LEAF EXTRACT OF *COMBRETUM HYPOPILINUM* (DIELS) OKAFOR (COMBRETACEAE)

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PHYTOCHEMICAL AND ANTICONVULSANT STUDIES ON THE METHANOL LEAF EXTRACT OF *COMBRETUM HYPOPILINUM* (DIELS) OKAFOR (COMBRETACEAE)

BY

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DECLARATION

I declare that the work in this dissertation entitled Phytochemical and Anticonvulsant Studies on the Methanol Leaf Extract of *Combretum hypopilinum* (Diels) Okafor (Combretaceae) has been carried out by me in the Department of Pharmaceutical and Medicinal Chemistry. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

BASHIR BAWA GARBA

_____ _____ _____

Signature Date

CERTIFICATION

This dissertation entitled PHYTOCHEMICAL AND ANTICONVULSANT STUDIES ON THE METHANOL LEAF EXTRACT OF*COMBRETUM HYPOPILINUM* (DIELS) OKAFOR (COMBRETACEAE) by Bashir Bawa GARBA meets the regulations governing the award of the degree of Master of Science in Pharmaceutical Chemistry of the Ahmadu Bello University, and is approved for its' contribution to knowledge and literary presentation.

Prof. (Mrs) H.S. Hassan

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Chairman, Supervisory Committee	Signature	Date

Dr. Y.M. Sani

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Member, Supervisory Committee	Signature	Date

Prof. A. Musa

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Head of Department	Signature	Date

Prof. S.A. Abdullahi

_____	_____	_____
Dean, School of Postgraduate Studies	Signature	Date

DEDICATION

This work is dedicated to Almighty ALLAH SWT for His infinite mercies, guidance, protections and the means for making this work successful.

ACKNOWLEDGEMENT

All praises are due to ALMIGHTY ALLAH SWT for guiding me throughout the program. I wish to express my profound gratitude to my supervisors Prof. (Mrs) H.S. Hassan and Dr. Y.M. Sani, who despite their schedules, still had time to guide me, and effect corrections throughout the conduct of this research. I will also like to acknowledge the assistance rendered by Prof. A.M. Musa and Dr. M.G. Magaji, may Allah SWT reward them abundantly. I will also acknowledge the co-operation and relentless encouragements of helping hands received from the technical staff of Departments of Pharmaceutical and Medicinal Chemistry, Pharmacology and Therapeutics, Pharmacognosy and Drug Development of Ahmadu Bello University, Zaria.My sincere gratitude goes to my parents Alhaji Ahmed Bawa Garba and Hajiya Hauwa Ahmed Garba for the moral and financial support they offered which made me who I am today. May Allah (SWT) grant them long healthy and blissful life in this world and the hereafter. Not at all have I forgotten my siblings, uncles, aunts may Allah SWT bless you all. I will also appreciate the efforts of my friends and colleagues for their supports, advice and knowledge we have impacted on one another directly or indirectly. May ALLAH SWT reward us all in abundance.

ABSTRACT

Combretum hypopilinum is used by the traditional medicine practitioners for the treatment of epilepsy, hepatic disorders, snake bites, diarrhoea, relief of pains and headache. This study was aimed at investigating the anticonvulsant potentials in chicks and mice and to isolate compound(s) present from the methanol leaf extract (MLE) of *Combretum hypopilinum*. Preliminary phytochemical screening and oral acute toxicity studies were conducted using standard methods. Anticonvulsant studies were conducted using maximal electroshock test (MEST) in chicks and pentylenetetrazole induced-seizure (PTZ) in mice. The phytochemical screening revealed the presence of terpenoids, steroids, carbohydrates, cardiac glycoside, saponins, flavonoids, tannins and alkaloids. The column chromatographic analysis of the n-hexane fraction from the MLE led to the isolation of lupeol and lupenone which were characterized by physicochemical tests and spectroscopic analysis and by comparison with data from literature. The oral median lethal dose (LD_{50})of the MLE was found to be greater than 5000 mg/kg body weight in mice. The MLE at all tested doses (125, 250 and 500 mg/kg) did not exhibit significant effect on the mean recovery time from seizure in the MEST and none of the chicks were protected against tonic hind limb extension (THLE). However in the PTZ-induced seizure test, MLE at the highest dose of 500 mg/kg significantly ($p<0.05$) increased the mean onset of seizures. In conclusion, the result of this study suggests that the methanol leaf extract of *Combretum hypopilinum* contains compound(s) that possess anticonvulsant activity, thus, providing scientific rationale for the plant's ethnomedicinal use in the management of epilepsy.

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Abbreviations

Latin Abbreviation	English Translation
TLC	Thin Layer Chromatography
WHO	World Health Organisation
LD_{50}	Median Lethal Dose
NMR	Nuclear Magnetic Resonance
R_f	Retention Factor
^{13}C NMR	Carbon-13 Nuclear Magnetic Resonance
^{1}H NMR	Proton Nuclear Magnetic Resonance
$CDCl_3$	Deuterated Chloroform
HMBC	Heteronuclear Multiple Bond Correlation
HQSC	Heteronuclear Spin Quantum Correlation
DEPT	Distortionless Enhancement by Polarization Transfer
*Sc*PTZ	Subcutaneous Pentylenetetrazole
MEST	Maximal Electroshock Test
MLE	Methanol Leaf Extract
nHF	n-Hexane fraction
CF	Chloroform fraction
EF	Ethyl acetate fraction
C.	*Combretum*
VPA	Sodium Valproate
PHT	Phenytoin
AEDs	Anti-epileptic Drugs
THLE	Tonic Hind Limb Extension
ILAE	International League Against Epilepsy
GABA	Gamma Amino Butyric Acid

CHAPTER ONE

1.0 INTRODUCTION

1.1 Natural Product

A natural product is a chemical compound produced by living organisms found in nature (Khaleel, 2018). Natural products represent rich sources of molecular diversity in compound libraries, as an important part of our achievement in scientific research, and have greatly promoted the development of medicine, synthetic chemistry, and ecological systems. (Newman and Cragg, 2020).The major challenges associated with natural products are mainly isolation, purification, and structure elucidation. Structure elucidation is generally considered a vital task in natural product chemistry research (Brown and Lawrence, 2017). Natural products have received constant attention from scientific communities due to their relevance in drug discovery, chemical ecology and molecular biology in general (Sorokina and Steinbeck, 2020). Global demand for medicinal plants and fungi has threatened certain species, contributing to biodiversity loss and depletion of natural resources that are important for the health of humanity (Howes*et al.*, 2020). Today, plants and fungi are embedded in global healthcare systems as sources of pharmaceuticals (Newman and Cragg, 2020) or as traditional/complementary medicines, and are often associated with cultural and social significance (WHO, 2019). Scientists, governments, and other stakeholders must establish functional and equitable agreements to ensure that with respect to therapeutics from nature, there is compliance with the Nagoya Protocol and Associated Access and Benefit Sharing Legislation and Consideration of the Value and Origins of any Specimens Collected (Pérez-Escobar *et al.*, 2020).

Phytochemicals are non-nutritive active plant chemical or bioactive compounds and are responsible for protecting the plant against infections. Some are responsible for the colours, aromas and other organoleptic properties (Alamgir, 2018). Phytochemicals have been designated as one of the important area of traditional system of medicine and are extensively used worldwide due to wide-spread applicability and multiple pharmacological applications (Bawa *et al*., 2019).

1.2 Traditional Medicine

Traditional medicine (TM) also referred to as folk medicine or indigenous medicine, comprises medical aspects of traditional knowledge that developed over generations within various societies before the era of modern medicine. The World Health Organization (WHO) has defined traditional medicine as the sum total of the knowledge, skills, and practices based on the theories and experiences indigenous to different cultures, whether it is explainable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness (WHO, 2008).

The development and use of TM has a very long historical background that corresponds to the Stone Age (Ezekwesili-Ofili and Okaka, 2019). African traditional medicine is a form of holistic health care system that is organized into three levels of specialty, which includes; divination, spiritualism, and herbalism, though these may overlap in some situations (Mahomoodally, 2017).

In some Asian and African countries, up to 80% of the population relies on TM for their primary health care needs (WHO, 2008). When obtained outside its traditional culture, TM is often considered a form of alternative medicine. The WHO noted that, the inappropriate use of traditional medicines or practices can have negative or dangerous

effectsand also, further research is needed to ascertain the efficacy and safety of several practices and medicinal plants used by TM systems (WHO, 2008). Some researchers stated that many of the alternative treatments are statistically perceived from an inert treatment (The Economist, 2011).Many countries have practices TM which may co-exist with official, science-based, and institutionalized systems of medical practice represented by the conventional medicine (WHO, 2018). Some examples of TM includes; Traditional Chinese medicine (TCM), Traditional Korean medicine (TKM), Arabic indigenous medicine (AIM), Uyghur traditional medicine (UTM), Japanese Kampō medicine (JKM), Traditional Aboriginal bush medicine (TABM), and Georgian folk medicine (GFM), among others (WHO, 2013).The term African traditional medicine (ATM) is not comparable with the alternative and complementary medicine. ATM is the African natural system of health care, therefore cannot be seen as an alternative,the dosage are not clearly expressed, and some of the medicines are prepared under unclean conditions as evidenced by microbial contamination of many herbal preparations sold in the markets (Ezekwesili-Ofili and Okaka, 2019).

Plants have been the primary source of most medicines in the world, and they still continue to provide mankind with new remedies (Mbele *et al.*, 2017). Natural products and their derivatives represent more than 50% of all drugs in clinical use, of which higher plants contribute more than 25%. A number of African plants have found their way in modern medicine. These plants which had been used traditionally for ages have through improved scientific expertise been the sources of important drugs. A host of many African plants with promising pharmaceutical potentials includes;*Cajanus cajan, Balanites aegyptiaca, Acanthospermum hispidum, Calotropis procera, Jatropha curcas,* among others, as potential sources of anticancer agents (Mbele *et al.*, 2017).

1.2.1 Medicinal plants and epilepsy

They are considered to be thebackbone of traditional medicine and are widely used to treat acute and chronic diseases (WHO, 2013). The World Health Organization estimated that perhaps 80% of the inhabitants of the world rely on traditional medicines (WHO, 2013). It therefore, approved the use of herbal products for national policies and drug regulatory measures in order to strengthen research and evaluation of the safety and efficacy of herbal products (WHO, 2013).It was also reported that 17 medicinal plants including; *Uncaria rhynchophylla, Cannabis*(Cannabinoid compound),*Desmodium triflorum, Viscum album, Morus alba, Berberis integerrima, Mussaenda philippica, Justicia pectoralis, Gladiolus dalenii, Ficus religiosa, Withania somnifera, Lobelia nicotianaefolia, Marsilea quadrifolia, Passiflora incarnate, Mondia whitei, Gastrodia elata*and*Phytol*have shown anticonvulsant activity in animal models (Stephen, 2018).It was reported that, in Africa many plants such as,*Cannabis sativa, Canscora decussate, Cinchona officinalis, Mognolia officinalis, Nardastachys jatamansi, Panax ginseng, Rauwolfia serpentina, Vaeriana officinalis, Ergot* (clavicepsperpu) (Ahirrao *et al.*, 2017) possessed anticonvulsant activities.

1.3 Statement of Research Problem

Epilepsy constitutes the fourth most common neurological disorders for all ages, and also the most common chronic neurological disorders in childhood in many countries (Giannakaki, 2020).Epilepsy is a major public health problem in low and middle income countries of which more than 80% of people living with epilepsy are found (Igwe, 2016). Individuals living with epilepsy in the low and middle income countries receive inadequate or no treatment which often leads to huge treatment gap (Igwe and Aronu, 2021). A proper diagnosis of epilepsy is one of the greatest challenges facing epilepsy care in the low and middle income countries (Igwe, 2016). The first contact for patients with epilepsy usually

involves all health sectors which includes; the herbalists, spiritualist and the orthodox medical practitioners in primary care settings (Igwe, 2017). Epilepsy is a disorder that is associated with lots of social stigma and diverse cultural beliefs as its causation and management (Igwe, 2016). In Nigeria, the management of epilepsy has been inadequate as a result of widespread poverty, illiteracy, beliefs in the causes of epilepsy, social stigma, inequity in the availability of specialized care centres, and inefficient health care system (Ojinnaka *et al*., 2019).Recent studies have shown that older persons with epilepsy are more likely to suffer from mental dysfunctionsand might be bi-directional relationship between epilepsy and dementia (Sen *et al.,* 2018).

1.4 Justification for the Study

Epilepsy accounts for a significant proportion of the world's disease burden, affecting more than50 million people worldwide. The estimated proportion of the general population with active epilepsy at a given time is between 4 and 10 per 1000 people. However, some studies in low and middle income countries suggested that the proportion is higher, between 7 and 15 per 1000 people.It was estimated globally that 2.4 million people are diagnosed with epilepsy each year. In high-income countries, new annual cases are between 30 and 50 per 100,000 people in the general population. While, in low-and middle-income countries, this figures can be up to two times higher. This is likely due to the increased risk of endemic conditions such as malaria or neurocysticercosis, the higher incidence of road traffic injuries, birth-related injuries, and variations in medical infrastructure, availability of preventative health programmes and accessible care (WHO Factsheet, 2019).

About 80% of people with epilepsy live in lowand middle-income countries (WHO factsheet, 2019).However, studies combining detailed management, treatment outcomes

and the cost of treatment of epilepsy are rare in the country particularly in the northern part of Nigeria (Ipingbemi, 2015). Additionally, 20-30% of those patients are resistant to treatments with synthetic drugs so, it is necessary to discover new treatments by using the natural medicine to reduce the complications or sideeffects of antiepileptic drugs (Herrera-Calderon *et al.*, 2018). The scientific investigations of the anticonvulsant activity on the leaf of *Combretum hypopilinum* will confirm or otherwise disprove its traditional use.

1.5 Aim and Objectives

1.5.1 Aim

To isolate and characterize some of the compound(s) and scientifically validate the ethnomedicinal claim for the use of *Combretum hypopilinum* in the treatment of epilepsy.

1.5.2 Specific objectives

1. To identify the phytochemical constituents present in the methanol leaf extract and partitioned fractions of *Combretum hypopilinum* using standard methods.
2. To isolate and characterize some of the compounds present using chromatographic methods and spectral analysis.
3. To determine the oral median lethal dose LD_{50} (Acute toxicity) using mice.
4. To determine the anticonvulsant activity using the MES and *sc*PTZ test in chicks and mice respectively.

1.6 Null Hypothesis

The methanol leaf extract of *Combretum hypopilinum*does not contain phytochemical constituents with anticonvulsant activity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Plant *Combretum hypopilinum*(Diels) Okafor

2.1.1 Botanical description of *Combretum hypopilinum*

The plant *Combretum hypopilinum* Diels (Combretaceae) is a synonym of *Combretum collinum* Fresen sub-specie *hypopilinum* (Diels) Okafor (Idoh *et al.*, 2018). It is a small to medium-sized tree with several stems, deciduous, up to 12-17 m tall (Idoh *et al*., 2018).

2.1.2 Ecological and geographical distribution of *Combretum hypopilinum*

The plant is highly dominant where itoccurs and is widespread, extending from West Africa (Gambia, Nigeria, Ghana, Guinea, and Togo), Central Africa Republic to East Africa (Uganda) and Sudan. It grows in various soils, with semi-arid to moderate rainfall conditions (Idoh*et al.*, 2018).

2.1.3 Taxonomy of *Combretum hypopilinum*

The genealogy of the plant is as follows;

Kingdom: Plantae

Phylum: Magnoliopsida

Order: Myrtales

Class: Streptophyta

Family: Combretaceae

Genus: Combretum Loefl.

