CULCASIA FALCIFOLIA ETHANOL LEAF EXTRACT EFFICACY AND SAFETY IN THE TREATMENT OF EPILEPSY INDUCED BY PENTYLENETETRAZOLE IN LABORATORY MICE

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DECLARATION

DECLARATION

This thesis is my original work and has not been submitted for any academic award in any institution; and shall not be reproduced in part or full, or in any format without prior written permission from the author and/or University of Eldoret.

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DEDICATION

This thesis is dedicated to my beloved husband Dr. Ramesh Francis, my children Angelyn Joanah, Alwyn Johanson and Adwyn Johanan, and my parents Mr. Anthony Doss and Mrs. Rathna Anthony Doss for their love, support and prayers.

ABSTRACT

Culcasia falcifolia is a therapeutic plant conventionally used in the form of ash in Nandi County, Kenya in the treatment of epilepsy. The study intended to evaluate the antiepileptic activity of ethanol extract of *C. falcilofia* against pentylenetetrazole induced seizure in laboratory mice. The target of the study were: to valuate the anticonvulsant action of extract by valuating the levels of endogenous antioxidant enzymes and levels of neurotransmitters in brain tissue of mice; then the depressant action *of the* central nervous system. The procedure of the Organization for Economic Co-operation and Development 423 was used to lead the toxicity study. The antiepileptic activity was assessed using four groups of six mice treated at the dose levels 200 and 400 mg/kg body weight against pentylentetrazole induced seizure.. The antiepileptic activity was assessed using four groups of six mice treated at the dose levels 200 and 400 mg/kg body weight against pentylentetrazole induced seizure. By the end of the antiepileptic study, the animals were decapitated and the brains were seperated, homogenized and centrifuged to estimate the amount of endogenous antioxidant enzyme such as superoxide-dismutase, catalase, glutathione-reductase, glutathione-peroxidase and lipid-peroxidation, dopamine, serotonin, noradrenaline, and gamma aminobutyric acid. Rota-rod and act-photometer were used to measure central nervous system depressant effect of the extract at 200 and 400 mg/kg. The data were inferred by one-way analysis of variance followed by poshoc Dunnett test by means of Graph Pad version 3. The results of the present study uncovered that the extract was not toxic up to 2000 mg/kg body. Antiepileptic study exhibited that the extract significantly ($F_{1,5}$=65.56, p<0.001) increased the latency and decreased the duration of convulsion and provided 100% defense (400mg/kg body weight) against mortality. The extract significantly increased the levels of ($F_{1,5}$=20.1,p<0.01) superoxide dismutase, ($F_{1,5}$=34.9,p<0.01) catalase, ($F_{1,5}$=39.2,p<0.01) glutathione reductase, ($F_{1,5}$=35.3,p<0.01) glutathione peroxidase and significantly ($F_{1,5}$=7.7, p<0.001) decreased lipid peroxidation; gamma amino-butyric acid ($F_{1.5}$= 342.4,p=0.000), dopamine ($F_{1.5}$= 1793.14, p=0.001), serotonin ($F_{1.5}$=282.2,p=0.000) and noradrenaline ($F_{1.5}$= 1158.1,p=0.000). The extract offered depressant action. The existence of alkaloids, flavonoids, tannins, saponins, phenolic, sterols and cardiac glycosides was shown. Flavonoids such as myrrcetin, kaempferol, isorhamnetin, spigenin, rutin, quercetin and chlorogenic acid eluted using ethyl acetate. The findings showed that extract possesses anticonvulsant activity by increasing latency and decreasing duration of convulsions; restored the levels of gamma aminobutyric acid, dopamine, serotonin and noradrenaline, provided protection against oxidative stress by increasing levels of antioxidant enzymes and decreased lipid peroxidation. The ethanol extract of *Culcasia falcifolia* is safe and efficacious in the treatment of epilepsy.

ABBREVATIONS

AED	Antiepileptic Drug
ANOVA	analysis of variance
EECF	ethanolic extract of *Culcasia falcifolia*
Ca^{+}	calcium ions
CAT	catalase
CDNB	1-chloro 2, 4 dinitrobenzene
CNS	Central Nervous system
DA	dopamine
DTNB	5-5'-dithiobis-2-Nitrobenzoic acid
EDTA	ethylene diamine tetra acetic acid
$FeCl_3$	ferric chloride
GABA	gamma amino butyric acid
$GABA_A$	gamma amino butyric acid A receptor
GSH	reduced glutathione
GPx	glutathione peroxidase
H_2O_2	hydrogen peroxide
H_2SO_4	hydrogen sulphate
HCl	hydrochloric acid
5-HT	seratonin
i.p	intraperitoneal
IBE	International Bureau for epilepsy
ILAE	International league Against Epilepsy

K^+	potassium ions
kg	kilogram
LPO	lipid peroxidation
M	mole
MDA	malondialdehyde
MES	Maximal electroshock
mg	milligram
ml	milliliter
NA	Noradrenalin
Na^+	sodium ions
NaOH	sodium hydroxide
NBT	nitro blue tetrazolium
nm	nanometer
NSE	National Society for Epilepsy
PTZ	pentylentetrazole
r.p.m	resolution per minute
RNS	reactive nitrogen species
ROS	reactive oxygen species
SEM	Standard error mean
SOD	superoxide dismutase
TBARS	thiobarbituric acid reactive substances
TCA	trichloro acetic acid
TM	Traditional Medicine
WHO	World Health Organization

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CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Epilepsy is a chronic, neurological condition which is regarded as repetition of seizures which is occurs as a result of excessive, outburst of electrical signals transmission in the brain. The neurons could fire five hundred times faster than normal during epileptic seizure. Epilepsy occurs as a result of abnormal rise in the excitatory neurotransmitter, glutamate levels while reduction in inhibitory neurotransmitter, gamma amino-butyric acid levels (Czapinsk *et al*., 2005).

Epilepsy is categorized into different types, subject to the section of the brain that is affected and causing seizure. All types of epilepsies have the same symptoms; characterized by convulsions and seizures. Epilepsy is a neurologic condition which can occur commonly in the complex and well developed brains of mammalian species or possibly in all mammalian species. There is no boundaries for the occurance of epilepsy, it is found evenly spread around the world regardless of any ethnic, topographical, community or class limitations, both sexes, at all ages, specifically in juvenile, youth and progressively in elderly people (Renolds, 2002; Bettina, 2002). Epilepsy is a disorder affecting nearly eighty percentage of the population in developing countries (Yemadje *et al.,* 2011). Nearly, five percentage of the world's people cultivates epilepsy in their lifetime (Raza *et al*., 2000). In the developing countries the use of traditional systems of medicines and around eighty percentage of the population depends on the folk remedies for crucial wellbeing requirements. Traditional therapeutic system is an affordable health care management and aids as a

vital basis of living for most populations (Roberson, 2010). The existence of a large number of bio-active components, traditional medicinal herbs/plants have great potential to treat epielpsy (Kavvadias *et al.,* 2004).

Culcasia falcifolia a perennial climber belongs to the family Araceae. *Culcasia falcifolia* leaves are used in the form of ash (taken internally) to treat epilepsy in the traditional medicinal system in Nandi county, Kenya (Pascaline *et al.,* 2008). *Culcasia falcifolia* is consumed as a stimulant or consumed mixed with porridge. Chepnamobon/ Kipnamobon is the vernacular name of *Culcasia falcifolia*. From the currently available literature there is no documented indication for the therauptic application of *Culcasia falcilofia* in the management of epilepsy.

1.2 Statement of the problem

Anew obtainable antiepileptic drugs with the goal to reduce recurrence of seizure have been promoted wide-reaching in the course of the past decades and are accessible to treat diverse categories of seizures. The older and newer antiepileptic drugs do not assure to be competent in inhibiting all types of seizures in all epileptic patients (Schmidt & Rogawski, 2002). All antiepileptic drugs have performed to be operative in short-range in patients with uninhibited epilepsy. Antiepileptic drugs are connected with reactions, including chronictoxicity, teratogenicity, and antagonistic properties on understanding and performance of patients treated (Mathur *et al*., 2010). Amongst seventy to eighty percentage of individuals are effectively treated with one of the existing antiepileptic drugs and achievement rates mainly be determined by on the reason for the source of seizure disorder. The remaining twenty to thirty percentage of patients have uncontrolled seizures or intractable seizures or undergo substantially diverse adjacent problems to medication (French *et al*., 2004). All the

conventional and anew antiepileptic drugs are not consistently effective in medicational therapy of epilepsy and are all related to multiple adverse reactions leading to reduced acceptance (McNamara *et al*., 2011).

There is necessity for antiepileptic drug that will subdue seizures without causing side effects or fewer side effects. Therefore, is essential to examine for an ancillary antiepileptic manager that is extremely effectual and harmless in terms of drug associated injuries. The substitute for the treatment of epilepsy couldbe bythe usage of therapeutic compounds in plants (Hassan *et al*., 2012). Herbal medicine is acknowledged world-wide and widely applied in antiepileptic treatment because; they are inexpensive and available for human health care services; fewer side effects and less toxcic conpared to conventional medicines.

There is incredible dependence on traditional herbal medication as there are numerous individuals in the developing countries for their fundamental health care needs. There is a deficiency of confirmation for how efficiently most herbal plants are used in the treatment of epilepsy. Therfore, it turn out to be appropriate to examine for potential and generally benign plant medicine.

1.3 Objectives

The objective of the study was to determine the antiepileptic activity of the ethanolic extract of *Culcasia falcifolia* on epilepsy induced in laboratory Swiss albino mice.

1.3.1 Specific objectives of the study

1. To investigate the acute toxicity of the ethanolic extract of *Culcasia falcifolia* for fourteen days of oral administration of the extract at the amounts of 5 mg/kg, 50mg/kg, 300 mg/kg and 2000mg/kg in Swiss albino mice.

2. To ascertain the phytochemicals present in the ethanolic extract of *Culcasia falcifolia* after the process of ethanol extraction.

3. To evaluate the anticonvulsant activity of ethanolic extracts of *Culcasia falcifolia* on Pentylenetetrazole-induced seizures in Swiss albino mice after twenty one days of oral administration of the extract at 200 and 400 mg/kg.

4. To determine the levels of antioxidant enzyme in brain tissue of Swiss albino mice after the oral administration of extract for twenty one for the assessment of the antiepileptic outcome of ethanolic extract of *Culcasia falcifolia* on pentylenetetrazole induced seizure in mice.

5. To determine the levels of neurotransmitters in brain tissue of Swiss albino mice after the oral administration of the extract for twenty one days for evaluation of the antiepileptic effect of ethanolic extract of *Culcasia falcifolia* on Pentylenetetrazole–induced seizure in mice.

6. To examine the central nervous system depressant action of Swiss albino mice on rota rod and actophotometer after fourteen days of administration of the ethanolic extract of *Culcasia falcifolia* by oral route.

1.4 Hypothesis

1. The ethanolic extract of *C. falcifolia* is not safe as an antiepileptic agent in mice.

2. The phytochemicals present in the ethanolic extract of *C. falcifolia* do not relate to its antiepileptic activity.

3. The ethanolic extracts of *C. falcifolia* does not have antiepileptic activity on pentylenetetrazoleinduced seizures in Swiss albino mice.

4. The ethanolic extract of *C. falcifolia* does not have any effect on the endogenous antioxidant enzymes on pentylenetetrazoleinduced seizure in Swiss albino mice.

5. The ethanolic extract of *C. falcifolia* does not have any effect on the levels of neurotransmitters in brain homogenate of on pentylenetetrazoleinduced seizure in Swiss albino mice.

6. The ethanolic extract of *C. falcifolia* doe not have central nervous system depressant effect Swiss albino mice on the rota rod and actophotometer.

1.5. Significance of the study

Medicinal plants are broadly used in the therapeutic treatment of ailments due to their wide-ranging applicability and healing effectiveness with mild antagonistic properties. Antiepileptic activities of numerous medicinal plants have been researched for their antiepileptic activity using various animal models. *Culcasia falcifolia* leaves are used in the form of ash (taken internally) to treat epilepsy in the traditional medicinal system in Nandi county, Kenya (Pascaline *et al.*, 2008). *Culcasia falcifolia* is consumed as a stimulant or consumed mixed with porridge. The thesis provides scientific documentation for the antiepileptic activity of *Culcasia falcifolia*. In the wider frame of the ongoing research on the treatment of epilepsy, the present study is the initial work done on the anti-epileptic activity of *Culcasia falcifolia*. The finding of the indications might be useful for the future in the development of new antiepileptic drug of higher efficacy and minimal toxicity.

1.6. Justification of the study

In the management of epilepsy different plant extracts had been effectively used in distinct epileptic animal models. The phytochemicals in various plant extracts have the promising impact on epilepsy management. In the therapy and management of epilepsy, there is proof of clinical effectiveness and minimal side effects of herbal drugs. A number of medicinal plants were reported to possess antiepileptic activity in various folklore cultures were discovered to have active compounds when experimented using modern bioassays for discovering their anticonvulsive activities (Mohsin, 2000). The determination of this work was to valuate the antiepileptic action of *Culcasia falcifolia* on pentylenetetrazole induced seizure in mice. In the direction of identifing the potential of *Culcasia falcifolia* expected to be an alternative drug for the treatment of epilepsy, which is a disease of sociological and economic importance, a scientific source is essential. The current study aimed to establish the mechanism of antiepiletic activity of *Culcasia falcifolia* and and attempted to provide the scientific documentation for the practical application of *Culcasia falcifolia* in the traditional treatment of epilepsy.

1.7. Scope and limitations of the study

There is lack of literature referring to the antiepileptic activity of *Culcasia falcifolia* to support the present study as no similar studies have been done before. Therefore the work attempted to study the anti-epileptic action of *Culcasia falcifolia* ethanolic leaf extract. This piece of work was carried out only on the ethanolic leaf extract of the *Culcasia falcifolia* at different doses to evaluate the antiepileptic activity only in the laboratory Swiss Albino mice. In the leaf extraction of *Culcasia falcifolia* the solvent ethanolic was used. The observations of the study were limited to acutely generated seizure on laboratory Swiss Albino mice using pentylenetetrazole injection, which

models clonic seizures. Pentelynetetrazole- induced seizure test is an accepted *in vivo* model for screening antiepileptic drugs. This investigation was performed to study the efficacy and safety of ethanolic extract of the leaf of *Culcasia falcifolia* on PTZ induced seizure.

CHAPTER TWO

LITERATURE REVIEW

2.1. Epilepsy

Epilepsy is a central nervous system disorder categorized by short-term events of seizures. It is typically related with loss of consciousness, forceful spasmodic contractions of skeletal muscles (convulsions) and autonomic hyperactivity (Ramesh *et al.,* 2007). Physiological studies have showed that seizures constitute brief, unusual and synchronous of the brain neuronal population. This type of brain dysfunction (seizures) may be accompanied by motor, sensory and autonomic abnormalities depending on the region of the brain involved in the cause and/or range of seizures (Wladyslaw, 2013).

The mechanisms of seizure at the cellular level involve regular or tonic excitation or the synchronized and repeated interaction between excitatory and inhibitory neurons and conductance of the membrane. In reaction to a loss of balance between stimulating neurotransmission and inhibitory neurotransmissions, seizures result in frequent bursts (McCormick, 2001). Neurochemical studies show that seizures can be produced in a given population of neuronal cells in the brain from excessive excitatory processes or from hypoactivity of neuronal inhibition. The seizure mechanism consists of the hyperactivity of the glutamatergic transmission and the perational disruption of the ligand-gated or voltage gated sodium channels and calcium channels. Deficiency in the inhibitory processes is associated with inadequate gamma amino butyric acid receptor-mediated neurotransmission and extracellular potassium currents. Alterations of the ligand-gated and/or voltage-gated sodium,

potassium, chloride and calcium channels, inflammatory processes, imbalance in the cellular energetics leads to seizures (Armijo, 2006).

2.2. Etiological classification of epilepsy

2.2.1. Genetic/Idiopathic epilepsy is predominately genetic in origin, in which there are no gross neuroanatomical or neuropathological abnormalities

2.2.2. Symptomatic epilepsy is acquired or genetic epilepsy associated with gross abnormalities neuroanatomically and neuropathologically indicating underlying disease condition.

2.2.3. Provoked epilepsy is epilepsy in which an environmental factor is the principal source of seizures, and there are no great changes anatomically and pathologically. Some provoked epilepsies will have genetic basis and some an acquired basis.

2.2.4. Cryptogenic epilepsy is epilepsy in which the cause is not apparent (Shorvon, 2011).

2.3. Epileptic seizure types

Epilepsy is a group of diverse types of seizures that varies broadly in the origin, severity, consequence, and management. Seizures are divided into the subsequent groups:

2.3.1. Partial Seizures*:* Partial seizures begin in one hemisphere of the brain, with signs such as twitching in an arm or face, a sensory alteration, or even the focal form of memory loss. The following are the types of partial seizures:

a) *Simple Partial Seizures***:** affects only one part of the brain with symptoms like shaking, unusual movement of the skull, numbness, and misconceptions in seeing hearing or smelling things that are not around.

b) *Complex Partial Seizures**:*** affects one lobe of the cerebrum. A person with complex partial seizures may have warnings for the seizure, such as feeling nausea, feeling warm, being unable to respond or difference in sensory perceptions.

c) *Secondarily Generalized Seizures:* Seizures transmission starts at a particular part of the brain as partial seizures then spread across the entire brain and evolve into generalised seizure (Dekker, 2002).

2.3.2. Generalized Seizures begins at both the right and the left cerebral hemispheres. This type of seizures originates in deep structures of the brain and spreads to the cortical surface. There is loss of consciousness occuring in generalized seizures. The following are the categories of generalized seizures:

(a) *Absence Seizures**:*** Absence seizures are usually briefer and recovers is quicker. Absence seizures previously were called petit mal seizures. Absence seizures present with staring spells lasting several seconds, fluttering eyelids or head nodding.

(b) *Tonic-Clonic Seizures**:*** usually continues from 1 to 3 minutes. Generalized tonic-clonic seizures formerly were called as grand mal seizures. In tonic-clonic seizures there is loss of consciousness, stiffening of the limbs (tonic activity) which are followed by rhythmic jerking (clonic activity) of the limbs.

