for anti-inflammatory activity (Chuan-Chung *et al.,* 2008). Other studies on *Erythrophleum fordi* by Jing *et al.* (2007) which was aimed to develop an effective analytical method for structural identification of new trace natural products in plants has led to characterization of structure of newly isolated compounds on the basis of HPLC/HRMS, HPLC-DAD/ESI-MS(n), HPLC/(1)H NMR and HPLC/(1)H-(1)H COSY.



(4β,5β,8β,9β,10a,13a,14β)-5,9,13-trimethyl-24,25,26-trinoroleanan-3-one,DA-friede-leananan-3-one, friedelan-3-one (8).

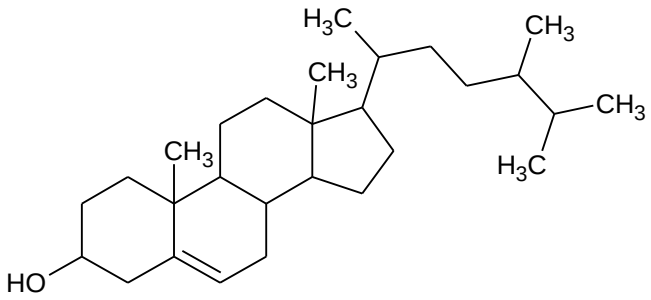
A phytochemical investigation of the bark of *Erythrophleum fordii* has led to isolation of six new cassaine diterpenoid amides, together with two known compounds of the same skeleton, and their structures were mainly established on the basis of spectroscopy analysis, and the compound 1, 2, 4, 6 - 8 exhibited selective cytotoxic activities against human cancer cell (Jing *et al.,* 2006).



Cassaine diterpenoid amides (9)

The antioxidant activities, total phenolic contents and chemical compositions of extracts from four Cameroonian woods were investigated and the *Erythrophleum suaveolens* exhibited highest antioxidant properties compared to *Distemonanthus benthamianus, Pterocarpus soyauxii* (Taub) and *Baillonella*

toxisperma respectively, triterpenes were the major compound extracted (Jean-Bosco *et al.,* 2012; Kahinde *et al.,* 2012).



Sistosterol (10)

The investigation of extract of *Erythrophleum succirubrum* by Miyagawa *et al.* (2009) has led to the isolation of four new cassaine diterpenoid and their structures were elucidated by spectral data.

Studies on the antidote activities of aqueous extract of stem bark of *Tamarindus indica* and aqueous leaves extract of *E. africanum* on albino rats were carried out (Hassan *et al.,* 2001). The *E. africanum* treated group of albino rats showed a significant decrease in the serum enzyme and total bilirubin concentration. When the *Tamarindus indica* stem bark extract was administered, it offered some protection to albino rats against the toxicity of aqueous leaf extracts of *E. africanum* (Hassan *et al.,* 2001).

Mohammed *et al.* (2014) Studied the phytochemical and antimicrobial properties of the fractions of the leaf extract of *E. africanum* obtained by partitioning with petroleum ether, chloroform, ethylacetate and n-butanol exhibited diverse antimicrobial activities against different microbes due to the presence of phytochemical constituents which were responsible for the treatment of sores, boils, wounds, dysentery, diarrhoea and sexually transmitted infection.

The detailed phytochemical and cytotoxic investigation of the leaf extracts of *Erythrophleum fordii* resulted to the isolation of 13 compounds and their structures were determined by NMR spectra and ESI-MS methods. Some isolated compounds exhibited significantly selective cytotoxic activities against human cancer cells compared to other compound (Dan Du *et al.,* 2010).

The leaf decoction of *E. africanum* is well known by the traditional healers in Congo, Zaire, Eastern province of Cameroun and India who used it empirically for several ailment including cardiovascular disease, various inflammation, diabetes, simple goiter, dysentery, diarrhea and as an astringent (Dalziel, 1999).

These *Erythrophleum* species were reported to contained flavonoids and anthocyanidins and as such was used as a tooth pick for oral hygiene (Nwude and Chineme, 1981; Burkill, 1995). Some alkaloids from the leaf of this plant have been implicated to be gastrointestinal tract irritants, cholinesterase inhibitors and also affect the nervous system by causing drowsi-ness, salivation, labored breathing, trembling, and loss of consciousness, coma and death due to paralysis (Roberts and Wink, 1998; Ahmad *et al.,* 1994). In the northern part of Nigeria (Africa) the leaf decoction with natron, Gwaska as it is called by the Hausas is taken for the treatment of sexually transmitted disease, as an abortificient agent and is also used in the treatment of leprosy (Jinju, 1990). The aqueous leave extract of this plant is also used to cure cancer of the blood and mentally related sickness in human (Jinju, 1990; Dalziel, 1999). Consequently, the leaf part of this plant was screened with a view to validate the folkloric claim (Mohammed *et al.,* 2014).

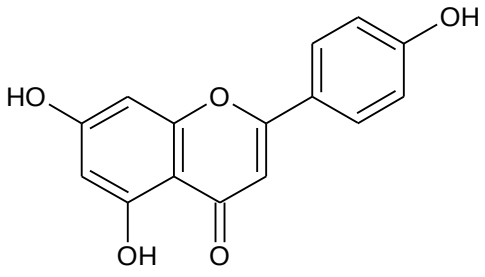
Studies have revealed that *Erythrophleum* species are extremely toxic to livestock all over the world especially to goats, sheep and cows. Several species which include *E. quineense, E. invorense, E. lasicanthum,* and *E. chlorostachys* are known to be poisonous (Dalziel, 1959; Griffin *et al.,* 1971). This plant is reported for its uses as an ordeal poison for executing capital punishment for witches and also

to kill or scare away stubborn pest from cultivated farm lands (Dalma, 1970; Loder *et al.,* 1974; Mattocks and Jukes, 1987; Kaita, 1998; Dongmo *et al*., 2001; Tulikki, 2003; Adeoye and Oyedapo, 2004; Agaie *et al*., 2007).

2.2.4 Chemical Constituents and Antimicrobial Studies of *E. Africanum*

Studies on the methanol extract from the leaf of *E. africanum* showed the presence of saponins, cardiac glycosides, tannins (true tannins and pseudotannins), flavonoids, glycosides, free flavonoids and alkaloids while the aqueous leaf extracts of *E. africanum* showed the toxicity effect at 2000-3000 mg kg^{-1} in albino rat which is attributed to the combined effect of phytochemical constituents (Hassan *et al.,* 2001).

Kadja *et al.* (2011) studied the total flavonoids and antioxidant properties of the stem of *E. africanum* used as toothpicks showed the presence of flavonoids, anthocyanidins and radical scanvenging activity, flavonic aglycones and the total flavonoid content is responsible to 94.4 % antioxidant properties of the stem.



Flavone (11)

CHAPTER THREE

3.0 MATERIAL AND METHODS

3.1 Materials for Extraction and Phytochemical Test

i. Soxhlet extractor apparatus

ii. Rotary evaporator

iii. Vacuum pump

iv. Steam bath

v. Anti-bumping granules

vi. Heating mantle

vii. Test tubes

viii. Beakers

ix. Separatory funnel

3.1.1 Reagent for Extraction and Phytochemical Screening

i. Ethanol

ii. Petroleum ether

iii. Chloroform

iv. Ethyl acetate

v. n-Butanol

vi. Sodium hydroxide (10 %)

vii. Hydrochloric acid (concentrated, 10 %)

viii. Dilute sulphuric acid

ix. Acetone

x. Ammonia solution (28 %)

xi. Acetic anhydride

xii. Picric acid

xiii. Magnesium metal

xiv. Fehling solution A ($CuSO_4$ + H_2O)

xv. Fehling solution B (KOH 25 % + Sodium potassium tartarate)

xvi. Wagner's reagent (2 g of iodine + 3 g of potassium iodide in water)

xvii. Dragendoff's reagent (potassium bismuth iodide solution)

xviii. Mayer's reagent (potassium mercuric iodide solution)

xix. Molisch reagent (solution of α- naphthol 10 % in alcohol)

xx. Liebermanns Burchard reagent (sulphuric acid + nitrous acid)

The reagents were prepared as described by Brain and Turner (1975).

3.2 Material for Chromatography (TLC & CC)

i. TLC plates 20 cm x 20 cm

ii. Chromatographic tank

iii. Ultraviolet lamp

iv. Gallenkamp spreader

v. Capillary tubes

vi. Pre-coated TLC plates

vii. Silica gel (60-120 mesh)

3.3 Methodology

3.3.1 Preparation of Plant Material

The bark of *E. africanum* was collected from Badeggi, Katcha local government area of Niger State and was identified with voucher No. 2897, in the Herbarium unit of Biological Sciences

Department, Ahmadu Bello University, Zaria. The plant material was air-dried on the laboratory bench and then ground into powdered form using wooded pestle and mortar. The powdered sample was stored in sealed container.

3.3.2 Extraction

The air-dried powdered material (2 kg) was extracted at room temperature with ethanol to exhaustion. The combined ethanol extracts were filtered under pressure and the filtrate was concentrated at room temperature using a rotary evaporator. The concentrated ethanol extract was suspended in water and then filtered. The filtrate was partitioned with petroleum ether, ethyl acetate, chloroform and n-butanol respectively. The various fractions were concentrated at room temperature. Fig. 3.1 shows the extraction scheme of the powdered plant material from the stembark of *E. africanum*.

Extraction Procedure

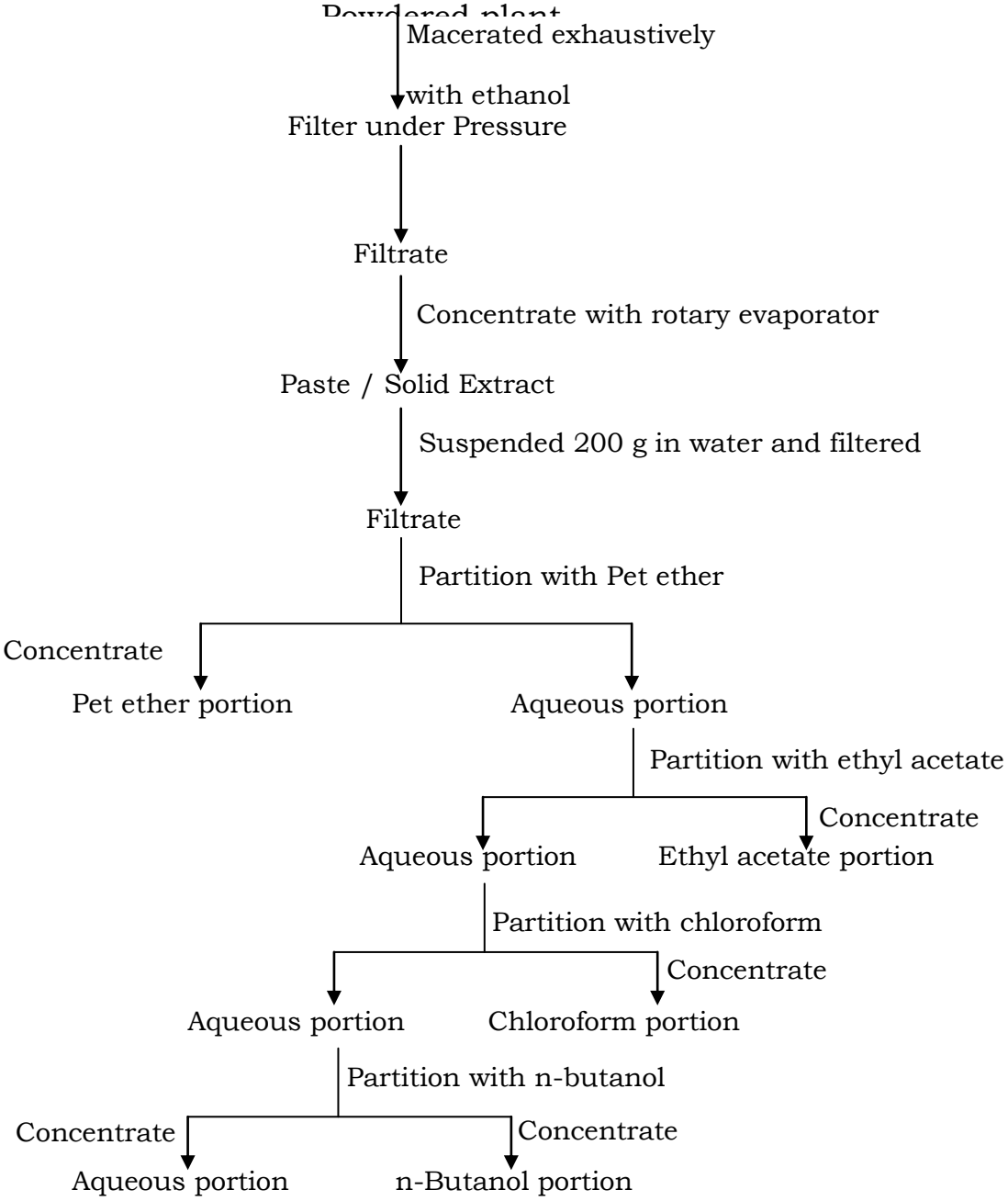


Fig 3.1: The extraction scheme of the plant material

3.4 Phytochemical Screening

The crude extracts of the bark of *Erythrophleum africanum* was subjected to phytochemical analysis using standard procedure described by Brain and Tuner (1975), Trease and Evans (1978), Sofowora (1993), Oyedapo *et al.* (1999), to test for carbohydrate, saponins, glycoside, tannins, flavonoids, cardiac glycosides and alkaloids.

3.4.1 Test for carbohydrates

a. General test (Molisch's)

The ethanol extract (0.5 g) was dissolved in distilled water and filtered to obtained a clear solution, few drops of Molisch reagent was added to the filtrate, followed by addition of 1 ml of conc. sulphuric acid down the side of the test tube .The mixture was allowed to stand for 2 minutes and diluted with 5 ml of distilled water. The appearance of red colour at the interphase of two layers, confirm the presence of carbohydrate (Brain and Tuner, 1975).

b. Aniline acetate reaction

Approximately (1 g) of a solid sample of ethanol extract was heated, over a flame, and the vapour was allowed to react with aniline inpregnated on a filter paper placed on the vapour. The appearance of red colour indicated the presence of heterocyclic aldehyde produced from carbohydrate dehydration (Brain and Tuner, 1975).

c. Barfoed's test (test for sugar)

The ethanol extract (0.5 g) was dissolved in distilled water in a test tube and 1 ml of distilled water containing in a test tube, 1 ml of Barfoed's reagent was added. The mixture was heated on a water bath

for 2 minutes. A red precipitate of Cu_2O was observed which indicate the presence of sugars (Trease and Evans, 1989).

d. Test for reducing sugar (Fehling test)

The ethanol extract (0.5 g) was in dissolved water in a test tube, equal volume (5 ml) of Fehling solution A and B was added and heat on a water bath. Red precipitate of Cu_2O observed indicate the presence of reducing sugar (Brain and Tuner, 1975).

3.4.2 Test for flavonoids

a. Shinoda's test

Approximately (1 g) of ethanol extract was dissolved in ethanol in a test tube and warmed on a water bath, the mixture was filtered and three to four pieces of magnesium chip was added to the filtrate with a few drops of concentrated hydrochloric acid (HCl). The pink colouration was observed which indicate the presence of flavonoids (Trease and Evans, 1989).

b. Ferric chloride test

The ethanol extract (0.5 g) was dissolved in ethanol in a test tube and few drops of 10 % ferric chloride ($FeCl_3$) solution was added and boiled on a water bath. Violet coloured was observed which indicated the presence of a phenolic hydroxyl group (Trease and Evans, 1989).