Species: hypopilinum(Diels).

2.1.4 Common names*Combretum hypopilinum*

Bush willow (English)

Jar Taramniya (Hausa)

Katankara (Kanuri)

Aro (Yoruba)

Dagbani (Ghana)

Pupiong (Togo)

Plate I:*Combretum hypopilinum* in its Natural Habitat (Giwa Local Government Area, Kaduna State-Nigeria)

2.1.5 Ethnomedicinal uses of *Combretum hypopilinum*

The plant (*Combretum hypopilinum*) has many uses in Africa, especially in traditional medicine. The whole parts of the plant are used forthe treatment of epilepsy (Muazu and Kaita, 2008).Infusion of fresh or dried leaves and decoction of root bark are taken as any agent that promotes the discharge of bile, diuretic, purgative and also to treat gastrointestinal disorders, including diarrhoea, dysentery and stomach aches (Fyhrquist *et al.*, 2004).

Decoction of leaves and twigs is taken as a drink, and fresh roots are chewed to treat lung problems such as cough, bronchitis, and tuberculosis and also to treat snake bites and jaundice (Elof, 1999). Maceration or decoction of root bark is taken to treat gonorrhoea and infertility in women and men. In addition, the infusion of leaves and roots is taken as a blood tonic (Abreu *et al.*, 1999). The gum exuded from injured branches are edible and used to cure toothache or to plug a tooth with caries (Burkill, 1985).A twig bark and root decoction is given to relief pains and headache (Adamu *et al.*, 2005).The decoction of the root bark is used to alleviate various ailments including hepatic disorder (Idoh *et al.*, 2018).

2.1.6 Pharmacological properties of *Combretum hypopilinum*

Extract of *Combretum hypopilinum* has been shown to have antimicrobial properties against both gram positive and gram negative organisms and also have antineoplastic properties (Pettic, 1995). It has been reported that an aqueous extract of stem bark and root of *Combretum hypopilinum* showed antibacterial activity against *Proteus mirabilis,* while different leaf extracts showed weak antifungal activities *in vitro* (Fyhrquist *et al.*, 2004).Antifungal activity was reported in models that used *Epidermophyton floccosum*, *Microsporum gypseum*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Sporothrix schenckii* and

Microsporum canis (Baba-Moussa *et al.*, 1999; Masoko *et al.*, 2007). *Combretum molle* was also able to inhibit the growth of *Mycobacterium tuberculosis* (Lall and Meyer, 1999). Anti-trypansomal andanthelmintic activities of different extracts have also been reported (Atindehou *et al.,* 2004; Ademola and Elof, 2010).

In the case of compounds obtained from *Combretum molle*, the analgesic and anti-inflammatory properties of mollic acid glucoside (MAG), a 1-α-hydroxycycloartenoid extracted from *Combretum molle* leaves, have been investigated in mice and rats (Ojewole, 2008). Chika and Bello, (2010) demonstrated an antidiabetic effect for the aqueous leaf extract of *Combretum micranthum.* The Antibacterial studies of *Combretum molle* have demonstrated its activity against *Staphylococcus aureus* and *Helicobacter pylori* at different extract concentrations (Njume *et al.*, 2011).The protective effectof the ethanol root bark extract of*Combretum hypopilinum* Diels of against carbon tetrachloride CCl_4-induced acute hepatotoxicity in Wistar rat was also reported (Idoh *et al.*, 2018).

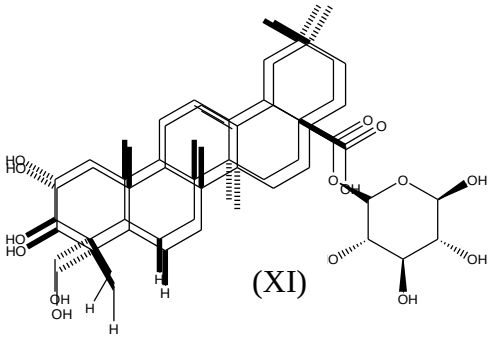
2.1.7 Phytochemistry of thegenus *Combretum*

The phytochemical studies carried out in the genus *Combretum* have demonstrated the occurrence of many classes of constituents, including triterpenoids, flavonoids, lignans and non-protein amino acids, among others (Pietrovski *et al.*, 2012). Bisoli *et al.* (2008)isolated 11 triterpenes and their glycosides from *Combretum laxum* including; oleanane, ursane and lupane-type triterpenoids such as arjunolic acid (XI), arjunglucoside II (XII), bellericoside (XIII), chebuloside II (XIV), quadranoside IV (XV), asiatic acid (XVI) and betulinic acid (XVII) (Bisoli *et al.*, 2008). Cycloartane dienone lactone was isolated from *Combretum quadrangulare* (Banskota *et al.,* 2000).Moreover, a series of amino acids have been

isolated from gum exudates, the most important being aspartic acid (XVIII), glycine (XIX), glutamic acid (XX), and alanine (XXI) (Anderson *et al.*, 1987).

Some flavonoids, rhamnoctrin (XXII), quercetin-5, 3'-dimethylether (XXIII), ramnazin (XXIV) and kaempferol (XXV) were isolated from *Combretum erythrophyllum* (Martini *et al.*, 2004), as well as quercetin (XXVI), kaempferol and pinocembrin (flavanone) (XXVII) from *Combretum apiculatum* (Aderogba *et al.*, 2012). Cardamonin (chalcone) (XXVIII) was also isolated from *Combretum apiculatum* (Aderogba *et al*, 2012) and ellagic acid derivatives from *Combretum kraussii* (Chaabi *et al.*, 2008).*Combretum hypopilinum*was found to contain choline, vitexin, oxalic, mallic and gallic acid (Muazu and Kaita, 2008).

To the best of our literature search, there is no report on isolation and characterization of any compound from the leaf of this plant.



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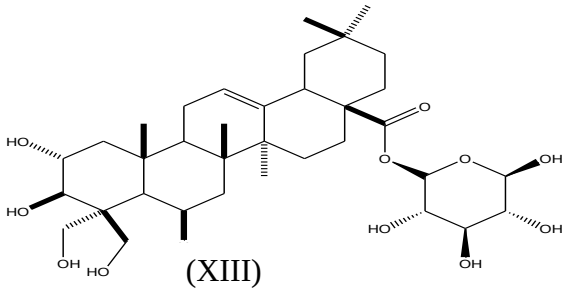

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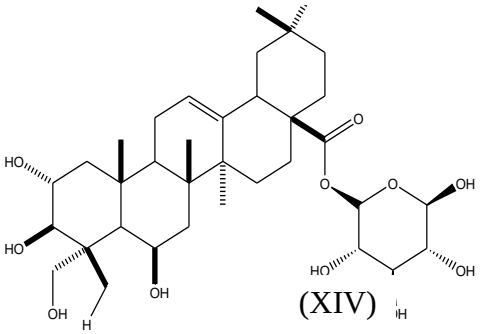

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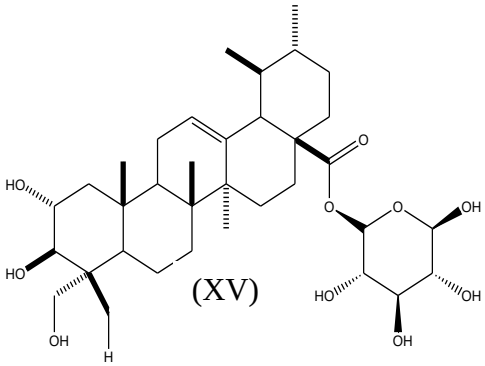

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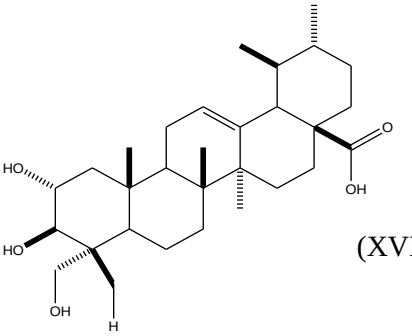

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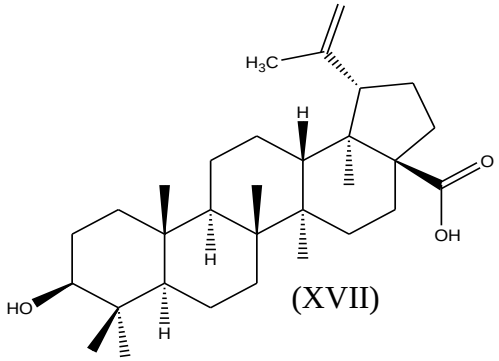

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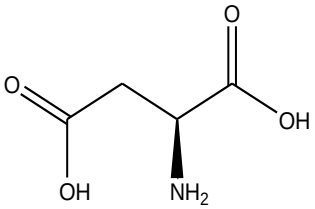

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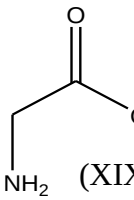

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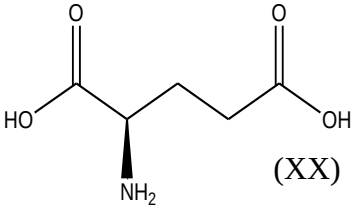

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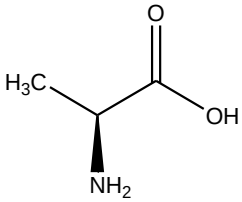

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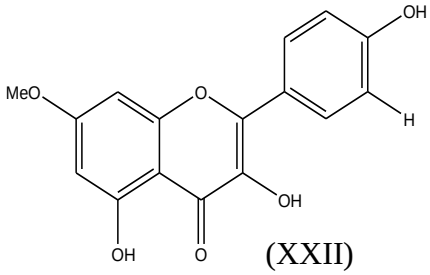

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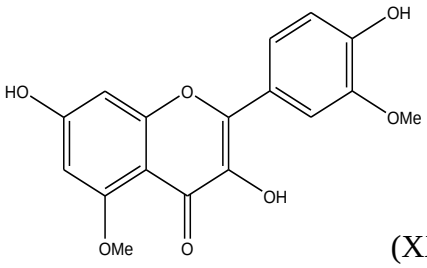

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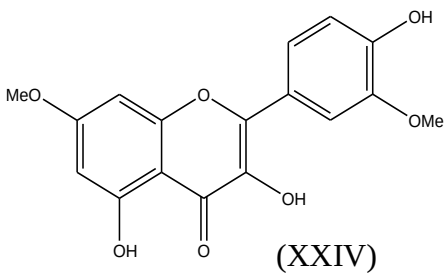

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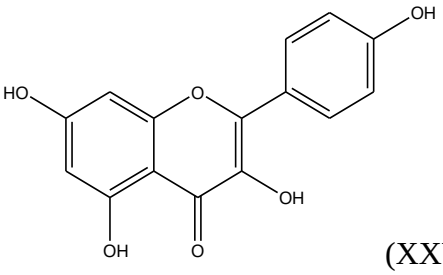

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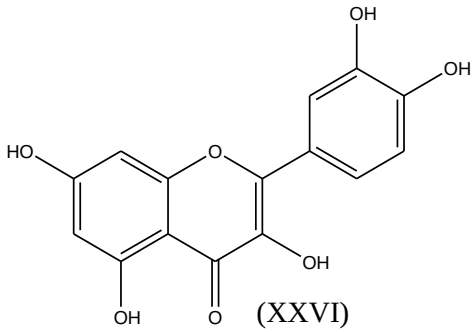

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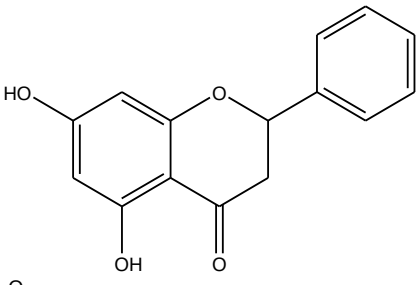

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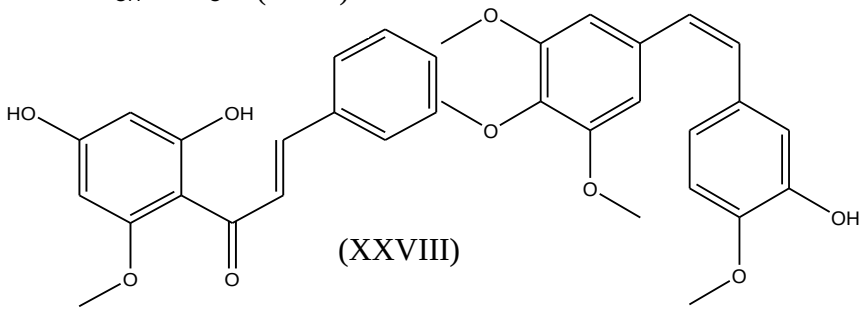

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2.2 Epilepsy

Epilepsy is a chronic disorder of the brain that affects people worldwide (WHO Factsheet, 2018). It is characterized by recurrent seizures, which are brief episodes of involuntary movement that may involve a localize part in the body or the entire parts body, and are sometimes accompanied by the loss of consciousness and control of bowel or bladder function (WHO Factsheet, 2018).

Seizure episodes are a result of excessive electrical discharges in a group of brain cells. Different parts of the brain can be the site of such discharges. Seizures can vary from the briefest lapses of attention or muscle jerks to severe and prolonged convulsions and it can also vary in frequency, from less than one per year to several per day (WHO Factsheet, 2019). One seizure does not signify epilepsy (up to 10% of people worldwide have one seizure during their lifetime (WHO Factsheet, 2019). Fear, misunderstanding, discrimination and social stigma have surrounded epilepsy for centuries. This stigma continues in many countries today and can impact on the quality of life for people with the disease and their families (WHO Factsheet, 2019).

Multiple studies have shown an increased risk of premature death in people with epilepsy as compared with the general population. This has been summarized in a recent systematic review prepared by the Mortality Task Force of the International League against Epilepsy (Thurman *et al.*, 2017). Sudden unexpected death in epilepsy and status epilepticus are important causes of epilepsy-related death but these generally account for fewer than 5% of deaths. The majority of underlying causes of death are related to somatic comorbidities. The most frequent of these are non-cerebral neoplasm, cardiovascular disease, and cerebrovascular disease (Keezer *et al.*, 2016). Interestingly, however, among those who are seizure-free, the risk of premature mortality remains elevated. In a set or group of 695 individuals with a history of epileptic seizures, even among those with only a single notified seizure, the risk of an early death compared with the general population after almost 25 years of follow-up was increased by between 49% (in those with an unknown cause) and 72% (in those with a putative etiology), controlling for differences in age, sex, and calendar year (Bell *et al.*, 2016).

The most important associations include structural and functional diseases of the central nervous system such as stroke, dementia, and migraine, but non-neurological disorders are also increased. For instance, heart disease, hypertension, chronic obstructive pulmonary disease, and neoplasm are more likely to occur in people with epilepsy than in the general population (Keezer *et al.*, 2016).

2.2.1 Etiologyof epilepsy

Epilepsy is not contagious (WHO Factsheet, 2019). The most common type of epilepsy which affects 6 out of 10 people with the disease, is called *idiopathic epilepsy* and has no identifiable cause, while epilepsy with a known cause is called secondary epilepsy or *symptomatic epilepsy*. The causes of secondary (symptomatic) epilepsy includes: brain damage from prenatal or perinatal causes (e.g. a loss of oxygen or trauma during birth, low birth weight), congenital abnormalities or genetic conditions with associated brain malformations, a severe head injury, a stroke that restricts the amount of oxygen to the brain, an infection of the brain such as meningitis, encephalitis, neurocysticercosis, certain genetic syndromes, and a brain tumour (WHO Factsheet, 2019).