(c) *Atonic Seizures:* A person with atonic seizures grows into drooping and might tumble to the ground abruptly. Atonic seizures occur in adults with prevalent brain injuries or in children.

(d) Myoclonic Seizures: A Myoclonic seizure is a lasting briefly with unprolonged jerk series and is different from the rhythmic jerks which occur during a generalized tonic-clonic seizure.

(e) Tonic Seizures: Tonic seizures involve stiffening of muscles as the primary seizure symptom. Arms or legs may extend forward or up into the air and stiffen. Consciousness may possibly or may not be gone. This type of seizure there is absence of the jerking (clonic) phase.

(f) Mixed Seizure Types: seizure starts as one seizure type and may progress into another type of seizure as the electrical activity spreads throughout the brain. It begins as a simple partial seizure and advances into complex partial seizure to a secondarily generalized seizure after the electrical activity has spread to the whole brain (Dekker, 2002).

2.4. Epidemiology and prevalence of epilepsy

Approximately 1% of the world's population is affected by epilepsy for this reason that epilepsy is classified one among the most encountered neurological conditions (Hirtz *et al.,* 2007). In developed countries, prevalence rates for epilepsy ranges from 0.027–0.1 per 1000 individuals have been reported (Banerjee *et al*., 2009). The prevalence of epilepsy is forty-four per one hundred thousand people in a year according to the age group of patients. High number of epilepsy is identified in the elderly people at the time of diagnosis out of 125,000 new epilepsy cases each year (Gidal *et al.,* 2005). According to the reports presented by the National Sentinel Audit of Epilepsy-Related Deaths nearly one thousand deaths which occur as a result of epilepsy are associated with seizure in the U.K every year and most of those deaths were possibly avoidable (Hanna *et al.,* 2002).

2.5 Pathophysiology of epileptic seizure

The normal function of the brain is to have a balance between excitation and inhibition of neuronal transmission. A seizure results when a sudden imbalance that occurs between the excitatory (glutamatory signaling) and inhibitory (GABAergic signaling) signaling within the network of neurons at the synaptic level. Seizures originate from the gray matter of any cortical or sub cortical area. Firstly a small number of neurons fire abnormally and then additional excitability spread either to nearby neurons to produce a focal seizure or spread more widely to neurons throughout the entire brain to produce a generalized seizure. Seizure is produced when the normal conductance of ions across the membrane and the inhibitory synaptic current transmission is interrupted. This onset of seizure spreads physiologic pathways to involve adjacent to remote areas. Deformity in the voltage stimulated ion channels, a malfunction of potassium conductance, or a deficiency in the membrane ATPase that are related to ion transport might cause instability in the neuronal membrane and cause a seizure. There are certain neurotransmitters such as glutamate, norepinephrine augment the excitability and spread of neuronal activity, while gamama amino butyric acid and dopamine inhibit neuronal activity and spread of abnormal excitability. When the seizure period is prolonged the brain may be weakened and the blood circulation might be disturbed which may contribute to neuronal loss or damage to the parts of the brain (Gidal et al., 2005). Figure 1 shows the pathophysiology ofepileptic seizures.

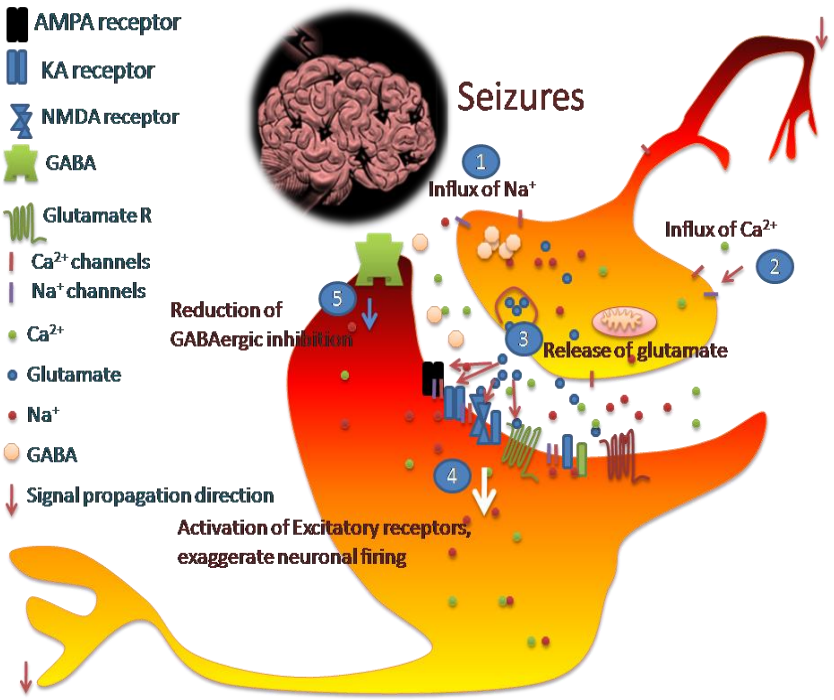

Figure 1: Pathophysiology of epileptic seizures
Source: Epilepsy: A Brief Review. Available from:
https://www.pharmatutor.org/articles/epilepsy-brief-review

2.6 Pathogenesis of epilepsy

The key role of gamama amino butyric acid is to regulate the neuronal excitability. The deficiency of gamama amino butyric acid (GABA) causes siezure. Most rapid inhibition of neurotransmission in the brain is produced by gamama amino butyric acid by increasing neuronal membrane conductance for chloride ions causing hyperpolarization. Gamama amino butyric acid binds to $GABA_A$ receptor which is a gated ion channel used by gamama amino butyric acid for the mechanism of inhibitory effect. Gamama amino butyric acid type A receptor is goal for various significant neuroactive medications including antiepileptic drugs. It is believed that GABA exposure to postsynaptic receptors results in the production of post synaptic inhibitory currents. Postsynaptic inhibitory currents mediated by Gamma amino-

butyric acid type A receptors has key role in the prevention of development of neuronal hyperexcitability (David, 2010).

2.7. Antiepileptic drugs

The drugs that are used for the treatment of epilepsy are Antiepileptic drugs (AED). The goal of all the currently available and old antiepileptic drugs is to subdue the fast and excessive firing of neurons that result in a seizure. An efficient antiepileptic drug would prevent the spread of the seizure within the brain and provide protection against toxic effects which might result in the damage of the brain (Nirmala, 2015). The antiepileptic drugs remain neither protective nor remedial besides they are merely as a source of governing symptoms (Brodie *et al.*, 2011).

2.7.1. Targets and treatment approaches for anti-epileptic drugs

The anti-epileptic drugs have different objectives for their mechanism of action. The following are the targets for anti-epileptic drugs: a) to inhibit the excitatory neurotransmitter, Glutamate; or b) to enhance the inhibitory neurotransmitter gamma-aminobutyric acid (GABA); or c) to block the voltage-gated positive currents of Na^+ and Ca_2^+; or d) to increase the outward positive current, K^+ (Meinardi, 2000). Figure 2 shows the mechanism of action of antiepileptic drugs that are currently available in the treatment of epilepsy.

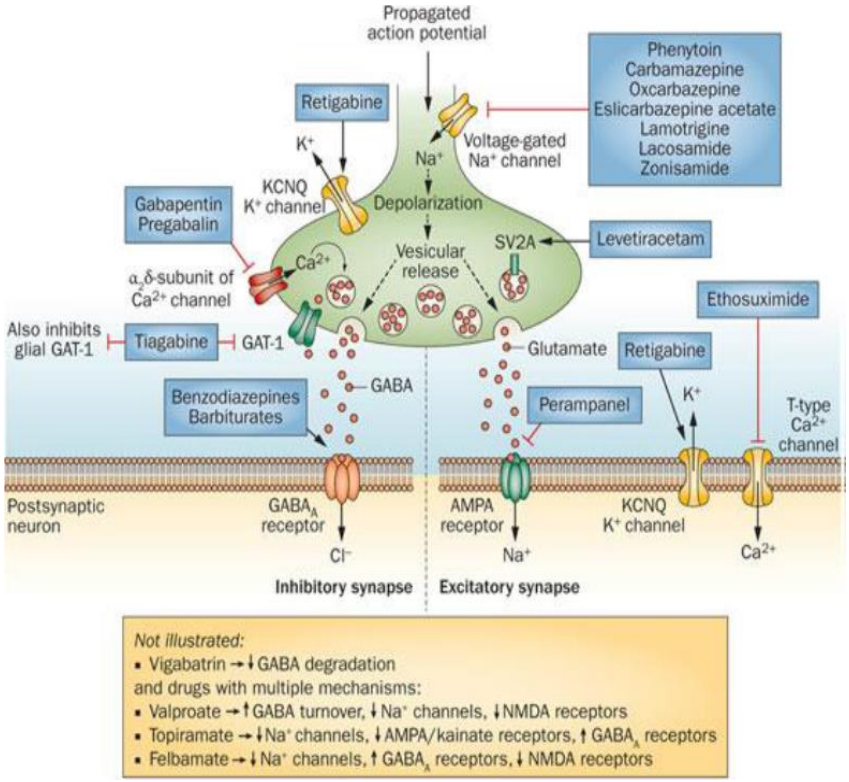

Figure 2: mechanism of action of antiepileptic drugs

Source: New drug classes for the treatment of partial onset epilepsy: Available from: https://www.researchgate.net/figure/Mechanisms-of-action-of-antiepileptic-drugs-Clinically-approved-antiepileptic-drugs-such_fig1_250925467

The treatment of epilepsy with presently existing antiepileptic drugs show chronic toxicity, side effects which are dose-related and affect almost each organ of the human body system. Furthermore, presently accessible antiepileptic drugs are likely to produce adverse effects on behavior and cognition of the individuals treated (Duncan, 2002). Table 1 shows the list of currently available antiepieltpic drugs, their clinical applications and the adverse effects caused by the antiepileptic drugs (Brunton *et al.*, 2007; Katzung *et al.*, 2012)

Table 1: Antiepileptic drugs, clinical applications and their adverse effects

Antiepileptic Drug	Used in the treatment of	Adverse side effects caused by the antiepileptic drug
Phenytoin	Partial seizures and	Lack of voluntary cordination, double vision,

	generalized tonic clonic seizures	involuntary eye movement, skin rashes, agranulocytosis, aplasticanemia
Phenobarbital	Generalized tonic clonic seizures and Partial seizures	Drowsiness, fatigue, difficulty in speech, skin rashes, osteomalacia
Ethosuximide	Absence seizures	GI changes, drowsiness, tiredness, temper variations
Carbamazepine	tonic-clonic seizures and partial seizures	Double vision, Lack of voluntary cordination, nystagmus, skin rashes, agranulocytosis, weight gain, osteomalacia,
Diazepam	Status epilepticus	Drowsiness, lethargy, drowsiness, dizziness, hyper salivation
Gabapentin	Generalized seizures and partial seizures	Drowsiness, dizziness, Lack of voluntary cordination, lethargy, hyperactivity (in children), weight gain
Pregabalin	Partial seizures	Weight gain, peripheral edema, giddiness, headache
Vigabatrin	Partial seizures and infantile spasms	Drowsiness, dizziness, involuntary eye movement, shiver, weariness, sleeplessness, phobia, weight gain
Valproate	Generalized seizures, partial seizures, absence seizures and myoclonic seizures	Weight gain, indigestion, heart burns, peripheral edema, inflammation of pancrease, shivers
Lamotrigine	Generalized tonic-clonic seizures, partial seizures and absence seizures	Dizziness, drowsiness, headache, double vision, involuntary eye movement, skin rash
Lacosamide	Generalized tonic-clonic seizures and partial seizure	Dizziness, nausea, double and blurred vision, vomiting, headache, shakes

2.8. Traditional medicinal plants

Traditional medicinal practices have continued to remain as a significant element of health care systems of many societies inspite of the accessibility of well-known substitutes (Ndoye, 2005). Practices of traditional medicinal plants are ethnically customary. Traditional systems are unappreciated from the time when the modern health care services were introduced for the modern health care system provides quick

healing. Medicinal plants are the abundant sources of bioactive compounds that are used in the production of pharmaceutical products and also used in the traditional system of treating various ailments. The current researches are all focused on the separation and identification of the active compounds from medicinal plants for those diseases where presently available drugs are not effective. Herbal medicines are currently going through resurgence as many people are turning their attention from modern drugs toward traditional herbal medicine systems which are also known as alternative medicine (Aslam & Ahma, 2016). Medicinal plants contain several phytochemical compounds that act in a synergitic and in an additive manner to improve health (Shutz *et al*., 2006). The scientific communities have recently paid attention to the benefits of folk medicine and have begun to consider the medicinal properties of natural products as accepted by the traditional system of medicine plants (Barbosa-Filho *et al*., 2006). Conventional drugs are synthysized from medicinal plants for the production of drugs for the treatment of several human diseases because of their effective healing with less or no side effects, even though there are numerous advancements in synthetic drug production (Sharma, 2008). Medicinal plants have great dependence for the treatment of diseases as well as for the potential drug discovery; it has become important to the search for powerful, efficient and comparatively safe plant medicinal products and to the scientific validation of success claims concerning medicinal plants already being used (David, 2010).

2.9. Culcasia falcifolia

Plate 1: Leaves of Culcasia falcifolia
Source: https://alchetron.com/*Culcasia*

2.9.1. Systematic classification

Kingdom: Plantae

Phylum: Tracheophyta

Class: Liliopsida

Order: Arales

Family: Araceae

Genus: Culcasia

Species: *Culcasia falcifolia*

2.9.2. Description of *Culcasia falcifolia*

C. falcifolia belongs to the family Araceae, is a perennial climber, epiphytic on trees, stem with adventitious roots, penetrating bark with short clasping roots at the nodes showed in plate 1. The leaves are oval or elliptic to oblong, dark green and shiny, about 30cms long. Spathe (leaflike bract that encloses the flower) is waxy about six centimeters long; and long spadix which is yellowish. The flowers appear yellow or

green short clusters, distinct branches, enclosed by a heavy rubbery. Fruits grow as clusters of obovoid berries which are orange-red in color when ripen (Ghogue, 2010).

2.9.3. Ecological and geographical distribution

Culcasia falcifolia is found to grow in shady damp evergreen forest, swamp and riverine or marshy forest. *Culcasia falcifolia* is found growing in Kenya, Ethiopia, Malawi, Tanzania, Uganda, Zambia, Zimbabwe (Ghogue, 2010).

2.9.4. Traditional uses of *Culcasia falcifolia*

In the traditional medicinal practice of Nandi County, Kenya, the leaves of *Culcasia falcifolia* are used in the form of ash which consumed internally for the treatment of epilepsy and edema**.** *Culcasia falcifolia* leaves of are consumed in the form of ash mixed with with porridge or consumed as a stimulant. *Culcasia falcifolia* is called as Chepnamobon/ Kipnamobon in Aldai division of south Nandi district (Pascaline *et al.*, 2008).

2.10. Antiepileptic plant extracts

Medicinal plant extracts for the treatment of epilepsy can be an essential natural source of less harmful medicines. Medicinal plants are used to treat epileptic seizures, possibly since they are appropriate to the cultures of people; are less expensive; have low side effects; have no contraindications; and have no interactions with drugs that are consumed concurrently (Malvi *et al.,* 2014). Several researches have showed promising anticonvulsant activities of medicinal plants that are already in use in the traditional medicinal practice which were done using animal models to screen for their anticonvulsant properties. These medicinal plants can be an invaluable source for search for new

antiepileptic compounds (Stafford *et al.*, 2008). Molecular level researches have been done on medicinal plants for their antiepileptic activity as a result of these studies numerous signficant phyto-chemicals have been isolated (Md Asif, 2014). The phytochemicals isolated from more than thirty herbal extracts that are used in the traditional Indian, Chinese and Japanese herbal remedies have been researched using different models of epileptic animal for their antiepileptic activities. In both *in vivo* and *in vitro* screening for antiepileptic activities nearly 2/3rd phytochemicals derived from those plant extracts had showed efficient anticonvulsant activity (Steven, 2009).

2.10.1. Studies on antiepileptic activities of medicinal plant extracts against Pentylenetetrazole-induced seizure in rats/mice

Ojewole *et al.,* (2006) reported antiepileptic screening studies on the aqueous leaf extract of *Persea americana* (avocado). The results of the study showed that the extract delayed the seizure onset and supressed PTZ-induced seizure. *Persea americana is* used in the traditonal medicinal system in African countries to treat and manage childhood convulsions and epilepsy. Ojewole and his colleauges concluded from their study that the extract of *P. americana* produced its anticonvulsant effect by augmenting the GABA neurotransmission in the brain of mice. Arumugam and his colleauges (2009) studied the antiepileptic activity of the ethanolic extract of inner bark of *Guettarda speciosa*. Their results of their study showed that the extract delayed the seizure induced by PTZ and the ethanolic extract of *Guettarda speciosa* probably exhibited its antiepileptic activity mediated through chloride channel of the GABA receptor complex. *Guettarda speciosa* belongs to the family: Rubiaceae which is broadly dispersed from Asia to East Africa commonly along the seashore and low land forests. According to the study conducted by Sankari *et al.* (2010) on the ethanolic leaf extract of *Aegle marmelos*, the ethanolic extract of *Aegle marmelos*

blocked tonic clonic seizure induced by PTZ. The study suggests that the extract has its positive effect on GABA-ergic neurotransmission to show its anticonvulsant effect. Sankari and her colleauges quoted in addition, that the presence of flavonoid attributed to their anticonvulsant action of the ethanolic extract of *Aegle marmelos*.

The study conducted by Jain and his colleauges (2011), showed that the methanolic extract of stems of *Artocarpus heterophyllus* have anticonvulsant activity against PTZ induced clonic convulsions. The results of their study on the anticonvulsant activity of the extract documented that *Artocarpus heterophyllus* probably involves noradrenergic/ GABA-ergic pathways to block PTZ induced seizure. This is because PTZ induces convulsion either by inhibiting GABA-ergic pathway in CNS or by increasing the central noradregenic activity. Patrick *et al*., 2012 stated that *Synedrella nodiflora* a medicinal plant that belongs to the family Asteraceae used in traditional Ghanaian medicine, the whole plant is boiled and the aqueous extract is consumed as much as essential for the treatment of epilepsy. The results of the study presented by Patrick and his colleagues reported that *S. nodiflora* demonstrated anticonvulsant by enhancing gamma-aminobutyric acid neurotransmission.