3.4.3 Test for tannins

Approximately (2 g) of ethanol extract was boiled in 10 ml of distilled water in a test tube on a water bath and cooled, the mixture was filtered and the filtrate was use for the following test.

a. To 1 ml of filtrate in first test tube, few drops of lead acetate was added to it in test tube, a white precipitate was observed which indicate the presence of tannins (Trease and Evans, 1989).

b. To 1 ml of the filtrate in second test tube, three drops of 1 % of ferric chloride ($FeCl_3$) solution was added and the blue–black, green or blue-green precipitate was observed to show the presence of tannins (Trease and Evans, 1989).

c. Approximately (2 ml) of Ethanoic acid and 2 ml of 10 % lead acetate each was added to 5 ml of filtrate in third test tube and a white precipitate was observed which indicates the presence of tannins (Trease and Evans, 1989).

d. Approximately 3 drops of 10 % HCl and 1 drop of methanol were added to 2 ml of filtrate in fourth test tube and mixture was boiled on a water bath. A red precipitate was observed which indicate the presence of tannins (Trease and Evans, 1989).

3.4.4 Test for saponins

Frothing test

Approximately (1 g) of ethanol extract was shaken in 5 ml of distilled water in a test tube and warm on water bath, the persistence frothing was observed which indicate the presence of saponins (Sofowora, 1993).

3.4.5 Test for alkaloids

Sample of ethanol extract (1 g) was dissolved 5 ml of 1 % aqueous hydrochloric acid (HCl) in a test tube and the mixture was stirred on the water bath and filtered, 3 ml of the filtrate was taken and divided into 3 portions 1 ml each.

a. Dragendoff"s reagent

To the 1 ml of the first portion of filtrate in a test tube Dragendoff"s reagent was added and orange red precipitate was observed which show the presence of alkaloid.

b. Mayer's reagent

To the 1 ml of the second portion of filtrate, Mayer's reagent was added and a buff colouration was observed which indicate the presence of alkaloid.

c. Wagner's reagent

To the 1 ml of the third portion of filtrate, Wagner's reagent was added and a dark brown precipitate was observed.

The colours changed observed in a, b and c, confirmed the presence of alkaloid (Brain and Tuner, 1975).

3.4.6 Test for steroids

Libermann Burchard test

Approximately (0.5 g) of extract was dissolved in 2 ml of chloroform in a test tube and filtered. Acetic acid (2 ml) was added to the filtrate and the mixture was cooled in iced. Concentrated tetraoxosulphate (VI) acid was added to the mixture down the side of the test tube in iced and violet coloured changed to bluish green was observed which indicate the presence of steroidal nucleus (i.e aglycone portion of the cardiac glycoside (Sofowora, 1993).

3.4.7 Test for glycosides

General test

Two grammes (2 g) of ethanol extract was dissolved in 25 ml of 2.5 M tetraoxosulphate (VI) acid in a beaker and boiled on a water bath, the mixture was neutralized with 20 % potassium hydroxide. 5 ml of equal volume of Fehling solution A and B was added to a neutralized mixture in beakers and boiled. Brick red precipitate was observed which indicated the presence of glycosides (Trease and Evans, 1989).

Keller – Killiani test

Ethanol extract (0.5 g) was dissolved in distilled water in a test tube; 1 ml of glacial acetic acid and few drops of ferric chloride solution were added to the aqueous solution of the ethanol extract. 1 ml of concentrated sulphuric acid was added to the mixture at an angle 45 $^{\circ}$C to the wall of the test tube. The mixture was allowed to stand for some minutes, and a purple ring colour was observed at the interphase, which indicates the presence of glycoside (Trease and Evans, 1978).

3.5 Microbial Media and Test Organisms for Antimicrobial Screening

3.5.1 Microbial Media for Antimicrobial Screening

a. Nutrient broth

b. Nutrient agar and

c. Sabouroud Dextrose agar

d. Mueller Hinton agar

3.5.2 Test Organisms for Antimicrobial Screening

i. Gram-positive organism and gram-negative *micro* organism such as *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabills, Enterobacter cloacae, Staphylococcus aureus* and *Streptococcus faecalis.*

ii. Fungi e.g. *Candida albicans* and *Candida stealloida.*

3.6 Antimicrobial Studies

The anti-microbial screening of the stembark of *E. africanum* extracts was carried out using selected test bacteria and fungi mension above using standard techniques described by Kinsbury and Wagner (1990) and Bauer *et al.* (1996).

3.6.1 Test microorganisms

The test microorganisms were isolates obtained from the Medical Microbiology Department, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.

3.6.2 Zone of inhibition of the crude ethanol extract

All the isolates were checked for purity and were maintained on slants of nutrient agar for the bacteria and slant of Sabouraud dextrose agar for the fungi. Well diffusion method was used to determine the antimicrobial activities of the extracts from the plant. Approximately 0.2 g of the extract was dissolved in 10 ml of DMSO to obtain the initial concentration of the extract and serial dilusions of extract were made to obtain the concentration of 20, 10, 5, 2.5 and 1.25 mg/ml respectively. The positive controls used were sparfloxacin (2 mg/ml) for the bacteria and fluconazole (5 mg/ml) for the fungi. This was the initial concentration used to check the antimicrobial activities of the extracts from the plant.

Mueller Hinton agar and Sabouraud dextrose agar were the media used as growth media and were prepared according to the manufacturer's instructions (Oxoid England), boiled to dissolve and were sterilized at 121 °C for 15 minutes, the media were cooled to 45 °C and the sterile medium (20 ml) were poured into sterile Petri dishes, the medium was then covered to cool and solidify. Each medium was then inoculated with 0.1 ml of the standardised test microorganism; the inoculums were spread evenly over the surface of the medium by the use of a sterile swab (NCCLS, 1999).

3.6.3 Minimum inhibitory concentration (MIC) of the crude ethanol extract

The minimum inhibitory concentration of the bark extract against the test micro- organisms were determined using broth dilution techniques of Kinsbury and Wagner (1990) and Bauer *et al*. (1996). Mueller-Hinton broth was prepared according to manufacturer's instruction using 10 ml of the broth to dispense into test tubes. The broth was sterilized at 121 °C for 15 minutes after it was allowed to cool. Test microbes were inoculated and incubated at 37 °C for 6 h. Mcfarland's turbidity scale number 0.5 was prepared to give a turbid suspension of the microorganisms. Dilution of the test microbes was done in normal saline until the turbidity matches with that of the Mcfarland's scale by visual comparison against printed material. The initial concentration of extract was obtained by dissolving 0.5 g of extract in 50 ml of sterile broth. Two fold serial dilutions of the extract were made in the sterile broth to obtain the concentrations of 50, 25, 12.5, 6.25 and 3.125 mg/ml respectively. About 0.1 ml of each test microbe in the normal saline was then inoculated into the different concentrations. After incubation at 37 °C for 24 h, each test tube was observed for turbidity. The lowest concentration of the extract for which no turbidity was recorded was the minimum inhibition concentration (Cowan, 1999; Osadebe and Ukwueze, 2004).

3.6.4 Minimum bactericidal and fungicidal concentrations (MBC/MFC) of the crude ethanol extract

Minimum bactericidal and fungicidal concentration of the extract was carried out to check whether the test microbes were killed. Mueller Hinton and Sabouraud dextrose agar were prepared, boiled to dissolve and were sterilized at 121 $^{\circ}$C for 15 minutes. The media were cooled to 45 $^{\circ}$C and 20 ml each was measured into sterilised petri dishes. The plates were covered and allowed to cool and solidify. Loopful contents of each tube of the MIC in the serial dilution were inoculated on the surface media, the media were inoculated for 24 h, at 37 $^{\circ}$C for the bacteria and at 30 $^{\circ}$C for 1-7 days for the fungi, after which the plates were observed for colonies of growth. The MBC/MFC was the plate with lowest concentrations of the extract without microbial growth (Ogbulie *et al.,* 2007).

The same tests were repeated for the pure isolated compound coded N_A though at different concentrations (20, 10, 5, 2.5, 1.25 mg/ml) and standard antibiotics drug 5mg/ml of cefuroxin and 2 mg/ml fluconazole were used as control.

3.7 Chromatography Techniques

3.7.1 Thin Layer Chromatography

This technique was used to determine the numbers of spots/compounds/fractions as well as suitable solvents used in sepereation. Little portion of n- butanol fraction dissolved in n-butanol was spotted on a pre-coated plate (4 x 4 cm), and developed in a tank using methanol/ethyl acetate (7/3 v/v). After elution, the plate was removed, the solvent front was marked and the plate was dried rapidly. The dried plate was viewed under UV light and the R_f values of the prominent spots were calculated (0.21, 0.17 and 0.12 cm). The n-butanol fraction was chromatographed by preparative TLC technique. Fractions with similar R_f values was collected on the basis of their TLC pattern and combined.

3.7.2 Preparation of preparative TLC plates

Preparative TLC plates (20 × 20 cm) were prepared by adding 50 g of silica gel in 100 ml of distilled water and shaking for 1 minute, the slurry was spread on the plates using a spreader. The plates were air dried for 30 minutes and activated in oven for 1.5 h, and allowed to cool in the oven for another 30 minutes.

3.7.3 Isolation of isolate N_A from n-Butanol fraction

Dried n-butanol fraction (8 g) was dissolved in n-butanol, and the fraction was spotted on the previously prepared preparative TLC plate (20 × 20 cm). The solvent system [ethyl acetate/methanol (70:30 v/v)] was allowed to travel up the plates (developed) for 20 minutes, in a saturated chamber (tank). The plates were removed from tank, and their solvent front was immediately marked and dried as rapid as possible to avoid diffusion of the spots. The dried plates were viewed under UV light (366 nm) and the position of the spots on the plates were marked and their R_f values was calculated. Fractions with the similar R_f values (0.85, 0.67 and 0.48 cm) were collected and combined. The combined fractions were washed (dissolved in methanol) and filtered, the filtrate was concentrated and then coded A, B and C. The purest fraction C, was purified further using preparative TLC techniques and a yellowish amorphous compound coded N_A was obtained and then subjected to NMR spectral analysis.

3.8 Basic Principle behind the Operation of NMR Machine

The nuclear magnetics reasonance (NMR) is an analytical procedure that operate based on the magnetic properties of certain atomic nuclei, it is one of the most important and powerful tool available for identification and structural determination of the organic molecules. When the nucleus of certain atoms e.g ^{1}H, ^{13}C, ^{15}N and ^{31}P which are both electrical charge (positively charge) and mechanically

spinning were placed in electromagnetic field, the spinning of these nucleus generate tiny bar magnets called induced magnetic field which move along the axis of the spinning, when the external magnetic field (applied field) is applied, the nucleus distributed themselves into a number of quantized orientation with respect to the applied field. Some of these orientations are either against (opposes) the external field while others are reinforces (aligned) the external field, the energy is absorbed and such nucleus is said to be shielded or deshielded. Hence the number of orientation available to a nucleus varies depending on the spin quantum number (I) which may have values of 0, ½, 1, 3/2, 5/2 e.t.c. Nucleus of spin quantum number I has 2I + 1 orientation when placed in an external field and the nucleus for which I = 0 are not NMR active e.g ^{12}C, ^{14}N, ^{16}O. If I = 0 therefore: 2 (0) + 1 = 0 + 1 = 1 such nucleus do not reasonate, has no chromophor and there will be no transition state for such nucleus.

3.9 Spectral Analysis of the Isolated Compound N_A

The isolated compound N_A was subjected to spectroscopic analysis using a Bruker Advance (600 MHz) NMR spectrometer for ^1H and ^{13}C analysis using CD_3OD as solvent and TMS as internal standard. DEPT, COSY, NOESY, HMBC and HSQC were all carried out at South Africa to allow for full structural analysis of the isolated compound.

CHAPTER FOUR

4.0 RESULTS

4.1 Results of Extraction

The pulverized plant material was macerated exhaustively with ethanol; the mixture was then filtered, concentrated and the crude extract was weighed. The weight of crude extract was found to be 200 g.

4.1.1 Percentage (%) Extractable Estimation of Fractions from Crude Ethanol Extract

The 200 g of the crude ethanol extract obtained was partitioned with petroleum ether, ethyl acetate, chloroform and n-butanol. The quantities of the different fractions obtained from this process was weighed and also expressed in terms of percentage (%) yield. The results are presented in Table 4.1.

Table 4.1: Percentage (%) extractable yield fraction from crude ethanol extract

Solvent medium	Yield fraction (g)	Percentage (%) estimation
Petroleum spirit	7.2	3.6
Ethyl acetate	19.6	9.8
Chloroform	8.2	4.1
n-Butanol	9.4	4.7

4.2 Phytochemical Screening of the Crude Ethanol Extract

The phytochemical screening of the crude ethanol extract showed the presence of secondary metabolites which include glycosides, cardiac glycosides, saponins, triterpenes, flavonoids, tannins and alkaloids as presented in Table 4.2.

4.2 Result of phytochemical screening

Table 4.2: The results of phytochemical screening of the crude ethanol extract

Phytochemicals	Tests	Inference
Carbohydrates	Molisch's	+

	Fehling		+
Glycosides	Fehling		+
	Ferric chloride		+
Anthraquinones	Free anthraquinone	-	
	Combine anthracene		-
Cardiac glycosides	Kelle killiani	+	
	Kadde's		-
	Salkwoski's		+
Saponins	Frothing		+
Steroid and triterpenes	Steroid		-
	Triterpene		+
Flavonoids	Shinoda's		+
	Sodium hydroxide		+
Tannins	Ferric chloride		+
	Lead acetate	+	
	Bromine water		+
Alkaloids	Mayer's		+
	Dragendoff's	+	
	Wagner's		+

Key: + = Present, - = Absent

4.3 Result of Antimicrobial Studies

4.3.1 Antimicrobial sensitivity studies of crude ethanol extract

Table 4.3 shows the result of the antimicrobial sensitivity test of the crude ethanol extract. It was observed that the extract had strong activity against *E. cloacae* and *S. faecalis* while moderate to weak activity against *S. aureus, C. stellatoidea* and *P. mirabili*s was observed. *P. aeruginosa, E. coli, C. ulcerans* and *C. albicans* were resistant. The disc potency for each antibiotics drug was 5 mg/ml for bacteria and 2 mg/ml for fungi. Hence increase in concentrations of the extract tends to increase the sensitivity of the micro-organisms to the crude extract.

Table 4.3: Sensitivity test of the crude ethanol extracts with standard antibiotics drugs

Test organisms	**Ethanol Extract**	**CEF**	**SPA**	**ERY**	**FLU**
S. aureus	*S*	*S*	*S*	*R*	*R*
S. faecalis	*S*	*S*	*S*	*S*	*R*
C. ulcerans	*R*	*R*	*S*	*R*	*R*
E. coli	*R*	*S*	*S*	*R*	*R*
E. cloacae	*S*	*R*	*R*	*R*	*R*
P. aeruginosa	*R*	*R*	*S*	*S*	*R*
P. mirabilis	*S*	*R*	*S*	*S*	*R*

C. stellatoidea	*S*	*R*	*R*	*R*	*S*
C. albicans	*R*	*R*	*R*	*R*	*S*

Key: S = Sensitive, R = Resistance, CEF = Cefuroxin, SPA = Sparfloxacin, ERY = Erythromycin, FLU = Fluconazole

4.3.2 Zone of inhibition of crude ethanol extract

The zone of inhibition of the crude ethanol extract was measured against *S. aureus, S. faecalis, E. cloacae, P. mirabilis* and *C. stellatoidea* to be 25, 27, 29, 22 and 24 mm respectively. It was found that

the growth of *P. aeruginosa, E. coli* and *C. albicans* ware not inhibited. *E. cloacae* was not inhibited by all the standard antibiotics used in this study (disc potency of antibiotics was 5 mg/ml for bacteria and 2 mg/ml for fungi) but it was inhibited by crude ethanol extract as presented in Table 4.4.

