ANTICONVULSANT STUDIES OF THE METHANOL EXTRACT AND FRACTIONS OF CASSIA SIAMEA LAM. (FABACEAE) IN MICE AND CHICKS

By

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AUGUST, 2014

DECLARATION

I declare that the work in the thesis entitled "**Anticonvulsant studies of the methanol extract and fractions of *Cassia siamea* lam. (Fabaceae) in mice and chicks"** has been performed by me in the Department of Pharmacology and Therapeutics under the supervision of Dr. N. M. Danjuma and Professor O. A. Salawu. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for the award of another degree or diploma at this or any other institution.

BELLO OMENESA RAMATU		**07 August, 2014**
Name of Student	**Signature**	**Date**

CERTIFICATION

This dissertation entitled **"ANTICONVULSANT STUDIES OF THE MTHANOL EXTRACT AND FRACTIONS OF *CASSIA SIAMEA* LAM. (FABACEAE) IN MICE AND CHICKS "** by RAMATU OMENESA BELLO, meets the regulations governing the award of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated

To Almighty Allah (S. W. A)

then

To my husband, Alhaji (Engr.) Bello Yahya.

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ABBREVIATIONS AND SYMBOLS

ADD	Anticonvulsant Drug Development
AEDS	Anti-epileptic drugs
AIDS	Acquired immune deficiency syndrome
AIS	Axon Initial Segment
AMPA	2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid
ANOVA	Analysis of Variance
ASP	Anticonvulsant Screening Programme
BBB	Blood brain barrier
Ca^{2+}	Calcium ion
Cl^{-}	Chloride ion
CSME	Cassia siamea methanol extract
CT	Computerized tomography
CYP 450	Cytochrome P 450 enzyme
EAF	Ethyl acetate fraction
EEG	Electroencephalograh
EPs	Epileptiform patterns
GABA	Gamma- aminobutyric acid
GAD	Glutamic acid decarboxylase
HLTE	Hind limb tonic extension
K+	Potassium ion
KOR	Kappa opioid receptor
LD_{50}	Median lethal dose
ME	Methanol extract
MEST	Maximal electroshock test
MRI	Magnetic resonance imaging

MRS	Magnetic resonance spectroscopy
Na^+	Sodium ion
Nal	Naloxone
NAPRI	National Animal Production Research Institute
NBF	n-butanol fraction
NMDA	N-methyl-D-aspartate
PHB	Phenobarbital
PLWE	People living with epilepsy
RAF	Residual aqueous fraction
ROS	Reactive oxygen species
ScPTZ	Subcutaneous Pentylenetetrazole
SE	Status Epilepticus
SEM	Standard Error of Mean
SPECT	Single Photon Emission Computerized Tomography
TBI	Traumatic Brain Injury
TLC	Thin Layer Chromatography
TLE	Temporal Lobe Epilepsy
WHO	World Health Organization
ZNS	Zonisamide

ABSTRACT

Cassia siamea is a shrub belonging to the Fabaceae family, native of Southeast Asia. The plant is commonly used in traditional medicine to treat hypertension, malaria and diabetes. Due to the easy cultivation of the plant, its widespread and also remarkable biological activities, Cassia siamea has become a worldwide medicine. The study was conducted to assess the anticonvulsant potential of the methanol extract of Cassia siamea (CSME) and its fractions (EAF, NBF and RAF) using Pentylenetetrazole (PTZ) induced seizures in mice and Maximal electroshock (MES) induced seizures in chicks. Acute toxicity was carried out on CSME and fractions. The possible mechanism(s) involved in anticonvulsant action were determined using Picrotoxin and naloxone. The preliminary phytochemical screening of the methanol extract revealed the presence of alkaloids, flavonoids, polyphenols, saponins, steroids, terpenoids and tannins. The LD_{50} of CSME, its EAF and NBF was found to be greater than 5000mg/kg intraperitoneally *(i.p*) in chicks and mice, suggesting a non toxic profile of the extractst, while the LD_{50} of the RAF *(i.p*) was found to be 1095mg/kg in mice and 3807 mg/kg in chicks implying the RAF is mildly toxic via the intraperitoneal route. The CSME and its EAF were found to have varied anticonvulsant activities; the EAF at 250mg/kg dose protected 40% of mice against hind limb tonic extension induced by maximal electroshock and in convulsed chicks a significant ($P < 0.05$) decrease in mean recovery time was noted. The ethyl acetate fraction at 250mg/kg and 500mg/kg doses produced a significant ($P < 0.05$) delay in mean onset of seizures and offered mice a 2/6 and 5/6 quantal protection against mortality, valproic acid the standard anticonvulsant drug used produced a 100% protection against seizures. The EAF did not protect mice against pirotoxin induced seizures indicating lack of activity on chloride ion channels of the $GABA_A$ receptor complex. Naloxone did not reverse the anticonvulsant activity of the EAF

against Pentylenetetrazole-induced seizures, suggesting lack ogf involvement of $GABA_A$-BDZ receptors and opioid receptors in the anticonvulsant effect of EAF. The CSME and EAF at 100mg/kg dose significantly ($P < 0.05$) prolonged the total duration of Diazepam induced sleeping time in mice without affecting the mean onset of sleep, indicating sedative action of the extract. The results suggests the presence of bioactive component(s) that posess anticonvulsant and sedative activities. The data may provide pharmacological basis for the use of the plant alone or in combination with other plant(s) in the management of febrile convulsions and insomnia in West African countries including Nigeria.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Epilepsy is one of the most common afflictions of human beings (Muralidharan *et al*., 2009). It affects approximately 50 million people worldwide and accounts for about 1% of the global burden of disease (Reynolds, 2002). Epilepsy is coined from the Greek word "*epilembanein"* which means "to seize". It is a chronic neurological disorder affecting both sexes (Blume *et al*., 2001). It is the second most common chronic neurological condition seen by neurologists worldwide (Sridharan, 2002). It is characterized predominantly by recurrent and unpredictable interruptions of normal brain function, called epileptic seizures which arise due to sudden, excessive and rapid discharge of cerebral neurons in the grey matter of the brain. It is a diverse family of disorders, having in common an abnormally increased predisposition to seizures reflecting underlying brain dysfunction that may result from different causes (Fischer *et al.,* 2005).

Among brain disorders, epilepsy stands out not only because of its high prevalence and incidence rates, but in particular because of the myths and beliefs attached to the condition in various cultures and the resulting impacts on the individual, the family, and the community as a whole (Jamison *et al*., 2006). Epilepsy remains among the most stigmatized brain disorders. The associated stigma is more obvious in developing countries because of illiteracy and misinformation regarding the actual nature of the condition. The

shame and fear associated with the disorder prevent many affected individuals from seeking treatment. As a result, their epilepsy becomes uncontrolled, with consequences for education, employment opportunities, and social acceptance (Birbeck, 2000).

Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral – based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well being (WHO, 2003).

The use of natural products with healing properties is as old as human civilization (Rates, 2001). Natural products from folk remedies have contributed significantly in the discovery of modern drugs and are now considered an alternative source for the discovery of antiepileptic drugs with novel structures and better safety and efficacy profiles (Raza *et al.*, 2003). Many natural products and synthetically modified natural product derivatives have been successfully developed for clinical use to treat human diseases in almost all therapeutic areas (Newman *et al.*, 2007). The African continent has a long history of the use of plants as therapeutic agents and in some African countries phytotherapy still plays an important role in the management of diseases mainly among populations with low income (Geoffrey and Kirby, 1996).

The World health organization estimated that 80% of people in developing countries rely on medicinal plants for their primary health care needs (Farnsworth, 1998). This can be attributed to the high cost of acquiring synthetic drugs and the side effects associated with their use in a setting where traditional medicines are readily available and affordable.

It is estimated that out of over 30,000 human diseases or disorders, only one third can be managed to some extent symptomatically with the existing drugs and at a great socio-economic cost (Wangchuck *et al*., 2007). These diseases include infections like the Acquired immunodeficiency syndrome (AIDS), tuberculosis and chronic debilitating disorders like cancers as well as neurological disorders including epilepsy hence, these diseases are of special concern to communities worldwide (Wangchuk, 2008). Strengthening and developing traditional medicines through evidence-based research for use against these diseases especially the chronic ones is pertinent to the development of newer and safer therapeutic agents.

1.2 Statement Of Research Problem

Epilepsy is a global problem; eight people per 1000 of the world population have epilepsy (WHO, 2001). Most people with epilepsy reside in developing regions (Mac *et al.*, 2007). Epilepsy is the most prevelant neurologic disorder in sub-Saharan Africa (Eisenberg, 1997; Leonardi and Ustun, 2002). It is estimated that one quarter of the 40 million individuals estimated to be have epilepsy in the developing world live in Africa (Onwuekwe *et al.*, 2009). The world health organization (WHO) estimates that 10 million people in Africa live with epilepsy, and 8 million (80%) are not receiving adequate treatment (WHO, 2004). The incidence of epilepsy varies greatly with age, with high rates in early childhood, low levels in early adult life and a second peak in people over 65 years old from as high as 560 cases per100, 000 of the population per year for infants to as low as 20.3 cases per 100,000 per

year for ages 15 to 30 years. However, above 30 years the incidence of epilepsy rises. Among people with epilepsy under thirty-five years of age, mortality rates were 50 times that of people without epilepsy, with most deaths related to seizures or seizure-associated injuries (Mu *et al*., 2012). Approximately 80% of people with epilepsy are found in resource-poor countries i.e. many low and middle-income countries like Tanzania where there is usually low availability of antiepileptic drugs, in some instances only one antiepileptic drug might be routinely available in the public sector and where the available antiepileptic drugs are sold at near prohibitive cost approximately 9 out of 10 people with epilepsy (PWE) go untreated (Birbeck, 2012).

Although data on epilepsy in Nigeria are non-detailed, from the small number of community-based studies available the point prevalence of epilepsy in Nigeria varies from 5.3 to 37 per 1000 (Osuntokun *et al.,* 1989, Akinsulore and Adewuya, 2010). Life span and prognosis of patients with epilepsy depends on the seizure type, severity of seizures as well as the underlying cause of the seizures (Darwin, 1989).

Epilepsy is among the disorders that are strongly associated with significant psychological and social consequences for everyday living (Baker, 2002). Stigmatization leads to discrimination and people with epilepsy experience prejudicial and discriminatory treatment in many spheres of life and across many cultures (Pahl and Boer, 2005). Currently available antiepileptic drugs (AEDs) in clinical use are synthetic molecules and pharmacotherapy involving the use of these agents is entirely symptomatic *vis a vis* neither

effective prophylaxis nor cure is available for patients suffering from epileptic seizures (Hema *et al*., 2009)

Although about 70-80% of people living with epilepsy (PLWE) may be provided with adequate seizure control, many patients with epilepsy fail to experience adequate seizure control despite optimal use of the available AED's, other patients do so only at the expense of significant toxic side effects. The existing lack of seizure control in the remaining 20-25% of PLWE is worrisome and has propelled intensive research for novel antiepileptic drugs (Czapin'ski *et al.*, 2005). A study by Cheuk and Wong (2009) suggests that 70% of people with epilepsy will find seizure relief on one or more anti-epileptic drugs or go into remission. The remaining patients will continue to have seizures despite treatment with adequate doses of AEDs and are considered drug resistant. This has lead to constant search for newer drugs and alternative therapies to treat epilepsy.

The available antiepileptic drugs not affect epileptogenesis, various studies have attributed serious side effects with the use of these agents including teratogenicity (orofacial clefts, congenital heart defects, skeletal malformations/malfunctions, central nervous system malformations/malfunctions), chronic toxicity as well as adverse effects on cognition and behavior (Samren *et al.*, 1997).

1.3 Justification of the Study

Despite the successful development of various new antiepileptic drugs in recent decades, the search for new therapies with better efficacy and tolerability remains an important goal (Bialer and White, 2010). Current therapy with antiepileptic drugs only suppresses seizures but do not alter the underlying epileptogenic process (Shinner and Berg, 1996). As yet, prevention of epilepsy in patients at risk is an unmet clinical need, there is growing concern that the efficacy of drug treatment of epilepsy has not substantially improved with the introduction of new antiepileptic drugs. Due to the chronic nature of the disorder, compliance with therapy is a major problem for most patients because of the need for long term therapy coupled with unwanted effects ranging in severity from minimal effects like gingival hyperplasia to death from Aplastic anaemia or hepatic failure (McNamara, 2006).

Traditional medicines enjoy a wide patronage and acceptance (Stanley, 2005). The treatment and control of diseases by the use of available medicinal plants in a locality will continue to play significant roles in medical health care implementation in the developing countries of the world (Akharaiyi and Boboye, 2010). It therefore becomes pertinent to validate the folkloric claims of medicinal plants used in traditional healing practices in order to provide a scientific basis for their use and also to optimize their safety and efficacy probably through isolation of their bioactive principles responsible for the observed therapeutic effect. Isolation of active anticonvulsant principles from medicinal plants may provide potent, cheap, easily accessible and readily available compounds for the management of epilepsies and even refractory types.

1.4 Theoretical Framework

Experimentally induced seizures and animal models of epilepsy have been used extensively to study the nature of seizures and epilepsy and to develop new therapies for epilepsy (Fischer, 1989). The discovery of novel antiepileptic drugs (AEDs) relies upon the preclinical employment of animal models to establish efficacy and safety prior to the introduction of the AEDs in human volunteers (Löscher and Schmidt, 2006). Clearly, the more predictive the animal model for any given seizure type or syndrome, the greater the likelihood that an investigational AED will demonstrate efficacy in human clinical trials (Smith *et al*., 2007).

Initial preclinical evaluation of a compound's anticonvulsant potential is accomplished by the Anticonvulsant Screening Programme (APS). The APS screens new compounds through a series of *in vivo* and *in vitro* tests (Stables and Kupferberg, 1997). There are three *in vivo* models that are routinely used by most AED discovery programs, they include the maximal electroshock (MES) test, the Pentylenetetrazole (PTZ) seizure test, and the kindling model. Of these, the MES and the subcutaneous PTZ seizure models represent the two animal seizure models most widely used in the search for new AEDs (White *et al.*, 2002). It has been found empirically that drugs which inhibit PTZ-induced convulsions and raise the threshold for production of electrically induced seizures are generally effective against absence seizures, whereas those that reduce the duration and spread of electrically induced convulsions are effective in focal types of epilepsy, such as tonic-clonic seizures. The maximal electroshock test (MEST) and the subcutaneous Pentylenetetrazole (scPTZ)

induced seizures were used in this study as preliminary anticonvulsant screening tests conducted on the crude methanol whole plant of *Cassia siamea* and its fractions to determine the fraction with the most potent anticonvulsant activity for further investigations using other specific models of epileptic seizures (Rang *et al*., 2005).

1.5 Aim and Objectives

1.5.1 Aim of the study

The aim of the study is to establish the anticonvulsant potential of the methanol extract of *Cassia siamea* Lam. and its fractions in mice and chicks.

1.5.2 Objectives of the study

The specific objectives of this study are to:

i. To establish the *in-vivo* anticonvulsant potential of the methanol extract of *Cassia siamea* and its fractions using animal models of epilepsy.

ii. To establish the central nervous system depressant activity of the methanol extract and its most active fraction in mice

1.6 Research Hypothesis

Cassia siamea plant contains bioactive constituent(s) which possess' anticonvulsant activity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Etiology and Risk Factors for Developing Epilepsy

Epileptic conditions are multifactorial disorders in which the action of more than one gene together with environmental factors contributes to the disease phenotype (Todorova *et al*.**,** 1999**).** Epilepsy is characterized by abnormal synchronized discharge of neurons leading to alterations in electroencephalograph activity and behavior; it may result from long lasting plastic changes in the brain affecting neurotransmitter release and transport, the properties of receptors and channels, synaptic reorganization and astrocyte activity (Sierra-Paredes, 2007).

Three important factors have been implicated in the etiology of epilepsy. The first factor is predisposition, or threshold. The ease with which a seizure can be provoked, or an epileptic condition can be induced, is referred to as a threshold. Individual differences in threshold are largely attributable to genetic variations but could also be acquired via different means e.g. certain types of perinatal injuries, which can alter threshold. Threshold is a dynamic phenomenon (Omer *et al*., 2011), which varies throughout the day, and it also changes in relation to hormonal influences during the menstrual cycle in women. Patients with a high seizure threshold can experience severe epileptogenic brain injuries and precipitating factors but never have seizures, while those with low seizure thresholds can develop epilepsy with minimal insults and, in many, from precipitating factors alone (provoked seizures).

Stimulant drugs lower seizure threshold and sedative drugs increase it: however, withdrawal from sedative drugs can lower threshold and provoke seizures. Antiepileptic drugs work by increasing seizure threshold (Fischer *et al*, 2005). In a case-control study in which Nigerian epileptic patients were compared with age and sex – matched controls, febrile convulsions and head trauma were significant risk factors for epilepsy (Ogunniyi *et al.*, 1987).

