

**PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF THE ROOT BARK
EXTRACTS OF *ACACIA ATAXACANTHA* DC (FABACEAE)**

BY

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NIGERIA**

MAY, 2015

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BY

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**DEPARTMENT OF CHEMISTRY,
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NIGERIA**

MAY, 2015

DECLARATION

I declare that the work in this thesis entitled “PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF THE ROOT BARK OF *ACACIA ATAXACANTHA* DC” has been carried out by me in the Department of Chemistry. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

Aba, Ophelia Yakandi

Name of Student

Signature

Date

CERTIFICATION

This thesis entitled “PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF THE ROOT OF *Acacia ataxacantha* DC (FABACEAE)” by Ophelia Yakandi ABA meets the regulations governing the award of the degree of Master of Science in Chemistry, Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To the Glory of God the Father, Son and Spirit

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ABSTRACT

The root-bark of the plant *Acacia ataxacantha* DC of the family Fabaceae was investigated for medicinal values. The Phytochemical screening gave positive results for the presence of flavonoids, carbohydrates, glycosides, saponins, steroids/triterpenes, tannins and alkaloids. The antimicrobial screening of the crude methanol, ethyl acetate, chloroform and petroleum ether extracts showed that the plant roots could inhibit the growths of *Bacillus subtilis*, *Streptococcus pneumonia*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Salmonella typhi*, *Escherichia coli*, *Candida albicans* and *Candida krusei* but were not active on *Corynebacterium ulcerans*, *Streptococcus faecalis*, *Proteus mirabilis* and *Candida tropicalis*. Ethyl acetate extract had the widest diameter of zone of inhibition of 30 mm, followed by chloroform with 25 mm, then methanol with 24 mm and petroleum ether with 19 mm against the various micro-organisms. The Minimum Inhibitory Concentration (MIC) of the extracts was determined for the organisms whose growths were inhibited. Methanol and chloroform fractions had MIC values of 5 mg/ml and petroleum ether had 10 mg/ml for all the test organisms, while ethyl acetate was the most active with 2.5 mg/ml for *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumonia*. The Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) showed that the ethyl acetate extract had cidal effect against *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae* at a concentration of 5 mg/ml. The

Ethyl acetate extract was purified using chromatographic techniques and two pure compounds (ABA and ABA 1) were isolated and characterized using spectral techniques. Based on available spectral data and comparison with existing data bank, the compounds were established to be α - amyrenol and lupeol respectively. The antimicrobial activity of α - amyrenol was determined with the same test organisms. The MIC and MBC/MFC were found to be 12.5 and 25 $\mu\text{g/ml}$ respectively against *B.subtilis*, *E.coli* and *S.typhi*, thus justifying the numerous folkloric uses of the plant.

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CHAPTER ONE

1.0 INTRODUCTION

Ever since ancient times, people looked for drugs in nature in search for cure for their diseases. In view of the fact that at that time there was no sufficient information either concerning the reasons for the illnesses or concerning which plant and how it could be utilized as a cure, everything was based on experience. In time, the reasons for the usage of specific medicinal plants for treatment of certain diseases were being discovered; thus, medicinal plants' usage gradually abandoned the empiric framework and became founded on explicatory facts (Petrovska, 2012). The use of the medicinal herbs for curing diseases has been documented in the history of all civilizations. The drugs were used in crude forms like expressed juice, powder, decoction or infusion (Amritpal, 2011).

The World Health Organization (WHO) defined medicinal herbs as finished, labelled medicinal products that contain active ingredients aerial or underground parts of plants, or other plant material, or combinations thereof, whether in the crude state or as plant preparations. Herbal medicines may contain excipients [inert additives such as starch used to improve adhesive quality in order to prepare pills or tablets] in addition to the active ingredients (WHO, 1996).

[Ethnobotany](#) (the study of traditional human uses of plants) is recognized as an effective way to discover future medicines. In 2001, researchers identified 122 compounds used in modern medicine which were derived from "ethnomedical" plant sources; 80% of these have had an ethnomedical use identical or related to the current use of the active elements of the plant (Fabricant and Farnsworth, 2001). Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including [aspirin](#), [digitalis](#), [quinine](#), and [opium](#). Numerous medicines in use today were extracted from plants. About 50 to 60%

of pharmaceutical drugs are either of natural origin or obtained through use of natural products as starting points in their synthesis (Verlet, 1990; Balandrin *et al.*, 1993).

Traditional medicine has a long history, it is the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses (WHO, 2000). The widespread use of plants in folk medicine by traditional medicinal practitioners has continued to create awareness in the study of plants and medicinal plants. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as [insects](#), fungi and herbivorous [mammals](#). At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total (Lai and Roy, 2004; Tapsell *et al.*, 2006).

The world's tropical rain forests are especially rich in biodiversity but there is rapid depletion of these natural resources worldwide, and in Nigeria in particular, the pressures from degradation, unsustainable arable land use, urbanization and industrialization are taking their toll as well (Obute and Osuji, 2002; Ayodele, 2005). The vegetable world comprises three main groups of plants; the Superior, Intermediary and the Inferior. These encompass bacteria, microscopic algae, mushrooms, ferns, bushes and trees among others (Sofowora, 1993). Medicinal plants and herbs contain substances known to modern and ancient civilization for their healing properties. Until the development of organic compounds in the nineteenth century, medicinal plants and herbs were the sole source of active principles capable of curing man's ailments. Modern pharmaceuticals rely heavily on the same active principles, be they natural or synthetic. These active principles differ from plant to plant due to their biodiversity (Sofowora, 1993). Plants have continued to be major sources of medicine either in the form of traditional medicine preparations or as pure active principles. This has made it

important to identify plants with useful therapeutic action for possible isolation and characterization of their active constituents (Ndukwe *et al.*, 2007). The need to identify active chemical constituents in plant extracts requires phytochemical and analytical techniques. Different phytoconstituents have different degrees of solubility in different types of solvents depending upon their polarity and structure (El-Mahamood and Doughari, 2005). Phytochemical surveys are being seen as the first step towards the discovery and structural elucidation of useful natural organic constituents for medicinal applications (Hostettmann *et al.*, 2000). Many plants are chemically different depending on the locality where they are found with some of the constituents occurring only at certain seasons of the year (Adelani, 2007).

The continued investigation of their secondary metabolites has led to important breakthrough in pharmacology and has helped tremendously in the development of modern pharmacotherapeutics in Africa and other parts of the world (Nwaogu *et al.*, 2007). Plants are particularly interesting because they have the broadest spectrum of biosynthetic capability, and produce a wide variety of compounds. It is against this background that it has become necessary to investigate the active ingredient(s) of *Acacia ataxacantha*; which is a plant used extensively as herbal remedy in some parts of Nigeria and West Africa.

1.1 AIM AND OBJECTIVES OF THE RESEARCH

1.1.1 Aim of the Research

The aim of the research is to screen the root-bark of *Acacia ataxacantha* of the family Fabaceae for bioactive constituents, together with the structural elucidation of such constituents.

1.1.2 Objectives of the Research

- i. Collection, proper botanical identification, drying and pulverizing of the roots of the plant.

- ii. Extraction of the pulverized plant material using different solvents based on the eluotropic series i.e. from non-polar (petroleum ether 60-80°C) to polar (methanol).
- iii. Phytochemical screening for bio-active compounds using the crude extracts.
- iv. Antibacterial and Antifungal screening of the extracts.
- v. Analytical separations involving several consecutive steps of chromatographic techniques and purification.
- vi. Verification of the purity of the isolated compounds. Structural elucidation and characterization of the isolated compounds using available spectral techniques such as FTIR, ¹H NMR, ¹³C NMR and DEPT

1.2 JUSTIFICATION OF THE RESEARCH

The choice of *Acacia ataxacantha* as the plant of interest in this work is based on its medicinal importance among traditional medicine practitioners. Although oral evidence indicates that the plant is implicated in the treatment of chicken pox, headache, pneumonia, constipation, excessive cough, toothache, respiratory diseases, yellow fever and dysentery; and there is no documented evidence to support such uses. Therefore, there is need for a scientific validation of these claims.

1.3 SCOPE AND LIMITATION OF THE RESEARCH

The scope of this research work involved the following:

- i. Phytochemical screening.
- ii. Antibacterial/antifungal screening
- iii. Isolation
- iv. Characterization and structural elucidation. However, not all the active components were elucidated due to limited laboratory equipment.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 GENERAL BOTANICAL FEATURES OF THE FABACEAE FAMILY

The Fabaceae or Leguminosae, commonly known as the legume, pea, or bean family, are a large and economically important [family](#) of [flowering plants](#). It includes [trees](#), [shrubs](#), and [herbaceous plants](#) [perennials](#) or annuals, which are easily recognized by their [fruit \(legume\)](#) and their compound [stipulated](#) leaves. This family is widely distributed and is the third-largest [land plant](#) family in terms of number of species, behind only the [Orchidaceae](#) and [Asteraceae](#), with 630 genera and over 18,860 species (Stevens, 2001). The five largest of the 630 legume genera are [Astragalus](#) (over 2,000 species), [Acacia](#) (over 1000 species), [Indigofera](#) (around 700 species), [Crotalaria](#) (around 700 species) and [Mimosa](#) (around 500 species), which constitute about a quarter of all legume species. About 18,000 [legume species](#) are known, amounting to about 7% of flowering plant species. Fabaceae is the most common family found in tropical rain forest and in dry forests in the [Americas](#) and [Africa](#) (Magallon and Sanderson, 2001).

The Fabaceae have a wide variety of [growth forms](#) including trees, shrubs or herbaceous plants or even [vines](#) or [lianas](#). The herbaceous plants can be annuals, [biennials](#) or perennials, without basal or terminal leaf aggregations. They are upright plants, [epiphytes](#) or vines. The latter support themselves by means of shoots that twist around a support or through cauline or foliar [tendrils](#). Plants can be heliophytes, [mesophytes](#) or [xerophytes](#) (Watson and Dallwitz, 2007).

2.1.1 Taxonomic Position of Family Fabaceae/ Leguminosae:

Division: Angiospermae
Class: Dicotyledonae
Sub Class: Polypetalae
Series: Calyciflorae
Order: Rosales
Family: Fabaceae (Leguminosae)

2.1.2 Characteristic Features of Fabaceae Family (Common to all Sub- Families)

Habit: Legumes are trees (Acacia, Laburnum), shrubs (gorse, furze) and (majority) herbs (clovers, medicago). They may be runner, climbers or with twining stems.

Habitat: Contains about 690 genera and over 18,000 species. Found in varying habitats based on their sub family characteristics.

Roots: Roots contain nodules. Nitrogen fixing bacteria harbour at these roots in a symbiotic manner.

Leaves: compound, alternate, simple and pulvinate (swollen leaf base)

Inflorescence: Predominantly racemose type (that is, a cluster of stalked flowers are borne on a main stalk in an upward and arranged fashion; older flowers below and younger above)

Flowers: zygomorphic (irregular arrangement), hypogynous (ovary is above other floral parts) , heterochlamydeous (distinct calyx and corolla present), and pentamerous (floral members arrangement in fives)

Androecium: Stamens ten in number; monadelphous (stamens fused fully or partially into a tube), diadelphous (stamens are fused in two groups) or free

Gynoecium: Monocarpellary (single carpel), unilocular (single cavity) with marginal placentation (ovule placed at the side of the ovary).

Fruit: Legume or lomentum (Mittra, 2010).

The Fabaceae are placed in the order Fabales according to most taxonomic systems, including the Angiosperm Phylogeny Group (APG) III system (APG, 2009). The family includes three sub-families:

- i. **Mimosoideae:** 80 genera and 3,200 species. Mostly tropical and warm temperate Asia and America (*Mimosa*, *Acacia*)
- ii. **Caesalpinioideae:** 170 genera and 2,000 species, cosmopolitan. (*Caesalpinia*, *Senna*, *Bauhinia*, *Amherstia*)
- iii. **Faboideae (Papilionoideae):** 470 genera and 14,000 species, cosmopolitan. (*Astragalus*, *Lupinus*).

2.2 ACACIA

Also known as a thorn tree, [whistling thorn](#) or wattle, is the second largest genus in the Fabaceae/Leguminosae family, comprising more than 1,200 species worldwide, with members found in almost all habitats. Out of the 1,200 *Acacia* species, approximately 800 are found in Australia, 130 in Africa, 20 in India, a smaller number in Asia, and the remaining species in the New World (Ross, 1979). They are [pod](#)-bearing, with sap and leaves typically bearing large amounts of [tannins](#) and [condensed tannins](#) that historically in many species found use as pharmaceuticals and preservatives. The flowers are very fragrant and are often made in to an essential oil that is used in aromatherapy (Ratsch 1998).

Acacia trees are very tolerant of dry climates and can actually restore fertility to the soil where they grow. They enjoy sunny places and coarse, dry soil containing sand, gravel and rock dust. *Acacia* trees only require moderate watering, and are fungus-sensitive. They are very hardy and quite tolerant of both cold and heat (Ratsch 1998).

2.2.1 Species of the Genus *Acacia*

The genus *Acacia* comprises about 1200 species; the most common ones are listed below:

adunca, *aneura*, *aphylla*, *ashbyae*, *falcata*, *longifolia*, *angustifolia*, *macracantha*, *baileyana*, *calamifolia*, *catechu*, *coringera*, *maidenii*, *cunninghamii*, *myrtifolia*, *lunata*, *macracantha*, *obtusifolia*, *sieberiana*, *tortilis*, *pycnantha*, *horrida*, *retinodes*, *salicina*, *notabilis*, *pendula*, *confusa*, *dodonaeifolia*, *eburnea*, *erubescens*.

It has been reported that much care should be taken with the preparation and consumption of unknown species of *acacia*, as many contain poisonous cyanogenic glycosides and have been known to poison livestock (Dewick, 2009). Closely related species also often interbreed, which may make identification of the alkaloids present in the plant very challenging. Therefore, it is not recommended that one consume any part of an *Acacia* plant until the species is confirmed (Voogelbreinder 2009). Some *Acacia* species containing cyanogens include; [Acacia erioloba](#), *Acacia cunninghamii*, *Acacia obtusifolia*, *Acacia sieberiana* and *Acacia sieberiana var. woodii* (David and John, 1987).

2.2.2 Medicinal Uses of Some *Acacia* Species

Numerous *Acacia* species have been used for medicine and as entheogens, as well as for making incense.

In Mexico, the root of *Acacia angustifolia* is used as an additive to pulque, a fermented psychoactive [agave](#) beverage. The Aztecs called this small tree *ocpatl* – pulque drug. This beverage is thought to have interesting psychoactive effects (Ratsch, 1998).

The bark of *Acacia campylacantha* is used in West Africa as a psychoactive additive to a psychoactive beverage known as *dolo*, which is brewed from sorghum and pennisetum, as

well as honey. *Dolo* is consumed ceremonially as well as recreationally. The beverage is said to impart strength and lift the mood (Ratsch, 1998).

Acacia catechu is found in India, Indonesia and Malaysia. The inner wood is boiled in water to create an extract known as catechu, which is an odourless substance used both for tanning and as an additive to [betel quids](#). In India, *Acacia catechu* is used as a tonic for digestive ailments, to treat skin disorders, and to treat ulcers in the mouth, inflamed throats, and toothaches. *A. catechu* contains a great deal of tannins, and so is suitable for treating inflammations.

Traditionally, Chinese uses *Acacia confusa* as a medicine to treat blood disorders and as a muscle relaxant (Voogelbreinder 2009).

In India, the gum of *Acacia nilotica* is fried in ghee and taken as an aphrodisiac, and the tree is considered sacred and is not to be cut down. *Acacia nilotica* has been used in Sudan to treat a variety of different inflammatory disorders. The Masai use a decoction of the stem bark and root to acquire courage and as a stimulant.

The flower infusion of *Acacia farnesiana* is used as an aphrodisiac and muscle relaxant (Ratsch 1998). It is used in India to treat insanity, epilepsy, rabies and convulsions (Voogelbreinder 2009).

Acacia cornigeris is a South-American species used in the preparation of the Mayan ritual drink known as *balche*. The Maya of Belize uses the root and bark to treat snakebites, and as a tea to treat impotence. It is also used to treat asthma and headaches (Voogelbreinder 2009).

In Africa, *Acacia ataxacantha* root is combined with other herbs and used to treat wounds. The leaf is an analgesic. The Masai, who uses *Acacia ataxacantha* to stimulate themselves for battle or hunting prepare the plant by making a water infusion of the bark and roots and then

consume meat that has been cooked with an extract of the same plant. Milk is not to be consumed at the same time as this combination, in order to avoid illness. The Masai also occasionally chew the bark to produce stimulation and courage. The boiled leaves, shoots and seeds of many *acacia* trees are edible, and the roots can be tapped for water or used as medicine (Voogelbreinder 2009).