Table 4.4: Zone of inhibition (mm) of crude ethanol extract and the antibiotic drugs

Test organisms	**Ethanol Extract**	**CEF**	**SPA**	**ERY**	**FLU**
S. aureus	*25*	*32*	*36*	*0*	*0*
S. faecalis	*27*	*29*	*32*	*22*	*0*
C. ulcerans	*0*	*0*	*32*	*0*	*0*
E. coli	*0*	*30*	*35*	*0*	*0*
E. cloacae	*29*	*0*	*0*	*0*	*0*
P. aeruginosa	*0*	*0*	*29*	*22*	*0*
P. mirabilis	*22*	*0*	*0*	*22*	*0*
C. stellatoidea	*24*	*0*	*0*	*0*	*39*
C. albicans	*0*	*0*	*0*	*0*	*32*

Key: CEF = Cefuroxin, SPA = Sparfloxacin, ERY = Erythromycin, FLU = Fluconazole

4.3.3 Minimum inhibitory concentration of the crude ethanol extract

Table 4.5 show the minimum inhibitory concentration of the crude ethanol extract at 5 mg/ml against *S. aureus, S. faecalis, E. cloacae, P. mirabilis* and *C. stellatoidea,* these organisms also showed scanty to moderate colony growth at 2.5 to 1.25 mg/ml of the crude extract. It was noted that even at 20 mg/ml *P. aeruginosa, E. coli, C. ulcerans* and *C. albicans* were resistant.

Table 4.5: Minimum inhibition concentration of the crude ethanol extract

Test organisms	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**
S. aureus	-	-	*OA*	+	++
S. faecalis	-	-	*OA*	+	++
C. ulcerans	*R*	*R*	*R*	*R*	*R*
E. coli	*R*	*R*	*R*	*R*	*R*
E. cloacae	-	-	*OA*	+	++
P. aeruginosa	*R*	*R*	*R*	*R*	*R*
P. mirabilis	-	-	*OA*	+	++

C. stellatoidea	-	-	*OA*	*+*	*++*
C. albicans	*R*	*R*	*R*	*R*	*R*

Key: - = No colony growth, OA = Minimum inhibitory concentration (MIC), + = Scanty colony growth, + + = Moderate colony growth, R = Resistance.

4.3.4 Minimum Bactericidal/Fungicidal Concentration of the Crude Extract

The minimum bactericidal/fungicidal concentration of the crude ethanol extract was 20 mg/ml against *S. aureus* and *P. mirabilis*, but *S. faecalis, E. cloacae* and *C. stellatoidea* showed no colony growth at 10 mg/ml. *P. aeruginosa, E. coli, C. ulcerans* and *C. albicans* were resistant, as presented in Table 4.6.

Table 4.6: MBC/MFC of the crude ethanol extract against the test organisms

Test organisms	20 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml
S. aureus	*OA*	*+*	*++*	*++ +*	*++++*
S. faecalis	*-*	*OA*	*+*	*++*	*+++*
C. ulcerans	*R*	*R*	*R*	*R*	*R*
E. coli	*R*	*R*	*R*	*R*	*R*
E. cloacae	*-*	*OA*	*+*	*++*	*+ ++*
P. aeruginosa	*R*	*R*	*R*	*R*	*R*
P. mirabilis	*OA*	*+*	*++*	*+++*	*++++*
C. stellatoidea	*-*	*OA*	*+*	*++*	*+++*
C. albicans	*R*	*R*	*R*	*R*	*R*

Key: - = No colony growth, OA = Minimum Bacteriacidal/Fungicidal (MBC/MFC) concentration, + = Scanty colony growth, + + = Moderate colony growth, + + + = Heavy colony growth, + + + + = Very heavy colony growth, R = Resistant.

4.3.5 Antimicrobial activities of the isolated compound N_A and antibiotic drugs

The sensitivity test of the isolated compound, $N_{A,}$ was carried out and the sensitivity test for *S. aureus, S. faecalis, E. cloacae, E. coli, E. cloacae, P. aeruginosa, P. mirabilis, C. stellatoidea* and *C. albicans* were similar to that of the crude ethanol extract. The sensitivity result of isolated compound N_A was compared with two antibiotics drug used as standard in Table 4.7.

Table 4.7: Sensitivity test of the isolated compound N_A with antibiotics drug as standard

Test organisms	**N_A Isolate**	**Cefuroxin**	**Fluconazole**
S. aureus	*S*	*S*	*R*
S. faecalis	*S*	*S*	*R*
C. ulcerans	*R*	*R*	*R*
E. coli	*R*	*S*	*R*
E. cloacae	*S*	*R*	*R*
P. aeruginosa	*R*	*R*	*R*
P. mirabilis	*S*	*R*	*R*
C. stellatoidea	*S*	*R*	*S*

C. albicans	*R*	*R*	*S*

Key: S = Sensitive, R = Resistance, N_A = Isolated compound

4.3.6 Measurement of zone of inhibition of isolated compound N_A and antibiotic drugs against test organism.

Table 4.8 shows that the zone of inhibition of the isolated compound N_A exceeded that of the crude ethanol extract against *S. aureus* (26), *S. faecalis* (29), *E. cloacae* (30), *P. mirabilis* (26) and *C. stellatoidea* (29) mm, while *P. aeruginosa, E. coli, C. ulcerans* and *C. albicans* were resistant. The zone of inhibition of isolated compound N_A was compared with two standard antibiotic drugs with disc potency of 5 mg/ml for bacteria and 2 mg/ml for fungi.

Table 4.8: Zone of inhibition of the isolated compound N_A with antibiotics

Test organisms	**N_A Isolate**	**Cefuroxin**	**Fluconazole**
S. aureus	*26*	*37*	*0*

S. faecalis	*29*	*39*	*0*
C. ulcerans	*0*	*35*	*0*
E. coli	*0*	*35*	*0*
E. cloacae	*30*	*32*	*0*
P. aeruginosa	*0*	*34*	*0*
P. mirabilis	*26*	*27*	*0*
C. stellatoidea	*29*	*0*	*35*
C. albicans	*0*	*0*	*32*

4.3.7 MIC of the isolated compound N_A against the test organisms

Table 4.9 shows the minimum inhibitory concentration of the isolated compound N_A against *S. aureus* and *P. mirabilis* at 5 mg/ml and for *S. faecalis, E. cloacae* and *C. stellatoidea* at 2.5 mg/ml while *P. aeruginosa, E. coli, C. ulcerans* and *C. albicans* were not inhibited even at 20 mg/ml.

Table 4.9: MIC of the isolated compound N_A against the Test Organisms

Test organisms	**20 mg/ml**	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**
S. aureus	-	-	*OA*	*+*	*++*
S. faecalis	-	-	-	*OA*	*+*
C. ulcerans	*R*	*R*	*R*	*R*	*R*
E. coli	*R*	*R*	*R*	*R*	*R*
E. cloacae	-	-	-	*OA*	*+*
P. aeruginosa	*R*	*R*	*R*	*R*	*R*
P. mirabilis	-	-	*OA*	*+*	*++*
C. stellatoidea	-	-	-	OA	*+*
C. albicans	*R*	*R*	*R*	*R*	*R*

KEYS: - = No turbidity (growth), OA = MIC, + = Turbidity (light growth), + + = Moderate turbidity, R = Resistant.

4.3.8 MBC/MFC of the isolated compound N_A Against the test organisms.

The minimum bactericidal/fungicidal concentration of the isolated compound N_A against *S. aureus, P. mirabilis* and *C. stellatoidea* was observed at 10 mg/ml while *S. faecalis* and *E. cloacae* was at 5 mg/ml. these organisms showed moderate to heavy colony growth at 2.5 to 1.25 mg/ml in Table 4.10.

Table 4.10: MBC/MFC of the isolated compound N_A against the test microbes

Test organisms	20 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml
S. aureus	-	*OA*	*+*	*++*	*+++*

S. faecalis	-	-	*OA*	+	++
C. ulcerans	*R*	*R*	*R*	*R*	*R*
E. coli	*R*	*R*	*R*	*R*	*R*
E. cloacae	-	-	*OA*	++	++
P. aeruginosa	*R*	*R*	*R*	*R*	*R*
P. mirabilis	-	*OA*	+	++	+++
C. stellatoidea	-	*OA*	+	++	+++
C. albicans	*R*	*R*	*R*	*R*	*R*

KEYS: - = Colony growth, OA = MBC/MFC, + = Scanty colonies growth, + + = Moderate colonies growth, + + + = Heavy colonies growth, R = Resistance.

4.4 Results of Spectral Analysis of the Isolated Compound N_A

4.4.1 The ^{1}HNMR spectrum of isolated compound N_A

The ^{1}HNMR spectrum of the isolated compound N_A (Fig 4.1), shows signals at δ_H 1.93, 2.08, 3.84 ppm all indicating the presence of methylene protons while the signal of methyl protons is observed at δ_H 1.16 ppm. The characteristic signal of an anomeric protons of cyclopentano pyran ring system is observed at δ_H 5.81 ppm and the signal observed at δ_H 7.45 ppm result from an iridoid protons present on a cyclopentano pyran ring system. The characteristic signals of other protons were listed in Table 4.11 and discussed in section 5.2.1.

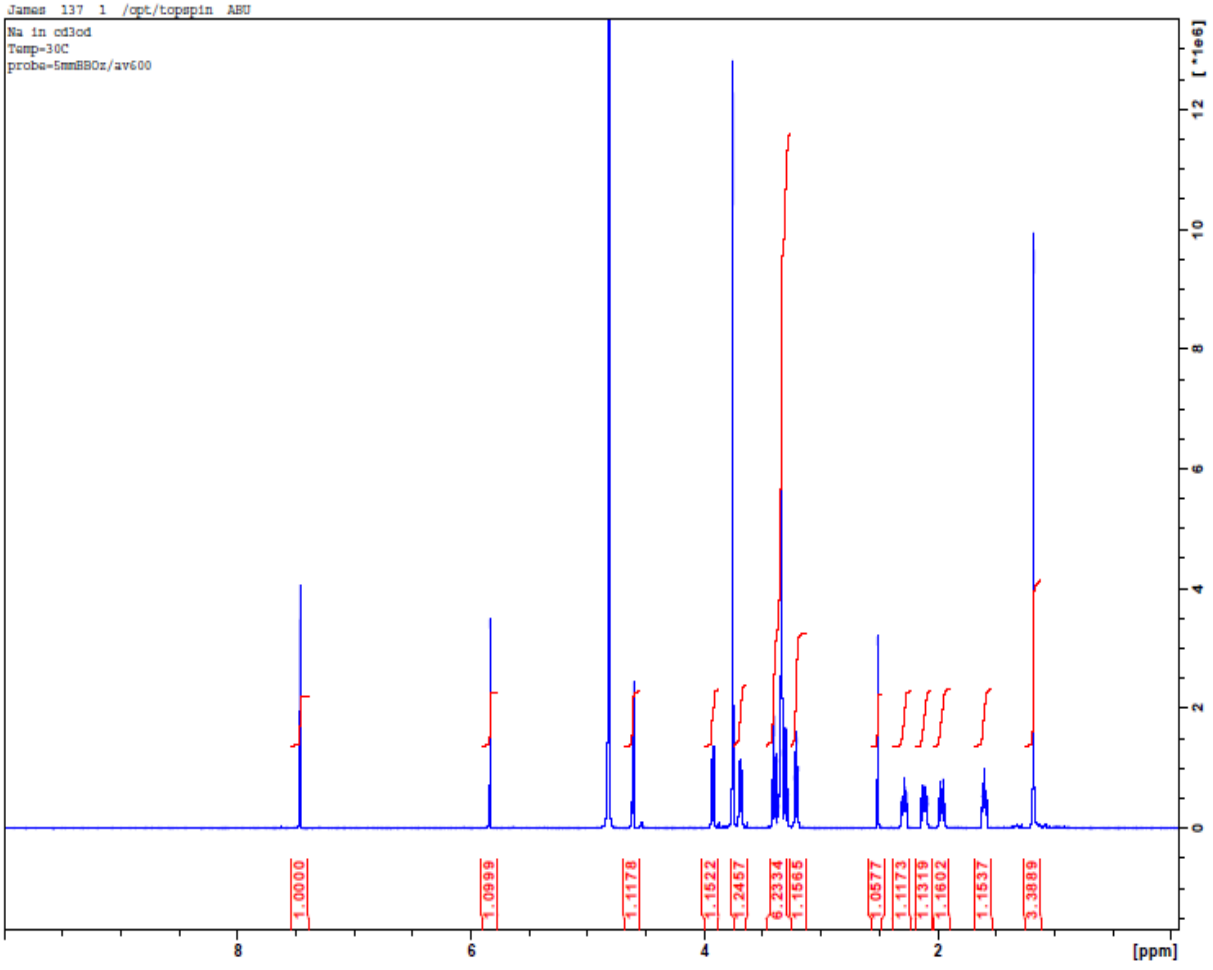


Fig 4.1: The ^{1}HNMR Spectrum of Isolated Compound N_A

4.4.2 The ^{13}CNMR spectrum of isolated compound N_A

The carbon-13 nuclear magnetic resonance (^{13}CNMR) spectral of the isolated compound N_A (Fig. 4.2) gives a total number of seventeen (17) carbon atom signals of which eleven (11) may be attributed to the presence of an aglycone and six (6) to glucopyranose moiety.