2.2.2 Pathophysiologyof epilepsy

Epileptic seizures arise from an excessively synchronous and sustained discharge of a group of neurons, and the single feature of all epileptic syndromes is a persistent increase of neuronal excitability. Abnormal cellular discharges may be associated with a variety of causative factors such as trauma, oxygen deprivation, tumours, infection, and metabolic derangements. However, no specific causative factors are found in about half of the patients suffering from epilepsy(Engelborghs *et al.*, 2000).

2.2.2.1 Disorders of neuronal migration

Neuronal migration is a remarkable process in which new-born neurons in the developing brain move to their final destination where they will make proper short and long distance contacts and will form functional neuronal circuits. The human brain is far larger than that of the mouse, way more complex, and develops over a longer time scale. Whereas in humans, develop- mental neuronal migration occurs during months of gestation, and continues in the early postnatal period, in rodents this process is completed within days (Reiner *et al*., 2020).

2.2.3 Classification of epilepsy

The most recent classification of seizures and epilepsies was the International League Against Epilepsy (ILAE), 2017. This new classification is better organized with a clear elucidation of terminologies and list some new seizure types. Better diagnosis and management of seizures and epilepsy is achieved when classified and grouped into similar entities as different drugs which are usually effective for different types of seizure (Dhinakaran and Devendra, 2019). In this classification by ILAE, the clinical features of epilepsy are categorized into three, which includes; the seizures, epilepsies, and epilepsy syndromes. Emphasis have been made to consider etiology and comorbidities at each level (Zuberi and Brunklaus, 2017).

Epilepsy was also declared as a curable disease rather than a disorder. It is said to be resolved after ten years of the seizure-free period with the last five years spent without medications, or the patient is no longer at risk for age-related epilepsy syndrome (Fisher and Bonner, 2018). The older terminologies such as; convulsion, dyscognitive, simple partial, complex partial, psychic, and secondarily generalized were removed from the new classification due to serious criticism, as they were not completely understood by the

patient and the public, since these were the ones that watch the seizure event, not the physician in most of the cases (Brodie*et at.*, 2018). Focal seizure with secondary generalization was replaced by focal to bilateral tonic-clonic terminology as it better describes the propagation pattern of the seizure (Dhinakaran and Devendra, 2019). Dyscognitive was replaced by focal impaired awareness, Grand mal was replaced by generalized tonic-clonic, unknown by onset tonic-clonic and infantile spasm replaced by epileptic spasm (Fisher *et al*., 2017).

Other newer terminologies includes emotional seizure, cognitive seizure, absence with eyelid myoclonia, myoclonic, atonic, focal myoclonic, focal tonic, focal epileptic spasms, behaviour arrest, unaware, and unclassified seizures (Sarmast *et al.*, 2020). The term unconsciousness was avoided in the new classification, because, it could be confusing as to whether the patient has really lost consciousness. Therefore, aware was introduced because a person may be fully conscious but not aware of what happened (Zuberi and Brunklaus, 2017).

Absence seizure, which used to be called petit mal, is when an individual loses awareness of the surroundings which usually last up to 15 seconds and is accompanied by the loss of memory (National Health Service, 2020). It mainly affects children below the age of 14, but can also occur at any age, in rare case (National Health Service, 2020). During an absence seizure, the individual may stare blankly into space, look like they are is daydreaming, fluttering or blinking of the eyes and also making slight jerking movement of the body or limbs (National Health Service, 2020).

2.2.4 Status epilepticus

Status epilepticus (SE) is a neurological emergency requiring immediate evaluation and management to prevent significant morbidity or mortality (Xu, 2019).

Status epilepticus maybe convulsive, non-convulsive, focal motor, myoclonic and any can become refractory. Convulsive status epilepticus consists of generalized tonic-clonic movements. Focal motor status epilepticus involves the refractory motor activity of a limb or a group of muscles on one side of the body with or without the loss of consciousness is known as myoclonic status epilepticus. Refractory status epilepticus refers to continuing seizures (convulsive or non-convulsive) despite appropriate antiepileptic drugs(Won *et al*., 2019). Status epilepticus is the most common paediatric neurological emergency (Matricardi *et al*., 2019).

2.2.5 Management of epilepsy

The aim of the management of epilepsy is to reduce the frequency or to eliminate the seizures using the following steps:

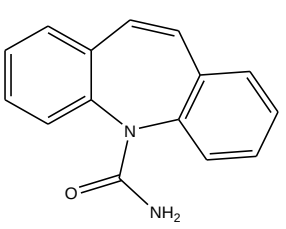
i. Determine whether a phenomenon is a seizure or not,
ii. Determine whether the seizure has an immediate identifiableand correctable cause (provoked) OR epilepsy as discussed above,
iii. Classify and treat epilepsy, look for co-morbidities andworkup for a cause for the condition, and
iv. Identify syndromes,it should be noted that status epilepticus, provoked or with a background of thechronic condition of epilepsy is an emergency.

It is worth notingthat a good description of the clinical presentation helps further in the assessment of electroencephalograms (EEGs) and might be a reason to initiatetherapy on its own merits (Koutroumanidis*et al.*, 2017).

2.2.6 Antiepileptic drugs (AEDs)

Antiepileptic drugs (AEDs) are widely used for the treatment of epilepsy, a condition which affects approximately ten million children globally (Atanaskovic-Markovic *et al*.,

2019).The effect of AEDs on mental dysfunction has been elucidated (Sen *et al*., 2018). Several newer-generation of AEDs have undergone comparative trials demonstrating efficacy equal to or and tolerability at least equal to or better than older AEDs as first-line therapy (Abou-Khalil, 2016). AEDs are classified according to their chemical structure, namely; aromatic and non-aromatic, examples of the aromatic AEDs includes; Lamotrigine (XXXIII), Carbamazepine (XXXI), Phenobarbital (XXXV), Phenytoin (XXXIV), oxcarbazepine, Benzodiazepine (XXXVI), felbamate, zonisamide, primidone.Examples of the non-aromatic AEDs includes; Sodium valproate (XXXII), Topiramate (XLII), levetiracetam (XXXVIII), clobazam, Ethosuximide (XXXVII), gabapentin (XXXIX), Pregabalin (XL), Vigabatrin (XLI) and lacosamide (Atanaskovic-Markovic *et al*., 2019).



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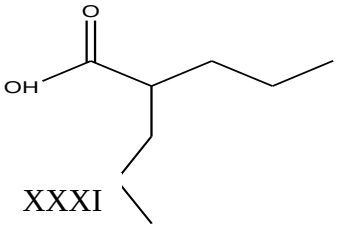

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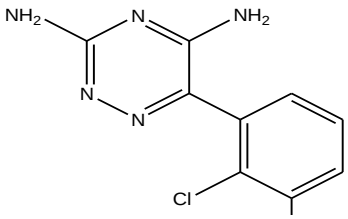

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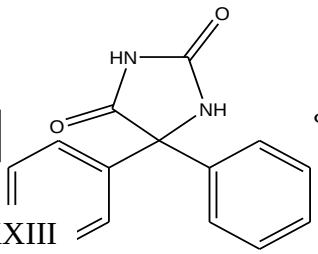

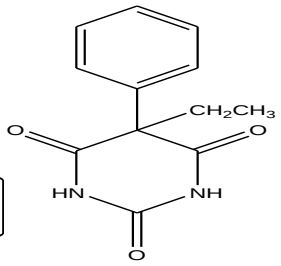

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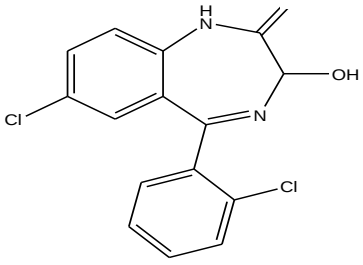

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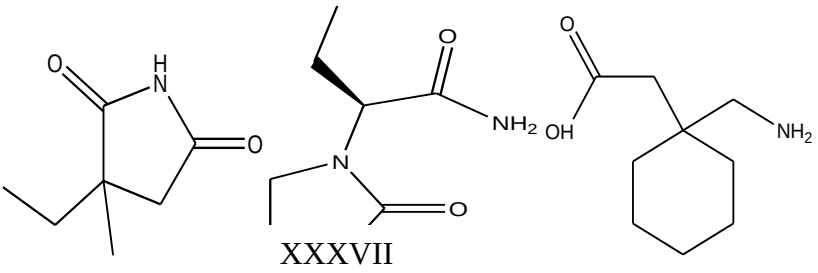

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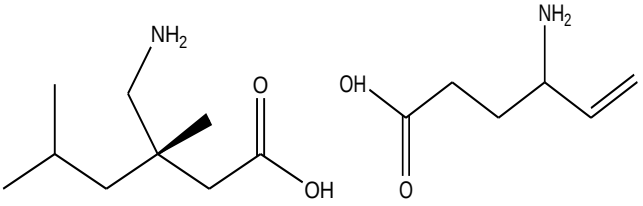
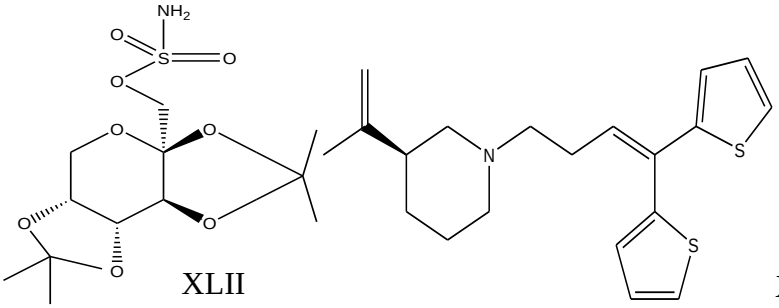


XL

XLI



XLII

XLIII

2.3 Experimental Models for Screening Anticonvulsant Agents

In the late 1800s, researchers began to elicit seizure activity in dogs using electrical stimulation and chemoconvulsants derived from naturally occurring chemicals such as pentylenetetrazole (PTZ), which was found to be a gamma-amino butyric acid A (GABAA) receptor antagonist (Loscher, 2017).

Despite these encouraging research developments, there was no accepted procedure for drug screening, and it was not feasible to screen for many compounds given the cost and

labour-intensive nature of seizure induction in those early models. Furthermore, the experimental protocols employed did not lead to consistent results (Rho and White, 2018).

However, in the early 1930s it changed when Merritt and Putnam adapted a simple and reliable electroshock seizure model test in cats, in using this new test, they were able to screen many compounds, and also discovered phenytoin which was found to be more effective and less sedating than either potassium bromide or phenobarbital, which remained the primary treatment for various forms of epilepsy for decades to come (Rho and White, 2018).

In 1944, Everett and Richards used the PTZ seizure model in mice, which was able to block absence seizures in humans, unlike phenytoin, that was ineffective in the PTZ model. Therefore, the combination of the electroshock model used by Merritt and Putnam and the PTZ model by Everett and Richards laid the foundation for later efforts and adaptations of these early animal models for more expanded drug-screening approaches, notably the U.S. National Institutes of Health (NIH), National Institute of Neurological Disorders and Stroke (NINDS)sponsored Anticonvulsant Screening Program (ASP) in the 1970s (Porter and Kupferberg, 2017).

2.4 Acute Seizure Model

Acute seizures in patients with epilepsy are a potential of source of neurological damage; their causes must be researched (Valdes-Galvan *et al.*, 2019).Acute seizure exacerbation is defined as an abrupt increase in seizure frequency, such seizuresrepresent an increase in disease severity with a low chance of remitting without treatment and may also lead to status epilepticus and neuronal damage (Hantus, 2016). Factors that cause seizure exacerbations are defined as those that are associated with an increased risk of seizures in a

relatively brief and defined period of time, examples of some common causes include; emotional stress or anxiety, sleep deprivation, dosage omission or treatment non-adherence, menses-related and alcohol use (Valdes-Galvan *et al*., 2019).

For this study, seizures were induced using the maximal electroshock test (MEST), the pulse was applied with a headphone electrodes placed on the head of the chicks with frequency (50 to 60 Hz) and short duration (0.2 sec) was applied, the extension of the hind limb tonic component was used as the end point in the test (Garrido-Acosta*et al*., 2016).

Seizure were also induced in mice using pentylenetetrazole subcutaneously, by administering 90 mg/ kg body weight in each mice, suppression of clonic spasms and death were considered the end point (Garrido-Acosta*et al*., 2016).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Solvents, reagents and chromatography materials

The solvents used were of analytical grade (Sigma Chemical Company St. Louis, USA) which included; methanol, n-hexane, ethyl acetate and chloroform. Reagents used for the phytochemical screening were freshly prepared. They includes; Salkowski's reagent, Molisch's reagent, Dragendorf's reagent, Mayer's reagent, Shinoda's reagent, Wagner's reagent and Borntrager's reagent. Chromatographic materials included TLC plates (aluminium), Silica gel (60-120 mesh) (Merck KGA, Darmstadt, Germany), Chromatographic tanks and open column (85 cm by 6 cm and 75cm by 3.5cm).

Standard drugs and chemicals used for the anticonvulsant studies included; Phenytoin sodium (Parker-Davis and Co Limited, Detroit), Sodium Valproate (Sanofi-Aventis, UK), distilled water, and Pentylenetetrazole (PTZ) (Sigma Chemical Co. St. Louis, USA). Other materials were; syringes and needles, scissors and razor blades, mortar and pestle, spatula, beakers, conical flask, test tubes, sample bottles, animals (mice and chicks) and plant materials.

