

**PHYTOCHEMICAL AND ANTICONVULSANT STUDIES ON METHANOL EXTRACT  
OF THE ROOTBARK OF *FUVARIA CHAMAEP.* BEAUV. (ANNONACEAE)**

**BY**

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ZARIA, NIGERIA**

**MARCH, 2017**

## DECLARATION

I declare that the work in this dissertation entitled **PHYTOCHEMICAL AND ANTICONVULSANT STUDIES ON METHANOL EXTRACT OF THE ROOT BARK OF *UVARIA CHAMAE* P. BEAUV. (ANNONACEAE)** 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.

FATIMA IGANYA SULEIMAN

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Signature

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Date

## CERTIFICATION

This dissertation entitled **PHYTOCHEMICAL AND ANTICONVULSANT STUDIES ON METHANOL EXTRACT OF THE ROOTBARK OF *UVARIA CHAMAEP.BEAUV.* (ANNONACEAE)** by FATIMA IGANYA SULEIMAN 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.

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## **DEDICATION**

This work is dedicated to my husband, Dr. Suleiman S.who is my source of inspiration.

## ACKNOWLEDGMENT

All praises are due to Almighty Allah (SWT) for giving me all it takes to complete this work. I wish to express my profound gratitude to my supervisors Prof. M. I. Sule and Prof. (Mrs) H.S. Hassan, who despite their tight 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 Dr. Y. M. Sani throughout the course of this work and i will forever remain grateful to him. May Allah reward you abundantly.

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Not at all have I forgotten my siblings especially my elder brother and my fellow M.Sc and Ph.D students in the Department of Pharmaceutical and Medicinal Chemistry especially Garba with whom I shared ideas, thank you for your tremendous help to the success of this work. If I continue I will not finish. May Allah bless those who contributed in one way or the other to make this work a success.

## ABSTRACT

*Uvariachamae* P. Beauv. (Annonaceae) is widely distributed in Africa, the plant species are used in traditional medicine as an anti-inflammatory, antimalarial, and analgesic. It is also used for treatment of jaundice, epilepsy and microbial infections. The powdered root bark of *U.chamae* was extracted with methanol using maceration method and the resulting crude methanol extract (CME) was solvent fractionated to give hexane (HF), chloroform (CF), ethylacetate (EF) and n-Butanol (BF) fractions. Chloroform fraction was step-wisely eluted in a silica gel packed column to afford eleven fractions S<sub>1</sub>-S<sub>12</sub>. Fraction S<sub>6</sub> was subjected to Preparative Thin Layer Chromatography (PTLC), using hexane: ethyl acetate (5:3) as solvent system led to the isolation of Quercetin. The structure of the compound was elucidated using chemical test and spectroscopic techniques (UV and 1D-NMR) and by comparison with reference spectral data. The preliminary phytochemical screening of CME revealed the presence of carbohydrates, alkaloids, flavonoids, saponins, tannins, cardiac glycosides, steroids and triterpenes. The acute toxicity study was carried out using Lorke's method and the anticonvulsant activity was studied using maximal electroshock induced seizure test (MES) and subcutaneous pentylenetetrazole induced seizure test (Sc. PTZ). The LD<sub>50</sub> of the crude methanol extract (CME) was estimated to be 1131.37mg/kg in mice. The crude methanol extract (CME) was found to increase the mean time of recovery from seizures in MEST compared to negative control. The absence of anticonvulsant activity in MEST suggests that CME may not be useful in the treatment of generalized tonic clonic and partial seizures. In Sc.PTZ test, the CME at dose of 30mg/kg protected 80% of mice against pentylenetetrazole and significantly ( $P \leq 0.05$ ) delayed the onset of seizure. Standard antiepileptic drugs such as valproic acid are thought to produce their effects by enhancing GABA mediated inhibition in the brain. In this regard, the CME have demonstrated anticonvulsant activity and may be useful in treatment of generalized absence seizure.

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## ABBREVIATION

WHO	World Health Organization
LD <sub>50</sub>	Median Lethal Dose
<i>U.</i>	<i>Uvaria</i>
TLC	Thin-Layer Chromatography
1D	One Dimensional
UV	Ultraviolet
HF	Hexane Fraction
EF	Ethyl-acetate Fraction
CF	Chloroform Fraction
BF	n-Butanol Fraction
CME	Crude Methanol Extract
NMR	Nuclear Magnetic Resonance
CNS	Central Nervous System
PTLC	Preparative Thin-Layer Chromatography
R <sub>f</sub>	Retention Factor
H-NMR	Proton Nuclear Magnetic Resonance
MeOD	Deuterated Methanol
GABA	Gamma Amino Butyric Acid
MES	Maximum Electro Shock
MEST	Maximal Electro Shock Test
Sc.PTZ	Subcutaneous Pentylenetetrazole
i.p	Intraperitoneal

Sc.	Subcutaneous
EEG	Electroencephalogram
MRI	Magnetic Resonance Imaging
CT	Computerized Tomography
HTLE	Hind Limb Tonic Extension
PTZ	Pentylentetrazole
ADHD	Attention deficit Hyperactivity Disorder
FDA	Food and Drug Agency

## CHAPTER ONE

### 1.0 INTRODUCTION

#### 1.1 Natural product

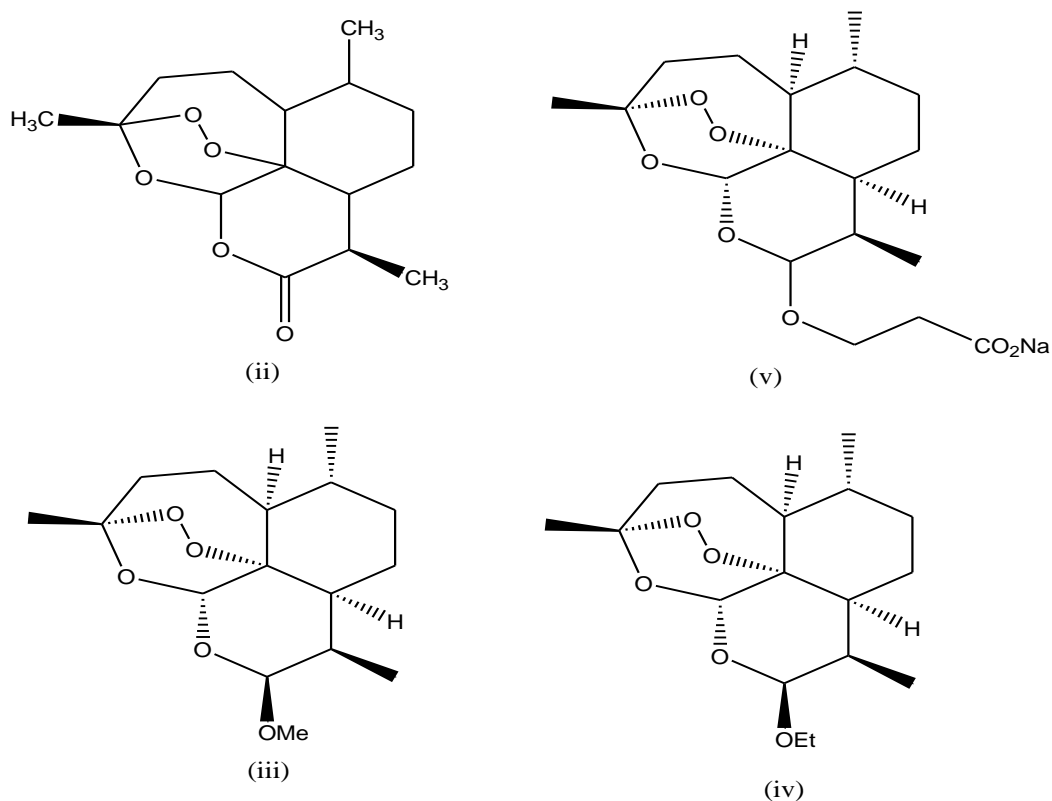
Natural products have been and still are an inexhaustible source of drugs. The medicinal properties of plants predate human history and are the basis of much of modern medicine. Traditional medicine has been defined as the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness (WHO, 2013). For many years, traditional medicine has proven to be effective in healthcare, disease management and prevention (Philipson, 2001). The use of traditional medicine is not limited to developing countries. Statistically, 70% of medical doctors in France and Germany regularly prescribe traditional medicine (Murray and Pizorno, 2000). Examples of drugs used in orthodox medicines that are derived from plants are: (i) Quinine: obtained from *Cinchona officinalis* bark, an antimalarial which lead to the synthesis of 8-amino and 4-amino quinoline antimalarial; (ii) Artemisinin: from *Artemisia annua* served as a lead for the synthesis of (iii) artemether, (iv) arteether, and (v) artesunate as antimalarials; (vi) Morphine: a narcotic analgesic from *Papaver somniferum* served as a lead compound to the synthesis of naloxone and buprenorphine; (vii) Cocaine: from *Erythroxylon coca* leaves served as a lead for more potent and less toxic local anaesthetics such as procaine and lidocaine; (viii) Digoxin: from foxglove (*Digitalis purpurea*, *D. lanata*) is still the best drug for the treatment of myocardial dysfunction; there is yet no synthetic drug capable of replacing the constituents of *Digitalis* (Olaniyi, 1989). The use of herbal medicine is becoming increasingly popular. There are various reasons for this upsurge; these are safety, availability, ease of accessibility or convenience, and low cost as compared to conventional medicine (Winslow and Kroll,

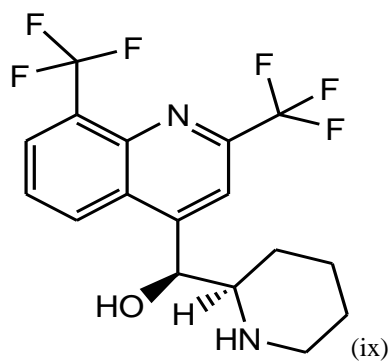
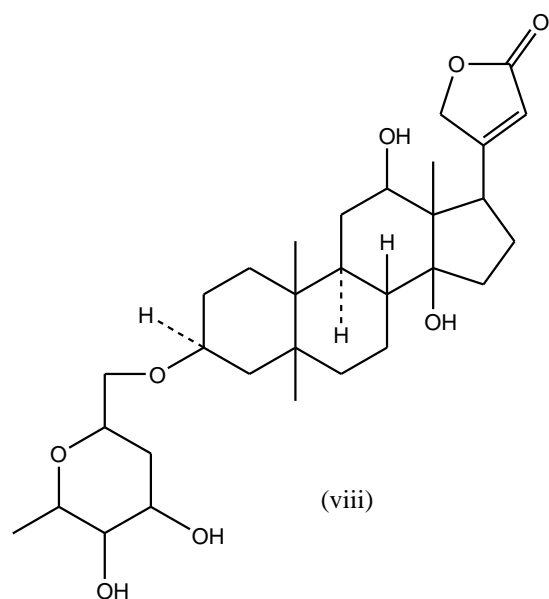
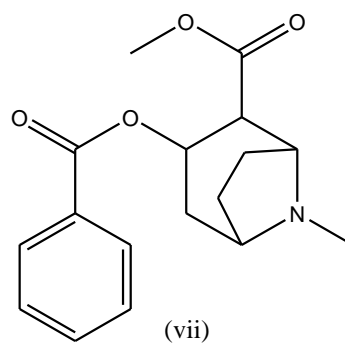
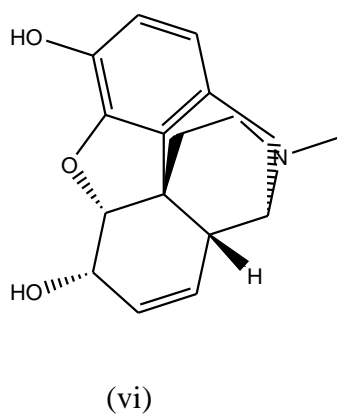
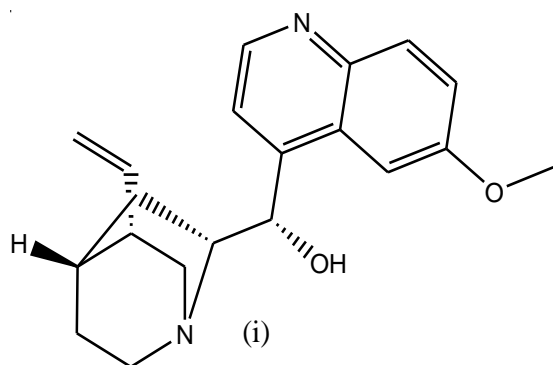
1998).