In Australia, a wide variety of native *Acacias* are used as food and to treat a number of illnesses. The leaves are also often burned as a “smoking medicine”, meaning that the smoke from the burning plant is inhaled to treat illness (Ratsch 1998).

There are a number of positive reports of using the leaves and bark of *Acacia phlebophylla*, an Australian species and *Acacia maidenii*, a species native to California, as a part of ayahuasca analogs (Voogelbreinder 2009).

2.2.3 Chemical Composition

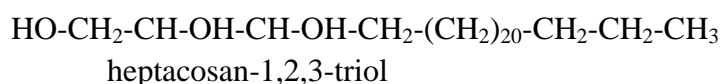
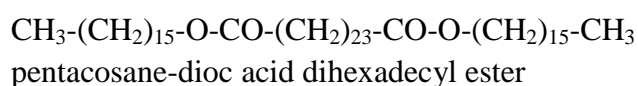
Many species of *Acacia*, particularly Australian ones, contain dimethyltryptamine (DMT) [I] and other tryptamines, and are therefore suitable as ingredients in ayahuasca analogues (Ratsch 1998).

Watson and Dallwitz (2007) reported that the Fabaceae are rarely cyanogenic however, where they are, the cyanogenic compounds are derived from [tyrosine](#), [phenylalanine](#) or leucine. They frequently contain alkaloids. Proanthocyanidins can be present either as cyanidin or delphinidine or both at the same time. Flavonoids such as kaempferol[3,5,7-Trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one][II], quercetin[2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one][III] and myricetin [3,5,7-Trihydroxy-2-(3,4,5-trihydroxyphenyl)-4-chromenone][IV] are often present.

Ratsch (1998) reported that the leaves of *Acacia campylacantha* contain *N, N*-dimethyltryptamine (*N, N*-DMT) [I] and other tryptamines.

Chang *et al.*, (2001) reported that the crude extracts of *Acacia confusa* bark not only contain a wide variety of phenolic compounds but also show an excellent antioxidant activity. Eleven phenolic compounds were isolated and identified as 3,4- dihydroxybenzoic acid; 3-hydroxy-4-methoxybenzoic acid; 3,4-dihydroxybenzoic acid methyl ester; 3,4-dihydroxybenzoic acid ethyl ester; 4-hydroxybenzoic acid ethyl ester; 4-hydroxybenzoic acid; 4-hydroxy-3,5-dimethoxybenzoic acid; 4-hydroxy-3,5-dimethoxybenzoic acid ethyl ester; 3,4-dihydroxy-*trans*-cinnamic acid ethyl ester; 3,4-dihydroxy-*trans*cinnamic acid pentyl ester; and 4-hydroxy-3-methoxybenzoic acid (Chang *et al.*, 2007).

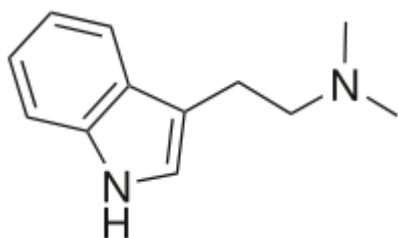
Banso (2009) reported that the phytochemical screening of the stem bark of *Acacia nilotica* revealed the presence of terpenoids, alkaloids, saponins and glycosides. The compounds pentacosane-dioic acid dihexadecyl ester (C₅₇H₁₁₂O₄) and heptacosan-1, 2, 3-triol (C₂₇H₅₆O₃) were isolated from the plant (Deshpande *et al.*, 2006).



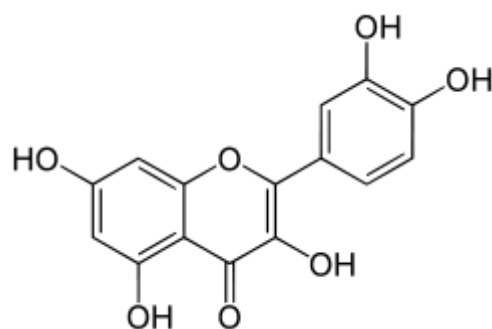
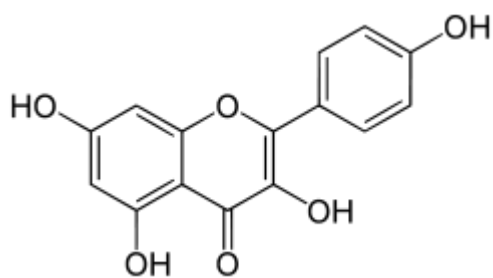
Vagias *et al.*, 2007 reported that the pentacyclic triterpenoids: (20*R*)-3-oxolupan-30-al (**1**), (20*S*)-3- oxolupan-30-al (**2**) and (20*R*)-28-hydroxylupen-30-al-3-one(**3**), along with (20*S*)-3β-hydroxylupan-30-al (**4**),[**V**] were isolated from *Acacia mellifera*. In addition, the known metabolites 30-hydroxylup-20-(29)-en-3-one (**5**), 30-hydroxylup-20-(29)-en-3β-ol (**6**), atranorin, methyl 2, 4-dihydroxy-3,6 dimethyl benzoate, sitosterol-3β-O-glucoside and linoleic acid were found in the analyzed plant species.

Imperato (1982) isolated a new yellow pigment from the flowers of *Acacia dealbata* and was shown to be chalcononaringenin 2'-[O-rhamnosyl-(1→4)-xyloside]; a chalcone

glycoside[VI]. Four lupine -type triterpenes were isolated from the leaves, flowers and seeds, these are lupenone (Lup-2O (29)-en-3-one) (1), lupeol (Lup-2O (29)-en-3 β -ol) (2), lupenyl palpimate (3) and lupenyl cinnamate (4) (Pereira *et al.*, 1996).

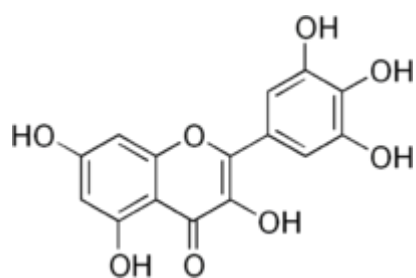


[I] *N,N*-Dimethyltryptamine (DMT or *N,N*-DMT)



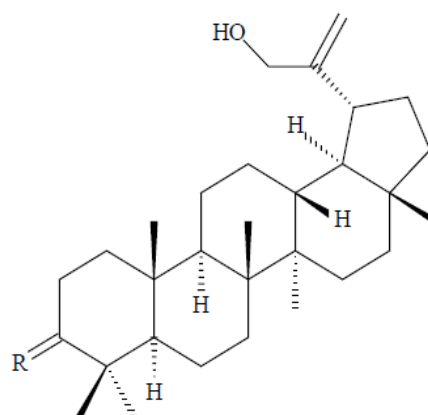
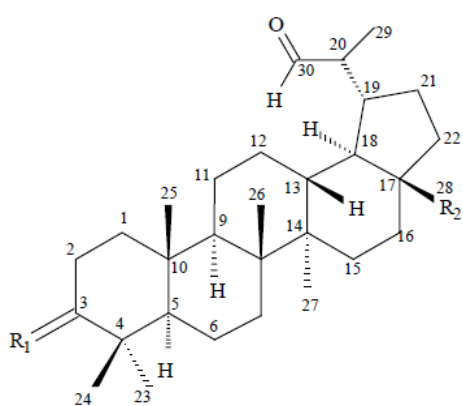
[II]

I Kaempferol



III Quercetin

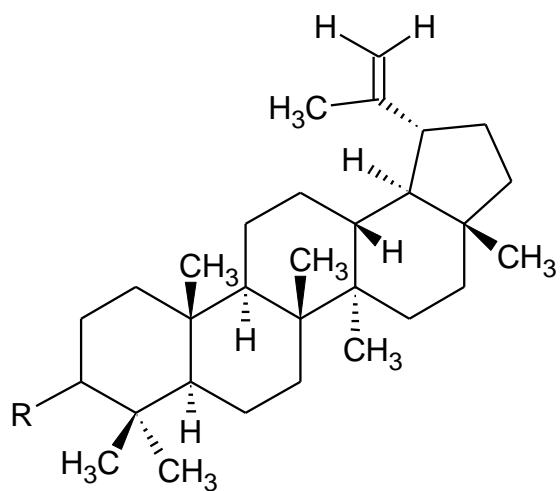
IV Myricetin



	R ₁	R ₂	C-20 configuration
1	O	CH ₃	20R
2	O	CH ₃	20S
3	O	CH ₂ OH	20R
4	-β OH	CH ₃	20S

	R
5	O
6	-β OH

V Isolated lupane triterpenes from *A. mellifera*.



Comp. No.	R
1	= O
2	β -OH
3	$\beta\text{-O}-\overset{\overset{1'}{\text{O}}}{\underset{\underset{\text{O}}{\parallel}}{\text{C}}}-\overset{2'}{\text{CH}_2}-\overset{7'}{(\text{CH}_2)_{14}\text{CH}_3}$
4	

[VI] Isolated triterpenes from *Acaciadealbata*

STRUCTURES OF COMPOUNDS ISOLATED FROM SOME ACACIA SPECIES

2.3 ACACIA ATAXACANTHA DC

Common Names:

English - Flame thorn, Benin rope acacia, [whistling thorn](#) or wattle

Yoruba - *Epinpin, Ewon*

Igbo - *Uke*

Hausa - *Bagaruwar kasa, Sarƙaƙiyaa or Kwiiyaa* (depending on the geographical area)

Others include *Bosoni* (Guinea), *Ango-ka* (Ghana), *Wonje* (Sierra Leone) and *Acharam* (Senegal) (Burkill, 1985).

Botanical Name - *Acacia ataxacantha* DC

2.3.1 Scientific Classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliatae or Rosidae

Order: Fabales

Family: Fabaceae

Sub-family: Mimosoideae

Genus: *Acacia*

Species: *Ataxacantha*

Binomial Name: *Acacia ataxacantha* [DC](#)

2.3.2 Botanical Description of *Acacia ataxacantha* [DC](#)

Acacia ataxacantha (flame thorn) is a common, widespread tree occurring in subtropical Africa from Senegal in the west to Sudan in the north-east, extending southwards into Namibia and South Africa. It may be found through much of the former Transvaal province, KwaZulu-Natal and Swaziland. In drier areas it is usually confined to water courses and ravines, but in higher rainfall areas it may be encountered as a normal bush constituent or even forest margin situations. The flame thorn often forms impenetrable thickets - particularly in disturbed areas; it is fairly untidy, many-stemmed shrub up to 3-5 m in height, often scrambling. It may on occasion form a small tree with a stem diameter of up to 20-30 cm in diameter. The bark is greyish, sometimes with a brownish tinge, and is fissured longitudinally, often with coarse flaking. Young stems are fairly smooth with longitudinal striations on which numerous unpaired, hooked prickles up to 8 mm are borne. Small prickles are also found on the underside of the leaf axis. The large, fairly droopy, compound leaves are comprised of many tiny leaflets. The foliage is generally dark green and fairly dense, with the new growth often purple-tinged. The leaf stalk is hairy and bears a distinctive stalked gland (Turner, 2001).

2.3.3 Products and Uses

The plant is grown as a hedge, and to make an impenetrable stockade around villages. Suitably cited with ditches and ingress and egress passages, it becomes a formidable defensive barrier - it is good for cattle enclosures (Burkill, 1985). The thorns are used as fish hooks, the stems are cut into walking sticks and bows; the bark is free of gum but contains a

very tough fibre good for making rope; the strongest kind used in Southern part of Nigeria. The foliage is recorded as being uneaten by horses but that it is greatly relished by other livestock (Burkill, 1985).

2.3.4 Medicinal Uses of *Acacia ataxacantha*

The stem-bark sap is used for treating chicken pox (Oladunmoye and Kehinde, 2011); the root is used for treating headache, bleeding and pneumonia, and used by some Shona traditional healers in Zimbabwe against constipation (Cheikhyoussef *et al.*, 2011). The bark is dissolved in boiling water and the filtrate taken for pneumonia (Hedimbi and Chinsebu, 2012); whole plant is used for treating excessive cough, and yellow fever. (Dambatta and Aliyu, 2011); the young leaves are taken for dysentery and backache (Kadiri *et al.*, 2008). It is used medicinally against syphilis, boils, helminthiasis, wound treatment, headache, toothache, respiratory diseases. The Wolof and Serer of Senegal take the powdered leaf, with various other drug plants, for syphilis. In Ubangi the dried and powdered leaf is applied to chancres [syphilitic] on the penis. Bark-infusion is prepared in the Soudano-guinean region as a mouth wash for carious and aching teeth, the mouth being rinsed out with copious quantities to relieve the pain; the leaf is used in fumigations for maladies of the respiratory tract, especially when accompanied by chest-pains (Burkill, 1985). An aqueous macerate of the root in association with *Capparis tomentosa* (Capparaceae) and *Securidaca longipedunculata* (Polygalaceae) is taken in draughts and for embrocation by Fula in Senegal for hernia, helminthiasis, sores and wounds (Burkill, 1985). This finding suggests that *Acacia ataxacantha* may have antibacterial properties.

2.4 INCREASING POPULARITY OF MEDICINAL PLANTS

The use of [herbs](#) to treat [disease](#) is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals. The [World](#)

[Health Organization](#) (WHO) estimates that 80 percent of the populations of some Asian and African countries presently use herbal medicine for some aspect of primary health care. Studies in the United States and Europe have shown that their use is less common in clinical settings, but has become increasingly more in recent years as scientific evidence about the effectiveness of herbal medicine has become more widely available. The annual global export value of pharmaceutical plants in 2011 accounted for over US \$2.2 billion. (<http://www.traffic.org/medicinal-plants/>).

Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects. (Lai and Roy, 2004; Tapsell *et al.*, 2006). The high costs of western pharmaceuticals put modern health care services out of reach of most of the world's population, which relies on traditional medicine and medicinal plants to meet their primary health care needs. Even where modern medical care is available and affordable, many people prefer more traditional practices. This is particularly true for First Nations and Immigrant populations, who have tended to retain ethnic medical practices. (Duke, 1993; Cox and Balick 1994).

In the last decade, there has been considerable interest in resurrecting medicinal plants in western medicine, and integrating their use into modern medical systems. The reasons for this interest are varied, and include:

- i. **low cost:** herbals are relatively inexpensive and the cost of pharmaceuticals to governments and individuals is rising
- ii. **drug resistance:** the need for alternative treatments for drug-resistant pathogens

- iii. **limitations of medicine:** the existence of ailments without an effective pharmaceutical treatment
- iv. **medicinal value:** laboratory and clinical corroboration of safety and efficacy for a growing number of medicinal plants
- v. **cultural exchange:** expanding contact and growing respect for foreign cultures, including alternative systems of medicine
- vi. **commercial value:** growing appreciation of trade and other commercial economic opportunities represented by medicinal plants

However, the pace of re-adopting the use of traditional medicinal plants is by no means uniform in western medicine (Duke 1993, Cox and Balick, 1994).

2.5 PLANT CONSTITUENTS OF PHARMACOLOGICAL IMPORTANCE

All plants produce chemical compounds as part of their normal metabolic activities. These phytochemicals are divided into (1) primary metabolites such as sugars and fats, which are found in all plants; and (2) secondary metabolites—compounds which are found in a smaller range of plants, serving a more specific function (Meskin, 2002). The medicinal qualities of plants are due to these chemicals. Primary metabolites are critical to their existence. These includes compounds that are necessary for cellular processes such as amino acids, nucleic acids, lipids and simple sugars that serve a variety of purposes indispensable for sustenance and reproduction, not only for the plants themselves, but also for animals that feed on them (Cseke *et al.*, 2006). Secondary metabolites are also additional components synthesized by plants. These include compounds that are produced in response to stress that is induced by abiotic (e.g. heat, drought) and biotic (e.g. herbivores, pathogens, humans) factors on the plant (Keeling and Bohlmann, 2006). Many secondary metabolites are "antibiotic" in a broad sense, protecting the plants against fungi, bacteria, animals, and even other plants. Some

secondary metabolites are toxins used to deter predation and others are pheromones used to attract insects for pollination. It is these secondary metabolites and pigments that can have therapeutic actions in humans and which can be refined to produce drugs (Meskin, 2002).

Often, secondary metabolites are referred to as natural products, as these compounds exhibit effects on other organisms; some of these compounds have been reported as exhibiting curative effects on the human body (Zwenger and Basu, 2008; Njoku and Obi, 2009).

Secondary metabolites produced by higher eukaryotes such as plants are highly toxic (Rasoanaivo, *et al.*, 2011). These toxins are said to be stored in specific vesicles or in the vacuole of the plant; this kind of storage functions have been found as a detoxification of the plant itself and generates a reservoir of, for example, nitrogen-rich molecules (Roze *et al.*, 2011). Even though some secondary plant products are very common, not every plant can produce every product. Some secondary metabolites are restricted to single species, others to related groups, but they are nearly always found only in certain specific plant organs, also they are often generated only during a specific developmental period of the plant (Sawada *et al.*, 2009; Mounet *et al.*, 2009).