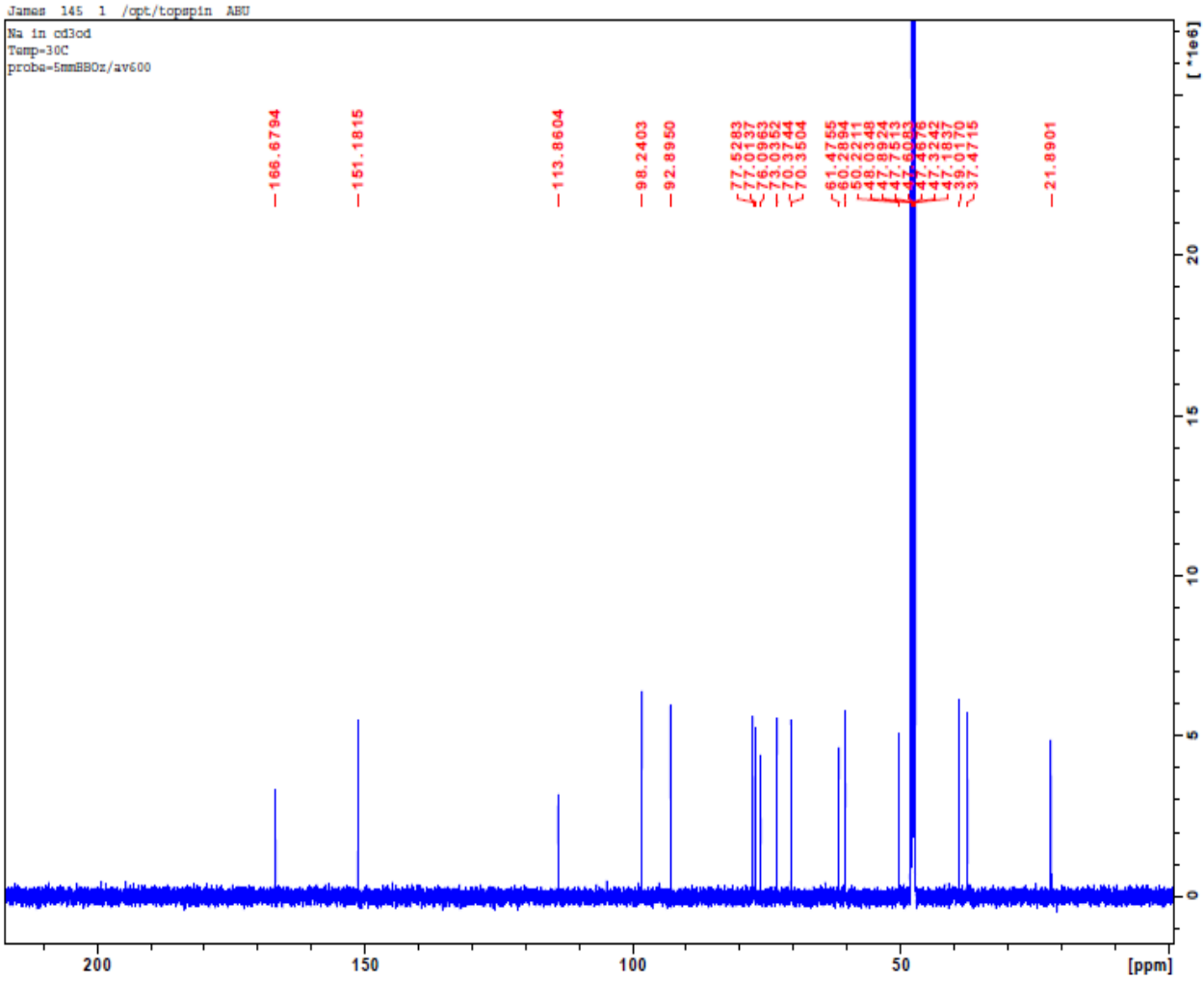


Fig 4.2: The ^{13}CNMR Spectrum of Isolated Compound N_A

4.4.3 The DEPT spectrum of isolated compound N_A

The distortionless enhancement by polarization transfer (DEPT) spectra (Fig. 4.3) gave a characteristic signal of a total number of eight 8 methine (-CH-) group, three 3 methylene ($-CH_2$) which may be assigned to C-6, C-7 and C-6^1 , two 2 methyl (CH_3) group, this also gave signals accounting to a total number of four 4 quaternary carbon atom assumed to be C-4, C-5, C-8 and C-11.

\

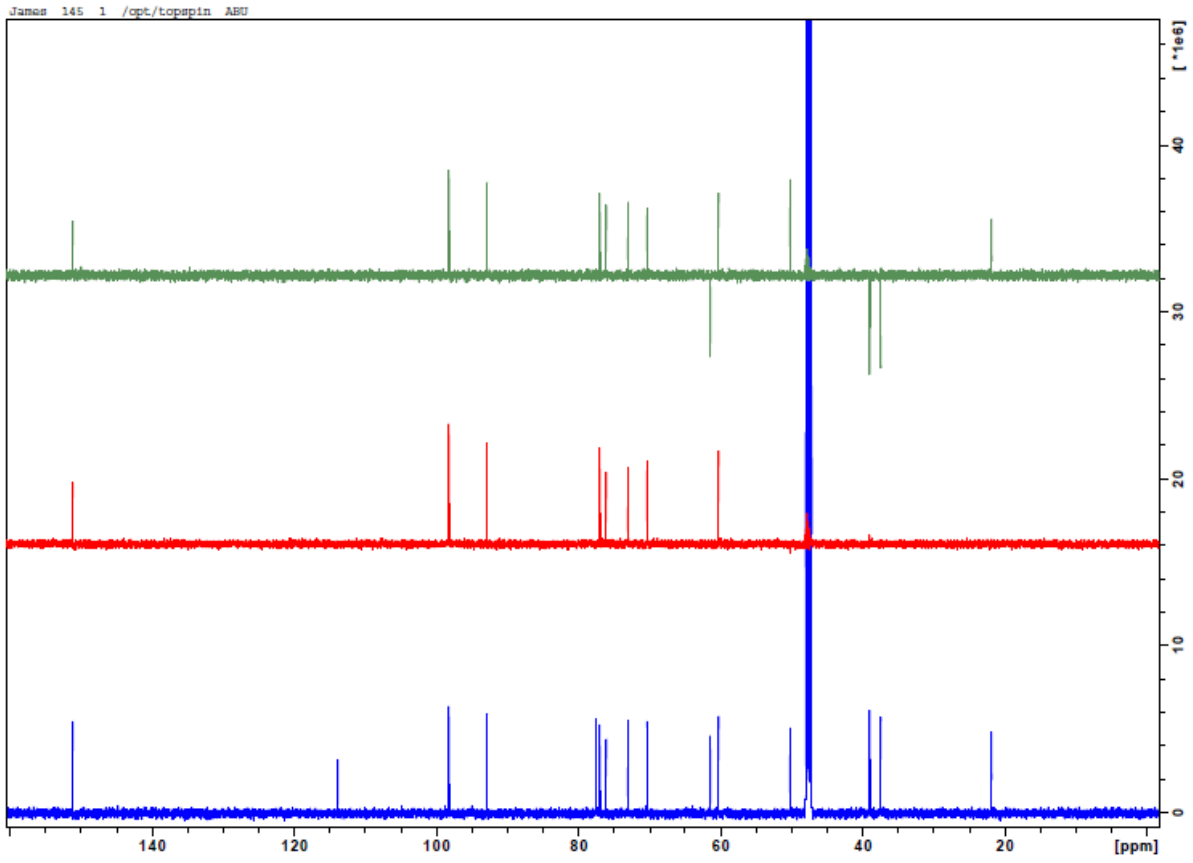


Fig 4.3: The DEPT Spectrum of Isolated Compound N_A

4.4.4 The H-H correlation spectroscopy (COSY) of isolated compound N_A

The proton-proton correlation spectroscopy (COSY) indicating characteristic signals of nuclei of one proton couple to another proton. Thus, this established the correlation of proton (H-1) of cyclopentano pyran ring system and of sugar moiety at δ_H 5.8 ppm and 4.5 ppm in (Fig 4.4).

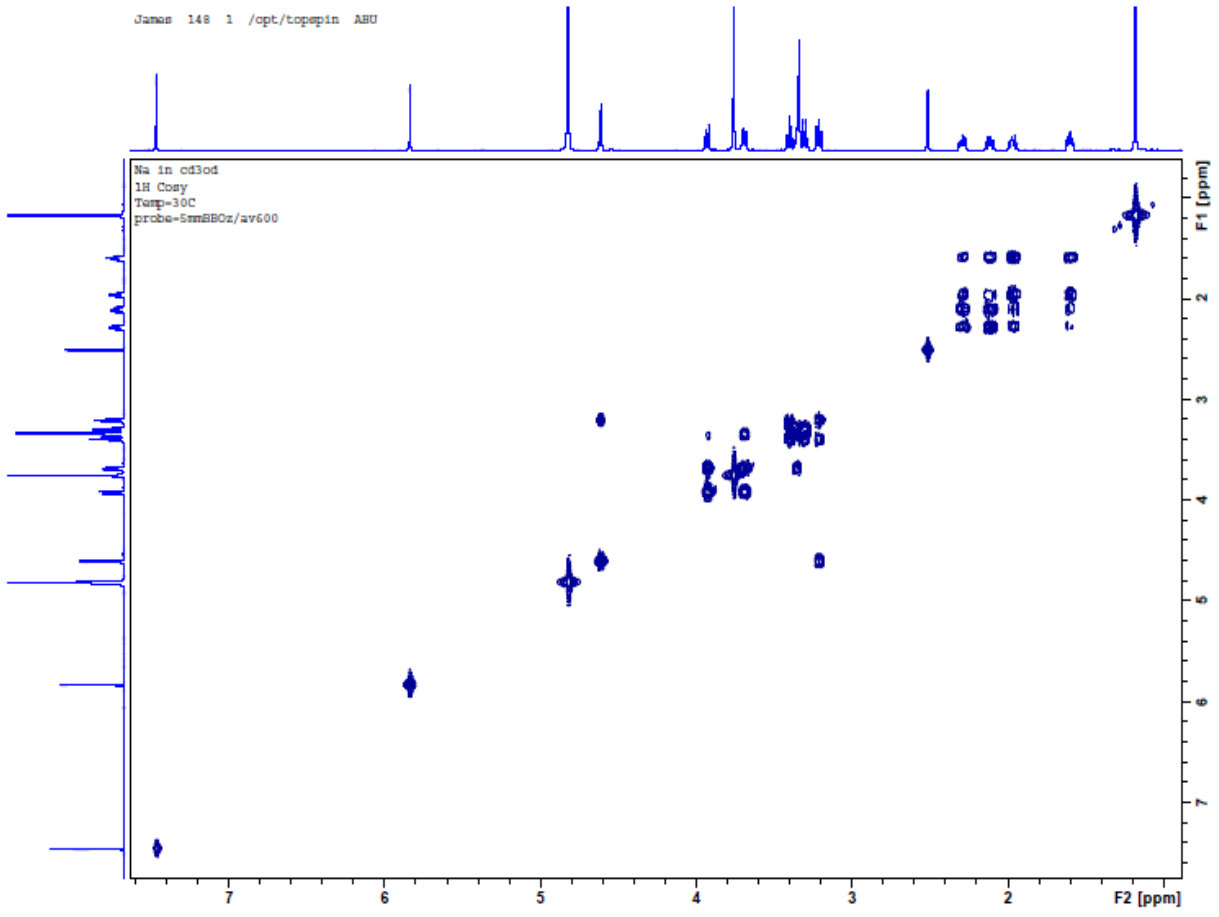


Fig 4.4: The H-H COSY Spectrum of Isolated Compound N_A

4.4.5 The HMBC spectrum of isolated compound N_A

The heteronuclear multiple bond correlation spectra (HMBC) of isolate N_A in (Fig 4.5) showing some characteristic signals suggesting the correlation of iridoid proton at δ_H/δ_C 7.45/151.30 ppm with those protons on δ_C 70.20, 93.20, 113.90 and 166.90 ppm.

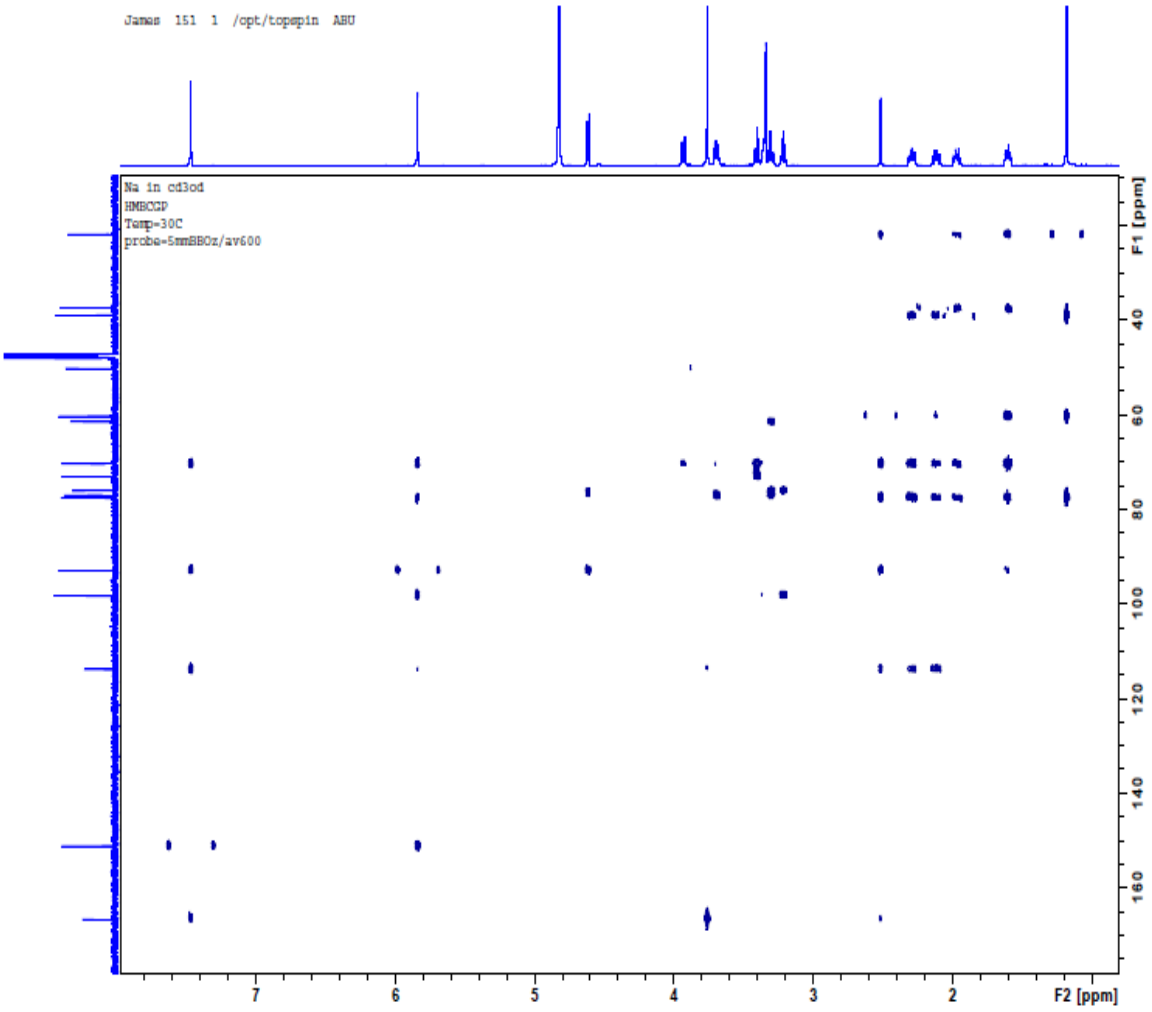


Fig 4.5: The HMBC Spectrum of Isolated Compound N_A

4.4.6 The HSQC spectrum of isolated compound N_A

The heteronuclear spin quantum correlation (HSQC) spectra of isolate N_A in (Fig 4.6) displayed characteristic signal suggesting the correlation of anomeric proton at δ_H 5.81 ppm to δ_C 93.2 ppm of cyclopentano pyran ring system and δ_H 4.59 ppm to δ_C 99.02 ppm of proton of sugar moiety.