Sandeep *et al*., (2015) recognized the presence of antiepileptic phytochemical combinations of flavonoids, saponins, tannins, terpenes in *Biophytum sensitivum* (Linn.) leaf extract. Sandeep and his colleauges demonstrated that the ethanolic extract of *Biophytum sensitivum* at various portions, for example, 50, 100 and 200 mg/kg body weight critically decreased the length and beginning of spasms in mice. *Biophytum sensitivum* is herbaceous plant that has a place with the family Oxalidaceae. It is found in tropical Africa and Asia. As indicated by Saikia and colleauges (2016) distribution on the anticonvulsant action of methanolic leaf extract

of *Lawsonia inermis* the concentrate altogether dense the span and intensified the inactivity of spasms in the PTZ induced mice model. The anticonvulsant activity of *Lawsonia inermis* leaves acknowledges that the flavonoid compounds present in the extract might be liable for the anticonvulsant action of *Lawsonia inermis* by connecting with the GABA-ergic neurotransmission. *Lawsonia inermis* develops in tropical and subtropical zones of North Africa and Southern Asia including India. The plant has been customarily utilized in the treatment of epilepsy and varoius different illnesses, for example, jaundice, rheumatoid joint inflammation. Oscar *et al.* (2017) assessed the antiepileptic impact of ethanolic extract of the leaves of *C. articulatus* on PTZ initiated seizures in mice. The aftereffect of the examination indicated huge abatement in the beginning of seizures, decline in the length of seizures and recurrence of seizure. *Cyperus articulatus* L. (Cyperaceae) has been utilized for a considerable length of time in African and Asian drug. Studies show that its conventional use for migraine and epilepsy might be because of its impact on synapses in the cerebrum. There are no scientific evidences on the antiepileptic activity of the ethanolic extract of *Culcasia falcifolia*. The thesis worked on the antiepieltpic activity of *Culcasia falcifolia* against pentylenetetrazole induced seizures in mice.

2.11. Oxidative stress and epilepsy

Oxidative stress is a consequence of the deteriorated oxidant burden which devastates the endogenous antioxidants and a consequence of diminished endogenous antioxidants. (Halliwell & Gutteridge, 2007). Oxidative stress is the most prominent mechanism in the development and progression of epilepsy (Ramalingam, 2013). Studies have reported that oxidative stress occurs when free radicals are produced and membrane lipid peroxidation occurs during an epileptic attack which cause tissue

damage (Ilhan *et al.,* 2005). Generation of seizure is connected with the homeostatic imbalance between antioxidants and oxidants (Gluck, 2001). Epileptic seizure causes influx of calcium through voltage-gated ion channels that exaggerate intracellular calcium ions and bring about biochemical changes. High levels of intracellular calcium can induce reactive oxygen species which leads to seizures (Patel, 2004). Antioxidant reduce oxidative stress, have attracted much attention in treatment for epilepsy (Sudha *et al.,* 2001). A number of studies reported the production of reactive oxygen species in different brain regions following experimental seizures (Devi *et al.,* 2008). The imbalance between free radicals and antioxidants (i.e. too many free radicals and too few antioxisdants) is the major cause for oxidative stress. As a result of oxidative stress oxidation of biomolecules such as lipids, proteins, and nucleotides can lead to cell death or cellular damage (Sudha *et al.,* 2001). Increased oxidative stress disrupts biological membranes structure and function which is lipid peroxidation. (Poonam *et al.,* 2004). There are various phenomena which occurs in the brain including high oxygen consumption by neurons; low concentration of endogenous antioxidants; the abundance of polyunsaturated fatty acids comprising brain lipids, and excess of neurotransmitter glutamate marks the brain susceptibility to oxidative stress (Zecca, 2003). The brain has a range of defense system to counterbalance the reactive oxygen species, these include enzymatic and non-enzymatic antioxidants that lower the concentration of free radicals and repair oxidative cellular damage. The anitoxidant enzymes plays an important role in scavenging free radicals. Superoxide dismutase, catalase, and glutathione are antioxidant enzymes found in the brain. Superoxide dismutase catalyzes the dismutation (is a process of simultaneous oxidation and reduction). Catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen (Rodrigues, 2013).

Glutathione removes intracellular hydrogen peroxide and protects the cell and its components from damage caused by oxidative stress (Patil *et al.,* 2011).

Numerous studies have been conducted to study the influence of plant extract on enzymatic antioxidant activity, and Malondialdihyde levels that have shown neuroprotective effects against oxidative stress in different animal models of seizures. Therefore, in the present study the effect of ethanolic extract of *Culcasia falcifolia* in the levels of superoxidedismutase, catalase, glutathione then malondialdehyde on pentylenetetrazole induced seizures in mice were evaluated.

2.11.1. Studies on the plant extracts against oxidative stress markers in pentylenetetrazole induced seizures in rats/ mice brain tissue.

Golechha *et al.* (2010) reported the effect of hydro-alcoholic fruit extract of *Emblica officinalis* on PTZ induced seizure had neuroprotective effects against oxidative stress exerted by reducing free radicles and thus against oxidative stress markers. Vaibhav (2013) stated that the aqueous leaf extract of *D. triflorum* exerted antioxidant effect on PTZ induced seizure by reducing the the duration of convulsion generation of free radicles and thus showed neuroprotective effects against oxidative stress markers and oxidative stress markers. According to the report produced by Choudhary (2013) *Glycyrrhiza glabra* exhibited its anticonvulsant potential by increased levels of SOD and CAT in the brain homogenate of PTZ induced seizure rats. Reza *et al. (*2015) demonstrated that the hydro-alcoholic extract of Ariel parts of *Coriandrum sativum* possess antiepileptic and antioxidant effect in mice brain tissue significantly reduced the free radical-mediated lipid peroxidation indicated by a decrease in the MDA levels. The leaves extract of *Cyprus articulatus* was shown to provide protection against lipid peroxidation by inhibiting malondialdihyde level on mice

brain. The ethanolic extract from *C. articulatus* leaves showed anticonvulsant effect on Pentylenetetrazole induced seizure and antioxidant activity *in vitro* and *in vivo.* The leaves extract exerted protection against neuronal damage produced by PTZ injection (Oscar *et al.,* 2017). There are no scientific evidences on the antioxidant activity of the ethanolic extract of *Culcasia falcifolia.* The thesis worked on the effect of ehtanolic extract of *Culcasia falcifolia* against oxidative stress markers such as SOD, CAT, GSH and MDA on pentylenetetrazole induced seizures in mice.

2.12. Role of Neurotransmitters in epilepsy

Transmission signals from one neuron to another is throught the release of neurotransmitters. A swift disproportion amongst the inhibitory neurotransmitter (gamma amino butyric acid) and excitatory neurotransmitter (glutamate) in the brain causes epileptic seizures. There are other neurotransmitters being involved in causing seizures, noradrenaline, serotonin, and dopamine respectively. Several researches had shown that dopamine and serotonin have effects in the pathophysiology of seizures. Equally, pathophysiological studies and pharmacological studies have shown that the deficit in amount of serotonin and noradrenaline neurotransmitters have been recognized in some humans with epilepsy, besides in animal models of epilepsy. Augmentations of both noradrenalin and serotonin neurotransmission can evade occurrence of seizure, whereas reduction will have the contrary outcome. Experiments conducted on plant extracts on experimentally induced deficiencies in the amount of gamma amino butyric acid, noradrenaline, dopamine and serotonin are associated with the onset and continuation of seizure. Several studies have shown the effect of plant extracts on the neurotransmitter levels on the brains of pentylenetetrazole induced seizures in mice. (Clinckers, 2005).

2.12.1 Studies on the effect of medicinal plant extracts on the modulation of neurotransmitter levels on pentelynetetrazole induced seizures in rats/mice brain tissue

Kumar and Gandhimathi (2009) stated that the ethanolic extracts of *Guettarda speciosa* showed significant effect on biogenic amines concentrations in rat brain after induction of seizures by PTZ. The extract increased the monoamines levels in forebrain of rats. Dilipkumar (2009) stated that the levels of catecholamine in the brain homonenate of mice treated with ethanolic extract of the aerial parts of *Cynodon dactylon* and roots & rhizomes of *Cyperus rotundus* against convulsions induced by PTZ in mice. After six week of the exrtact treatment the amount of GABA, increased significantly in mice brain homogenate. Their findings of the study revealed that the extract presented a significant anticonvulsive property by altering the level of catecholamine such as dopamine and norepinephrine and brain amino acids in mice. *Cassytha filiformis* belongs to the Family Cassythaceae. It is widely distributed throughout the pacific and tropic region. It is used to treat convulsion. Govardhan *et al.* (2011) showed that the ethanolic extract of *C. filiformis* increased noradrenaline level, dopamine level, serotonin level, GABA level in PTZ induced rat brains. The results of the study showed that the *C. filiformis* ethanolic extract significantly restored biogenic amine levels and increased the seizure threshold and decreased the susceptibility to PTZ induced seizures in rat. Porwal *et al.* (2013) demonstrated that the ethanolic extract of the leaves of *Annona squamosa* protected the mice against convulsions induced by chemo convulsive agents. The levels of dopamine, noradrenalin and adrenalin were significantly. Results of the study revealed that the processed extract has significant anticonvulsive property by altering the level of biogenic amines in mice. Mahakalayanaka Ghrita (MKG) polyherbal extract,

pretreatment of MKG showed anticonvulsant activity against PTZ induced seizure. Kasthuri *et al.* (2015) demonstrated that the polyherbal extract significantly increased the levels of GABA, dopamine, and decrease in the levels of glutamate in mice treated with the extract. The result suggests that MKG might have possible action by blocking calcium ion dependent potassium conductance or MKG might have acted on glutamate transporters and inhibited the glutamate mediated excitatory effects by blocking NMDA receptor. Senthil (2010) showed that the methanolic extract of *Oxalis corniculata* on PTZ induced rat brains showed significant increase in the biogenic amine such as dopamine, noradrenaline, GABA and serotonin. The results of the study showed that the methanolic extract significantly restored biogenic amine levels and increased the seizure threshold and decreased the susceptibility to PTZ induced seizures in rats. Kishore *et al.* (2017) showed that the ethanolic extract of *Capsicum annuum* has significant increase in the dopamine, serotonin and noradrenalin level in the fore brains of extract treated mice. The result of the study suggests that administration of *Capsicum annuum* administration significantly increased the brain levels of serotonin, dopamine and noradrenaline, which could have attributed to the significant protection offered against induced seizures. Therefore, in the present study the effect of ethanolic extract of *Culcasia falcifolia* on the levels of neurotransmitter in mice induced pentylenetetrazole were evaluated to provide scientific substantiation for the extracts effect on neurotransmitter for its antiepileptic activity.

2.13. Neuromuscular coordination

Neuromuscular coordination can be described as the ability of the central nervous system (CNS) to regulate the muscles when performing of multi-limb functional movements (Jose, 2018). Locomotor coordination is most commonly assessed using a

Rota rod. This test provides an estimate of the animal's level of neuromuscular coordination. Drugs which are known to perturb neuromuscular coordination reduce the time the animals stay on the rod. The Rota rod test is one of the oldest used in the behavioral assessment of drug action. It provides a simple first estimation whether a test substance has any effect on neuromuscular coordination (Gerhard, 2006). Most of the CNS acting drugs influence the locomotor activities in man and animals. The CNS depressant drugs reduce the motor activity; while the stimulants increase the activity. Increase in the motor activity indicates CNS stimulant property of the drug while reduction in the motor activity indicates CNS depressant property of the drug (Anand, 2012). There are some reports on plants showing potential central nervous system depressant activity.

2.13.1. Studies on the effect of medicinal plant extracts on motor coordination and spontaneous locomotor activity of rats/mice

Bryophyllum pinnatum (Lam.) is a perennial herb growing widely and used in folkloric medicine in tropical Africa, India, China, Australia and tropical America. Presence of active compounds, including flavonoids, glycosides, steroids, bufadienolides and organic acids, has been identified in the aqueous leaf extract of *B. pinnatum*. Salahdeen and Yemitan (2006) studied the effects of the aqueous leaf extract of *B. pinnatum* on central nervous system activities in mice. It was reported that there was a significant loss of coordination and decrease muscle tone in animals treated intraperitoneally with *B. pinnatum* aqueous extract in a dose dependent fashion. The extract also showed anticonvulsant activities against chemically induced seizure in mice (Salahdeen & Yemitan, 2006). *Amorphophallus paeoniifolius* is known as Elephant foot yam which is found growing in south East Asia. The effect of petroleum ether extract of *Amorphophallus paeoniifolius* tuber on central nervous

system activity in mice was evaluated using an Actophotometer and Rota rod apparatus. The petroleum ether extract of *Amorphophallus paeoniifolius* tuber showed significant decrease in locomotor activity and grip test in a dose-dependent manner (Das *et al*., 2009). *Dichrostachys cinerea* is a shrub that belongs to the Fabaceae family. The alcoholic root extract of *D. cinerea* was tested for its CNS depressant activity. Motor coordination test was carried out using Rota rod apparatus. Locomotor activity was measured using Actophotometer. The results of the study shown the presence of glycosides, saponins, steroids, carbohydrates and tannins. Rota rod and Actophotometer test revealed a significant loss of muscle coordination and showed significant decrease in spontaneous motor activity. The results suggest that the alcoholic root extract of the *D. cinerea* root has CNS depressant activity (Ramya & Thaakur, 2009). *Flaveria trinervia* is an herb which belongs to the Asterecae family found growing only in alkaline soil. The methanolic and aqueous extracts of the whole plant of *F. trinervia* were screened for CNS depressant activity by actophotometer, Rota rod. The results showed that the administration of the methanolic and aqueous extracts of the whole plant spontaneously depressed the animals in locomotor and muscle coordination. The findings of the evaluation suggest that the extracts of *F. trinervia* have significant central nervous system depressant effect (Joy *et al.,* 2011). Jayashree *et al.* (2012) assessed the muscle relaxation and motor coordination activity of aqueous extract of *Sapindus trifoliatus* on in mice. The outcome of the spontaneous motor activity showed that extract reduced the motor activity then decreased the time spent by the mice on the rota-rod test. Jayasree *et al.* (2015) studied the effect of aqueous extract of *N. oleander* flowers on the skeletal muscle relaxant activity in albino rats. The phytochemical examination of the extract showed the presence of carbohydrates, flavonoids, saponins, tannins, and alkaloids.

The outcome of the study showed that the aqueous extract of *N. oleander* flower has muscle relaxant action. The study suggests that the central nervous system depressant effects of extract could be due to the interactions of the phytochemical of the extract with the GABA receptor complex in brain. *Acacia nilotica* commonly known as gum Arabic tree belonging to the genus of Acacia, family Mimosaceae. Chakraborty *et al.* (2016) researched on the ethanolic extract of the bark of *A. nilotica* to evaluate the CNS-depressant property of the extract on the motor activity in mice. The results of the study showed that the ethanolic extract of *Acacia nilotica* bark had significant muscle relaxant property and reduced the spontaneous activity in mice. *Chromolaena odorata* belongs to the family Asteraceae. Akash *et al.* (2017) measured the skeletal muscle relaxant activity of the ethanolic extract of leaves of *Chromoleana odorata* with Rota-rod. The study showed that the extract demonstrated significant skeletal muscle relaxant activity by hindering the NMDA receptors. There is no scientifc evidance on the central nervous system depressant activity of ethanol extract of *Culcasia falcifolia* till present date. Therefore, the present effort attemted to the assess the effect of ethanol extract of *Culcasia falcifolia* on the depressant activity of the central nervous system.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Site of Experiment

This study was conducted in the Post Graduate Research Laboratory; Biological sciences department, department of Chemistry at the University of Eastern Africa Baraton, Eldoret, Kenya and Nandha Research Institute, India.

3.2. Experimental Design

The study was a randomised controlled experimental Design. Swiss albino mice were selected through random sampling technique for this study. Twenty four animals were chosen randomly (by numbering 1-4) and separated into four groups indicated as group I, group II, group III and group IV comprising of six animals per group. Each group had a specific treatment. Group I acted as negative control received 0.1% CMC. Group II was the positive control received diazepam weighing 5mg/kg. Group III had 200 mg/kg body weight of ethanol extract of *C. falcifolia* extract. Group IV weighed 400 mg/kg of ethanol extract of *C. falcifolia*

3.3. Collection and Processing of *Culcasis falcifolia*

Whole leaves of *Culcasia Falcifolia* were collected from along the River Kiṅgwal of Kaptildil Nandi County, in Rift Valley, Kenya. The National Herbarium Kenya verified the authencity of the leaves, (voucher number PS 22/05). The leaves were then thoroughly washed with tap water to rid off dust, then undesirable materials collected from their usual surroundings. The leave free from dust were then allowed to dry under shade in the laboratory for two weeks then grounded to powder with laboratory electric blender. The powder was further passed through a sieve N0. 180

with the aperture 0.180mm to acquire finer powder and stored in a clean dry labeled glass beaker up until required used for extract preparation and analysis.

3.4. *Culcasia falcifolia* ethanolic extract preparation

The sample was mixed and macerated with absolute ethanol (100 g in 1 litre of solvent) for 7 days. Then the extract was filtered through Whatman No 1 filter paper followed by evaporation of the supernatant using the BUCHI Switzerland rotary evaporator to remove the ethanol and to obtain concentrated, oily extract. The crude extract was stored in sterile universal glass bottle until further use.

3.5. Experimental Animals

Swiss Albino male mice ranging between 20-25g of 18 weeks were used for the study. The animals were placed in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{o}C and relative humidity of 30 – 70 %. A 12:12 light: dark cycle was followed. All animals were supplied water and food *ad libitum*. The animals were procured from KEMRI and Nandha Research Institute, India. They were permitted to adjust to the laboratory conditions, and handling for one week.