3.1.2 Equipment

Gallenkamp melting point apparatus at the Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Ohaus digital weighing balance (Champ 11 CH15R, Ohaus Corporation, Pine brook NJ, USA), ABB-MB3000 Bruker AVANCE III NMR spectrometer (400MHz) at the Natural product laboratories, Strathclyde Institute of

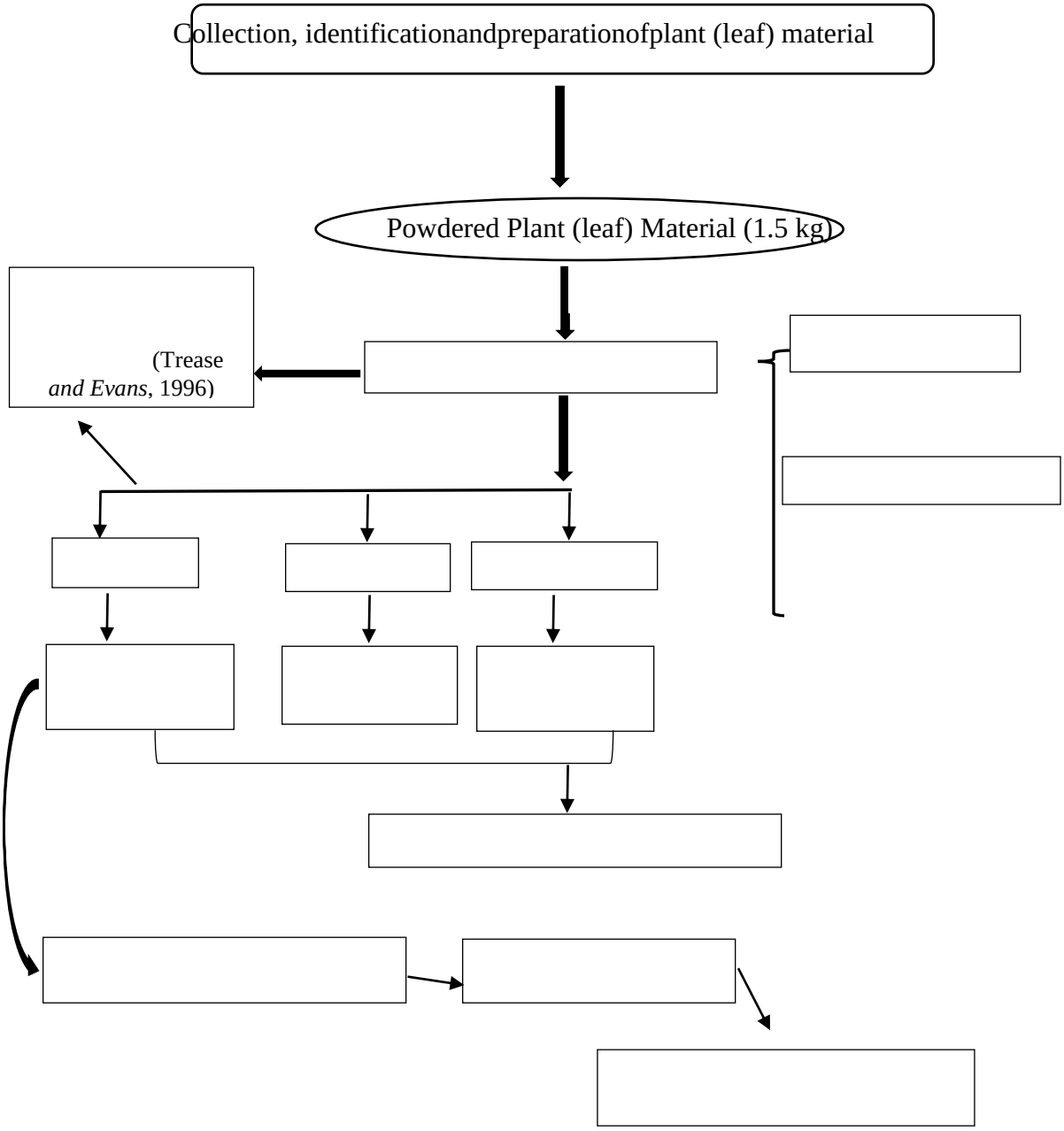
Pharmacy and Biomedical sciences (SIPBS), University of Strathclyde,Scotland, United Kingdom.

3.1.3 Experimental animals

Locally bred adult Swiss albino mice of either sex weighing between 17-22gwere obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria and were used for the study. In addition, day old ranger cockerels weighing between (26-31g)were obtained from Chi Farms Ltd along Ibadan-Lagos express way, Ibadan, Oyo state. The animals were maintained and fed with standard animal feed and water *ad libitum*, were housed in polypropylene cages at room temperature throughout the study.

3.2 Methods

3.2.1 Chart for research methodology



3.2.2 Collection, identification and preparation of plant material

*Combretum hypopilinum*leaves werecollected from Giwa, Kaduna State, Nigeria in April, 2018. The plant sample was authenticated by Mallam Namadi Sunusi of the Herbarium section of the Botany Department, Faculty of Life Sciences, Ahmadu Bello University, Zaria, by comparing it with a standardspecimen with voucher number 090143. The leaves were shade dried, pulverized maually using a wooden mortar and pestle to reduce the sizes.

3.2.3 Extraction and partitioning

One thousand five hundred grams (1500g) of the pulverized leaves of the plant were macerated with 80% of methanol with occasional shaking for 72 hours and the solvent was removed using rotary vacuum evaporator to afforded a dark green coloured extract (183.03g), subsequently referred to as methanol leaf extract (MLE). The MLE (170.03g) was partitioned (solid-liquid partitioning) with n-hexane, chloroform and ethyl acetate to give n-hexane (nHF), chloroform (CF) and ethyl acetate (EF) respectively.

3.2.4 Preliminary phytochemical screening

Preliminary phytochemical screening was carried out on the MLE and partitioned fractions according to standard procedures as follows:

3.2.3.1 Test for carbohydrates (Molisch's test)

To a small portion of the MLE in a test tube, 3 drops of Molisch's reagent were added followed by concentrated sulphuric acid. The formation of a reddish colour ring at the interface indicates the presence of carbohydrates (Trease and Evans, 1996).

3.2.3.2 Test for anthraquinones (Bontrager's Test)

A small portion of the MLE was dissolved in 5ml chloroform,shaken and filtered. To the filtrare, an equal volume of 10% ammonia solution was added with continuous shaking,

bright pink colour in the aqueous upper layer indicates the presence of anthraquinone (Trease and Evans, 1996).

3.2.3.3 Test for terpenoids/steroid

i. Salkowski's Test

A small portion of the MLE was dissolved in 2ml of chloroform, 3 drops of concentrated sulphuric acid was added at the side of the test tube. A reddish brown coloration at the interface indicates the presence of terpenoids (Trease and Evans, 1996).

ii. Liebermann-Burchard's Test

To the portion of the MLE, equal volume of acetic anhydride was added and mixed gently. Concentrated sulphuric acid (1 ml) was added down the test tube. This was observed for instant colour changes and over a period of one hour. Blue to blue-green color in the upper layer and a redish,pink or purple colour at the junction of the two layers indicates the presence of triterpenes(Trease and Evans, 1996).

3.2.3.4 Test for cardiac glycosides (Keller-Kiliani Test)

A small portion of the MLE was dissolved in 1ml glacial acetic acid containing traces of ferric chloride solution. The solution was then transferred into a dry test tube to which an equal volume of sulphuric acid was added, a brown ring obtained at the interface indicates the presence of deoxy sugars(Trease and Evans, 1996).

3.2.3.5 Test for tannins

Lead sub-acetate test

To a small portion of the MLE, 4 drops of lead sub-acetate solution was added, the formation of a cream colour precipitate indicates presence of tannins (Trease and Evans, 1996).

3.2.3.6 Test for flavonoids

i. Shinoda test

The MLE was dissolved in 2ml of methanol and pieces of metallic magnesium chips were added followed by few drops of concentrated hydrochloric acid, the formation of a pink, orange or red to purple colouration indicates the presence of flavonoid (Trease and Evans, 1996).

ii. Sodium hydroxide test

Two drops of 10% sodium hydroxide was added to the solution of the MLE, yellow colouration indicates the presence of flavonoids (Trease and Evans, 1996).

iii. Ferric chloride test

An amountof 2 to 3 drops of ferric chloride solution were added to the solution of the MLE. The colour was observed (Trease and Evans, 1996).

3.2.3.6 Test for alkaloids

i. Dragendoff's Test

The MLE (0.2g) was dissolved in 2 ml of 1% aqueous hydrochloric acid with continuous stirring in a water bath. The mixture was filtered and few drops of Dragendoff's reagent was added, rose red precipitate indicates the presence of alkaloids (Trease and Evans, 1996).

ii. Mayer's Test

To a 2ml solution of the MLE in a test tube, few drops of Mayer's reagent were added, a cream precipitate indicates the presence of alkaloids (Trease and Evans, 1996).

3.2.3.8Test for saponins (Frothing test)

To a small portion of the MLE in test tube, 10 ml of distilled water was added and then shaken continuously for 30 seconds. The solution was allowed to stand for 5minutes, the formation of a persistent froth indicates the presence of saponins (Trease and Evans, 1996).

3.2.5 Chromatographic procedures

3.2.4.1Thin layer chromatographic analysis (TLC)

Pre-coated TLC plates were used to carry out thin layer chromatography by one way ascending technique. Capillary tubes were used to manually to apply spots on the TLC plates,and the chromatogram of the MLE and the partitioned fractions was developed in an air tight chromatographic tank at room temperature,using different solvent system as follows;n-Hexane: ethyl acetate 9:1, 7:3 and 5:1. The spots were visualized under UV (254-366nm) after been sprayed with 10% sulphuric acid followed by heating in an oven at 105^{o}C for about 5 minutes.

3.2.4.2 Column chromatography of n-hexane fraction

The column (85 cm by 6 cm) was packed using slurry method. Asmall piece of cotton wool was placed at the bottom end of the column, n-hexane was added and the tap was opened to ensure it runs well. The column was packed with 60-120 mesh size silica gel suspended in n-hexane and allowed to settle. Another piece of cotton wool was placed on the silica gel bed with solvent slightly above the bed to avoid drying and cracking of the column. Ten grams (10 g) of the n-hexane fraction was dissolved withn-hexane in a 100ml beaker, and small amount of 60-120 mesh size silica gel was added and mixed together using a plastic steering rod. The mixture was transferred to a petri-dish and allowed to dry for few hours. The dried mixture was crushed using a clean pistil on the petri-dish to obtain a finely powdered mixture. The finely powdered mixture was then loaded on thecotton wool slightly above the 60-120 mesh size silica gel bed in the column. It was eluted with n-

hexane, ethyl acetate and methanol gradient-wise. The n-hexane fraction was initially eluted with 100% n-hexane. The polarity was gradually increased with the addition of ethyl acetate as follows; n-hexane: ethyl acetate 98:2, 96:4, 94:6, 92:8, 90:10, 85:15, 80:20, 50:50. The column was finally washed with 100% methanol. A total of one hundred and thirty collections were made. Eight pooled fractions coded A(11-15), B (16-20), C (21-28), DE (29-40), F (41-47), J (48-85), L (86-96) and M (97-125) were made based on their TLC profiling.

3.2.4.3 Column chromatography of column fraction A and C.

The pooled fraction A (11-15) was chromatographed over 60-120 mesh size silica gel in a smaller capillary open column (75 cm by 3.5 cm). It was eluted with n-hexane, ethyl acetateand methanol gradient-wise. The elution was initiated with 100% n-hexane and gradual increase in the polarity by the addition of ethyl acetate as follows; n-hexane: ethyl acetate 95:5, 90:10, 85: 15, 80: 20, 50:50 and then finally washed the column with 100% methanol. A total of seventy four collections were made. Four pooled fractions coded AA (11-21), AB (22-33), AC (34-44) and AD (45-74) were made based on their TLC profiling.The pooled fraction AA was further purified over 60-120 mesh size silica gel using a smaller capillary column. It was eluted with n-hexane, ethyl acetate and methanol gradient-wise. The elution was initiated with 100% n-hexane, and gradual increase in the polarity by the addition of ethyl acetate as follows; n-hexane: ethyl acetate 99:1, 98:2, 97:3, 96:4, 95:5, 50:50 and finally washed the column with 100% methanol. A total of eighty one collections were made. Seven pooled fractions coded AAA (35-40), AAA2 (41-46), AAA3 (47-51), AAA4 (52-64), AAA5 (65-70), AAA6 (71-75) and AAA7 (76-81) were made based on their TLC profiling.The pooled fraction AAA (35-40) was further purified over

60-120 mesh size silica gel in a small capillary open column. It was eluted with n-hexane, ethyl acetate and methanol gradient-wise. The elution was initiated with 100% n-hexane and gradual increase in the polarity by the addition of ethyl acetate as follow; n-hexane: ethyl acetate 99:1, 90:10, 50:50 and finally washed with 100% methanol. A total of ninety five collections were made. Four pooled fractions coded A_2 (38-41), A_3 (42-51), A_4 (52-71), A_5 (72-85) and A_6 (86-95) were made based on their TLC profiling.A_5 (72-85) gave a single spot on the TLC plate and also afforded a white amorphous powder (3 mg) that was completely soluble in chloroform.

The pooled column fraction coded C (21-28) was further purified over 60-120 mesh size silica gel, using the isocratic elution technique, with 24ml volume of a gradient mixture of n-hexane: ethyl acetate 5:1. A total of twenty collections were made. Three pooled fractions coded C_1 (6-7), C_2 (8-17) and C_3 (18-20) were made based on their TLC profiling. The pooled fraction C_2 (8-17) was recrystallized with n-hexane to afforded a white crystalline solid (8 mg) that gave a single spot on the TLC plate and was found to be completely soluble in chloroform.

3.2.6 Physical and chemical tests

i. Solubility test:

The solubility of compound C_2 and A_5 were observed in methanol and chloroform.

ii. Chemical test:

The isolated compounds C_2 and A_5 were subjected to theSalkowski's and Liebermann Burchard's test.

iii. Melting point determination:

The melting point of the isolated compounds were determined using Gallenkamp melting point apparatus.

3.2.7 Spectral analysis

The isolated compounds $C_2$and A_5 were subjected to IR and NMR analysis.The NMR spectral were obtained on a Bruker AVANCE (400 MHz for ^{1}H and ^{13}C) spectrometer, using the residual solvent peaks as internal standard. Chemical shift values (δ) were reported in parts per million (ppm) relative to appropriate internal solvent standard. The NMR solvents used for this measurements was deuterated chloroform ($CDCl_3$).

3.2.8 Anticonvulsant studies of methanol leaf extract (MLE)

3.2.7.1 Acute toxicity studies

The Lorke's method (1983) was adopted for the study, which was carried out in two phases.

In the first phase, nine mice were divided into three groups consisting of three mice in each group. They were administered with varying doses of the MLE(10, 100 and 1000mg/kg body weight) via the oral route and observed for any signs of toxicity and mortality for 24 hours.

In the second phase, based on the outcome of the first phase, three (3) groups with one mice each were treated with the doses at 1600, 2900 and 5000mg/kg of the MLE orally and observed for signs of toxicity and death. The median lethal dose was estimated as a geometric mean of the highest non-lethal dose (with no death) and the lowest lethal dose (where death occurred).

$$LD_{50} = \sqrt{mnimum\ lethal\ dose \times maximum\ tolerated\ dose}$$

3.2.7.2 Maximal electroshock convulsion test in chicks

The methods of Swinyard and Kupferberg (1989) was employed. Day old chicks weighing between 26-31 g were randomly divided into five groups of ten each. The first group were treated with normal saline 10 ml/kg orally. The second, third and fourth groups were treated with 125mg/kg, 250mg/kg and 500mg/kg doses of the MLE via the oral routerespectively, while the fifth group were treated with phenytoin 20 mg/kg via the oral route, as positive control. Sixty minutes later, maximal electroshock was administered to induce seizure in all the groups using Ugo Basil electroconvulsive machine (Model 7801) with corneal electrodes placed on the upper eyelids of the chicks. The current, shock duration, frequency and pulse width was maintained at 80mA, $0.8s^{-1}$, 100 pulse per second and 0.6ms respectively. The chicks were observed for tonic hind limb extension (THLE). An episode of THLE was regarded as full convulsion while lack of THLE was regarded as protection. For the unprotected animals, their recovery time were recorded.

3.2.7.3Subcutaneous Pentylenetetrazole (scPTZ) induced convulsion test in mice

The method of Swinyard et al., (1989) was employed. Thirty mice of either sex weighing between 17 to 22 g were randomly divided into five groups of five mice each. Mice in group one were treated with normal saline 10 ml/kg orally.The second, third and fourth groups were treated with 125, 250 and 500mg/kg doses of the MLE via the oral routerespectively, while the fifth group was treated with 200 mg/kg sodium valproate orally. Sixty minutes later, mice in all groups were treated with 90 mg/kg body weight of freshly prepared PTZ subcutaneously. The mice were then observed for the presence or absence of seizure of at least 5 seconds duration. The absence of seizure was considered as against the MLE ability to protect against PTZ-induced seizures.

3.2.9 Statistical analysis

Statistical analysis was performed using SPSS software version 20.The mean onset seizure and the mean recovery time were presented as mean ± standard error mean (SEM). The mean values of the control group were compared with the mean values group treated with the test extracts using one-way analysis of variance(ANOVA) followed by Dunnette post-hoc test for multiple comparison.The result was considered significant at $P<0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Extraction and Partitioning

The extraction of 1500g of *Combretum hypopilinum* afforded a yield of 183.03g of the leaf extract (12.20 %). The percentage yield from the partitioned methanol leaf extract (170.03g) are presented below in Table 4.1.