Nevertheless, herbal medicine can be harmful. They are heterogeneous in composition and impose challenges to quality control, assurance and the regulatory processes. Some of them contain heavy metals such as mercury, lead, corticosteroids and poisonous organic compounds in harmful amounts (Sanjoy and Yogeshwer, 2003). Thus the need to purify and isolate only the active components.

## 1.2 Artemisinin based analogues

Artemisinin (ii) is a sesquiterpinelactone isolated from the plant *Artemisia annua* and its semi-synthetic derivatives are such as, artemether (iii), arteether (iv), artesunate (v) are group of drugs that possess the most rapid action of all current drugs against *Plasmodium falciparum* malaria (White, 1997). In some areas of South-East Asia, combinations of artemisinins (ii) and mefloquine (ix) offer the only reliable treatment for uncomplicated malaria, due to the development and prevalence of multidrug resistant falciparum malaria (White, 2004).





### 1.3 Prevalence of Epilepsy

Epilepsy is a chronic neurological disorder of periodic and unpredictable seizures (provoked and unprovoked) from abnormal excessive episodic electrical activity in the brain. It is

characterized by signs and symptoms such as convulsion and loss of consciousness (McNamara, 2006; Blume *et al.*, 2001).

It accounts for 0.5% of the global burden of diseases with close to 80% of the cases worldwide found in developing regions (WHO, 2012). It occurs in all ages but most commonly in children and the elderly. Epilepsy is a non-communicable disease and was previously known as "Falling Sickness" (WHO Factsheets, 2009). Most patients with this disease suffer migraine, anxiety, hyperactivity disorder, infertility, serious injuries and suicide-related behaviours with depression (Walczak *et al.*, 2001; Pliophys *et al.*, 2007). People with epilepsy have more days off work, low self-esteem and poor academic performance (Scott *et al.*, 2001). The social stigmatization surrounding epilepsy is often more difficult to overcome than the seizure itself (WHO Factsheets, 2009).

The treatment of epilepsy is based on neuroprotection to reduce seizure duration (recovery) and suppress its occurrence (onset) (Arzimanoglou *et al.*, 2002). Many plant-based drugs have been used traditionally in the management of epilepsy of which only a limited number have been evaluated for their potential effects on the central nervous system including epilepsy (Arzimanoglou *et al.*, 2002).

The clinical effectiveness, minimal side effect profiles and relatively low cost of herbal drugs are the reasons for their various applications in traditional medicine (Valiathan, 1998). Their use in modern medicine need to be established by scientifically justifying their validation.

#### **1.4 Statement of Research Problems**

Epileptic seizures may lead to serious injuries and suicide-related behaviours with depression thus there is need for medical intervention (Walczak *et al.*, 2004; Pliophys *et al.*, 2007).

The WHO estimated in 2006 that neurological disorder and their direct consequences affect as many as one billion people in the world and identified health inequalities and social stigma or

discrimination as major factors contributing to the associated disability and suffering. Currently available antiepileptic drugs are confronted with problems such as, pharmacoresistance in approximately 30% of epileptic patients (Sander, 2004), as well as toxicity and serious side effects. In addition, continuous medication is necessary even after the seizures have long been suppressed.

In view of these drawbacks, most Epileptologists agree on the need for more selective and less toxic antiepileptic drugs.

### **1.5 Justification of the Study**

About 50 million people in the world have epilepsy (WHO Factsheets, 2009).and about one-third of them are not being adequately managed with the currently available antiepileptic drugs because of non-compliance as a result of long-term therapy, unwanted side effects, high cost and even unavailability of these drugs (McNamara, 2006).

Approximately 80% of the World inhabitants rely on traditional medicine for their primary health care and plants play important role in healthcare system (Cragg *et al.*, 1999). Plants which are readily available and naturally eaten as food could be used in the treatment of epilepsy. WHO encourages the inclusion of herbal medicine of proven safety and efficacy in health care problems of developing Countries (Amos *et al.*, 2001). Scientific research is therefore needed to provide evidence of the safety and efficacy of beneficial medicinal plants. Epilepsy is the most common non neurological public health issue especially in developing African countries. As many as 9 out of 10 people in Africa go untreated (WHO, 2012). This treatment gap greatly increases the burden of epilepsy and disability.

Antiepileptic drugs are expensive and beyond the reach of the common man in Nigeria and other African countries. The scientific investigations of the anticonvulsant activity of the root bark extract of *Uvaria chamae* will confirm or otherwise disprove its traditional use.

## **1.6 Aim and objectives of the Study**

### 1.6.1 Aim

The aim of this research work is to establish the phytochemical constituents present in the root bark of *U. chamae*, to isolate some compounds using chromatographic techniques and determine the anticonvulsant activities the crude methanol extract (CME) of the plants root bark.

### 1.6.2 Objectives

- i) To carryout preliminary phytochemical screening on the CME root bark of *U.chamae*.
- ii) To screen for the anticonvulsant activity of *U. chamae* crude methanol extract (CME).
- iii) To isolate some of the compounds through chromatographic techniques.
- iv) To characterize the isolated compounds using spectroscopic techniques.

## **1.7 Research Hypothesis**

The CME of the root bark of *U. chamae* has phytochemical constituents that have anticonvulsant activity

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 The Plant *Uvaria chamae*

The plant *Uvaria chamae* belongs to the *Uvaria* genus which is in the family *Annonaceae*. It is a climbing shrub or small tree growing up to 4 metres tall. The tree is used locally being harvested from the wild forest for its edible fruit, medicinal and other uses. The fruits are yellow when ripe and have a sweet pulp. The fruit carpels are finger-like clusters and the shape given rise to many vernacular names translated as bush banana or the like implying wildness (Burkill, 1985).

##### 2.1.1 Habitat

*Uvaria chamae* is a climbing large shrub or small tree native to Tropical West and Central Africa where it grows in wet and dry forest and coastal scrublands (Burkill, 1985).



Plate I: *Uvaria chamae* in its natural habitat.

### 2.1.2 Botanical features

The plant is commonly called bush banana, 'Kaskaifi' by the Hausas, 'Okoaja' by the Yorubas and 'Mmimiohia' by the Igbos in Nigeria (Burkill, 1985). The Igala people of Kogi State call it 'Ailoko'.

The genealogy of the plant is given as follows;

Kingdom: Plantae

Phylum: Magnoliophyta

Order: Magnoliales

Class: Magnoliopsida

Family: Annonaceae

Genus: Uvaria

Species: Chamae

### 2.1.3 Ethnomedicinal uses of *U. chamae*

The root bark of *U. chamae* is used as an astringent and galactagogue. It is taken orally in the treatment of inflammation of the mucous membranes, bronchitis and gonorrhoea (Burkill, 1985). It is also used in the treatment of epilepsy, dysentery, piles, epistaxis, haematuria, haematemesis and haemoptysis. It is boiled with spices and the decoction is taken for the treatment of fever that are classed locally as yellow-fever (Burkill, 1985).

The root is used in the treatment of fever, stomach ache, amenorrhoea, purgative and vermifuge. It is also used to prevent miscarriage and to relieve the pains of childbirth (Burkill, 1985). The sap of the leaves, roots and stems is widely used on wounds and sores and is said to promote rapid healing. A leaf-infusion is used as eyewash and a leaf-decoction as a febrifuge (Burkill, 1985; Achigan, 2009). The leaves are used for the treatment of malaria (Koudouvo *et al.*, 2011). Other species of *Uvaria* have also found use in folklore medicine and these includes; *U. doeringii*, *U. tortilis*, *U. scabrida*, *U. thomasi*. *U. doeringii* leaves decoction

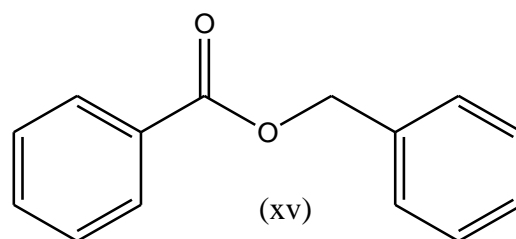
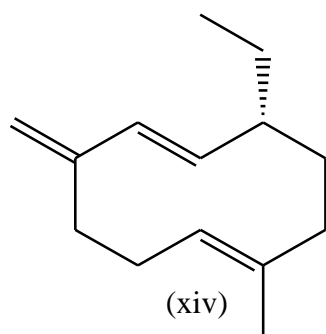
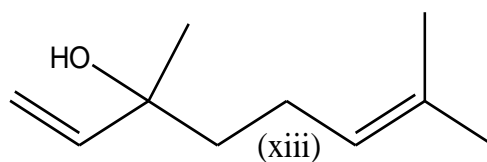
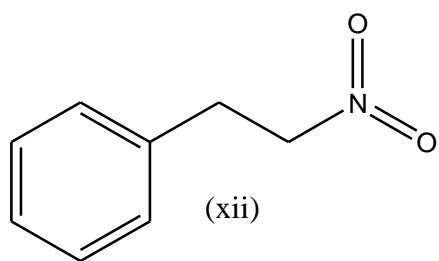
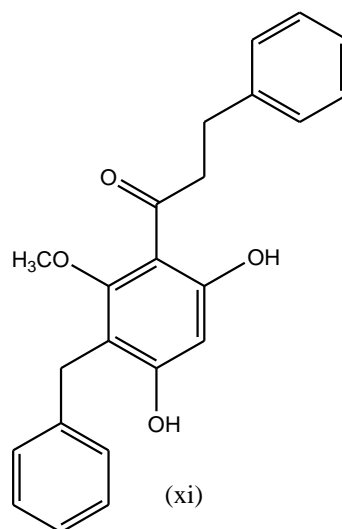
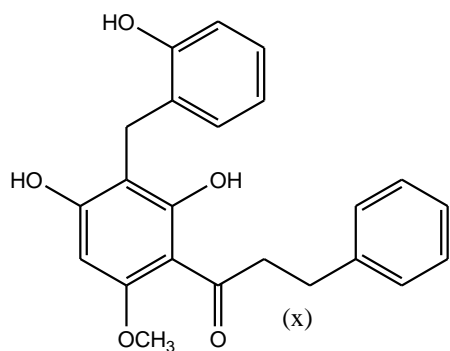
is taken for piles, palpitations and pains (Burkill, 1985). *U. tortilis* is used in the treatment of amenorrhoea (Borquet and Debray, 1974). *U. scabrida* is used in the treatment of insanity while *U. thomasi* used in the form of a leaf decoction for catarrh and colic (Kerharo and Adam, 1974).