Every plant species contains chemicals that can affect some animals or micro-organisms negatively, strongly supporting the interpretation that secondary metabolites play a vital role in combating diseases and herbivores. Plants have been a rich source of medicines because they produce a host of bioactive molecules, most of which probably evolved as chemical defences against predation or infection (Cox and Balick 1994).

Most animals, including humans, have adapted over millions of years to a regular diet of plants. Consequently, the human system is adapted to a regular intake of plant constituents. Essential dietary constituents of plants are reasonably well understood, but the possible therapeutic role of most components of plants is not. Plants synthesize a bewildering variety

of [phytochemicals](#) but most are derivatives of a few biochemical motifs (Springbob and Toni, 2009)

2.5.1 Alkaloids

Alkaloids are a group of naturally occurring [chemical compounds](#) that contain mostly one [basic nitrogen](#) atom (in a ring). This group also includes some related compounds with neutral and even weakly [acidic](#) properties. Some synthetic compounds of similar structure are also attributed to alkaloids. In addition to [carbon](#), [hydrogen](#) and [nitrogen](#), alkaloids may also contain [oxygen](#), [sulfur](#) and more rarely other elements such as [chlorine](#), [bromine](#), and [phosphorus](#).

Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals, and are part of the group of natural products (also called secondary metabolites). Many alkaloids can be purified from crude extracts by acid-base extraction. Alkaloids are significant sources of pharmaceutical drugs. More than 12,000-alkaloids are known to exist in green flora and only few have been exploited for medicinal purposes (Amritpal, 2011). They are used as recreational drugs, or in entheogenic rituals. Examples are the local anesthetic and stimulant cocaine; the psychedelic psilocin; the stimulant caffeine; nicotine; the analgesic morphine; the antibacterial berberine; the anticancer compound vincristine; the antihypertension agent reserpine; the cholinomimetic galatamine; the spasmolysis agent atropine; the vasodilator vincamine; the anti-arrhythmia compound quinidine; the anti-asthma therapeutic ephedrine; and the antimalarial drug quinine. Although alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly invoke a bitter taste (Joseph, 2002).

Alkaloids are [generated](#) by various living organisms, especially by [higher plants](#) – about 10 to 25% of those contain alkaloids. Therefore, in the past the term "alkaloid" was associated with

plants. The alkaloids content in plants is usually within a few percent and is inhomogeneous over the plant tissues. Depending on the type of plants, the maximum concentration is observed in the leaves ([black henbane](#)), [fruits](#) or [seeds](#) ([Strychnine tree](#)), root ([Rauwolfia serpentina](#)) or bark ([cinchona](#)). Furthermore, different tissues of the same plants may contain different alkaloids (Herbert, 1999).

Beside plants, alkaloids are found in certain types of [fungi](#), such as [psilocybin](#) in the fungus of the genus [Psilocybe](#), and in animals, such as [bufotenin](#) in the skin of some toads. Many marine organisms also contain alkaloids. Some [amines](#), such as [adrenaline](#) and [serotonin](#), which play an important role in higher animals, are similar to alkaloids in their structure and biosynthesis and are sometimes called alkaloids. The role of alkaloids for living organisms that produce them is still unclear. It was initially assumed that the alkaloids are the final products of [nitrogenmetabolism](#) in plants, as [urea](#) in mammals. It was later shown that alkaloid concentrations vary over time, and this hypothesis was refuted (Akanda, 2012).

Most of the known functions of alkaloids are related to protection. For example, [aporphine](#) alkaloid [liriodenine](#) produced by the [tulip tree](#) protects it from parasitic mushrooms. In addition, presence of alkaloids in the plant prevents insects and [chordate](#) animals from eating it. However, some animals adapted to alkaloids and even use them in their own metabolism. Such alkaloid-related substances as [serotonin](#), [dopamine](#) and [histamine](#) are important [neurotransmitters](#) in animals. Alkaloids are also known to regulate plant growth (Akanda, 2012).

2.5.2 Flavonoids

Flavonoids are a class of [plantsecondary metabolites](#). They are water-soluble polyphenolic molecules containing 15 carbon atoms. Flavanoids can be visualized as two benzene rings which are joined together with a short three carbon chain. One of the carbons of the short

chain is always connected to a carbon of one of the benzene rings, either directly or through an oxygen bridge, thereby forming a third middle ring, which can be five or six-membered. Chemically, they have the general structure of a 15-carbon skeleton, which consists of two phenyl rings (A and B) and heterocyclic ring (C) (McNaught and Wilkinson, 1997).

The flavonoids consist of 6 major subgroups: chalcone, flavone, flavonol, flavanone, anthocyanins and isoflavonoids. Together with carotenes, flavonoids are also responsible for the coloring of fruits, vegetables and herbs.

According to the [IUPAC](#) nomenclature, they can be classified into:

- i. *flavonoids* or *bioflavonoids*, derived from 2-phenylchromen-4-one (2-phenyl-1,4-benzopyrone) structure
- ii. *isoflavonoids*, derived from 3-phenylchromen-4-one (3-phenyl-1,4-benzopyrone) structure
- iii. *neoflavonoids*, derived from 4-phenylcoumarine (4-phenyl-1,2-benzopyrone) structure

These three flavonoid classes are all [ketone](#)-containing compounds, and as such, are [anthoxanthins](#) ([flavones](#) and [flavonols](#)). This class was the first to be termed bioflavonoids (Akanda, 2012).

Flavonoids are widely distributed in plants, fulfilling many functions. Flavonoids are the most important [plant pigments](#) for flower coloration, producing yellow or red/blue pigmentation in petals designed to attract [pollinator](#) animals.

In higher plants, flavonoids are involved in UV filtration, symbiotic nitrogen fixation and floral pigmentation (Galeotti *et al.*, 2008).

Flavonoids have been shown to have a wide range of biological and pharmacological activities in [in vitro](#) studies. Examples include anti-[allergic](#),(Yamamoto and Gaynor, 2001)

[anti-inflammatory](#) (Yamamoto and Gaynor, 2001; Cazarolli *et al.*, 2008), [antioxidant](#) and anti-[cancer](#), (Cazarolli *et al.*, 2008), [anti-microbial](#) [[antibacterial](#)],(Cushnie and Lamb, 2011; Manner *et al.*, 2013), [antifungal](#), and [antiviral](#) (Cushnie and Lamb, 2005; Friedman, 2007)], and anti-[diarrheal](#) activities

2.5.3 Steroids

Steroids are compounds possessing the skeleton of cyclopentanophenanthrene[VII] or a skeleton derived therefrom by one or more bond scissions or ring expansions or contractions.

A steroid is a type of [organic compound](#) that contains a characteristic arrangement of four [cycloalkane](#) rings joined to one another. The steroid core is composed of seventeen [carbon](#) atoms bonded together in the form of four fused rings: three [cyclohexane](#) rings and one [cyclopentane](#) ring. Individual steroids vary, first and primarily, by the [oxidation state](#) of the carbon atoms in the rings and by the chains and functional groups attached to this four-ring system; second, steroids can vary more markedly via changes to the ring structure. [Sterols](#) are a particularly important form of steroids, with sterols having a [cholestane](#)-derived framework and a [hydroxyl](#) group at the C-3 ring position being the most prominent. Hundreds of distinct steroids are found in [animals](#), [fungi](#), [plants](#), and elsewhere. All [natural](#) steroids are made in living cells, either from the sterol [lanosterol](#) (animals and fungi) or from [cycloartenol](#) (plants) (Moss 1989).

2.5.4 Glycosides

A glycoside is a molecule in which a sugar group is bonded through its [anomeric carbon](#) to another group (a non-carbohydrate moiety, usually a small organic molecule) via a [glycosidic bond](#). Glycosides can be linked by an O- (an O-glycoside), N- (a [glycosylamine](#)), S-(a thioglycoside), or C- (a C-glycoside) glycosidic bond. The sugar group is known as the

glycone and the non-sugar group as the [aglycone](#) or genin part of the glycoside. The glycone can consist of a single sugar group ([monosaccharide](#)) or several sugar groups ([oligosaccharide](#)). Glycosides play numerous important roles in living organisms; many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis, which causes the sugar part to be broken off, making the chemical available for use. Many such plant glycosides are used as medications (Srivastava and Lambert, 1996).

In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body. An example is the cyanoglycosides in cherry pits that release toxins only when bitten by a herbivore.

2.5.5 Saponins

Saponins [VIII] are glucosides with foaming characteristics. They consist of polycyclic aglycones attached to one or more sugar side chains. The aglycone part, also called sapogenin is either a steroid (C₂₇) or a triterpene (C₃₀). Their foaming abilities are caused by the combination of a hydrophobic (fat soluble) sapogenin and a hydrophilic (water soluble) sugar part. Saponins are a class of chemical compounds found in particular abundance in various plant species. They are [amphipathic glycosides](#) grouped [phenomenologically](#) by the soap-like foaming they produce when shaken in [aqueous](#) solutions, and structurally by having one or more [hydrophilic](#) glycoside moieties combined with a [lipophilic triterpene](#) derivative. Saponins are natural glycosides which possess a wide range of pharmacological properties including cytotoxic activity. They exert a wide range of pharmacological activities including expectorant, anti-inflammatory, vasoprotective, hypocholesterolemic, immunomodulatory, hypoglycaemic, molluscicidal, antifungal, antiparasitic, hyperglycaemia, anti-oxidant, anti-cancer, weight loss and serve as natural antibiotics (Podolak *et al.*, 2010).

2.5.6 Tannins

Tannins are [polyphenolic](#) compounds that binds to and [precipitates proteins](#) and various other organic compounds including [amino acids](#) and [alkaloids](#). They are large [polyphenolic](#) compounds containing sufficient [hydroxyls](#) and other suitable groups (such as [carboxyls](#)) to form strong complexes with various [macromolecules](#). The tannins are widely distributed in many species of plants, where they play a role in protection from predation, and also as pesticides, and in plant growth regulation. The [astringency](#) from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripened fruit or red wine. Tannins have [molecular weights](#) ranging from 500 to over 3,000 ([gallic acid esters](#)) and up to 20,000 ([proanthocyanidins](#)).

Tannins are the most abundant secondary metabolites made by plants, commonly ranging from 5% to 10% dry weight of tree leaves. Tannins can defend leaves against insect herbivores by deterrence and/or toxicity. Contrary to early theories, tannins have no effect on protein digestion in insect herbivores. By contrast, in vertebrate herbivores tannins can decrease protein digestion (Barbehenn and Peter, 2011). Tannins are found in leaf, bud, seed, root, and stem tissues. An example of the location of the tannins in stem tissue is that they are often found in the growth areas of trees, such as the secondary phloem and xylem and the layer between the cortex and epidermis. Tannins may help regulate the growth of these tissues. When incubated with red grape juice and [red wines](#) with a high content of condensed tannins, the poliovirus, [herpes simplex virus](#), and various enteric viruses are inactivated. In tissue-cultured cell assays tannins have shown antiviral, antibacterial and antiparasitic effects (Akiyama *et al.*, 2001). Tannins are used as antiseptic and this activity is due to presence of the phenolic group. Tannin-rich medicinal plants are used as healing agents in a number of diseases. In Ayurveda, formulations based on tannin-rich plants have been used for the treatment of diseases like leucorrhoea, rhinorrhoea and diarrhea (Amritpal, 2011).

The anti-inflammatory effect of tannins help control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders Tannins have been used for immediate relief of sore throats, diarrhea, dysentery, hemorrhaging, fatigue, skin ulcers and as a cicatrizant on gangrenous wounds. Tannins not only heal burns and stop bleeding, but they also stop infection while they continue to heal the wound internally. The ability of tannins to form a protective layer over the exposed tissue keeps the wound from being infected even more (Akanda, 2012).

2.5.7 Terpenes

Terpenes are a large and diverse class of organic compounds, produced by a variety of plants, particularly conifers, which are often strong smelling and thus may have had a protective function. They are the major components of resin, and of turpentine produced from resin. (The name "terpene" is derived from the word "turpentine"). They are classified as hemiterpene (C₅), monoterpene (C₁₀), sesquiterpene (C₁₅), diterpene (C₂₀), sesterterpenes: C₂₅ (rare), triterpenes: C₃₀ and Carotenoids: C₄₀. Terpenes are major biosynthetic building blocks within nearly every living creature. Steroids, for example, are derivatives of the triterpene squalene. When terpenes are modified chemically, such as by oxidation or rearrangement of the carbon skeleton, the resulting compounds are generally referred to as *terpenoids* (sometimes called isoprenoids). Terpenes and terpenoids are the primary constituents of the essential oils of many types of plants and flowers. Essential oils are used widely as natural flavor additives for food, as fragrances in perfumery, and in traditional and alternative medicines such as aromatherapy. Synthetic variations and derivatives of natural terpenes and terpenoids also greatly expand the variety of aromas used in perfumery and

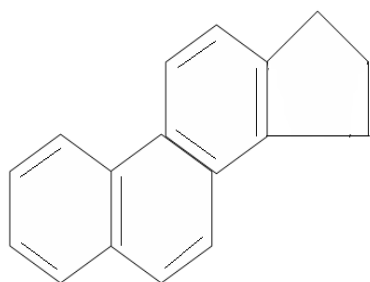
flavors used in food additives. Vitamin A is an example of a terpene. The fragrances of rose and lavender are due to monoterpenes. The carotenoids produce the reds, yellows and oranges of pumpkin, corn and tomatoes. The function of terpene is generally considered to be both ecological and physiological (Amritpal, 2011; Cseke *et al.*, 2006). They play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic, and other pharmaceutical functions (Akanda, 2012)

2.5.8 Carbohydrates

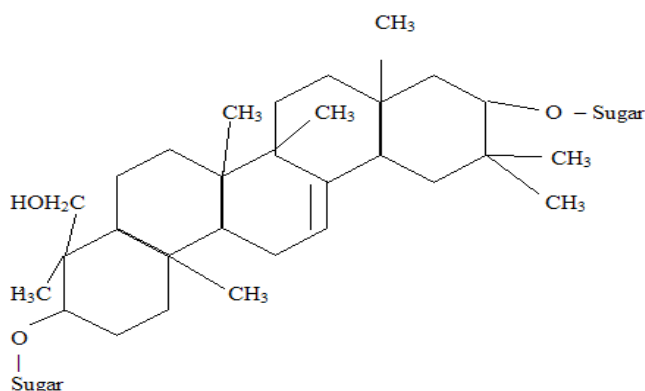
A carbohydrate is a large biological molecule, or macromolecule, consisting of carbon (C), hydrogen (H), and oxygen (O) atoms, usually with a hydrogen:oxygen atom ratio of 2:1 (as in water); in other words, with the empirical formula $C_m(H_2O)_n$ (where m could be different from n). Carbohydrates are technically hydrates of carbon; structurally it is more accurate to view them as polyhydroxy aldehydes and ketones. The term is most common in biochemistry, where it is a synonym of saccharide (Flitsch and Ulijn, 2003).

The carbohydrates (saccharides) are divided into four chemical groups: monosaccharides, disaccharides, oligosaccharides, and polysaccharides. In general, the monosaccharides and disaccharides, which are smaller (lower molecular weight) carbohydrates, are commonly referred to as sugars. While the scientific nomenclature of carbohydrates is complex, the names of the monosaccharides and disaccharides very often end in the suffix -ose. For example, grape sugar is the monosaccharide glucose, cane sugar is the disaccharide sucrose, and milk sugar is the disaccharide lactose. Carbohydrates are the most abundant class of organic compounds found in living organisms. They originate as products of photosynthesis, an endothermic reductive condensation of carbondioxide requiring light energy and the pigment chlorophyll.

Carbohydrates perform numerous roles in living organisms. Polysaccharides serve for the storage of [energy](#) (e.g., [starch](#) and [glycogen](#)), and as structural components (e.g., [cellulose](#) in plants and [chitin](#) in arthropods). The 5-carbon monosaccharide [ribose](#) is an important component of coenzymes such as adenosine triphosphate [ATP](#), flavin adenine dinucleotide [FAD](#), and Nicotinamide adenine dinucleotide [NAD](#); and the backbone of the genetic molecule known as ribonucleic acid, [RNA](#). The related deoxyribose is a component of deoxyribonucleic acid, DNA. Saccharides and their derivatives include many other important biomolecules that play key roles in the [immune system](#), fertilization, preventing [pathogenesis](#), blood clotting, and [development](#) (Flitsch and Ulijn, 2003).