Table 4.1: Weight and Percentage Yield of MLE and Partitioned Fractions of*Combretum hypopilinum*

Solvent	Weight (g)	Percentage yield (%)
n-hexane	20.12	11.76
Chloroform	4.28	2.52
Ethyl acetate	0.54	0.32

4.2 Preliminary Phytochemical Constituents

Preliminary phytochemical screening of the methanol leaf extract revealed the presence and absence of carbohydrates, anthraquinones, steroids, triterpenoids, flavonoids, tannins, cardiac glycosides, saponins and flavonoids as shown in Table 4.2.

Table 4.2: Phytochemical Constituents of Methanol Leaf Extract (MLE)

Constituents	**Test**	**Observations (Extracts)**			
		MLE	**HF**	**CF**	**EF**
Alkaloids	Mayer	+	-	-	+
	Dragendoff	+	--	+	
Anthraquinones	Bontrager	-	- -	-	
Carbohydrates	Molisch	+	-	-	+
Cardiac glycoside	Keller-Kiliani	+	-+	+	
Flavonoids	Sodium hydroxide	+	-	-	+
Saponins	Frothing	+	-	-+	
Steroids	Salkwoski	+ +	+	-	
Tannins	Ferric chloride	+	-	- +	
Triterpenoids	Liebermann Buchard	+	+	+	-

Key: + = present, - = absent

4.3 Results of Thin-Layer Chromatography

Thethin-layer chromatography profile of MLEand partitioned fractions (nHF, CF and EF) gavethe chromatographic profiles as shown in Plates II-IV using 10% Sulphuric acid as spraying reagent. Table 4.3 shows the summary of the TLC plates.

Plate II:n-Hex: EA 9:1

Plate III: n-Hex: EA 7:3

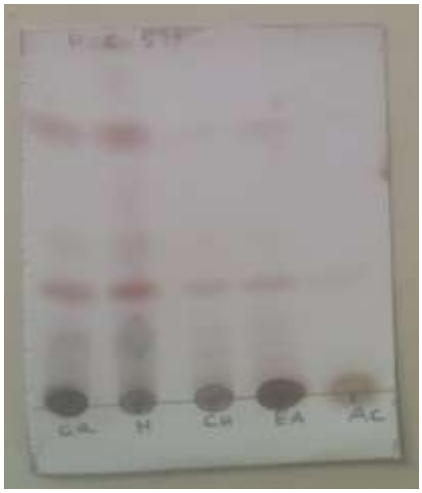


Table 4.3: Summary of TLC Profiles of Partitioned Fractions

Fractions	Number of spots	R_f values of spots	Solvent systems
n-Hexane	7	0.12, 0.22, 0.31, 0.41 0.51, 0.65, 0.82	n-Hexane:Ethyl acetate (9:1)
Chloroform	5	0.25, 0.32, 0.42, 0.52, 0.71	n-Hexane:Ethyl acetate (7:3)
Ethyl acetate	5	0.28, 0.41, 0.50, 0.63, 0.76	n-Hexane:Ethyl acetate (5:1)

4.4 Column Chromatography of n-Hexane Fraction

Table 4.4 shows the column chromatography of n-hexane fraction and the TLC profiles as shown in plates V-IX. Table 4.5 shows the pooled column fractions and their coding, while plate X shows the TLC profile of pooled column fractions.

Table 4.4: Column Chromatography of n-Hexane Fraction

No of Beakers collected	Elution solvent(%)	Number of spots
1-7	N-Hexane 100	Nil
8-10	N-Hex: Ethyl acetate 98:2	Nil
11-12	N-Hex: Ethyl acetate 96:4	7
13-15	N-Hex: Ethyl acetate 94:6	7
16-20	N-Hex: Ethyl acetate 94:6	8
21-28	N-Hex: Ethyl acetate 94:6	9
29-33	N-Hex: Ethyl acetate 94:6	7
34-40	N-Hex: Ethyl acetate 94:6	6
41-47	N-Hex: Ethyl acetate 94:6	5
48-85	N-Hex: Ethyl acetate 94:6	4
86-94	N-Hex: Ethyl acetate 94:6	7
95-96	N-Hex: Ethyl acetate 92:8	7
97-102	N-Hex: Ethyl acetate 90:10	7
103-105	N-Hex: Ethyl acetate 90:10	7
106-109	N-Hex: Ethyl acetate 85:15	7
110-114	N-Hex: Ethyl acetate 80:20	7
115-119	N-Hex: Ethyl acetate 50:50	7
120-125	Ethyl acetate 100	7
126-130	Methanol 100	Nil

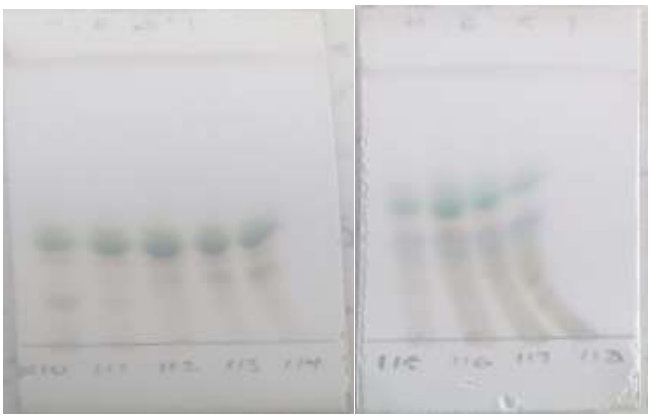


Plate V: (110-114) **Plate VI: (115-118)**
N-Hex: EA (5:1) **N-Hex: EA (5:1)**

Plates V-VI: TLC Profiles of Some of the Collections from Column Chromatography of n-Hexane Fraction

Table 4.5: Column Chromatography of Pooled Column Fractions

Pooled fractions	Code names	No of spots/ TLC Status
11-15	**A**	**7**
16-20	B	8
21-28	**C**	**9**
29-40	DE	7
41-47	F	5
48-85	J	4
86-96	L	6
97-125	M	7

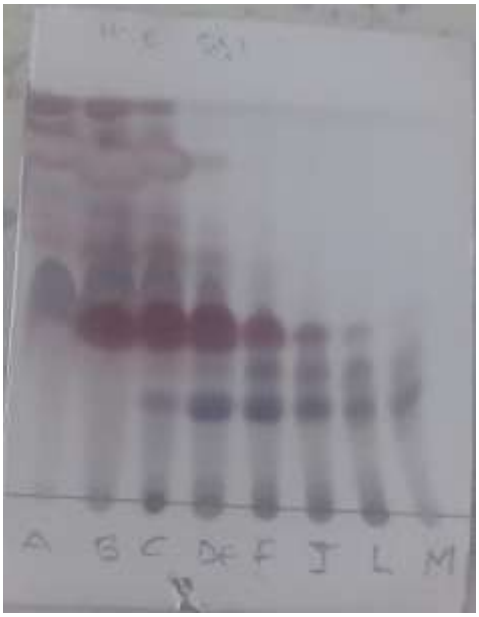


Plate VII:TLC of pooled column fractions

N-Hex: EA (5:1)

4.5 TLC Profiles of Isolated Compound C_2 and A_5

TLC profiles of isolated compound C_2 in N-Hex: EA (9:1) RF=0.31 (Plate XI), (5:1) RF=0.50 (Plate XII) and Compound A_5 in N- Hex: EA (9:1) RF=0.42 (Plate XIII) using 10% H_2SO_4 as spraying agent respectively.

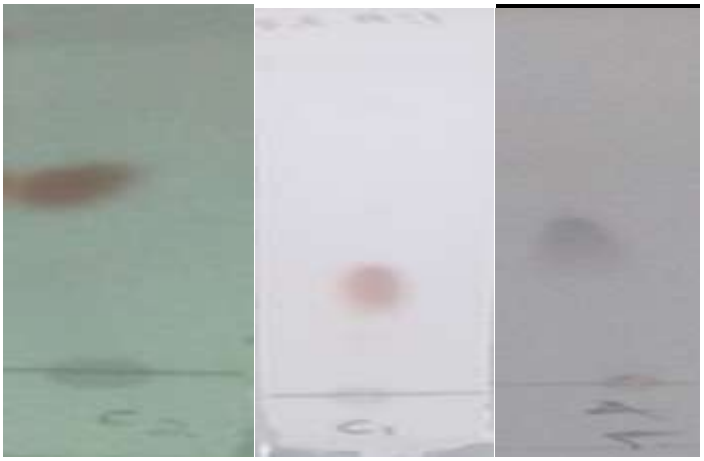


Plate VIII:n-Hex: EA (5:1)Plate IX:n-Hex: EA (9:1)Plate X:n-Hex: EA (9:1)

4.6 Characterization of Compound C_2

4.6.1 Solubility profile of compound C_2

Compound C_2 was found to be completely soluble in chloroform.

4.6.2 Melting point of compound C_2

The isolated compound C_2 was found to melt between 214-216^{O}C.

4.6.3 Chemical test of compound C_2

Compound C_2 produce reddish colouration when subjected to Liebermann Burchard's test and reddish brown colour at the interface when subjected to Salkowski's test (Trease and Evans, 1996).

4.6.4 Colour of compound C_2

Compound C_2 was found to be a white solid crystals.

4.7 Spectral Analysis of Compound C_2

4.7.1 IR spectrum of compound C_2

Some important IR absorption frequencies for compound C_2shown at (cm^{-1}); 3362.1, 2922.2, 2855.1, 1640, 1453.7, 1379.1, 1032.5 (Figure 4.1).

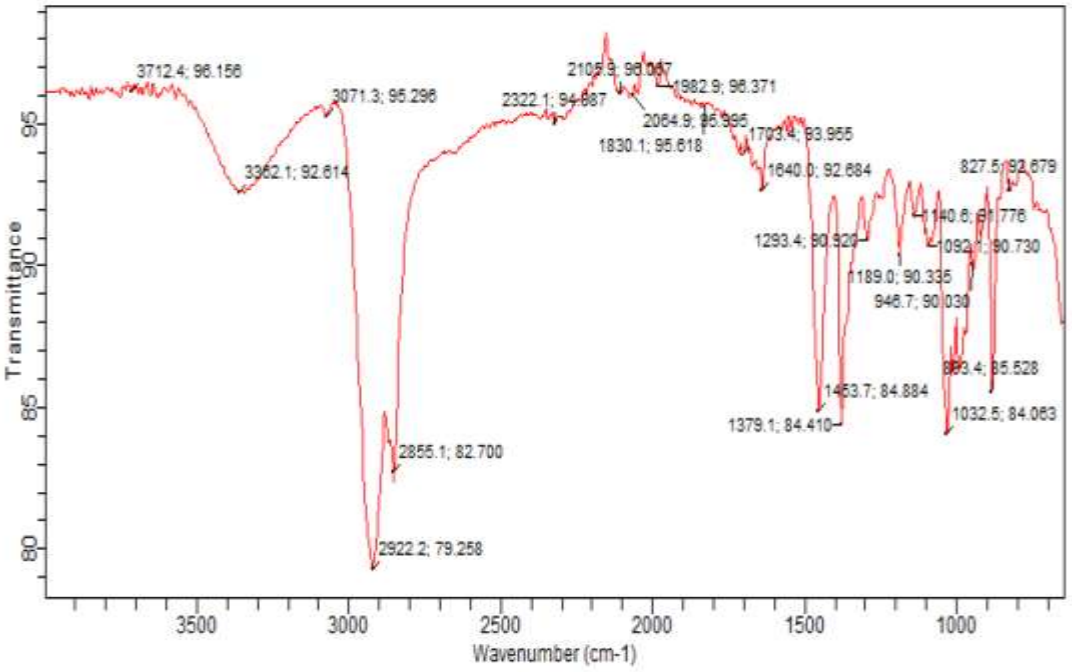


Figure 4.1: IR Spectrum of Compound C_2

4.7.2 ^{1}H NMR spectrum of compound C_2

The ^{1}HNMR spectrumof C_2($CDCl_3$, 400MHz)revealed the following chemical shift values/ integrations δ ppm at:0.79 (3H, s, H-24), 0.76 (3H, s, H-28), 0.83 (3H, s, H-25), 0.97 (3H, s, H-27), 1.01 (3H, s, H-23), 1.07 (3H, s, H-26), 1.68 (3H, s, H-30), 3.20 (1H, m, H-3), 2.41 (1H, m, H-19), 1.37 (1H, dd, H-18), 1.91 (2H, m, H-21), 1.26 (1H, m, H-22), 1.62 (1H, m, H-13), 0.99 (1H, m, H-15), 0.69 (1H, m, H-5), 1.20 (1H, m, H-6), 1.71 (1H, m, H-12), 1.41 (1H, m, H-11), 1.63 (1H, m, H-2), 1.28 (1H, dd, H-9), 0.94 (1H, m, H-1), 1.39 (1H, m, H-7), 4.68 (1H, dd, H-29a), 4.57 (1H, dd, H-29b) (Figure 4.2).

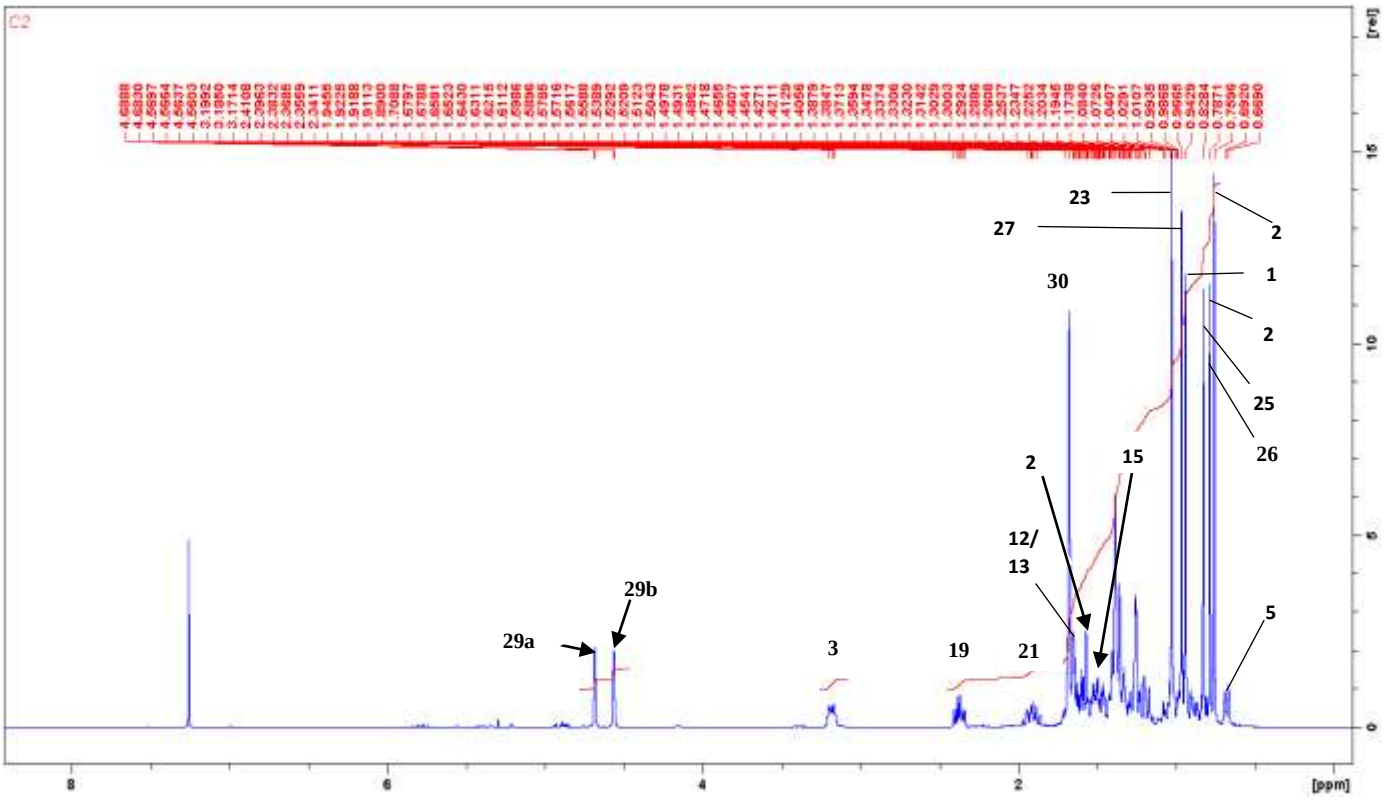


Figure 4.2:^{1}H NMR Spectrum of Compound C_2 in $CDCl_3$

4.7.3 ^{13}C NMR spectrum of compound C_2

The ^{13}C NMR spectrum of compound C_2 ($CDCl_3$, 400MHz) revealed the presence of 30 carbon atomsat δ ppm; 39.08 (C-1), 27.64 (C-2), 79.22 (C-3), 38.93 (C-4), 55.51 (C-5), 18.54 (C-6), 34.50 (C-7), 41.05 (C-8), 50.66 (C-9), 37.39 (C-10), 21.15 (C-11), 25.36 (C-12), 38.27 (C-13), 43.05 (C-14), 27.66 (C-15), 35.80 (C-16), 43.22 (C-17), 48.52 (C-18), 48.21 (C-19), 151.20 (C-20), 30.07 (C-21), 40.22 (C-22), 28.21 (C-23), 15.59 (C-24), 16.34 (C-25), 16.19 (C-26), 14.77 (C-27), 18.22 (C-28), 109.54 (C-29), 19.52 (C-30) (Figure 4.3).