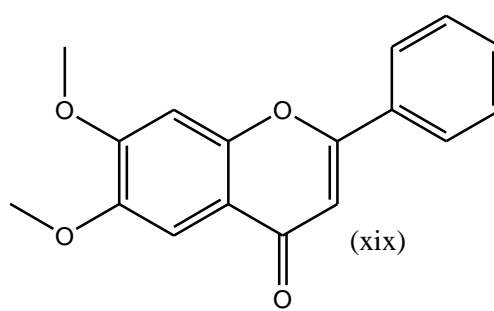
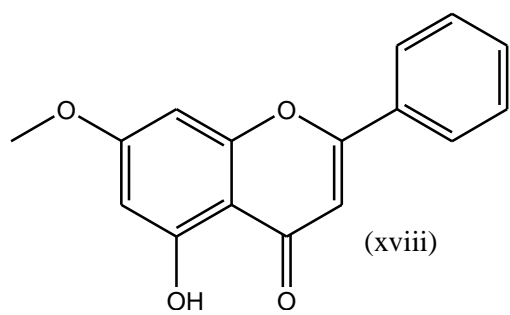
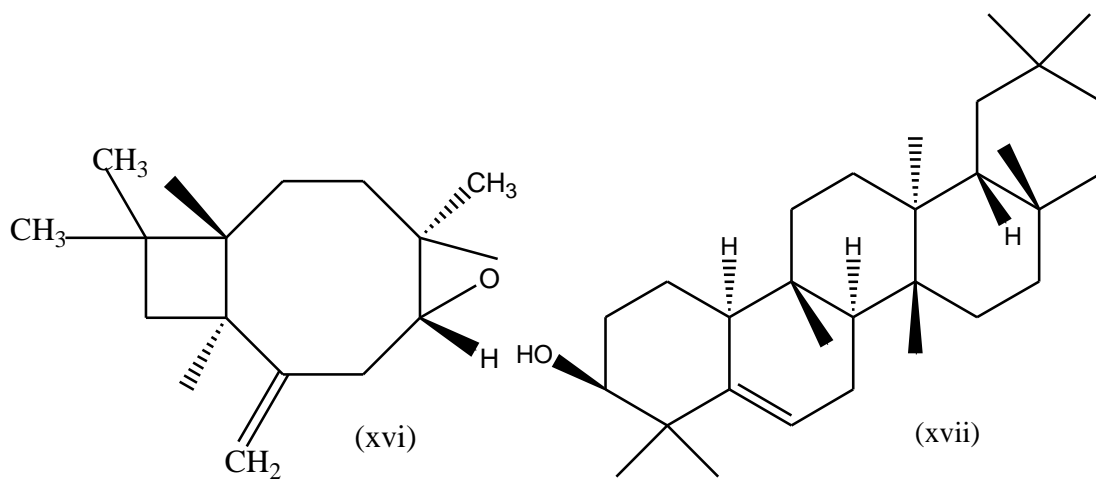
## **2.2 Pharmacological Actions of the Plants from the Genus *Uvaria***

The pharmacological actions of *Uvaria* species are numerous. *Uvaria chamae* has been reported to have sedative, analgesic, cardio-protective, anti-inflammatory, antiplasmodial and immuno modulatory activities (Okwu, 2007). It was also reported to have antibacterial and antifungal activities (Oluremi *et al.*, 2010; Okwuosa *et al.*, 2012). A related specie *Uvaria afzelli* has been reported to have anti-parasitic activity (Okpekon *et al.*, 2004). To the best of my search, there is no report in the literature on the anticonvulsant activity of *U. chamae*.

## **2.3 Chemical Constituents of *U. chamae***

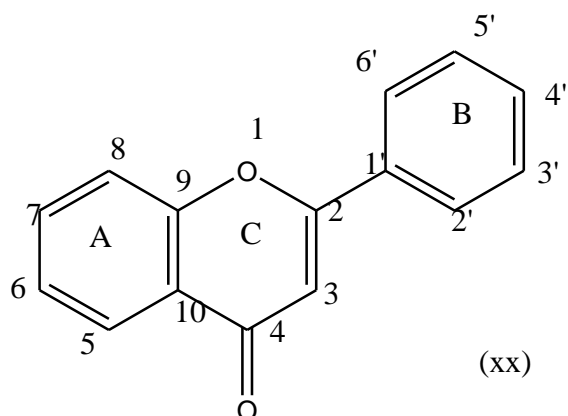
Previous phytochemical screening on *Uvaria chamae* leaves and root bark revealed the presence of alkaloids, saponin, tannins, flavonoids, cardiac glycoside, terpenoids, and steroid (Osuagwu and Ihewosu, 2014). A large number of flavonoids and other chemical compounds have been isolated from the seeds, leaves, stem bark and root of plant in the Annonaceae family (Hairin *et al.*, 2013). Epicatechin (Flavan-3-ols) was isolated from the leaves of *U. chamae* (Bila H., 2016). Uvaretin (x) and isouvaretin (xi) have been isolated from the stem bark of *Uvaria chamae* (Hufford and Laswell, 1976). 1-nitro-2-phenylethane (xii), linalool (xiii) and germacrene D (x) were isolated from the leaf oil of the plant (Moses *et al.*, 2013; Oguntimein *et al.*, 1989). Benzyl benzoate (xiv), caryophyllene oxide (xv), glutinol (xvi), 5-hydroxy-7-methoxyflavone (xvii), 5-hydroxy-6,7-dimethoxyflavone (xviii) have been isolated from *Uvaria rufa* (Rosandy *et al.*, 2013 ).





### 2.3.1 Flavonoids

Flavonoids are a large family of compounds synthesized by plants. Chemically they have the general structure of a 15-carbon skeleton, which consists of two phenyl rings (A and B) and heterocyclic ring (C) that contain one oxygen atom. The general structure of flavonoids (xvi)



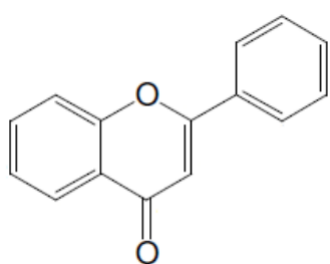
Flavonoids are secondary metabolites of plants with polyphenolic structure. They are synthesized by the polypropanoid pathway and the starting component is phenylalanine molecule, all flavonoids share the basic C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub> structural skeleton, consisting of two aromatic C<sub>6</sub> rings (A and B) and a heterocyclic ring (C) that contains one oxygen atom (xvi).

They have been classified into six subgroups:

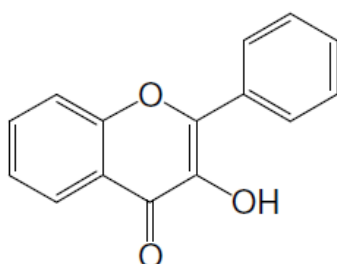
1. Flavones (xvii): (luteonin, apigenin, tangeritin).
2. Flavonols (xviii): (quercetin, kaemferol, myricetin, isorhamnetin, pachypodol).
3. Flavanones (xix): (hesteretin, naringenin, eriodictyol).
4. Flavan-3-ols (xx): (catechins and epicatechins).
5. Isoflavones (xxi): (genistein, daidzein, glycitein).
6. Anthocyanidins compounds (xxii): (cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin).

Other common flavonoid groups include chalcones, aurones, xanthenes, and condensed tannins (Manach *et al.*, 2004; Dahan and Altman, 2004).

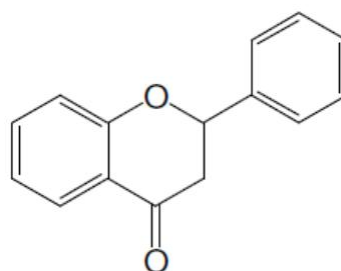
The catechins and leuco anthocyanidins are structurally similar and only rarely exist as their glycosides. Most flavonoids are present in our daily life (Manach *et al.*, 2004; Dahan and Altman, 2004). To date, about 6000 flavonoid compounds have been isolated and identified and many are common in higher plants (Tolonen *et al.*, 2002; Austin and Noel, 2003). Most flavonoid compounds which are often accumulated in the vacuoles of plant cells are glycoside (Aaron *et al.*, 2016).



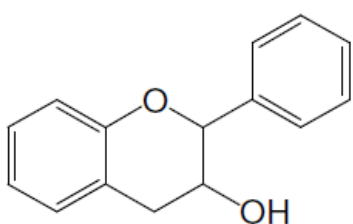
(xxi)



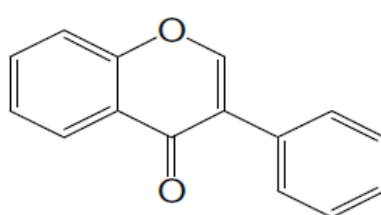
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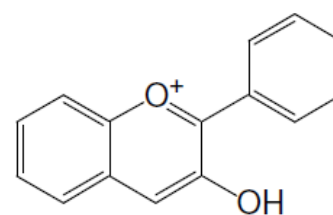
(xxiii)



(xxiv)



(xxv)



(xxvi)

## 2.4 Epilepsy

Epilepsy was believed to be a sacred disease characterized by momentary throwing of a person to the ground in a convulsive manner (hence the name falling Sickness). Thus convulsive fit (shaking, muscle contraction and relaxation of the body) are symptoms of epilepsy. The name lunatic was applied to sufferers of the disease while "maniacs" referred to mad people but it is now known that both terms refer to mentally incapacitated people (Randall *et al.*, 2012).

According to WHO definition, epilepsy is a chronic disorder of the central nervous system (CNS) of various aetiology due to alteration in the body system that causes excessive discharge of the central neurons. Epileptic seizures are recurrent, other seizures that occur once in a lifetime occasionally due to acute illness, hypoglycaemia, intentional chemical or electrical induction, etc should not be classified as epilepsy (WHO, 2012).

### 2.4.1 Pathophysiology

A rhythmic repetitive hyper synchronous discharge of neurons in the brain from a focus occurs if there is a change in normal neuronal discharge pattern resulting from damage, injury, chemical or metabolic assault which may either be localized in a portion of the brain or generalized throughout the brain (Sander, 2004).

### 2.4.2 Diagnosis

The diagnosis is clinical and by blood test, lumbar puncture and toxicological screening can be done to rule out underlying causes like drug withdrawal (e.g. alcohol and benzodiazepines), CNS infections (e.g. Meningitis), drug overdose, cardiac causes (e.g. cardiac arrhythmias), endocrine causes (hypoglycemia), syncope, cataplexy and cerebral hypoxia.

Acute seizure diagnosis depends on medical history i.e. finding out about previous convulsions or head trauma. Neurological examination and use of diagnostic techniques such as electroencephalogram (EEG), computerized tomography (CT) scan and magnetic

resonance imaging (MRI) should be done. EEG is the most critical in identifying the specific seizure type. The CT scan can be used to assess newly diagnosed patients, for structural causes of the disorder but MRI is preferred to CT scan as it locates brain lesions or other anatomical defects not detected by conventional radiography or CT scan (Sander, 2004).

#### 2.4.3 Signs and Symptoms

Temporary symptoms can occur such as loss of awareness or consciousness, disturbances of movement and sensation including vision, hearing, taste, mood or mental disturbance. People with epilepsy tend to have more physical problems such as fractures, bruise, cuts and injuries (WHO Fact sheets, 2009).

#### 2.4.4 Classification of Seizures

Seizures are mainly classified as generalized or partial. Either of these two is further classified as simple if consciousness is not lost and complex if consciousness is lost (Rang *et al.*, 2003).

##### 2.4.4.1 Generalized Seizures

i Generalized Tonic-Clonic Seizure (Major or Grand mal epilepsy)\_- It is the commonest form of epilepsy that last 1-2 minutes. It has a sequence of an aura (warning sign) and a cry that precedes unconsciousness that causes falling followed by tonic spasm and clonic jerking of the body muscles and the depression of all CNS functions (Rang *et al.*, 2003).

ii Absence Seizures (Minor or Petit mal epilepsy) - This is more common in children and lasts for about 30 seconds. It is characterized by a momentary loss of consciousness with no muscular component involved and little or no bilateral jerking.

There is also the infantile spasm characterized by intermittent muscle spasm and progressive mental deterioration (Rang *et al.*, 2003).

iii Atonic Seizure (Akinetic epilepsy) - There is loss of consciousness with relaxation of all muscles that may lead to fall due to excessive inhibitory discharge (Rang *et al.*, 2003).

iv Myoclonic epilepsy\_- There is shock-like momentary contraction of muscles of a limb or the whole body (Rang *et al.*, 2003).

#### 2.4.4.2 Partial Seizures

i Simple Partial Seizures\_- This is cortical focal epilepsy that usually lasts for about 30 to 60 seconds. The convulsions are confined to a localized group of muscles or senses depending on the area of the cortex involved and often without loss of consciousness (Rang *et al.*, 2003).

ii Complex partial seizures (Psychomotor or Temporal Lobe epilepsy) - It is preceded by an aura phase and characterized by bizarre and confused behaviour of purposeless movements and emotional changes in mood lasting 1-2 minutes with impairment of consciousness (Rang *et al.*, 2003).

iii Simple or Complex Partial Seizures (Secondary generalized)\_- Here, partial seizure occurs first and then degenerates into tonic-clonic seizures with loss of consciousness. This is an overlap of the two main classes of seizures. Status epilepticus is a dangerous state in which a person has frequent seizures without recovery or consciousness between each episode and if not treated might lead to brain damage or death (Rang *et al.*, 2003).