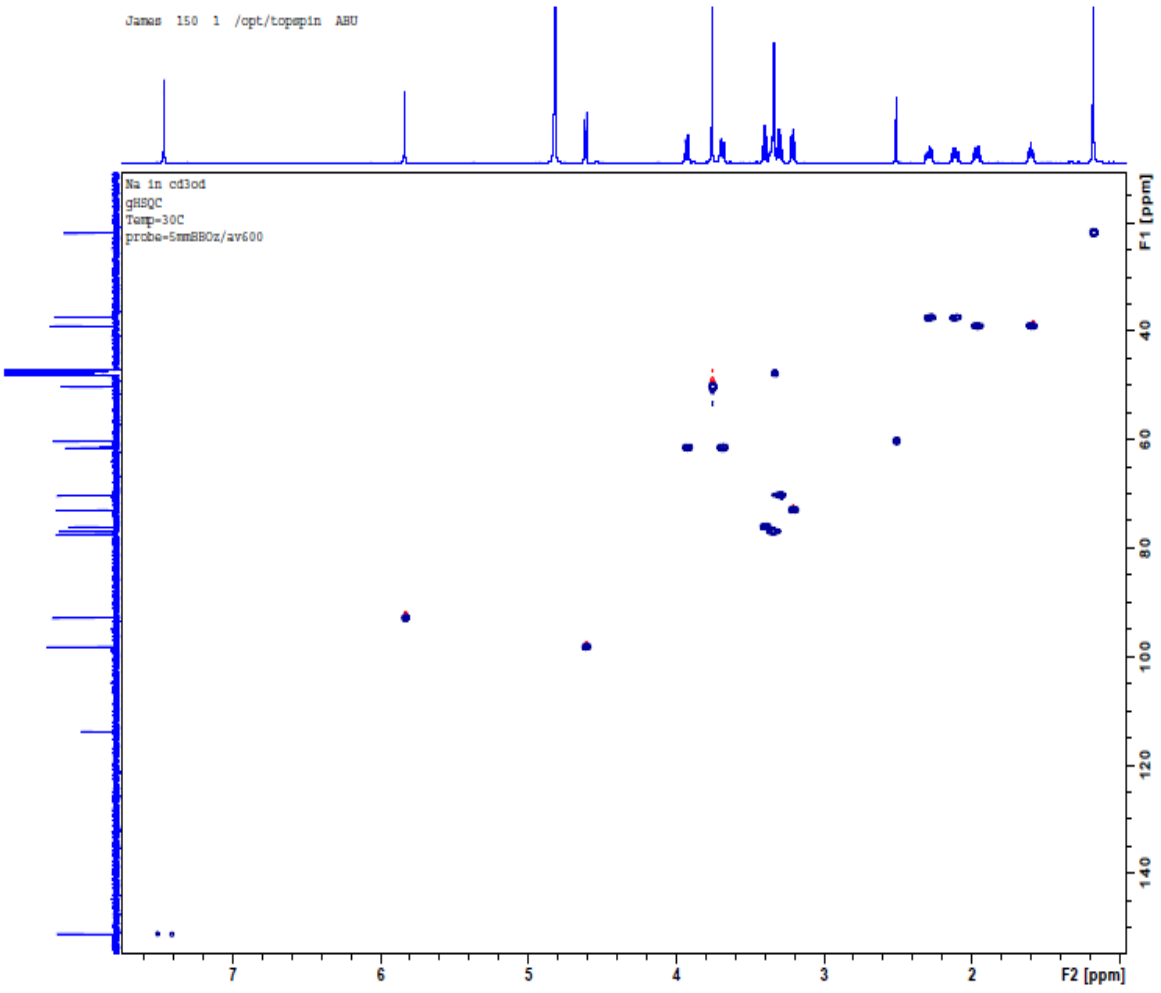


Fig 4.6: The HSQC Spectrum of Isolated Compound N_A

4.4.7 The NOESY spectrum of isolated compound N_A

The nuclear overhauser enhancement spectroscopy (NOESY) spectra of isolate N_A in (Fig 4.7) demonstrated that, nuclei of some protons of isolate N_A are in closed spatial proximity within the molecule. Thus, the spectral showed characteristic signals suggesting the correlation of proton at δ_H 5.81 ppm → δ_H 1.16 ppm and δ_H 5.81 ppm → δ_H 4.59 ppm.

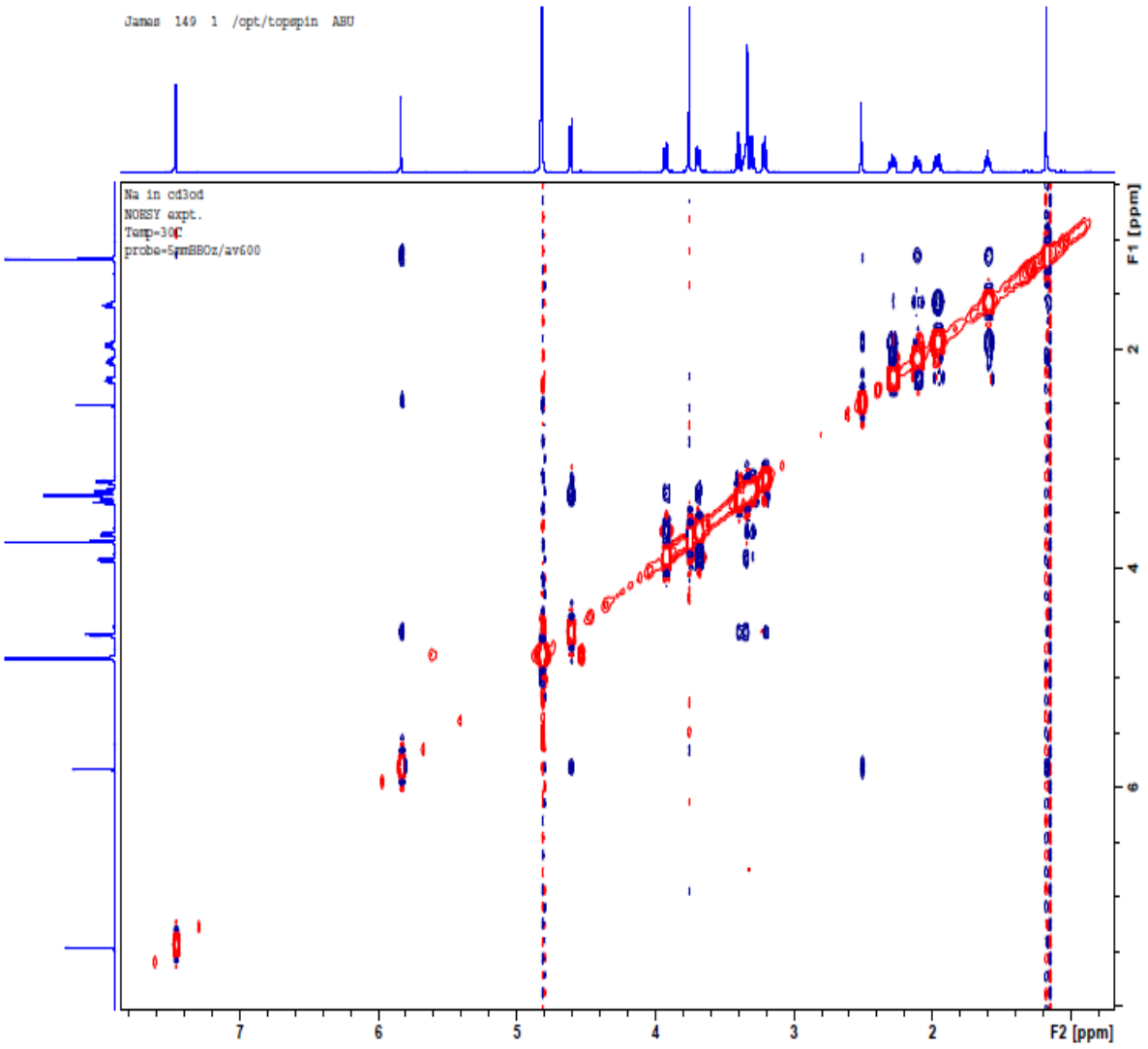


Fig 4.7: The NOESY Spectrum of Isolated Compound N_A

4.4.8 Comparism of ^{13}CNMR and ^{1}HNMR data of isolated compound N_A and ipolamiide

Table 4.11 showed the comparism of ^{13}CNMR and ^{1}HNMR spectral data of an isolate N_A with the ipolamiide.

Table 4.11: Comparison of ^{13}CNMR and ^{1}HNMR spectral data of an isolated compound N_A with ipolamiide (reference *data)

Position of N_A carbon/proton	δ_C of N_A	δ_H of N_A	*δ_C of ipolamiide	*δ_H of ipolamiides
1	CH = 93.20	5.81(1H, d, J=1.1Hz, H-1)	93.0	5.80(1H, d, J= 1.1Hz,H-1)
2				
3	CH = 151.30	7.45(1H,s, H-3)	151.4	7.43(1H, s, H-3)
4	C = 113.90	-	114.0	-
5	C = 70.36	1.93(IH, m,H_a-6)	70.6	1.92(IH, m, Ha-6)
6	CH2 = 37.80	1.57(1H, m, H_a- 7), 2.08(1H, m, H_b-7)	37.6	1.56(1H, m, H_a- 7), 2.08(1H, m, H_b-7)
7	CH2 = 39.20	2.08(1H,m,H_b-7)	39.6	2.08(1H, m, H_b-7)
8	C = 77.70	-	77.7	-
9	CH = 60.50	2.48(1H,bs, H-9)	60.5	2.47(1H,bs, H-9)
10	CH3 = 22.01	1.16(3H,s, H-10)	22.01	1.14 (3H,s, H-10)
11	C = 166.90	-	166.8	-
OCH_3	50.80	3.75(3H, s, COOMe)	50.4	3.72(3H, s, COOMe)
1^1	CH = 99.02	4.59(1H, d, J=8.0Hz, H-1^1)	98.4	4.5(1H, d, J=8.0Hz, H-1^1)
2^1	CH = 73.50	3.21(1H, dd, J=	73.2	3.17(1H, dd, J=

		9.1/8.0Hz, H-2^1)		9.1/8.0Hz, H-2^1)
3^1	CH = 76.02		76.2	
4^1	CH = 70.20		70.6	
5^1	CH = 77.09	3.22-3.37(3H, m, H-3^1, H-4^1, H-5^1)	77.1	3.23-3.38(3H, m, H-3^1, H-4^1, H-5^1)
6^1	CH2 = 61.70	3.84(1H, dd, J=12.0/5.5Hz, H_b-6^1), 3.65(1H, dd,J=11.90/2.2Hz, H_b-6^1)	61.5	3.89(1H, dd, J=12.0/5.5Hz, H_b-6^1), 3.65(1H, dd,J=11.90/2.2Hz, H_b-6^1)

*(Zuhal *et al.,* 2005)

CHAPTER FIVE

5.0 DISCUSSION

5.1 Phytochemical Screening of the Plant Extract

The crude ethanol extract of *E. africanum* plants was screened phytochemically, and the extract showed the presence of secondary metabolites which included glycosides, cardiac glycosides, saponins, triterpenes, flavonoids, tannins steroids and alkaloids; these secondary metabolites were tested positive in the crude methanol extract of the leaf of *E. africanum* when studied by Mohammed *et al.* (2014). It was observed in the report by Iwu *et al.* (1999), that plants have limitless ability to synthesize secondary metabolites, which according to Cowan (1999), these metabolites serve as plants defensive mechanism against microbes, insects and herbivores. In other words these secondary metabolites were known to confer protection against wood decaying microorganism (Barry *et al.,* 2002). Terpenoids, essential oils and some flavonoid compounds were reported to exhibit antifungal activities on some brown and white rot fungi (Cobb *et al.,* 1968; Carll and Highley, 1999).

Saponins were tested positive in crude ethanol extract of *E. africanum*, although researchers demonstrated several important biological properties such as antimicrobial, pesticidal, anti-inflammatory, spermicidal, adaptogenic, antipyretic, analgestic, antifeedant, antiviral, anticancer and immunostimulant activities have been attributed to saponins (Price *et al.,* 1987; Riguera, 1997; Yoshiki *et al.,* 1998). In folk medicine, the stem bark decoction of *E. africanum* is used as an emetic and purgative, as an anaesthetic, antihelmintic, anti-malarial, analgesic and disinfectant. The extracts are utilized in cases of skin disease, oedemas, gangrenous wounds, rheumatism and arthritis. It is also reported to be used as a poison or as a repellant against rodent, insects and some aquatic animals and also in tannins hides and as dye (Dalma, 1970; Burkill, 1985). The alkaloids were among the phytochemical constituents present in the crude ethanol extract of *E. africanum*, and earlier studies have speculated that alkaloids were responsible for the toxic effects of *Erythrophleum* species and some saponins have also been reported to be highly toxic (Nwude and Chineme, 1980; Nwude, 1981; Adeoye and Oyedapo, 2004). Studies by Attele *et al.* (1999) and D'Mello (1997) reported that saponins were among the components that elicited toxic pharmacological properties which include gastroenteritis, cellular damage, and modification of tissue permeability and induction of irritation of mucous membranes and such findings raised the possibilities of saponin contribution to the overall toxicity of *E. suaveolens* stembark.

The phytochemical constituents present in crude ethanol extract of the bark of *E. africanum* were the same metabolites in stem bark extract of *E. suaveolens,* this include alkaloids, saponins, tannins, glycosides and flavonoids (Adeoye and Oyedapo, 2004). The stembark extracts of *E. suaveolens* have been reported to show anaesthetic and convulsant effects as studied by Mgbeka and Ejiofor (1999), Harborne and Baxter (1993), the procyanidins from the stem was implicated to possess potent anti-inflammatory and analgesic properties reported by Dongomo *et al.* (2001), while alkaloidal fractions markedly alter the serum metabolites and activities of the hepatic enzymes. Saponins are surface-active,

high molecular-weight and chemically complex group of compounds that occur naturally in plants, and to a lesser extent in lower marine organisms and bacteria (Hostettman and Martson, 1995). Studies on diterpenoid alkaloids showed potent antimicrobial activities against bacteria and yeasts (Adeoye and Oyedapo, 2004; Ngounou *et al.,* 2005, Aiyegoro *et al.,* 2007).

5.2 Antimicrobial Screening Result

Results obtained in this study indicated that the crude ethanol extracts of the bark of *E. africanum* inhibited the growth of some of the micro-organism except *P. aeruginosa, E. coli, C. ulcerans* and *C. albicans*. It was observed that increase in the concentrations of the ethanol extract tended to increase the zone of growth inhibition of the sensitive micro-organisms. The antimicrobial studies by Mohammed *et al.* (2014) on the leaf extract of *E. africanum* showed similar sensitivity effects with crude ethanol extract on the test organism in the present study. The microorganisms showed different responses at various concentrations of the crude ethanol extract, this was demonstrated by other workers that the extracts of plants inhibited the growth of various microorganisms at different concentrations (Osadebe and Ukwueze, 2004). The observed antimicrobial effects of crude ethanol extract of *E. africanum* on the test microbes could be attributed to the presence of saponins, glycosides, flavonoids, tannins, alkaloids and terpenoids which was also reported by Cowan (1999). The presence of these secondary metabolites in the crude ethanol extract has suggested the great potential for the use of *E. africanum* plant as a source of phytomedicines. The presence of alkaloids in plants is known for decreasing the blood pressure and balancing of the nervous system in case of mental illness. The effectiveness in wound healing and as anti-parasitic may be attributed to the presence of tannins. The presence of terpenes suggests it can be used as an anti-tumor and anti-viral agent as some terpenes are known to be cytotoxic to tumor cells, while the saponins in plants are believed to have antioxidant, anti-cancer, anti-inflammatory and anti-viral effects (Balas and Agarwal, 1980; Yoshida *et al.,* 1990). The crude ethanol

extract tested positive for alkaloids, tannins, terpenes and saponins, this suggests that *E. africanum* can also be used in the treatment of various disease listed above.

The zone of inhibition exhibited by the isolated compound N_A exceeded that of the crude ethanol extract for *S. aureus* (25 to 26), *S. faecalis* (27 to 29), *E. cloacae* (29 to 30)*, P. mirabilis* (22 to 26) and *C. stellatoidea* (24 to 29) mm. Studied by Kaushik and Goyal (2008) on the aqueous and organic extracts of various parts of *Datura innoxia,* showed that the intensity of inhibition depended largely upon the plant part, extracting solvent and the test organism, it has also been observed that organic extracts showed more potent antibacterial activity than the aqueous extracts. Urinary tract diseases, toothaches, meningitis, endocardiatis are diseases caused by *S. feacalis* reported by Conly (2002) and *S. aureus* have been implicated by Braude (1982) in cases of boils, sores, wounds and pimples, while *E. cloacae* and *P. mirabilis* are gram negative bacteria responsible for respiratory tract infection, intra abdominal diseases, osteomyelitis, endocardiatis, ophthalmic infection, pneumonia, gastrointestinal infection (Deal *et al.,* 2007). This justified the uses of *E. africanum* plant by the traditional medical practitioners in the treatment of various diseases mentioned above, since the crude ethanol extract and the isolated compound from *E. africanum* plant was capable of inhibiting the growth of these micro-organisms.