[VII] Cyclopentanophenanthrene



[VIII] Saponin

2.6 TEST MICRO-ORGANISMS

Bacillus subtilis - known also as the hay bacillus or grass bacillus, is a [Gram-positive](#), [catalase-positive bacterium](#). *B. subtilis* is only known to cause disease in severely immune-compromised patients, and can conversely be used as a [probiotic](#) in healthy individuals. It causes food poisoning, fatal pneumonia and bacteraemia, dermatitis, respiratory ailments, spoilage in the food industry and has the ability to liquefy gelatin, fibrin or coagulated serum to produce indole or H₂S and to digest milk and meat. *B. subtilis* has proven highly amenable to genetic manipulation, and has become widely adopted as a model organism for laboratory studies, especially of sporulation, which is a simplified example of [cellular differentiation](#) (Logan, 1988).

Candida albicans- *Candida* species are among the most frequently isolated microorganisms in clinical microbiology laboratories and are becoming increasingly important, especially as causes of opportunistic and hospital-acquired infections. *Candida albicans* is a [diploid fungus](#) that grows both as [yeast](#) and [filamentous](#) cells and a causal agent of [opportunistic oral](#) and

[genital](#) infections in humans, and [candidal onychomycosis](#), an infection of the [nail plate](#). Systemic fungal infections ([fungemias](#)) including those by *C. albicans* have emerged as important causes of [morbidity](#) and [mortality](#) in [immunocompromised](#) patients. *C. albicans* is [commensal](#) and a constituent of the normal [gut flora](#) comprising microorganisms that live in the human mouth and [gastrointestinal tract](#). *C. albicans* lives in 80% of the human population without causing harmful effects, although overgrowth of the fungus results in [candidiasis](#) (Aboelli and Al-Tuwaijri, 2010).

Escherichia coli (commonly abbreviated *E. coli*) is a Gram-negative, facultative anaerobic, [rod-shaped bacterium](#) of the genus [Escherichia](#) that is commonly found in the lower [intestine](#) of [warm-blooded](#) organisms (endotherms). Most *E. coli* [strains](#) are harmless, but some [serotypes](#) can cause serious [food poisoning](#) in their hosts, and are occasionally responsible for [product recalls](#) due to [food contamination](#). The harmless strains are part of the [normal flora](#) of the [gut](#), and can benefit their hosts by producing [vitamin K₂](#), and preventing colonization of the intestine with [pathogenic](#) bacteria. The bacterium can be grown easily and inexpensively in a laboratory setting, and has been intensively investigated for over 60 years. *E. coli* is the most widely studied [prokaryotic model organism](#), and an important species in the fields of [biotechnology](#) and [microbiology](#), where it has served as the [host organism](#) for the majority of work with [recombinant DNA](#). Under favourable conditions it takes only 20 minutes to reproduce. Infections include enteric/diarrhoeal disease, urinary tract infections (UTIs) and sepsis/meningitis (Kaper *et al.*, 2004).

Klebsiella pneumoniae is a [Gram-negative](#), non-motile, [encapsulated](#), [lactose-fermenting](#), [facultative anaerobic](#), rod-shaped [bacterium](#). In the clinical setting, it is the most significant member of the [Klebsiellagenus](#) of [Enterobacteriaceae](#). Although it is found in the normal flora of the mouth, skin, and intestines, it can cause destructive changes to human lungs if aspirated. In addition to pneumonia, *Klebsiella* can also cause infections in the [urinary](#) tract,

lower [biliary](#) tract, and surgical wound sites. The range of clinical diseases includes pneumonia, [thrombophlebitis](#), [urinary tract infection](#), liver abscess complicated by endophthalmitis, [cholecystitis](#), [diarrhea](#), upper [respiratory](#) tract infection, wound infection, [osteomyelitis](#), [meningitis](#), bacteremia and [septicemia](#). *K. pneumoniae* has been reported as one of the most common Gram-negative pathogens, after *P. aeruginosa* (Vuotto *et al.*, 2014).

Pseudomonas aeruginosa - It is a [Gram-negative](#), [aerobic](#), [coccobacillus bacterium](#) with [unipolar motility](#). It is the [type species](#) of the genus *Pseudomonas* (Migula). It is citrate, catalase, and oxidase positive. It is found in soil, water, [skin flora](#), and most man-made environments throughout the world. It thrives not only in normal atmospheres, but also in [hypoxic](#) atmospheres, and has, thus, colonized many natural and artificial environments. An [opportunistic](#), [nosocomial](#) pathogen of [immunocompromised](#) individuals, *P. aeruginosa* typically infects the pulmonary tract, [urinary tract](#), gastrointestinal tract, [burns](#), [wounds](#), and also causes pneumonia and other [blood infections](#) (Gellatly and Hancock, 2013).

Salmonella is a [genus](#) of rod-shaped, [gram-negative bacteria](#). There are only two species of *Salmonella*, [Salmonella bongori](#) and [Salmonella enterica](#). *Salmonella* species are found worldwide in both cold-blooded and warm-blooded animals, and in the environment. They are facultative [intracellular pathogens](#). Many infections are due to ingestion of contaminated food. They can be divided into two groups—typhoidal and nontyphoidal *Salmonella* serovars. Nontyphoidal serovars are more common, and usually cause self-limiting gastrointestinal disease. They can infect a range of animals, and are [zoonotic](#), (i.e. they can be transferred between humans and other animals). Typhoidal serovars include *Salmonella typhi* and *Salmonella paratyphi* A, which are adapted to humans and do not occur in other animals. *Salmonella typhi* causes illnesses such as typhoid fever, [food poisoning](#), muscle pains, diarrhoea with abdominal pains, cough and sore throat.

Staphylococcus aureus is a [Gram-positive coccobacterium](#) that is a member of the [Firmicutes](#), and is frequently found in the human respiratory tract and on the skin. *S. aureus* can cause a range of illnesses, from minor skin [infections](#), such as [pimples](#), [impetigo](#), [boils](#) (furuncles), [cellulitis](#) folliculitis, [carbuncles](#), [scalded skin syndrome](#), and [abscesses](#), to life-threatening diseases such as [meningitis](#), [osteomyelitis](#), [endocarditis](#), [toxic shock syndrome](#) (TSS) and [sepsis](#). *Staphylococcus aureus* is the leading cause of hospital-acquired infections. It is the primary cause of lower respiratory tract infections and surgical site infections and the second leading cause of nosocomial bacteremia, pneumonia, and cardiovascular infections. Its incidence ranges from skin, soft tissue, respiratory, bone, joint, endovascular to [wound infections](#). Infections with *S. aureus* are especially difficult to treat because of evolved resistance to antimicrobial drugs (Klein *et al.*, 2007).

Streptococcus pneumoniae, or pneumococcus, is a [Gram-positive](#), [alpha-hemolytic](#), [aerotolerant](#), [aerobic](#) member of the [genus Streptococcus](#). A significant human [pathogenic bacterium](#), *S. pneumoniae* was recognized as a major cause of [pneumonia](#) in the late 19th century, and is the subject of many [humoral immunity](#) studies. Despite the name, the organism causes many types of [pneumococcal infections](#) other than [pneumonia](#). These invasive pneumococcal diseases include [bronchitis](#), [rhinitis](#), [acute sinusitis](#), [otitis media](#), [conjunctivitis](#), [meningitis](#), [bacteremia](#), [sepsis](#), [osteomyelitis](#), [septic arthritis](#), [endocarditis](#), [peritonitis](#), [pericarditis](#), [cellulitis](#), and [brain abscess](#) (CDC, 1996).

Streptococcus pyogenes is a [spherical](#), [Gram-positive bacterium](#) that is the cause of [Group A streptococcal infections](#). [Streptococci](#) are [catalase](#)-negative. It is an infrequent, but usually pathogenic, part of the [skin flora](#). *S. pyogenes* is the cause of many important human diseases, ranging from mild superficial skin infections to life-threatening systemic diseases. Infections typically begin in the throat or skin. Examples of mild *S. pyogenes* infections include

[pharyngitis](#) (strep throat) and localized skin infection ([impetigo](#)). [Erysipelas](#) and [cellulitis](#) are characterized by multiplication and lateral spread of *S. pyogenes* in deep layers of the skin. *S. pyogenes* invasion and multiplication in the [fascia](#) can lead to [necrotizing fasciitis](#), a life-threatening condition requiring surgery. Throat infections associated with release of certain toxins lead to [scarlet fever](#). Other toxigenic *S. pyogenes* infections may lead to streptococcal [toxic shock syndrome \(STSS\)](#), which can be life-threatening (Lynskey *et al.*, 2011).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 APPARATUS, INSTRUMENTS AND REAGENTS

3.1.1 Apparatus and Instruments

- i. Rotary Evaporator
- ii. Oven
- iii. Autoclave
- iv. Pre-coated Chromatographic Plates (20 × 20 cm) and Tank
- v. Silica gel (60 – 120 mesh)
- vi. Chromatographic Column
- vii. Separating Funnel
- viii. Incubator
- ix. Ultraviolet lamp (254 – 366 nm)
- x. Perkin Elmer Spectrum 8400S FT-IR Spectrometer
- xi. Bruker – Avance 400 MHz FT – NMR Spectrometer

3.1.2 Solvents

- i. Methanol
- ii. Ethyl acetate
- iii. Chloroform
- iv. Petroleum ether (60 – 80 °C)

All solvents and reagents used were of high analytical grade supplied by Sigma- Aldrich. The solvents were distilled before use.

3.1.3 Micro-organisms

Gram-positive bacteria

Bacillus subtilis

Corynebacterium ulcerans

Streptococcus pneumoniae

Streptococcus faecalis

Streptococcus pyogenes

Staphylococcus aureus

Gram-negative bacteria

Klebsiella pneumoniae

Proteus mirabilis

Pseudomonas aeruginosa

Salmonella enteritidis

Salmonella typhi

Escherichia coli

Fungi

Candida albicans

Candida tropicalis

Candida krusei

3.2 COLLECTION AND PREPARATION OF PLANT MATERIAL

3.2.1 Sample Collection and Identification

The plant parts of *Acacia ataxacantha* was collected in Edumoga; Okpokwu Local Government Area of Benue state, Nigeria in the month of March, 2013. They were properly identified and authenticated by Mallam U.S. Gallah; the Curator of the Herbarium, Department of Biological Sciences A.B.U Zaria and a voucher specimen number 1707 was deposited there.

3.2.2 Preparation of Pulverized Plant Material

The root part of the plant was washed and air-dried in the laboratory at room temperature, pulverized into coarse powder and stored in polythene bags until needed for work.

3.3 EXTRACTION

The coarse - powdered sample (1 kg) was subjected to cold extraction (maceration) using 2.5 litre methanol for 5 days, then filtered and concentrated using rotary evaporator at 45⁰C.

3.3.1 Fractionation

Fractionation procedure as reported by Hostettmann *et al.*, (2000) was used. The methanol extract was resuspended in water and extracted with petroleum ether (pet. ether), chloroform (CHCl₃) and ethyl acetate (EtOAc) in that order. The resulting extracts were then concentrated using a rotary evaporator, at reduced pressure.

3.4 PHYTOCHEMICAL SCREENING

The various extracts were subjected to phytochemical screening using standard techniques as described by Harborne (1984), Sofowora (1993), and Trease and Evans (1989). The metabolites tested for were alkaloids, saponins, glycosides, tannins, cardiac glycosides, flavonoids and steroids. A negative reaction does not exclude the presence of any compound by reason of the fact that such a compound may occur in too low concentration for unambiguous detection (Richard and Cannell, 1998).

3.4.1 Test for Carbohydrates

Since most of carbohydrates' containing drugs are disaccharides or polymers, elucidation of their constituents requires hydrolysis as an initial step of investigation followed by specific tests.

3.4.1.1 Molisch's Test

Few drops of Molisch's reagent were added to a small portion of each extract in a test tube followed by conc. H_2SO_4 which was added down the side of the test tubes. A red coloured ring at the interphase resulted.

3.4.1.2 Fehling's Test

To a small portion of each of the extracts in a test tube, an equal mixture of Fehling's solutions A and B were added and boiled in a water bath. A brick red precipitate resulted.

3.4.2 Test for Glycosides

Glycosides are hydrolysed by dilute mineral acid or by enzymatic action.

To a portion of each extract, dilute H_2SO_4 (5 ml) was added and boiled for 15mins. The solutions were cooled, neutralized with 20% KOH and FeCl_3 (3 ml)solution was added. A bluish solution resulted due to the release of phenolic aglycones as a result of hydrolysis.

3.4.3 Test for Cardiac Glycosides

3.4.3.1 Keller-Killiani Test

A portion of each extract was dissolved in glacial acetic acid (1 ml) containing a trace of FeCl_3 solution and transferred into a test tube. Conc. H_2SO_4 (1 ml) was added (with the tube inclined at 45°) to form a bottom layer. A brown ring was formed at the interface of the two liquids (lactone ring) and a pale green colour in the upper acetic acid layer resulted.

3.4.4 Test for Free Anthracene Derivatives (Bornträger's) Test:

To a portion of each extract in a test tube, chloroform (5 ml) was added and shaken for about 5mins; filtered and shaken with equal volume of 10% ammonia solution. No visible reaction was observed.

3.4.5 Test for Saponins

3.4.5.1 Frothing Test

Distilled water (10 ml) was added to a portion of each extract, shaken vigorously for 30 seconds and allowed to stand vertically for over 30 minutes. A frothing solution resulted.

3.4.6 Test for Tannins

3.4.6.1 FeCl_3 Solution

To a portion of each extract,3-5 drops of FeCl_3 solution was added. It gave a greenish-black precipitate (condensed tannins).

3.4.7 Test for Flavonoids

3.4.7.1 NaOH Test

Methanolic solution of the extract was mixed with few drops of 10% NaOH. A yellow-coloured solution resulted.

3.4.7.2 Shinoda's Test

A portion of each extract was dissolved in 50% methanol (2 ml) and heated in a water bath. Few pieces of metallic Magnesium chips were added followed by drops of conc. HCl which gave a reddish colouration.

3.4.8 Test for Alkaloids

3.4.8.1 Mayer's Test

Few drops of Mayer's reagent were added to a portion of each of the extracts. It gave a creamy white precipitate.

3.4.8.2 Dragendoff's Test

Few drops of Dragendoff's reagent were added to a portion of each extract and a reddish-brown precipitate resulted.

3.4.8.3 Wagner's Test

Few drops of Wagner's reagent were added to a small quantity of each extract resulting in a reddish-brown precipitate.

3.4.9 Test for Steroids and Triterpenes

3.4.9.1 Lieberman-Burchard Test

Equal volume of acetic anhydride was added to a portion of each extract and mixed gently. Conc. H₂SO₄(1 ml) was added down the side of the test tubes to form a lower layer. A reddish-brown ring was formed at the interface.

3.4.9.2 Salkowski's Test (for unsaturated Sterols)

A small portion of each extract was placed in a test tube and held at 45°, and then 2-3 drops of conc. H₂SO₄ were added down the side of the test tubes. A reddish-brown ring was formed at the interface of the two layers.

3.5 BIOASSAY ANALYSES OF THE PLANT EXTRACTS

In order to confirm scientifically and correlate with the ethno medicinal use of *Acacia ataxacantha*, antimicrobial sensitivity tests were conducted with the extracts. This was done to determine the sensitivity of the test organisms to a known concentration of the crude extracts and the potency of possible anti-microbial agents in solution.

3.5.1 Antimicrobial Sensitivity Test

Antimicrobial sensitivity test was carried out *in vitro* to determine if the extracts were active on some pathogenic organisms. The cork and bore diffusion method of Bauer *et al.*, (1966), and Barry and Thornsberry (1985) were used in the anti-microbial screening.

3.5.2 Determination of Zone of Inhibition.

The Zone of Inhibition of *Acacia ataxacantha* plant extracts were determined using selected pathogenic micro-organisms obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH) Zaria. All the clinical isolates were checked for purity and maintained in slants of nutrient agar. The extracts (0.2 g) were weighed and dissolved in dimethyl sulphoxide (DMSO) (10 mls) to obtain a concentration of 20 mg/ml. This was the initial concentration of the extracts used to check the antimicrobial activities of the plant. Mueller Hinton agar was the medium used as the growth medium for the microbes. The medium was prepared according to the manufacturers' instructions and

sterilized by autoclaving at 121°C for 15 mins. It was then transferred into petri dishes and allowed to cool and solidify. The sterilized medium was seeded with 0.1 ml of the standard inoculum of the test microbe, and was spread evenly over the surface of the medium by the use of a sterile swab and the plates were allowed to dry at 37°C for 30 min. By the use of a sterile standard cork borer of 6 mm in diameter, a well was cut at the centre of each inoculated medium. The extract (0.1 ml) of the concentration of 20 mg/ml was then introduced into each well on the inoculated medium, the plates were incubated at 37°C for 24 hrs, after which each plate of the medium was observed for the zone of inhibition of growth, the diameter of the zones were measured with a calliper after which the calliper was measured using a transparent rule and the result recorded in millimetres (mm).