3.6. Drugs and chemicals used in the study

3.6.1 Drugs used in the antiepileptic study: Pentylenetetrazole, Diazepam standard drug.

3.6.2 Chemicals used in the phytochemical analysis: Wagner's reagent; Dragendorff's reagent; magnesium metal; concentrated hydrochloric acid; 1.8% sodium chloride solution; 10% lead acetate solution; glacial acetic acid, 5% ferrous chloride and concentrated sulphuric acid; Molisch's regent; chloroform;

acetic anhydride; ninhydrin solution; 4% sodium hydroxide and 1% copper sulphate solution; ferric chloride solution.

3.6.3 Chemicals used in the estimation of neurotransmitters: Sodium acetate buffer iodine solution; Acetic acid; sodium sulphate solution; trichloro-acetic acid; O-Phthalaldehyde (OPT) reagent; copper tartrate reagent.

3.6.4 Chemicals used in the estimation of oxidative stress markers: Xantine and Xanthine oxidase; Potassium phosphate buffer; nitro blue tetrazolium; Hydrogen peroxide; dichromate-acetic acid; Sodium azide; thiobarbituric acid reactive substances.

3.6.5 Chemicals used in the identification and isolation of flavonoids: Silica gel, thin layer chromatography plates, ammonium hydroxide, hydrochloric acid, ethyl acetate, anhydrous calcium chloride, Ammonia. Chemicals were purchased from Sigma Aldrich.

3.7 Apparatus used

Electric blender; Water Bath; Electronic Heater; Electronic Balance; Refrigerator (LG, Korea), Rotatory Vacuum Evaporator (Buchi RII Switzerland); UV Chamber for TLC (Toshiba TM) Double Beam UV Spectrophotometer (Hitachi) Centrifuge, Rota rod (HV53), Actophotometer (TK EM27).

3.8. Acute Oral Toxicity Study of Ethanol Leaf Extract of *Culcasia Falcifolia*

Acute toxicity studies were performed under guidelines of Economic Organization and Cooperation Development (OECD-423). A total of three animals were used for the study. The animals were then fasted for 4 hours with only water access. Following the fasting period, and the extract was administered from the four fixed dose levels 5, 50, 300 and 2000 mg/kg body weight recommended by the OECD -423 guidelines for

a peroid of 14 days. The acute toxicity started with administration of a initial dose 5 mg/kg followed by 50mg/kg, 300mg/kg b. wt. of the extract was orally administered for all three mice and was frequently observed for impermanence, and signs of toxicity for the first 30 minutes, 24 hours (first 4 hours with special attention) and daily thereafter for 14 days. With the death of two animals out of the three, then the dose administered was considered as toxic. When mortality was observed in only one animal, out of three animals, then the same dose was re-administered again to confirm the toxic effect. When no mortality was observed, then higher (50, 300, 2000 mg/kg) doses of the plant extracts were administered for further toxicity studies. Three animals were used for each step. The following general behaviors were observed during the study: sedation; hypnotics; convulsion; ptosis; pain distress; stupor reaction; salivation; somatomotor activity; muscle relaxation; pilo erection; change in skin color; lacrimal secretion; lethargy; diahorreah (Ecobichon, 1997).

3.9 Qualitative phytochemical analysis of ethanol extract of *Culcasia falcifolia*

3.9.1. Test Alkaloids

i) Wagner's Test:1 ml of Wagner's reagent were added to 2 ml of the extract in a test tube. Formation of reddish brown precipitate indicates the presence of alkaloids (Kokate *et al*., 2001).

ii) Dragendorff's Tests: 1 ml of Dragendorff's reagent was added to 2 ml ethanol extract of *C. falcifolia* in a test tube. Formation of orange brown precipitate indicates the presence of alkaloids (Kokate *et al.*, 2001).

3.9.2. Tests for Flavonoids

i) Shinoda Tests: 0.5 grams of magnesium fragments were added to 2 ml of ethanol leaf extract of *C. falcifolia* in a test tube, then 5 drops (drop-wise) concentrate

hydrochloric acid. Were added. Appearance of orange red color indicateF presence of flavonoids (Kokate *et al.,* 2001).

ii) NaOH Tests*:* To 2 ml of Ethanol Leaf Extract of *C. Falcifolia*, 2 ml of 10% sodium hydroxide solution was added in a test tube. Formation of intense yellow color indicates the presence of flavonoids (Khandewal, 2008).

3.9.3. Test for Saponins

Foam Test: 20 ml of distilled water was diluted with 2 ml of ethanol leaf extract of *C. falcifolia* then shaked for 15 minutes in a graduated cylinder. Formation of one centimeter of foam layer specifies the existence of saponins (Kokate *et al.,* 2001).

3.9.4. Test for Tannins

Gelatin Test: aqueous solution of gelatin and Sodium Chloride are added to one milliliter of the ethanol leaf extract of *C. falcifolia*. Formation of white precipitate indicates the presence of tannins (Treare & Evans 1985).

3.9.5. Lead Acetate Test:

Five drops of 10% Lead Acetate solution were added to five milliliters of ethanol leaf extract of *C. falcifolia* in a test tube. Formation of yellow precipitate specified existence of tannins (Treare & Evans, 1985).

3.9.6. Liebermann-Burchard test:

Ten drops of aceticanhydride, five millilieter of concentrated sulphuricacid were added to two milliliter of the extract in a test tube. Existence of triterpenoids were confirmed by the formation of greenish color (Kokate *et al.,* 2001).

3.9.7. Test for Amino acid/ Protein

i) Biuret's Test: one milliliter of sodiumhydroxide, one milliliter of copper sulphate were added to three milliliter of extract. The existence of proteins in the extract was specified by modification of violet color to pink (Kokate *et al.,* 2002).

3.9.8. Tests for phenols

i) Ferric Chloride test: To 3 ml ethanol leaf extract of *C. falcifolia,* three milliliters of ferric acid was added then detected for the green or blue color formation which showed the presence of phenols (Kokate *et al.,* 2002)

3.9.9. Tests for Oils and Resins:

2 ml of ethanol leaf extract of *C. falcifolia* was applied on filter paper. The existence of oils and resins was confirmed by the transparent appearance on the filter paper indicated (Evans, 1996).

3.10. Identification and separation of flavonoids from the ethanol extract of *Culcasia falcifolia*

3.10.1. Preparative Silica Gel plates

Silica gel (TLC) plates were prepared with sample and standard and placed into the beaker closed and left for 5 minutes. The plate was removed, dried and visualized with the help of UV light at 254 nm in UV TLC viewer.

3.10.2. Extraction of Flavonoid Compounds present in ethanol extract of *Culcasia falcifolia*

Five milliliter of solvent (Butanol: acetic acid: water (BAW) water; 4: 1: 5, v/v/v), and Ethyl Acetate: Formic Acid: Acetic Acid: Water; volume ratio of 100:11:11:26 as mobile phase). was poured into the beaker with a lid along with a piece of filter paper.

3.10.3. Detection of flavonoids present in the ethanol extract of *Culcasia falcifolia*

The thin layer chromatography plates were visualized after drying and with the help of ultra violet light at 366 nm in ultra violet thin layer chromatography viewer. R_f values of reference flavonoids and flavonoids contained in the extracts were determined. R_f values were calculated and compared with that of the standards (Harborne, 1973).

3.11. Evaluation of antiepileptic activity of ethanol leaf extract of *culcasia falcifolia*

The animals were divided into four groups of six animals each**.** Group I served as negative control received 0.1% CMC. Group II served as positive control received diazepam (5mg/kg body weight). Group III received 200 mg/kg body weight of ethanol extract of *C. falcifolia* extract. Group IV received 400 mg/kg body weight

ethanol extract of *C. falcifolia*. Animals in Group II received the standard drug diazepam (5 mg/kg body wt.) only once (i.e. on the day of the experiment). All the test drugs were administered orally by suspending in 0.5% carboxy methyl cellulose solution**.** On the experimental day (21^{st} day), pentylenetetrazole (PTZ) (60 mg/kg body weight, i. p) was administered to all the groups to induce clonic convulsions. PTZ was administered 60 minutes after the administration of the two doses (200 and 400 mg/kg b. wt.) ethanol extract of *Culcasia falcifolia* to group III and IV. Pentylenetetrazole was injected 30 minutes after the administration of Diazepam to group II. Following the administration of PTZ, mice were placed in separate cages and were observed for the occurrence of seizures, primarily for thirty minutes, then up to twenty four hours. The subsequent factors were observed: Latency of convulsions (the time before the onset of tonic convulsions), duration of tonic convulsions, and mortality protection (percentage of deaths in 24 hours) were recorded (Fisher, 1989).

Percentage of animals protected by the drug from mortality was calculated by

$$= \frac{\text{Number of animals alive after 24 hours in the group}}{\text{Total number of animals in the group}} \times 100$$

3.12. Estimation of the levels of endogenous anti-oxidant enzymes

This test was done to evaluate the effect of the ethanol extract of *Culcasia falcifolia* on the endogenous enzymatic antioxidant activity in mice brain after induction of seizure by PTZ to further investigate the anticonvulsant activity of ethanol extract of *Culcasia falcifolia.*

3.12.1. Preparation of tissue homogenate

After the observation of convulsion all groups of mice were beheaded. Then the brains were immediately carefully seperated then washed with 0.9% cold normal saline two times and placed into a sterile cooled glass plate. Then the brains were separated and cut into pieces using small surgical scissors. The cut pieces were homogenized with motor driven Teflon coated homogenizer with 5 mL of ice-cold 0.1 M Tris-HCl buffer pH 7.4 to get 10% homogenate. The homogenate was centrifuged at 10,000 rpm for 20 min at 5˚C in order to separate the two phases. The overlaying organic phase was removed and the supernatant was collected and used for the estimation and *in-vivo* antioxidant activity. The readings were obtained using spectroflurimeter.

3.12.2. Lipid Peroxidation Assay

To 100 µL of the tissue homogenate, 2 mL of thiobarbituric acid (TBA) reagent was added and mixed. The mixture was incubated in a boiling water bath for 40 min, cooled to room temperature and centrifuged at 3500 rpm for 10 min. The pink colour developed was estimated at 535 nm against a reagent blank, in a spectrophotometer. Lipid peroxidation as expressed as nmol of MDA/mg protein (Fernandez *et al.,* 1997).

3.12.3. Assay for superoxidedismutase estimation

Assay was carried out by the way described by Sun and Oberly. Supernatants of brain tissue samples were incubated with xantine and xanthine oxidase in potassium phosphate buffer (pH 7.8, 37°C) for 40 min and Nitro blue tetrazolium chloride (NBT) was added. Blue formazan was then monitored spectrophotometrically at 550 nm. The inhibition of NBT reduction to 50% maximum by SOD enzyme was obtained and was defined as 1 nitrite unit (NU) of SOD activity (Sun & Oberley, 1988).

3.12.4. Estimation of Catalase

To 0.9 ml of phosphate buffer (0.01 M, pH 7.0), 0.1ml of tissue homogenate 0.4ml of hydrogen peroxide was added., 2.0 ml of dichromateacetic acid mixture was added after sixty seconds, then kept in boiling water bath for 10minutes, the fluorescence was read at 620nm. against blank reagent. Catalase was stated by means of µ mol /mg protein (Sinha, 1972).

3.12.5. Evaluation of Glutathione

2.5 ml of 0.02M Sodium acetate buffer (pH 6.9) was added to two milliliters of homogenate, and shaken vigorously then four milliliters of cold purified water and one milliliter of trichloroacetic acid was added. Subsequently, centrifuged for 15 min. Next two milliliter of the supernatant was mixed with 0.4M tris buffer (pH 8.9) and mixed well and 0.1 mL of 0.01M 5, 5'-dithiobis-(2-nitrobenzoic acid (DTNB) was added, the absorbance was read at 412 nm against reagent blank. Glutathione was stated as µ mol /g (Sedlak & Lindsay, 1968).

3.12.6. Estimation of Glutathione Peroxidase

To 0.2 ml of Tris Buffer, (0.4M, pH 7.0), 0.2 ml of sodiumacetate buffer (pH 6. 9), 0.1 ml of Sodium Azide and 0.5 ml of tissue homogenate were added. To this mixture, 0.2 ml glutathione followed by 0.1 ml Hydrogen Peroxide were added. The contents were mixed and incubated in water bath at 37^0C for 10 then 0.5 ml of 10% Trichloro acetic acid was added and centrifuged then supernatant was assayed for glutathione (Ellman, 1959).

3.13. Estimation of neurotransmitters level in the mice brain tissue of Pentylenetetrazole-induced seizure in mice

One milliliter of supernatant was added to 2.5 ml heptane and 0.3ml HCl of 0.1M shaked vigorous then centrifuged for 10 minutes at 2000 r.p.m in order to separate the two phases. The overlaying organic phase was removed. The aqueous phase was taken for the assay. The reading was obtained using a spectroflurimeter.

3.13.1. Estimation of noradrenaline and dopamine

0.05 ml 0.4 M HCl and 0.1 ml of Sodium acetate buffer (pH 6. 9) were added to the 0.2 ml of aqueous phase, then 0.1 ml iodine solution (0.1 M in ethanol) was added. After two minutes 0.1 ml sodium sulphate was added then heated to hundred degree celcius for six minutes. The readings were recorded at 345 nm for dopamine and 425 nm for noradrenaline (Schlumpf, 1974).

3.13.2. Estimation of Serotonin

0.25 ml of O-phthaldialdehyde (OPT) reagent was added to 0.2 ml aqueous phase and heated at 100°C for 10 min. 0.25 ml concentrated. Hydrochloric acid without OPT was added to serotonin tissue blank. Then the readings were taken at 360 nm in the spectrofluorimeter (Schlumpf, 1974).

3.13.3. Estimation of GABA

0.2ml of 0.14 M Ninhydrin solution, 0.5M carbonate-bicarbonate buffer (pH 9.95) were mixed with 0.1ml of tissue extract then in kept in a water bath at sixty degree celcius for 30 minutes, then allowed to cooled at room temperature then five milliliter of copper tartrate reagent were added. The developed fluorescence was read at 450nm in a spectofluorimeter (Lowe *et al.*, 1958).

3.14. Assessment of the effect of ethanol extract of *Culcasia falcifolia*

This test was done to assess the effect of the ethanol extract of *Culcasia falcifolia* on the central nervous system depressant effect in mice. The animals were divided into four groups of five mice each. The same four groups of mice were used for Rota rod test and Actophotometer test.

3.14.1. Drug treatment

Group I served as control (0.1% carboxy methyl cellulose)

Group II served as positive control treated with diazepam (5mg/kg b. wt.)

Group III treated with ethanol extract of *C. falcifolia* (200 mg/kg b. wt.)

Group IV treated with ethanol extract of *C. falcifolia* (400 mg/kg b. wt.)

3.14.2. Assessment of muscle relaxation

The skeletal muscle relaxation induced by a test compound could be evaluated by testing the ability of mice to remain on a revolving rod (rota rod). The Mice were then placed on revolving rod. The mice that remain on rotrod for 3 min were employed for the assessment. Twenty four mice weighing between 20 and 30 g are assessed. The speed of the rotating rod was adjusted to 25 rotations per minute. One hour after drug administration, the animals were positioned on the rotarod individually and the time taken by the animals to fall off the rotating rod was noted. Fall off time is the period of time for which the animal remains on the Rota rod before falling off. The alteration in the time of falling off from rotating rod before then after drug administration were considered as an key for muscle relaxation. Percentage decrease in fall off time was calculated using the formula ([Time b–Time a)/Time b] × 100 where Time b and Time a are fall off time before and after drug administration, respectively (Vogel, 2007).

3.14.3. Assessment of locomotor activity

Locomotor activities of the four groups of mice were monitored using actophotometer. The animals from each group were placed on the actophotometer individually, and basal activity score was recorded over the period of 5 min. Each animal was from each group were treated with respective drugs, and activity score was recorded after 30 minutes after the administration of diazepam 5mg/kg body weigh to group II and 1 hour after extract administration to group III and IV. The counts correspond to locomotor activity. Decreased activity score were considered as key for central nervous system depression. The differences in the locomotor action, before drug administration and after drug administration, were recorded. Calculations were done using the following formula to find out the percentage change in locomotor activity of mice

Change in Motor Activity = (A-B)/A x 100

Where; A: score before drug administration, B: Score after drug administration (Kulkari, 1999)

3.15. Analysis of data

All the experiments were done with the sample size of, 6 (n=6) was used. All the values of the results obtained were expressed as mean ± SEM. To compare the differences bewteen the means of the groups data were analyzed by using one way analysis of variance (ANOVA) followed by Dunnett's test. Graph Pad version 3 was used to analyse all the results. P values may be $p< 0.05$, $p<0.01$, $p<0.001$ were considered as significant.

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CHAPTER FOUR

RESULTS

4.1. Effects of acute oral toxicity of the ethanol extract of *Culcasia falcifolia*

The acute oral toxicity test of ethanol extract of *Culcasia falcifolia* did not show mortality on the dosages of 5, 50, 300 then 2000 mg/kg b. wt. in the mice used during the period (72 hours) of observation. The extract did not show any signs of changes in all the activities generally observed such as diahorreah, hypnosis, convulsions, stupor reaction, piloerection, changes in skin color, lacrimation, allergic reactions, and mortality in mice on the dosage of 5, 50, 300 mg/kg b. wt. However, at the higher dose of 2000 mg/kg b. wt. the extract showed signs of sedation and ptosis after 4 hours of administration of the dose. Death was not witnessed at the higher dosage 2000 mg/kg body weight. Hence, one tenth (200 mg/kg body weight) and one fifth (400 mg/kg body weight) of 2000 mg/kg body wt. was selected intended for further studies.