The second important factor for epilepsy is the epileptogenic abnormality itself. Epilepsies attributable to identifiable brain defects are referred to as symptomatic epilepsies. Symptomatic epilepsies can be caused by a variety of disorders, including brain malformations, infections, vascular disturbances, neoplasms, scars from trauma, including strokes, and disorders of cerebral metabolism. Treatment for symptomatic epilepsy is most effective if it is directed at the underlying cause. The most common symptomatic epilepsy is temporal lobe epilepsy (TLE), usually associated with a characteristic lesion called "hippocampal sclerosis". Hippocampal sclerosis appears to be caused by cerebral injury within the first few years of life in individuals with a genetic predisposition to this condition. Some forms of epilepsy are unassociated with identifiable structural lesions or diseases and are usually unassociated with other neurological or mental deficits. These are genetically inherited, generally easily treated with medications without sequelae, and referred to as idiopathic epilepsies (Sheth and Hermann, 2007).

The third important factor is the precipitating condition, which determines when seizures occur. Common precipitating factors include fever for children with febrile seizures, alcohol and sedative drug withdrawal, hypoglycemia, anoxia sleep deprivation, stimulant

drugs and in some patients stress. Reflex seizures are precipitated by specific sensory stimuli. The most common are photosensitive seizures induced by flickering light, but some patients have very specific reflex epilepsy with seizures precipitated by such stimuli as being startled, particular types of music, certain visual patterns, reading, eating and hot-water baths. Identification of precipitating factors is helpful if they can be avoided, but in most patients specific precipitating factors are not apparent, and may not exist at all.

2.2 Classification of seizures

Optimum treatment of seizure disorders requires accurate classification of seizure type as well as appropriate choice and use of medication (Dreifuss, 1989). Many different types of seizures can be identified on the basis of their clinical phenomena (Loscher, 1998). The seizure classification used is based on the international classification of epileptic seizures (Dreifuss, 1989):

2.2.1 **Partial seizures** (Focal or local seizures)

2.2.1.1 Simple partial seizures: there is transient local high frequency discharges. These have various manifestations, without impairment of consciousness. They may include convulsion confined to a single limb or muscle group (motor epilepsy), specific and localized sensory disturbances (sensory epilepsy) and other limited signs and symptoms depending upon the particular cortical area producing the abnormal discharge.

2.2.1.2 Complex partial seizures: These attacks result in confused behavior, with impairment of consciousness. They have a wide variety of clinical manifestations associated with bizarre generalized electoencephlographic (EEG) activity during the seizure but with evidence of anterior temporal lobe focal abnormalities even in the inter-seizure period in many cases.

2.2.2 Generalized seizures (Convulsive or non convulsive)

These are high frequency discharges that spread across the entire brain. The main categories are:

2.2.2.1 Absence seizures

a) *Atypical absence seizures*: Such attacks have a slower onset and cessation than is usual for absence seizures and are associated with a more heterogeneous EEG.
b) *Typical absence seizures*: These seizures are brief and abrupt. The resultant loss of consciousness is associated with high-voltage, bilaterally synchronous, 3-per-second spike-and-wave pattern in the EEG, usually with some symmetrical clonic motor activity varying from eyelid blinking to jerking of the entire body, sometimes with no motor activity.

2.2.2.2 Myoclonic seizures: are isolated clonic jerks associated with brief bursts of multiple spikes in the EEG.

2.2.2.3 Clonic seizures: are rhythmic clonic contractions of all muscles. They result in loss of consciousness and marked autonomic manifestations.

2.2.2.4 Tonic seizures: are opisthotonus and result in a loss of consciousness and marked autonomic manifestations.

2.2.2.5 Tonic-clonic (grand mal) seizures are major convulsions, usually a sequence of maximal tonic spasms of all body musculature, followed by synchronous clonic jerking and a depression of all central functions.

2.2.2.6 Atonic seizures: These are characterized by loss of postural tone, sagging of the head and/or falling

2.2.3 Unclassified epileptic seizures: these include seizures that cannot be classified as they do not fall under any of the categories previously mentioned. These include;

i. febrile convulsions (e.g. accompanied by high grade fever in children aged 5years and below, characterized by rhythmic eye movements and swimming movements)

ii. Status epilepticus: this refers to continuous or intermittent (but frequent) seizures lasting more than 5 minutes in which one fit follows another so closely without full recovery of consciousness between seizures (Cherian and Sanjeev, 2009).

2.3 Mechanism of Epileptogenesis

Epileptogenesis is the process by which the previously normal brain is functionally altered and biased towards the generation of abnormal increase in electrical activity that sub serves chronic seizures. The concept of 'mechanism of epilepsy' refers to any biological feature of the brain that drives molecular, anatomical or circuit level alterations, such as cell death or dysregulation of an ion channel or neurotransmitter receptor (Goldberg and Douglas, 2013). A widely accepted hypothesis holds that during the interval between brain injury and the appearance of clinically obvious seizures (latent period) which characterizes many (if not

all) cases of symptomatic epilepsy, there is a cascade of poorly understood changes that transform the nonepileptic brain into one that generates spontaneous recurrent seizures (Pitkänen and Lukasiuk, 2009). This insult-induced process, which is of variable length in different patients and ultimately leads to chronic epilepsy, is called epileptogenesis.

2.3.1 Basic mechanisms of focal seizure initiation and propagation

The hypersynchronous discharges that occur during a seizure may begin in a very discrete region of the cortex and then spread to neighboring regions. Seizure initiation is characterized by two concurrent events namely:

i) high-frequency bursts of action potentials

ii) hypersynchronization of a neuronal population (Bromfield *et al*., 2006).

The synchronized bursts from a sufficient number of neurons result in a so-called spike discharge on the EEG. At the level of single neurons, epileptiform activity consists of sustained neuronal depolarization resulting in a burst of action potentials, a plateau-like depolarization associated with completion of the action potential burst, and then a rapid repolarization followed by hyperpolarization. This sequence is called the paroxysmal depolarizing shift. The bursting activity resulting from the relatively prolonged depolarization of the neuronal membrane is due to influx of extracellular Ca^{2+}, which leads to the opening of voltage-dependent Na^{+} channels, influx of Na^{+}, and generation of repetitive action potentials. The subsequent hyperpolarizing after-potential is mediated by GABA receptors and Cl^{-} influx, or by K^{+} efflux, depending on the cell type (Lee *et al*., 2011).

Seizure propagation is the process by which a partial seizure spreads within the brain. It occurs when there is sufficient activation to recruit surrounding neurons. This leads to a loss of surrounding inhibition and spread of seizure activity into contiguous areas via local cortical connections, and to more distant areas via long association pathways such as the corpus callosum. Epileptogenesis is promoted by both nonsynaptic and synaptic mechanisms that affect synchronicity as well as signal amplification by cerebral neurons (Engelborghs *et al*., 2000). Acquired epilepsy may involve a wide range of mechanisms including alterations in neurotransmitter receptors (e.g. gamma aminobutyric $acid_A$ receptors) and/or voltage dependent currents e.g. sodium channels (Dyhrfield *et al.*, 2010).

2.3.1.1Non-synaptic Mechanisms

Nonsynaptic mechanisms exert a powerful influence on seizure threshold. The regulation of intracellular and extracellular ions is necessary for establishing ionic gradients required for the operation of neuronal ion channels (Payne *et al*., 2003). It is well established that nonsynaptic epileptiform activity can be induced in hippocampal slices by reducing extracellular Ca^{2+} concentration (Marom *et al.*, 2001)

Changes in ionic concentrations observed during hyperexcitation such as increased extracellular K^+ or decreased extracellular Ca^{2+} may be caused by decreases in extracellular size or volume. Failure of Na^+-K^+ pump due to hypoxia or ischemia is known to promote epileptogenesis in animal models, and interference with Cl^--K^+ transport, which controls intracellular Cl and regulates GABA-activated inhibitory Cl currents, may lead to enhanced excitation (Wang *et al*., 2005). Excitability of synaptic terminals depends on the extent of depolarization and the amount of neurotransmitter released. Synchronization following abnormal bursts of spikes in the axonal branching of thalamocortical relay cells plays a key

role in epileptogenesis. Ephaptic interactions that occur between neighboring neurons separated by small extracellular spaces also contribute to increased synchronization (Jefferys, 1995).

2.3.1.2 Synaptic Mechanisms

Synaptic pathophysiology of epilepsy and epileptic disorders primarily involves reduced GABAergic inhibition or enhanced glutamatergic excitation (Bromfield *et al*., 2006).

2.3.2 Gamma aminobutyric acid (GABA) - receptor inhibition

Occurrence of Gamma aminobutyric acid (GABA) in the central nervous system was demonstrated in 1950 and in the same decade GABA was shown to inhibit seizure activity after its direct cerebral application in dogs (Meldrum, 1978).

The major inhibitory neurotransmitter GABA interacts with 2 major subtypes of receptor: $GABA_A$ (ionotropic) and $GABA_B$ (metabotropic) receptors. $GABA_A$ receptors are found postsynaptically, while $GABA_B$ receptors are found presynaptically, and can thereby modulate synaptic release. In the adult brain, $GABA_A$ receptors are permeable to Cl^- ions; upon activation Cl^- influx hyperpolarizes the membrane and inhibits action potentials. Neuronal circuits that are epileptic are known for being hyperexcitable and for lacking the normal balance of glutamatergic neurons (those that usually increase excitation) and GABAergic ones - those that decrease it (Aroniadou *et al.,* 2008). In addition, the levels of GABA and the sensitivity of $GABA_A$ receptors to the neurotransmitter may decrease, resulting in less inhibition (Armijo *et al.,* 2002). Therefore, substances which are $GABA_A$

receptor agonists, such as barbiturates and benzodiazepines, are well known to suppress seizure activity (Bromfield *et al.*, 2006).

The action of GABA in the mammalian brain is mediated via the $GABA_A$ and $GABA_B$ receptors, these receptors differ in terms of their distribution in the brain, pharmacological profile and their mechanisms of signal transduction (De Sarro *et al.*, 2000). The functional role of $GABA_B$ receptors is to regulate the release of excitatory and inhibitory neurotransmitters. Both $GABA_A$ and $GABA_B$ are involved in the control of neuronal excitability in the brain and invariably play a major role in epileptogenesis (Kaila *et al*., 2014).

GABA levels have been shown to be reduced in the cerebrospinal fluid (CSF) of patients with certain kinds of epilepsy, such as infantile spasms, untreated generalized tonic-clonic seizures, and in excised epileptic tissue from patients with drug-resistant epilepsy, suggesting that these patients have decreased inhibition (Loscher and Siemes, 1985). Dogs with epilepsy have been shown to have low CSF levels of GABA, and mice genetically susceptible to audiogenic seizures have a lower number of GABA receptors than non-seizure prone animals. Reduced GABA binding to GABA receptors has been reported in human brain tissue, and low glutamic acid decarboxylase levels have been shown in kindled rats and in excised human epileptic tissue, suggestive of decreased GABAergic inhibition (Engelborghs *et al*., 2000).

2.3.3 Glutamate Receptors Activation

Glutamate is the predominant excitatory neurotransmitter in the motor and sensory systems of the central nervous system. Glutamate interacts with a range of specific receptor and transporter systems to produce fast and sustained synaptic excitation. It initiates various calcium dependent processes in target cells including the production of nitric oxide (Bienvenu *et al.*, 2002). Three main glutamate receptor subtypes are N-methyl-D-aspartate (NMDA), non-NMDA (alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid and kainate receptors. Epilepsy may result from excessive release of glutamate from central nerve terminals (Leonard, 2003). Research has shown that excessive stimulation of glutamate receptors cause excitotoxicity, a phenomenon implicated in both acute and chronic neurodegenerative diseases (ischemia, Huntington's disease and amyotrophic lateral sclerosis). Several lines of evidence indicate that excessive stimulation of glutamate receptors, perhaps due to impairment of the glutamate-transport system could lead to Ca^{2+} overload in mitochondria, resulting in overproduction of reactive oxygen species (ROS) and oxidative stress–mediated motor-neuron damage (Kong,1998).

Hippocampal recordings from conscious human brains have shown sustained increases in the levels of extracellular glutamate levels during and preceding seizures. GABA levels remain low in the epileptogenic hippocampus, but during seizures, GABA concentrations increase, although mostly in the non-epileptogenic hippocampus. This leads to a toxic increase in extracellular glutamate due to reduced inhibition in the epileptogenic areas (During and Spencer, 1993).

2.3.4 Extra-neuronal (extrinsic) factors

2.3.4.1 Changes in extracellular ion concentration due to variations in the volume of the extracellular space.

Decreased extracellular volume leads to increased extracellular K^+ concentration, resisting the outward movement of K^+ ions needed to repolarize the cell, thereby effectively increasing excitability. The repititive depolarization and repolarization of the nerve membrane eventually leads to an energy-deprived state within the cell, thereby preventing the restoration of the cell membrane potential. Depolarization would lead to an influx of Ca^{2+} ions and an efflux of K^+ ions which if it persists would eventually result in cell (neuronal) death (Leonard, 2003).

2.3.4.2 Remodeling of synaptic contacts.

Movement of an afferent axon terminal closer to the target cell body increases the likelihood that inward ionic currents at the synapse will bring the target neuron to threshold. The coupling between the pre- and post-synaptic elements can be made more efficient by shortening of the spine neck. In addition, previous synaptic experience such as a brief burst of high frequency stimulation (e.g., long-term potentiation) also increases the efficacy of such synapses, increasing their excitability.

2.3.5 Glutamate mediated excitation

Glutamate binds to multiple receptor sites that differ in activation and inactivation time courses, desensitization kinetics, conductance, and ion permeability transmitter metabolism

by glial cells. Excitability increases, for example, if glial metabolism or uptake of excitatory transmitters such as glutamate or acetylcholine decreases (Bromfield, 2006).

2.4 DIAGNOSIS OF EPILEPSY

For a first seizure, it is important to distinguish between an isolated event that is caused by an unusual stress, such as alcohol withdrawal, high fever or hypoglycemia and an unprovoked event that may be an initial manifestation of a recurrent seizure disorder (Mosewich, 1996).

Despite the fact that the diagnosis of epilepsy is largely based on clinical data, the evaluation of patients with suspected seizures requires the use of investigative modalities such as electroencephalography and neuroimaging facilities.

2.4.1 Electroencephalography

Electroencephalograph (EEG) can supply supportive evidence for the diagnosis of epilepsy and also provide critical clues to the classification of epileptic seizures and syndromes. In addition, it may help in anatomical localization of an underlying cerebral pathology, but neuroimaging techniques provide more useful information concerning structural abnormalities. EEG indirectly aids in the selection of appropriate antiepileptic drug(s) and in certain circumstances, also helps in formulating a prognosis, since it is extremely valuable in the determination of seizure type. Most routine EEGs in epileptic patients are obtained in the interictal state and the diagnostically useful finding is the epileptiform

patterns (EPs) which suggests the presence of real epileptogenic process (Lodder *et al*., 2014).

2.4.2 Neuro-imaging computerized tomography

Computerized tomography (CT) is very useful in the detection of structural lesions and the determination of the exact location of such lesions. It is also used in the determination of cerebral atrophy, which is the commonest abnormality demonstrated in epileptic patients. The proportion of abnormalities in CT is higher in late onset epilepsy as localization related epileptic syndromes are commoner in the adult population. A descriptive study of 103 consecutive children with epilepsy for over 5 year period showed abnormal films in 51.5% with most patients having hydrocephalus followed by cerebral atrophy and infarct. A high incidence of abnormal findings was reported in those with partial seizures. The CT findings in 75 adult Nigerian patients reported by Ogunniyi *et al*., (1994) showed normal findings in 54.7 %, cerebral atrophy in 21.3% while cerebral tumours, vascular lesions and porencephaly accounted for the remaining 24%.

2.4.3 Plain radiology

Plain skull X-ray still has a definite place in developing countries although the development of advanced imaging techniques has made it almost irrelevant. It is still cheap, widely available and relatively innocuous despite its inferiority in both sensitivity and specificity to newer techniques. It is useful in the detection of bony changes (as seen in raised intracranial pressure and tumours) and abnormal calcification (commonly associated with cerebral tumours, arteriovenous malformations and infections like cysticercosis, toxoplasmosis and cytomegalovirus).

2.4.4 Single photon emission computerized tomography (SPECT)

This is also not commonly used in the evaluation of epilepsy. It is both a structural and functional neuro radiological investigation as it reveals the presence of structural lesions and disturbances in metabolism. It utilizes radioisotope scanning to delineate cerebral perfusion and structural abnormalities.