P. aeruginosa, E. coli and *C. ulcerans* are gram negative bacteria which proved to be resistant to both the crude ethanol extract and the pure isolated compound, due to double cell membrane as described by Deal *et al.* (2007). In some cases Taylor *et al.* (2001) attributed the resistance of microbes to plant extracts due to minute quantity of active compounds to show activity with dose level, although according to Farnsworth (1993) lack of activity sometime are proven by using large doses. Studies of extracts of *B. pinnatum* by Ellof (1998) showed moderate to weak activity against test microbes. This was attributed to the loss of some plant's active principle when drying or the inability of the solvents to dissolve some of the active principles of this plant. The antifungal activities of various plants has been

studied, the ethanol extract of *Moringa oleifera* and *E. suaveolens* was reported to be active against *T. rubrum* and *T. mentagrophytes* (Nwosu and Okafor, 1995; Ficker *et al.,* 2003). Mahmoudabadi and Nasery (2009) reported that the fresh juice of shallot (*Allium ascalonicum*) used worldwide as a spice, food and folk medicine showed remarkable antifungal activity against *Candida* species and dermatophytes (*T. rubrum, T. tonsurans and M. audouinii*).

The MIC exhibited by the *E. africanum* plant extract against *E. cloacae* is of great importance in the health care delivery system, since it could be used as an alternative to antibiotic drugs in the treatment of infections caused by the organisms it was active against, especially as they frequently develops resistance to known antibiotic drugs (Singleton, 1999). The uses of *E. africanum* plant will also reduce the cost of obtaining health care delivery. The inability of the crude ethanol extract and the isolated compound to inhibit *P. aeruginosa, E. coli, C. ulcerans* and *C. albicans* may be due to a bacterial mechanism to detoxifying the active principle contained in the extract (Singleton, 1999). Some bacteria are known to possess mechanisms by which they convert substances that inhibit their growth to non-toxic compounds (Singleton, 1999). Therefore, the observed antimicrobial properties of some pathogens on this plant *E. africanum* are in agreement with its uses in folkloric medicine.

5.3 Spectroscopic Analysis

5.3.1 The ^{1}HNMR Analysis

The ^{1}HNMR spectral of the isolated compound N_A (Fig 4.1 and Table 4.10) showed signal at δ_H (7.45, s) confirming the presence of tetra substituted enol ether system typically of an iridoid which was also reported by Zuhal *et al.* (2005) in their studies. A characteristic signal at δ_H 3.75 and δ_H 1.16 ppm depicting the presence of proton from methoxy and methyl group, thus the signal at δ_H 4.59 ppm indicates the presence of anomeric proton of β-glucopyranose unit. The H-1 signal δ_H 5.81 ppm may be as a result of glycosidic linkage, indicating the point union of glucopyranose unit with iridoid moiety unit,

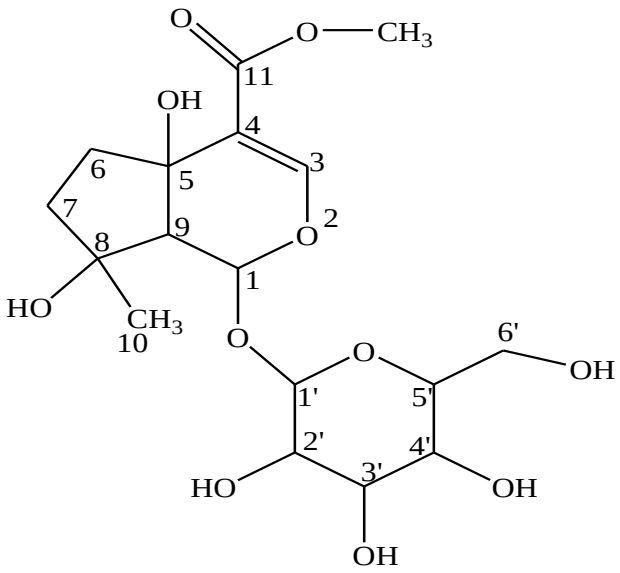
while the signal observed at δ_H 3.21, 3.41, 3.46, 3.64. 3.75, 3.88 and 4.59 ppm are characteristic signals of protons which could be attributed to glucopyranose unit, all of this signals can be compared to those reported by Zuhal *et al.* (2005). The point of attachment of β-glucopyranose unit (C-1) was confirmed by HMBC and the correlation between (H-1^1/C-1) at δ_H 4.59 ppm/δ_C 93.7 ppm and (H-1/C-1^1) at δ_H 5.81 ppm /δ_C 99.02 ppm was established and the correlation was also reported by Masaki *et al*. (2001).

The H-H COSY showed the correlation of H-1 of cyclopentano pyran ring system to H-1, H-12 and H-10. The H-1 was found to be correlated to H-5, H-4 and H-1 respectively (Nan-Zhang *et al.,* 2008). The HSQC correlation confirmed the attachment of carbon (C-1/C^1) to proton (H-1/H^1) at δ_C 93.7 ppm/δ_C 99.02 ppm and δ_H 5.81 ppm/δ_H 4.59 ppm, this was compared to the once reported by Tayfun *et al.* (2001).

5.3.2 The ^{13}CNMR Analysis

The ^{13}CNMR (Fig 4.2 and Table 4.11) peaks were assigned based by considering the chemical shift and comparison with reference data of glucopyranose. The ^{13}CNMR spectrum of N_A showing the presence of seventeen (17) characteristic signals out of which 11 were suggested to be aglycone and the remaining 6 to be β-glucopyranosyl moiety. The observed signal at δ_C 99.02, 73.50, 77.70, 70.20, 76.02 and 61.70 ppm are characteristic signals for glucopyranose unit carbon atom (Tayfun *et al.,* 2001; Yong and Peng, 2003). The β-anomeric configuration for the glucose was confirmed by it coupling constant (J_{HZ} 8.00 ppm) and the correlation between C-1/H-1, H-1/C-11 and H-1/C-11 confirmed the attachment of β-glucopyranose unit to (C-1) position of aglycone unit (Ik *et al.,* 2004). The chemical shift values and the splitting pattern of H-3 (δ_H 7.45, s) and H-9 (δ_H 2.46, s) was suggestive of C-4, C-5, and C-8 to be positioned at C-4, due to the highly desheilded signal of the H-3 proton, and the quaternary carbon resonate at δ_C 70.20 ppm which could be attributed to C-5 (Zuhal *et al .,* 2005).

The complete analysis of ^{1}H NMR and ^{13}C NMR spectral data of the isolated compound N_A allowed the assignment of the multiple signals observed at δ_H 2.08/2.48 ppm and δ_H 1.57/2.08 ppm to methylene protons at C-6 (δ_C 37.80 ppm) and C-7 (δ_C 39.20 ppm) respectively. The multiplicity of H-9 was due to the presence of totally substituted C-8. Thus, the chemical shift values of tertiary methyl group (δ_H 1.16, s) confirmed its attachment at C-8. Hence the chemical shift values of both C-8 (δ_C 77.70 ppm) and H-10 depicting the presence of tertiary hydroxyl functionality a C-8 position (Robert, 1989; Zuhal *et al.,* 2005). Based on the result obtained from the ^{1}HNMR and ^{13}CNMR on isolate N_A and comparison with those given in the literature, the isolate N_A was confirmed to be the same Ipolamiide as in the work of Tayfun *et al.* (2001) and Zuhal *et al.* (2005). Thus, the isolate has molecular formula $C_{17}H_{26}O_{11}$ and named as 4a,7–dihydroxy–7–methyl–1–3,4,5–trihydroxy–6–hydromethyl–tetrahydropyran–2–yloxy)–1–4a,5,6,7,7a–hexahydrocyclopentapyran–4–carboxylic acid methyl ester.



Name: Ipolamiide

Molecular Formular: $C_{17}H_{26}O_{11}$

Molecular Weight: 406.38174

Studies of *Weidemannia orientalis* plant by Zuhal *et al.* (2005) led to the isolation of many compounds, which include ipolamiide that showed anti-inflammatory properties, this anti-inflammatory property could be related to the anti-inflammatory property of the crude ethanol extract of *E. africanum,* from which the n-butanol fraction was obtained and the same ipolamiide was isolated. It has also been reported in the literature, that the leaves of *E. africanum* possessed many properties including anti-inflammatory activity (Dalziel, 1999).

CHAPTER SIX

6.0 Summary, Conclusion and Recommendation

6.1 Summary

The stembark of *E. africanum* plant collected from Badeggi, Katcha local government area of Niger State was identified with voucher number 2897 in Herberium unit of Biological Science Department, A.B.U Zaria. The stembark was air dried, pulverized, and extracted with ethanol by maceration, the bulk extract was filtered, then concentrated and dried. Litle portion of the dried ethanol extract was screened phytochemically and antimicrobially, while about 200 g of the remaining extract was dissolved in distilled water and then partitioned with petroleum ether, ethyl acetate, chloroform and n-Butanol. The n-Butanol fraction was loaded on the preparative TLC plates and a yellowish amorphous coded N_A obtained was subjected to spectroscopic techniques called NMR analysis whose data revealed to be ipolammide.

6.2 Conclusion

The extract of the stembark of *E. africanum* provides a promising solution in the ethno medicine practice of disease control. Hence this can be used to replace the synthetic antibiotics used in the treatment of related ailments as phytochemicals from natural sources are generally considered safer, available and affordable compared to the synthetic drugs in the treatment of infectious diseases. Various workers have demonstrated already that Gram positive bacteria are more susceptible towards plant extracts as compared to Gram negative bacteria (Lin *et al.,* 1999; Parekh and Chanda, 2006). These differences may be attributed to the fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram negative bacteria cell wall is multilayered (Yao *et al.,* 1995). Alternatively, if the active principle is present in reasonable quantities, there could be other constituents exerting antagonistic

effects or negating the positive effects of the bioactive agents (Jager *et al.,* 1996). With no antibacterial activity, extracts may be active against other bacterial species which were not tested (Shale *et al.,* 1999).

Alternatively, the passage of the active compound through the Gram negative cell wall may be inhibited. It is thought that observed differences may result from the doses used in this study. In addition, microorganisms show variable susceptibility to chemical substances related to different resistance levels between strains (Cetin and Gurler, 1989). Further trials using solvents of various polarities will explore the effects of solvent composition on extract efficacy (Romero *et al.,* 2005). It also explains why plants give similar therapeutic result when they are used in spite of their uniqueness (Sofowora, 1993).

Finally, the spectroscopy result was used to establish the structure of the isolated compound N_A and the spectroscopic analysis carried out included ^{13}C nuclear magnetic resonance (^{13}C NMR) and Proton nuclear magnetic resonance (^{1}H NMR), through the help of ^{1}H NMR and ^{13}C NMR parameters the structure of the isolated compound was properly elucidated as indicated above based on the comparison of the result of both the ^{1}H and ^{13}C NMR with those in the literature.

6.3 Recommendations

The research has provided a reliable evidence for the use of *E. africanum* has claimed by ethno medicinal practices in disease control. This can be used to supplement the synthetic antibiotic drugs used in the treatment of related ailments as secondary metabolites from natural sources are generally considered safer, readily available and affordable compared to the synthetic drugs in the treatment of infectious diseases.

Further trials on the test organisms which proved to be resistant in this study should be carried out using solvents of various polarities to explore the effects of solvent composition of extract on both the test and other organisms.

More research such as phytochemical screening, antimicrobial screening, isolation and characterization of other compounds should be conducted on other parts or portions of the plant e.g stem, root, seed and leaves.

Both government and non-governmental organization should encourage the researchers with the basic laboratories equipments e.g chemicals, solvents, apparatus and equipments (GC-MS, FTIR, U.V spectrophotometer, ^{1}H and ^{13}C NMR e.t.c).

REFERENCES

Abdel-Hameed, E.S.S. (2009). Total Phenolic contents and free radical scavenging activity of certain Egyptian *Ficus* species leaf samples. *Food Chem*istry, **114:** 1271-1277.

Adedotun, A.A., Salawu, O.T., Morenikeji O.A. and Odaibo, A.B. (2003). Plasmodia Infection and Haematological Parameters in Fibrile patients in Hospital in Oyo town, South-Western Nigeria. *Journal of Public Health and Epidemiology*, **5(3):** 144-148.

Adeoye, B.A. and Oyedapo, O.O. (2004). Toxicity of *Erythrophleum guineense* stem-bark: Role of Alkaloidal fraction. *African Journal of Tradition Complementary Alternative Medicine*, **1:** 45.

Agaie, B.M., Salisu, A. and Ebbo, A.A. (2007). A survey of common toxic plants of livestock in Sokoto State, Nigeria. *Science Resources. Essay*, **2:** 40-42.

Ahmad, W., Ahmad, Z., Najumul, S., Kazim, H. and Malik, A. (1994). Pyrrolizidine alkaloid content of the genus *Senecio*. *Journal of Chemical Society Pakistan,* **16:** 64-81.

Aiyegoro, O.A., Akinpelu, D. A. and Okoh, A. I. (2007). *In vitro* antibacterial potentials of the stem bark of red water tree (*Erythrophleum suaveolens*). *Journal of Biological Science,* **7:** 1233-1238.

Aiyegoro, O.A., Akinpelu, D.A., Afolayan, A.J. and Okoh, A.L. (2008). Antibacterial Activity of Crude Stem Bark Extracts of *Distemonanthus benthamianus Bail. Journal of Biological Science,* **8(2):** 356-361.

Akerele, O., Heywood, V. and Synge, H. (1991). Conservation of Medicinal Plants. Cambridge University Press, UK. P. 543

Al-Kassien, G.A.M. (2009). Influence of two plants extracts derived from thyme and cinnamon of broiler performance. *Pakistan Vetenary Journal*, ***29*****(4):** 169-173.

Antwi-Boaskiako, C. and Damoah, D. (2010). Investigation of Synergistic effects of Extracts from *Erythrophleum suaveolens*, *Azadirachta indica and Chromoloena odorata* on the durability of *Antiaris toxicaria. International Biodeterior. Biodegradation,* **64:** 97-103.

Arbonnier, M. (2004). Trees, shrubs, and lianas of West African Dry Zones. Africa-biodiversity. Flora tree. P. 574

Attele, A.S., Wu J.A. and Yuan, C.S. (1999). Ginseng Pharmacology: Multiple based bisglycosides from *Anemone raddeana Regel*. *Phytochemistry*, **45:** 1031–1033.

Balas, R.K. and Agarwal, R. (1980). Chemical Examination of E. hirta. In Book of abstracts II Micher, E. Reinhard E. (Eds). International Research Congress on Natural Products as Medicinal Agents Strasbourg, France. P. 25.

Barbour, K.E., Yaghi, H.R., Jaber, S.L., Shaib, A.H. and Harakeh, S. (2010). Safety and antiviral activity of essential oil against Avian influenza and Newcastle disease viruses. *International Journal Applied Resource. Vetenary Medicine, **8*****(1):*** 60-64.

Barry, K.M., Davies, N.W. and Mohammed, C. L. (2002). Effect of season and different fungi on Phenolics in response to xylem wounding and inoculation in *Eucalypus nitens. Forestry Pathology,* **32(1):** 321-327.

Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turck, M. (1996). Antibiotic susceptibility testing by standardized single disc method. *American Journal of Clinical Pathology,* **44:** 493-496.

Begum, S., Hassan S.I., Siddiqui, B.S., Shaheen, F. and Ghaayur, A.H. (2002). Trierpenoid from the leaves of *Psidium guajava*. *Phytochemistry*, **61:** 399-403.