3.5.3 Determination of Minimum Inhibitory Concentration (MIC) of the extracts.

The Broth Dilution method of Thornsberry and Donald(1975) was employed in this determination. Mueller Hinton broth was prepared, 10 ml was dispensed into test tubes and sterilized at 121°C for 15 min, and the broth was allowed to cool. Mc-Farlands turbidity scale number 0.5 was prepared to give turbid solution. Normal saline (10 ml) was dispensed into sterile test tubes and the test microorganisms were inoculated into the different test tubes containing the normal saline. Incubation of the normal saline was done at 37°C for 6 hrs. Dilution of the test microorganisms in the normal saline was done continuously until the turbidity matched that of the Mc-Farland's scale by visual comparison. At this point the test microbe has a concentration of about 1.5×10^8 cfu/ml.

Two-fold serial dilution of the extracts in the sterile broth was made to obtain the concentrations of 20, 10, 5, 2.5 and 1.25 mg/ml. The initial concentration was obtained by dissolving 0.2 g of the extracts in 10 ml of the sterile broth. Having obtained the different concentrations of the extracts in the broth, 0.1 ml of the test microorganism in the normal

saline was then inoculated into the different concentrations; incubation was done at 37°C for 24 hrs after which the broth was observed for turbidity (growth). The lowest concentration (highest dilution) of the extract in the broth which inhibited 100% of visible growth was recorded as the minimum inhibitory concentration (MIC). At this dilution the extract is said to be [bacteriostatic](#) (prevents the bacteria from multiplying).

3.5.4 Determination of Minimum Bactericidal and Fungicidal Concentration (MBC/MFC)

Minimum Bactericidal and Fungicidal Concentration (MBC/MFC) was determined to check whether the test microbes were killed or only their growth was inhibited. Mueller Hinton agar was prepared, sterilized at 121°C for 15 min, 20 ml was transferred into sterile petri dishes and allowed to cool and solidify. The contents of the MIC tubes in the serial dilution were sub-cultured into the prepared medium. Incubation was done at 37°C for 24 hrs, after which the plates were observed for colony growth. The MBC/MFC was the plates with lowest concentration (highest dilution) of the extract without any bacterial growth ([bactericidal](#)).

3.6 ISOLATION AND PURIFICATION OF ACTIVE COMPONENTS

Thin layer, column and preparative thin layer chromatography were the major techniques employed in isolating and purifying the crude extracts. Ethyl acetate extract was used.

3.6.1 Thin Layer Chromatography

The technique was used to determine the appropriate solvent combinations required to separate the components in the ethyl acetate extract. Commercially available pre-coated silica gel plates were used. The ethyl acetate extract was dissolved in minimal amount of methanol, and then spotted at the base of the plate. These were developed using several solvent systems but the solvent systems of chloroform /ethyl acetate (8:2 and 7:3) gave better separation of

the components, and were therefore used in the TLC monitoring of the column chromatography. Visualisation was carried out under ultraviolet (UV) light.

3.6.2 Column Chromatography

The separation of the respective extracts into different chemical components was carried out using the column chromatographic technique. Ethyl acetate extract was loaded into the column already loaded with silica gel (60 – 120 mesh), elution was carried out first with 100% n-hexane. The polarity of the solvent was increased by mixing with ethyl acetate and fractions were collected and spotted on the pre-coated TLC plates. Fractions with similar R_f - values were combined and re-spotted. Two compounds coded ABA with R_f -value of 0.588 and ABA 1 with R_f -value of 0.713 were isolated.

3.6.3 Preparative Thin Layer Chromatography (PTLC)

The compounds isolated were further purified using PTLC by dissolving each in chloroform. ABA was developed in a mixture of chloroform and ethyl acetate (8:2) while ABA 1 was developed in a mixture of n-hexane and ethyl acetate (8:2).

3.7 SPECTROSCOPY

The final products (ABA and ABA 1) were analysed using Fourier-transformed Infrared spectroscopy (FTIR) and Nuclear magnetic resonance (NMR) spectrometer (in $CDCl_3$).

3.8 ANTIMICROBIAL SCREENING OF ABA

The antimicrobial activity of ABA was determined using the same pathogenic microorganisms used on the crude extracts. This was done to determine the medicinal properties of the isolated compound.

Sensitivity Test, Zones of Inhibition, Minimum Inhibitory Concentration (MIC) And Minimum Bactericidal/ Fungicidal Concentration (MBC/MFC) was done as described previously except for the concentrations. The compound (1×10^{-3} mg) was weighed and dissolved in 10 ml of Dimethyl sulphur oxide (DMSO) to obtain a concentration of 1×10^2 $\mu\text{g/ml}$. This was the initial concentration used to check the antimicrobial activities of the isolated compound. Two-fold serial dilution of the extracts in the sterile broth was made to obtain the concentrations of 100, 50, 25, 12.5 and 6.25 $\mu\text{g/ml}$. The initial concentration was obtained by dissolving 1×10^{-3} mg of the compound in 10 ml of the sterile broth.

CHAPTER FOUR

4.0 RESULTS

4.1 EXTRACTION

Extraction of the pulverised root of *Acacia ataxacantha* (1 kg) and fractionation gave various weights as shown in Table 4.1

4.2 RESULTS OF PHYTOCHEMICAL SCREENING EXPERIMENTS

The crude plant extracts were screened using the methods of Harborne (1984), Sofowora (1993), and Trease and Evans (1989) and the results obtained are recorded in Table 4.2

4.3 RESULTS OF THE ANTIMICROBIAL SCREENING EXPERIMENTS

The fractionated samples of petroleum ether, chloroform, ethyl acetate and methanol were subjected to antimicrobial screenings and the results obtained are as shown in Tables 4.3 – 4.12.

4.4 ANTIMICROBIAL SCREENING OF PURE COMPOUND (ABA)

The isolated compound (ABA) was subjected to antimicrobial screenings and the results obtained are as recorded in Tables 4.13 – 4.16

4.5 SPECTROSCOPY

The Fourier Transformed Infrared Spectroscopy (FTIR) of ABA and ABA 1 was run on Perkin Elmer Spectrum 8400S FT-IR Spectrometer and the results obtained are as shown in Figures 4.1 and 4.5.

Similarly, the proton, Carbon – 13 and DEPT NMR were recorded in Bruker – Avance 400 MHz FT – NMR Spectrometer using deuterated chloroform as solvent and the results obtained are as shown in Figures 4.2- 4.4 and 4.6 -4.8

Table 4.1: Extraction values of the root of *Acacia ataxacantha*

Extract	Weight (g)	Percentage recovery (%)
Methanol	38.30	3.83
Ethyl acetate	7.40	0.74
Chloroform	4.80	0.48
Petroleum ether	13.20	1.32

Table 4.2: Phytochemical Screening of the root extracts of *Acacia ataxacantha*

Test	Petroleum ether	Chloroform	Ethyl acetate	Methanol
Flavonoids	+	+	+	+
Glycosides	+	+	+	+
Anthraquinones	-	-	-	-
Saponins	+	+	+	+
Tannins	+	+	+	+
Carbohydrates	-	+	+	+
Alkaloids	+	+	+	+
Steroids/ Triterpenes	+	+	+	+

Key: - → Absent + → Present

Table 4.3: Antimicrobial Sensitivity Test of the Crude Extracts and Control

Test organisms	PE	CH	EA	ME	SF	CX	FZ
<i>Staphylococcus aureus</i>	S	S	S	S	S	S	R
<i>Streptococcus pyogenes</i>	S	S	S	S	S	S	R
<i>Streptococcus faecalis</i>	R	R	R	R	S	S	R
<i>Streptococcus pneumoniae</i>	S	S	S	S	S	S	R
<i>Corynebacterium ulcerans</i>	R	R	R	R	S	R	R
<i>Bacillus subtilis</i>	S	S	S	S	S	S	R
<i>Escherichia coli</i>	S	S	S	S	S	S	R
<i>Salmonella typhi</i>	S	S	S	S	S	R	R
<i>Salmonella enteritidis</i>	S	S	S	S	S	S	R
<i>Proteus mirabilis</i>	R	R	R	R	S	R	R
<i>Pseudomonas aeruginosa</i>	S	S	S	S	R	S	R
<i>Klebsiella pneumoniae</i>	S	S	S	S	S	S	R
<i>Candida albicans</i>	S	S	S	S	R	R	S
<i>Candida tropicalis</i>	R	R	R	R	R	R	S
<i>Candida krusei</i>	S	S	S	S	R	R	S

Key: PE →Petroleum ether; CH→Chloroform; EA→Ethyl acetate; ME→Methanol; SF→Sparfloxacin; CX→ Cefuroxime; FZ→ Fluconazole
S → sensitive R → resistance

Table 4.4: Diameter of Zone of Inhibition of the Crude extracts and Control against the Test Organisms (mm)

Test organisms	PE	CH	EA	ME	SF	CX	FZ
<i>Staphylococcus aureus</i>	18	22	25	20	36	32	0
<i>Streptococcus pyogenes</i>	17	21	25	21	30	30	0

<i>Streptococcus faecalis</i>	0	0	0	0	32	29	0
<i>Streptococcus pneumoniae</i>	18	22	26	23	35	32	0
<i>Corynebacterium ulcerans</i>	0	0	0	0	32	0	0
<i>Bacillus subtilis</i>	19	25	29	23	45	40	0
<i>Escherichia coli</i>	18	25	30	24	35	30	0
<i>Salmonella typhi</i>	18	22	27	20	30	0	0
<i>Salmonella enteritidis</i>	17	20	25	20	32	30	0
<i>Proteus mirabilis</i>	0	0	0	0	27	0	0
<i>Pseudomonas aeruginosa</i>	17	21	24	21	0	32	0
<i>Klebsiella pneumonia</i>	19	24	28	22	47	40	0
<i>Candida albicans</i>	18	20	23	20	0	0	32
<i>Candida tropicalis</i>	0	0	0	0	0	0	29
<i>Candida krusei</i>	18	21	20	20	0	0	34

Key: PE →Petroleum ether; CH→Chloroform; EA→Ethyl acetate; ME→Methanol; SF→Sparfloxacin; CX→ Cefuroxime; FZ→ Fluconazole

Table 4.5: Minimum Inhibitory Concentration (MIC) of Methanol Extract

Test organisms	← Concentrations (mg/ml) →				
	20	10	5	2.5	1.25
<i>Staphylococcus aureus</i>	-	-	0+	+	++
<i>Streptococcus pyogenes</i>	-	-	0+	+	++

<i>Streptococcus pneumonia</i>	-	-	0+	+	++
<i>Bacillus subtilis</i>	-	-	0+	+	++
<i>Escherichia coli</i>	-	-	0+	+	++
<i>Salmonella typhi</i>	-	-	0+	+	++
<i>Salmonella enteritidis</i>	-	-	0+	+	++
<i>Pseudomonas aeruginosa</i>	-	-	0+	+	++
<i>Klebsiella pneumonia</i>	-	-	0+	+	++
<i>Candida albicans</i>	-	-	0+	+	++
<i>Candida krusei</i>	-	-	0+	+	++

Key: - → no turbidity (no growth), 0+ → MIC, + → turbid(light growth),
 ++ → moderate turbidity, +++ → high turbidity

Table 4.6: Minimum Inhibitory Concentration (MIC) of Ethyl acetate Extract

Test organisms	← Concentrations (mg/ml) →				
	20	10	5	2.5	1.25
<i>Staphylococcus aureus</i>	-	-	0+	+	++
<i>Streptococcus pyogenes</i>	-	-	0+	+	++
<i>Streptococcus pneumonia</i>	-	-	0+	+	++
<i>Bacillus subtilis</i>	-	-	-	0+	+
<i>Escherichia coli</i>	-	-	-	0+	+
<i>Salmonella typhi</i>	-	-	-	0+	+

<i>Salmonella enteritidis</i>	-	-	0+	+	++
<i>Pseudomonas aeruginosa</i>	-	-	0+	+	++
<i>Klebsiella pneumonia</i>	-	-	-	0+	+
<i>Candida albicans</i>	-	-	0+	+	++
<i>Candida krusei</i>	-	-	0+	+	++

Key:- → no turbidity (no growth), 0+ → MIC, + → turbid(light growth),
 ++ → moderate turbidity, +++ → high turbidity

Table 4.7: Minimum Inhibitory Concentration (MIC) of Chloroform Extract

Test organisms	←———— Concentrations (mg/ml) ———→				
	20	10	5	2.5	1.25
<i>Staphylococcus aureus</i>	-	-	0+	+	++
<i>Streptococcus pyogenes</i>	-	-	0+	+	++
<i>Streptococcus pneumonia</i>	-	-	0+	+	++
<i>Bacillus subtilis</i>	-	-	0+	+	++
<i>Escherichia coli</i>	-	-	0+	+	++
<i>Salmonella typhi</i>	-	-	0+	+	++
<i>Salmonella enteritidis</i>	-	-	0+	+	++
<i>Pseudomonas aeruginosa</i>	-	-	0+	+	++

<i>Klebsiella pneumonia</i>	-	-	0+	+	++
<i>Candida albicans</i>	-	-	0+	+	++
<i>Candida krusei</i>	-	-	0+	+	++

Key: - → no turbidity (no growth), 0+ → MIC, + → turbid(light growth),
 ++ → moderate turbidity, +++ → high turbidity

Table 4.8: Minimum Inhibitory Concentration (MIC) of Petroleum ether Extract

Test organisms	← Concentrations (mg/ml) →				
	20	10	5	2.5	1.25
<i>Staphylococcus aureus</i>	-	0+	+	++	+++
<i>Streptococcus pyogenes</i>	-	0+	+	++	+++
<i>Streptococcus pneumoniae</i>	-	0+	+	++	+++
<i>Bacillus subtilis</i>	-	0+	+	++	+++
<i>Escherichia coli</i>	-	0+	+	++	+++
<i>Salmonella typhi</i>	-	0+	+	++	+++
<i>Salmonella enteritidis</i>	-	0+	+	++	+++
<i>Pseudomonas aeruginosa</i>	-	0+	+	++	+++
<i>Klebsiella pneumonia</i>	-	0+	+	++	+++
<i>Candida albicans</i>	-	0+	+	++	+++
<i>Candida krusei</i>	-	0+	+	++	+++

Key:- → no turbidity (no growth), 0+ → MIC, + → turbid(light growth),
 ++ → moderate turbidity, +++ → high turbidity

Table 4.9: Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of Methanol Extract

Test organisms	← Concentrations (mg/ml) →				
	20	10	5	2.5	1.25
<i>Staphylococcus aureus</i>	0+	+	++	+++	++++
<i>Streptococcus pyogenes</i>	0+	+	++	+++	++++
<i>Streptococcus pneumonia</i>	-	0+	+	++	+++
<i>Bacillus subtilis</i>	-	0+	+	++	+++
<i>Escherichia coli</i>	-	0+	+	++	+++
<i>Salmonella typhi</i>	0+	+	++	+++	++++
<i>Salmonella enteritidis</i>	0+	+	++	+++	++++
<i>Pseudomonas aeruginosa</i>	-	0+	+	++	+++
<i>Klebsiella pneumonia</i>	-	0+	+	++	+++
<i>Candida albicans</i>	0+	+	++	+++	++++
<i>Candida krusei</i>	0+	+	++	+++	++++

Key:- → no turbidity (no growth), 0+ → MBC/MFC, + → turbid(light growth) ++
 → moderate turbidity, +++ → high turbidity

Table 4.10: Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of Ethyl acetate Extract

Test organisms	← Concentrations (mg/ml) →				
	20	10	5	2.5	1.25
<i>Staphylococcus aureus</i>	-	0+	+	++	+++
<i>Streptococcus pyogenes</i>	-	0+	+	++	+++
<i>Streptococcus pneumonia</i>	-	0+	+	++	+++
<i>Bacillus subtilis</i>	-	-	0+	+	++
<i>Escherichia coli</i>	-	-	0+	+	++
<i>Salmonella typhi</i>	-	0+	+	++	+++
<i>Salmonella enteritidis</i>	-	0+	+	++	+++
<i>Pseudomonas aeruginosa</i>	-	0+	+	++	+++
<i>Klebsiella pneumonia</i>	-	-	0+	+	++
<i>Candida albicans</i>	-	0+	+	++	+++
<i>Candida krusei</i>	-	0+	+	++	+++

Key:- → no turbidity (no growth), 0+ → MBC/MFC, + → turbid(light growth),
 ++ → moderate turbidity, +++ → high turbidity

Table 4.11: Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of Chloroform Extract

Test organisms	← Concentrations (mg/ml) →				
	20	10	5	2.5	1.25
<i>Staphylococcus aureus</i>	-	0+	+	++	+++