2.4.5 Magnetic resonance imaging (MRI)

This technique is superior to CT in the detection of most structural lesions, including tumours, and especially in the evaluation of demyelinating diseases, lesions in the posterior *fossa* and many white matter disorders. It is not readily available in many developing countries. In patients suffering from intractable temporal lobe epilepsy, in whom pre-surgical localization of the epileptic focus is one of main problems, studying the seizure pattern and EEG findings complemented by MRI results and neuropsychological assessment have proved inadequate. Magnetic resonance spectroscopy has proved superior in pre-surgical localization and it has been used to detect N – acetyl aspartate reduction in the affected temporal lobe and this positively correlates with clinical EEG and structural lateralization.

2.5 Classification of Antiepileptic drugs on the basis of their mechanism(s) of action

AEDs are neither preventive nor curative and are employed solely as a means of controlling symptoms (i.e. suppression of seizures).

Three major mechanisms are recognized (Graeme, 2005): modulation of voltage-gated ion channels; enhancement of gamma-aminobutyric acid (GABA)-mediated inhibitory neurotransmission; and attenuation of glutamate-mediated excitatory neurotransmission. The principal pharmacological targets of currently available AEDs are as follows

2.5.1 Voltage-Gated Sodium Channel blockers

Sodium channel blockade is the most common and best-characterized mechanism of currently available antiepileptic drugs (Taylor *et al*., 1999). Voltage-gated sodium channels are responsible for depolarisation of the nerve cell membrane and conduction of action potentials across the surface of neuronal cells. They are expressed throughout the neuronal membrane, on dendrites, soma, axons, and nerve terminals. Density of expression is highest in the axon initial segment (AIS) where action potentials are generated. Each sodium channel dynamically exists in the following 3 states:

- A resting state, during which the channel allows passage of sodium into the cell
- An active state, in which the channel allows increased influx of sodium into the cell

- An inactive state, in which the channel does not allow passage of sodium into the cell

During an action potential, these channels exist in the active state and allow influx of sodium ions. Once the activation or stimulus is terminated, a percentage of these sodium channels become inactive for a period known as the refractory period. With constant stimulus or rapid firing, many of these channels exist in the inactive state, rendering the axon incapable of propagating the action potential. Some antiepileptic drugs stabilize the inactive configuration of sodium (Na^+) channel, preventing high-frequency neuronal firing.

AEDs that target the sodium channels prevent the return of these channels to the active state by stabilizing them in the inactive state. In doing so, they prevent repetitive firing of the axons. Anticonvulsant drugs that act via this mechanism include; Carbamazepine and Phenytoin.

2.5.2 Voltage-gated Calcium channel blockers

Calcium channels exist in 3 known forms in the human brain: L, N, and T.These channels are small and are inactivated quickly. Voltage-gated calcium channels contribute to the overall electrical excitability of neurons, are closely involved in neuronal burst firing, and are responsible for the control of neurotransmitter release at pre-synaptic nerve terminals. The influx of calcium currents in the resting state produces a partial depolarization of the membrane, facilitating the development of an action potential after rapid depolarization of the cell.

Calcium channels function as the "pacemakers" of normal rhythmic brain activity. This is particularly true of the thalamus. T-calcium channels have been known to play a role in the spike-and-wave discharges of absence seizures. AEDs that inhibit these T-calcium channels are particularly useful for controlling absence seizures (Lowestein, 2001).

Low-voltage calcium (Ca^{2+}) currents (T-type) are responsible for rhythmic thalamocortical spike and wave patterns of generalized absence seizures. Some antiepileptic drugs (e.g ; Ethosuximide and Valproic acid) block these channels, inhibiting underlying slow depolarizations necessary to generate spike-wave bursts.

2.5.3 GABA ergic agents

Gamma-aminobutyric acid (GABA) has 2 types of receptors, A and B. When GABA binds to a $GABA_A$ receptor, the passage of chloride, a negatively charged ion, into the cell is facilitated via chloride channels. This influx of chloride increases the negativity of the cell (i.e., a more negative resting membrane potential) and causes the cell to have greater difficulty reaching the action potential. The $GABA_B$ receptor is linked to a potassium channel (Porter and Meldrum, 1995).

Gamma-aminobutyric acid ($GABA_A$) receptor mediates chloride (Cl^-) influx, leading to hyperpolarization of cell and inhibition. Antiepileptic drugs may act to enhance Cl- influx or decrease GABA metabolism. The GABA system can be enhanced by binding directly to $GABA_A$ receptors, by blocking presynaptic GABA uptake, by inhibiting the metabolism of GABA by GABA transaminase, and by increasing the synthesis of GABA. GABA is produced by decarboxylation of glutamate mediated by the enzyme glutamic acid decarboxylase (GAD). Some AEDs such as valproate act as modulators of this enzyme,

enhancing the production of GABA and down-regulating glutamate. Some AEDs (eg, tiagabine) function as an agonist to chloride conductance, either by blocking the reuptake of GABA or by inhibiting its metabolism as mediated by GABA transaminase (eg, vigabatrin), resulting in increased accumulation of GABA at the postsynaptic receptors. (Hussaini, 2006).

2.5.4 Glutamate blockers

Glutamate receptors bind glutamate, an excitatory amino acid neurotransmitter. Upon binding glutamate, the receptors facilitate the flow of both sodium and calcium ions into the cell, while potassium ions flow out of the cell, resulting in excitation.

The glutamate receptor has 5 potential binding sites, as follows:

- The alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) site
- The kainate site
- The *N* -methyl-D-aspartate (NMDA) site
- The glycine site
- The metabotropic site, which has 7 subunits

AEDs that act via these receptors are antagonistic to glutamate. Responses to glutamate antagonists differ, depending on the site being affected (Chapmann, 1998).

2.5.5 Carbonic anhydrase inhibitors

Inhibition of the enzyme carbonic anhydrase increases the concentration of hydrogen ions intracellularly and decreases the pH. The potassium ions shift to the extracellular compartment to buffer the acid-base status. This event results in hyperpolarization and an increase in seizure threshold of the cells.

Acetazolamide has been used as an adjunctive therapy in refractory seizures with catamenial pattern (i.e., seizure clustering around menstrual period) while topiramate and zonisamide (ZNS) have been shown to be weak inhibitors of the carbonic anhydrase enzyme.

2.6 Limitations associated with the use of existing antiepileptic drugs

Modern treatment of seizures started in 1850 with the introduction of bromides, in 1910, phenobarbital (PHB), which then was used to induce sleep, was found to have antiseizure activity and became the drug of choice for many years. More than 150 years after bromide was introduced as the first antiepileptic drug, adverse effects remain a leading cause of treatment failure and a major determinant of impaired health-related quality of life in people with epilepsy. Adverse effects can be broadly classified into those that are reversible and dose dependent (e.g., ataxia, sedation, dizziness, cognitive dysfunction), those that are chronic and non-rapidly reversible (e.g., changes in body weight, hirsutism, gingival hyperplasia), and those that are idiosyncratic (e.g., skin rashes, blood dyscrasias, liver toxicity) (Battino *et al.*, 2000). Adverse effects can develop acutely or many years after

starting treatment, in the past two decades, many efforts have been made to reduce the burden of antiepileptic drug toxicity (Perruca & Gilliam, 2012).

Drug interactions are a major consideration in the clinical use of AEDs, for a number of reasons (Patsalos and Perucca, 2003). Antiepileptic drugs are usually prescribed for prolonged periods, often for a lifetime, and the probability of coadministration with medications used to treat comorbid conditions is considerable; polypharmacy is relatively common, both in epilepsy and in other indications. Many AEDs interfere with the activity of cytochromes P450 (CYP) and glucuronyltransferases, and many also are substrates of these enzymes. Due to the narrow therapeutic ratio of AEDs, interactions resulting in changes in serum drug concentration can easily result in toxicity or in loss of response, and as a result of the partly overlapping mechanisms of action of these agents, a potential exists for pharmacodynamic interactions (Patsalos and Perucca, 2003).

2.7.1 Medicinal plants and epilepsy

Plants have a long history in the management of epilepsy. A typical example of which is *Valariana officinalis* used as a herbal treatment of epilepsy in Europe and America (Murray, 1998). A number of African medicinal plants have been reported to posess bioactive constituents capable of exerting anticonvulsant action hence making them relevant in the management of seizure disorders. These plants include; *Glycyrrhyza glabra* (Ambawade *et al*., 2002), *Dalbergia saxatilis* (Yemitan and Adeyemi, 2006), *Cassia occidentalis, Heliotropium indicum, Xylopia aethiopica* (Mann *et al.,* 2003).

2.7.2 Plant description: *Cassia siamea*



Plate I: *Cassia siamea* in its natural habitat



Plate II: Aerial portion of *Cassia siamea*

Cassia siamea also known as **Kassod Tree**, **Cassod Tree** and as **Cassia tree** is a legume in the subfamily Caesalpinioideae. It is native to South and Southeast Asia, introduced and now naturalized in Africa although its exact origin is unknown.

2.7.3 Botanical description

Cassia siamea Lam. (Irwin and Barneby- *Cassia siamea* Lam.) (Fabaceae) is a medium sized evergreen tree having many branches. The leaves are arranged in cascades and the flowers hang in bunches similar to grapes. The petals are yellow and are from 5 to 7 cm in length. The plant grows exclusively in forests in tropical South-East Asia and in Thailand they are found mainly in low-lying (sea level) regions.

Synonym: *Senna siamea*

2.7.3 Ethno medical uses of *Cassia siamea* plant

Cassia siamea plant (commomly known as *Malga* in Hausa language) is used by traditional medical practitioners for the management of constipation associated with surgery, childbirth and the use of narcotic pain relievers (Hill, 1992). A decoction of the leaves and bark is used locally by the people of Okeigbo in Ondo state, Nigeria as a remedy for Malaria (Lose *et al*., 2000; Odugbemi *et al*., 2007). Matured fruit of the plant is used to charm away intestinal worms and to prevent convulsion in children (Kiepe, 2001). In Burkina faso, Ghana and Nigeria, the decoction of the whole stem or stem's bark is drunk or used for body bath against Malaria and liver disorders (Asase *et. al*., 2010). The same uses were reported in Malaysia (Al-Adhroey *et. al*., 2010). Dried stems of C. siamea mixed with the fruit of *Xylopia aethiopica* is pulverized and administered as laxative (Kiepe,

1995) *Cassia siamea* has been reported to be used in the management of constipation, diabetes, insomnia (Tripathii and Gupta, 1991), hypertension, asthma, typhoid fever and dieresis (Hill, 1992), used for treatment of diabetes, lymph node swelling, urine stones, general deficiency conditions- Beri Beri , classic deficiency / lack of vitamin B1 (thiamine) in gastrointestinal disorders - malabsorption - meals taken with polished rice etc. For the treatment of Gonorrhea and syphilis, roots of *Cassia siamea* are peeled, pounded, boiled (Maregesi *et. al.,* 2007).

The leaves are used for malaria therapy in Okeigbo Ondo state (Odugbemi *et al.,* 2007). Stem barks of *Cassia siamea* is used by traditional medicine practitioners in the treatment of tuberculosis (Tabuti *et al.,* 2010). It has been reported that chewing a stem of cassia siamea is effective in the maintenance of dental hygiene (Kayode and Omotoyinbo, 2009).

Non medicinal uses include as fodder plant, in intercropping systems, for erosion control and as windbreaks. Young leaves are eaten as a vegetable and curry dishes are made with young leaves and flowers. In addition, *Cassia siamea* is also used in the production of honey and tannins.

2.7.4 Pharmacological activity of *Cassia siamea*

Previous scientific research that have been carried out on *Cassia siamea* revealed that the alcoholic extract of *Cassia siamea* flowers exert antioxidant activity (Gupreert *et al*., 2006). Barkol a compound extracted from cassia siamea plant has been shown to exert laxative properties via the stimulation of chloride ion secretion in rat colon (Chatsri *et al*., 2005).

The plant has also been shown to posses bioactive principles capable of exerting antibacterial action against pseudomonas aeruginosa (Otimeyin *et al*., 2010). The leaves of cassia siamea have been shown to posses antihyperglyceamic activity (Jangiti *et al*., 2013).

2.7.5 Chemical composition of the plant

The chemical composition of Cassia siamea plant has been extensively reported. The plant yields chromones (Chromone alkaloids, chromones glycosides, dihydronaphthalenone compounds, bischromone), polyphenols (anthrax quinines, bianthraquinones, anthrone, flavonoids, isoflavonoids, phenolics, tannins), alkaloids, saponins, steroids, carotenoids, oxalates, phytate, reducing sugars, vitamins, minerals and enzymes and its derivatives.

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CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Plant Material Collection and Authentication

The roots, stems and aerial parts of *Cassia siamea* plant were collected within the vicinity of the Ahmadu Bello University staff residential quarters in July, 2012. The various parts collected were washed thrice under running tap water to remove dirt and adhering material(s) and other associated contaminants after which they were taken for authentication in the herbarium section of the Department of Biological Sciences, Ahmadu Bello University Zaria. The plant was identified and authenticated by Mallam Umar .S. Gallah of the Herbarium Section of the Department of Biological Sciences, A. B. U Zaria by comparing with existing Voucher Specimen (No. 168).

3.1.1 Plant extraction

The previously washed roots, stems and ariel parts of *Cassia siamea* plant were air dried under shade until attainment of constant weight. Equal portions (200 g each) of the root, stems and ariel portion of the air dried plant were then crushed into a coarse powder with the aid of a mortar and pestle. A portion (500 g) of the resulting powdered plant was extracted with 2 Litres of aqueous methanol (70% methanol: 30% water) for 72 hours using cold maceration technique. The extract was collected from the separating funnel by filteration using Whatmann filter No. 25. The solvent was evaporated from the resulting filtrate on a water bath set at low temperature (40^{o}C). The solutions of the extract were always freshly prepared for each study by dissolution of the appropriate amount required in deionized water under standard laboratory conditions.

3.2 Experimental Animals

Male and female swiss albino mice weighing between 18-25 g were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria- Nigeria. Day old Ranger cockerels (25 – 38 g) were obtained from the National Animal Production Research Institute (NAPRI), Shika-Nigeria. The animals were housed in propylene cages situated in well ventilated rooms at the Pharmacology and Therapeutics Animal House Facility. Both mice and chicks were maintained on standard laboratory animal feed and water *ad libitum*. All experiments performed on laboratory animals were in accordance with the Ahmadu Bello University Research Policy Guidelines.

3.3 Drugs and Chemicals

Aluminum chloride (Bhd Ltd Poole England), Diazepam 10mg in 2ml (Roche®, Pakistan), Dragendorff reagent (Bhd Ltd Poole England), Ethyl acetate, Glacial acetic acid (Searle Essex, England), Hydrochloric acid (Bhd Ltd Poole England), Methanol (Sigma-aldrich, St. Louis U.S.A), n-Hexane (Sigma, St. Louis U.S.A), Naloxone (BIOMOL Research Lab PA. U.S.A), N-butanol (Sigma-aldrich, St. Louis U.S.A), P-anisaldehyde (Sigma, St. Louis U.S.A), Pentylenetetrazole (Sigma-aldrich, St.louis U.S.A), Phenobarbitone (Lab Renaudin, France), Phenytoin sodium capsules (Parke-Davis and Co. Ltd), Picrotoxin (Sigma, St. Louis U.S.A), Sodium valproate (Sanofi St.Surrey, Canada), Sulphuric acid (Bhd Ltd Poole England), Tween 80 (Cole-Parmer illinois U.S.A).

3.4 Equipment

Animal cages (Propylene-type), conical flasks**,** cotton wool, capillary tubes, evaporating dish, glass funnel, glass chromatography tank, glass storage jars and stopper, maximal electroshock apparatus with corneal electrodes (Ugo Basile 7801), measuring cylinder, porcelain crucibles , porcelain pestle and mortar, separating funnel, syringes and needles (1ml, 2ml, 5ml and 10ml), thin layer chromatography precoated plates**,** Ultra violet lamp (Hanovia Lamps, Slough-England) water bath, weighing balance (Mettler U.S.A)**,** Whatman filter paper**.**

3.5 Fractionation of *Cassia siamea* crude extract.

The method described by Deng *et al*., (2007) was employed in the fractionation of the methanol extract of *Cassia siamea* (Fig.3.1). 30 grams of the methanol extract of *Cassia siamea* was dissolved in 100 ml of distilled water, 300 ml of ethyl acetate was used to partition the dissolved extract. The resulting ethyl acetate portion was fractionated with the aid of a separating funnel, it was then concentrated to dryness by gentle heating over a water bath set at low temperature (40^0C) to obtain the Ethyl acetate fraction (EAF). The aqueous portion remaining was further partitioned with 300 ml of n-butanol, the n-butanol portion was then collected using a separating funnel and also denoted as the n-butanol fraction (NBF). The remaining aqueous portion also evaporated to dryness over a water bath set at low temperature and was denoted residual aqueous fraction (RAF).