Bisignano, G., Sanogo, R., Marino, A., Aquino, R., D'Angelo, V., Germanò, M.P., De Pasquale, R. and Pizza, C. (2000). Antimicrobial activity of *Mitracarpus scaber* extracts and isolated constituents. *Leter of Applied Microbiol*ogy, **30:** 105-108.

Bouamama, H., Noel, T., Villard, J., Benharref, A. and Jana, M. (2006). Antimicrobial activities of the leaf of the leaf extract two Moroccan *Citus L*. species. *Journal of Ethnopharmacology,* **104:** 104-107.

Braude, A.I. (1982). W.B. Microbiology. Sauders Company, London. Pp 32-36

Brain, D.R. and Tuner, T.D. (1975). Extraction procedures. In the practical evaluation of phytopharmaceuticals. Wright scenetechnical, Britol. UK. P. 98.

Brenan, J.P.M. (1967). Leguminosae-Subfamily Caealpinioideae. In: Flora of Tropical East Africa, Milne-Redhead, E. and R. M. Polhill (Eds.). White Friars Press, London, P. 230.

Brummitt, R.K., Chikuni, A.C., Lock, J.M. and Polhill, R.O. (2007). *Erythrophleum africanum* (*Welw. ex Benth.*) *Harms* in *Leguminosae*, Vol. **3:** 675-678.

Burkill, H.M. (1995). The Useful Plants of West Tropic Africa Royal Botanical Gardens. London. **3:** 101.

Carll, C.G. and Highley, T.L. (1999). Decay of Wood and Wood Base Products aboved Ground in Building. *Journal of Testing and Evaluation* (*JTEVA*). **2:** 150-158

Caro, T.M. (2003). Umbrella species: Critique and lesson from East Africa. *Animals Conservation*, **6:** 171-181.

Cetin, T.E. and Gurler, N. (1989): Bakterilerin antibiyotiklere duyaarlilik deneyinin yapilmasi. Kukem Dergisi, **12:** 2-5.

Chopra, R .N. (1958). Indigenous Drugs of India, Dhar UN and Sons, Calcutta. Pp 23-27

Chuan-Chung, T., Yuh-Chiang, S., Chung-Ren, S., Chia-Ying, L., Meei-Jen, L., Nguyen-Xuan, D. and Tian-Shung, W. (2008). New diterpenoids and the bioactivity of *Erythrophleum fordii*.*Bioorg. Med. Chem.* Department of Chemistry, National Cheng Kung University, No. 1 University Road, Tainan 701, Taiwan.

Clarke, E.G.C. (1970). The forensic Chemistry of Alkaloids. In "Alkaloids Chemistry and Physiology" Ed. R.H.F. Academia Press, New Yolk. Vol. **12:** 514-589

Cobb, B.G., Wolf, J.A. and Ansell, J. S. (1968). Congenital Structure of the Proximal Urethra Bulb. *Journal of Urology*, **99:** 629-631

Conley, J. (2002). Antimicrobial resistance in Canada. *Canadian Medical Association Journal,* **167:** 885-891.

Cordell, G.A. (2000). Biodiversity and drug discovery a symbiotic relationship. *Journal of Phytochemistry,* **55:** 463-480.

Cowan, M. M. (1999). Plant materials as antimicrobial agents. *Clinical medical review,* **12 (4):** 564-582.

Craig, J.W. (1999). Health promoting properties of common herbs. *American Journal of Clinical Nutrition,* ***70*** **(3):** 491-499.

Dalma, G. (1970). The *Erythrophleum* Alkaloids. In "The A1ka1oids" Ed. Manske, H. F. Academic Press, New York. **4:** 265-273.

Dalziel, J.M. (1959). The useful plants of West Tropical Africa. An appendix to the flora of West Tropical Africa. Crown Agents of Overseas Government and Administrative, London. Pp. 192-194.

Dalziel, J.M. (1999). The useful plants of West Tropical Africa. Crown Agents London. Pp. 432-434.

Dan Du , Jing Qu, Jia-ming, W., Shi-Shan Yu, Xiao-Guang Chen, Song Xu, Shuang-Gang Ma, Yong Li, Guang-zhi, D. and Lei Fang. (2010). Cytotoxic cassaine diterpenoid–diterpenoid amide dimers and diterpenoid amides from the leaves of *Erythrophleum fordii*. Phytochemistry, **71(14-15):** 1749-1755.

Deal, E.N., Micek. S.T., Ritchie, D.J., Reichley, R.M., Dunne, W.M. and jr, Kollef, M.H. (2007). Predictor of hospital mortality for blood stream infection cause by enterobacter species or *Citrobacter freundii*, **27(2):** 191-199.

Dean, D.A. and Burchard, K.W. (1996). Fungal infection in surgical patients *American Journal Surgery*, **171:** 374-382.

D'Mello, J.P.F. (1997). Handbook of Plant and Fungal Toxicants. CRC Press, Boca Raton, FL, USA, Pp 191-204.

Donatus, E.O. and Rosemary, U. (2009): Isolation, Characterization and Antibacterial Activity Screening of Methoxyamine Tetrahydroxyanthocyanidines from *Detarium Senegalense Gmelim* Stem bark. *African Journal of Pure and Applied Chemistry,* **3:** 1-5.

Dongmo, A.B., Kamanyi, A., Achang, M.S., Chunga-Anye, N.B. and N'jamen, D. (2001). Anti-inflammatory and analgesic properties of the stem bark extracts of *Erythrophleum suaveolens (caesalpiniaceae)*, Guillemin and Perrottet. *Journal of Ethnopharmacology,* **77:** 137-141.

Ellof, J.N. (1998). Which extract should be used for the screening and isolation of antimicrobial components from plants, *Journal of Ethnopharmacology,* **60:** 1-8.

Evans, W.C. (1997). Trease and Evans, Pharmacognosy. 15th Edition, Elsevier India, Pp. 137-204.

Farnsworth, N.R. (1993). Biological approaches to the screening and evaluation of natural products. In: Rasoanaivo, P, Ratsimamanga-Urverg, S (Eds). Biological Evaluation of Plants with Reference to the Malagasy Flora, Madagascar, Pp. 35-43.

Ferguson, L.R. (2001). Role of plant polyphenols in genomeric stability. *Mutation Research*, **475:** 89-111.

Firas, A.A. and Hassan, F.A. (2008). Antibacterial and antifungal activities of different Parts of *Tribulus terrestris L*. Growing in Iraq. *Journal of Zhejiang University of Science,* B. **9(2):** 154-159.

Ficker C., Smith, M.L., Akpagana, K., Gbeasor, M, and Zhang, J (2003). Bioassay Guided Isolation and Identification of Antifugal compounds from Ginger. *Phytother. Research,* **17:** 897-902.

Frankič, T., Voljč, M., Salobir, J. and Rezar, V. (2009). Use of herbs and spices and their extracts in animal nutrition. *Acta agriculturae Slovenica,* **94(2):** 95-102.

Gadekar, R., Singour, P.K., Chaurasiya, P.K., Pawar, R.S. and Pati, U.K. (2010). A potential of some medicinal plants as an antiulcer agents. *Pharmacognostics Rev*iew, **4:** 136-146.

Girma, M.W., Maya, P.S., Williams, M.M. and Barbara, N.T. (2003). Constituents of Antibacterial Extract of *Caesapinia Puraguariensis* Burk. *Z. Naturforsch*. **58c:** 70-75.

Griffin, W.J., Phippard, J.H., Culvenor, C.C.J., Loder, J.W. and Neam, R. (1971). Alkaloids of the leaves of *Erythrophelum chlorostachys*. *Phytochemistry,* **10:** 2793-2797.

Ik, D., Poothullil, A.M. and Conwey, R.M. (2004). Low dose proton and carbom beam therapy for circumscribed choroidal. *Journal of organic chemistry*, **9**: 132-134

Hammer, K.A., Carson, C.F. and Riley, T.V. (1999). Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbial*ogy, **86:** 985-990.

Harborne, J.B. and Baxter, H. (1993). Phytochemical Dictionary, Taylor and Francis, London,UK, P. 791.

Harborne, J.B. (1973). Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. 3rd Edn., Chapman and Hall, London, Pp: 7-13, 60-89, 131-135, 186-188, 203, 279.

Harborne, J.B. (I972). Phytochemical Ecology. 8th Edn., Academic Press Inc., London, New York, Pp: 182-195.

Harleen, K.S., Mohanjit, K., Bimlesh, K. and Sunil, P. (2011). An update on *Nyctanthes Arbortritis Linn, Internationale Pharmaceutica Sciencia,* **1(1):** 77-86.

Hassan, S.W., Ladan, M.J., Dogondaji, R.A., Umar, R.A., Bilbis, L.S., Hassan, L.G., Ebbo, A.A. and Matazu, I.K. (2001). Phytochemical and Toxicological Studies of Aqueous Leaves Extracts of *Erythrophleum africanum, Journal of Ethnopharmacology*, **77(2-3):** 137-141.

Hostettmann, K. and Marston, A. (1995). Chemistry and pharmacology of Natural products. Cambridge University Press, Cambridge, Pp 284-286.

Hostettmann, K., Pettei, M. J., Kubo, I. and Nakanishi, K. (1977). Direct obtaining of pure compounds from crude plant extracts by preparative liquid chromatography Lloydia. *Helv Chim Acta*. **60:** 670-672.

Iwu, M. W., Dunncan, A. R. and Okunji, C.O. (1999). New antimicrobials of plant origin. In: Perspectives on new crops and new uses. J. Janick (ed). ASHS Press, Alexandria, V.A. Pp 457-462.

Jager, A.K., Hutchings, A. and Van Staden, J. (1996): Screening of Zulu medicinal plants for prostaglandinsynthesis inhibitors. *Journal of Ethnopharmacol*ogy, **52:** 95-100.

Jean-Bosco, T. S., Daouda, A., Stéphane, D., Maurice, K.N., Philippe, G. and Joseph, N.N. (2012). Antioxidant activities, total phenolic contents and chemical compositions of extracts from four

Cameroonian woods: *Padouk (Pterocarpus soyauxii Taubb), tali (Erythrophleum suaveolens), moabi (Baillonella toxisperma), and movingui (Distemonanthus benthamianus).* Laboratory of Physico-Chemistry of Wood, University of Yaounde 1, Cameroon.

Jigna, P. and Chanda, S. (2007). Antibacterial and Phytochemical Studies on Twelve Species of Indian Medicinal Plants. *African Journal Biomedical Research,* **10:** 175-181.

Jing, Q., You-Cai, H., Shi-Shan ,Y., Xiao-Guang, C. and Yan, L. (2007). Rapid structural etermination of new trace cassaine-type diterpenoid amides in fractions from e*rythrophleum fordii* by liquid chromatography-diode-array detection/electrospray ionization tandem mass spectrometry and liquid chromatography/nuclear magnetic resonance. *Planta Medical*, **72 (5):** 442-449.

Jing, Q., You-Cai, H., Shi-Shan, Y., Xiao-Guang, C. and Yan, L. (2006). New Cassaine Diterpenoid Amides with Cytotoxic Activities from the Bark of *Erythrophleum fordii*. *Planta Medicine,* **50 (5):** 212-219.

Jing, Q., Ying-Hong, W., Jian-Bei, L., Shi-Shan, Y., Yuan, L. and Yun-Bao, L. (2007). Rapid Commun Mass Spectrum, **6-21(13):** 2109-2119 .

Jinju, M.H. (1990). African Traditional Medicine. A case study of Hausa Medicinal Plants and Therapy. Gaskiya Corpn. Ltd. Zaria. Nigeria. Pp 43-50.

Jonathan, E.K., Anna, K. J. and Johannes, V.S. (2000). Zulu Medicinal Plants with antibacterial activity. *Journal of Ethnopharmacology*, **69:** 241-261.

Justin, R.S. and Baby, J. (2011). Pharmacognistic and Phytochemical Properties of *Ficus carica Linn.* An Overview. *International Journal of PharmTech Research,* **3(1):** 8-12.

Justin, R.S. (2011). Pharmocognistic and Traditional Properties of *Cissus quadrancularis Linn.* An Overview. *International Journal of Pharmacology* and *Bioscience,* **2:** 135-139.

Kadja, A.B., Mamyrbekova-Bekro, J.A., Benie, A., Boua, B.B., N'gaman, K.C. and Bekro, Y.A. (2001). *Erythrophleum africanum Afzel,* an African Toothpick: Phytochemical Screening, Total Flavonoid Content and Antioxidant Activity. *Journal of Medicinal Plant Research*, **5(27):** 6273-6277.

Kaushik, P. and Goyal, P. (2008). Invitro Evaluation of *Datura innoxia* (thorn-apple) for potential antimicrobial activity. *Indian Journal of Microbiology*, **48(3):** 353-357.

Katasini, D., Srinu, B. and Bala, R. (2011). Phytochemical Screening, Quantitative estimation of Total Phenolic, Flavonoid and Antimicrobial Evaluation of *Trachyspermum ammi. Journal of Atoms and Molecule,* **1(1):** 1-8.

Kaita, B. (1998). VeNseNtation post-culturale enzone Soudanienne au Senegal. Influence des pratiques culturales et des facteurs anthropiques Surla reconstitution VeNgeNtale apreNs abandon cultural. Doctorate, Thesis, UniversiteN coarse (Pascal Paoli), P. 169.

Kehinde, T.K., Alli, S.O. and Atayese, A.O. (2012). Antibacterial effect of *Distemonanthus Benthamianus* Extract Against some Oral Pathogens. *International Journal of Applied Science and Technology,* **2:** 114-118.

Kingsbury, D.T. and Wagner, G.E. (1990). Microbiology. Williams Wilkins, Maryland. Pp 213-216.

Kirby, G.C. (1996). Medicinal plants and the control of parasites. *Trans. Royal Society of Tropical Medicine Hyg*ene, **90:** 605-609.

Kumar, A., Lingadurai, S., Jain, A. and Barman, N.R. (2010). *Erythrina variegata Linn*: A Review on Morphology, Phytochemistry and Pharmacological Aspects. *Pharmacognostic Review,* **4:** 147-152.

Kumar, V.P., Chauhau, N.S., Padh, H. and Rajani, M. (2006). Search for Antibacterial and Antifungal Agents from selected Indian Medicinal Plants (Electronic Version). *Journal of Ethnopharmacology,* **107(2):** 182-188.

Latha, L.S. and Reddy, P.N. (2009). Antimicrobial, Antidiarrhoeal and Analysis of Phytochemical Constituents of *Sphaeranthus amaranthoides. Indian Journal of Science and Technology,* **2(3):** 45-48.

Lawrence, R., Tripath, J. and Jeyakumar, E. (2009). Isolation, purification and evaluation of antibacterial agents from *Aloe vera*. *Brazil. Journal of Microbiol*ogy, **40:** 906-915.

Lin, J., Opoku, A.R., Geheeb-Keller, M., Hutchings, A.D., Terblanche, S.E., Jager, A.K. and Van Staden, J. (1999): Preliminary screening of some traditional zulu medicinal plants for anti-inflammatory and antimicrobial activities. *Journal of Ethnopharmacol*ogy, **68:** 267-274.

Lock, J.M. (1990). *Cassia Sens. Lat. (leguminosae-calsapinioideae*) in Africa. *Kew Bulletin.* **43(2):** 333-342.

Loder, N.J., Culrenor, C.J., Nearn, R.H., Russel, G.B. and Stranton, F.W. (1974). Tumur Inhibitory Plants New Alkaloids from bark of *E. chlorostachys* (*leguminosae*). *Australian Journal of Chemistry*, **27:** 179-185

Mabberley, D.J. (1997). The Plant-Book. 2nd Eds., Cambridge University Press, Cambridge. Pp. 212-217.