Table 2:Effects of acute oral toxicity study of the ethanol leaf extract of *Culcasia falcifolia* at 2000 mg/kg b. wt. in mice

General Behavior	5 mg/kg b. wt.	50 mg/kg b. wt.	300 mg/kg b. wt.	2000 mg/kg b. wt.
Sedation	-	-	-	+
Hypnosis	-	-	-	-
Convulsion	-	-	-	-
Ptosis	-	-	-	+
Pain distress	-	-	-	-
Stupor reaction	-	-	-	-
Allergic reactions	-	-	-	-
Pilo Erection	-	-	-	-
Changes in skin color and fur	-	-	-	-
Lacrimation	-	-	-	-
Diahorreah	-	-	-	-
Mortality	-	-	-	-
Lethargy	-	-	-	-
Difficulty in breathing	-	-	-	-

Note: '+' = present; '-' = absent

4.2. The phytochemicals in the ethanolic extract of *Culcasia falcifolia*

Preliminary qualitative phytochemical screening of the ethanol extract of the leaves of *Culcasia falcifolia* revealed the presence of alkaloids, flavonoids, saponins, tannins, phenols sterols and cardiac glycosides (Table 3).

Table 3: Phytochemical analysis of the ethanol extract of *Culcasia falcifolia.*

Phytochemicals	Observation
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Steroids	–
Terpenoids	–
Phenols	+
Sterols	+
Cardiac glycosides	+
Triterpenoids	–
Fixed oils and resins	–

Note: '+' = present; '-' = absent

4.3. Identification and separation of the bioactive phytochemical in the ethanol extract of *Culcasia falcifolia*

As a result of thin layer chromatography seven spots (spots A-G) were observed under ultraviolet light. The Rf values of separated components of *Culcasia falcifolia* extract were measured. Spot A was measured at Rf 0.27, Spot B was measured at Rf 0.36, Spot C was measured at Rf 0.45, Spot D was measured at Rf 0.54, Spot E was measured at 0.63, Spot F was measured 0.85 and Spot G was measured at 0.95. As a result of the comparison of Rf values, and colour changes under UV light, the flavonoid compounds in *Culcasia falcifolia* were identified as myrrcetin, kaempferol, isorhamnetin, apigenin, rutin, quercetin and chlorogenic acid eluted using ethyl acetate: formic acid: acetic acid: water (100:11:11:26 v/v/v/v as mobile phase) (Table 4).

Table 4: TLC results for ethanol extract eluted using ethyl acetate: formic acid: acetic acid: water in volume ratio 100: 11: 11: 26 as mobile phase

Spot	Trail	Trial 2	Trial 3	Mean Rf values	Flavonoids present	Colour in U.V + Ammonia
A	0.27	0.27	0.27	0.27		
B	0.36	0.36	0.36	0.36	Rutin	Bright Yellow
C	0.45	0.44	0.45	0.45	Myricetin	Bright Yellow
D	0.54	0.54	0.54	0.54	Isorhamnetin	Bright Yellow
E	0.65	0.65	0.65	0.63	chlorogenic acid	Bright Yellow
F	0.85	0.86	0.85	0.85	Apigenin	Bright Yellow
G	0.96	0.92	0.96	0.95	Quercetin	Bright Yellow

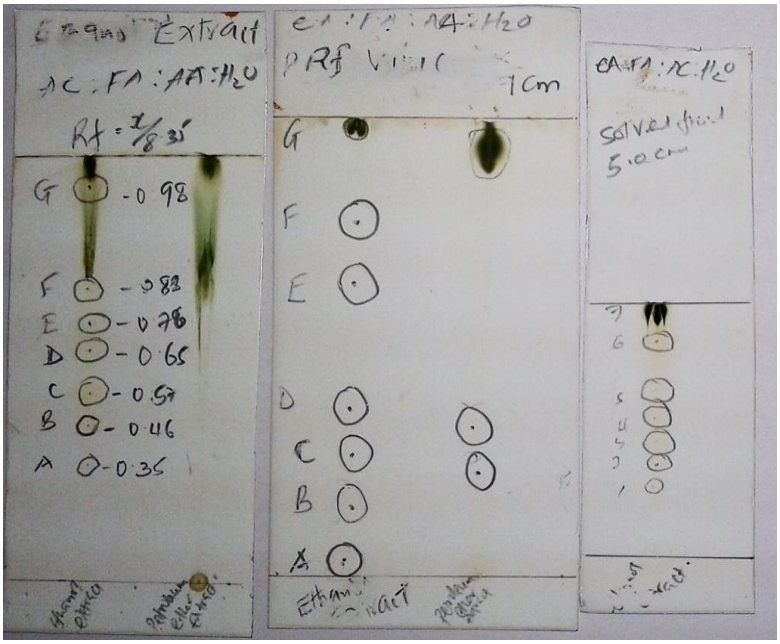

Plate 2: Chromatogram of flavonoids in the ethanol extract of Culcasia falcifolia

4.4. Effects of ethanol extract of *Culcasia falcifolia* and standard drug diazepam on pentylenetetrazole-induced seizure in mice

4.4.1. Effect of the extract of *Culcasia falcifolia* on the latency of tonic convulsions on Pentylenetetrazole-induced seizure in mice

There was a significant ($p<0.01$**, $p<0.001$***) increase in the latency of tonic convulsions in the groups pre-treated with the extract at the doses of 200 and 400 mg/kg body weight (243.72±6.90** and 402.56±5.52***) when compared to the control group (125.37±5.66). The standard drug diazepam used as a positive control showed significant ($F_{1.5}$= 65 .56, p=0.000) increase in the latency of tonic convulsion (Table 5).

4.4.2. Result of *Culcasia falcifolia* on the duration of tonic convulsions on Pentylenetetrazole-induced seizure in mice

There was a significant ($p<0.01$**, $p<0.001$***) decrease in the duration of tonic convulsions in the groups pre-treated using the extract at the dosages of 200 and 400 mg/kg body weight (192.62±7.72** and 158.99±8.66***) when compared to the control group (392.98±6.43). The standard drug diazepam used as a positive control showed significant ($F_{1.5}$= 249, p=0.000) decrease in the duration of tonic convulsion (Table 5).

4.4.3. Effects of the extract of *Culcasia falcifolia* on the percentage of protection on mortality on Pentylenetetrazole-induced seizure in mice

The groups pre-treated with the extract at the doses of 200 and 400 mg/kg body weight showed 33.33% and 100% protection on mortality of mice when compared to the control. The standard drug diazepam used as a positive control showed 100% protection on mortality of mice (Table 5).

Table 5 :Effects of ethanol extract of Culcasia falcifolia and standard drug diazepam on the latency and duration of tonic- clonic convulsion on Pentylenetetrazole-induced seizure in mice

Group	Drug treatment	Latency of Tonic Convulsion (sec)	Duration of Tonic Convulsion (sec)
Group I	Control 0.1 % CMC +PTZ	125.37±5.66	392.98±6.43
Group II	Diazepam 5mg/kg + PTZ	492.59±7.32***	135.77±6.78***
Group III	EECF 200mg/kg +PTZ	243.72±6.90**	192.62±7.72**
Group IV	EECF 400mg/kg + PTZ	402.56±5.52***	158.99±8.66***

Note: EECF: Ethanol extract of *Culcasia falcifolia;* CMC: Carboxy methyl cellulose**;** Values are in mean ± SEM (n=6); $p<0.01$, $p<0.001$ Statistical significant test for comparison was done by ANOVA, followed by Dunnett test.

Table 6: Effects of the extract of Culcasia falcifolia and standard drug diazepam on the percentage of protection on mortality in mice on Pentylenetetrazole-induced seizure in mice

Group	Drug treatment	(no. of animals alive after 30 min)	(no. of animals alive after 24 hours)	% Protection
Group I	Control 0.1% CMC +PTZ	0	0	0
Group II	Diazepam 5 mg/kg + PTZ	6	6	100
Group III	EECF 200 mg/kg +PTZ	6	2	33.33
Group IV	EECF 400 mg/kg +PTZ	6	6	100

Note: EECF: Ethanol extract of *Culcasia falcifolia;* CMC: Carboxy methyl cellulose**;** number of animals =6.

4.5. Effects of ethanol extract of *Culcasia falcifolia* and standard drug diazepam on the oxidative stress markers in the brain tissue on pentylenetetrazole-induced seizure in mice

4.5.1. Effect of the extract and standard drug diazepam on Malondialdehyde (MDA) levels on Pentylenetetrazole-induced seizure in mice

There was a alteration establish in the brain malondialdehyde levels between all the groups when compared to the control. The post Dunnett's analysis revealed the MDA level was increased in PTZ treated group (3.89± 0.47**) when matched to control group (1.62±0.32). Reversal in the increased MDA levels were observed in extract treated (200 and 400 mg/kg b. wt.) groups IV and V ($F_{1.5}$= 7.7, p=0.0003) in comparison to PTZ treated group (3.89± 0.47*) which were statistically significant (Table 7).

Table 7: Effects of ethanol extract of Culcasia falcifolia and standard drug diazepam on levels of Malondialdehyde (MDA) on Pentylenetetrazole-induced seizure in mice

Group	Drug treatment	Lipid peroxidation nmol MDA/mg protein
Group I	Control 0.1% CMC	1.62±0.32
Group II	PTZ 1ml/100gms	3.89± 0.47**
Group II	Diazepam (5mg/kg) + PTZ 60 mg/kg	4.24±0.52
Group III	EECF (200mg/kg)+PTZ 60 mg/kg	2.52± 0.28*
Group IV	EECF (400 mg/kg)+PTZ 60 mg/kg	1.97 0.45*

Note: EECF: Ethanol extract of *Culcasia falcifolia;* Values are expressed as mean ± SEM; p<0.05*, p<0.01**, Statistical significant test for comparison was done by ANOVA, followed by Dunnett test.

4.5.2. Result of the ethanol extract of *Culcasia falcifolia* and standard drug diazepam in levels of superoxide dismutase (SOD) on Pentylenetetrazole-induced seizure in mice

Low SOD activity was observed in PTZ treated group (9.13±0.46**). Animals treated with the extract at the doses of 200 and 400mg/kg body weight showed significant ($F_{1.5}$= 20.61, p=0.000) increase in SOD activity (12.47±0.62* and 13.59±0.62*) when compared to PTZ (9.13±0.46*) and diazepam treated group (9.62±0.46*) (Table 8).

4.5.3. Result of the ethanol extract of *Culcasia falcifolia* and standard drug diazepam in levels catalase on Pentylenetetrazole-induced seizure in mice

There was a significant ($p<0.05$) decrease in the levels of catalase in the PTZ treated group (15.45 ± 0.33**) when compared to control (20.47±0.34). The extract at the doses of 200 and 400mg/kg body weight showed significant ($F_{1.5}$= 34.96,p= 0.000) rise in catalase levels (18.63 ± 0.53* and 19.13 ± 0.26*) when compared to PTZ (15.45±0.33*) and diazepam treated group (16.05 ± 0.27*) (Table 8).

4.5.4. Result of the ethanol extract of *Culcasia falcifolia* and standard drug diazepam in levels of glutathione reductase on Pentylenetetrazole-induced seizure in mice

There was a significant ($p<0.01$**) decrease in the levels of glutathione reductase in the PTZ treated group (22.86±0.54**) when compared to the control group (29.12 ±0.41). The extract treated groups at the doses of 200 and 400 mg/kg body weight significantly ($F_{1.5}$= 39.28, p=0.0000) increased glutathione reductase levels (26.49±0.41* and 27.63±0.67*) when compared to the PTZ (22.86±0.54**) and diazepam treated group (21.98±0.36**) (Table 9).

4.5.5. Result of the ethanol extract of *Culcasia falcifolia* and standard drug diazepam in the levels of glutathione peroxidase (GPX) on Pentylenetetrazole-induced seizure in mice

There was a significant ($p<0.01$**) decrease in the levels of glutathione peroxidase in the PTZ treated group (17.43±0.29**) when compared to the control group (23.13±0.28). The extract treated groups at the doses of 200 and 400 mg/kg body weight significantly ($F_{1.5}$= 35.3. p=0.000) increased the glutathione peroxidase levels (21.43±0.57* and 22.74 ±0.49*) when compared to PTZ (17.43± 0.29**) and diazepam treated group (17.72± 0.49**) (Table 9).

Table 8: Effects of ethanol extract of *Culcasia falcifolia* and standard drug diazepam on SOD and CAT levels in the brain tissue on PTZ-induced seizure in mice

Group	Drug treatment	Superoxide dismutase (SOD) U/mg protein	Catalase (CAT) units/mg protein
Group I	Control 0.1% CMC	14.42±0.53	20.47±0.34
Group II	PTZ (SCMC) 1ml/100gms	9.13 ±0.31**	15.45± 0.33**
Group III	Diazepam (5mg/kg)+ PTZ 60mg/kg	9.62±0.46**	16.05±0.27**
Group IV	EECF (200mg/kg)+ PTZ 60 mg/kg	12.47± 0.62*	18.63± 0.53*
Group V	EECF (400mg/kg)+ PTZ 60 mg/kg	13.59± 0.62*	19.13± 0.26*

Note: EECF: Ethanol extract of the leaf of *Culcasia falcifolia*; Values are expressed as mean ± SEM; *p<0.05;**p< 0.01, Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test.

Table 9: Effects of ethanol extract of *Culcasia falcifolia* and standard drug diazepam on GSR and GPx levels in the brain tissue on PTZ-induced seizure in mice

Group	Drug treatment	Glutathione reductase (GSR) µmol/mg protein	Glutathione peroxidase (GPx) µmol/mg protein
Group I	control 0.1% CMC	29.12±0.41	23.13±0.28
Group II	PTZ (SCMC) 1ml/100gms	22.86 ±0.54**	17.43 ±0.29**
Group III	Diazepam(5mg/kg) + PTZ 60 mg/kg	21.98±0.36**	17.72±0.49**
Group IV	EECF (200mg/kg) + PTZ 60 mg/kg	26.49± 0.41*	21.43± 0.57*
Group V	EECF (400 mg/kg) + PTZ 60 mg/kg	27.63± 0.67*	22.74 ±0.49*

Note: EECF: Ethanol extract of the leaf of *Culcasia falcifolia*; data were expressed as mean ± standard error of means; *p<0.05;**p< 0.01 considered significant; Statistical analysis were performed by ANOVA, followed by posthoc Dunnett's test.

4.6. Effects of the ethanol extract of *Culcasia falcifolia* and standard drug diazepam on neurotransmitters oncentrations levels in the brain tissue on pentylenetetrazole-induced seizure in mice

4.6.1. Effects of ethanol extract and standard drug diazepam on GABA levels on Pentylenetetrazole-induced seizure in mice.

The levels of GABA in the PTZ treated group was decreased (195.27±2.53^{ns}) when compared to the control group (298.41±2.49). The extract pretreated groups at the doses of 200 and 400 mg/kg body weight significantly ($F_{1.5}$= 342.4,p=0.000) increased the GABA levels (248.52±1.73** and 274.29±1.49**) when compared to PTZ (195.27±2.53^{ns}) (Table 10).

4.6.2. Effect of the extract and standard drug diazepam on Serotonin levels on Pentylenetetrazole-induced seizure in mice

The levels of serotonin in the PTZ treated group was decreased (107.5±2.37^{ns}) when compared to the control group (203.1±1.31). The extract pretreated groups at the doses of 200 and 400 mg/kg body weight significantly ($F_{1.5}$=282.2,p=0.000) increased the serotonin levels (117.24±2.44** and 153.68±3.28**) when compared to PTZ (107.5±2.37). (Table 10).

4.6.3 Effect of the extract and standard drug diazepam on dopamine levels on Pentylenetetrazole-induced seizure in mice

The levels of dopamine in the PTZ treated group was decreased (522.41±3.24^{ns}) when compared to the control group (824.10±2.43). The extract pretreated groups at the doses of 200 and 400 mg/kg body weight significantly ($F_{1.5}$= 1793.14, p=0.001) increased the dopamine levels (703.21±3.27** and 791.48±2.32**) when compared to PTZ (522.41±3.24^{ns}). (Table 10).

4.6.4. Effect of the extract and standard drug diazepam on noradrenaline levels on Pentylenetetrazole-induced seizure in mice

The levels of noradrenaline in the PTZ treated group was decreased (437.17±2.63^{ns}) when compared to the control group (696.62±3.57). The extract pretreated groups at the doses of 200 and 400 mg/kg body weight significantly ($F_{1.5}$= 1158.1,p=0.000) increased the dopamine levels (573.61±2.26** and 707.42 ±4.71**) when compared to PTZ (437.17±2.63^{ns}) (Table 11).

Table 10: Effects of the ethanol extract of *Culcasia falcifolia* and standard drug diazepam on GABA and serotonin concentrations levels in the brain tissue of Pentylenetetrazole-induced seizure in mice

Group	Drug treatment	GABA Picogram/mg of wet tissue	Serotonin Picogram/mg of wet tissue
Group I	control 0.1% CMC	298.41±2.49	203.1±1.31
Group II	PTZ (SCMC) 1ml/100gms	195.27±2.53^{ns}	107.5±2.37^{ns}
Group III	Diazepam (5mg/kg) + PTZ 60 mg/kg	286.54±2.58**	164.54±1.46**
Group IV	EECF (200mg/kg) + PTZ 60 mg/kg	248.52±1.73**	117.24±2.44**
Group V	EECF (400 mg/kg) + PTZ 60 mg/kg	274.29±1.49**	153.68±3.28**

Note: EECF: ethanol extract of *Culcasia falcifolia;* Comparison between a- group I vs. group II; b- group III vs. group IV and group V**;** Values are expressed as mean ±SEM; ns- non significant, p< 0.01**, Statistical significant test by ANOVA, followed by Dunnett's t' test.

Table 11: Effects of the ethanol extract of *Culcasia falcifolia* and standard drug diazepam Dopamine and noradrenaline concentrations levels in the brain tissue Pentylenetetrazole-induction seizure in mice

Group	Drug treatment	Dopamine Picogram/mg of wet tissue	Noradrenaline Picogram/mg of wet tissue
Group I	Control 0.1% CMC	824.10±2.43	696.62±3.57
Group II	PTZ (SCMC) 1ml/100gms	522.41±3.24^{ns}	437.17±2.63^{ns}
Group III	Diazepam (5mg/kg) + PTZ 60 mg/kg	867.31±4.42**	629.83±2.34**
Group IV	EECF (200mg/kg) + PTZ 60 mg/kg	703.21±3.27**	573.61±2.26**
Group V	EECF (400 mg/kg) + PTZ 60 mg/kg	791.48±2.32**	707.42±4.71**

*Note***:** EECF: ethanol extract of *Culcasia falcifolia**;*** Comparison between a- group I vs. group II; b- group III vs. group IV and group V**;** Values are expressed as mean ±SEM; ns- non significant, p< 0.01**, Statistical significant test for comparison was done by ANOVA, followed by Dunnett's t' test.