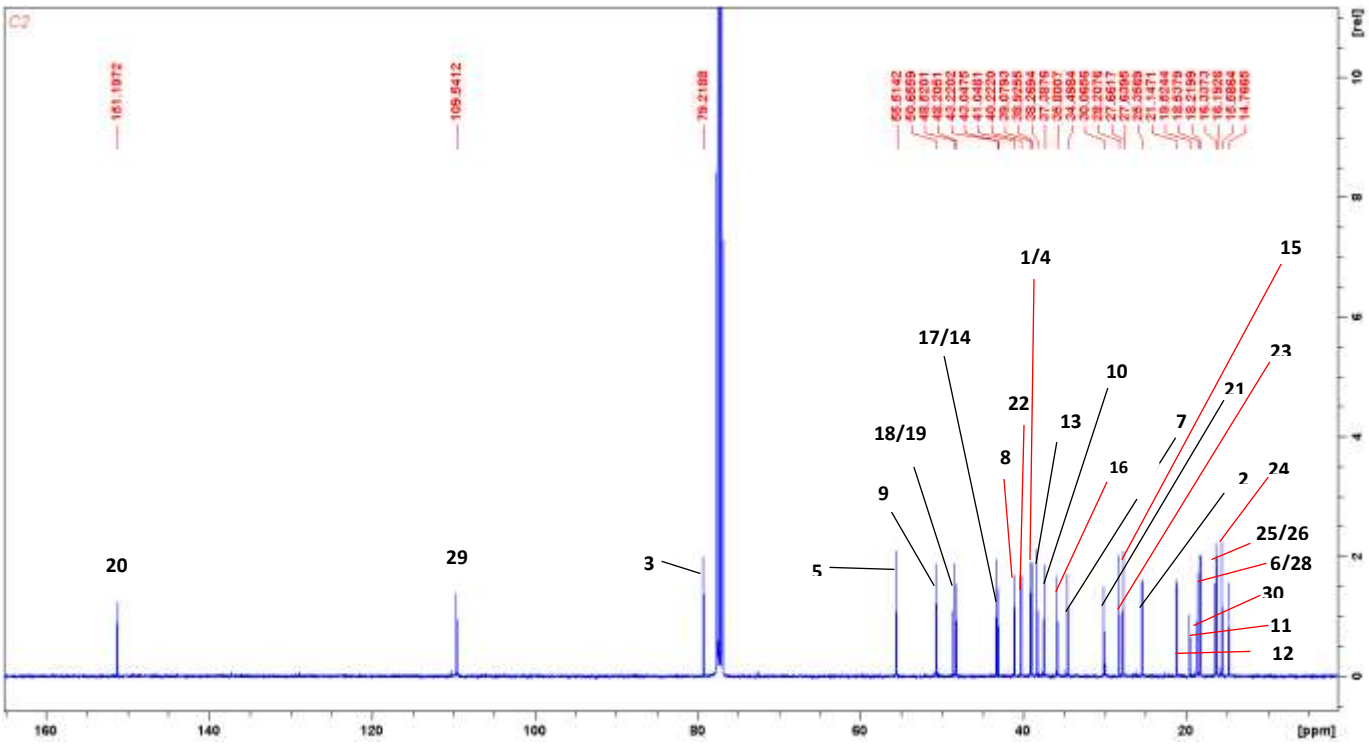


Figure 4.3:^{13}C NMR Spectrum of Compound C_2 in $CDCl_3$

4.7.4 DEPT spectrum of compound C_2

The Distortion less Enhancement by Polarization Transfer (DEPT) revealedmethyl carbon atoms at δ ppm;28.21 (C-23),15.59 (C-24), 16.34 (C-25),16.19 (C-26),14.77(C-27), 18.22 (C-28), and 19.52 (C-30).

Methylene carbon atoms at δ ppm; 39.08 (C-1),27.64 (C-2), 18.54 (C-6),34.50 (C-7),21.15 (C-11), 25.36 (C-12), 27.66 (C-15), 35.80 (C-16), 30.07 (C-21), 40.22 (C-22),109.54 (C-29).

Methine carbon atoms at δ ppm; 79.22 (C-3), 55.51 (C-5), 50.66 (C-9), 38.27 (C-13), 48.52 (C18), 48.21 (C-19)(Figure 4.4).

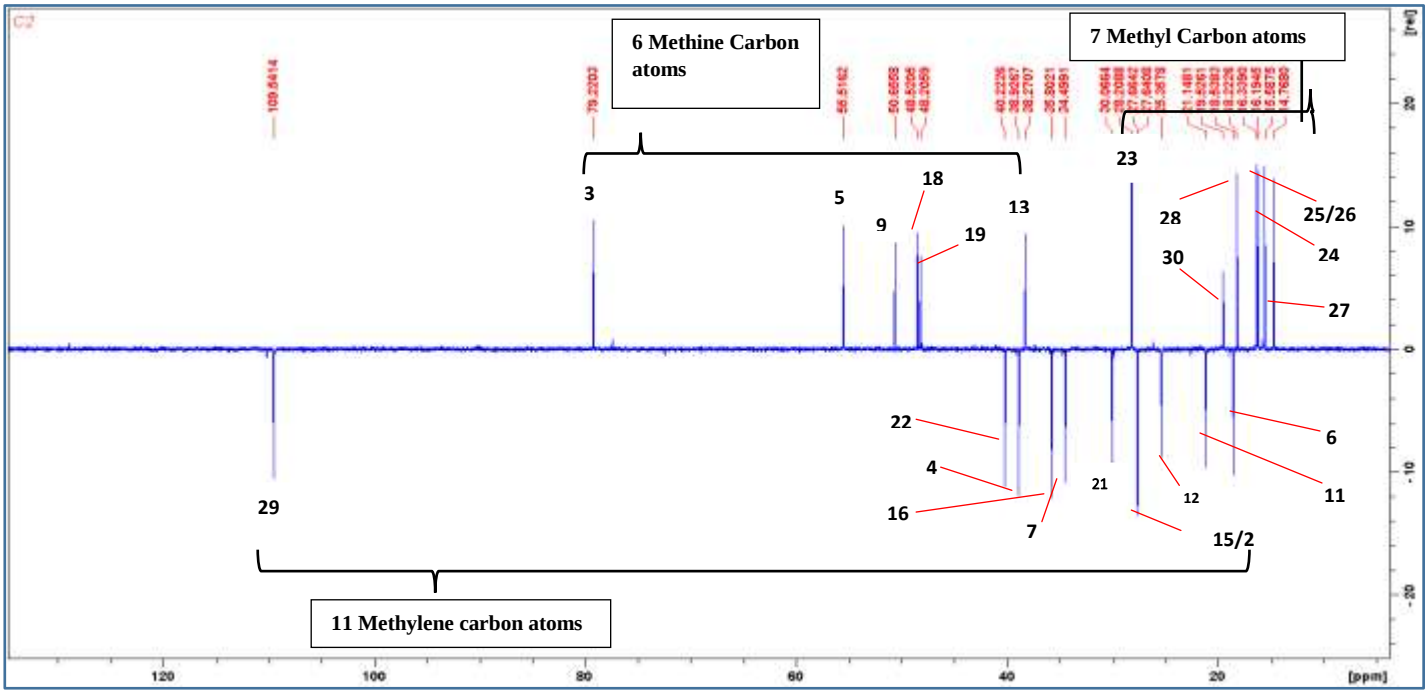


Figure 4.4:DEPT Spectrum of Compound C_2 in $CDCl_3$

4.7.5 HSQC spectrum of compound C_2

The Heteronuclear Single Quantum Coherence (HQSC) established attachment among others are as follows δ ppm;

4.68 (H-29a) and4.57 (H-29b) # 109.54 (C-29), 2.41 (H-19) # 48.21 (C-19), 1.91 (H-21)# 30.07 (C-21),1.37 (H-18) #48.52 (C-18), 1.29 (H-9) #50.66 (C-9), 3.20 (H-3) #79.22 (C-3) and 0.69 (H-5) #55.51 (C-5), 1.68 (H-30) # 19.52 (C-30) (Figure 4.5).

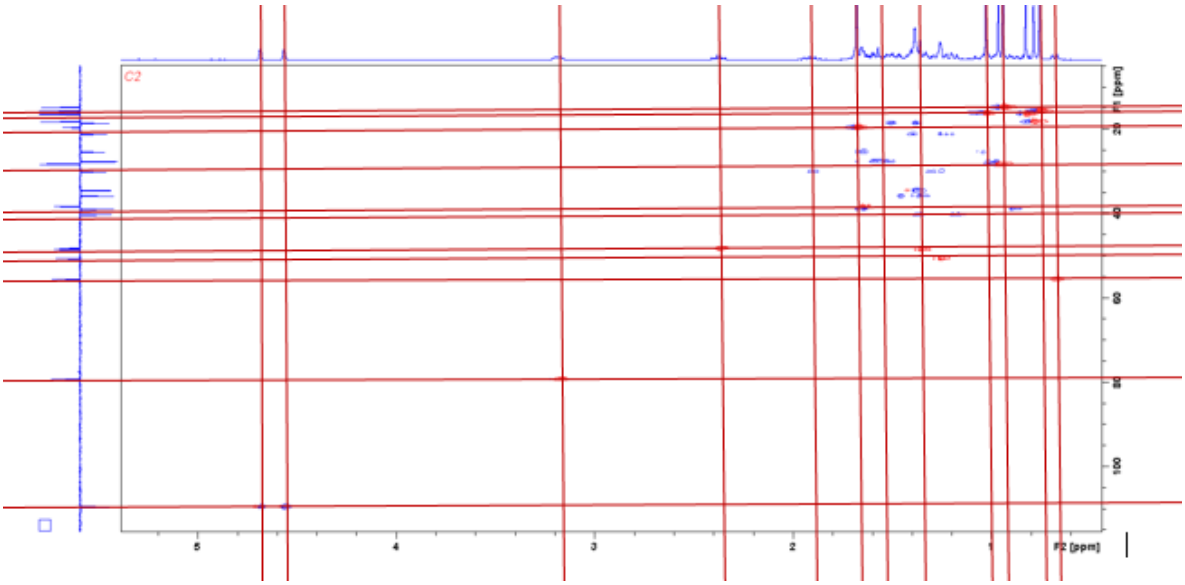


Figure 4.5:HSQC Spectrum of Compound C_2 in $CDCl_3$

4.7.6 HMBC spectrum ofcompound C_2

The Heteronuclear Multiple Bond Coherence (HMBC) established correlation among others are as follows; δ_H4.68 (H-29a), δ_H4.57 (H-29b) correlate withδ_C19.52 (C-30) and δ_C48.21 (C-19), δ_H2.41 (H-19) correlate withδ_C19.52 (C-30), δ_C109.54 (C-29),δ_C151.20 (C-20) andδ_C48.52 (C-18), δ_H1.68 (H-30) correlate withδ_C48.21 (C-19),δ_C79.22,δ_C109.54 (C-29) and δ_C151.20 (C-20) (Figure 4.6).

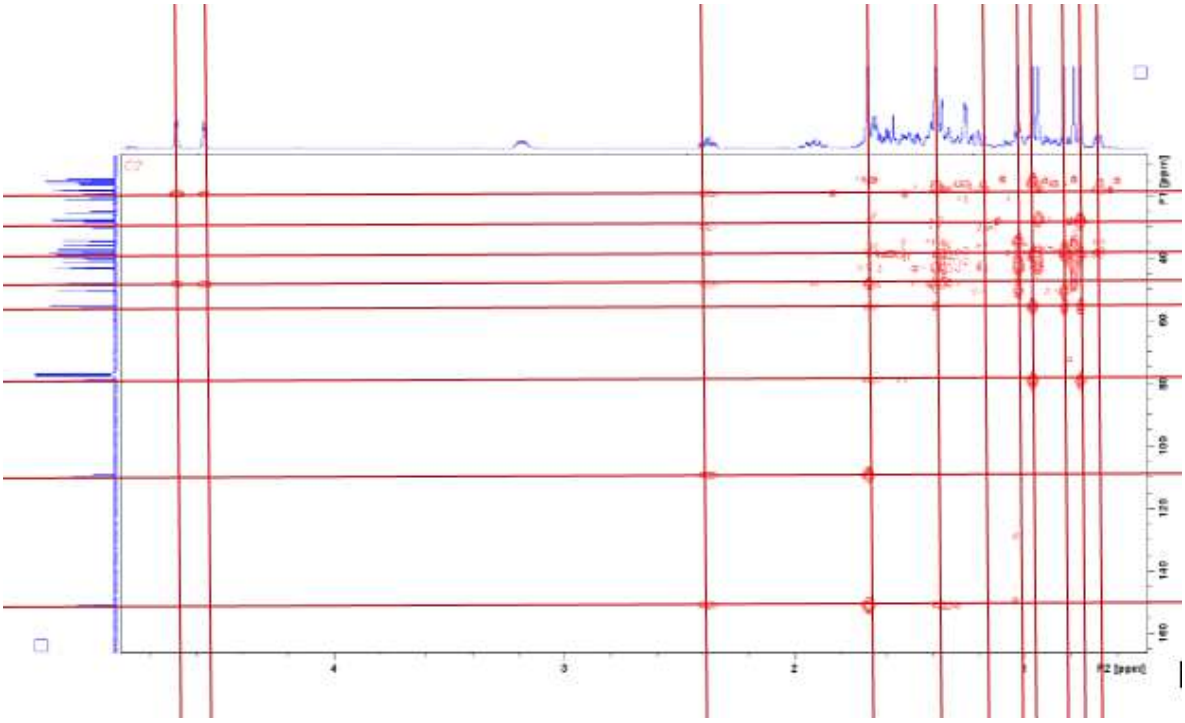


Figure 4.6:^{1}H – ^{13}C HMBC Spectrum of Compound C_2in $CDCl_3$

4.7.7 Summary of spectral data compared with literature values

Table 4.6. Summary of Spectral Data for Compound C_2 ($CDCl_3$, 400 MHz)