<i>Streptococcus pyogenes</i>	0+	+	++	+++	++++
<i>Streptococcus pneumonia</i>	-	0+	+	++	+++
<i>Bacillus subtilis</i>	-	0+	+	++	+++
<i>Escherichia coli</i>	-	0+	+	++	+++
<i>Salmonella typhi</i>	-	0+	+	++	+++
<i>Salmonella enteritidis</i>	0+	+	++	+++	++++
<i>Pseudomonas aeruginosa</i>	-	0+	+	++	+++
<i>Klebsiella pneumonia</i>	-	0+	+	++	+++
<i>Candida albicans</i>	0+	+	++	+++	++++
<i>Candida krusei</i>	0+	+	++	+++	++++

Key:- → no turbidity (no growth), 0+ → MBC/MFC, + → turbid(light growth),
 ++ → moderate turbidity, +++ → high turbidity

Table 4.12: Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of Petroleum ether Extract

Test organisms	←———— Concentrations (mg/ml) —————→				
	20	10	5	2.5	1.25
<i>Staphylococcus aureus</i>	0+	+	++	+++	++++
<i>Streptococcus pyogenes</i>	0+	+	++	+++	++++
<i>Streptococcus pneumonia</i>	0+	+	++	+++	++++

<i>Bacillus subtilis</i>	0+	+	++	+++	++++
<i>Escherichia coli</i>	0+	+	++	+++	++++
<i>Salmonella typhi</i>	0+	+	++	+++	++++
<i>Salmonella enteritidis</i>	0+	+	++	+++	++++
<i>Pseudomonas aeruginosa</i>	0+	+	++	+++	++++
<i>Klebsiella pneumonia</i>	0+	+	++	+++	++++
<i>Candida albicans</i>	0+	+	++	+++	++++
<i>Candida krusei</i>	0+	+	++	+++	++++

Key:- → no turbidity (no growth), 0+ → MBC/MFC, + → turbid(light growth),
 ++ → moderate turbidity, +++ → high turbidity

Table 4.13: Antimicrobial Sensitivity Test of ABA and Control

Test organisms	ABA	Sparfloxacin	Cefuroxime	Fluconazole
<i>Staphylococcus aureus</i>	S	S	S	R
<i>Streptococcus pyogenes</i>	S	S	S	R
<i>Streptococcus faecalis</i>	R	S	S	R
<i>Streptococcus pneumonia</i>	S	S	S	R
<i>Corynebacterium ulcerans</i>	R	S	R	R
<i>Bacillus subtilis</i>	S	S	S	R
<i>Escherichia coli</i>	S	S	S	R
<i>Salmonella typhi</i>	S	S	S	R

<i>Salmonella enteritidis</i>	S	S	R	R
<i>Proteus mirabilis</i>	R	R	S	R
<i>Pseudomonas aeruginosa</i>	S	R	S	R
<i>Klebsiella pneumonia</i>	S	S	S	R
<i>Candida albicans</i>	S	R	R	S
<i>Candida tropicalis</i>	R	R	R	S
<i>Candida krusei</i>	S	R	R	S

Key: S → sensitive R → resistance

Table 4.14: Diameter of Zones of Inhibition of ABA and Control Against the Test Organisms (mm)

Test organisms	ABA	Sparfloxacin	Cefuroxime	Fluconazole
<i>Staphylococcus aureus</i>	28	37	38	0
<i>Streptococcus pyogenes</i>	26	35	34	0
<i>Streptococcus faecalis</i>	0	36	37	0
<i>Streptococcus pneumoniae</i>	24	37	35	0
<i>Corynebacterium ulcerans</i>	0	35	0	0
<i>Bacillus subtilis</i>	31	42	40	0
<i>Escherichia coli</i>	28	35	37	0
<i>Salmonella typhi</i>	29	35	37	0
<i>Salmonella enteritidis</i>	25	37	0	0
<i>Proteus mirabilis</i>	0	0	35	0
<i>Pseudomonas aeruginosa</i>	27	0	34	0

<i>Klebsiella pneumonia</i>	25	37	39	32
<i>Candida albicans</i>	25	0	0	0
<i>Candida tropicalis</i>	0	0	0	37
<i>Candida krusei</i>	24	0	0	35

Table 4.15: Minimum Inhibitory Concentration (MIC) of ABA

Test organisms	← Concentrations (µg/ml) →				
	100	50	25	12.5	6.25
<i>Staphylococcus aureus</i>	-	-	-	0+	+
<i>Streptococcus pyogenes</i>	-	-	0+	+	++
<i>Streptococcus pneumoniae</i>	-	-	0+	+	++
<i>Bacillus subtilis</i>	-	-	-	0+	+
<i>Escherichia coli</i>	-	-	-	0+	+
<i>Salmonella typhi</i>	-	-	-	0+	+
<i>Salmonella enteritidis</i>	-	-	0+	+	++
<i>Pseudomonas aeruginosa</i>	-	-	-	0+	+
<i>Klebsiella pneumonia</i>	-	-	0+	+	++
<i>Candida albicans</i>	-	-	0+	+	++
<i>Candida krusei</i>	-	-	0+	+	++

Key:- → no turbidity (no growth), 0+ → MIC, + → turbid(light growth),
 ++ → moderate turbidity,

Table 4.16: Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of ABA

Test organisms	← Concentrations (µg/ml) →				
	100	50	25	12.5	6.25
<i>Staphylococcus aureus</i>	-	0+	+	++	+++
<i>Streptococcus pyogenes</i>	-	0+	+	++	+++
<i>Streptococcus pneumonia</i>	-	0+	+	++	+++
<i>Bacillus subtilis</i>	-	-	0+	+	++
<i>Escherichia coli</i>	-	-	0+	+	++
<i>Salmonella typhi</i>	-	-	0+	+	++
<i>Salmonella enteritidis</i>	-	0+	+	++	+++
<i>Pseudomonas aeruginosa</i>	-	0+	+	++	+++
<i>Klebsiella pneumonia</i>	-	0+	+	++	+++
<i>Candida albicans</i>	-	0+	+	++	+++
<i>Candida krusei</i>	-	0+	+	++	+++

Key:- → no colony growth), 0+ → MBC/MFC, + → scanty colonies growth,
 ++ → moderate colonies growth, +++ → heavy colonies growth

FTIR ANALYSIS RESULT NARICT,ZARIA

FTIR- 8400S FOURIER TRANSFORM
INFRARED SPECTROPHOTOMETER

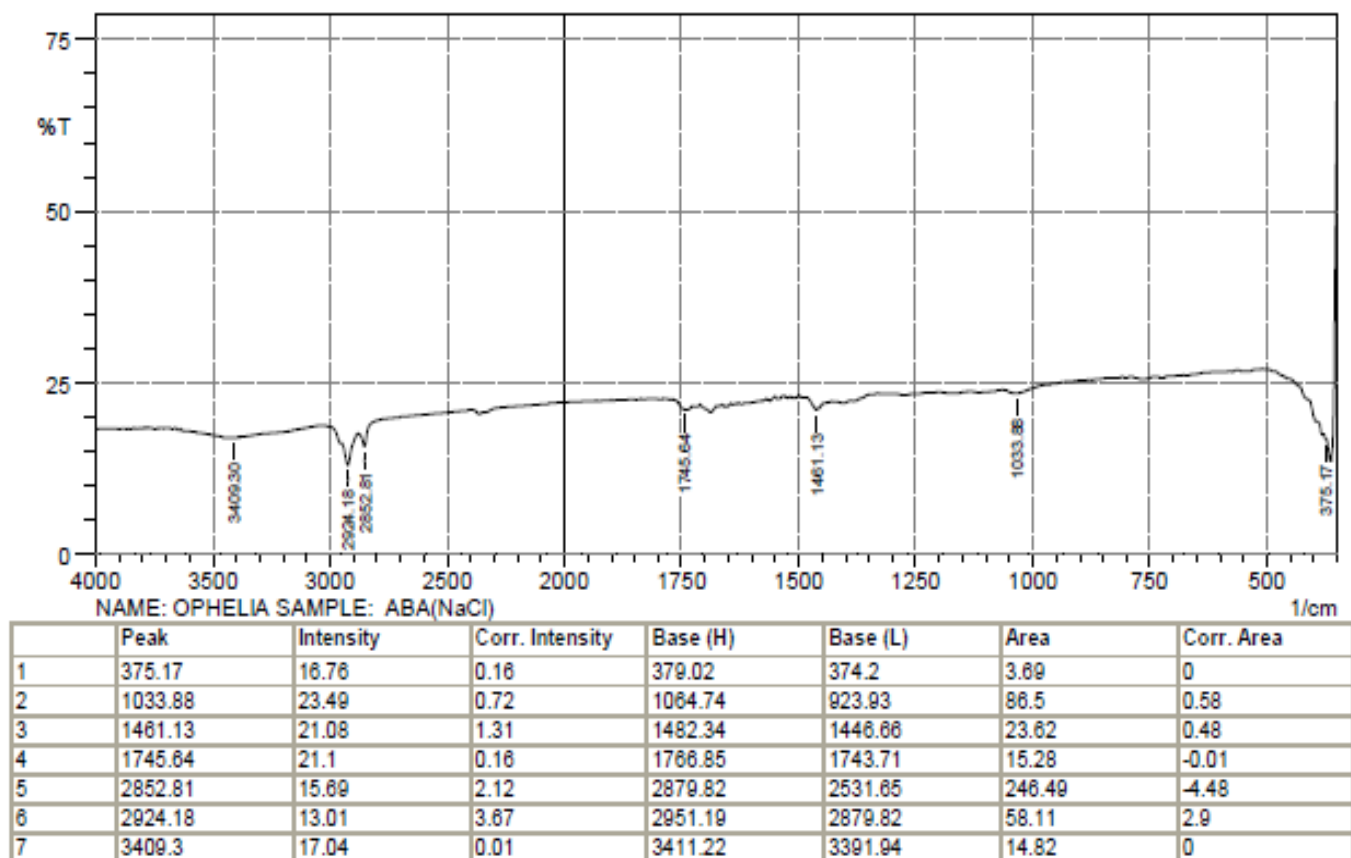


Figure 4.1: FTIR of ABA

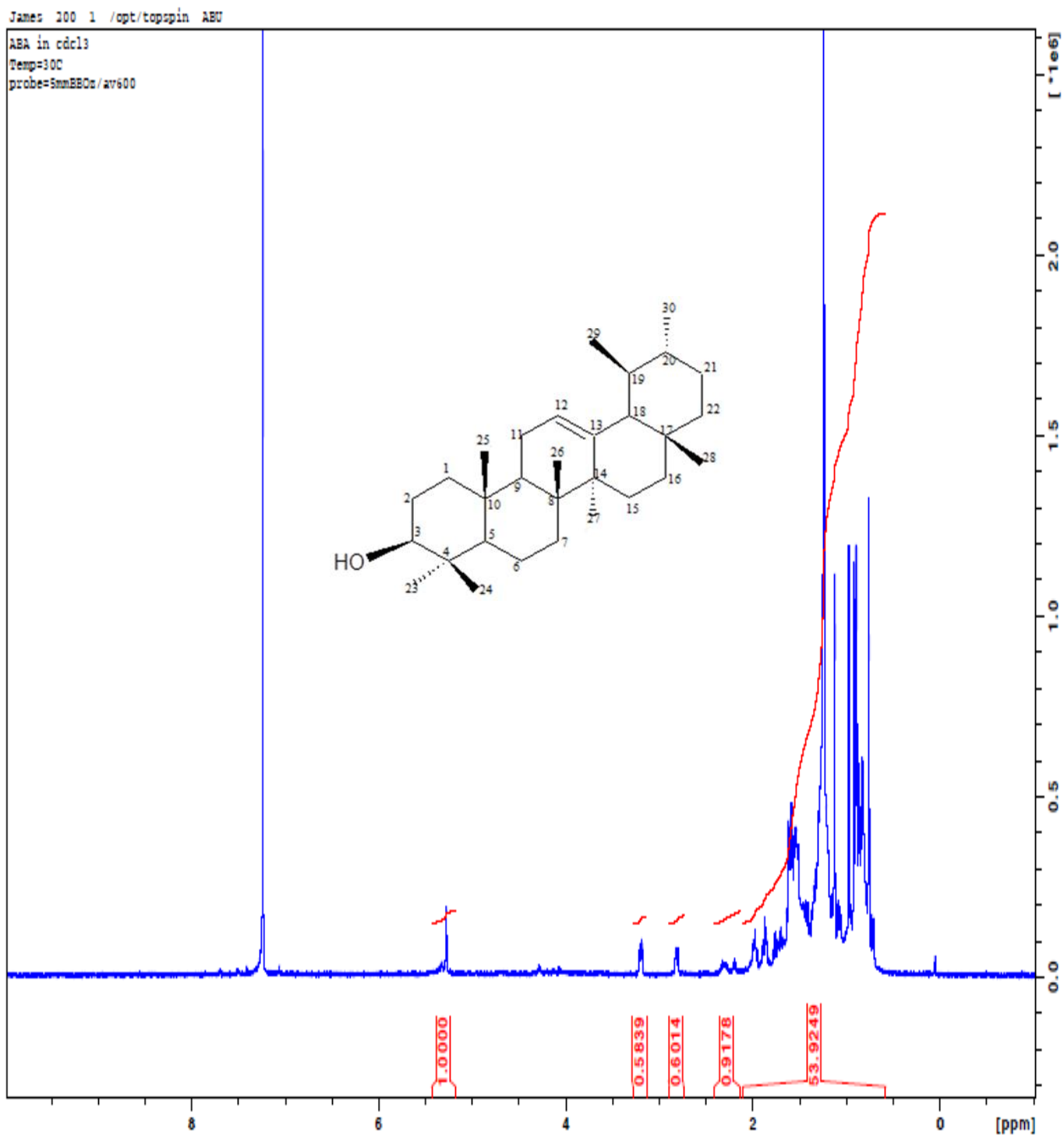


Figure 4.2: ^1H -NMR of ABA

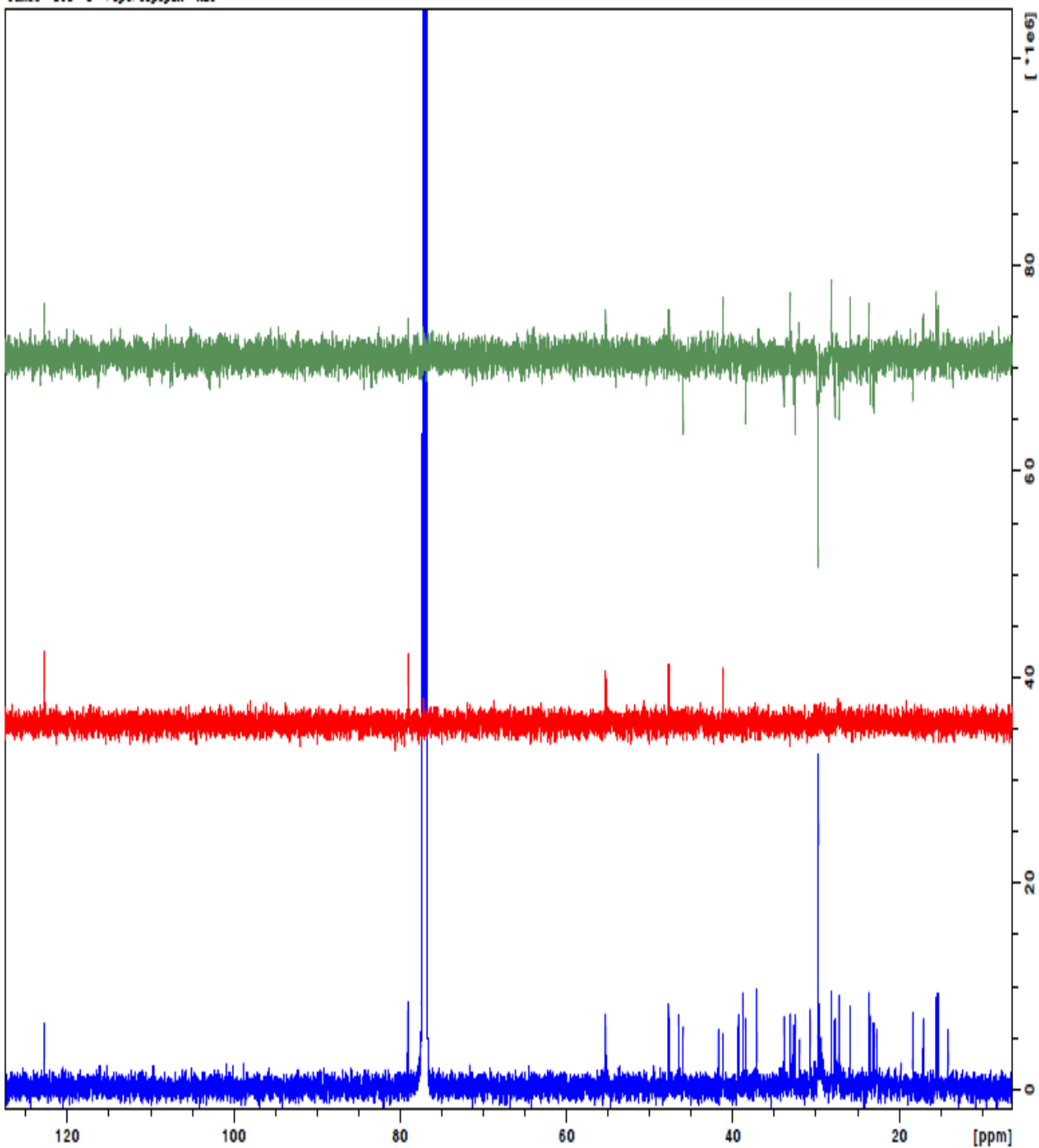


Figure 4.4: DEPT of ABA

Table 4.17: ^{13}C -NMR and ^1H -NMR Assignment of ABA in CDCl_3 (δ in ppm)