4.7. Result of *Culcasia falcifolia* and standard drug diazepam on spontaneous locomotor activity and muscle relaxation activity in mice

4.7.1. The effect of ethanol extract of *Culcasia falcifolia* and standard drug diazepam on Rotarod Performance

The groups treated with ethanol extract of the leaves of *Culcasia falcifolia* at the doses 200 and 400 mg/kg body weight showed significant (p<0.01** and p<0.001***) reduction in the number of seconds spent on the Rota rod (55.06** and 77.37***) when compared to the control (Table 12).

Table 12: Effect of ethanol extract of *Culcasia falcifolia* and standard drug diazepam on muscle relaxant activity in mice on Rota rod

Drug Treatment	Time taken to fall from rotating rod (sec)		% of muscle griping
	Before Drug Treatment	After 60 minutes of Drug Treatment	
Control0.1 % CMC	59.31±2.27	61.88±2.46	-
Diazepam (5mg/kg)	62.77±1.58	9.82±0.41	84.36***
EECF (200mg/kg)	62.07±2.49	27.89±1.79	55.06**
EECF (400mg/kg)	63.71±2.93	14.42±1.02	77.37***

Note: EECF: Ethanol extract of the leaves of *Culcasia falcifolia;* CMC: Carboxy methyl cellulose; Values are in mean ± SEM (n=6); **p<0.01, ***p<0.001 vs. Control

4.7.2. Effect of ethanol extract of *Culcasia falcifolia* and standard drug diazepam on Actophotometer

The groups treated with the two doses of ethanol extract of the leaves of *Culcasia falcifolia* i.e. 200 and 400 mg/kg body weight indicated substantial (p<0.05* and p<0.01**) decrease in the locomotor activities (36.58* and 47.37**) when compared to the control (Table 13).

Table 13: Effect of ethanol extract of *Culcasia falcifolia* in mice on Actophotometer

Drug Treatment	Locomotor Activity in 10 minutes		% Change in Activity
	Before Drug Treatment	After 60 mts of drug Treatment	
Control 0.1 % CMC	307.66±12.92	284.41±16.03	-
Diazepam (5mg/kg)	296.37±9.87	137.62±11.22	53.56***
EECF (200mg/kg)	289.51±10.46	183.61±10.42	36.58*
EECF (400mg/kg)	299.42±15.52	157.58±9.05	47.37**

Note: EECF: Ethanol extract of the leaves of *Culcasia falcifolia;* CMC: Carboxy methyl cellulose**;** Values are in mean ± SEM (n=6); *P < 0.05, **P<0.01.

CHAPTER FIVE

DISCUSSION

5.1. Oral acute toxicity of the ethanolic extract of *Culcasia falcifolia*

The aim of the acute toxicity test was to determine the therapeutic index, which is the ratio between the lethal dose and the effective dose of ethanolic extract of *Culcasia falcifolia*. Acute toxicity test was carried out to measure the relative safety of the ethanolic extract of *Culcasia falcifolia* in the treatment of epilepsy induced by Pentylenetetrazole in mice. The findings of the acute toxicity study exhibited that the extract at 2000 mg/kg dose was mildly toxic as it showed signs of sedation and muscle relaxation.

The extract was considered as no death was observed at 2000 mg/kg body weight. Globally Harmonized System (GHS) of Classification and labeling of Chemicals, considers the substances having an lethal dose 50% (LD_{50}) value higher than the dosage of 2000 mg/kg b. wt. as relatively safe (GHS, 2005). According to Konan et al. (2007) therapeutic herbal extracts having LD_{50} values greater 2000 mg/kg b. wt. and as per the Globally Harmonized system standards, these extracts have been considered to be relatively safe which is in agreement with the finding of this study.

Acute toxicity study of ethanolic extract of *Culcasia falcifolia* provided very important data on the toxicity profile that might be useful for any future study of this plant medicine. *Culcasia falcifolia* ethanolic extract was found to be less toxic when oral acute toxicity test in mice were performed. These findings showed that the use of extract of *Culcasia falcifolia* is safe to use in the treatment of epilepsy in mice.

5.2. Phytochemical properties of the ethanolic extract of *Culcasia falcifolia*

The findings of the phytochemical analysis of the ethanolic extract of *Culcasia falcifolia* revealed the presence of of alkaloids, flavonoids, saponins, tannins, phenols, sterols and cardiac glycosides. The results suggest that the phytochemicals identified in the ethanolic extract of *Culcasia falcifolia* attributes to its antioxidant and antiepileptic activity against pentylenttetrazole induced seizure in mice. The findings are in agreement with Hui-Ling Zhu et al. (2014) that the phytochemicals in plant extracts like alkaloids, flavonoids, saponins have been reported to possess anticonvulsant activity on Pentylenetetrazole-induced seizure.

5.3. Phytoflavonoids recognized in *Culcasia falcifolia*

The findings of thin layer chromatography showed the presence phytoflavonoid compounds such as the myrrcetin, kaempferol, isorhamnetin, apigenin, rutin, quercetin and chlorogenic acid. The results of the present study showed that the phytoflavonoids identified in the ethanolic extract of *Culcasia falcifolia* are the bioactive compounds responsible its antiepileptic activity against Pentylenetetrazole-induced seizure in mice. According to Devi et al. (2008) among all the phytochemical found in natural products, phytoflavonoids are the most important bioactive product isolated from traditional herbs in the treatment of epilepsy.

Phytoflavonoids like rutin, apigenin, kaempferol, myrricetin, and quercetin isolated from different plant extracts exert their mechanism of action and acts as agonitic to GABA receptors, blocker of Na^+ ion channels, block Ca^+ ion channel, antogonistic to glutamate receptors and also possess antioxidant activity thereby act as anticonvulsant.

The findings of the research conducted by Zongo et al. (2013) showed that the flavonoids epicatechin, quercetin, kaempferol identified in the extract *Waltheria indica* L possesses therapeutic potential in the prevention of oxidative stress which is concurrent with the findings of the present study. that the phytoflavonids identified such as myrrcetin, kaempferol, isorhamnetin, apigenin, rutin, quercetin.

The mechanism of seizure generated in epilepsy is inhibited by flavonoids in different means; decrease influx of sodium ion by inhibiting voltage gated sodium ion channels; by increasing potassium ion outflow; by inhibition of of gamma amino butyric acid receptors which results in chloride ion influx causin hyperpolarization in neurons (Paramdeep, 2014). The findings of the present study infer that the identified flavonoids such as myrrcetin, kaempferol, isorhamnetin, apigenin, rutin, quercetin could be responsibe in various ways for the antiepileptic activity of the ethanolic extract of *Culcasia falcifolia.*

5.4. Anti-epileptic action of *Culcasia falcifolia*

In the present study, the effect of the ethanolic extract of *Culcasia falcifolia* was evaluated on Pentylenetetrazole-induced seizure in mice. The results demonstrated that *C. falcifolia* ethanolic extract produced potent anticonvulsant activity on pentylenetetrazole-induced seizures in mice. *Culcasia falcifolia* significantly delayed the beginning and reduced length convulsion. The dosage of the extract that showed lowest or no mortality and reduced the duration of clonic seizures was considered as the most effective dose for the protection against pentylenetetrazole induced seizures. In the present study, the group treated with the dosage, that is, 400 mg/kg body weight of ethanolic extract of *C. falcifolia* showed 100% protection against mortality, whereas, the group administered with the dosage 200 mg/kg body weight of *Culcasia*

falcifolia showed 33.33% protection against mortality. Therefore, 400 mg/kg of the ethanolic extract of *C. falcifolia* is taken as the effective dosage that produced antiepileptic activity. Likewise in the present study, ethanolic extract of *C. falcifolia* at the dose of 400 mg/kg body weight showed significant activity by delaying the onset of clonic convulsions and decreasing duration of convulsions and offered 100% protection against mortality which is comparable to the action of the standard drug, diazepam. Studies have shown that diazepam, a standard anticonvulsant drug, act as anticonvulsant against Pentylenetetrazole-induced seizures in mice by activation of gamma amino butyric acid receptors and facilitate the GABA mediated opening of chloride channel (Mohamed & Ojewole, 2006).

Pentylenetetrazole is an antagonist of gamma-amino butyric acid (GABA) at $GABA_A$ receptor which has been widely implicated in epilepsy (Mishra *et al.*, 2011). The $GABA_A$ receptors are ligand-gated ion channels, which mediate the most common inhibitory transmission in synapses. The $GABA_A$ receptor function not only prevents the development of epilepsy, but also inhibits the development of convulsive activity throughout the cerebral cortex tissues (Fisher, 2005). Studies have shown that drugs which protect animals against the seizure induced by PTZ reduce the T-type of Ca^{++} currents or inhibit GABA-mediated neurotransmission, elevate the seizure threshold and are effective in myoclonic and absence seizures (Mishra *et al.*, 2011). Prevention of PTZ-induced seizures in laboratory animals is the most commonly used preliminary screening test for characterizing potential anti-convulsive drugs. The test is assumed to identify anticonvulsant drugs effective against generalized clonic seizures. The antiepileptic drug should increase the threshold for clonic and tonic convulsions. The mechanism by which PTZ exert its convulsive action is by acting as an antagonist at the $GABA_A$ receptor complex. Drugs that offer protections against

tonic–clonic seizures induced by PTZ in rodents are considered to be useful to control of myoclonic and absence seizures in humans (Khosla & Pandhi, 2001).

The findings of the study showed that the ethanolic extract at 400 mg/kg b. wt. increased latency of convulsion produced by PTZ and decreased duration of convulsion produced by PTZ to the maximum like the standard drug diazepam. Therefore, the extract acts as an anticonvulsant by modulating $GABA_A$ receptor mediated inhibitory neurotransmission. PTZ may be exerting their convulsant effects by inhibiting the activity of gamma amino butyric acid (GABA) at $GABA_A$ receptors (Sankari *et al.*, 2010). The enhancement of GABA neurotransmission will attenuate convulsion and inhibition of GABA neurotransmission will enhance convulsion (Gale, 1992). The ethanolic extract of *Culcasia falcifolia* is able to enhance GABA neurotransmission at $GABA_A$ receptor complex to attenuate the convulsion caused by pentylenetetrazole. Studies have shown that diazepam, act as anticonvulsant against PTZ-induced seizures in mice by activation of $GABA_A$ receptors and facilitate the GABA mediated opening of chloride channel (Mohamed & Ojewole, 2006).

Hema and colleagues showed that the anticonvulsant activity of the Drosera burmanni may be attributed due to flavonoids causing enhancement of $GABA_A$ receptor activity and blocking of glutametargic excitation (Hema *et al*., 2009). In another study the activity of ethanol extract of the Abelmoschus manihot on PTZ induced convulsion the extract showed anticonvulsant effect. The potential active components of extract identified were isoquercitrin, hyperoside, hibifolin, quercetin-3-O-glucoside, quercetin, and isorhamnetin (Guo *et al.*, 2011). The outcomes of the anticonvulsant activity exerted by the *Culcasia falcifolia* displayed anticonvulsant action against Pentylenetetrazole-induced seizure in mice is due to the presence of the flavonoids

such as kaempferol, myricetin, isorhamnetin and quercetin may act via reduction of T-type calcium currents, enhancement of $GABA_A$-BZD receptor activity and blocking of glutametargic excitation mediated by NMDA receptors.

5.5. Antioxidant activity of ethanolic extract of *Culcasia falcifolia*

The present study showed increase in malondialdehyde (MDA) level and decrease in endogenous antioxidant enzyme level such as superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR) and glutathione peroxidase (GPx) in the Pentylenetetrazole treated group when compared to the control group brain tissue homogenate. The ethanolic extract of *Culcasia falcifolia* at 200 and 400 mg/kg body weight showed significant decrease in MDA level and increase in the antioxidant enzyme level, which specifies reduction of lipid peroxidation and intensification of antioxidant defence.

In the present study, it was observed that the extract increased the superoxide dismutase relative to PTZ treated mice. This result shows that the ethanolic extract with antioxidant effect is able to preserve the antioxidant enzyme SOD. A significant low level of catalase in PTZ treated mice was observed. Catalase is an endogenous antioxidant enzyme protects the cells against hydrogen peroxide generated inside the cells as a result of oxidative stress (Usui *et al*., 2009).

In the present study, it was observed that the extract increased the glutathione peroxidase and glutathione reductase levels relative to PTZ treated mice. The oxidative stress in the brain is a common mechanism of cellular damage in many acute neurological attacks, such as seizure activity (Oliver *et al.,* 1990). In the normal body system, the harmful effects of oxidative stress and free radical are controlled to some extent by antioxidant systems such as SOD enzyme (Freitas, 2009).

The findings of this study showed that the *Culcasia falcifolia* ethanolic extract weaken the oxidative stress initiated during seizure induced by pentylenetetrazole. The increase in superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase levels indicates the neuroprotective property of the ethanolic extract of *C. falcifolia*. The extract reduced the free radical-mediated lipid peroxidation indicated by a decrease in the malondialdihyde levels and the extract exerted antioxidant effect by reducing the generation of free radicles. The ethanolic extract of *C. falcifolia* exerts its antiepileptic activity on Pentylenetetrazole-induced seizure in mice which is mediated via attenuation of oxidative stress by combating with the free radicals and augmentation of enzymatic antioxidant defence.

5.6. *Culcasia falcifolia* ethanolic extract and Neurotransmitter levels in mice

The findings shows, *Culcasia falcifolia* ethanolic extract at the dosage of 200 then 400 mg/kg body weight significantly increased the level of inhibitory neurotransmitter GABA also significantly increased the levels of dopamine, noradrenalin then serotonin when related to PTZ treated group. Many drugs that increase the brain contents of GABA have exhibited anticonvulsant activity against seizures induced (Sandhyarani *et al.,* 2014). Slight deficiencies in GABA-ergic transmission may lead to hyper excitability and pathological neuronal discharges leading to epilepsy. GABA is an endogenous agonist at $GABA_A$ receptor (ionotropic receptor) thereby opening the channels to chloride ions in the neuronal membrane (Katzung, *2009*).

Epileptic action of PTZ involves disruption of GABA-ergic neurotransmission in the central nervous system. Several anti-epileptic drugs, in current clinical use facilitate GABA neurotransmission by different mechanisms: benzodiazepines, such as diazepam modulate the action of GABA by enhancing chloridecurrents in channels linked to different receptor sites (Amoreaux, 2010). The extract showed significant

increase GABA content in brain. This suggests that the anticonvulsant activity of *Culcasia falcifolia* extract is probably through elevation of brain GABA content. The increase in the serotonergic transmission raises the threshold of pentylenetetrazole induced seizures in many animal test systems, thereby protecting against PTZ induced convulsions (Srinivasan *et al.,* 2009). The brain serotonin is thought to have an epileptic effect and antidepressant drugs-like selective serotonin reuptake inhibitors-have proved to be useful in seizure control (Abdel-Reheim *et al*., 2008). Noradrenaline has also a role to play in the control of seizures, but less significantly when compared with other biogenic amines, as it is mainly concerned with blood pressure regulation (Saravana & Gandhimathi, 2009). Sandhyarani *et al.,* (2014) showed that *Barringtonia acutangula L.* ethanolic leaf extract increased neurotransmitter levels significantly in rat's forebrain. The extract increased the seizure threshold and decreased the susceptibility to MES induced seizure in rats. The study suggested that ethanolic extract of leaves of *B. acutangula* possess antiepileptic properties that may be due to restored biogenic amines in rat brain. Senthil (2016) observed that the methanolic extract of *Oxalis corniculata* significantly reestablished the levels of brain gmma aminobutyric acid, dopamine, noradrenaline then serotonin in mice brain. The study suggested that methanolic extract of *O. corniculata* increased the levels of biogenic amine on rat brain, which diminished the predisposition to pentylenetetrzole induced seizure.

The findings of the present study suggests that the antiepileptic activity offered by the ethanolic extract of *Culcasia falcifolia* against Pentylenetetrazole-induced seizure may be due to the increase in GABA, noradrenaline, dopamine and serotonin levels in mice. The ethanolic extract of *C. falcifolia* decreased seizure susceptibility to

Pentylenetetrazole-induced seizure in mice due to restoration of GABA, serotonin, dopamine and noradrenaline concentration levels in mice brain.

5.7. Effect of the ethanolic extract of *Culcasia falcifolia* on motor coordination and locomotor activity in mice

Culcasia falcifolia ethanolic extract was evaluated the skeletal muscle relaxation (motor coordination) using Rota rod test and the locomotor activity using Actophotometer test to assess the central nervous system depressant property of the ethanolic extract of *C. falcifolia* in mice. The results of the present study showed that the ethanolic extract of *C. falcifolia* has muscle relaxant effect in mice on Rota rod test, the extract also showed central nervous system depressant effect by decrease in the locomotor activity of mice on Actophotometer.

Locomotor activity is considered as an index of alertness and a decrease in it is indicative of sedation. The antiepileptic effects of drugs such as benzodiazepines are accompanied by decreased locomotor activity. The extract reduced locomotor activity in this experiment. The extract showed central nervous system depressant activity to a lesser extent than diazepam. The ethanol extract of *Culcasia falcifolia* like other plant extracts with antiepileptic activity and decrease in locomotor activity and muscle relaxation and these include *Xeromphis niclotica* (Danjuma *et al.,* 2009), *Palisota hirsute* (Woode *et al*., 2010), *Balanites aegyptiaca* (Ya'u *et al.,* 2011), *Viscum album* (Gupta *et al.,* 2012) and *Zizyphus nummularia* (Goyal, 2014).