#### 2.4.5 Prevention of Epilepsy

Idiopathic epilepsy is not preventable but preventive measures can be applied to known causes of secondary epilepsy (WHO Fact sheets, 2009). Injuries leading to epilepsy can be prevented by wearing seatbelts, helmets and car seats for children to prevent head injury and trauma in case of an accident. Also, prescribing medications after first, second or febrile seizures may prevent epilepsy. Good prenatal care including treatment of high blood pressure and infections as well as the use of folic acid for neural tube defects can prevent brain damages in developing babies. Also identifying genes for neurological disorders can provide opportunities for genetic screening and prenatal diagnosis that may ultimately prevent seizures. Elimination of environmental parasites and education may be applicable to reduce epilepsy worldwide (WHO Fact sheets, 2009).

## 2.4.6 Management of Epilepsy

The management of epilepsy like most disorders involves both drug and non - drug management. Recent studies in both developed and developing countries have shown that up to 70% of newly - diagnosed epilepsy disorders can be successfully managed with antiepileptic drugs (AEDS). Such prompt intervention with use of AEDS for 2-5 years often ameliorates the disorder without relapses (WHO Fact sheets, 2009).

### 2.4.6.1 *Drug management of epilepsy*

Drugs commonly used in the management of epilepsy are referred to as antiepileptic drugs, anticonvulsant drugs or anti- seizure drugs. They includes;

- a) Aliphatic carboxylic acid e.g. Valproic acid
- b) Barbiturates e.g. Phenobarbital
- c) Benzodiazepines e.g. Clonazepam, Diazepam, Clobazam
- d) Cyclic GABA analogues e.g. Gabapentine
- e) Deoxybarbiturate e.g. Primidone
- f) Hydantoin, e.g Phenytoin
- g) Immunostibenes e.g. Carbamazepine
- h) Phenyltriazine e.g. Lamotrigine
- I) Succinimide e.g. Ethosuximide
- J) Newer agents include: Tiagabine, Levetiracetan, Vigabatrine, and Topiramate (WHO Fact sheets, 2009).

### 2.4.6.2 *Non Drug Management*

The non- pharmacological management of epilepsy include surgery, ketogenic diet and some other devices.

- a) Surgery:-This is an established option for epilepsies associated with lesions (neoplastic or vascular malformations) or temporal lobe epilepsy. Patients eligible for this are those with

clearly defined structural focus and history of partial seizure. Surgery is cost-effective especially when long term non-medical cost of uncontrolled epilepsy (such as treating fractures, skin grafting in case of burns, etc) are taken into account. The risk of surgery (such as that from anterior temporal lobectomy and multiple subpial transections) should always be balanced with the benefit (WHO Fact sheets, 2009).

b) Ketogenic Diet:-These are diet rich in fats but low in carbohydrates and so cause the body to breakdown fats rather than carbohydrates leading to ketosis. Studies have shown that maintaining children on such diets decreases the frequency of seizures. However, such diets may cause retarded growth due to nutritional deficiency and a buildup of uric acid in the blood leading to kidney stones. There has been no proof on how ketosis inhibits seizure in animals but a study showed that  $\beta$ -hydroxybutyrate, a by-product of ketosis inhibited seizures in animals (WHO Fact sheets, 2009).

c) Other Devices:- These are techniques used to stimulate specific areas of the CNS in an attempt to produce seizure control. Two of these techniques are currently undergoing development, one of which (the vagus nerve stimulation) has been approved by the Food and Drug Agency (F.D.A) in the United States of America since 1997 for medically intractable epilepsy. The intracranial magnetic stimulations is still being investigated. There is also hope of developing an implantable device that can deliver drugs to specific parts of the brain (WHO Fact sheets, 2009).

#### 2.4.7 Mechanism of action of anticonvulsants

These drugs generally suppress the discharge of neurons in seizure focus vis-a-vis propagation of seizure activity via:

- i) Enhancement of inhibitory neurotransmitters in the brain such as gamma aminobutyric acid (GABA).
- ii) Modification of ionic conductance ( $\text{Na}^+$  and  $\text{Ca}^{2+}$ )
- iii) Inhibition of excitatory neurotransmitters in the brain such as glutamine

*2.4.7.1 Enhancement of GABA:-* GABA is the predominant inhibitory neurotransmitter in the brain and has two receptor subtypes, GABA<sub>A</sub> with a Cl<sup>-</sup> channel within and GABA<sub>B</sub>, a G-protein coupled receptor. Benzodiazepines, barbiturates and valproic acid are antiepileptics specific for GABA<sub>A</sub> and potentiate GABA by inhibiting GABA transaminase (e.g. vigabatrine ), increasing GABA release at the synapses (e.g Gabapentin) and/or inhibiting GABA reuptake (e.g Tiagabine) ( Shastri *et al* 2005., Rang *et al.*, 2003).

*2.4.7.2 Modification of ionic conductance:-* this takes place via the following route;

Prolongation of sodium channel inactivation makes the neuronal membrane refractory to the generation of the next action potential thereby reducing neuronal discharge. Drugs such as phenytoin and valproic acid act via sodium channel inhibition and are often used for partial and secondary generalized seizures (Randall *et al.*, 2009). Inhibition of low threshold calcium channels of the T - type increases refractory period and reduce neuronal activity thus producing minor antiepileptic effects e.g. ethosuximide effective in absence seizure. Gabapentin in addition to its effect on GABA release also acts on L - type calcium channels, the effect of which on epilepsy is unknown (Rang *et al.*, 2003).

*2.4.7.3 Inhibition of Excitatory Neurotransmitters:-*Inhibition of the excitatory glutaminergic transmission via the blockage of glutamate channels e.g. Lamotrigine (Randall *et al.*, 2009).

#### 2.4.8 Prognosis

Once a substantial period of about 2-5 years of remission has been achieved, the risk of seizure is greatly reduced and has been the case in about 70 – 80 % of people with epilepsy while in others (20-30 %), it may be a lifelong condition requiring medication for life. This is more commonly seen in symptomatic epilepsy conditions, patients with more than one seizure type, associated learning disabilities, neurological or psychiatric disorders (Sander, 2004).

Some diseases occur at a higher rate than reported in people with epilepsy such as depression, anxiety disorder, migraine, infertility, decreased libido, dementia, mental retardation, multiple sclerosis, bowel disorders, urinary incontinence, Alzheimer's disease and attention deficit hyperactive disorder (ADHD) in children.

#### 2.4.9 Mortality

Although serious injuries can occur when seizure occurs during or when operating equipments, there is usually very low risk of sudden death with the disease. Most of those at high risk of epilepsy-related deaths have other neurological impairment or poorly-controlled seizures (WHO Factsheets, 2009).

### **2.5 Experimental Models for Screening of Anticonvulsants Agents**

There are a lot of experimental models used for screening both acute and chronic anticonvulsant activity of drugs in laboratory animals (such as rats, mice and day old chicks). The pentylenetetrazole (PTZ)-induced convulsion and maximal electroshock (MES) test are two most commonly used methods for acute evaluation.

i The MES test is most commonly used to screen for drugs effective in grand mal seizures whereby hind limb tonic extension (HLTE) are induced by electrical stimuli. The behavioural and electrographic seizures generated in this model are consistent with the human disorder of this type of seizures (Swinyard and Kupferberg, 1989). The apparatus with corneal or ear electrodes of Woodbury and Davenport is often used to deliver the stimuli of grand mal epilepsy and the potential of a compound to suppress or antagonize the induced HTLE is assessed.

ii The pentylenetetrazole is an agent most commonly used for chemical assay. This model identifies primarily compounds that raise the seizure threshold such that animals resist the cause of the seizure. The profile of the subcutaneous PTZ model is also consistent with human conditions (Swinyard and Kupferberg, 1989).

iii Picrotoxin is used to induce seizure so as to further evaluate central nervous system (CNS) active compounds. It is a gamma amino butyric acid (GABA)-A antagonist modifying the functions of the chloride ion channels of the receptor (Vogel and Vogel, 1997).

iv Strychnine -induced convulsions - Strychnine competitively antagonizes inhibitory effect of the neurotransmitter glycine.

v Pilocarpine - induced convulsion is used as a model for temporal lobe epilepsy and it also generates status epilepticus.

vi 4-Amino Pyridine - induced convulsion is used for compounds that act by opening K<sup>+</sup> channel (Rogawski and Porter, 1992).

vii Alumina cream model is used for chronic evaluation of anticonvulsants and it involves application of alumina cream on motor cortex of a monkey.

viii The Kindled model is also used for chronic evaluation.

## 2.6 Medicinal Plants and Epilepsy

Numerous medicinal plants are used in the treatment of epilepsy in Africa, (Bum *et al.*, 2011) in Cameroon established twenty three plants with anticonvulsant properties, eighteen of which showed moderate activity, five had very good anticonvulsant activity, while three plants were found to be very toxic, thus leaving twenty medicinal plants for safe use in treatment of epilepsy. These range from leaves, flowers, barks and roots of various plants such as *Annona muricata*, *Bidens pilosa*, *Citrus sinensis*, *Daniellia oliveri* and *Khaya senegalensis* amongst others.

Similarly, in a review of traditional plants for epilepsy amongst the Hausa Fulani of Northern Nigeria, (Muazu and Kaita, 2008) showed that plants such as *Securidaca longipedunculata*, *Mitragyna inermis* and *Celtis integrifolia* all had anticonvulsant activity.

### 2.6.1 Phytochemical constituents with anticonvulsant activity

In natural product chemistry, it is usually difficult to isolate sufficient quantity of phytochemical compounds for analysis and pharmacological activity. However, (Sayyah *et*

*al.*, 2004) opined that monoterpenoids isolated from *Artemisia drancunculus* may be responsible for the observed anticonvulsant effect of the essential oil obtained from the aerial parts of the plant.

Other phytochemical compounds with anticonvulsant activity includes Thymoquinone, Pinene,  $\alpha$  – asarone, Linalool, Eugenol, Citronellol, Piperitone, Eudesmol and Jatamasone, most of which are phenolic compounds.

## CHAPTER THREE

### 3.0 MATERIALS AND METHOD

#### 3.1 Materials

##### 3.1.1 Solvents/Reagents and chromatography materials.

Solvents used are of analytical grade obtained from Sigma, Aldrich's, Germany, they include; Methanol, Hexane, Chloroform, Ethylacetate, and reagents used include those from photochemical screening such as, Molisch's reagent, Salkowski's reagent, Dragendoff's reagent, Mayer's reagent, Wagner's reagent and Bontrager's reagent . Chromatographic materials used are TLC plates (Aluminium) Silica gel (60-120 mesh), Chromatographic tanks and columns.

##### 3.1.2 Equipment

Thermo electron UV machine and Gallenkamp melting point apparatus at the Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria was used for UV-spectroscopy and determination of melting point. Bruker Avance III NMR spectrometer (400MHz) at School of Chemistry, University of Malaya, Kuala Lumpur Malaysia, was used for 1D NMR spectroscopy.

##### 3.1.3 Experimental animals

Locally bred adult Swiss albino mice of either sex (15-30 g body weight) were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. Day old chicks were obtained from Mall Musa at kwangila. The animals were fed with laboratory diet and water *ad libitum* and maintained under standard conditions in propylene cages at room temperature.

##### 3.1.4 Collection and identification of plant material

The leaves and root bark of *Uvaria chamae* was collected in September, 2014 from Idah Local Government Area of Kogi State. It was confirmed and authenticated by mallam

Namadi Sanusi at the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University, Zaria by comparing it with a standard specimen with voucher number 3129.

## **3.2 Methods**

### **3.2.1 Extraction and partitioning**

The root bark (1 kg) of the plant was dried, powdered and extracted with methanol using the maceration method. 250 g of crude methanol extract (CME) was subjected to solvent partitioning by dissolving it in distilled water (100 ml) and fractionating successively with hexane (600 ml), chloroform (900 ml), ethylacetate (400 ml), and n-butanol (500 ml) which afforded HF, CF, EF, BF. respectively. The stepwise processes carried out is shown on scheme I.

### **3.2.2 Phytochemical Constituents**

The preliminary phytochemical screening was carried out on the crude methanol extract (CME) according to standard procedures as summarized below.