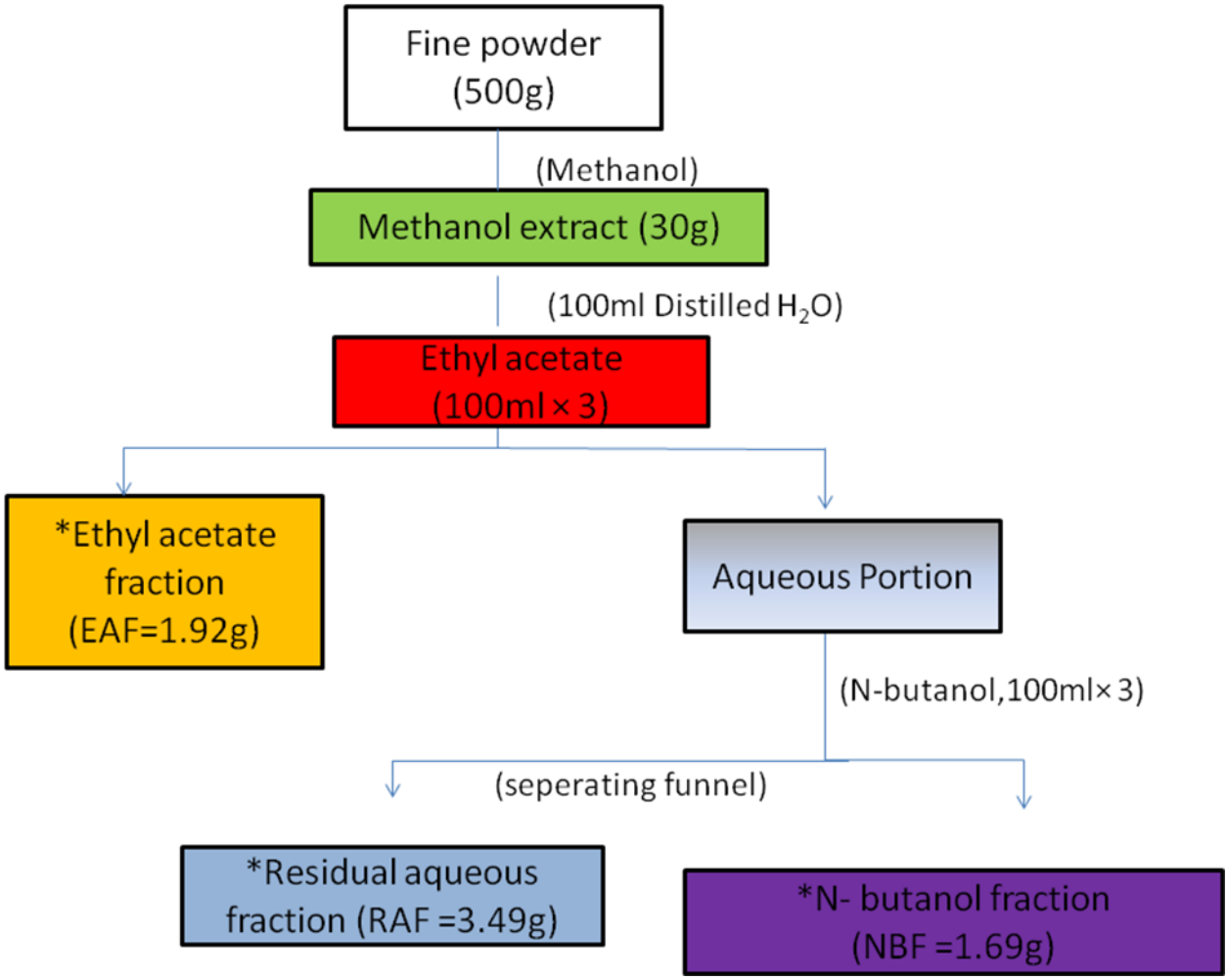


Figure 1: The fractionation of the methanol extract of *Cassia siamea* **(**Deng *et al.*, 2007).

*Fractions were subjected to preliminary anticonvulsant screening using to Maximal electroshock and Pentylenetetrazole induced acute seizure models.

3.6 Preliminary Phytochemical Studies

The following conditions were employed:

a) Technique used: one-way ascending

b) Spotting: Solutions of the crude plant extract were spotted manually with the aid of a capillary tube on silica gel precoated thin layer aluminum chromatographic plates. The plates were developed in a predetermined solvent system (ethylacetate: n-hexane). Each plate was sprayed with a different visualizing reagent.

c) TLC plate: Fluka Silica gel precoated aluminum plate 20cm × 20cm with layer thickness of 0.25mm.

A thin line about 2cm from the bottom of the TLC plate was drawn with the aid of a pencil. The crude methanol extract of *Cassia siamea* was dissolved in a minimum volume of deionized water to give a 20 mg/ml solution. The resulting solution of the extract was subsequently applied uniformly along the thin line previously drawn with the aid of a capillary tube. The plate was allowed to dry after which it was developed using Ethyl acetate: Hexane (8.5: 1.5) as solvent system. The developed plate was air dried in a fume cupboard after which it was sprayed with Anisaldehyde in sulphuric and various other detecting reagents to aid in the detection of some phytochemical constituents (Harborne, 1998).

3.6.1 Test for alkaloids

Dragendorff's reagent was used as the visualizing reagent. Orange spots would indicate the presence of alkaloids.

3.6.2 Test for flavonoids

1% ethanolic solution of aluminum chloride was used as visualizing reagent. Yellow fluorescence under UV light (360nm) would indicate the presence of flavonoids.

3.6.3 Test for phenols

A freshly prepared solution of 0.5ml p-anisaldehyde in 50ml glacial acetic acid, with application of gentle heat. Appearance of violet coloured spots indicates the presence of phenols.

3.6.4 Test for terpenes and steroids

A freshly prepared solution of 0.5ml p-anisaldehyde in 50ml glacial acetic acid, with application of gentle heat. Appearance of blue coloured spots indicates the presence of terpenes, while green coloured spots indicate presence of steroids

3.6.5 Test for Tannins

A solution of dilute Ferric chloride was used as detecting reagent. Appearance of blue-black or greenish black spots would indicate the presence of tannins.

3.7 Pharmacological Studies

3.7.1 Median lethal dose determination (LD_{50})

The method described by Lorke (1983) was adopted. The median lethal dose (LD_{50}) for the methanol extract of *Cassia siamea* as well as its Ethyl acetate, n-butanol and aqueous portion was determined in mice and chicks.

3.7.1.1 Acute toxicity Studies in Mice and Chicks

The study was divided into two phases. In the first phase, three groups each containing three mice (chicks) were treated intraperitoneally (*i.p*) with the methanol extract of *Cassia siamea* at doses of 10, 100 and 1000 mg/kg body weight. The animals were subsequently observed for signs of toxicity and death for a period of 24 hours after treatment. In the second phase, four groups each comprising one mouse (chick) was treated intraperitoneally with the methanol extract of *Cassia siamea* using four specific doses of the extract which depended on the outcome of the first phase. The LD_{50} value was determined as the square root of the product of the lowest dose that caused death and the highest non- lethal dose. i.e. the geometric mean of consecutive doses for which 0 and 100% survival rates were recorded.

The same procedure was repeated for the Ethylacetate, n-butanol and aqueous portions of *Cassia siamea* extract respectively. The median lethal dose was calculated as the square root of the product of the lowest dose that caused death and the highest non-lethal as shown below:

$$LD_{50} = \sqrt{\text{highest non-lethal dose x minimum lethal dose}}$$

3.7.2 Anticonvulsant Studies

3.7.2.1 Maximal electroshock-induced seizure test in chicks

The method described by Swinyard and Kupferberg (1985) was employed in this study. Fifty (50) one-day old Ranger cockerels were randomly divided into five groups each containing ten chicks. The first group of chicks received normal saline (10 ml/kg). The second, third and fourth groups received intraperitoneally 250, 500 and 1000 mg/kg of the methanol extract respectively. The fifth group was administered 20 mg/kg phenytoin. Thirty minutes later, maximal electroshock was administered to all chicks to induce seizure in the chicks using Ugo basile electroconvulsive machine with corneal electrodes placed on the upper eyelid of the chicks. The shock duration, frequency and pulse width were set and maintained at 0.8 s, 150 pulses s/s, 80 mA and 0.6 ms respectively. A current of 80 mA which produced tonic seizures in 90% of the negative control chicks was maintained and used throughout the study. Seizures were manifested as hind limb tonic extension (HLTE). The ability of the extract to prevent hind limb tonic extension was considered as an indication of anticonvulsant activity (Swinyard, 1969).

In unprotected animals the recovery time was recorded. The same procedure was repeated for the ethyl acetate (250, 500 and 1000 mg/kg), n-butanol (250, 500 and 1000 mg/kg) and residual aqueous portions (200, 400 and 800 mg/kg) of *Cassia siamea*.

3.7.2.2 *Pentylenetetrazole –induced seizure in mice*

The method previously described by Swinyard (1989) was employed. Thirty mice were randomly divided into five groups of six mice each. Group one representing the negative control group was given 10 ml per kg normal saline via the intraperitoneal route, while group two, three and four were pre-treated with the methanol extract of *Cassia siamea* at doses of 250, 500 and 1000 mg/kg body weight via the intraperitoneal route. The fifth group of mice (positive control group) was treated with 200 mg/kg valproic acid intraperitoneally. Thirty minutes after treatment with the extract, 90 mg/kg of pentylenetetrazole (CD_{100}) solution was administered subcutaneously to each mouse. The mice were observed for thirty minutes for presence or absence of threshold seizures (i.e. an episode of clonic spasm of at least five seconds duration)

The same procedure was repeated for the ethyl acetate, n-butanol and residual aqueous portion of the *Cassia siamea* whole plant extract.

3.7.2.3 Picrotoxin induced seizure in mice

This test was conducted on the most active fraction of the plant to investigate the effect of picrotoxin on its anticonvulsant activity. The method previously described by Salih and Mustafa (2008) was adopted. Thirty mice were grouped into five each consisting of six mice each. The first group received normal saline (10 ml/kg). The mice in the second, third and fourth groups received intraperitoneally 250, 500 and 1000 mg per kg body weight of the ethyl acetate fraction of *Cassia siamea* whole plant extract respectively. Mice in the fifth group received 10mg diazepam per kg body weight. Thirty (30) minutes post

treatment, 10mg Picrotoxin per kg body weight was administered to each mouse via the intraperitoneal route. The mice were subsequently observed for hind limb tonic seizures for thirty minutes. Absence of tonic hind limb extension or prolongation of the latency of tonic hind limb extension was considered as an indication of anticonvulsant activity.

3.3.2.4 *Effects of Naloxone on the Anticonvulsant Activity of the Ethyl acetate Fraction of Cassia siamea.*

This study was undertaken to investigate the probable modulatory properties of opioid receptors on the anticonvulsant activity of the ethyl acetate fraction obtained from the whole plant extract of *Cassia siamea*. In this study, naloxone was used as an opioid receptor antagonist (Lauretti *et al.*, 1994 ; Marjan *et al*., 2007). Fourty (40) mice were used. The experimental procedure comprised of four groups comprising of ten (10) mice each. Naloxone at a dose of (3 mg/kg) was administered 5 min before the administration of the ethyl acetate fraction of *Cassia siamea* (250 mg/kg) to group 1 mice. In group 2 the ethylacetate fraction alone was administered, in group 3 naloxone alone was administered while normal saline was administered to the fourth group of mice at a dose of 10ml/kg body weight. Thirty (30) minutes post treatment, pentylenetetrazole (90mg/kg) was administered to all groups. The anticonvulsant activity of the ethyl acetate fraction in groups pretreated with naloxone (i.e groups 1) was assessed and compared with animals pretreated only with the ethylacetate fraction (250 mg/kg), naloxone (3 mg/kg) and normal saline (10 ml/kg) groups.

3.8 Behavioral Study on the Ethyl acetate Portion of *Cassia siamea* Plant

3.8.1 Diazepam-Induced Sleep in Mice

The method described by Beretz *et al*., (1978) and modified by Rakotonirina *et al*., (2001) was adopted in this study. Mice were randomly divided into four groups containing 6 mice each. The first group received normal saline (10 ml/kg) intraperitoneally. The second, third and fourth groups received 250, 500 and 1000mg ethylacetate fraction per kg body weight via the same route. 30 minutes post treatment mice in all the groups received diazepam at 20mg/kg *i.p*. The mice were placed individually in propylene cages for observation. The onset and duration of sleep were determined for each animal. The time interval between the administration of Diazepam to the loss of rightening reflex was recorded as the onset for sleep while the interval between the loss and the recovery of righting reflex was regarded as the duration of sleep (Soulimani *et al*., 2001).

3.9 Statistical Analysis

Results were expressed as Mean ± Standard Error of the Mean (SEM) and as percentages where appropriate. Statistical analysis was performed using analysis of variance (ANOVA); a post hoc Dunnets test was performed for multiple comparisons when statistically significant results were obtained with ANOVA. Values of $P<0.05$ were considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 The Plant Extract and Fractions

4.1.1 Fractionation of the Crude Methanol Plant Extract

Fractionation of the crude methanol extract of *Cassia siamea* with different solvents of increasing polarity resulted in three (3) fractions namely; Ethyl acetate (EA), n-butanol (NB) and Residual aqueous fraction (RAF). The yield of the methanol extract was obtained to be 14.7%w/w. The n-butanol fraction had the highest yield (10.6%w/w), followed by the ethyl acetate fraction (5.81%w/w), while the residual aqueous fraction had the least yield (5.12%w/w). The yield of the various fractions (grams & percentage) is as shown (Table 4.1).

All the fractions obtained were solid; no oil (s) was obtained. In physical appearance the Ethyl acetate fraction (EAF) was a sticky dark brown coloured solid, the n-butanol fraction (NBF) was a dark brown powder, while the residual aqueous fraction (RAF) was dark brown in color, hygroscopic and very sticky.

Table 4.1 Percentage yield of fractions obtained from the methanol extract of *Cassia siamea*

Fractions	**Yield (g)**	**Yield (%w/w)**
Ethyl acetate fraction	1.92	5.81
n-butanol fraction	3.49	10.6
Residual Aqueous Fraction	1.69	5.12

4.2 Phytochemical Constituents of *Cassia siamea*

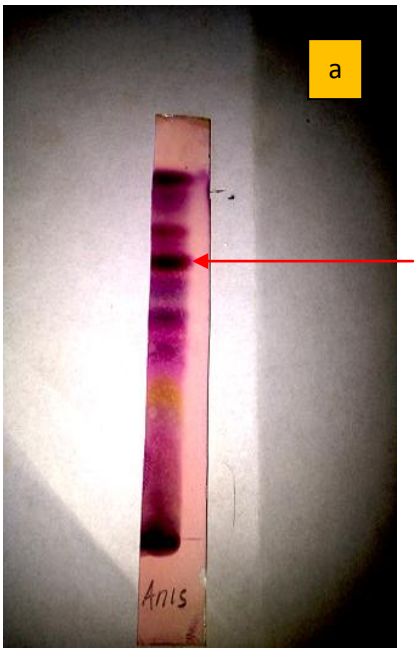
Preliminary phytochemical screening of the methanol plant extract of *Cassia siamea* and its ethyl acetate fraction using thin layer chromatographic method and various detecting reagents revealed the presence of alkaloids, flavonoids, polyphenols, terpenes, steroids, saponins and tannins in the methanol extract. Anthraquinones were found to be absent in both the methanol extract and its ethylacetate fraction while polyphenols were found to be absent in the ethylacetate fraction (Table 4.2).

Table 4.2 Phytochemical Constituents of the Methanol Extract of *Cassia siamea* (CSME) and its Ethyl acetate fraction (EAF)

Constituents	Extract	
	C.SME	EAF
Alkaloids	+	+
Anthraquinones	-	-
Flavonoids	+	+
Polyphenols	+	-
Saponins	+	+
Steroids/Terpenoids	+	+
Tannins	+	+

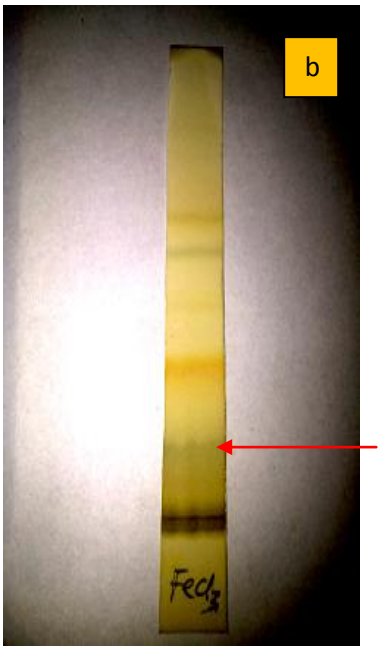
Key: + = present , − = absent ;

CSME = *Cassia siamea* methanol extract ; EAF = ethyl acetate fraction



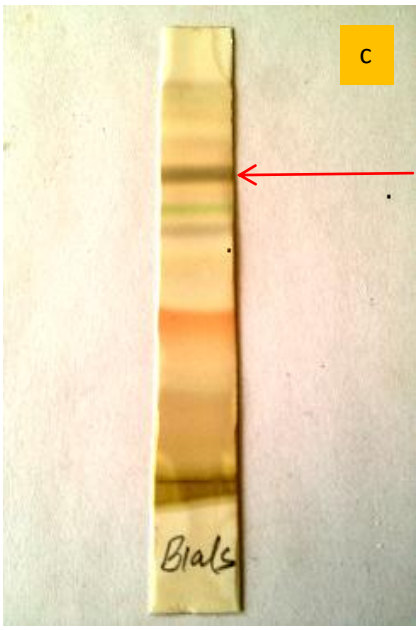
a-Phenolics

Plate III: TLC of CSME , ethyl acetate: n-hexane (8.5:1.5 v/v), visualizing agent:p-anisaldehyde in glacial acetic acid followed by heating 105^0C.