The receptor complex gamma amino butyric acid type A is involved in the central nervous system depressant activity. Diazepam, follows on the $GABA_A$ receptors, causing membrane hyperpolarization, in the end leads to decrease in the firing rate of neurons in the brain or by directly acting on the GABA receptor where increase in the

neurotransmission of gamma amino butyric acid has a restriction effect on the stimulatory pathways causing a psychologically calming effect" (Kolawole *et al.,* 2007). The gamma amino butyric acid type A receptor complex consist of a chloride channel and binding sites for several drug compounds, (Korpi *et al.,* 2002). Flavonoids are able to impact central nervous system action both by binding to the site on the $GABA_A$ receptor resulting in sedation or anti-convulsive effects (Choudhary *et al*., 2011; Guo *et al.,* 2011). Many flavonoids were found to be ligands for the gamma amino butyric acid tye A receptors in the CNS; and to bind to the benzodiazepine binding site with resulting depressant actions in mice (Marder & Paladini, 2002). The decrease in locomotion is due to increase in GABAergic transmission (Chitra *et al*., 2014). Different types of anxiolytic, muscle relaxant; sedative-hypnotic drugs have explicated their mode of action through $GABA_A$ receptor (Khatun *et al.,* 2011). Flavonoids have been reported by several researchers to be responsible for sedative and inhibition of spontaneous motor activity in mice (Viswanatha *et al*., 2006; Musa *et al*., 2008).

The study found that the ethanolic extract of *C. falcifolia* exerts central nervous system calming action by reducing the locomotor action on actophotometer and reducing the time spent on rota rod showing skeletal muscle relxant action owing to the increase in the level of gamma amino butyric acid level.

CHAPTER SIX

CONCLUSION AND RECOMENDATION

6.1. Conclusion

From the research that has been carried out, it could be concluded that the ethanol extract of *Culcasia falcifolia* exerts its antiepileptic activity by increasing the latency and decreasing the duration of seizure on Pentylenetetrazole-triggered seizures in mice. The ethanol extract provided safety against oxidative stress in pentylenetetrazole induced mice brain by breaking the free radical formed and by the stimulation of endogenous antioxidant enzymes. The ethanolic extract of *Culcasia falcifolia* showed antiepileptic activity due to restoration of GABA, serotonin, dopamine and noradrenaline concentration levels in mice brain. The extract also expressed central nervous system depressant effect. The phytochemical screening of the ethanolic extract of *Culcasia falcifolia* showed the presence of of alkaloids, flavonoids, saponins, tannins, phenols, sterols and cardiac glycosides.The ethanolic extract of *Culcasia falcifolia* showed the presence of flavonoid compounds such as myrrcetin, kaempferol, isorhamnetin, apigenin, rutin, quercetin and phenolic compound such as chlorogenic acid that were recognized as the active components.

6.2. Recommendation

The conclusions of the study reveals that *Culcasia falcifolia* is potent and nontoxic in the traditional therapy of epilepsy. Additional researches must be led on further acute and chronic models of epilepsy using diverse epileptic agents to explore antiepileptic activity of the extract and for the subsequent progress of new antiepileptic medicine with advanced effectiveness.

REFERENCES

Abbès, S., Ouanes, Z., Ben Salah-Abbès, J., Houas Z., Oueslati R, Bacha, H., & Othman, O. (2006). The protective effect of hydrated sodium calcium aluminum silicate against hematological, biochemical and pathological changes induced by Zeara lenone in mice. *Toxicon* 47, 567-574.

Abdel-Reheim, E. S., Ahmed R. R., & Mohamed E.T. (2008). Modulatory effects of pumpkin seed oil on pilocarpine – model of epilepsy in rats compared to topiramate as a common antiepileptic drug. *Journal of the Egyptian German Society of Zoology*, 57C, 151-195.

Akdogan, I., & Yonguc, N.G. (2011). Experimental epilepsy models andmorphologic alterations of experimental epilepsy models in brain and hippocampus,underlying mechanisms of epilepsy. Croatia: *In Tech*; p. 269-82.

Aji, B.M., Effraim, K.D., & Onyeyili, P.A. (2001). Anti-stress activities of *Mitragyna Africana* (Wild) stem bark extract, *The Sciences*.105-107.

Almeida, E.R., Rafael K.R.O., Couto G.B.L., & Ishigami A.B.M. (2009). Anxiolytic Anticonvulsant Effects on Mice of Flavonoids, Linalool, and α-Tocopherol Presents in the Extract of Leaves of *Cissus sicyoides* L.(Vitaceae). *Journal of Biomedicine and Biotechnology.* Article ID 274740. 10.1155/2009/274740.

Almeida, J.R.G.S., & Quintans-Júnior L.J. (2006). Natural products inhibitors of the acetylcholinesterase. *Rev Bras Farmacogn 16*, 258-285.

Agra, M.F., França P.F., & Barbosa-Filho J.M. (2007). Synopsis of the plants known medicinal and poisonous in Northeast of Brazil. *Rev Bras Farmacogn 17*, 114-140.

Ahmadiani, A., Mandgary, A., & Sayyah, M. (2003). Anticonvulsant effect of flutamide on seizures induced by pentylenetetrazole: involvement of benzodiazepine receptors. *Epilepsia.* 44, 629–35.

Akash, K.S., Vinutha, N., Sagar, F., Udasimath., Kalleshappa, C.M., Krupanidhi, Akshara (2017). Evaluation Of Skeletal Muscle Relaxant Activity Of Ethanol Extract of *Chromoleana Odorata*. *IOSR J. Pharm*. 7 (3)1, 92-95

Amabeokua, G.J., Green, I. & Kabatende, J. (2007). Anticonvulsant activity of *Cotyledon orbiculata* L. (Crassulaceae) leaf extract in mice. *J Ethnopharmacol* 112: 101-107.

Amos, S., Kolawole, E., Akah, P., Wambebe, C., & Gamaniel, K. (2001) . Behavioral effects of the aqueous extract of *Guiera senegalensis* in mice and rats. *Phytomedicine*. 8, 356-361.

Anand, B. (2012). Actophotometer standard procedure. https://pharmawiki.in/actophotometer-standard-operating-pocedure.

Anne, T. B., & John J. M. (2013) "The 2010 Revised Classification of Seizures and Epilepsy" *Continuum (Minneap Minn)* 19(3),571–59

Armijo, J.A., Valdizán, E.M., De Las Cuevas .I., & Cuadrado A. (2002). "Advances in the physiopathology of epileptogenesis: molecular aspects". *Rev Neurol*. 34, 409–429.

Arumugam, S., Palanivelu ,A., Retnasamy, G., & Ramaiyan, D. (2009). Study on the antiseizure activities of inner bark of *Guettarda Speciosa*. *Iran J Pharmacol Thers*.8,73-76.

Aseervatham, G. S. B., Sivasudha, T., Sasikumar, J. M., Christabel, H., Jeyadevi, P. Ananth, D. A. (2014). Antioxidant and hepatoprotective potential of *Pouteria campechiana* on acetaminophen-induced hepatic toxicity in rats," *Journal of Physiology and Biochemistry,* 70(1) 1, 1–14.

Ayyildiz, M., Coskun, S., Yildirim, M. & Agar E. (2007). The effects of acid on penicillin-induced epileptiform activity in rats. *Epilepsia* 48, 1388-1395.

Bagdy, G., Kecskemeti, V., Riba, P.,& Jakus, R. (2007). Serotonin and epilepsy. *J. Neurochem.*100, 857-873.

Barbosa-Filho, J.M., Medeiros, K.P., Diniz, M.M., Batista, L.M, Athayde- Silva M., Cunha, E., Barnham, K. J., Masters C. L.,& Bush, A. I. (2004). Neurodegenerative and oxidative stress. *Nature Reviews Drug Discovery* 3(3), 205–214.

Banerjee, P.N., Filippi, D., Allen Hauser, W. (2009).The descriptive epidemiology of epilepsy a review. *Epilepsy Res*. 85 (1),31–45.

Brodie, M. J., & Kwan, M. (2012) Current position of phenobarbital in epilepsy and Future. *Epilepsia*, 53(Suppl. 8):40–46. DOI: 10.1111/epi.12027

Brunton, L.L., Goodman L.S., Blumenthal, D., & Buxton I. (2007). Goodman and Gilman's manual of pharmacology and therapeutics. United States: McGraw-Hill Professional. Chapter 19 Pharmacotherapy of the Epilepsies; 321-337.

Cecilia, B., Martina, D.F., Alessio, F., Susanna, P.& Massimiliano, T. (2013). Flavonoids as Antioxidants and Developmental Regulators: Relative Significance in Plants and Humans. *Int J Mol Sci*. 14(2), 3540–3555.

Chakraborty, P., Bala, N.N., & Das, S. (2016). Evaluation of the locomotor and skeletal muscle relaxant activities of ethanolic extracts of *Acacia nilotica* Linn. BARK. (Mimosaceae). *Indian J Pharm & Pharmacol*. 3(4),206-209.

Chitra, K.K., Babitha, S., Sharanbasappa, D., Thippeswamy, B.S., Veerapur, V.P., & Badami S. (2014). Anti-epileptic and Anti-psychotic Effects of *Ipomoea reniformis* (Convolvulaceae) in Experimental Animals. *Journal of Natural Remedies*. 14 (2), 153-163.

Choudhary, N., Bijjem, K.R., & Kali, A.N. (2011). Antiepileptic potential of flavonoids fraction from the leaves of *Anisomeles malabarca*. *Journal of Ethnopharmacology*, 135(2), 238-242.

Chowdhury, B., Subrat, K. B., & Mangala, C. D. (2013). Anti-convulsant action and amelioration of oxidative stress by Glycyrrhiza glabra root extract in pentylenetetrazole- induced seizure in albino rats. *Indian J Pharmacol*. 45(1), 40–43. DOI: 10.4103/0253-7613.106433

Czapinski, P., Blaszczyk, B. & Czuczwar, S.J. (2005). Mechanisms of Antiepileptic Drugs. *Current Topics in Medicinal Chemistry* (5)1, 3-143, ISSN 1568-0266.

Das, K, Tiwari, R.K.S.,& Shrivastava, D.K. (2010). Techniques for evaluation of medicinalplant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research*. 4(2), 104-111.

David, A. (2010). Anticonvulsant And Neurobehavioural Effects of the Aqueous Leaf Extract of *Leea Guineensis* G. Don (Family: Leeaceae). M.phil thesis.

Dilipkumar, P., Abhijit, S., Sumanta, G., Sandip, J. & Soumit, M. (2011). CNS Depressant activities of roots of *Coccos nucifera* in mice, *Acta Poloniae Pharaceutica. Drug Research*, 68, 249- 254.

Devi, P.U., Manocha, A., Vohora, D. (2008). Seizures, antiepileptics, and oxidative stress:an insight for researchers. *Expert Opin Pharmacother*. 9(3),169–3177.

Duncan, J.S. (2002). The promise of new antiepileptic drugs. *Br J Clin Pharmacol* 53(2)**,** 123-131.

Ecobichon, D.J., The basis of toxicology testing. CRC Press, New York. 1997;4386.

Ellman, G.L. (1959). Tissue sulfhydrl groups. *Arch. Biochem. Biophys*.80,70-77.

Elzbieta, P., Pawe, Z., Fergus, N., Jadwiga, K., Henryk, B., Tuomas, W., Pekka, K., Sakaewan, O., Markus, K., & Faik A. (2013). Antioxidant agents and physiological responses in adult epileptic patients treated with lamotrigine. *Pharmacological Reports.* 65: 99-106

Eraković, V., Zupan, G., Varljen, J., Laginja, J., & Simonić, A. (2001). Altered activities of rat brain metabolic enzymes caused by pentylenetetrazol kindling and pentylenetetrazol--induced seizures. *Epilepsy Res*. 43(2),165-73.

Farnsworth, N.R. (1989). Screening plants for new medicines. *In: Ed, Biodiversity, Part II. National Academy Press, Washington*. 83-97.

Fisher, R.S. (1989). Animal models of the epilepsies. *Brain Res Rev*. 14, 245-78.

Fisher, R.S., Van Emde Boas, W., Blume, W., Elger, C., Genton, P., Lee, P., & Egel. (2005). Epileptic seizures and epilepsy: Definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia*. 46:470-472.

French, J.A. (2007). Refractory epilepsy: clinical overview. *Epilepsia* ;48(1), 3–7.

Golechha, M., Bhatia, J., Arya, D.S. (2010). Hydro alcoholic extract of *Emblica Officinalis* Gaertn. affords protection against PTZ- induced seizure, oxidative stress and cognitive in rats". *Ind J Exp Biol 48*, 474-478.

Gidal, B.E., & Garnett, W.R. (2005). Epilepsy. In: Dipiro J.T, Talbert R.L, Yee G.C, Matzke G.R, Wells B.G, Posey L.M, eds. Pharmacotherapy: a pathophysiologic approach. USA: McGraw-Hill Companies Inc, 6th ed.1023-46.

Govardhan, P., Jagdeesh, B., Suresh, B., & Praveen, K. (2011). In vitro evaluation of effect of *Cassytha Filiformis* L. extracts on Brain neurotransmitters in Albino rats. *Journal of science* 1(1), 21-25.

Guillemain, J. & Tetau, M. (1980). "Contribution à l'étude d'un "tranquillisant Tiliatomentosa Bourgeons. *Cahiers de Biothérapie*, 68, 1-8.

Ghogue, J.P., & Ali, M. (2010). *Culcasia falcifolia*. The IUCN Red List of Threatened Species http://dx.doi.org/10.2305/IUCN.UK.2010 3.RLTS.

Gluck, M.R., Jayatilleke, E., Shaw, S., Rowan, A.J., & Haroutunian, V. (2000). CNS oxidative stress associated with the kainic acid rodent model of experimental epilepsy**.** *Epilepsy Res*, 39 (1), 63–71.

Guo, J., Xue, C., Duan, J.-A., Qian, D., Tang, Y., & You Y. (2011). Anticonvulsant, antidepressant-like activity of *Abelmoschus manihot* ethanol extract and its potential active components *in vivo* . *Phytomedicine*. 18(14),1250–1254.

Hanna, N.J., Black, M., & Sander, J.W. (2002). The national sentinel audit of epilepsy related death. The Stationary Office, London. 1-135.

Hanneke, M., De Boer., Marco Mula., Josemir. W., & Sander, (2008). "The global burden and stigma of epilepsy". 12:4:540-546.

Halliwell, B., Gutteridge, J. (2007). *Free Radicals in Biology and Medicine* 4th Ed., Chapter 4: Cellular responses to oxidative stress: adaptation, damage, repair, senscence and death. 2007.

Hegde, K., Thakker, S.P., Joshi, A.B., Shastry, C.S., & ChandraShekha,r K.S. (2009). Anticonvulsant Activity of *Carissa carandas* Linn.Root Extract in Experimental mice. *Tropical Journal of Pharmaceutical Research*. 8 (2),117-125.

Hirtz, D., Thurman, D.J., Gwinn-Hardy, K., Mohamed, M., Chaudhury, A.R.,& Zalutsky, R. (2007). How common are the 'Common' neurologic disorders? *Neurology* 68 (5),326-37.

Jäger, A. K., Krydsfeldt, K., & Rasmussen, H. B. (2009). Bioassay-guided isolation of apigenin GABA-benzodiazepine activity from *Tanacetum parthenium* . *Phytotherapy Research.* 23(11),1642–1644.

Jager, A.K., Gauguin, B., Adsersen, A., & Gudiksen, L. (2006). Screening of plants used in Danish folk medicine to treat epilepsy and convulsions. *J Ethnopharmacol.* 105,294–300.

Jawaid, T., Argal, S., Singh, S. (2011). Botanicals and herbs: A traditional approach in treatment of epilepsy. *J Pharm Res.* 4, 1138 - 1140.

Joy, H., Krishna, V., Santhosh, K., Shyam, K., & Mallikarjuna, G. (2011). CNS depressant Activity of extracts from *Flaveria trinervia* Spring C. Mohr. *Phytopharmacology* 1(4), 100-107.

Joy, P.P., Thomas, J., Mathew, S., & Skaria, B.P. (2001). *Medicinal plants.* In: Bose Kabir J, Joy PP, ed. Tropical Horticulture. Vol 2. Kolkata, India: Naya

Kabir, M., Iliyasu, Z., Abubakar, I.S., Kabir, Z.S., & Farinyaro, A.U. (2005). Knowledge, Attitude and Beliefs about epilepsy among adults in a northern Nigerian urban community. *Annals Afr Med*. 4,107-112.

Karunamoorthi, K., Jegajeevanram, K., Jerome, X., Vijayalakshmi, J., & Melita, L. (2012). Tamil traditional medicinal system—siddha: an indigenous health practice in the international perspectives. *Int J Genuine Trad Med* 2(2), 1-11.

Kasthuri, S., Kavimani, S., Devi, R., Sundhararajan, R. & Deepa, N. (2015) Anticonvulsant activity of *Mahakalayanaka ghurita* in maximal electro-shock and pentylenetetrazole induced seizure in rats. *Int. Res. J. Pharm*. 6(10), 715-719.

Katzung, B., Masters, S.B., & Trevor, A.J. (2009). Antiseizure drugs. In: Basic and clinical pharmacology, 11th edn. Tata Mcgraw Hill, India. pp. 399

Katzung, B., Masters, S.B., & Trevor, A.J. (2012). Basic and clinical pharmacology.

New York: McGraw-Hill Medical. Chapter 24 Antiseizure drugs;403-426.

Khandelwal, K.R. (2008) Practical pharmacognosy. Pragati Book Pvt Ltd, India.

Khatun, M. H., Islam, M. R., Mamun, A., Nahar, L., & Luthfunnesa, U. (2011). In Vivo Evaluation of CNS Depressant and Antinociceptive activities of Methanol Extract of Hibiscus sabdariffa Fruits. *Journal of Applied Sciences Research*. 7 (6), 798-804.

Kolawole, O.T., Makinde, J.M., & Olajide, O.A. (2007). Central Nervous System Depressant Activity of *Russelia equisetiformis* O.T. Niger. *J. Physiol.Sci* 22, 59-73

Kokate, C. K., Purohit, A. P., & Gokhale, S.B. (2001). Carbohydrate and derived Products, drugs containing glycosides, drugs containing tannins, lipids and protein alkaloids. Text book of Pharmacognosy, 7 edition: 133 -166.