S/No	^{1}H NMR Abdullahi *et al*, 2013	^{1}H NMR	^{13}C NMR Abdullahi *et al*, 2013	^{13}C NMR	DEPT 135 Abdullahi *et al*, 2013	DEPT
1	0.93	0.94	38.80	39.08	CH_2	CH_2
2	1.60	1.63	27.20	27.64	CH_2	CH_2
3	3.19	3.20	79.30	79.22	CH	CH
4	-	-	39.10	38.93	C	C
5	0.68	0.69	55.30	55.51	CH	CH
6	1.40m 1.19m	1.20	18.30	18.54	CH_2	CH_2
7	1.38	1.39	34.20	34.50	CH_2	CH_2
8	-	-	41.10	41.05	C	C
9	1.28	1.28	50.60	50.66	CH	CH
10	-	-	37.31	38.39	C	C
11	1.40	1.41	21.10	21.15	CH_2	CH_2
12	1.68	1.71	25.50	25.36	CH_2	CH_2
13	1.63	1.62	38.30	38.27	CH	CH
14	-	-	43.20	43.05	C	C
15	0.97	0.99	28.10	27.66	CH_2	CH_2
16	1.45	1.45	35.20	35.80	CH_2	CH_2
17	-	-	43.30	43.22	C	C
18	1.38 (1H, dd)	1.37	48.30	48.52	CH	CH
19	2.36 (1H, m)	2.41	48.10	48.21	CH	CH
20	-	-	150.9	151.20	C	C
21	1.28 (2H, m)	1.91	30.00	30.07	CH_2	CH_2
22	1.17	1.26	40.50	40.22	CH_2	CH_2
23	0.99	1.01	28.00	28.21	CH_3	CH_3
24	0.75	0.79	15.60	15.59	CH_3	CH_3
25	0.80	0.83	16.20	16.34	CH_3	CH_3
26	1.02	1.07	16.00	16.19	CH_3	CH_3
27	0.91	0.97	14.80	14.77	CH_3	CH_3
28	0.75	0.76	18.20	18.22	CH_3	CH_3
29	4.67 (1H, dd) 4.56 (1H, d)	4.68 4.57	109.50	109.54	CH_2	CH_2
30	1.68 s	1.68	19.40	19.52	CH_3	CH_3

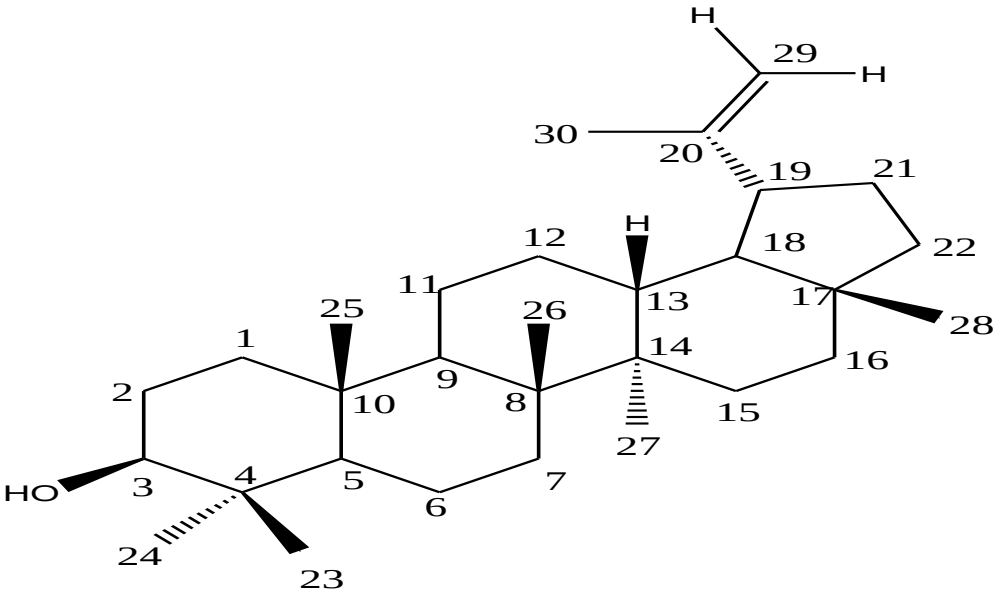


Figure 4.7: Chemical Structure of Compound C_2 (Lup-20(29)-en-3-ol)

4.8 Characterization of CompoundA_5

4.8.1 Colour of compound A_5

Compound A_5 was found to be a white amorphous powder.

4.8.2 Solubility profile of compound A_5

Compound A_5 was observed to be completely soluble in chloroform.

4.8.3 Melting point of compound A_5

The isolated compound A_5 was found to melt at 168 – 170^{o}C.

4.8.4 Chemical test on compound A_5

The compound A_5produce reddish colouration when subjected to Liebermann Burchard's test (Trease and Evans, 1996).

4.9 Spectral Analysis of Compound A_5

4.9.1 IR spectrum of compound A_5

Some important IR absorption frequencies for compound A_5 shown at (cm^{-1}); 2922.2, 2855.1, 1707.1, 1457.4, 1379.1 (Figure 4.8).

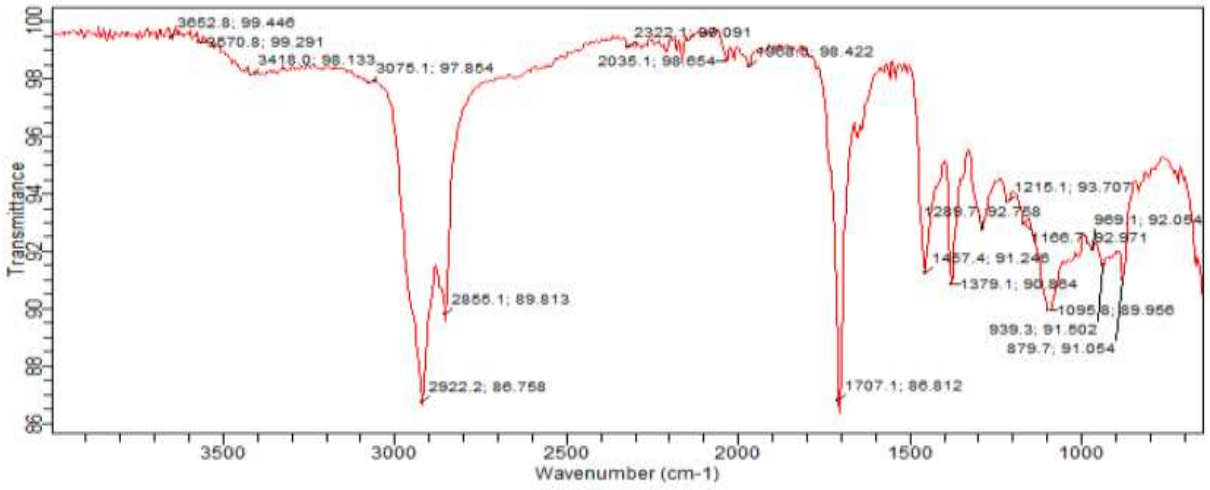


Figure 4.8: IR Spectrum of Compound A_5

4.9.2 ^{1}H NMR spectrum of compound A_5

The [1]HNMR spectrum of compound C_2 ($CDCl_3$, 400MHz) revealed the following chemical shift values/ integrations δ ppm at:1.68 (3H, s, H-30), 0.81 (3H, s, H-28), 0.95 (3H, s, H-27), 1.05 (3H, s, H-26), 0.84 (3H, s, H-25), 0.80 (3H, s, H-24), 0.94 (3H, s, H-23), 4.69 (1H, d, H-29a) and 4.57(1H, d, H-29b), 2.41 (1H, m, H-19), 0.93 (1H, m, H-1), 1.58 (1H, m, H-2), 0.72 (1H, m, H-5), 1.40 (1H, m, H-6), 1.37 (1H, m, H-7), 1.25 (1H, dd, H-9), 1.43 (1H, m, H-11), 1.66 (1H, m, H-12), 1.63 (1H, m, H-13), 1.04 (1H, m, H-15), 1.48 (1H, m, H-16), 1.37 (1H, m, H-18), 1.97 (2H, m, H-21) as shown in Figure 4.9.

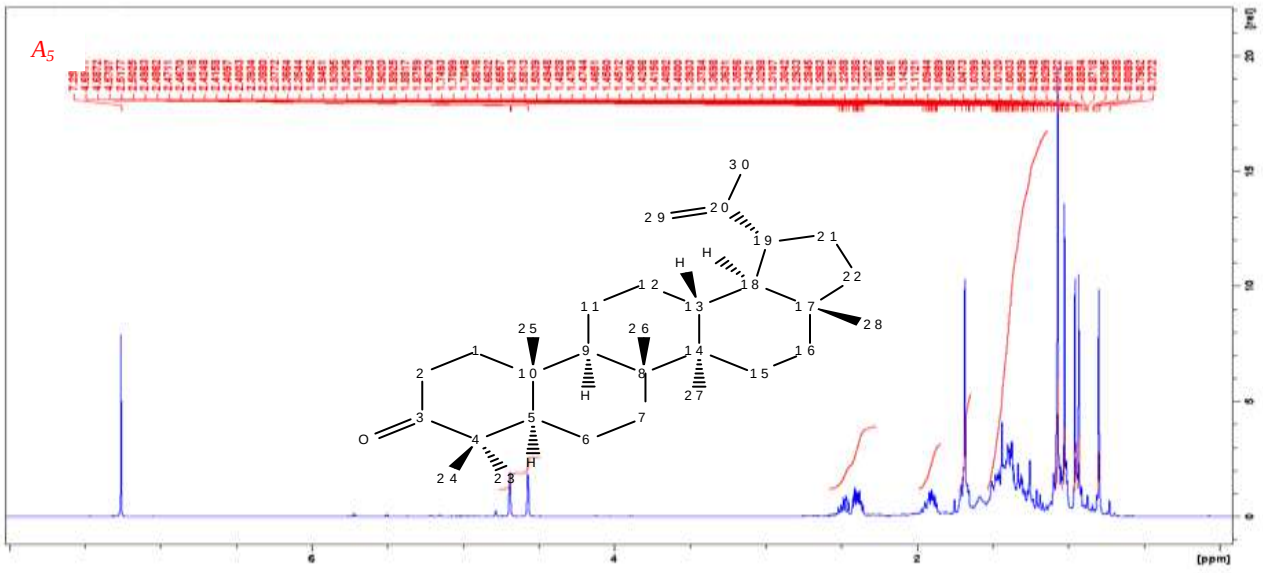


Figure 4.9: [1]H NMR Spectrum of Compound A_5 in $CDCl_3$

4.9.3 [13]C NMR spectrum of compound A_5

The ^{13}C NMR spectrum ($CDCl_3$, 400MHz) of compound A_5**r**evealed 30 carbon signals δ ppm at; 38.39 (C-1), 27.65 (C-2),218.45 (C-3), 39.84 (C-4), 55.15 (C-5), 19.90 (C-6), 34.08 (C-7), 43.12 (C-8), 50.01 (C-9), 38.39 (C-10), 21.26 (C-11), 26.87 (C-12), 37.10 (C-13), 43.21 (C-14), 30.05 (C-15), 35.74 (C-16), 47.56 (C-17), 48.49 (C-18), 48.18 (C-19), 151.11 (C-20), 33.79 (C-21), 41.00 (C-22), 25.38 (C-23), 19.53 (C-24), 14.70 (C-25), 16.19 (C-26), 16.01 (C-27), 18.23 (C-28), 109.61 (C-29), 21.69 (C-30) as shown in Figure 4.10.

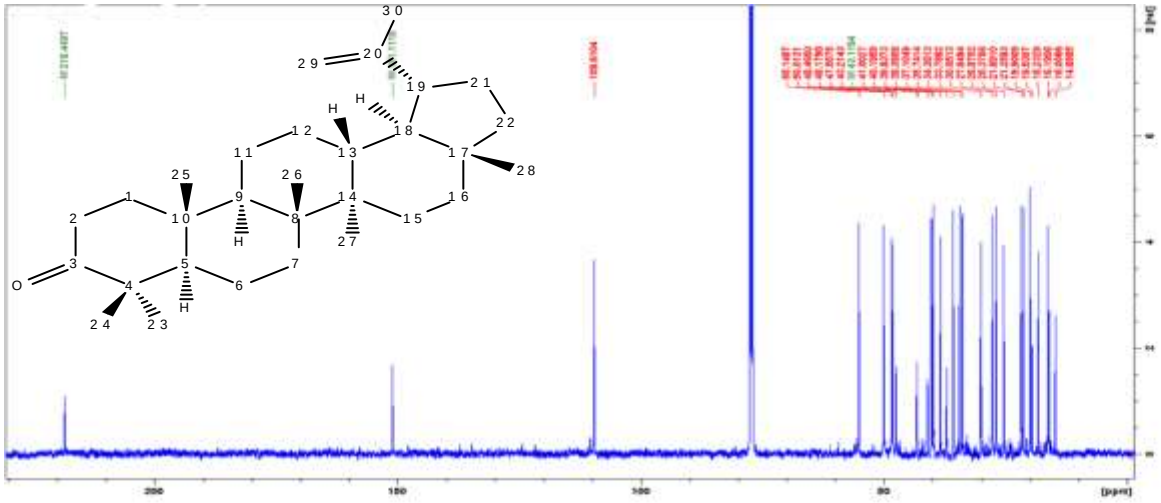


Figure 4.10:^{13}C NMR Spectrum of Compound A_5 in $CDCl_3$

4.9.4 Summary of spectral data of compound A_5 compared with literature values

Table 4.7:Summary of Spectral Data for Compound A_5 ($CDCl_3$, 400 MHz)

S/No	^{1}H NMR Robert*et al*, 2015	^{1}H NMR	^{13}C NMR Robert*et al*, 2015	^{13}C NMR
1	1.65 m, 0.9 m	0.93 m	38.70	38.39
2	1.55 m	1.58 m	27.90	27.65
3	-	-	218.00	218.45
4	-	-	40.00	39.84
5	0.60 m	0.72 m	55.40	55.15
6	1.51 m, 1.40 m	1.40 m	20.18	19.90
7	1.34 m	1.37 m	34.90	34.08
8	-	-	41.30	43.12
9	1.22 dd	1.25 dd	50.20	50.01
10	-	-	37.40	38.39
11	1.40 m, 1.12 m	1.43 m	21.40	21.26
12	1.64 m, 1.08	1.66 m	26.90	26.87
13	1.64 m	1.63 m	37.40	37.10
14	-	-	43.40	43.21
15	1.71 m, 1.01	1.04	29.90	30.05
16	1.47	1.48	36.00	35.74
17	-	-	43.50	47.56
18	1.35 dd	1.37 dd	48.70	48.49
19	2.4	2.41	48.50	48.18
20	-	-	151.60	151.11
21	2.03, 1.31 m	1.97m	32.50	33.79
22	1.37, 1.02	1.06s	40.40	41.00
23	0.92 s	0.94 s	23.20	25.38
24	0.74 s	0.80 s	20.10	19.53
25	0.84 s	0.84 s	14.40	14.70
26	1.04 s	1.05 s	16.10	16.19
27	0.97 s	0.95 s	14.80	16.01
28	0.79 s	0.81 s	18.00	18.23
29	4.74 4.60	4.69 dd 4.57 d	109.60	109.61
30	1.68 s	1.68 s	21.90	21.69

Figure 4.11: Chemical Structure of Compound A_5(Lup-20(29)-en-3-one)

4.10 Anticonvulsant Studies

4.10.1 Acute toxicity studies in mice

The oral route median lethal dose of the methanol leaf extract (MLE) of *Combretum hypopilinum* was found to be above 5000 mg/kg body weight inmice which is relatively safe.