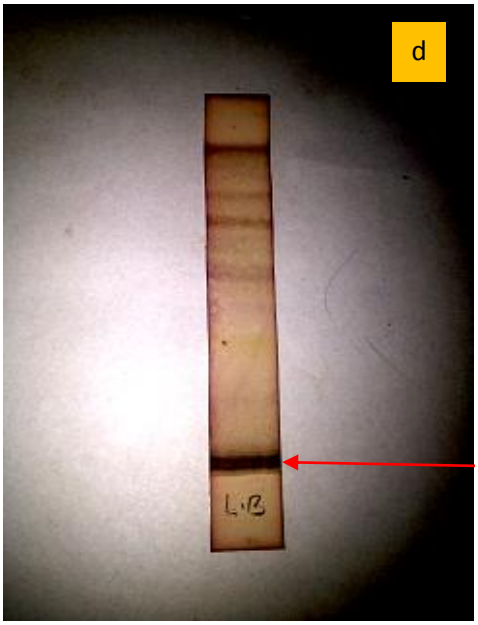
b- Tannins

Plate IV: TLC of CSME ethyl acetate: n-hexane (8.5:1.5 v/v), visualizing reagent: ferric chloride.



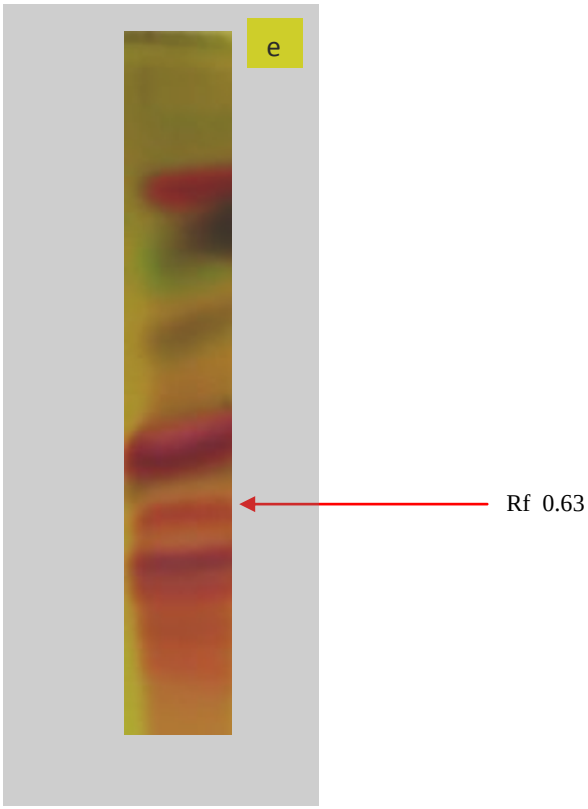
c- Pentose sugar

Plate V: TLC of CSME , ethyl acetate: n-hexane (8.5:1.5 v/v), visualizing agent: Bials reagent.



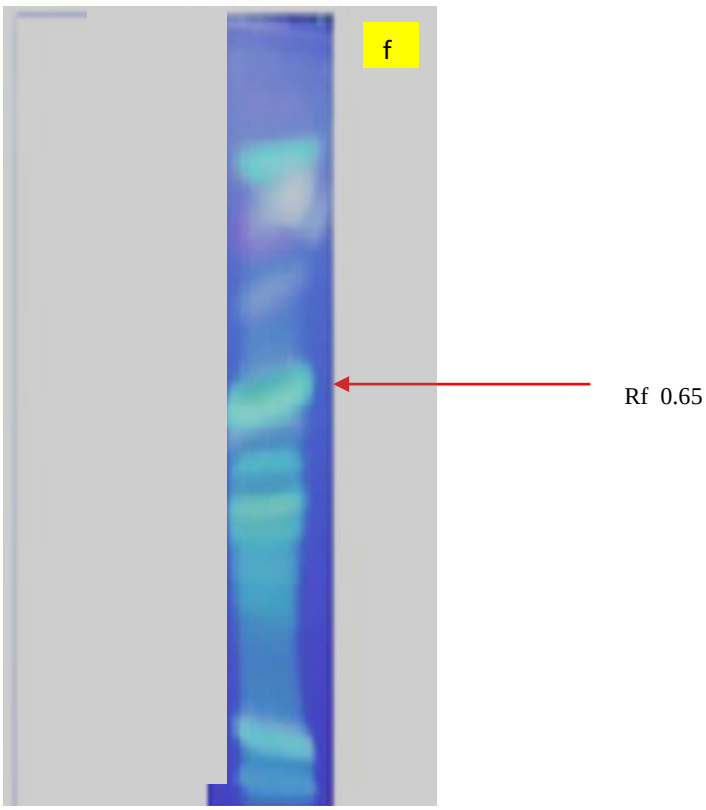
d- triterpenes

Plate VI: TLC of CSME , ethyl acetate: n-hexane (8.5:1.5 v/v), visualizing agent:- Liebermann –Bucchards.



e- Alkaloids

Plate VII: TLC of CSME , Ethyl acetate: n-hexane (8.5:1.5 v/v), visualizing agent: Dragendorff reagent.



f- Flavonoids

Plate VIII: TLC of CSME, Ethyl acetate: n-hexane (8.5:1.5 v/v), visualizing agent: I% ethanolic Aluminum chloride solutiojn under UV 360nm.

4.3 MEDIAN LETHAL DOSE (LD_{50}) VALUE IN MICE AND DAY-OLD CHICKS

The intraperitoneal median lethal dose (LD_{50}) values of the methanol, ethyl acetate and n-butanol extracts of *Cassia siamea* whole plant in mice and chicks were found to be >5000 while a value of 3,807 mg/kg and 1095 mg/kg were obtained as the intraperitoneal median lethal dose of the residual aqueous fraction in mice and day-old chicks respectively (Table 4.3).

Table 4.3: Intraperitoneal Median Lethal Dose (LD_{50}) Values of the Methanol extract of *Cassia siamea* and its fractions in mice and chicks

Fractions/Whole extract	LD_{50} Value (mg/kg) in Mice	LD_{50} Value (mg/kg) in Chicks
CSME	> 5000	> 5000
EAF	> 5000	> 5000
NBF	> 5000	> 5000
RAF	3807	1095

CSME= *Cassia siamea* methanol extract; EAF = ethyl acetate fraction; NBF = n-butanol fraction; RAF = residual aqueous fraction.

4.4 Anticonvulsant Studies

4.5 Effect of the methanol extract of *Cassia siamea* and its fractions on maximal electroshock-induced seizures in chicks

The Methanol extract of *Cassia siamea*, its n-butanol and residual aqueous fraction offered no protection to the chicks against hind limb tonic extension (HLTE) induced by maximal electroshock. The methanol whole plant extract of *Cassia siamea*, its n-butanol and residual aqueous fraction did not reduce the mean recovery time of the chicks after electroshock treatment compared to the control group. However, the ethyl acetate fraction (250 mg/kg) produced a significant decrease ($P< 0.05$) in the mean recovery time of the chicks (3.50 ± 0.40 mins) after electroshock treatment compared to the values obtained for the control group. Similarly, it also offered a 40% protection against hind limb tonic extension (HLTE) induced by maximal electroshock at the same dose

The standard anticonvulsant drug used, phenytoin (20 mg/kg) protected all the chicks (100%) from hind limb tonic extension induced by maximal electroshock (Table 4.5).

Table 4.5 Effect of Intraperitoneal administration of the methanol extract of *Cassia siamea* and its fractions on maximal electroshock-induced seizures in chicks

Treatment (mg/kg)	Quantal Protection	% Protection	Mean Recovery time (min)
Normal Saline	0/10	00.00	6.30 ± 0.54
CSME(1000)	0/10	00.00	5.2 ± 1.24
CSME (500)	0/10	00.00	7.30 ± 0.68
CSME (250)	2/10	20.00	9.38 ± 0.82
EAF (1000)	0/10	00.00	6.80 ± 1.42
EAF (500)	0/10	00.00	5.70 ± 1.00
EAF (250)	4/10	40.00	3.50 ± 0.40*
NBF (1000)	1/10	10.00	7.75 ± 1.03
NBF (500)	1/10	10.00	11.00 ± 2.08
NBF (250)	0/10	00.00	5.63 ± 0.75
RAF (800)	0/10	00.00	8.70 ± 1.56
RAF (400)	2/10	20.00	6.00 ± 1.49
RAF (200)	0/10	00.00	5.17 ± 0.91
Phenytoin (20)	10/10	100	-

*Data presented as Mean ± SEM, * = $P < 0.05$; n = 10; CSME = *Cassia siamea* Methanol Extract; EAF = Ethyl acetate Fraction; NBF= N-Butanol Fraction; RAF= Residual Aqueous Fraction.

4.6 Effect of the Methanol whole plant extract of *Cassia siamea* and its fractions on Pentylenetetrazole- induced seizures in mice

The methanol extract of *Cassia siamea*, its n-butanol and residual aqueous fractions at the doses tested offered no protection against seizures induced by pentylenetetrazole (90 mg/kg) 30 minutes after administration of the extract (and its fractions). The ethyl acetate fraction however at doses of 250 and 500 mg/kg produced a significant increase ($P < 0.05$) in the mean latency to onset of seizure compared to the control group. The ethyl acetate fraction at a dose 500 mg/kg protected the mice (83.3%) against mortality. The 250 mg/kg dose of the ethyl acetate fraction offered 33.3% protection against mortality. The methanol extract (1000 and 500 mg/kg), the n-butanol fraction (1000 mg/kg) and residual aqueous fraction (800 and 400 mg/kg) did not produce any significant increase in the mean onset of seizures. The methanol extract and the ethyl acetate fraction each at a dose of 1000 mg/kg offered mice 66.7% and 50% protection against mortality (Table 4.6).

Table 4.6: Effect of the methanol extract of *Cassia siamea* and its fractions on subcutaneous pentylenetetrazole- induced seizures in mice

Treatment (mg/kg)	Mean onset of Seizures (min)	Quantal protection against seizures	Quantal protection against mortality	% Protection against Mortality
Normal saline	4.5 ± 0.43	0/6	1/6	16.67
CSME (1000)	8.0 ± 0.82	0/6	3/6	50
CSME (500)	10.56 ± 0.23	2/6	2/6	33.33
EAF (1000)	6.20 ± 0.74	1/6	4/6	66.67
EAF (500)	11.25 ± 0.5*	3/6	5/6	83.33
EAF (250)	11.20 ± 0.9*	1/6	2/6	33.33
NBF (1000)	7.67 ± 0.62	0/6	3/6	50
NBF (500)	4.00 ± 0.52	0/6	2/6	33.33
NBF (250)	4.83 ± 1.01	0/6	1/6	16.67
RAF (800)	10.40 ± 1.69	2/6	3/6	50
RAF (400)	8.67 ± 1.69	2/6	3/6	50
RAF (200)	9.60 ± 0.12	1/6	1/6	16.67
V.A (200)	0.00 ± 0.00	6/6	0/6	100

CSME = *Cassia siamea* methanol extract; EAF = Ethyl Acetate Fraction; NBF = N-Butanol fraction RAF = Residual Aqueous Fraction; V.A = Valproic Acid; Values expressed as Mean ± SEM, n=6. *P < 0.05

4.7 Effect of the Ethyl acetate fraction of the methanol extract of *Cassia siamea* on picrotoxin-induced seizure in mice

The ethyl acetate fraction at all the doses tested (1000, 500 and 250 mg/kg) did not protect the animals against picrotoxin-induced seizure. There was no significant difference between the mean onsets of seizure in unprotected mice compared to the control group. Diazepam (2 mg/kg), the standard drug used, afforded a 100% protection of the mice against picrotoxin-induced seizure.

The ethyl acetate fraction at a dose of 500 mg/kg also afforded mice a 66.7% protection against mortality (Table 4.7).

Table 4.7 Effect of the Ethyl acetate Fraction of *Cassia siamea* on Picrotoxin-induced Seizures in Mice

Treatment (mg/kg)	Quantal protection against seizure	Mean onset of seizures (min)	Quantal protection against mortality	% Protection against Mortality
Normal saline	0/6	13.00 ± 0.97	0/6	0
EAF (250)	0/6	14.67 ± 0.60	2/6	33.33
EAF (500)	0/6	13.50 ± 1.43	4/6	66.67
EAF (1000)	0/6	14.83 ± 1.05	2/6	33.33
DZP (2)	6/6	0.0 0.00	6/6	100

EAF = Ethyl Acetate Fraction; DZP = Diazepam; Values expressed as Mean ± SEM, n=6.

4.8 Effect of Naloxone on anticonvulsant activity of the ethyl acetate portion of *Cassia siamea* in pentylenetetrazole (S.cPTZ) –induced convulsion in mice.

Administration of Naloxone (0.3 mg/kg) 5 minutes prior to the administration of the ethyl acetate fraction (250 mg/kg) failed to abolish the prolongation of seizure latency against subcutaneous pentylenetetrazole induced seizures offered by the ethyl acetate fraction. There was a moderate increase in the mean onset of seizures in mice previously treated with naloxone followed by the ethyl acetate fraction (250 mg/kg) compared to the control group. Similarly the protection against mortality offered by the ethyl acetate fraction alone was somewhat decreased by pretreatment with naloxone (Table 4.8)

Table 4.8 Effect of Naloxone on anticonvulsant activity of the Ethyl acetate portion of *Cassia siamea* in Pentylenetetrazole (S.cPTZ) –induced convulsion in mice

Treatment (mg/kg)	Mean onset of seizures (min)	% Protection against seizures	% Protection against mortality
Normal saline	3.33 ± 0.30	0.00	40
EAF (250)	10.5 ± 0.31	0.00	100
EAF(250) + Nal(0.3)	6.00 ± 0.70	0.00	80

EAF = Ethyl acetate fraction; Nal = Naloxone; Values are the Mean ± S.E.M. n=10 $^{*}P < 0.001$, compared to Normal saline group.

4.9 Effect of the Methanol extract and Ethyl acetate fraction of *Cassia siamea* on Diazepam induced Sleep

The ethyl acetate fraction at the doses tested significantly ($P < 0.05$, $P < 0.01$) increased the duration of diazepam induced sleep in mice. (Table 4.9)

Table 4.9: Effect of the Methanol extract and Ethylacetate Fraction of *Cassia siamea* whole plant extract on Diazepam-induced sleep

Treatment (mg/kg)	Mean onset of sleep (min)	Mean Duration of sleep (min)
Normal saline	3 ± 1.0	107.4 ± 0.9
EAF (1000)	3 ± 0.2	316.9 ± 0.66*
EAF (500)	3 ± 0.5	309 ± 0.6**
EAF (250)	5 ± 0.1	203.8 ± 0.15*
CSME (1000)	3 ± 0.3	171 ± 0.7*
CSME (500)	5 ± 0.1	150 ± 0.8*
CSME (250)	4 ± 0.1	143 ± 0.6*

EAF: Ethyl Acetate Fraction; CSME = *Cassia siamea* Methanol Extract. Values expressed as Mean ± SEM, n=6. *P < 0.05, **P <0.01

CHAPTER FIVE

5.0 DISCUSSION

It was earlier reported by Mohammed *et al*., (2013) that cassia siamea plant contains, alkaloids, glycosides, polyphenols, flavonoids, saponins, steroids, tannins, reducing sugars minerals, vitamins and enzymes. The preliminary phytochemical screening of the methanol extract of *Cassia siamea* and its ethyl acetate fraction revealed the presence of alkaloids, flavonoids, polyphenols, saponins, steroids, triterpenoids and tannins. Phenolic compounds were found to be absent in the ethyl acetate fraction.