#### *3.2.2.1 Test for anthraquinones*

Bontrager's Test: the extract (CME) 0.2 g was dissolved in 5 ml chloroform, shaken and filtered. To the filtrate, an equal volume of 10 % ammonia solution was added with continuous shaking. The formation of a pink red or violet colour indicates the presence of anthraquinones (Trease and Evans, 1996).

#### *3.2.2.2 Tests for alkaloids*

- a. Dragendoff Test: The extract (CME) 0.2g was weighed and 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. The formation of a red precipitate indicates the presence of alkaloids (Trease and Evans, 1996)

- b. Mayer's Test: To 1 ml acidic solution of the extract (CME) in a test tube, few drops of Mayer's reagent were added. The formation of a cream precipitate indicates the presence of alkaloid (Sofowora, 1982).

#### 3.2.2.3 Test for Carbohydrates

Molisch Test: To 2 ml aqueous portion of the extract (CME) in a test tube, few drops of freshly prepared Molisch reagent followed by concentrated sulphuric acid were added down the test tube in a slanting position. The formation of a red coloured ring at the interphase indicates the presence of carbohydrates (Trease and Evans, 1996).

#### 3.2.2.4 Test for Cardiac Glycosides

Keller-Kiliani Test: A small portion of the extract (CME) was dissolved in 1 ml 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 will indicate the presence of a deoxy sugar (Trease and Evans, 1996).

#### 3.2.2.5 Test for Saponins

Frothing Test: 10 ml of distilled water was added to a portion of the extract (CME) in a test tube and shaken vigorously for 30 seconds. The tube was allowed to stand for 30 minutes. The formation of a persistent froth indicates the presence of saponins (Trease and Evans, 1996).

#### 3.2.2.6 Tests for Flavonoids

- a. Sodium hydroxide Test: To 0.2 g the extract (CME), 10 % sodium hydroxide solution was added. This was followed by few drops of Hydrochloric acid. The formation of yellow colouration indicates the presence of flavonoids (Silva *et al.*, 1998).
- b. Shinoda Test: A small quantity of the extract (CME) was dissolved in methanol. Some

pieces of magnesium chip were added followed by five drops of concentrated hydrochloric acid. The formation of a red colouration indicates the presence of flavonoids (Silva *et al.*, 1998).

#### *3.2.2.7 Tests for Tannins*

a. Ferric chloride test: An aqueous solution of the extract (CME) was prepared. 4 drops of ferric chloride solution was added to the extract in a test tube. The formation of a green precipitate indicates the presence of hydrolysable tannins (Trease and Evans, 1996).

b. Lead sub-acetate test: A solution of 1 % lead acetate solution was added to 5 ml solution of the extract in a test tube. The formation of a coloured precipitate indicates the presence of tannins (Trease and Evans, 1996).

#### *3.2.2.8 Test for Steroids/Triterpenes*

a. Liebermann - Buchard Test: A chloroform solution of the extract was prepared to which equal volume of acetic anhydride was added and mixed gently. 1ml concentrated sulphuric acid was added down the test tube. This was observed for instant colour changes. Blue to blue-green color in the upper layer and a redish pink or purple color at the junction of the two layers indicates the presence of triterpene (Trease and Evans, 1996).

b. Salkowski test: A small portion of the extract was dissolve in 1ml of chloroform, 2-3 drops of concentrated sulphuric acid was added at the side of the test tube. The appearance of red color indicates the presence of steroids (Sofowora, 1983).

### 3.3 Chromatographic Procedures

#### 3.3.1 Thin-layer chromatography (TLC) of CME, and different fractions (HF, CF, EF, and BF)

Pre-coated TLC plates were used to carry out thin layer chromatography by one way ascending technique. Capillary tubes were used to manually apply spots and the chromatogram was developed in an air tight chromatographic tank at room temperature, employing hexane ethylacetate (7:3). The spots were visualized after been sprayed with 10 % sulphuric acid followed by heating in an oven for 5 minutes at a temperature of 105<sup>0</sup>C.

#### 3.3.2 Column chromatography of chloroform fraction

The column was packed using the slurry method. Cotton wool was placed at the bottom end of the column and solvent was added. The tap was then open to be sure it runs. The column was packed with silica gel suspended in hexane and allowed to settle. Cotton wool was also placed on the bed of the silica gel. The chloroform fraction was dissolved in chloroform, and silica gel was added to form a powdered mixture which was allowed to dry. The dried mixture was then packed on the cotton wool on the silica gel bed.

The column was gradiently eluted starting with hexane (100 %) a gradient mixture of hexane and chloroform through chloroform (100 %) to chloroform – ethylacetate (80 : 20) before the collection of the target compound in a less complicated mixture, as monitored by thin layer chromatography. Different column fractions collected were pooled together into twelve major combinations depending on the similarity of their TLC profiles. The twelve different combinations of the fractions are shown on Table 3.1.

#### 3.3.3 Preparative thin-layer chromatography (PTLC) of fraction S<sub>6</sub>

Preparative thin layer chromatography was performed using pre-coated silica gel glass plate (20 x 20 cm and 0.25 mm) thickness. Fraction S<sub>6</sub> was dissolved in methanol and capillary tubes were used to uniformly apply the dissolved sample on a thin line marked with pencil

about 2.5 cm from the bottom of the plate and allowed to dry. The dried plates were developed in a chromatographic tank using hexane : ethyl acetate (5 : 3) solvent system in sufficient quantity. The region containing the band of interest was marked and scrapped off after a part of the plate was cut off and sprayed with 10 % sulphuric acid and heated in an oven to visualize the spot. The scrapped sorbent was dissolved in methanol, filtered using syntax glass and repeatedly extracted with the mixture of hexane: ethyl acetate (5 : 3); the solution obtained was evaporated to afford a brown compound coded FS.

### **3.4 Characterization of Compound FS**

#### a. Solubility test:

The solubility of FS was observed in Acetone and Methanol.

#### b. Chemical test

The isolated compound FS was subjected to Shinoda test.

#### c. Melting point determination

The melting point of the isolated compound FS was determined using melting point apparatus.

### **3.5 Spectral Analysis of Compound**

The isolated compound FS was subjected to UV and 1D NMR analysis.

### **3.6 Anticonvulsant Studies**

#### 3.6.1 Acute toxicity studies

Nine mice were divided randomly into 3 groups of 3 mice each. In the first phase, varying doses of the extract (10, 100 and 1000 mg/kg body weight) were administered ip to groups 1, 2 and 3 respectively and observed for 24 hours for any sign of toxicity and mortality. In the second phase, doses of 200 mg/kg, 400 mg/kg, 800 mg/kg, and 1600 mg/kg were administered to 4 fresh mice through the same route and observed also for 24 hours (Lorke, 1983). The median lethal dose was calculated using the formula:

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

### 3.6.2 Anticonvulsant screening

#### 3.6.2.1 Maximal electroshock induced seizure test in chicks

For the MES screening, UGO Basile electroconvulsive unit ECT 800 fitted with ear clip electrodes, with shock duration 0.8 seconds, frequency 100 seconds, pulse width 0.8 m/s, current 80 mA, was used. The method of (Swinyard and Kupferberg, 1989) was employed. Day-old white ranger cockerels (50) were randomly divided into five groups of 10 chicks each. Group 1 (negative control) were given normal saline intraperitoneally (i.p). Group 5 (positive control) were given 20 mg/kg phenytoin. Group 2-4 were given 7.5 mg/kg, 15 mg/kg and 30 mg/kg of CME i.p respectively.

Thirty minutes later, maximum electroshock was delivered to induce seizure in all the groups. An episode of HLTE was regarded as full convulsion while lack of tonic extension of the hind limb was regarded as protection. In unprotected animals the recovery time was recorded.

#### 3.6.2.2 Pentylentetrazole (PTZ) Induced Seizure in Mice

The method of (Swinyard and Kupferberg, 1989) was also employed. 25 mice were divided into 5 groups of 5 mice each. Group 1 (negative control) received normal saline (10 mg/kg) i.p, group 5 (positive control) received valproic acid 200 mg/kg i.p. Groups 2-4 were given 7.5 mg/kg, 15 mg/kg and 30 mg/kg of crude methanol extract (CME) i.p respectively.

After thirty minutes, all the groups were administered 20 mg/kg freshly prepared PTZ subcutaneously. All the mice were observed for a period of 30 minutes after *sc.* PTZ administration for episodes of clonic spasm of the hind limbs of at least 5 seconds duration with loss of righting reflex. The time of onset of seizure and time of death, were observed and recorded and used for analysis. The absence of seizures was considered as protection.

### 3.6.3 Statistical analysis

The mean onset of seizure and mean recovery time were presented as mean  $\pm$  standard error of mean (SEM). The mean values of the control group were compared with the mean values

of the groups treated with the test extracts using one - way ANOVA followed by Dunnette post-hoc test for multiple comparison. The result was considered significant at  $p \leq 0.05$

## CHAPTER FOUR

### 4.0 RESULTS

#### 4.1 Extraction and Partitioning

Extraction and Partitioning of 1 kg root bark of *Uvaria chamae* gave yields as presented in Table 4.1

Table 4.1: Weight and Percentage yield of extract and Partitioned fractions of *U. chamae*

Solvent	Weight (g)	Yield (%)	Colour
Crude methanol extract	68.52	6.85	brown
Hexane	10.07	1.01	oily brown
Chloroform	18.78	1.88	orange brown
Ethyl acetate	4.25	0.43	dark brown
n-butanol	5.63	0.56	Brown

## 4.2 Phytochemical Constituents

Preliminary phytochemical screening of the crude methanol extract revealed the presence of Carbohydrates, flavonoids, tannins, saponins, steroids, alkaloids, cardiac glycosides and terpenes (Table 4.2).

Table 4.2: Phytochemical Constituents of the Crude Methanol Extract (CME)

Constituents	Test	Observation
Anthraquinones	Bontrager	-
Alkaloids	Dragendoff	+
	Mayer	+
Carbohydrates	Molisch	+
Cardiac Glycosides	Keller-Kiliani	+
Saponins	Frothing	+
Flavonoids	Sodium Hydroxide	+
	Shinoda	+
Tannins	Ferric chloride	+
	Lead sub-acetate	+
Triterpenes/Steroids	Liebermann-Burchard	+
	Salkowski	+

**Key :** + = present, - = absent

### 4.3 Thin-Layer Chromatography

4.3.1 The Thin-layer Chromatographic Profiles of CME, and different Fractions (HF, CF, EF, and BF), using hexane-ethyl acetate, (5:3) are shown in Table 4.3 and Plate II.

Table 4.3: Summary of TLC Profiles of Extracts/Fractions of *U. chamae*

Extract / Fractions	No. of Spots	R <sub>f</sub> values of spots
Crude methanol extract (CME)	3	0.21, 0.38, 0.47
Hexane (HF)	2	0.62, 0.75
Chloroform (CF)	4	0.18, 0.39, 0.52, 0.61
Ethyl acetate (EF)	Nil	Nil
n-butanol (BF)	Nil	Nil



Plate II: TLC profile of CME, HF, CF, EF and BF in hexane-ethyl acetate (5:3).

### 4.3.2 Thin-layer Chromatography of Various Fractions of CF Obtained from Column Chromatography

The column chromatography of CF gave twelve pooled fractions, the TLC profile of these column fractions were coded S<sub>1</sub> □ S<sub>12</sub> as shown in Table 4.4 and 4.5, they were developed using hexane: ethylacetate (7:3) as solvent system, the TLC revealed various spots as shown in Table 4.5 and plates III-VIII.