4.10.2 Maximum electroshock-induced convulsion in chicks

The MLE of *Combretum hypopilinum*did not show significant effect in the mean recovery time from seizure and noprotection was offered to the chicks against tonic hind limb extension (THLE) at all tested doses(125 mg/kg, 250 mg/kg and 500 mg/kg) when compared withthe standard anticonvulsant drug. The positive control group (PHT 20 mg/kg)gave 100% protections against THLE.

Table 4.8: Effect of Methanol Leaf Extract of *Combretum hypopilinum* on Maximal Electroshock-induced Convulsion in Chicks

Treatments	Mean recovery Time (min)	Quantal protection of THLE
NS 10 ml/kg	10.70 ± 1.21	0/10
MLE 125 mg/kg	8.10 ± 0.38	0/10
MLE 250 mg/kg	6.70 ± 0.58	0/10
MLE 500 mg/kg	6.4 ± 0.87	0/10
PHT 20 mg/kg	_	10/10

Mean recovery time expressed as mean ± SEM; protection against THLE expressed as Quantal protection; n = 10 per group; at $p < 0.05$statistical significant difference was observed between a control group and the values of treated groups;one-way ANOVA followed by Dunnett's post hoc test was used for statistical analysis; min = minutes; NS = Normal saline; MLE = Methanol leaf extract; PHT = Phenytoin; THLE = Tonic Hind limb extension.

4.10.3 Subcutaneous Pentylenetetrazole (*Sc*PTZ) induced Seizure in Mice

However, the MLE gave protection to some mice against clonic spasm at all tested doses of 125 mg/kg, 250 mg/kg and 500 mg/kg, thereby offering 60% maximum protection against the seizure. At the dose500 mg/kg of the MLE, and a dose-depended significant increase in the mean onset of seizure was observed when compared with the normal saline control group with statistical significant difference at $*p < 0.05$ as shown in Table 4.9.

Table 4.9: Effect of Methanol Leaf Extract of *Combretum hypopilinum* on Pentylenetetrazole-induced Convulsion in Mice.

Treatments	Mean onset of Seizure (min)	Quantal Protection against Seizure	% Protection against Seizure	Quantal Protection against mortality
NS 10 ml/kg	5.00 ± 0.55	0/5	0	0/5
MLE 125 mg/kg	6.80 ± 0.58	1/5	20	2/5
MLE 250 mg/kg	7.20 ± 0.58	2/5	40	4/5
MLE 500 mg/kg	9.80 ± 2.33*	3/5	60	4/5
VPA 20 mg/kg	_	5/5	100	5/5

Mean onset of clonic spasm expressed as Mean ±SEM; protection against clonic spasm expressed as Quantal and percentage protection; n = 5; protection against mortality are expressed as Quantal protection; at *$p<0.05$ statistical significant differences was observed between a control group and the values of treated groups; min=minutes; MLE = Methanol leaf extract; NS = Normal saline; VPA = Sodium valproate.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Phytochemical Screening and Spectral Analysis

The preliminary phytochemical screening carried out on the methanol leaf extract (MLE) and the partitioned fractions of *Combretum hypopilinum* revealed the presence of saponins, carbohydrates, cardiac glycosides, triterpenoids, steroids, tannins, flavonoids and alkaloids. The triterpenoids and steroids, among other phytochemical have been reported to have anticonvulsant activities (Barua *et al.*, 2013).

The column chromatography of the hexane fraction followed by recrystallization of column fraction C led to the isolation of a white solid crystals coded C_2(8 mg) which gave a single homogenous spot on the TLC using two different solvent systems. The melting point of compound C_2 was found to be at the range of 214-216^{o}C.

The IR spectrum of C_2 showed a characteristic absorption frequencies at 3362.1 cm^{-1}due to the hydrogen bonding of the hydroxyl group (OH). The corresponding C=C vibrations was observed at 1640 cm^{-1} as weakly intense bands due to the olefinic unsaturation.The corresponding C-O vibration was also shown as weak intense band at 1032.5 cm^{-1}. The stretching absorption frequencies at 2922.2 and 2855.1 cm^{-1}respectively were results of asymmetric and symmetric vibrations due to methyl and methylene groups. Absorption band at 1453.7 and 1379.1 cm^{-1} are typical CH_2 and CH_3 bending vibrations (Haruna *et al.*, 2017).

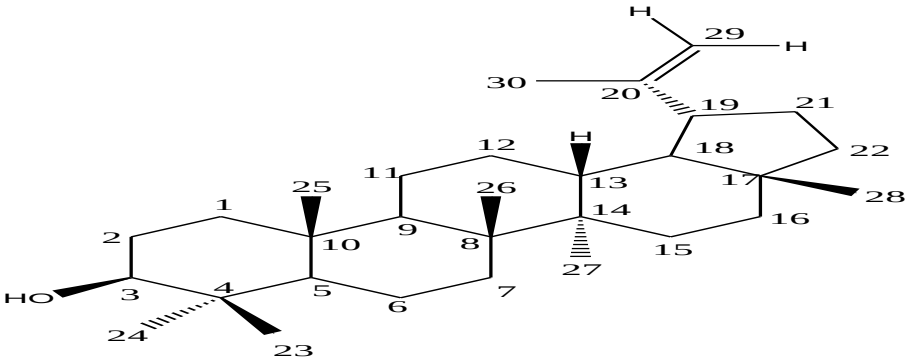
The ^{1}H NMR spectrum of compound C_2 revealed the presence of seven tertiary methyl proton signals as singlets at δ 1.01, 0.79, 0.83, 1.07, 0.97, 0.96 and 1.68 ppm respectively (integrated for 3H-each) due to the overlapping of the methyl and methylene protons

(Abdullahi *et al.,* 2013). A methine signal at δ 2.34 ppm was attributed to the proton at C-19 position. The H-3 proton showed a multiplet at δ 3.20 ppm while a pair of doublets at δ 4.68 and δ 4.57 ppm (IH, each) was indicative of olefinic protonsignals δ at (H-29 a and b) respectively (Figure 4.2). These assignments are in good agreement for the structure of lupeol (Abdullahi *et al.,* 2013; Haruna *et al.,* 2017).

The structural assignment of compound C_2 was further confirmed by the ^{13}C NMR experiments which revealed a total of 30 carbon atoms, which includes a carbon bonded to the hydroxyl group at C-3 position which appeared at δ 79.22, the olefinic carbons of the exocyclic double bond appeared at δ 151.20 and δ 109.54 ppm respectively, the spectrum also help in revealing the presence of quaternary carbons as shown in Figure 4.3. With the aid of DEPT spectrum of compound C_2 seven methyl, eleven methylene and six methine carbon atoms were assigned. The de-shielded signal δ at 79.22ppm was due to C-3 with a hydroxyl (OH) group attached to it as shown in Figure 4.4.The HSQC spectrum of compound C_2,which helped in correlating a hydrogen atom to its corresponding carbon atom, from the spectrum. The proton atom at δ 0.69 (H-5) which is a methine proton is correlating with the carbon atom at δ55.51(C-5) which shows that H-5 is bonded to C-5. The protons at δ 4.68 and δ 4.57 which methylene protons at position H-29a and H-29b respectively are bonded to C-29. The proton signal at δ 3.20 (H-3) correlated with methine carbon at δ 79.22 (C-3) which shows that H-3 is bonded to C-3 and also revealed that it is an oxymethine proton due to the presence of the OH group attached at C-3 on the structure of the compound. Long range correlation of ^{1}H ^{13}C of HMBC was used to prove bonding structure relationship of compound C_2. Long range correlation of proton at δ 1.68 (H-30) with quaternary carbon at δ 151.20 (C-20), methylene carbon at δ 109.54 (C-29) and methine carbon at δ 48.21 (C-19) revealed that the methyl carbon atom C-30 binds to the

quaternary carbonC-20. HMBC correlation of methyl protons at δ 1.01 (H-23) with methine carbons at δ 79.22 (C-3), δ 55.51 (C-5), quaternary carbon δ 38.93 (C-4), methyl carbon at δ 15.59 (C-24) and the methyl protons at δ 0.79 (H-24) correlating with quaternary carbon at δ 38.93 (C-4), methine carbons at δ 55.51 (C-5), δ 79.22 (C-3) and methylene carbon at δ 27.64 (C-2), supported the dimethyl position at C-4 as shown in Figure 4.6.

Comparing the1D and 2D spectraldata of compound $C_2$with the reported literature values, compound C_2 is suggested to be a lupeol, a pentacyclic triterpenes of the lupane group.



Chemical structure of compound C_2 (lup-20(29)-en-3-ol)

Column fraction A from the hexane fraction was re-chromatographed over 60-120 mesh size to silica gel to obtained a white amorphous powder coded A_5 (3 mg), which showed a single homogenous spot on a TLC plate using a solvent system. The melting point was found to be 168-170^{o}C.

IR spectrum of compound A_5 showeda characteristic absorption frequencies at 2922.2 and 2855.1 cm^{-1}respectively were results of asymmetric and symmetric vibrations due to methyl and methylene groups. A carbonyl absorption at 1707.1 cm^{-1}in the spectrum is an indicative of a saturated ketone. This was confirmed by the signal atδ 218.45 in the ^{13}C NMR spectrum.Absorption band at 1453.7 and 1379.1 cm^{-1} are typical CH_2 and CH_3 bending vibrations (Robert *et al.*, 2015).

The ^{1}H NMR spectrum of A_5 revealed the presence of seven tertiary methyl proton signals at δ 0.94, 0.80, 0.84, 1.05, 0.95, 0.81 and 1.68 respectively (integrated for 3H-each) due to the overlapping of the methyl and methylene protons (Robert *et al.*, 2015). A methine signal at δ 2.41 was attributed to the proton at C-19 position. However, the ^{1}H NMR spectrum of compoundA_5did not show the secondary hydroxyl group signal δ at 3.20 ppm (H-3) as it was in the case of compound C_2as shown in Figure 4.9**.**In addition, the ^{13}C NMR spectrum of compound A_5also revealed 30 carbon atoms similarly to that of compound C_2. It showed a de-shielded carbon atom signal at δ 218.45, which was assigned to the carbonyl carbon (saturated ketone) at C-3 position of compound A_5(Figure 4.10). These assignments are in good agreement for the structure of lupenone (Robert*et al.*, 2015). Comparing its^{1}H and ^{13}C spectral data with the reported literature, compound A_5 is suggested to be a lupenone, a pentacyclic triterpenoid of the lupane group.

Chemical structure of compound A_5 (Lup-20(29)-en-3-one)

Lupenone was also reported to have antimicrobial potential as it exhibited antifungal activity against *Candida albicans*, *Trichophyton mentagrophytes* and *Aspergillus niger*(Po-wei *et al.*, 2012). It was also reported to have exhibited lowantibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and partialinhibition of *Bacillus subtilis* (Po-wei *et al*.,2012).

5.2 Anticonvulsant Studies

The methanol leaf extract (MLE) was tested *in vivo* to ascertain its anticonvulsant activity. Both the MES and PTZ test models for anticonvulsant activity were employed. The results obtained were summarized on Table 4.8 and 4.9 respectively. The median lethal dose (LD_{50}) value of the methanol leaf extract of *Combretum hypopilinum* was found to be greater than 5000mg/kg body weight in mice. Thus, implies that the MLE is relatively safe, according to the toxicity classification by Lorke, (1983).

The MLE of *Combretum hypopilinum* did not give protection to the chicks against tonichind limb extension (THLE) at all tested doses of 125 mg/kg, 250 mg/kg and 500 mg/kg, while the standard anticonvulsant drug (Phenytoin) gave 100% protections against THLE in the positive control group. The MES test is a non-mechanistic seizure model that is clinically effective in identification of anticonvulsant drugs such as; phenytoin, carbamazepine, primidone, phenobarbital, sodium valproate and lamotrigine which all prevent the HLTE in MES test for the management ofgeneralized tonic-clonic and partial seizures (Rho and Sankar, 1999). The ability of an agent to prevent the THLE or to prolong the latency of the HLTE was considered as an indication of anticonvulsant activity (Sayyah *et al.,* 2002).Thus, the inability of the MLE to prevent the THLE suggests that, it may not be beneficial in the management of generalized tonic-clonic and partial seizure.

The MLE of *Combretum hypopilinum*at all tested doses of 125 mg/kg, 250 mg/kg and 500 mg/kg produced a dose-dependent anticonvulsant activity against PTZ-induced clonic spasm in mice. The MLE at the dose of 500 mg/kg gave 60% maximum protection against clonic spasm induced by the PTZ while the standard drug (sodium valproate) as the positive control group gave 100% protection against clonic spasmas shown on Table 4.9.

It has been found empirically that drugs which inhibit PTZ-induced seizures and raise the threshold for production of electrically induced seizures are generally effective against absence (Petit mal) seizures (Rang *et al.,* 2003). The dose-dependent anticonvulsant activity against PTZ-induced seizures suggests the presence of bioactive compounds effective in the therapy of absence (petit mal) or myoclonic seizures.It may also be plausible to suggest, that the anticonvulsant effect of the MLE may be due to the reduction of the T-type Ca^{2+} current, activation of GABA-ergic neurotransmission mediated by n-methyl-d-aspartate (NMDA) receptors or blockage of dopaminergic system in specific areas of the central nervous system (Magaji *et al.,* 2013).In conclusion, the findings of this study suggest that the MLE contain bioactive principles that may be beneficial in the management of absence (petit mal) seizure.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECONMMENDATIONS

6.1 Summary

The preliminary phytochemical screening of the MLE of *Combretum hypopilinum* revealed the presence of carbohydrates, cardiac glycosides, alkaloids, tannins, flavonoids, triterpenoids, steroid and saponins. The column chromatography of then-hexane fractionled to the isolation of lupeol and lupenone.

The anticonvulsant evaluation of the MLE of *Combretum hypopilinum*showed that the plant possesses significant activity effective in the therapy of absence (petit mal) seizure.

6.2 Conclusion

Lupeol and lupenone were isolated from the plant, the structure of which was identified on the basis of spectroscopic methods and comparing with spectral data reported in literature. The study validated the ethnomedicinal claim for the use of*Combretum hypopilinum* in the management of absence (petit mal) seizure.

6.3 Recommendations

i. There is need to carry out bioassay guided isolation with the view of isolating other bioactive constituents for the observed activity.

ii. Other fractions of the MLE should be subjected to column chromatography in other to isolate bioactive constituents as well as validating other ethnomedicinal claim.

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APPENDIX

I. Determination of oral median lethal dose (LD_{50}) of the methanol leaf extract of *Combretum hypopilinum.*

Dose mg/kg	**Number of mice used**	**Mortality**
10	3	0/3
100	3	0/3
1000	3	0/3

First Phase

Second Phase

Dose mg/kg	Number of mice used	Mortality
1600	1	0/1
2900	1	0/1
5000	1	0/1