Phytochemical screening provides basic information about the different classes of secondary metaboloites present in a plant and the medicinal importance of such plant extract (Shabbir *et al.,* 2013). Different species of the cassia genus are widely used in traditional medicine for treatment of a variety of illnesses some of which include; diabetes (Ahmed *et al*., 2012), as antimalarial therapy (Odugbemi *et. al*., 2007), as an anticancer remedy (Koyama *et. al*., 2002). The pharmacological potential of the cassia siamea plant in particular has been largely associated with the presence of different classes of secondary metabolites such as alkaloids, flavonoids, tannins and saponins (Edeoga *et. al*., 2005) Based on the results obtained from the phytochemical screening it is not possible to attribute with certainty the observed anticonvulsant effect of *Cassia siamea* to one or several active principles amongst those detected in the phytochemical screening. However, triterpenic steroids and saponins have been reported to possess anticonvulsant activity in some experimental seizure models such as MEST and ScPTZ (Kasture *et al*., 2002; Chauhan *et*

al., 1988). Some alkaloids and flavonoids have also been shown to exhibit protective effects against PTZ convulsions (Santos *et al*., 2005; Jhonston and Beart, 2004). Studies have revealed that flavonoids have neuroprotective effect against electrical kindling in rats (Tourandokht *et al*., 2010). The presence of these phytochemical constituents might be responsible for the observed pharmacological activities of the crude methanol whole plant extract and its ethyl acetate (EAF) fractions in the tests conducted. The notable anticonvulsant activity of the methanol extract and its ethyl acetate fraction as compared to the n-butanol and residual aqueous fraction imply that the anticonvulsant principles present in *Cassia siamea* plant are mostly non - polar.

Determination of median lethal dose value of plants used by traditional medicine practitioners using acute toxicity study is of paramount importance because it provides information regarding the margin of safety of the plant. The intraperitoneal median lethal dose (LD_{50}) values of the crude methanol whole plant extract, its n-butanol and ethyl acetate fractions were found to be greater than 5000 mg/kg body weight in both day-old chicks and swiss albino mice. This LD_{50} value implies that the crude methanol extract, its ethyl acetate and n-butanol fractions are relatively safe (Matsumura, 1995). The intraperitoneal median lethal dose (LD_{50}) of the residual aqueous fraction was found to be 3807 mg/kg body weight and 1095 mg/kg body weight in Swiss albino mice and day-old Ranger cockerels respectively, these values suggest that the residual aqueous fraction is moderately toxic intraperitoneally (Matsumura, 1995). The different LD_{50} values obtained for the residual aqueous fraction may be attributed to the fact that each of the fractions contain different phytochemical constituents which differ in biological activity as well as

toxicity on living systems. Doses of less than or equal to 30% of the LD_{50} which have been demonstrated to be relatively safe for ethnopharmacological research were used throughout the research procedure (Vongtau *et al*., 2004).

The causes of epilepsy are extremely diverse ranging from genetic, developmental defects, infections and traumatic occurrences to neoplastic and degenerative processes (Roger and Brain, 2004), hence it is highly unlikely that a single drug can be used as an effective treatment for the disorder

The inability of the methanol extract of *Cassia siamea*, its n-butanol (NBF) and Residual aqueous fractions (RAF) to protect day-old chicks against seizures induced by Maximal electro shock (MES) stimulus nor significantly affect the mean recovery time of the convulsed chicks post maximal electroshock stimulus nor did the fractions afford the animals any notable protection against seizures. The inability of these fractions (n-butanol and Residual aqueous fraction) to protect chicks from seizure induced by maximal electroshock may be as a result of fractionation with solvents of different polarity which may have separated the plant's bioactive principles capable of exerting anticonvulsant effect against electrically induced seizures.

The ability of the ethyl acetate fraction of *Cassia siamea* to significantly ($P < 0.05$) decrease the mean recovery time (3.50 ± 0.40 mins) of convulsed chicks compared to the

normal saline – control – group (6.30 ± 1.42 mins) post exposure to Maximal electroshock stimulus, showed that the ethyl acetate fraction probably antagonized the electrically induced seizures by inhibiting voltage gated Na^+ channels or by antagonism of glutamatergic excitation mediated by N-methyl-D- aspartate- receptor complex. Inhibition of Na^+ ion channels would invariably stabilize neuronal membranes protecting chicks against convulsion induced by maximal electroshock stimulus.

Maximal electroshock test for screening potentially active anticonvulsant agents (medicinal plants) is assumed to identify anticonvulsant agents that are protective against partial seizures and generalized seizures. The MES test model for anticonvulsant screening has a clearly defined (consistent) end point (inhibition of the tonic hind limb extension phase) and is highly reproducible (Ambawade *et al*., 2002). MEST is one of the best validated preclinical tests that predict drugs effective against generalized (Tonic – clonic seizures). Phenytoin and Carbamazepine have been shown to protect animals against hind limb tonic extension (HLTE) induced by maximal electroshock stimulus (Rho and Sankar, 1999). The behavioral and electrographic seizures generated in this model are consistent with the human disorder (Swinyard *et al.*, 1989). This model (MEST) essentially identifies those compounds which prevent seizure spread through neural tissue. The ability of the ethyl acetate fraction to inhibit seizures induced by electroshock stimulus and also shorten the recovery time of convulsed chicks infers that it is likely to exhibit activity against generalized tonic-clonic seizures (Ambawade *et al.* 2002). Research has show that agents which antagonize electrically induced seizures act by modulation of action potential ion current at the level of localized neurons or neural networks in the brain primarily by

inactivating voltage gated Na^+ channels or by antagonism of glutamatergic excitation mediated by N-methyl-D- aspartate- receptor complex. Inhibition of Na^+ion channels would invariably stabilize neuronal membranes thereby leading to a decrease in neuronal excitability.

Pentylenetetrazole (PTZ), a tetrazole derivative is the prototype agent in the class of "systemic" convulsants. (DeDyn *et. al.,* 1992). Pentylenetetrazole administration parenterally has consistent convulsant actions in mice, rats, cats and primates. PTZ initially produces myoclonic jerks which subsequently become generalized and may lead to a generalized tonic-clonic seizure. Pentylenetetrazole has been shown to diminish GABAergic tone (Macdonald and Barker, 1977). Gamma amino butyric acid is the major inhibitory neurotransmitter in the brain while glutamic acid is an excitatory neurotransmitter in the brain. The enhancement of GABA neurotransmission is reported to antagonize seizures while its inhibition promotes seizure (Rang *et al.*, 2005). Clonic seizures induced by PTZ are blocked by drugs that reduce T-type calcium currents and drugs that enhance the inhibitory neurotransmission by GABA receptors.

Compounds which are able to suppress PTZ-induced seizures are presumed to be effective in the treatment of absence seizures (McNamara, 2006). The PTZ model identifies agents that raise seizure threshold in the brain and the inhibition of PTZ-induced seizures has been shown to correlate quite well with clinical effectiveness against absence seizures (White *et al.,* 1995).

The ability of prior administration of the ethyl acetate fraction (250 mg/kg i.p and 500 mg/kg i.p.) to significantly ($P < 0.05$) attenuate pentylenetetrazole-induced seizure activity in mice measured in terms of onset time of seizures and Straub's tail phenomenon (a condition in which an animal carries its tail in a vertically erect or nearly vertical position), jerky body movements and convulsion implies that the ethyl acetate fraction of *Cassia siamea* has effects on GABAergic neurotransmission. A dopaminergic mechanism has also been implicated in PTZ-induced seizures. Dopamine has been shown to reduce seizure threshold in the brain and specific antagonists of dopamine have been shown to protect experimental animals against Sc.PTZ-induced seizures (Amabeoku, 1989). A study by Papp *et al*. (1987) found that pentylenetetrazole increases calcium influx and sodium influx, both of which depolarize the neuron. Because these effects were antagonized by calcium channel blockers, it was concluded that Pentylenetetrazole acts at calcium channels, and it causes calcium channels to lose selectivity and conduct sodium ions as well. (Papp *et al.* 1987; De Deyn *et al.*1992). PTZ was introduced as a screening test for anticonvulsants in part because the antiabsence drug Ethosuximide, which is effective against PTZ induced seizures, fails to alter maximal electroshock seizure (MES) thresholds.

The ability of the ethylacetate fraction to protect mice against PTZ- induced seizures suggests it may probably act by modulating GABA receptor mediated inhibitory neurotransmission and hence may be applicable as a potential antiabsence therapy for the management of petit mal (absence) epilepsy.

Picrotoxin is a non-competitive GABA antagonist which exerts its effect by binding to the picrotoxin binding site which is closely related to the chloride ionophore in the $GABA_A$ receptor complex. Picrotoxin antagonizes the $GABA_A$ receptor channel directly, which is a ligand-gated ion channel concerned chiefly with the passing of chloride ions across the cell membrane. Therefore picrotoxin prevents Cl- channel permeability and thus promotes an inhibitory influence on the target neuron. Picrotoxin reduces conductance through the channel by reducing not only the opening frequency but also the mean open time.

Drugs effective against picrotoxin – induced seizures have been shown to enhance GABA mediated neurotransmission. The protective effect of classical AED's against picrotoxin-induced seizures has been studied. Diazepam, Carbamazepine & Phenytoin have high protective efficacy against seizures induced by Picrotoxin, phenobarbitone has intermediate effectiveness, the antiabsence agents; Valproete & ethosuximide display low effectiveness. Inability of the ethyl acetate fraction at all the doses tested (1000, 500 and 250 mg/kg) to protect the animals against Picrotoxin-induced seizures suggests that the anticonvulsant action of the fraction may not involve interaction with the picrotoxin site(s) on the $GABA_A$ receptor complex.

The potential importance of endogenous opioids in modulating and regulating aspects of brain electrical activity has been described. (Adler., 1976). The results of the present study demonstrate that Naloxone (0.3mg/kg i.p) a specific opioid antagonist decreased the prolongation of seizure latency induced by the ethylacetate fraction of *Cassia siamea* whole

plant extract. However the anticonvulsant effect of the ethyl acetate fraction (250mg/kg i.p) against Pentylenetetrazole induced seizures was not completely reversed by naloxone (0.3mg/kg i.p). This finding suggests that the anticonvulsant effect of the ethyl acetate fraction (as assessed by its ability to delay the onset of Straub's tail phenomenon) in mice is only partly mediated by the release of endogenous opioids or activation of opioid receptors.

It has been documented that altered κappa opioid receptor (KOR) signaling has been observed in multiple seizure types (Beadles, 2006). It has also been reported that postsynaptic κ-opioid receptors bring about a reduction in the neuronal entry of $Ca^{2+,}$ which may contribute to inhibition of neuronal excitability due to $GABA_A$ receptor blockade caused by pentylenetetrazole (Meldrum, 1996). Presynaptic activation of KOR decreases N-, L- and P/Q-type Ca^{++} currents (Rusin *et al.*, 1997), resulting in reduction of glutamate release. It can thus be inferred that some part of the anticonvulsant effect due of the EAF related to activation of the opioid system was attenuated by naloxone.

Potentiation of the total sleeping time by the methanol extract and ethyl acetate fraction of *Cassia siamea* indicated the presence of sedative compounds. Sedation results from the activation of GABA receptors in the $GABA_A$ receptor complex thereby potentiating GABA mediated inhibitory action

At all the doses tested, the methanol extract and the ethyl acetate fraction produced a dose dependent increase in duration of Diazepam-induced sleep but did not significantly affect the onset time of diazepam induced sleep compared to the control group and the reference drug (Diazepam). By potentiating diazepam-induced sleep, the extract (and fraction) of *Cassia siamea* possess sleep-inducing properties (Rakotonirina *et al.*, 2001). The ability of

the extract to potentiate the sedative property of diazepam suggests that it may possibly act by interacting with GABA-mediated synaptic transmission. Sedation results from activation of GABA receptors in the $GABA_A$ receptor complex which results in an increase in GABA mediated synaptic inhibition either by directly activating $GABA_A$ receptors or by enhancing the action of GABA on $GABA_A$ receptors (Jhonston, 2005).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY

Cassia siamea is a commonly used medicinal plant in West Africa including Nigeria. The plant is well known in traditional medicine for its use in the treatment of hypertension, malaria and diabetes. Previous studies conducted on the crude extract of Cassia siamea show that the plant possesses antidiabetic, antimicrobial,antiprotozoal, anti tumour and laxative properties. In an attempt to characterize the active principles present in the plant, the crude extract was subjected to fractionation to obtain: ethyl acetate, n-butanol and residual aqueous fractions. Intraperitoneal median lethal dose values for the methanol, ethyl acetate and n-butanol fractions in mice and chicks was found to be greater than 5000mg/kg. While the the residual aqueous fraction had an LD50 value of 3807mg/kg and 1095mg/kg in mice and chicks respectively.

The crude extract and the ethyl acetate fraction showed varying anticonvulsant activity in Maximal electroshock and Pentylenetetrazole induced seizure models. The ethyl acetate fraction offered chicks a significant protection against maximal electroshock seizures and also shortened the mean recovery time of convulsed chicks, it also prolonged the mean onset time of seizures following the administration of Pentylenetetrazole. Naloxone was unable to antagonize the protective effect of the ethyl acetate fraction in the Pentylenetetrazole induced seizure model. In seizures induced by picrotoxin there was no significant activity by the ethyl acetate fraction although the EAF was able to afford mice a 66% protection against mortality.

The methanol extract and the Ethyl acetate fraction were subjected to preliminary phytochemical screening, alkaloids, flavonoids,Phenolic compounds,steroids, terpenoids and tannins were found to be present in both the extract (fraction).

6.2 CONCLUSION

From the results of the present study It may therefore be concluded that *Cassia siamea* plant possesses anticonvulsant and sedating actions in mice and chicks which may be responsible for use of the plant in the management of febrile convulsion and insomnia.. This activity of the plant was found to be particularly evident in its ethyl acetate fraction.

6.3 RECOMMENDATION

Based on the findings arrived at upon completion of this study, it is recommended that further studies should be conducted in order to isolate and purify and possibly elucidate the structure of the active compound(s) responsible for the observed anticonvulsant and sedating activity of the plant.

REFERENCES

Adler, M. W., Lin, C. H., Keinath, S. H, Braverman, S. and Geller, E. B. (1976). Anticonvulsant action of acute morphine administration in rats. *Journal of Pharmacology and Experimental therapeutics* 198: 655-660.

Ahmad - Alizaga, S.L. and Olayanju, S. (2007). Phytochemical screening of the leaf extracts of *Cassia siamea* Lam (Pop corn) and its antibacterial activity. *Biological and Environmental Sciences Journal for the Tropics, 4(2): 193 – 195*

Ahmed, S., Sabzwari, T, Hasam, M. M., and Azhar, I. (2012). Activity of leaves Extracts of five Leguminous plants. *International journal of research in Ayuverda and Pharmacy, 4:32.*

Akharaiyi, F. C and Boboye B. (2010). Antibacterial and phytochemical evaluation of three medicinal plants. *Journal of Natural Products*, 3 : 27–34.

Akinsulore, A and Adewuya. A. (2010). Psychosocial aspects of Epilepsy in Nigeria. *African Journal of Psychiatry*, 13 (5): 351- 6.

Al-Adhroey, A. H., Nor, Z. M., Al- Mekhlafi, H. M and Mahmud, R. (2010). Ethnobotanical study on some Malaysian antimalarial plants: A community based survey. *Journal of Ethno pharmacology*, 132: 362-364.

Aliyu, B.S. (2006). Common Ethno medicinal Plants of the Semi-Arid Regions of West Africa: Their Descriptions and Phytochemicals. Triumph Pub. Co. Ltd., Kano, Nigeria, pp: 93-94.

Amabeoku, G. J. (1989). Neuropharmacological profile of Quinine: An unpublished PhD (Pharmacology) Dissertation, Ahmadu Bello University, Zaria.

Ambawade, S. D., Kasture, V.S., and Kasyure, S. B. (2002). Anticonvulsant activity of roots and rhizomes of Glycyrrhiza glabra. *Indian journal of Pharmcology*, 34: 251-255.

André .V., Dubé .C., François .J., Leroy .C., Rigoulot .M. A., Roch .C., Namer .I. J. and Nehlig .A. (2007). Pathogenesis and pharmacology of epilepsy in the lithium-pilocarpine model. *Epilepsia*, 48 (Suppl 5):41–47.

Aroniadou, A. V., Fritsch, B., Qashu, F. and Braga, M. F. (2008). Pathology and pathophysiology of the amygdala in epileptogenesis and epilepsy. Epilepsy Research. 78: 102-116.

Armijio, J. A., Valdizan, E. M., De Las Cuevas, I. and Cuadrado, A. (2002) Advances in the Physiopathology of epileptogenesis: molecular aspects. *Neurology Reviews*, 34:409-429.

Asase, A., Akwetey, G. A and Achel, D. G. (2010). Ethnopharmacological use of herbal remedies for the treatment of malaria in the Dangme West District of Ghana. *Journal of Ethno pharmacology*, 129: 367-376.