Mahmoudabadi, A.Z. and Nasery, M.K.G. (2009). Antifungi activity of *Shallot, Allium ascalonium Linn.(liliaceae), invitro. Journal of Medicinal Plants Research,* **3:** 450-453

Manimozhi, D.M., Sankaranarayanan, S. and Sampathkumar, G. (2012). Evaluation of the Antibacterial Activity of Flavonoids Extract from *Ficus Benghalensis. International Journal of Pharmaceutical and Biological Research (IJPBR).* **3(1):** 7-18.

Manfouo, N.R., Lonts, D., N'gounou, N.F., Ngadjui, B.T. and Sondengam, B.L. (2005). Clinical Studies of Three Herbal Soaps in management of Superficial Fungal Infections. *Bulletin of Chemical Societyof Ethiopia*, **19:** 81.

Martin, K.R. and Appel, C.L. (2010). Polyphenols as dietary supplements: A double-edged sword. *Nutritional and Dietary Supplementary*, **2:** 1-12.

Masaki, M., Ikeda, A. and Kazautsumi, Y. (2001). "Identification and characterization of the functional domains in mixed lineage kinase LZK" FCBS *letter*. **488(3)**: 190-195

Mattocks, A.R. and Jukes, R. (1987). Improved Field Tests for Toxics Pyrrolizidine Alkaloids. *Journal of Natural Products,* **50:** 161-166.

Meenakshi, S., Jain, D.C., Darokar M.P. and Sharma, R.P. (2001). Antibacterial activity of Ailanthus excels (Roxb). *Phytotherapy Research*, **15:** 165-166.

Mgbeka, B.O. and Ejorfor, E.N. (1999). Effects of extract of dried leaves of *Erythrophleum suaveolens* as Anesthesia on Clarrid catfish. *Journal of Applied Aquaculture,* **8:** 73- 80.

Middleton, Jr. E., Kandaswami, M. and Theoharides, T.C. (2000). The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease and cancer. *Pharmacology Review,* **52:** 673-751.

Miller, L.P. (1973). Plants as organic laboratories in phytochemistry.Vol.I(miller.,p.edn) Reinhold publishing corporation New Yolk, U.S.A. Pp. 1-4.

Miyagawa, T., Ohtsuki, T., Koyano, T. and Kowithayakorn, T. (2009). Cassaine diterpenoid dimmers isolated from *Erythrophleum succirubrum* with TRAIL-resistance overcoming activity. *Planta Medicinal,* **50(32):** 4658-4662.

Mohammed, M., Musa, A.M., Garba, M.A., Adeiza, A.A. and Hanwa, U.A. (2014). Phytochemical and antimicrobial study on the leaf extracts of *E. Africanum. African Journal of Biotechnology.* **13(4):** 598-600.

Moghaddam, M.N, Khajeh-Karamoddin, M. A. and Ramezani, M. (2009). *In vitro* anti-bacterial activity of sweet basil fractions against *Helicobacter pylori. Journal Biological Science*, **9:** 276-279.

National Committee for Clinical Laboratory Stan-dards (NCCLS) (1999). Performance standards for antimicrobial susceptibility testing; ninth infor-mational supplement. Wayne, Pensilvania document M100-S9, Vol. **19:** 43-49.

Nandagopal, S. R. and Kumari, B. D. (2007). Phytochemical and Antibacterial Studies of *Chicory (Cichorium intybus L.)* - A Multipurpose Medicinal Plant. *Advanced Biological Research,* **1(1-2):** 17-21.

Nan-Zhang, S., Ling, Z. and Sumin, T. (2008). Evidence for an intermidiate line region in AGN's inner torus region and its evolution from narrow to braodline seyfert I galaxies. *Journal of Astrophysics.* **700:** 1173-1189

Neuwinger, H.D. (2000). African Tradition Medicine: a Dictionary of Plant uses and Applications. Mykosen, **26(2):** 94-99.

Newman, D.J., Cragg, G.M. and Snader, K .M. (2000). The influence of natural products upon drug discovery. *Natural Products Research*, **17:** 215-234.

Niber, B.T. (1994). The Ability of Powders and Slurries from Ten Species to Protect Stored Graibn from Attack by *Prostephanus truncatus Horn (Coleoptera: Bostrichidae)* and *Sitophilus Oryzae L. (Coleoptera: Curculionidae), Journal of Stored Production Research,* **30(4):** 297-301.

Ngane, A., Biyiti, L., Bouchet, p., Nkengfack, A. and Amivan zollo, P.H. (2003). Antifugal activity of *piper Guineense* of Camaroon Fititerapia, **74:** 464-468.

N'gouno, F.N., Manfouo, R.N., Tapndjuo, L.A., Lontsi, D., Kuete, V., Penlap, V.s, Etoa, F.X., Dubois, M. and Sondengam, B.L. (2005). Antimicrobial Diterpenoid Alkaloids fro *Erythrophleum Suaveolens* (Guill and Perr) Brenan. *Bulletin of Chemical Society of Ethiopia,* **19(2):** 22-226.

Nwude, N. (1981). Some stock poisoning plants of Nigeria. *Journal of Animal Production Research,* **2:** 109-122.

Nwude, N. and Chineme, C.N. (1981). Toxic effect s of the leaves of *E. africanum* (*Harms*) in sheep. *Bulletin of Animal Health Production Africa* (Kenya), **299:** 349-354.

Nwuso, M.O. and Okafor, J.I. (1995). Prelimary Studies of Antifungal Activities of some Medial Plants Againsts *Basidiobolus* and some other pathogenic fungi. *Mycoses*. **38(5-6):** 191-195

Ogbulie J.N., Ogueke, C.C., Okoli I.O. and Anyanwu, B.N. (2007). Antibacterial activities and toxicological potential of crude ethanolic extracts of *Euphorbia hirta African Journal Biotechnology,* **6(13):** 1544-1548.

Okemo, P.O., Bais, H.P. and Vivanco, J.M. (2003). In vitro activities of *Moesa lanceolata* extracts against fungal plant pathogens. *Fitoterapia,* **74:** 312-316.

Oladele, A.T., Ebujoba, A.A. and Oyelami, A.O. (2012). Clinical Studies of Three Herbal Soaps in Management of Superficial Fungal Infections. *Research Journal of Medicinal Plants.* **6:** 56-64.

Osadebe, P.O. and Ukwueze, S.E. (2004). A comparative study of the phytochemical and Antimicrobial properties of the Eastern Nigerian species of Africa Mistletoe (*Loranthus micranthus*) Sourced from different host trees. *Journal Biological Research Biotechnol*ogy, **2(1):** 18-23.

Osawa, K., Matsumoto, T., Maruyamu, T., Takiguchi, T., Okuda, K. and Takazoe, I. (1990). Studies of Antibacteria Activities of Plants Extracts and their Constituents Against *Periodontopathics* Bacteria. *Bulletin of Tokyo Dental Collection*, **31(1):** 17-21.

Oyedapo, O.O., Sab, F.C. and Olagunju, J.A. (1999). Bioactivities of Fresh Leeaves of *Lantana camara. Biomedical Letters*. **59:** 175-183

Parekh, J. and Chanda, S. (2006). In Vitro Antimicrobial Activities of Extract of *Launaea procumbens Roxb. (labiateae), Vitis Vinifera (Vitaceae)* and *Cyperus rotundus (Cyperaceae)*, *African Journal of Biomedical Research,* **9:** 89-93.

Pavrez, M, Mahboob, H.K, Zahuul, I. and Shek, M.H. (2005). Antimicrobial activities of the petroleum ether, methanol and acetone extracts of *Kaempferia galanga L. rhizome*. *Journal of Life Earth Science*, **1:** 25-29.

Perumal, S. R. and Ignacimuthu, S. (2000). Antibacterial activity of some of folklore medicinal plants used by tribals in Western Ghats of *India. Journal of Ethnopharmacology*, **69:** 63-71.

Poole, K. (2005). Efflux-mediated antimicrobial resistance. *Journal of Antimicrobial Chemother*, **56:** 20-51.

Price K.K., Johnson L.I. and Fenwick, D. (1987). The Chemical and biological significance of saponinsin food and feeding stuffs. *Food Science and Nutrition,* **26:** 27-35.

Rice-Evans, C.A., Miller, N.J., Bolwell, P.G., Bramley, P.M. and Pridham, J.B. (1995). The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radical Research*, **22:** 375-383.

Riguera, R. (1997). Isolating bioactive compounds from marine organisms. *Journal of Marine Biotechnology,* **5:** 187–193.

Robert, M.F. (1989). Isolation of alkaloids from *Moringa indica*. *Journal of Biological Research. Biotechnology*, **8(2):** 18-23.

Roberts, M.F. and Winks, M. (1998). Alkaloids: Biochemistry, Ecology and Medical Applications. Plenum Press, New York, Pp. 397-424.

Romero, C.D., Chopin, S.F., Buck, G., Martinez, E., Garcia, M. and Bixby, L. (2005): Antibacterial properties of common herbal remedies of the southwest. *Journal of Ethnopharmacology,* **99:** 253-257.

Ryno, F. (2009). An Investigation into the Antimicrobial and Anticancer Activities of *Geranium incanum, Artemisia afra and Artemisia absinthium.* Faculty of Health Science, Nelson Mandela Metropolitan University, South Africa. Pp 121-124.

Said, H.M. (1969). Hamdard Pharmacopoeia of Eastern Medicines, Times Press, Karachi, Pakistan, Pp 112-117.

Scalbert, A., Johnson, I.T. and Saltmarsh, M. (2005). Polyphenols: Antioxidants and beyond. *American Journal of Clinical Nutrition*, **81:** 215-217.

Shale, T.L., Strik, W.A. and Van Staden, J. (1999): Screening of plants used by southern African traditional healers in the treatment of dysmenorrhoea for prostaglandin-synthesis inhibitors and uterine relaxing activity. *Journal of Ethnopharmacol*ogy, **64:** 9-14.

Shariff, Z.U. (2001). Modern Herbal Therapy for Common Ailments. Nature Pharmacy Series, Spectrum Books Ltd., Ibadan, Nigeria in Association with Safari Books (Export) Ltd. UK, **1:** 9-84.

Singleton, P. (1999). Bacteria in Biology, Biotechnology and Medicine. 4th.Reinhold publishing corporation New Yolk, U.S.A. Pp. 1-7.

Sofowora, A. (1993). A Recent trend in Research into African Medicinal Plants. *Journal of Ethnopharmacology,* **38(2-3):** 209-214.

Sophon, R., Kasan, S., Nongmy, J., Narongsak, C. and Amoro, P. (2002). Crystal structure of Ipolamiide Monohydrate from *Stachytarpheta indica*. *Anal Science*, **18(9):** 1063-1064.

Taylor, R.B., Shakoor, O., Behrens, R.H., Everard, M., Low A.S., Wangboonskul, J., Reid, R.G. and Kolawale, J.A. (2001). Pharmacopaeial Quality of Drugs Supplied by Nigerian Pharmacies. **357(9272):** 1933-1936.

Tayfun, E., Harputs, U.S. and Colis, I. (2001). Iridoids, Phenylethanoids and Monoterpenes Glycosides from *Phlomis sieheana. Turky Journal of*\ *Chemistry,* **26:** 1-8.

Thomson, W.A.R. (1978). Healing plants. A Modern Herbal. Mi Grew Hill Company, U.K. Pp. 6 – 10.

Tomoko, N. and Takashi, A. (2002). Antibacterial activity of extracts prepared from tropical and sub-tropical plants on methicillin-resistant *Staphylococcus aureus, Journal Health Sci*ence, **48:** 273-276.

Trease, G.E. and Evan, W.C. (1989). Pharmacognosy. 13th(ed) ELBS/Bailiere Tindall, London. Pp 345-346, 535-536, 772-773.

Trease, G.E. and Evans, W.C. (1978). A Text Book of Pharmacology. 11th Edn. Bailliere Tindal, London, P. 530.

Treben, M. (1986). Health through God's pharmacy.Advice and experience within medicinal plants Wilhelm Ensthatersteys Autria. Pp. 6 – 20.

Tulikki, R. (2003). Defences and responses: Woody species and large herbivores in African Savannas. Ph.D Thesis, Swedish University of Agricultural Sciences. *Acta Universitatis Agriculturae Suecial, Silverstria*, P. 276.

Verotta, L., Aburjai, T., Rogers, C. B., Dorigo, P., Maragno, I., Fraccarollo, D., Santostasi, G., Gaion, R.M., Floreani, M. and Carpenedo, F. (1995). Chemical and pharmacological characterization of *Erythrophleum lasianthum* alkaloids. *Planta Medicine,* **61(3):** 271-274 .

Verpoorte, R., Harkes, P.A. and Tenhoupen, H.J.G. (1987). Plants cell culture as a tool in production of secondary metabolites in pharmaceutical sciences. Elservier Science Publishers, London. Pp. 263 – 282.

Walter, C., Gyebi, D., Yolandi, V.Z., Schoeman, C.J., Runner, T.M., Sam, O.Y., Jose, J., Petrina, T.K., Martha, K.S., Amanda, M. and Avrelija, C. (2011). Antibacterial and Anticandidal Activity of *Tylosema esculentum* (Marama) extracts. *South African Journal of Science*, **107(3-4):** 0038-2353.

Wills, R., Bone, K. and Morgan, M. (2000). Herbal products: Active components, mode of action and quality control. *Nutritional Research Review,* **13:** 47-77.

Windisch, W., Schedle, K., Plitzner, C. and Krois-mayer, A. (2008). Use of phytogenetic products as feed additives for swine and poultry. *Journal of Animal Science,* **86:** E140-E148.

Yao, J., Moellering, R., Murray, P., Baron E., Pfaller M., Tenover, F. and Yolken, R. (1995). Manual of Clinical Microbiology. Antibacterial Agents. ASM, Washington DC, **5:** 1281-1290.

Yik, L.C., Elaine, W.L., Pei, L.T., Yau, Y.L., Johnson, S. and Joo, K.G. (2011). Assessment of Phytochemical Content, Polyphenolic Composition, Antioxidant and Antibacterial Activities of *Leguminosae* medicinal plants in Peninsular Malaysia. *Complementary and Alternative Medicine,* **11:** 12.

Yong, K. and Peng, W. (2003). Theoritical Study on the Hydrogen Molecules on multi-walled Carbon Nanotube. *Acta physical Chemistry,* 19(2): 139-143

Yoshida, M., Kijima, M., Akita, M. and Beppu, T. (1990). Potent and Specifics Inhibition of Mammalian Histone Deacetylse both *in vivo and in vitro by Trichestatin A*. *Journal of Biological Chemistry*, **265(28):** 17174-17179

Yoshiki, Y., Kudou, S. and Okubo, K. (1998). Relationship between chemical structures and biological activities of triterpenoid saponins from soybean (Review). *Bioscience Biotechnology and Biochemistry*, **62:** 2291–2299.

Zakaria, Z.A., Mat, M.A., Jais, M., Mastura, S.H., Mat, J. and Mohamed, A.M. (2007). *In -vitro* antistaphylococcal activity of the extracts of several neglected plants in Malaysia. *International Journal of Pharmacology,* **3:** 428-431.

Zampini, I.C., Cuello, S., Alberto, M.R., Ordonez, R.M., Solorzano, E. and Isla M.I. (2009). Antimicrobial activity of seleted plant species from the *Argentine puna* against sensitive and multiresistant bacteria. *Journal of Ethnopharmacology,* **124:** 499-505.

Zuhal, G., Hilal, O., Turesin, U., Cavit, K. and Demirezer, L.O. (2005). Iridoids, Flavonoids and Phenylethanoids Glycosides From *Weidemannia orientalis*, *Turky Journal of Chemistry,* **30:** 391-400