Table 4.4: Column Chromatographic Studies of Chloroform Fraction

S/No	Eluting solvent (%)	Fractions	TLC Status	Codes
1.	n-Hexane 100	1-11	No profile	Nil
2.	Hex : Chloroform 97.5 : 2.5	12-19	No profile	Nil
3.	Hex : Chloroform 95 : 5	20-23	No profile	Nil
		24-32	No profile	Nil
4.	Hex : Chloroform 90 : 10	33-52	Similar profile	S <sub>1</sub>
		53-58	No profile	Nil
5.	Hex : Chloroform 80 : 20	59-66	Similar profile	S <sub>2</sub>
		67-69	No profile	Nil
		70-72	Similar profile	S <sub>3</sub>
6.	Chloroform 100	73	No profile	S <sub>4</sub>
7.	CHCl <sub>3</sub> : Ethyl acetate 90 : 10	74-75	Similar profile	S <sub>5</sub>
		76	No profile	
8.	CHCl <sub>3</sub> :Ethyl acetate 80 : 20	77-78	Similar profile	S <sub>6</sub>
9.	CHCl <sub>3</sub> : Ethyl acetate 75 : 25	79 – 81	Similar profile	S <sub>7</sub>
10.	CHCl <sub>3</sub> : Ethyl acetate 70 : 30	82 – 84	Similar profile	S <sub>8</sub>
11.	CHCl <sub>3</sub> :Ethyl acetate 80 : 20	85 – 89	Similar profile	S <sub>9</sub>
13.	CHCl <sub>3</sub> : Ethyl acetate 95 : 5	95-97	Similar profile	S <sub>11</sub>
14.	Ethyl acetate 100	98-101	Similar profile	S <sub>12</sub>

Table 4.5: Column Chromatography of the Chloroform fraction

<b>Fraction</b>	<b>Eluting solvent (%)</b>	<b>Fractions</b>	<b>No of clear spots</b>
S <sub>1</sub>	Hexane : Chloroform 90:10	33-52	4 spots
S <sub>2</sub>	Hexane : Chloroform 80:20	59-66	3 spots
S <sub>3</sub>	Hexane : Chloroform 80:20	70-72	2 spots
S <sub>4</sub>	Chloroform 100	73	No clear spots
S <sub>5</sub>	Chloroform : Ethyl acetate 90:10	74-75	4 spots
S <sub>6</sub>	Chloroform : Ethyl acetate 80:20	77-78	2 spots
S <sub>7</sub>	Chloroform : Ethyl acetate 75:25	79-81	No clear spot
S <sub>8</sub>	Chloroform : Ethyl acetate 70 : 30	82-84	2 spots
S <sub>9</sub>	Chloroform : Ethyl acetate 80 : 20	85-89	3 spots
S <sub>10</sub>	Chloroform : Ethyl acetate 90 : 10	90-94	2 spots
S <sub>11</sub>	Chloroform: Ethyl acetate 95 : 5	95-97	2 spots
S <sub>12</sub>	Ethyl acetate 100	98-101	2 spots

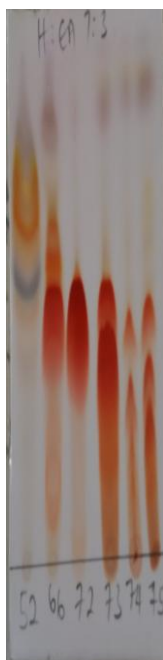


Plate III



Plate V

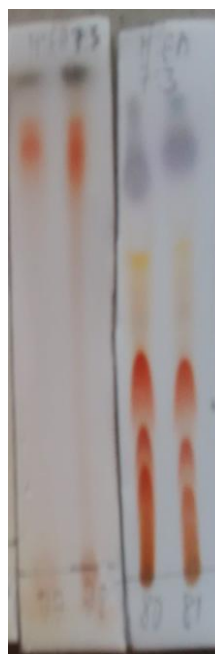


Plate IV

Plates III - IV: Thin-layer chromatography of various fractions of CF obtained from column chromatography.

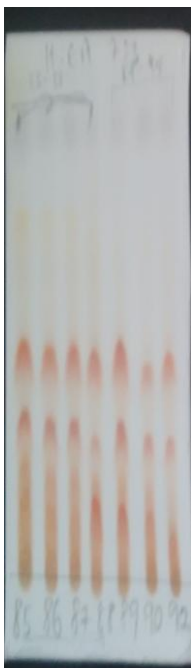


Plate VI

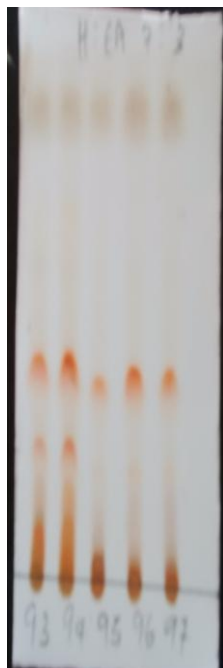


Plate V11

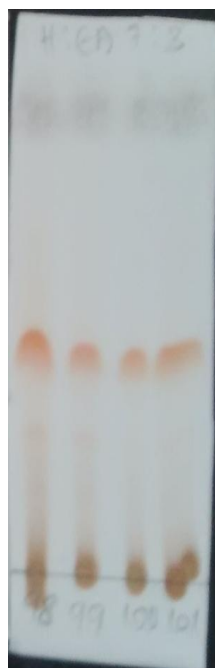


Plate VIII

Plates VI-VIII: Thin-layer chromatography of various fractions of CF obtained from column chromatography.

## 4.5 Isolation of Compound FS

### 4.5.1 Preparative thin-layer chromatography of column fraction S<sub>6</sub>

Fraction S<sub>6</sub> containing the compound of interest was isolated using preparative thin layer chromatography with hexane: ethyl acetate (5:3) as solvent system, the TLC is shown in Plate IX. The structure was elucidated using chemical test and spectroscopic analysis

( Figures 4.7.1 – 4.7.3 and Table 4.6 )

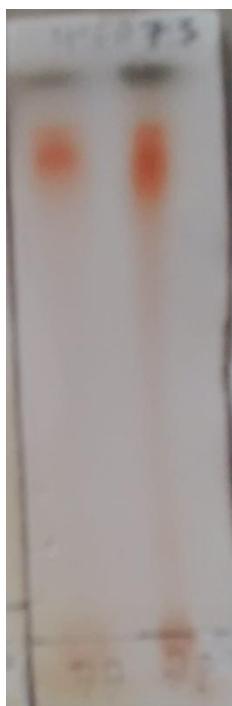


Plate IX : TLC profile of column fraction (S<sub>6</sub>)

#### 4.5.2 Thin-layer chromatographic analysis of compound FS

TLC analysis of the isolated compound FS using hexane: ethyl acetate (5:3) and (7:3) as solvent system, revealed single homogenous spot with  $R_f$  values 0.53 and 0.74 as shown in Plate X and XI respectively.



Plate X: Hexane: Ethylacetate (7:3)



Plate XI: Hexane: Ethylacetate (5:3)

Plate X-XI: TLC Profile of isolated compound FS

#### **4.6 Characterization of Compound FS**

FS was found to be soluble in methanol and acetone. The melting point of compound FS was found to be 314 - 316 °C. The compound FS gave orange-red coloured precipitate when subjected to Shinoda test.

## 4.7 Spectral Analysis of Compound FS

### 4.7.1 UV Spectrum of Compound FS

The wavelength of maximum absorption ( $\lambda_{max}$ ) of compound FS as shown on the UV spectrum (figure 4.1) below indicates the presence of a chromophore in the compound .

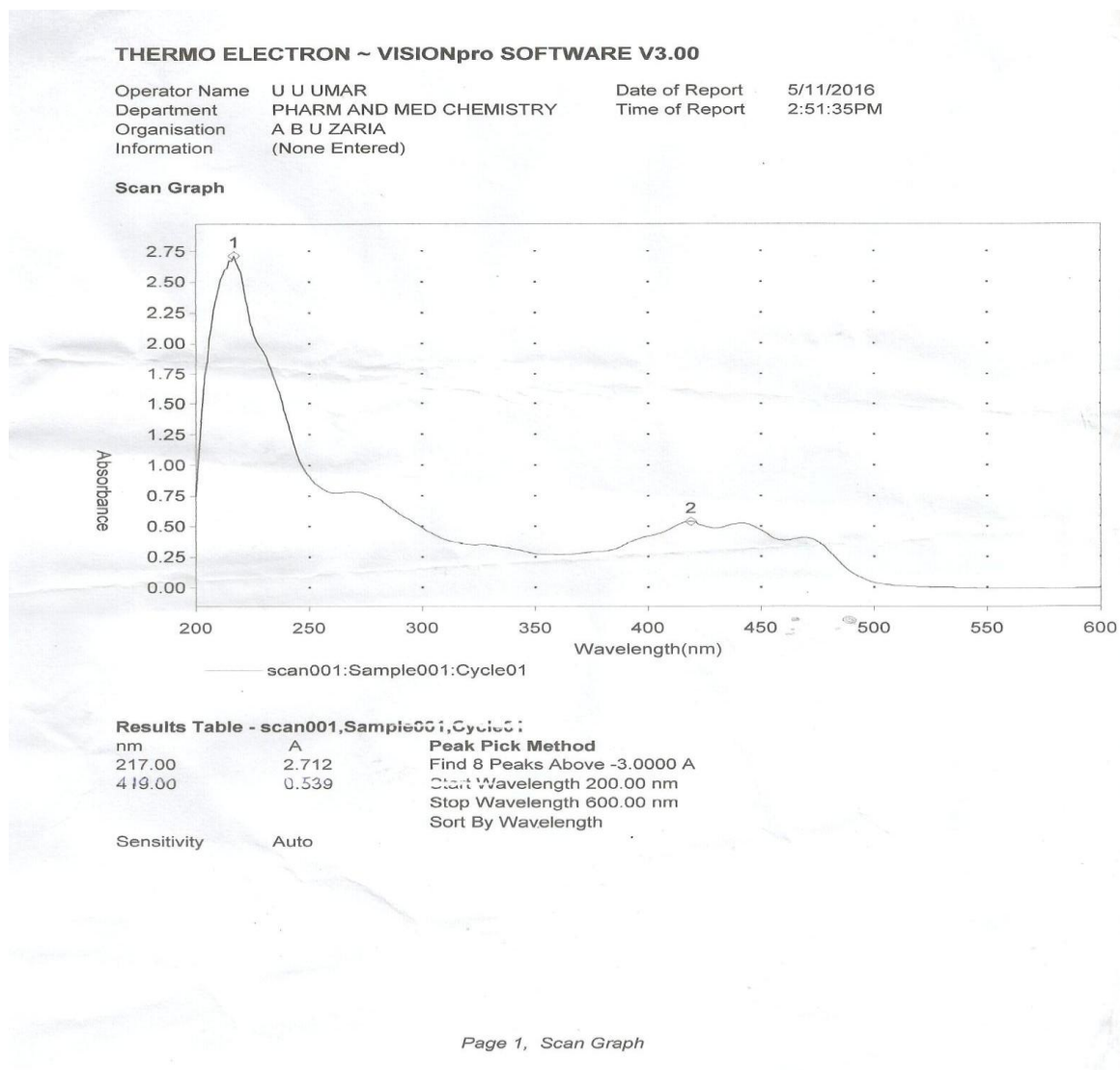


Figure 4.1: UV Spectrum of Compound FS

## 4.7.2 Proton Nuclear Magnetic Resonance Spectrum of Compound FS

The  $^1\text{H}$ NMR spectrum of FS in  $\text{CH}_3\text{OD}$  revealed the following chemical shift values/integrations  $\delta$  aromatic region; 6.21, 6.41, 6.92, 7.67, 7.76 (Figures 4.2).