Baker G. A. (2002). The psychosocial burden of epilepsy. *Epilepsia*, 43(Suppl. 6):26–30

Battino, D., Dukes, M. N. G and Perucca, E. (2000). Anticonvulsants. In: Dukes MNG, Aronson JK, eds. Meyler's side effects of drugs, 14th ed. Amsterdam: Elsevier Science BV, 2000:164–97.

Beadles–Bohling, A. S., Wiren, K. M. (2006). Alcohol and epileptogenesis. *Genes and Behaviour, 5 (6)*: 483 – 96.

Beretz, A., Haag-Berrurie, R. M and Anton, R. (1978). Choix de methods pharmacologiques pour l'etude des activities de l'aube pine. Plantes medicinales et Phytotherapie, 4: 305-314.

Bernasconi, R., Klein, M., Martin, P., Christen, P., Hafner, T., Portet, C., Schmutz, M. (1988).Gamma- Vinyl GABA: comparison of neurochemical and anticonvulsant effects in mice. *Journal of Neural Transmission.* **72**:213- 233

Berkovic S.F (1998). Genetics of epilepsy syndromes**.** In *Epilepsy: A comprehensive textbook*. *Volume 3*. Edited by Engel JJ and Pedley T A. Philadelphia, Lippincott-Raven**:** 217-224.

Bialer, M. and White, H. S. (2010). Key factors in the discovery and development of new antiepileptic drugs. National Review on Drug discovery, 9: 68-83.

Bienvenu, T., Poirier, K., Friocourt, G., Bahi, n., Beaumont, D., Fauchereau, F.and Gomot, M. (2002). ARX, a novel classed homeobox gene highly expressed in the telencephalon is mediated in X-linked mental retardation. Human Molecular Genetics, 11: 981-991.

Birbeck, G. L. (2000). Barriers to care for patients with neurological diseases in rural Zambia. *Archives of Neurology*, 57: 41414 - 41417.

Birbeck, G. L. (2012). Revising and refining the epilepsy classification system: Priorities from a developing world perspective. *Epilepsia* 53: 18-21.

Blume, W. T., Luders, H.O., Mizrahi, E., Tassinari, C., Emde Boas, W. and Engel, J. (2001). Descriptive terminology for ictal seminology: report of the ILAE task force on classification and terminology. Epilepsia. 42 (9): 1212-8.

Bromfield, E.B, Cavazos, J. E, Sirven, J. I. (2006). An introduction to Epilepsy; Basic Mechanisms underlying Seizures and Epilepsy. West Hartford (CT): American Epilepsy Society.

Available from:

http : // www.ncbi.nlm.nih.gov/books/NBK2508/

Bukar, A., Mukhtar, M. D and Hassan, A. S. (2009). Phytochemical Screening and Antibacterial Activity of Leaf Extracts of *Cassia siamea* (lam) on pseudomonas aeruginosa.- *International Journal of Pure and Applied Sciences Volume:* 2(1): 139-142.

Chapman, A. G. (19980. Glutamate receptors in epilepsy. *Progress in Brain Research.* 116: 371-383.

Chatsri, D., Sutthasinee, P., Watchareewan, T and Nateetip, K. (2005). Barakol extracted from *Cassia siamea* stimulates chloride secretion in Rat colon. *Journal of Pharmacology and Experimental Therapeutics.* 314: 732-737.

Chauhan, A. K., Dobhal, m. P., Joshi, B. (1988). Areview of medicinal plants showing anticonvulsant activity. *Journal of Ethno pharmacology*, 22:11-23.

Cherian, A and Sanjeev, V. T. (2009). Status epilepticus. *Annals of Indian Academy of Neurology*. 12 (3): 140-153.

Cheuk, D. K. L and Wong .V. (2009). Acupuncture for epilepsy (Review). Cochrane Database System Review, (4):CD005062.

Chindo, B. A. (1999). Neuropharmacological Properties of the Methanol Extract of *Ficus platyphylla* stem bark. An Unpublished Master in Pharmacology Research Thesis. Ahmadu Bello University, Zaria.

Czapinski, P., Blaszczyk, B and Czuczwar, S. J. (2005). Mechanisms of action of antiepileptic drugs, 5: 3-14.

Danesi M.A., Adetunji J.B. (1994). Use of alternative medicine by patients with epilepsy: a survey of epileptic patients in a developing country. *Epilepsia*, 35(2): 344-51.

Darwin, B. (1989). Epilepsy. In: Internal Medicine Board Review. 4th Ed. Department of Medicine, Emroy University, U. S. A. Pp 451.

De Dyn, P. P., D'Hooge, R., Marescau, B. and Yin-Quan, P. (1992). Chemical models of epilepsy with some reference to their applicability in the development of anticonvulsants. *Epilepsy Research,* 12: 87-110.

Deng, F., Chang, J., Zhang, J. S. (20070. New flavonoids and other constituents from Les pedeza cuneata. *Asian Journal of Natural Products research,* 9: 655-658.

DeSarro, G., Palma, E., Costa, N., Murra, R., Gratteri, S., Desarro, A and Rotiruli, D. (2000). Effects of the compounds acting on $GABA_B$ receptors in the Pentylenetetrazole kindling model of epilepsy in Mice. *Neuropharmacology*, 39: 2147-2161

Des Santos, J. G. Jr., Longo, B. M., Blanco, M. M., Menzes de Oliveira, M. G., and Mello, L. E. (2005). Behavioural changes resulting from the administration of cycloheximide in the plocarpine model of epilepsy. *Brain Research*, 1066: 37-48.

Devinsky, O. (1995). Cognitive and behavioural effects of antiepileptic drugs. *Epilepsia* 36: (Suppl.2),S46-S65.

Dichter, M. A. (2009) Emerging concepts in the pathogenesis of epilepsy and epileptogenesis. *Neurolology Archives*, 66:443–447.

Dreifuss, F. E., (1989). Classification of Epileptic Seizures and Epilepsy. *Paediatric Clinics of North America*, 36 : 265.

During, M. J. and Spencer, D. D., (1993). Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain. *Lancet* Neurology. 341:1607-10.

Dyhrfield-johnsen, J. Berdichersky, Y., Swierez, W., Sabolek, H. and Stanley, K. J. (2010). Interictal spikes precede ictal discharges in an organotypic hippocampal slice culture model of Epileptogenesis. *Journal of Clinical Neurophysiology*. 27: 418-424.

Easterford, K., Clough, P., Comish, S., Lawton, L. and Duncan, S. (2005). The use of Complementary Medicine and Alternative Practitioners in a cohort of Patients with Epilepsy. *Epilepsy Behaviour*. 6: 59-62.

Edeaoga, H. O., Okwu, D. E, Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journl of Biotechnology*, 4 (7): 685-688.

Eisenberg, L. (1997). Global burden of disease. *Lancet Neurology*, 350: 143.

Engel .J. Jr and Pedley .T.A., (2005). What is epilepsy? In: Epilepsy: A comprehensive textbook. Philadelphia: Lippincott-Raven, 1–11.

Engelborghs , S. , D'Hooge , R. and De Deyn, P.P ., (2000): Pathophysiology of epilepsy. *Belgium Neurological Society,*100: 201-13.

Fisher, R. S., Walter, E. B., Warren, B. Christian. E., Pierre, G., Phillip, L and Jerome, E. (2005). Epileptic seizures and epilepsy. Definitions proposed by the international league against epilepsy (ILAE) and the International Bureau for Epilepsy, 2005 (IBE). *Epilepsia,* 2005, 46:470-472.

Fischer, R. S. (1989). Animal models of the epilepsies. *Brain Research*, 14: 245-278.

Fransworth, N. R. (1998). Screening plants for New Medicines. In: Wilson, E. O. (Ed) Biodiversity, Washington, D. C., Natural Academy Press. Pp 83-97.

Harborne, J. B. (1998). A guide to modern technique of plant analysis. In: Chapman and Hall Ed. London.

Hill, A. R. (1992). Medicinal plants and tradition medicine in Africa. *Journal of International Medicine,* 39:42-45.

Geoffrey, C and Kirby, M. (1996). Medicinal plants and the control of protozoan disease with particular reference to malaria and hygiene, ISSN 0035-9203. London, Great Britain.

Golberg, E. M and Douglas A.C. (2013). Mechanisms of Epileptogenesis; a convergence on neural circuit dysfunction. *Nature Reviews Neuroscience,* 14: 337- 349.

Graeme, S. J. (20050. Pharmacogenetics of epilepsy: one step forward. *Epilepsy Currents*, 5: 236 – 238.

Hanneke, M. B.,Mareo. M. and Josemir. W. S (2008). Epilepsy and Behaviour. International league against epilepsy.(ILAE)

Hema, B., Bhupendra, S., Mohammed Saleem, T. S. and Gauthaman, K. (20090. Anticonvulsant effect of *Drosera burmanni* Vahl. *International Journal of Applied Research in Natural Products*, 2: 1-4.

Herman, S. T (2002). Epilepsy after brain insult: targeting epileptogenesis. *Neurology, 59:S21–S26.*

Hill, H.R., (1992). Medicinal plants and Traditional Medicine. *Journal of integrated Medicinal Research,* 39: 42-45.

Howes, M. J. R., Perry, N.S.L. and Houghton, P. J, (2003). Plants with Traditional Uses and Activities, Relevant to the Management of Alzheimer's Disease and Other Cognitive Disorders. *Phytotherpeutic. Research*, 17: 1-18.

Hussaini, I. M. (2006). Epilepsy, (unpublished) Lecture note: Advanced Central Nervous System. PHCL 807. Department of Pharmacology and Therapeutics. Ahmadu Bello University Zaria.

Irwin, H.S and Barneby, R. (1982). The American Cassinae. A synoptical revision of Leguminosae tribe Cassieae subtribe Cassinae in the New World. Memoirs of the NewYork Botanical Garden.

Jacobs, M. P., Leblanc, G.G., Brooks-Kayal, A., Jensen, F.E., Lowenstein, D. H., Noebels, J. L., Spencer, D. D and Swann, J. W. (2009). Curing epilepsy: progress and future directions. *Epilepsy Behaviour 14:*438–445*.*

Jamison, D. T., Mosely, H. W., Measham, A. R. and Bobadilla, J. L. (1993). Disease Control Priorities in Developing Countries. New York : Oxford University Press.

Jangiti, R. K., Battu, R. G., Majji, L. N and Talluri, R. M. (2013). Evaluation of Antidiabetic activity of *Cassia siamea* leaves in alloxan induced diabetic rats. *International journal of Phytopharmacology*, 4 (4): 237-240.

Jefferys, J.G. (1995) Nonsynaptic modulation of neuronal activity in the brain: electric currents and the extracellular ions. *Physiology Reviews,* 75 (4): 689-723.

Johnston, G. A. R. and Beart, P. M. (2004). Flavonoids: some of the wisdom of sage? *British Journal of Pharmacology,* 142: 809-810. doi: 10. 1038/sj. bjp.0705827.

Kaila, K., Ruusuvuori, E., Seja, P., Vaipio, J. and Puskarjov, M. (2014). GABA actions and ionic plasticity in epilepsy. *Current Opinion in Neurobiology*, 26: 34-41.

Kamboj, P., Singh, I., Mahadevan, N. and Chaudhary, G. (2009). Anticonvulsants from nature. *Pharmacognosy Reviews* ; 3(5):108–17.

Kasture, V. S., Kasture, S. B., Joshua, A. J., Damodaran, A. and Amit, A. (2002). Ootropic activity of BacoMind™, an enriched phytochemical composition from *Bacopa monnieri*. *Journal of Natural Remedies,* 7: 166-173.

Kayode, J., Omotoyinbo, M. A. (2009). Ethnobotanical Utilisation and Conservation of Chewing Sticks. Plants Species in Ekiti State, Nigeria. *Research Journal of Botany* 4(1): 1-9.

Kiepe, P. (1995). Effect of *Cassia siamea* hedgerows barriers on soil physical properties. *Geoderma,* 66:113-120.

Kiepe, P. L. (2001). Effect of *Cassia siamea* barriers on soil physical properties. *Geoderma journal of Intergrated Medicine***,** 68:113-720.

Kong, J, Xu, Z. (1998). Massive mitochondrial degeneration in motor neurons triggers the onset of amyotrophic lateral sclerosis in mice expressing a mutant SOC1. *Journal of neurological sciences,* 18: 3241-3250.

Koyama, R., Yamada, M. K., Fujisawa, S., Katoh-Semba, R., Matsuki, N. and Ikegaya, Y. (2002). Brain-derived neutrotrophic factor induces hyperexcitable reentrant circuits in the dentate gyrus. *Journal of Neuroscience, 24; 7215-7224.*

Lauretti, G. R, Ahmad, I., Pleuvry, B. J. (1994). The activity of opioid analgesics in seizure models utilizing N-methyl-DL-aspartate, Kainic acid, bicuculline and Pentylenetetrazole. *Neuropharmacology* 33: 155-160.

Lee, P., Paik, S. M., Shin, C. S., Huh, W. K. and Hahn, J. S. (2011). Regulation of yeast Yak1 kinase by PKA and autophosphorylation-dependent 14-3-3 binding. *Molecular Microbiology* 79(3): 633-46.

Lehmann, J., Hutchison, A., McPherson, S.E., Mondadori, C., Schmutz, M., Sinton, C.M., Tsai, C., Murphy, D.E., Steel, D.J., Williams, M., Cheney, D.L and Wood, P.L. (1988). CGS 19755, a selective and competitive N-methyl-D-aspartate-type excitatory amino acid receptor antagonist. *British Journal of Pharmacology*. 246:65-75.

Leonardi, M. and Olesen, J. (2003). The burden of diseases in Europe. *European Journal of Neurology,* 10: 471-477.

Leonardi, M. and Ustun, T. B. (2002). The global burden of epilepsy. *Epilepsia.,* 43:21-5.

Lodder, S. S., Ashkamp, J., van Putten, M. J. A. M. (2014). Computer-Assisted interpretation of the EEG Background Pattern: A Clinical Evaluation. PLos ONE 9(1): e85966.

Longe, A. C. and Osuntokun B. O. (1989).,Prevalence of neurological disorders in Udo, a rural community in Southern Nigeria. *Journal of Tropical and Geographical Medicine.*, 1989; 41(1): 36-40.

Lorke, D. A. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology* 54 : 275-287.

Loscher, W. and Siemes, H. (1985). Cerebrospinal fluid gamma-aminobutyric acid levels in children with different types of epilepsy: effect of anticonvulsant treatment. *Epilepsia,* 26:314-9.

Loscher, W., (1998). New visions in the pharmacology of anticonvulsants. *European Journal of Pharmacology,* 342: 1-13.

Löscher, W. (2002) Current status and future directions in the pharmacotherapy of epilepsy. Trends in Pharmacological Sciences, 23:113–118.

Loscher .W. and Schmidt .D. (2006). New horizons in the development of antiepileptic drugs: Innovative strategies. *Epilepsy Research Journal,* 69:183-272.

Lose, G. A. , Benardano, S. J. and Leihner, D. E. (2000). Studies on agroforestry hedgegrow system with *Senna siamea* rooting patterns and competition effects. *Journal of Science,* 38: 57-60.

Lowestein, G., Rick, S. and Cohen, J. D. (2001). Neuroeconomics. *Annual Reviews of Psychology,* 58:647-72.

Macdonald, R. L., Barker, J. L. (1977). Pentylenetetrazole and penicillin are selective antagonists of GABA-mediated post-synaptic inhibition in cultured mammalian neurons. *Nature 267:* 720-722.

Mac, T. L., Tran, D. S., Quet, F., Odermatt, P. and Preux, P. M. (2007). Epidemiology, aetiology and clinical management of epilepsy in Asia: a systematic review. *Lancet Neurology*, 6; 533-543.

Mann, A., Gbate, M. and Nda Umar, A. (2003). Medicinal and Economic plants Nupeland. Evans Books and publications, Bida, Nigeria. Pp. 1-276.

Maregesi, S. M., Ngassapa, O. D., Pieters, L. and Vlietinck, A. J. (2007). Ethnopharmacological survey of the Bunda district, Tanzania: Plants used to treat infectious diseases. *Journal of Ethno pharmacology,*113: 457 – 470.

Marjan, N. A., Shariati-Rad, S., and Zamansoltani, F. (2007). Anticonvulsant effect of aerial parts of *Passiflora incarnate* extract in mice: involvement of benzodiazepine and opioid receptors. *Complementary and Alternative Medicine*, 7-26.

Marom, B, Scott, C. B. and Dominique, M. D. (2001). Conditions sufficient for nonsynaptic epileptogenesis in the CA1 region of the hippocampal slices. *American journal of Neurophysiology.* 87(1): 62-71.

Mattson. R.H. (1995). Efficacy and adverse effects of established and new antiepileptic drugs. *Epilepsia* 1995 **36** (4), S41-S50.

McNamara, J. O. (2006). Pharmacology of Epilepsies. *In*; Brunton, L. L., Lazo, J. S. and Parker, K. L. (Eds) *Goodman and Gilman's, the pharmacological Basis of Therapeutics,* 11th Edition. Mc Graw-Hill Co. Inc. NewYork, USA. Pp.501-525.