Position	$\delta^{13}\text{C}$	$\delta^{13}\text{C}$ Lit.	CHn	^1H	^1H Lit.
1	38.7	38.7	CH ₂		
2	29.3	28.7	CH ₂		
3	79.0	79.6	CH	3.2	3.16
4	38.4	38.7	C		
5	55.2	55.1	CH	0.7	0.67
6	18.3	18.4	CH ₂		
7	32.4	32.2	CH ₂		
8	41.1	40.7	C		
9	47.6	47.7	CH		
10	37.1	36.6	C		
11	23.5	23.3	CH ₂		
12	122.7	124.4	CH	5.2	5.06
13	143.5	139.5	C		
14	41.7	42.0	C		
15	27.2	27.2	CH ₂	1.9	1.94
16	25.9	26.6	CH ₂	1.7	1.76
17	33.8	33.7	C		
18	55.2	59.0	CH		
19	39.3	39.6	CH		
20	39.3	39.6	CH		
21	30.9	31.2	CH ₂		
22	41.7	41.5	CH ₂		
23	27.7	28.1	CH ₃		
24	15.3	15.6	CH ₃		
25	14.0	15.6	CH ₃		
26	15.5	16.8	CH ₃		
27	23.4	23.2	CH ₃		
28	28.1	28.1	CH ₃		
29	17.0	17.4	CH ₃		
30	22.6	21.4	CH ₃		

FTIR ANALYSIS RESULT NARICT,ZARIA

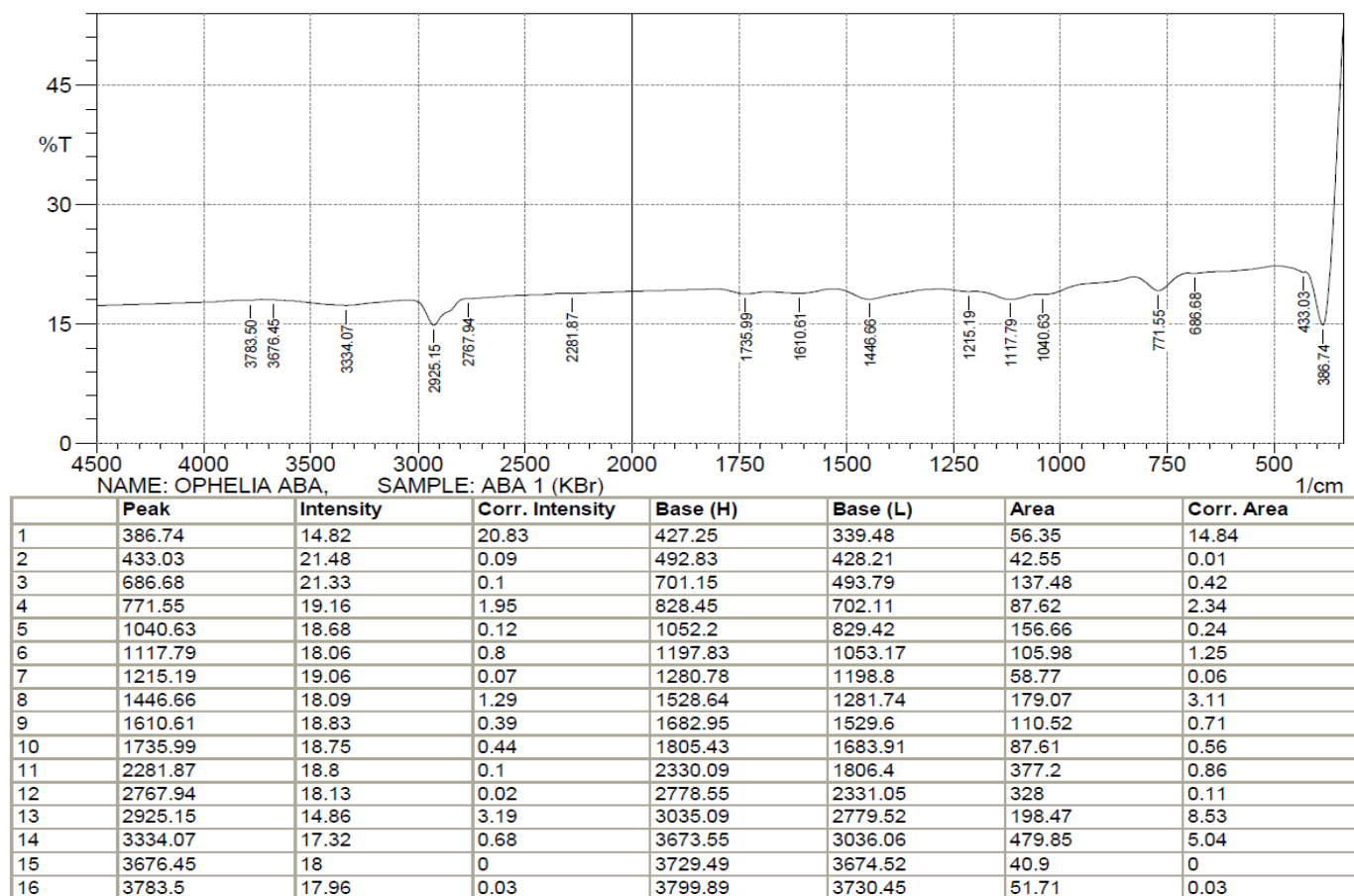
FTIR- 8400S FOURIER TRANSFORM
INFRARED SPECTROPHOTOMETER

Figure 4.5: FTIR of ABA 1

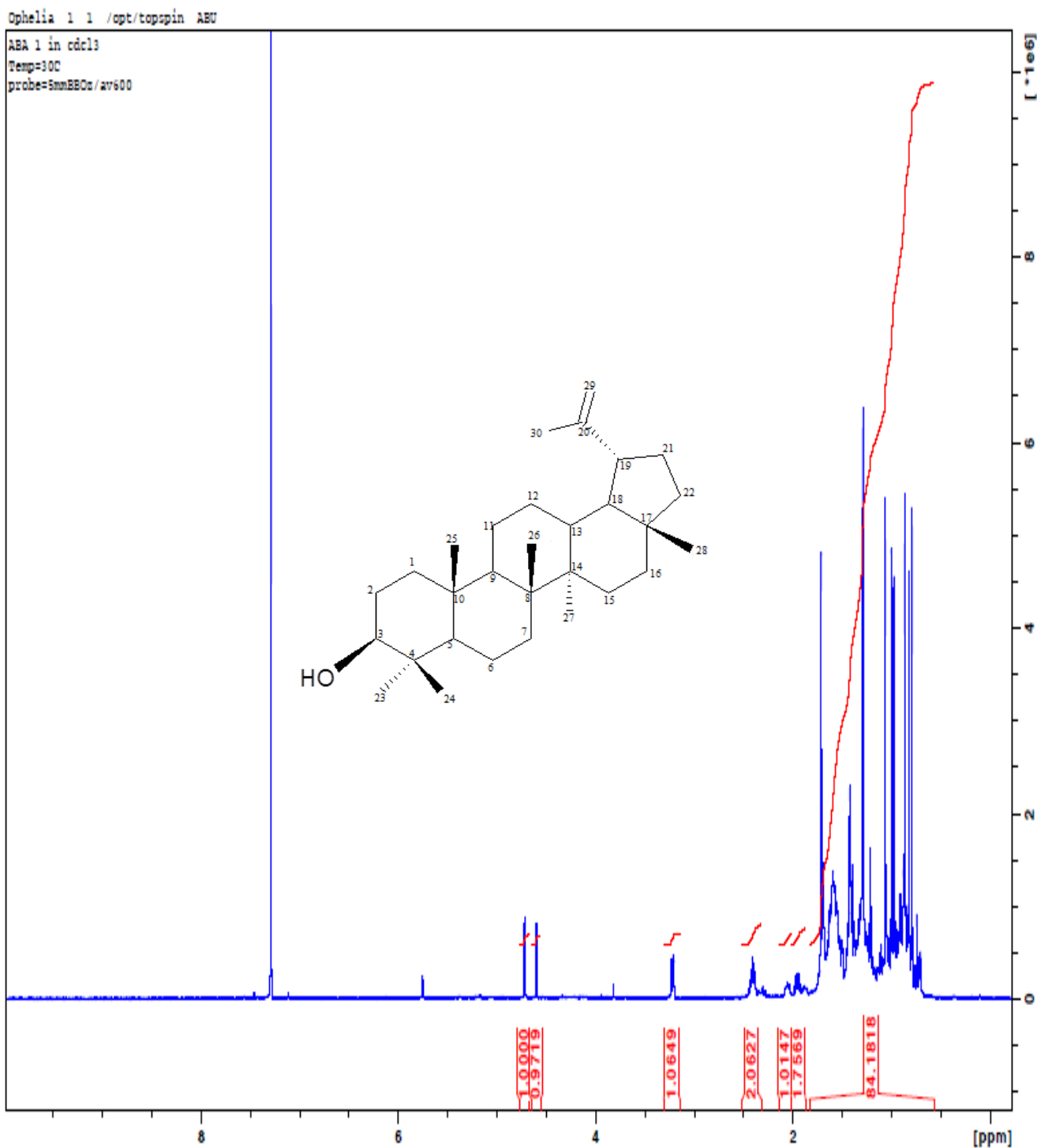


Figure 4.6: ^1H -NMR of ABA 1

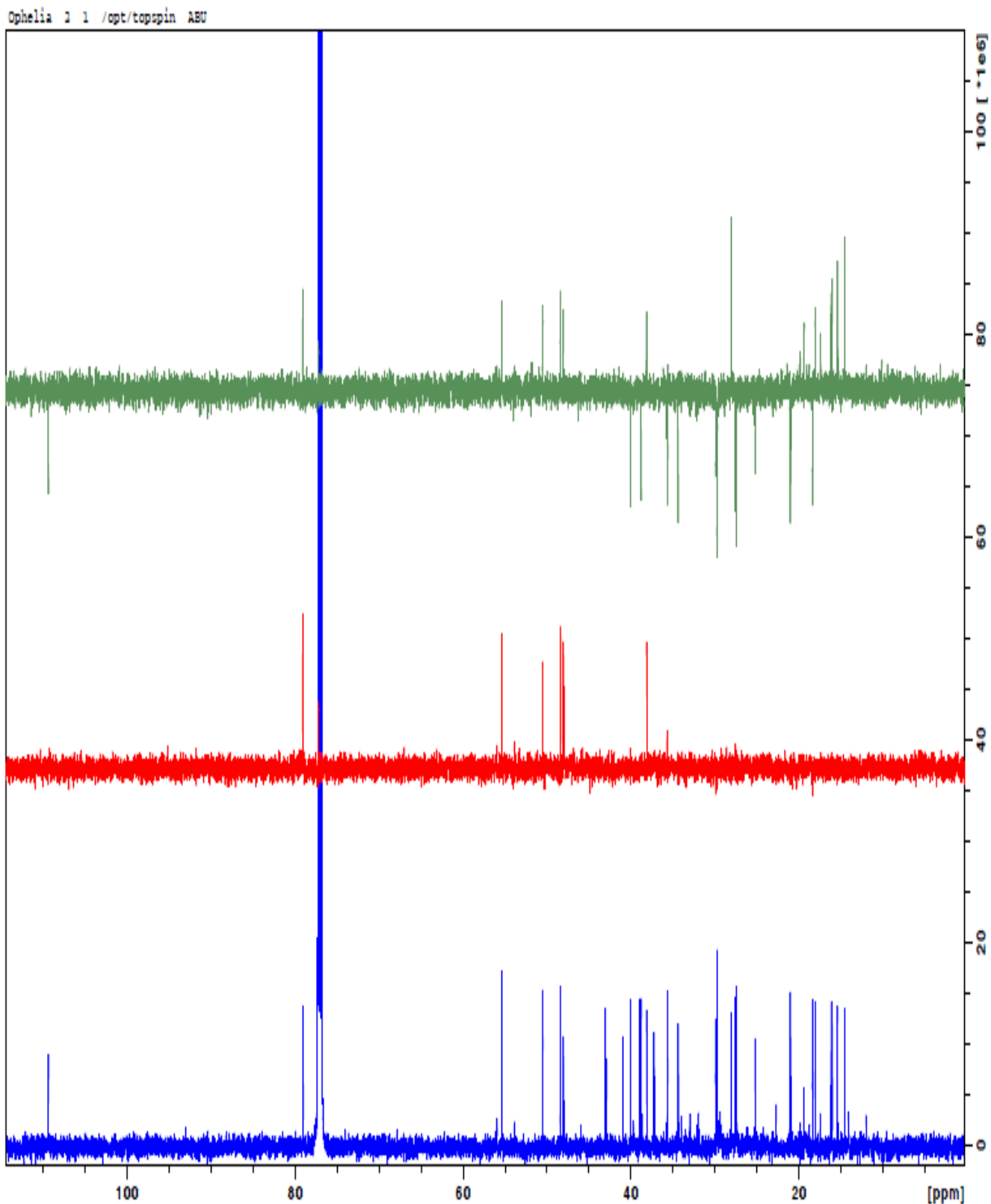


Figure 4.8: DEPT of ABA 1

Table 4.18: ^{13}C -NMR and 1H -NMR Assignment of ABA 1 in $CDCl_3$ (δ in ppm) .

Position	$\delta^{13}\text{C}$	$\delta^{13}\text{C}$ Lit.	CHn	^1H	^1H Lit.
1	38.7	38.7	CH ₂		
2	27.4	27.5	CH ₂		
3	79.0	79.3	CH	3.2	3.18
4	38.8	39.8	C		
5	55.3	55.5	CH		
6	18.3	19.0	CH ₂		
7	34.3	34.2	CH ₂		
8	40.8	41.1	C		
9	50.4	50.9	CH		
10	37.2	37.2	C		
11	20.9	21.2	CH ₂		
12	25.1	25.3	CH ₂		
13	38.1	38.5	CH		
14	42.8	42.8	C		
15	27.4	27.2	CH ₂		
16	35.6	35.9	CH ₂		
17	43.0	43.2	C		
18	48.3	48.5	CH		
19	48.0	47.8	CH	2.4	2.38
20	150.9	151.2	C		
21	29.8	30.1	CH ₂		
22	40.0	40.3	CH ₂		
23	28.0	28.4	CH ₃	0.95	0.97
24	15.3	15.6	CH ₃	0.75	0.77
25	16.1	16.2	CH ₃	0.82	0.84
26	15.9	16.1	CH ₃	1.1	1.04
27	14.5	14.8	CH ₃	1.0	0.96
28	18.0	18.1	CH ₃	0.8	0.8
29	109.3	109.5	CH ₂	4.6, 4.7	4.56, 4.68
30	19.3	19.8	CH ₃	1.7	1.7

CHAPTER FIVE

5.0 DISCUSSION

5.1 PHYTOCHEMICAL SCREENING

The antimicrobial activities of plant extracts have been linked to the presence of some bioactive compounds. These secondary metabolites also serve to protect the plants themselves against bacterial, fungal and viral infections (El-Mahmood and Amey, 2007). These bioactive compounds are known to work synergistically to produce various effects on the human and animal subjects (Amagase, 2006).

The screening of crude extracts of *Acacia ataxacantha* for phytochemical constituents has revealed the presence of flavonoids, steroids/ triterpenes, glycosides, tannins, saponins and alkaloids. Carbohydrate is present in all extracts except petroleum ether; while anthraquinones were absent in all the tested extracts. Steroids are important drugs used as cardiac depressants, hypotensive, sedatives and anti- dysenteric agents (Ghani, 1990).

Tannins have been reported to have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects (Trease and Evans, 2002). Alkaloids act as anti-malarial, anti-amoebic agents and astringents (Ghani, 1990). Flavonoids and tannins possess antimicrobial activity, the antimicrobial activity of flavonoids is due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell wall while that of tannins may be related to their ability to inactivate microbial adhesions, enzymes and cell envelop proteins (Cowan, 1999). This result is similar to the phytochemical screening results of the stem bark of *Acacia nilotica* as reported by Banso (2009).