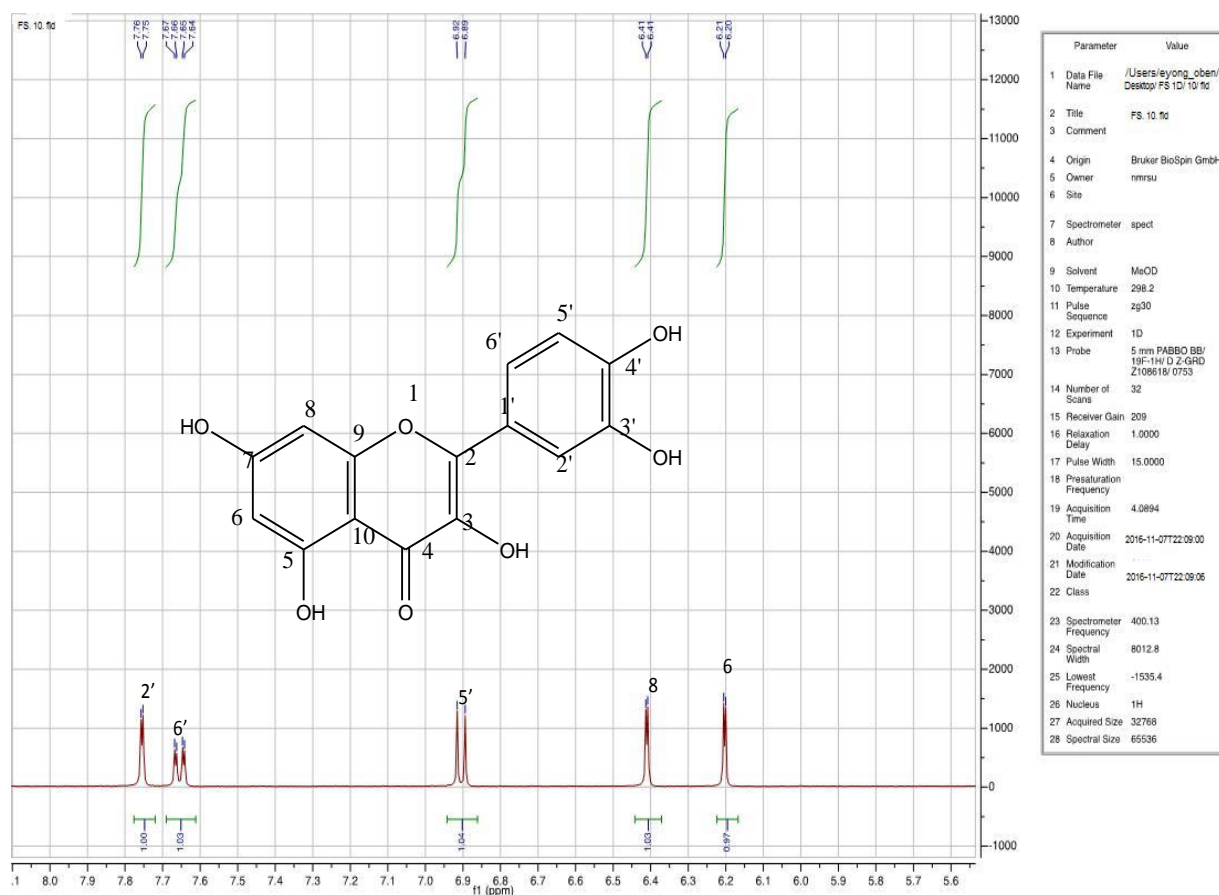


Figure 4.2:  $^1\text{H}$ -NMR Spectrum of Compound FS in  $\text{CH}_3\text{OD}$

### 4.7.3 $^{13}\text{C}$ NMR Spectrum Analysis of Compound FS

The  $^{13}\text{C}$ -NMR ( $\delta$  ppm, 400Hz,  $\text{CH}_3\text{OD}$ ) experiment showed the presence of 15 carbon atoms; 176.00, 164.20, 161.10, 156.82, 146.58, 145.00, 144.82, 135.82, 122.73, 120.25, 114.81, 114.57, 103.10, 97.83, 92.99, ( Figure 4.3 ).

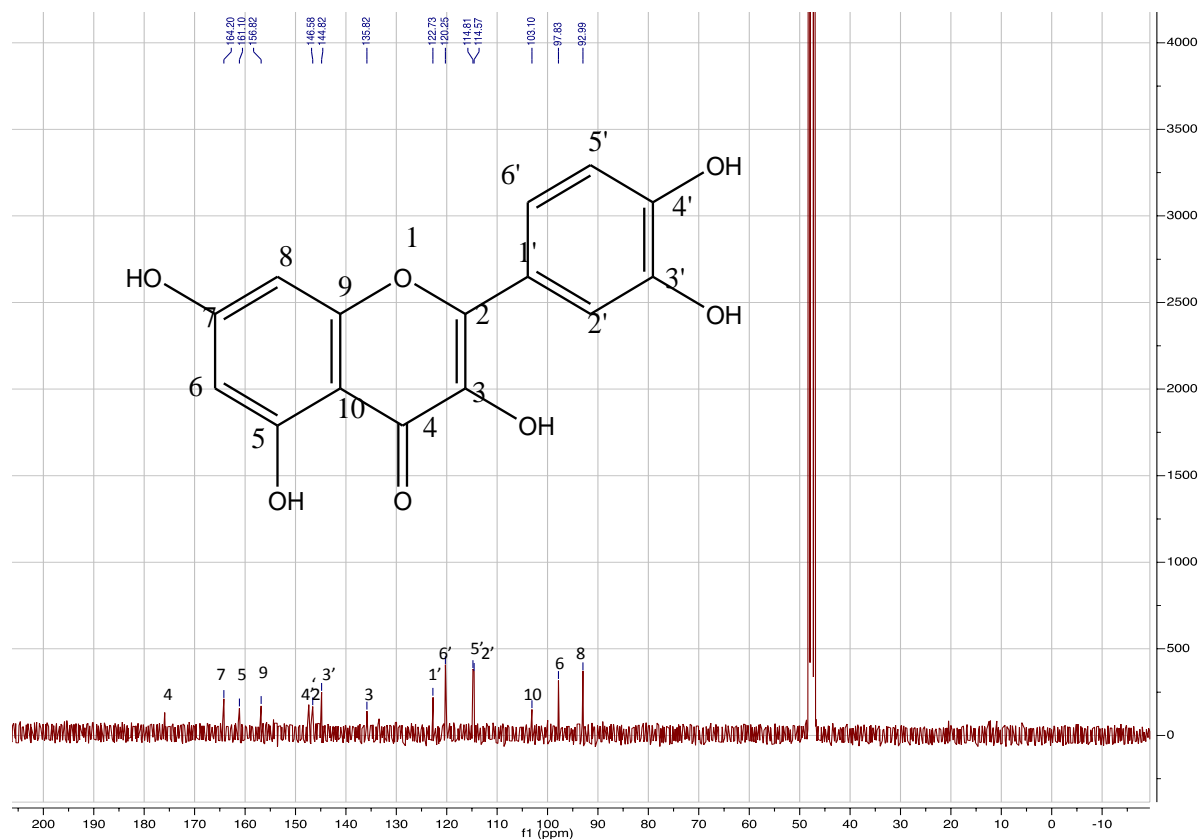


Figure 4.3:  $^{13}\text{C}$ -NMR Spectrum of Compound FS in  $\text{CH}_3\text{OD}$

## 4.8 Anticonvulsant Studies

### 4.8.1 Acute toxicity studies in mice

The median lethal dose was determined to be 1131.37 mg/kg

### 4.8.2 Maximal Electro Shock Induced Seizures in Chicks

The CME did not offer protection to the animals against tonic limbs extension at all the tested doses of 7.5 mg/kg, 15 mg/kg and 30 mg/kg respectively, but at 30 mg/kg there was statistically significant increase in the time of recovery from  $6.10 \pm 0.78$  mins in the normal saline group to  $11.17 \pm 2.19$  mins as shown in Table 4.6. PHT offered 100 % protection in the positive control group.

Table 4.7 Effect of CME and Phenytoin on Maximal Electro Shock Induced Seizures in Chicks

Treatment (mg/kg)	Recovery from Seizures(min)	Quantal Protection	Protection against Seizures (%)
N/Saline (10)	$6.10 \pm 0.78$	0/10	0.0
CME1 (7.5)	$7.20 \pm 0.89$	0/10	0.0
CME2 (15)	$7.00 \pm 0.42$	0/10	0.0
CME3 (30)	$11.17 \pm 2.19^*$	0/10	0.0
PHT (20)	-	10/10	100.0

Values are presented as Mean  $\pm$  SEM, n = 10 per group, at P  $\leq$  0.05 there was statistically significant difference in the mean recovery time between negative control and CME 30mg/kg using ANOVA test followed by Dunnett's post-hoc test (\*p  $\leq$  0.05). CME is crude methanol extract, PHT= Phenytoin

### 4.8.3 Subcutaneous Pentylentetrazole Induced Seizures in Mice

The CME offered maximum protection at 80 % against seizure to the animals at dose of 30 mg/kg, it also showed statistically significant increase in the mean onset of seizure.

Table 4.8: Effect of CME and Valproic acid on Subcutaneous Pentylentetrazole Induced Seizures in Mice

Treatment (mg/kg)	Mean Onset of Seizures (min)	Quantal Protection	Protection against Seizures (%)
N/Saline (10)	4.77 ± 0.66	1/5	20.0
CME1 (7.5)	5.00 ± 0.56	1/5	20.0
CME2 (15)	7.62 ± 0.37	3/5	60.0
CME3 (30)	8.00 ± 0.86*	4/5	80.0
VA (200)	-	5/5	100.0

Values are presented as Mean±SEM, n = 5 per group, there is statistically significant difference in the mean onset of seizures between negative control and CME3 analysed using one way ANOVA test followed by Dunnett's post-hoc test (\*p < 0.05). CME - Crude methanol extract, N/Saline = Normal Saline, VA = Valproic acid

## CHAPTER FIVE

### 5.0 DISCUSSIONS

Preliminary phytochemical studies of the crude methanol extract of the root bark of *Uvaria chamae* indicates the presence of steroids, triterpenes, tannins, alkaloids, cardiac glycosides, flavonoids and saponins. These phytochemical constituents have been reported to possess different kinds of pharmacological properties (Okwu, 2007) such as anti-inflammatory, (Okwuosa *et al.*, 2012), antibacterial and antifungal activities (Oluremi *et al.*, 2010) and antiparasitic activity (Okpekon *et al.*, 2004).

Preparative Thin-layer chromatography carried out on the column fraction S<sub>6</sub> collected from column chromatography of chloroform fraction obtained from the crude methanol extract of *Uvaria chamae* result in the isolation of a brown compound coded FS which gave single homogenous spot with two different solvent systems indicating the purity of the compound. The melting point of the compound was 314 °C – 316°C further suggesting that the compound was isolated pure, and it showed positive result with Shinoda test for flavonoid nucleus (Soumia *et al.*, 2012).

The <sup>13</sup>C NMR of FS revealed 15 C- signals, 176.00 (C-4), 164.20 (C-7), 161.10 (C-5), 156.82 (C-9), 146.58 (C-4'), 145.00 (C-2), 144.82 (C-3'), 135.82 (C-3), 122.73 (C-1'), 120.25 (C-6'), 114.81 (C-5'), 114.57 (C-2'), 103.10 (C-10), 97.83 (C-6), 92.99 (C-8), (Figure 4.3). The 15 carbon signals were due to the flavonoidal skeleton. The multiplicities of the carbon atoms were confirmed by <sup>13</sup>C NMR experiments, which revealed ten quaternary carbon atoms (9,10,7,5,4,4',3,3',2,1'). The oxygenated (quaternary) carbon signal at δ<sub>C</sub> 164.20 (7) and 161.10 (5) were due to phenolic carbon (Guvenalp *et al.*, 2006).

The <sup>1</sup>H NMR spectrum of FS showed the presence of five aromatic proton signals, the chemical shift values were as follows; 7.76 (2'), 7.67 (6'), 6.92 (5'), 6.41 (8), 6.21 (6)

(Figures 4.2). The  $^1\text{H}$ NMR spectrum showed meta coupled doublets at  $\delta_{\text{H}}7.67$  (1H, dd,  $J=8.0$ , 4.0 Hz, H-6'), and  $\delta_{\text{H}}7.76$  (1H, d,  $J=4\text{Hz}$  H-2') assignable to the tri-substituted benzene ring (B) (Saini and Ghosal, 1984; Samaraweera *et al.*, 1983; Biruk *et al.*, 2011), and  $\delta_{\text{H}}6.92$  (1H, d,  $J=12\text{Hz}$  H-5') suggesting ortho coupled on the B-ring, whereas 5,7-dihydroxylation of A-ring was assigned from the two meta-coupled doublets at  $\delta_{\text{H}}6.41$  (1H, d,  $J=4$  Hz,) and  $\delta_{\text{H}}6.21$  (1H, d,  $J=4.0$  Hz.). These pattern of coupling systems indicate a 3, 5, 7, 3', 4' penta-oxygenated flavonol typical of a quercetin nucleus (Mabry *et al.*, 1970; Markham *et al.*, 1978; Abdullahi *et al.*, 2011).