Matsumura, F. (1985). Toxicology of insecticides. 2nd Ed., Plenum Press, New York. Pp. 588.

Meldrum, B. S. (1978). Gamma-aminobutyric acid and the search for new anticonvulsant drugs. *Lancet Neurology,* 2: 304-306.

Mohammed, Z. K., Emine, K., Tanvir, U. S., Jonathan, M. and Dominique, M. D. (2013). Low frequency electrical stimulation of a fiber tract in temporal lobe epilepsy. *Annals of Neurology*. 74(2):223-231.

Mosewich, R. K. and So, E. L. (1996). A clinical approach to the classification of seizures and epileptic syndromes. *Mayo Clinic Proceedings.* 71(4): 405-14.

Muralidharan, P., Selvarajan, S., and Balamurugan G. (2009). Anti Epileptic Activity of Poly Herbal Extract from Indian Medicinal Plants. *Journal of Cell science Research*, 1(1): 153-159.

Murray, M. T. (1998). The Healing Power Of Herbs: The Enlightened Person's Guide to the Wonders of Medicinal Plants. 2nd ed. Rocklin, California: Prima Publishing. 40: 91.

Newman, D. J. and Cragg, G. M. (2007). Natural products as sources of new drugs over the last 25 years.*Journal of Natural Products.* 70(3): 461-77.

Ngo Bum, E., Dawack, D.L., Schmutz, M., Rakotonirina, A., Rakotonirina, S.V., Portet, C., Jeker, A., Olpe, H.R. and Herrling, P. (2004). Anticonvulsant activity of *Mimosa pudica* decoction. *Fitoterapia.* 75:310-315.

Ngo Bum, E., Naami, Y.F.C., Soudi, S., Rakotonirina, S.V. and Rakotonirina, A. (2005). *Psorospermum febrifugum* spach (Hypericaceae) decoction antagonized chemically-induced convulsions in mice. *International Journal of Pharmacol*ogy **1**:118-121.

Ngo Bum, E., Nkantchoua, G.N., Njifutie Njikam, Taiwe, G.S., Ngoupaye, G.T., Pelanken, M.M., Nanga, L., Maidawa, F., Rakotonirina, A. and Rakotonirina, S.V. (2010). Anticonvulsant and Sedative activity of leaves of *Senna spectabilis* in mice. *International Journal of Pharmacol*ogy, 6:123-128.

Nsour W.N., Lau C.B.S. and Wong I.C.K. (2000). Review on phytotherapy in epilepsy. *Seizure* 9: 96-107.

Obembe, A and Ahmed, M. H (1988) Epilepsy in Kaduna: A study of 164 Youths. *Nigerian Medical Practice*: 16 (2): 45-8.

Odugbemi, T. O., Akinsulire O. R., Aibinu, I. E. and Fabeku, P. O. (2007). Medicinal plants useful for malaria therapy in Okeigbo Ondo state, Southwest Nigeria. *African Journal of Traditional, Complementary and Alternative Medicines*. 4 (2): 191 – 198.

Ogunniyi, A., Oluwole, O.S. Osuntokun B.O, (1998). Two year remission in Nigerian epileptics. *East African Medical Journal*. 75 (7): pp 392-5.

Ogunniyi, A., Oluwole, O.S. Osuntokun B.O, (1998). Two year remission in Nigerian epileptics. *East African Medical Journal*. 75 (7): 392-5.

Ogunniyi, A.O, Adeyinka, A., Fagbemi, S.O., Orere, R., Falpoe, Z. F and Oyawole, O. S. A. (1994). Computerized tomographic findings in adolescent and adult Nigerian epileptics. *West African Journal of Med*icine. 13(2): 128-31.

Ogunniyi, A., Osuntokun, B.O., Adeuja, A.O., Bademosi, O., Olumide, A., Ige, O., Yaria, F., Bolis, C. L. and Schoenberg, B. S. (1987). Risk factors for epilepsy: Case-controlled study in Nigerians. *Epilepsia*. 28(30): 280-5.

Ömer, K. H., İlker, I., Ersin, E., Zeki, G., Zeki, O., (2011). Evaluating the feasibility of measures of motor threshold and cortical silent period as predictors of outcome after temporal lobe epilepsy surgery. *Seizure*. 20 : 775-778.

Onwuekwe, I. O., Onodugo, O. D., Ezeala-Adikaibe, B., Aguwa, e. N., Ejim, E. C. and Onyejizu, c. (2009). Patterns and presentation of epilepsy in Nigerian Africans: a study of trends in the southeast. *Journal of tropical Medicine,*103(8): 785-9

Osuntokun, B.O. (1972). Epilepsy in the developing countries: The Nigerian profile. *Epilepsia,* 13: 107-11.

Osuntokun, B.O., Adeuja, A.O. and Nottidge, V. A. (1987). Prevalence of epilepsy in Nigerian Africans: a community-based study. *Epilepsia*. 28 (3): 272 - 9.

Osuntokun, B.O., Bademosi O., Familusi J. B. and Oke, F. (1974). Electroencephalographic correlates of epilepsy in Nigerian children. Developmental Medicine and Child Neurology. 16(5): 659-63.

Otimenyin, S. O., Kolawole, J. A., Nwosu, M. (2010). Pharmacological basis for the continual use of the root of *Cassia siamea* in traditional medicine. - *International Journal of Pharmaceutical and Biological Sciences* (1) 3:S67.

Papp, A., Feher, O. and Erdelyi, L. (1987). "The ionic mechanism of the pentylenetetrazole convulsions". *Acta Biologica Hungarica.* **38** (3-4): 349-61.

Pahl, K., Boer, H. M. (2005). Epilepsy and rights. In: *Atlas: Epilepsy care in the world*. Geneva, World Health Organization, 72–73.

Patsalos, P. N and Perucca, E. (2003). Clinically important drug interactions in epilepsy: general features and interactions between antiepileptic drugs. *Lancet Neurology*, 2:347–56.

Payne, J. A., Rivera, C., Voipio, J. and Kaila, K. (2003). Cation- chloride co-transporters in neuronal communication, development and trauma. *Trends in Neuroscience.* 26:199-206.

Pitkänen, A. and Lukasiuk, K. (2009). Molecular and cellular basis of epileptogenesis in symptomatic epilepsy. *Epilepsy Behaviour*, 14 *(1):16–25*.

Porter, R. J and Meldrum, B. S. (1995). Antiepileptic drug. In: Katzung BG. Basic and Clinical Pharmacology. 6th Ed. London: Prentice Hall Inc. Ltd., p. 361-3.

Preux, P.M and Druet, C. M. (2005) Epidemiology and aetiology of epilepsy in sub-Saharan Africa. *Lancet Neurology*, 4:21–31.

Ramirez, B. E. D, Ruiz, N. N., Arellano, J. D.Q., Madrigal, B. R., Michel, M.T.V and Garzon, P. (1998) Anticonvulsant effects of *Magnolia grandiflora* l. in the rat. *Journal of Ethnopharmacol*ogy 61: 143-152.

Rang, H. P, Dale, M.M., Ritter, J. M and Moore, P. K. (2005) Rang and Dales Pharmacology, 5th ed. Churchill Livingstone, India. pp 456 – 473.

Rakotonirina, S. V., Ngo Bum, E., Rakotonirina, A. and Bopelet, M. (2001). Sedative properties of the decoction of rhizomes of *Cyperus Articulatus. Fitoterapia* 72**:**22-29.

Rates, S. M. K., (2001): Plants as source of drugs. *Toxicon* 39: 603-613.

Raza, M., Ahmad, M., Gado, A., Al-shabanah, O. (2003). A comparison of hepatoprotective activities of amino guanidine and N-acetylcysteine in rat against the toxic damageinduced by azathioprine. *Comparative Biochemistry and Physiology*, 134: 451-456.

Reynolds, E. H., ed. Epilepsy in the world. Launch of the second Phase of the ILAE/IBE/WHO Global Campaign against Epilepsy. *Epilepsia*, 43(6): 46.

Rho, J. M and Sankar, R. (1999). The Pharmacologic basis of antiepileptic drug action. *Epilepsia*, 40: 1471-1483.

Roger, J. P. and Brain, S. M. (2004). Antiseizure drugs. In: Bertram, G. K. (eds). *Basic and Clinical Pharmacology*. 9th ed. Lange medical books/McGraw Hill. New York. Pp379.

Rusin, K.I., Giovannucci, D.R., Stuenkel, E. L and Moises, H.C. (1997). Kappa-opioid receptor activation modulates Ca^{2+} currents and secretion in isolated neuroendocrine nerve terminals. *Journal of Neuroscience*, 17:6565-74.

Salih , M. A. M. and Mustafa, A. A. (2008). A substance in broad beans (*Vicia faba)* is protective against experimentally induced convulsions in mice. *Epilepsy Behaviour*, 12: 25-29.

Samren, E.B., Duijn, C.M, Koch, S, Christiaens, G. C, Hoffman, A., Lind-hout, D. (1997). Maternal use of antiepileptic drugs and risk of major congenital malformations: a joint European prospective study of human teratogenesis associated with maternal epilepsy. *Epilepsia* **38**: 981-990.

Sakulpanich, A and Gritsanapan, W. (2009). Laxative Anthraquinone Contents in Fresh and Cooked *Cassia siamea* leaves - *Southeast Asian Journal of Tropical Medicine and Public Health :* 40 (4) pp: 835-839 published: July 2009.

Schmidt, D. (2002). The clinical impact of new antiepileptic drugs after a decade of use in epilepsy. *Journal of Epilepsy research* **50**: 21-32.

Scott, A. R., Lhatoo, S. D and Sander, J.W.A.S. (2001). The treatment of epilepsy in developing countries: where do we go from here? *Bulletin of the World Health Organization*. 79(4): 344-51.

Schmutz, M., Portet, C., Jeker, A., Klebs, K., Vassout, A., Allgeier, H and Van Riezen, H. (1990). The competitive NMDA receptor antagonists CGP 37849 and CGP 39551 are potent, orally-active anticonvulsants in rodents. *Naunyn-Schmiedeberg's Archives of Pharmacology.* **342**:61-66.

Shabbir, M., Muhammad, R. K and Naima, S. (2013). Assessment of Phytochemicals, antioxidant, anti-lipid peroxidation and antihemolytic activity of extract and various fractions of *Mytenus royleanus. BMC Complementary and Alternative Medicine*, 13: 143.

Shamansky, S. L. and Glaser, G. H. (1992). Socioeconomic characteristics of seizure disorders. *Epilepsia* (33) 464-8.

Sheth, R. D., and Hermann, B. P. (2007) Bone in idiopathic and symptomatic epilepsy. *Epilepsy Research*, 78: 71-76.

Shinner, S., and Berg, A. T. (1996). Does antiepileptic drug therapy prevent the development of chronic epilepsy? *Epilepsia.* 37: 701-8.

Sierra, P. G. (2008). Recent advances in the neurochemistry of epilepsy. *European Neurological Review*. 3(1) ; 96-8.

Sierra, P. G. , Sierra, M. G., (2007). Extrasynaptic GABA and glutamate receptors in epilepsy.CNS Neurological Disorder Drug Targets; 6(4): 288-300.

Smith, M.C., Bleck, T.P. (1991). Convulsive disorders: toxicity of anticonvulsants. *Clinical Neuropharmacology* **14**: 97-115.

Smith, M., Wilcox, K. S and White, H. S. (2007). Dscovery of antiepileptic drugs. *Neurotherapeutics* 4: 12-17.

Soulimani, R., Younos, C., Jarmouni-Idrissi, S., Bousia, D., Khalouki, F. and Laila, A. (2001). Behavioural and pharmacotoxicological study of Papaver rhoeas L. in mice. *Journal of Ethno pharmacology*, 74: 265-274.

Sridharan, R. (2002). Epidemiology of epilepsy.*Current science*, 82(6): 664-670.

Stables, J. P., Bertram, E. H., White, H. S., Coulter, D. A., Dichter, M. A., Jacobs, M. P., and Noebels, J. L. (2002) Models for epilepsy and epileptogenesis: report from the NIH workshop, Bethesda, Maryland. *Epilepsia* 43:1410–1420.

Stables, J. P., and Kufferberg, H. J. (1987). The National Institute of Health Anticonvulsant Drug Development (ADD) Programme: Preclinical Anticonvulsant Screening Project, in Molecular and Cellular Targets for Antiepileptic Drugs (Eds. Avanzini, G., Regesta, G., Tanganelli, P., and Avoli, M. Pp191-198.

Stanley, J. L., Lincolin, R. J., Brawn, T. A., Macdonald L. M, Dawson, G. R and Reynolds (2005). The mouse beam walking assay offers improved sensitivity over the mouse rota- rod in determining motor co-ordination deficits induced by benzodiazepines. *Journal of Psychopharmacology*, 19 (3):221-227.

Swinyard, E. A. (1969). Laboratory evaluation of antiepileptic drugs: Review of laboratory methods. *Epilepsia,* 10: 107-119.

Swinyard , E. A. and Kufferberg, H. J. (1985) Antiepileptic drugs: detection, quantification and evaluation. *Federation Procedings,* 44: 39-43.

Swinyard, E. A., Woodhead, J. H., White, H. S and Franklin, M.R. (1989): General principles: experimental selection, quantification, and evaluation of anticonvulsants. In: *Antiepileptic Drugs,* Third Edition R.H. Levy, R.H. Mattson, B. Melrum, J.K. Penry and F.E. Dreifuss eds, pp. 85-102. New York: Raven Press.

Tabuti, J.R.S., Kukunda, C. B and P. J. Waako: (2010) Medicinal plants used by traditional medicine practitioners in the treatment of tuberculosis and related ailments in Uganda. *Journal of Ethnopharmacology*, Volume127, pp. 130–136.

Todorova, M. T., Burwell, T. J and Seyfried, T. N. (1999). Environmental risk factors for multifactorial epilepsy in EL mice. *Epilepsia*, **40:**1697-1707. Retrieved from PubMed Abstract.

Tourandokht, B. M and Roghani, M. (2010). Inhibitory Effect of High Dose of the Flavonoid Quercetin on Amygdala Electrical Kindling in Rats. *Basic and Clinical Neuroscience*, 1(3) 57-61.

Van Wyk, B.E., Van Oudtshoorn, B and Gericke, N. (1997). Medicinal plants of South Africa, 1st ed., Pretoria, South Africa, pp. 95-96.

Vongtau, H. O., Abbah, J., Mosugu, O., Chindo, B. A., Ngazal, I. E., Salawu, O. A., Kwanashie, H. O. and Gamaniel, K. S. (2004). Antinociceptive pfofile of the methanolic extract of *Neorautanania mitis* root in rats and mice. *Journal of Ethno pharmacology,* 92: 317-324.

Walker, M. C., White, H. S and Sander, J. W. (2002) Disease modification in partial epilepsy. *Brain 125:1937–1950.*

Wamil, A.W., Schmutz, M., Portet, C., Feldmann, K.F and McLean, M. J. (1994). Effects of Oxcarbazepine and 10-hydroxycarbamazepine on action potential firing and generalized seizures. *European Journal of Pharmacology.* **271**:301-308.

Wangchuck, P. , Wangchuck, D. and Aagaard-Hansen, J. (2007). Traditional Bhutanese medicine (gSo-ba rig-pa): An Integrated Part of the Formal Health Care Services. *South East Asian Journal of Tropical Medicine*.38(1): 161-67.

Wangchuck, P. (2008). Health impacts of Traditional Medicines and Bioprospecting: A World Scenario accentuating Bhutan's Perspective. *Journal of Bhutan Studies*, 18: 117-134.

World Health Organization (2003). Traditional Medicine. Factsheet. No. 134.

World Health Organization (2004). Epilepsy in the WHO African region: bridging the gap. the global campaign against epilepsy out of the shadows" World Health Organization. 2004.

World Health Organization. Fact sheet no. 134: Traditional medicine. 2008-12-01.

White, H. S., Woodhead, J. H., Wilcox, K. S., Stables, J. P., Kupferberg, H. J. and Wolf, H. H. (2002). Discovery and preclinical development of antiepileptic drugs. In: Levy, R. H, Mattson, R. H., Meldrum, B and Perucca, E, eds. *Antiepileptic drugs.* Philadelphia: Lippincott, 2002: 36-48.

White, H.S., Johnson, M., Wolf, H.H., Kupferberg, H.J. (1995): The early identification of anticonvulsant activity: role of the maximal electroshock and subcutaneous pentylenetetrazole seizure models. *Italian Journal of Science*, 16: 73-77.

Yemitan, O. K., Adeyemi, O. O. and Taiwo, A. E. (2006). Neurosedative and muscle relaxant activities of ethyl acetate extract of *Baphia nitida* AFZEL. *Journal of Ethno pharmacology*, 106: 312-316.