5.2 ANTIMICROBIAL SCREENING

5.2.1 Sensitivity Test of Extracts against selected Micro-organisms

The results of antimicrobial sensitivity test of petroleum ether, chloroform, ethyl acetate and methanol extracts of the root of *Acacia ataxacantha* showed that the test organisms *Bacillus subtilis*, *Streptococcus pneumonia*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Salmonella typhi*, *Escherichia coli*, *Candida albicans* and *Candida krusei* were sensitive to all the extracts while *Corynebacterium ulcerans*, *Streptococcus faecalis*, *Proteus mirabilis* and *Candida tropicalis* were resistant. This indicates that the extracts of *Acacia ataxacantha* have broad-spectrum of activity against both Gram positive, Gram negative bacteria and fungi (Table 4.3)

5.2.2 Diameters of Zones of Inhibition

All the crude extracts inhibited the growth of the micro-organisms at varying degrees except *Corynebacterium ulcerans*, *Streptococcus faecalis*, *Proteus mirabilis* and *Candida tropicalis*. Ethyl acetate extract had the highest inhibition zone ranging from 20-30 mm, implying that it is the more likely active fraction. Chloroform extract had a range of 20-25 mm, then methanol 20-24 mm. Petroleum ether extract had the least inhibition zone of 17-19 mm (Table 4.4). The antibacterial activity of the plant must be due to the phytochemicals identified in the extracts. This compares favourably with that of standard antibiotic drugs used as control i.e Sparfloxacin, Cefuroxime and Fluconazole.

5.2.3 Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) can be helpful in establishing the level of resistance of a particular bacterial strain and can substantially affect the decision to use certain antimicrobial agents. This was done to determine the minimum concentration of the extracts that can inhibit the growth of the microbes. From the results (Table 4.8); petroleum ether extract has MIC of 10 mg/ml methanol and chloroform extracts has 5 mg/ml (Table 4.5

and 4.7) for all the test organisms, while ethyl acetate extract has MIC of 5 mg/ml against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Candida albicans* and *Candida krusei*. The MIC value for *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumoniae* was found to be 2.5 mg/ml (Table 4.6) indicating that it is the most active (i.e. ethyl acetate extract) among the others. However, the MIC of the extracts on *Corynebacterium ulcerans*, *Streptococcus faecalis*, *Proteus mirabilis* and *Candida tropicalis* were not determined since they were resistant to all the extracts (Table 4.3). The effect of the plant extract on the MIC for the test microorganisms correlate with the report that microorganisms varied widely in the degree of their susceptibility (Emeruwa, 1982). Antimicrobial agents with a low activity against an organism have a high MIC while a highly active antimicrobial agent gives a low MIC.

5.2.4 Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

Determination of minimum bactericidal and fungicidal concentration (MBC/MFC) was carried out to check whether the test microbes were actually killed by the extracts or only their growth was inhibited. The results indicated that petroleum ether extract has MBC/MFC of 20 mg/ml for all the test microbes (Table 4.12); methanol and chloroform extracts has 10 mg/ml for *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae* (Tables 4.9 and 4.11) while ethyl acetate has 5 mg/ml for *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae* (Table 4.10). The results of the Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of methanol, ethyl acetate, chloroform and petroleum ether extracts revealed that the extracts has antibacterial and antifungal activity, ethyl acetate being the most active. Therefore, it can be said that the extracts of the root of *Acacia ataxacantha* can be used to treat infections like pneumonia,

cough, dysentery and respiratory diseases (caused by these microbes) as practiced by traditional healers.

5.3 SPECTROSCOPY

5.3.1 Spectral Analysis of ABA

The isolated compound ABA was subjected to FTIR spectroscopy (Figure 4.1) and the following absorption bands were observed. 3409.3 cm^{-1} which is characteristic of O-H stretching; 2924.18 cm^{-1} for C-H stretching; 1461.13 cm^{-1} for C=C stretching and 1033 cm^{-1} for C-O stretching (Coates, 2000 and David *et al.*, 2012). The ^1H NMR spectra (Figure 4.2) showed a peak at about 5.2 ppm corresponding to a methine proton; peak at about 3.2 ppm for oxy-methine ^1H ; 0.7 ppm (d) for ^1H of C-5. Peaks between 0.7-1.0 ppm revealed presence of methyl protons. The ^{13}C NMR spectra of ABA revealed the presence of thirty (30) carbon atoms (Figure 4.3). The chemical shift at $\delta 79.0$ signalled the presence of an oxy-methine carbon. Signals at $\delta 122.7$ and $\delta 143.3$ represented olefinic carbons. Methylene groups (CH_2) are responsible for signals observed at $\delta 38.7$, 29.3, 18.3, 32.4, 23.5, 27.2, 25.9, 30.9 and 41.7 while methyl groups (CH_3) are responsible for signals observed at $\delta 27.7$, 15.3, 14.0, 15.5, 23.4, 28.1, 17.0 and 22.6 (Table 4.17).

Distortionless Enhancement by Polarization Transfer (DEPT) experiment differentiates between methine (CH), methylene (CH_2) and methyl (CH_3) groups. The spectra at 135° angle gave fifteen (15) peaks for CH and CH_3 in a phase opposite to nine (9) peaks for CH_2 . Spectra at 90° angle gave seven (7) peaks for CH groups, while 45° angle gave all carbons with attached protons (regardless of number) in phase. Signals from quaternary carbons (C) are always absent due to the lack of attached protons thus are identified as the additional signals in the proton decoupled ^{13}C NMR spectra. The analysis thus indicates eight (8) methyl (CH_3) groups and six (6) quaternary carbon (C) groups (Figure 4.4).

All ^1H and ^{13}C NMR data signals were found to be in full agreement with those reported for α -amyrenol (Vazquez *et al.*, 2012; Niaz, 2013 and Fingolo *et al.*, 2013); a pentacyclic triterpenoid (ursane) which contains a double bond between positions 12 and 13 and in which the hydrogen at the 3β position is substituted by a hydroxyl group. The proposed structure for ABA is as in Figure 4.9

Structure of ABA

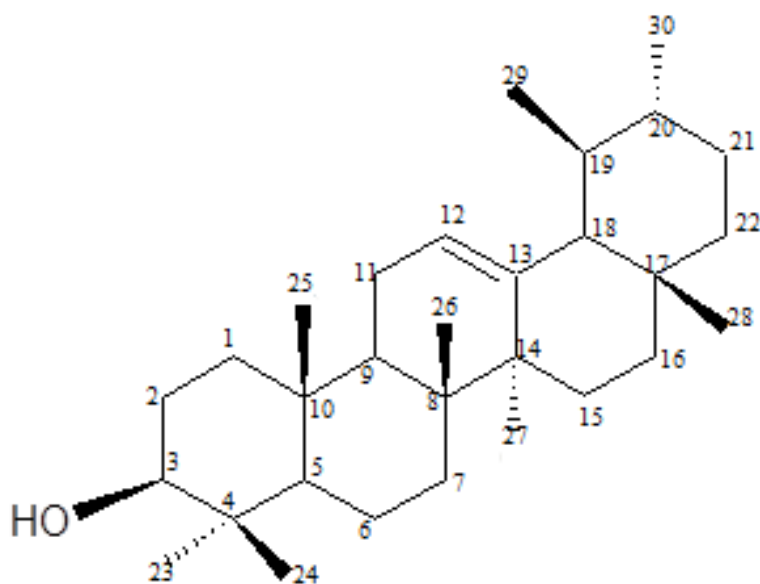


Figure 4.9: α -amyrenol $\text{C}_{30}\text{H}_{50}\text{O}$

IUPAC Name : (3 β)-Urs-12-en-3-ol or 12-Ursen-3 β -ol or Urs-12-en-3 β -ol

Other Names: Viminalol, α -amyrine, α -amyrin

5.3.2 Spectral Analysis of ABA 1

The IR spectrum of ABA 1 showed characteristic absorption frequencies at 3340.07cm^{-1} which is characteristic of O-H stretching; stretching vibrations due to methyl groups was represented by the band at 2925.15 cm^{-1} and the signal at 1446.66cm^{-1} was due to methylenic vibration; 1610.61 cm^{-1} for C=C stretching and 1040.63 cm^{-1} for C-O stretching; (Figure 4.5) (Cinta-Pinzaru *et al.*, 2012; Abdullahi *et al.*, 2013). The ^1H NMR spectrum showed one secondary hydroxyl group as doublet of doubles at δ 3.20. It also showed two olefinic protons at δ 4.6 and 4.7 representing the exocyclic double bond and seven methyl singlets at δ 0.95, 0.75, 0.82, 0.8, 1.0, 1.1 and 1.7 (Figure 4.6). The ^{13}C NMR spectra showed thirty (30) signals corresponding to 30 carbon atoms (Figure 4.7). Signals at δ 150.9 and δ 109.3 represented olefinic carbons. An oxygenated carbon shift was observed at δ 79.0 (Table 4.18).

DEPT experiment revealed seven (7) methyl (CH_3), eleven (11) methylene (CH_2), six (6) methine (CH) and six (6) quaternary carbon (C) groups (Figure 4.8). All ^1H and ^{13}C NMR data signals were found to be in full agreement with those reported for lupeol by Haba *et al.*, 2012; Prakash and Prakash, 2012 and Abdullahi *et al.*, 2013 which is a pentacyclic triterpenoid containing olefinic carbons of the exocyclic double bond at positions 20 and 29, and a hydroxyl group at C-3 position. The proposed structure for ABA 1 is as in Figure 4.10.

Structure of ABA 1

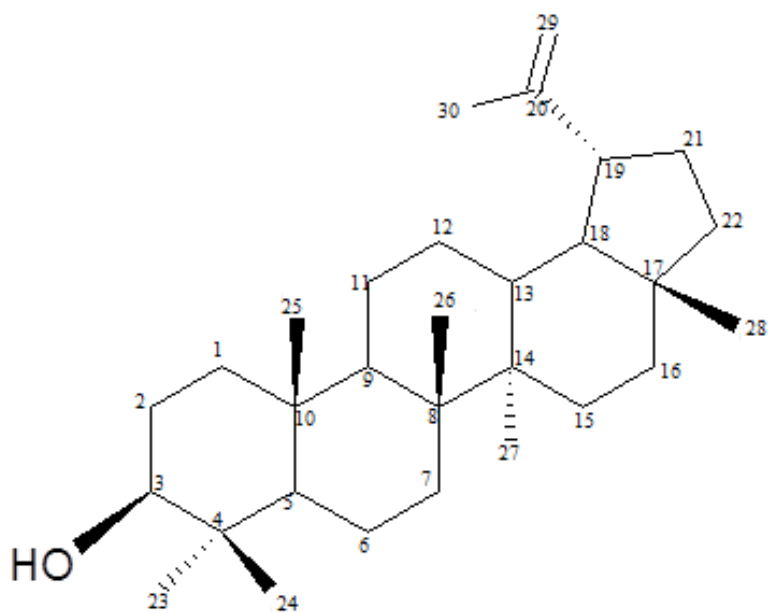


Figure 4.10: Lupeol $C_{30}H_{50}O$

IUPAC Name: (3 β)-lup-20(29)-en-3-ol; 20(29)-Lupen-3 β -ol; 3 β -Hydroxy-20(29)-lupene.

Other Names: Clerodol, Lupenol, Monogynol B, Fagarasterol, Farganasterol

5.4 ANTIMICROBIAL SCREENING OF ISOLATED COMPOUND (ABA)

The results showed that the test organisms *Bacillus subtilis*, *Streptococcus pneumonia*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Salmonella typhi*, *Escherichia coli*, *Candida albicans* and

Candida krusei were sensitive to the compound while *Corynebacterium ulcerans*, *Streptococcus faecalis*, *Proteus mirabilis* and *Candida tropicalis* were resistant just as in the case of the crude extracts (Table 4.13). The diameter of zone of inhibition showed a higher activity (as compared to the crude extracts) ranging from 24-31 mm (Table 4.14).

Table 4.15 showed the results of the Minimum Inhibitory Concentration (MIC). *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Salmonella enteritidis*, *Klebsiella pneumoniae*, *Candida albicans* and *Candida krusei* were affected by 25 µg/ml of the compound while *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* were affected by 12.5 µg/ml concentration. Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) results indicated that *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Salmonella enteritidis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Candida albicans* and *Candida krusei* were killed at concentration of 50 µg/ml while *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* were destroyed at concentration of 25 µg/ml (Table 4.16).

These bacteria have been known to cause typhoid fever, muscle pains, diarrhoea with abdominal pains (*S. typhi*); hair follicle infection, skin and wound infections, food poisoning, pimples (*S. aureus*); meningitis, eye infection, infection of wounds and burns (*P. aeruginosa*); diarrhea, gastroenteritis and urinary tract infection (*E. coli*); pneumonia, [thrombophlebitis](#), [cholecystitis](#), upper [respiratory](#) tract infection, wound infection, [osteomyelitis](#), [meningitis](#), bacteremia and [septicemia](#) (*K. pneumoniae*); [bronchitis](#), [rhinitis](#), [acute sinusitis](#), [otitis media](#) and [conjunctivitis](#) (*S. pneumoniae*) (Greenwood *et al.*, 1992). This shows that the plant extracts can be used as antibiotics. The result of Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) proves that *Acacia ataxacantha* could find its use in therapeutic preparations, especially in the diseases caused by *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumoniae*.

The compound isolated (α -amyrenol) was reported to have anti-inflammatory, antinociceptive, gastroprotective and hepatoprotective properties and acts as an antioxidant ([Melo et al., 2010](#)). Also used in the treatment of liver disorders ([Oliveira et al., 2005](#)), while lupeol displays [antiprotozoal](#), antimicrobial, anti-inflammatory, antitumor and [chemopreventive](#) properties ([Gallo and Sarachine, 2009](#)). It also possess significant antiurolithiatic activity ([Amritpal, 2011](#)), and is reported to reduce blood glucose by reducing the activity of alpha-amylase ([Adhyapak, 2014](#)).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMENDATION

6.1 SUMMARY

The root of *Acacia ataxacantha* DC was collected from Edumoga, Benue state Nigeria and was properly identified at the Herbarium of Biological Sciences A.B.U. Zaria with voucher number 1707. It was air-dried, pulverised and extracted. The crude extracts were subjected to phytochemical and antimicrobial screening. The Phytochemical screening gave positive results for flavonoids, glycosides, saponins, steroids/ triterpenes, tannins and alkaloids. The screening of the methanol, ethyl acetate, chloroform and petroleum ether extracts showed

that the plant root could inhibit the growths of *Bacillus subtilis*, *Streptococcus pneumonia*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Salmonella typhi*, *Escherichia coli*, *Candida albicans* and *Candida krusei* in varying degrees; but were not active on *Corynebacterium ulcerans*, *Streptococcus faecalis*, *Proteus mirabilis* and *Candida tropicalis*. The minimum inhibitory concentration (MIC) of the extracts were determined. Methanol and chloroform fractions had MIC of 5 mg/ml and petroleum ether had 10 mg/ml for all the test organisms, while ethyl acetate was the most active with 2.5 mg/ml for *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumonia*. The Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) showed that ethyl acetate extract could kill *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae* at a concentration of 5 mg/ml. Ethyl acetate extract was the most active, thus was subjected to chromatographic separations that led to the isolation of compounds ABA and ABA 1. The compounds were analysed spectroscopically (FTIR, ¹H NMR, ¹³C NMR and DEPT) and found to be α -amyrenol ((3 β)-Urs-12-en-3-ol) and lupeol ((3 β)-lup-20(29)-en-3-ol) respectively. The antimicrobial activity of ABA was determined with the same organisms. The MIC and MBC/MFC of ABA was found to be 12.5 and 25 μ g/ml respectively against *B.subtilis*, *E.coli* and *S.typhi*.

6.2 CONCLUSION

The phytochemical analysis of the root bark of *Acacia ataxacantha* was found to contain some components which are of medicinal value. They include alkaloids, glycosides, flavonoids, tannins and steroids/triterpenes. Antimicrobial screening of root extracts from the plant showed that the extracts were able to inhibit the growth of some bacteria and fungi such as *Bacillus subtilis*, *Streptococcus pneumonia*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Salmonella*

typhi and *Escherichia coli*; *Candida albicans* and *Candida krusei*. In conclusion, the findings in this research have justified the use of this plant in ethnomedical treatment of pneumonia, excessive cough, yellow fever, respiratory diseases, dysentery, and wounds which are caused by some of the organisms used in this study and other infections in which sparfloxacin and cefuroxime are used for treatment.

6.3 RECOMMENDATION

Further work can be carried out to isolate more bioactive compounds from the plant. There is need for biological and pharmacological studies of the isolated compound to determine their mode of action for possible use as chemotherapeutic drugs by humans.

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