The UV spectra of FS recorded in methanol showed absorption maxima at 217 nm and 419 nm indicate some level of conjugations in the compound. Indicate presence of chromophore (Soumia *et al.*, 2012).

Utilization of the 1D NMR spectra and comparison with literature data showed consistency with the reported literature values (Soumia *et al.*, 2012) and the structure of FS was assigned as quercetin, hence the proposed structure of FS [2-(3',4',-dihydroxyphenyl)-3,5,7-trihydroxy-4H'-chromen-4-one], figure 5.1.

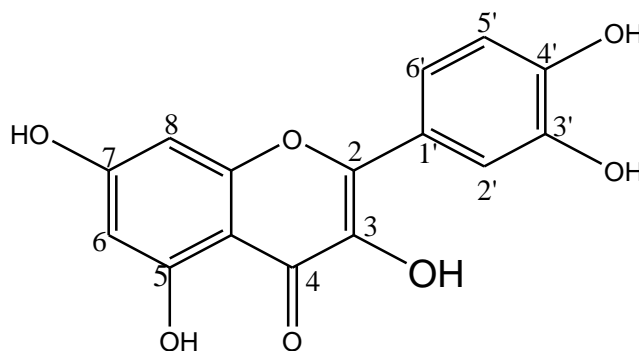


Figure 5.1: Proposed structure of FS [2-(3',4',-dihydroxyphenyl)-3,5,7-trihydroxy-chromen-4-one]

Diverse range of biological activities triggered by quercetin have been reported including, the protection against several degenerative diseases by preventing lipid peroxidation. (Alexandra, 2009). However, antiplasmodial effect of natural plant product has been

attributed to some of their active phytochemical component (Ayoola *et al.*, 2008; Soforawa 1980). Some of these phytochemical such as terpenes and flavonoids were reported to have antiplasmodial activity (Philipson and wright 1990; Christensen and Kharrami 2001; Go, 2003). Increase oxidation has also been shown to create an intracellular environment that is unfavorable to plasmodial growth (Boris and schaeffer1992; Lavender and Agar 1993). However, the lack of oxidizing action in some plant does not rule out antiplasmodial activity since they may be active through other biochemical mechanism. Flavonoids, (Choudhary N., *et al* 2011), triterpenes and steroids, among other phytochemicals have been reported to possess anticonvulsant activity (Musa *et al* 2014). Finally, Quercetin and its related compounds have been reported to possess some activity in the central nervous system (Choudhary N., *et al* 2011).

The crude methanol extract (CME) was tested *in vivo* to ascertain it's anticonvulsant activity. Both MES and ScPTZ test models for anticonvulsant activity was employed. The results obtained were summarized on Tables 4.7 and 4.8. The screening was done at the dose of 7.5 mg/kg, 15 mg/kg, and 30 mg/kg respectively. The respective doses were selected from the result obtained from the median lethal dose (Maiha *et al.*, 2009)

From the result on table 4.7, which shows MES test, it can be observed that at the dose of 30 mg/kg (CME3), there was significant increase in the mean time of recovery from seizures when compared to negative control, This indicates that the extract did not protect the animals against seizure. Maximal electroshock seizure can be prevented by sodium channel blockers such as phenytoin, vaproate felbamate and lamotrigine or agents which protect animals against MEST have been shown to be beneficial in the management of generalized tonic clonic seizure. Therefore, the absence of anticonvulsant ability in MES test suggest that CME may not be useful in the treatment of generalized tonic clonic seizures. On the other hand, the PTZ test result of Table 4.8, shows that there was a dose-dependent increase in the mean

onset time of seizure with CME compared to negative control with 80% maximum protection against seizure to the animals at dose of 30 mg/kg. Pentylenetetrazole is a widely used chemical convulsant in the screening of potential anticonvulsants and the mechanism of action involves GABA metabolism probably at the synapse where it acts at the GABA receptor complex in a way that decrease the inhibitory capacity of GABA (Randall *et al.*, 2012). Therefore protection against PTZ induced seizure suggests effects on GABAergic neurotransmission. Anticonvulsant activity in PTZ test identifies compounds that can raise seizure threshold in the brain (Randall *et al.*, 2012). Drugs useful in the treatment of absence seizures suppress PTZ induced seizures (McNamara, 2006). Therefore the activity of the crude methanol extract against the PTZ induced seizures suggests the presence of bioactive constituents such as flavonoids and the extract could be effective in the therapy of absence seizure.

## CHAPTER 6

### 6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

#### 6.1 Summary

Preliminary phytochemical screening of the methanol rootbark extract of *Uvaria chamae* revealed the presence of steroids, flavonoids, saponins, alkaloids, cardiac glycoside, tannins and carbohydrates. Column chromatography of chloroform fractions followed by preparative thin layer chromatography led to the isolation of quercetin a flavonoid compound.

Examination of the crude methanol extract activity against electrical and chemical model of convulsion revealed that the extract did not protect the animals against seizures in MES test but at dose of 30 mg/kg, it significantly increased the recovery time when compared to normal saline. The absence of anticonvulsant ability in MES test suggest that CME may not be useful in the treatment of generalized tonic clonic seizures. In Sc PTZ test the result shows that there was a dose-dependent increase in the mean onset time of seizure with CME compared to negative control, with 80 % maximum protection against seizure in mice at dose of 30 mg/kg. Therefore the activity of the extract against the PTZ induced seizures suggests the presence of bioactive constituents such as flavonoids and could be effective in the therapy of absence or myoclonic seizures. Therefore the methanol root bark extract of *U. chamae* possessed significant ( $p < 0.05$ ) anticonvulsant effect(s) in PTZ induced seizure model of epilepsy used and indicates a possible enhancement of GABA activity.

#### 6.2 Conclusion

The traditional use of *Uvaria chamae* for management of epilepsy has been confirmed by this study. The anticonvulsant study of the methanol root bark extract of *Uvaria chamae* might be linked to the quercetin compound isolated. To the best of our knowledge this is the first report of isolation of quercetin from the methanol root bark extract of *Uvaria chamae*. This work will therefore add to the global data base of natural product. Base on these findings, it

can be stated that the use of *Uvaria* in the management of epilepsy has been justified in this study.

### **6.3 Recommendation**

- I. Bioassay guided isolation should be carried out with a view of isolating the bioactive compounds responsible for the observed anticonvulsant activity.
- II. Other pharmacological studies should be carried out for various fractions.
- III. Chronic toxicity evaluation should be carried out on the plant to determine its safety upon use, in view of the common use of the plant in the management of diverse illness.

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## APPENDICES

Appendix I: Determination of intraperitoneal median lethal dose (LD<sub>50</sub>) of the crude extract of methanol Extract of *U. chamae*.

### First Phase

Dose (mgkg <sup>-1</sup> )	Number of mice used	Mortality
10	3	2/3
100	3	0/3
1000	3	0/3

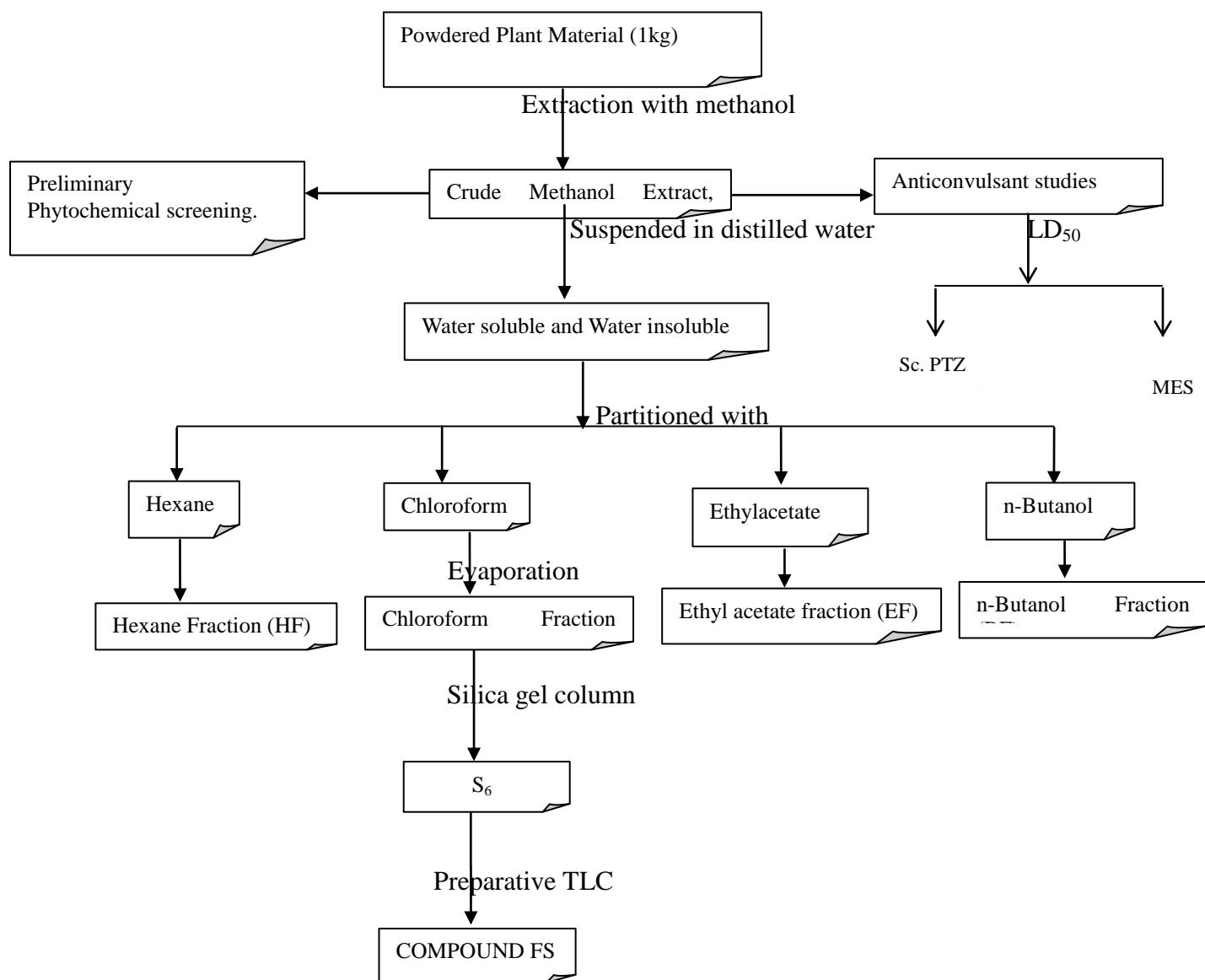
### Second Phase

Doses (mgkg <sup>-1</sup> )	Number of mice used	Mortality
200	1	0/1
400	1	0/1
800	1	0/1
1600	1	0/1

Appendix 2: Table 4.6: Comparison of 1D NMR Data of FS with Literature Data

Position	Compound FS (CH <sub>3</sub> OD)		Soumia <i>et al.</i> , 2012 (CH <sub>3</sub> OD)	
	$\delta^{13}\text{C}$	$\delta^1\text{H}$ , J (Hz)	$\delta^{13}\text{C}$	$\delta^1\text{H}$ , J (Hz)
2	145		148.2	
3	135.82		137.2	
4	176.00		177.5	
5	161.10		162.6	
6	97.83	6.21 (1H, d, J=4)	99.4	6.18 (1H, d, J=2.0)
7	164.20		165.7	
8	92.99	6.41 (1H, d, J=4)	94.6	6.39 (1H, d, J=2.0)
9	156.82		158.4	
10	103.10		104.7	
1'	122.73		124.3	
2'	114.57	7.76 (1H, d, J=4)	116.1	7.74 (1H, d, J=2.0)
3'	144.82		146.3	
4'	146.52		150.3	
5'	114.81	6.92 (1H, d, J=12)	116.1	6.88 (1H, d, J=8.4)
6'	120.25	7.67(1H, dd, J=4, 8)	121.8	7.62(1H, dd, J= 2.0,8.4)

Appendix 3: The Chart of Extraction and Isolation Protocol



Scheme I : The chart of extraction and isolation protocol