Kokate, C.K., Purohit, A.P. & Gohkale, S.B. (2002) *Pharmacognosy*. In: Terpenoids, 21st Edition, Nirali Prakashan, Pune, 377-37

Khosla, P., & Pandhi, P. (2001). Anticonvulsant effect of nimodipine alone and in combination with diazepam on PTZ induced convulsions. *Ind J Pharmacol* 33,208-11.

Konan, N.A., Bacchi, E.M., Lincopan, N., Varela, S.D. & Varanda, E.A. (2007). Acute, subacute toxicity and genotoxic effect of a hydroethanolic extract of the cashew (*Anacardium occidentale* L.). *J Ethnopharmacol*. 110(1),30-8.

Kulkarni, S.K., & Akula Dhir, A. (2008). Effect of *Withania somnifera* Dunal root extract against pentylenetetrazole seizure threshold in mice :Possible involvement of GABA-ergic system. *Indian Journal of Experimental Biology*. 47, 465-469.

Kumar, A.S., Gandhimathi. (2009). Effect of *Guettarda speciosa* extracts on biogenic amines concentrations in rat brain after induction of seizure. *International Journal of Pharmacy and Pharmaceutical Sciences*. 1(1), 237-243.

Kumar, A. P., Ramu, P. (2004). Anticonvulsant activity of *Benincasa hispida* fruit, methanol extract. *Journal of Natural Remedies*. 4(2), 195-198.

Kraus, G.L., Johnson, M.A., & Miller, N.R. (1998). Vigabatrin associated retinal cone

system dysfunction; electroretinogram and ophthalmologic findings. *Neurology*.50, 614-8.

Krivoshein, A.V., & Hess, G.P. (2006). The mechanism of alleviation by phenobarbital of the malfunction of an epilepsy-linked GABA (A) receptor. *Biochem*. 45, 11632–11641.

Jayasree, T., Navee, A., Chandrasekhar, N., Sunil, M., Kishan, P.V. & Jagan Rao, N.(2012). Evaluation of muscle relaxant activity of aqueous extract of *Sapindus trifoliatus* (pericarp) in swiss albino mice. *J. Chem. Pharm. Res*. 4(4),1960-1964

Luszczki, J.J. (2009). Third-generation antiepileptic drugs: mechanisms of action, Pharmacokinetics and interactions. *Pharmacol Rep*. 61(2), 197-216.

Malvi Rateesh, K., Bibonoya, P., & Sethi S. (2014). Medicinal plant used in the treatment of epilepsy. Available from: https://www.pharmatutor.org/.../herbal-remedies-used-in-the-treatment-of-epilepsy.

Mattson, R.H., Melrum, B., Penry, J.K., & Dreifuss, F.E. Antiepileptic Drugs. 3rd ed. New York: Raven Press p. 85-102.

Mathur, S., Sen, S., Ramesh, L., & Satish Kumar, M. (2010). Utilization pattern of antiepileptic drugs and their adverse effects in a teaching hospital. *Asian J Pharm Clin Res*. 3, 55–59.

McCormick, D.A., & Contreras, D. (2001). On the cellular and network bases of epileptic seizures. *Annu Rev Physiol*, 63, 815–846.

McNamara, J.O., Goodman., & Gilman's. (2011). The Pharmacological Basis of Therapeutics.12th ed. McGraw-Hill; Pharmacotherapy of the Epilepsies. In: Brunton L. L, Chabner B. A, knollmann B. C; p. 583.

Meinardi, H., *et al*., (2000). Selection of antiepileptic drug polytherapy based on mechanism of action; the evidence reviewed. *Epilepsia*. 41, 1364-1374

Mishra, G., Singh, P., Garg, V.K., Parvez, N. *et al*. (2011). Phytochemical screening and anticonvulsant activity of *Wedelia chinensis*. *Int J Pharm Sci Res* 2(1),39-43.

Mohamed Asif (2013). Anticonvulsant potentials of some medicinal plants and their beneficial properties. TANG / Volume 3 / Issue 4 / e27.

Mohsin, R., & Choudhary, M.I. (2000) Medicinal plants with anti convulsant activities *Studies in Natural products chemistry*. 22, 507-553.

Musa, A.M., Yaro, A.H., Usman, H., Magaji, M.G. & Habu, M. (2008). Phytochemical and Some Neuropharmacological Studies on the Methanolic Leaf Extracts of *Cissus cornifolia* [Vitaceae] in Mice. *Intl. J.Pharm*, 4, 145-148.

Ndoye, N. F.(2005). *Eur. J. Epilepsy* 14, 7.Newton, C.R., & Garcia, H.H. (2012). Epilepsy in poor regions of the world. *Lancet;* 380,1193–1201

Nirmala, D. (2015). Studies on Anticonvulsant activity of Annacyclus Pyrethrum in albino mice. *Asian J Pharm Clin Res*. 8(4), 178-187.

NSE. The national society for epilepsy, About Epilepsy. 2009. Available from: http://www.epilepsy Society. org.uk.

Ojewole, J.O., & Amabeoku, G.J. (2006). Anticonvulsant effect of *Persea americana* Mill (Avocado) leaf aqueous extract in mice. *Phytotherapy Research*. 20 (8), 696 - 700.

Oliver, C.N., Starke-Reed P.E., Stadtman, E.R., Lin G.J., Correy J.M., & Floyd R.A. (1990). Oxidative damage to brain proteins, loss of glutamine synthetase activity and production of free radicals during ischemia/reperfusion induced I njury to gerbil brain. *Proc. Natl. Acad. Sci*. USA. 87:514

Orhan, N., Orhan, D. D., Aslan, M., Ükürolu, M., & Orhan I. E. (2012). Analysis of *Galium spurium* towards its neuroprotective and anticonvulsant activities. *Journal of Ethnopharmacology*. ;141(1), 220–227.

Oscar, H.C., Rocio, S.A., Bertha, P.A., Edwin, E.R., & Vicente, M.C. (2017). Anticonvulsant effect of ethanolic extract of *Cyperus articulatus* L. leaves on pentylenetetrazol induced seizure in mice. *Journal of Traditional and Complementary Medicine* in Press. Available from:https://doi.org/10.1016/j.jtcme.2017.03.001

Patel, M. (2004) Mitochondrial dysfunction and oxidative stress: cause and consequence of epileptic seizures. *Free Radic. Biol. Med*. 3, 1951–1962.

Patil, C.D., Ahire, Y.S., Pathade, P.A., Pathade, V.V. & Mali, R.R. (2011). Free radicals, epilepsy and anti-oxidants: An overview. *Int. Res.J. Pharm*. 2(2), 64-71

Patrick, A., Woode, E., & Kombian S., (2012). Anticonvulsant and related neuropharmacological effects of the whole plant extract of *Synedrella nodiflora* (L.) Gaertn (Asteraceae). *J. Pharm Bioallied Sci*. 140-8

Pascaline, J., Catherine, L., George, O., Dennis, O., & Charles, M. (2008). Herbal Treatments in Aldai and Kaptumo Divisions in Nandi District, Rift Valley Province, Kenya. *African Journal of Traditional and Complementary and Alternative Medicine.* 5(1), 103-105.

Pal, D. K., & Nims,e S. B. (2006). *Asian J. Chem.*, , **13**, 3004–3008

Porwal, M., Komal, S.& Paras, M. (2011). Anticonvulsant effect of *Annona squamosa* leaves in mice. *Pharmacologyonline* 2, 44-52

Poon, H.F., Calabrese, V., Scapagnini G., & Butterfield, D.A. (2004). Free radicals and brain aging. *Clin. Geriatr. Med*. 20(2), 329–359.

Pueyo, I.U., & Calvo, M.I. (2009). Phytochemical Study and Evaluation of Antioxidant, Neuroprotective and Acetyl cholinesterase Inhibitor Activities of *Galeopsisladanum* L. extracts. *Pharmacognosy Mag*.5, 287–90.

Raza, M., Choudhary, M., & Atta-ur-Rahman. (2000). Medicinal plants with anticonvulsant activities. In Studies in Natural Product Chemistry, Atta-ur-Rahman (ed). Vol.22. Elsevier Science: 507–553.

Rang, H.P., Dale, M.M., Ritter, J.M., & Moore, P.K. (2007). *Pharmacology*, 6th ed. Churchill Livingstone, Edinburgh pp 457-475.

Ramesh, K., Goyal., Dessari., & Gandhi (2007). *Elements of pharmacology*. seventeenth edition: 2007; 288-289

Ramya, K. B., & Thaakur, S. (2009). Evaluation of Neuropharmacological effects of

Dischrostachys cinerea root. *International Journal of Pharmacological Science and Nanotechnology*. 1(4), 367-374.

Ramalingam, R., Nath, A., Madhavi, B., Nagulu, M., & Balasubramaniam, A. (2013). Free radical scavenging and antiepileptic activity of Leucas lanata. *Journal of pharmacy research*. 6(3), 368-372.

Rawi, S., Nadia, M. S., Arafa., & Mansour, M. El-Hazmi. (2011). Evaluation of the effects of ciprofloxcin or gatifloxacin on neurotransmitters levels in rat cortex and hippocampus. *African Journal of Pharmacy and Pharmacology*. 5(8), 993-1005.

Reynolds, E.H. (2002). Epilepsy in the world. Launch of the Second Phase of the ILAE/IBE/WHO Global Campaign Against Epilepsy. *Epilepsia*, 43 (6), 1–3.

Reza, H,M., Mohammad, H.M., Golnaz, E., & Sepehri, G.(2009). Effect of methonolic extract of *Hyoscymus niger* L. on the seizure indued by picrotoxin in mice. *Pakistan Journal of Pharmaceutical seizure.* 22(3), 308-12

Reza, K., Mahmoud, H., Toktam, M., Ahmad, G., Hamid, R.S., Hassan, R. (2015). Effects of hydroalcoholic extract of *Coriandrum sativum* on oxidative damage in pentylenetetrazole-induced seizures in rats. *Iran J Neurol*. 14(2), 59–66

Rodrigues, A. D., Scheffel, T. B., Scola, G. *et al.,* (2013). Purple grape juices prevent pentylenetetrazol-induced oxidative damage in the liver and serum of Wistar rats. *Nutrition Research.* 33(2), 120–125.

Roberson, E. (2010). Medicinal plants at risk. Nature's pharmacy, our Treasure: why We must conserve our natural heritage. http://www.biologicaldiversity.org/publications/papers/Medicinal_Plants_042008_lores.pdf.

Salahdeen, H.M., & Yemitan, O.K. (2006). Neuropharmacololgical effects of aqueous leaf extract of *Bryophyllum pinnatum* in mice. *African Journal of Biomedical Research*. 9, 101– 107.

Saikia, A., Purkayastha, A., Tigga, R., & Roy, D. (2016). Anticonvulsant Activity of the methanolic extract of *Lawsonia inermis* leaves in albino rats. *Int J Pharm Sci Res*. 7(7), 3068-72.

Sankari, M., Chitra, V., Silambujanaki, P., & Raju, D. (2010). Anticonvulsant activity of ethanolic extract of *Aeglemarmelos* (Leaves) in Mice. 2(1), 640-643.

Sandeep, K. K., Kota1, K., Tahashildar, J., Jameela, T. & Ragavendhra, P.(2015) Antiepileptic activity of ethanolic extract of Biophytum sensitivum (L.) DC. in Animal models. *Int. J. Curr. Res. Aca. Rev* 3(7), 23-30

Sandhyarani, G., Swathi, K., Gangarapu, K., & Praveen, K. (2014). Screening of phytochemical and role of *Barringtonai acutangula* extract on biogenic amines concentration in rat brain. *Asian journal of pharmaceutical science and technology*. 1(1), 61-64.

Satish Kumar, P., Sharvanabhava, B.S., Sainath Reddy, K., Rajitha B. (2013). Effect of *Momordica dioica Roxb*. fruits on pentylentetrazole induced convulsions and oxidative stress in mice. *American Journal of Drug Discovery and Development*. 3, 166-173.

Schmidt, D., & Rogawski, M. (2002). New strategies for the identification of drugs to prevent the development or progression of epilepsy. *Epilepsy Res*. 50, 71–78.

Schlumpf, M., Lichtensteiger, W., Langemann, H., Waser, P.G., & Hefti, F. (1974). fluorimetric dopamine micromethod for the simultaneous determination of serotonin, noradrenaline and in milligram amounts of brain tissue. *Biochemical Pharmacology*, 23, 237-46.

Sedlak, R.H., & Lindsay. (1968). Estimation of total protein bound and non-protein bond sulphydryl group in tissue with Ellman's reagent. *Anal Biochem*, 25, 192–197

Shutz, K., Carle, R., & Schieber, A. J. (2006). *Ethnopharmacol*. 107, 313-323.

Sharma, H.L., & Sharma, K.K. (2008). Principles of Pharmacology. Paras Medical Publication, New Delhi.

Senthil Kumar, K.K., & Rajkapoor, B. (2010). Effect of *Xanthium strumarium* L. extracts on biogenic amines concentrations in rat brain after induction of seizure. *Int. J. Precli. Pharma. Res.* 1(1), 50-53

Senthil, D. (2013). Effect of *Fagonia Cretica (L.)* Extracts on biogenic amines

concentrations in rat brain after induction of Seizure. *International Journal of Preclinical & Pharmaceutical Research*. 4(2), 81-84.

Simon, D. S. **(**2011). The etiologic classification of epilepsy. *Epilepsia*, Volume 52, issue 6; p 1052-1057 https://doi.org/10.1111/j.1528-1167.2011.03041.x

Sinha, A.K. (1972). Colorimetric assay of catalase. *Analytical biochemistry*. 47, 389-394.

Singh, B., Singh D., & Goel R. K. (2012). Dual protective effect of *Passiflora incarnata* in epilepsy and associated post-ictal depression. *Journal of Ethnopharmacology*.139(1), 273–279

Somjen, G.G., & Müller, M. (2000). Potassium-induced enhancement of persistent Inward current in hippocampal neurons in isolation and in tissue slices. *Brain Res*. 885, 102–110.

Sun, Y., Oberley L. W.,& Li Y. (1988). A simple method for clinical assay of superoxide dismutase. *Clinical Chemistry*. 34, 497–500.

Stafford, G.I., Pedersen, M.E., van Staden, J., & Jager, A.K. (2008). Review on plants with CNS-effects used in traditional South African medicine against mental diseases. *Journal Ethnopharmacol*. 119, 513–537.

Steven, C.S. (2009). Botanicals and Herbs: A Traditional Approach to Treating Epilepsy *Neurotherapeutics*. 6(2), 2009

Strzelczyk, A., Reese, J.P., Dodel, R., & Hamer, H.M. (2008). Cost of epilepsy: a systematic review. *Pharmacoeconomics*. 26 (6), 463–476.

Tapas, A.R., Sakarkar, D.M., & Kakde, R.B. (2008) Flavonoids as Nutraceuticals: A Review. *Tropical Journal of Pharmaceutical Research*. 7, 1089-1099.

Treare, G. E., & Evans, W. C. (1985). Pharmacognosy 17th edn., BahivTinal, London. P 149.

Trease, G.E., & Evans, W.C. (1996). Pharmacognosy 14th edition Ssuvnder Company limited, London. 191-293.

Tripathi, K.D. (2013). Essentials of medical pharmacology, *Jaypee brothers medical publishers (p) ltd*, 6, 401-402.

Uma Devi, P., Kolappa Pillai K., & Divya, V. (2006). Modulation of pentylenetetrazole-induced seizures and oxidative stress parameters by sodium valproate in the absence and presence of N-acetylcysteine. doi: 10.1111/j.1472-8206.2006.00401.x

Usui, S., Komeima, K., Lee S. Y. *et al*., (2009). Increased expression of catalase and superoxide dismutase 2 reduces cone cell death in retinitis pigmentosa. *Molecular Therapy*. 17(5), 778–786.

Vaibhav, B. (2013). Anticonvulsant and antioxidant activity of aqueous leaves extract of *Desmodium triflorum* in mice against pentylenetetrazole and maximal electroshock induced convulsion. *Brazilian Journal of Pharmacognosy*. 23(4), 692-698. DOI: 10.1590/S0102-695X2013005000047

Viswanatha, L., Nandakumar, K., Shylaja, H., Ramesh, C., Rajesh, S., & Srinath, R. (2009). Anxiolytic and anticonvulsant activity of alcoholic extract of heart wood of *Cedrus deodara* Roxb in rodents. *Journal of Pharmaceutical Research and Health Care*. 1(2), 217-239

Vogel, G. (2006). Drug Discovery and Evaluation: Safety and Pharmacokinetic Assays. Springer- Verlag Berlin Heidelberg, New York Also available as Electronic publication as: ISBN-10:3-540-25638-5.

Vyawhare, N.S., & Bodhankar, S.L. (2009). Anticonvulsant activity of *Agyeria speciosa* in mice. *Indian J. Pharm Sci*. 71, 131-4.

Władysław, L., & Monika, L. (2013). Effect of plant polyphenols on seizures – animal studies. *Journal of Epileptology* (21) 79–87. 10.1515/joepi-2015-0007

Wink, M., Schmeller, T., & Latz-Briining, B. (1998). Modes of action of allelochemical alkaloids: Intraction witneuroreceptors, DNA and other molecular targets. *Journal of chemical Ecology*. 2, 1888-1937.

Xianchun, L., Hui K., Nan, J., & Yinghe, H. (2005). Involvement of Scn1b and Kcna1 ion channels in audiogénico seizures and PTZ induced epilepsy. *Epilepsy Res. 66*, 155-163.

Ya'u Jamilu. (2011). Pharmacological rationale for the use of some medicinal plants as antiepileptic agents in Zaria, Nigeria. M.Sc. Thesis.

Zhu L.J., Chen Z., Zhang L.S., Xu S.J., Xu A., & Luo J.H. (2004). Spatiotemporal changes of the N-methyl-D-aspartate receptor subunit levels in rats with pentylenetetrazole-induced seizures. *Neurosci Lett*. 356(1), 53-6.

Zis, A.P., Nomkos, G.G., Brown, E.E., Damsa, G., & Fiberger. H.C. (1992). Neurochemical effects of electrically and chemically induce seizures: and in vivo microdialysis study in hippocampus. *Neuropsychopharmacology*. *7*,189–195.

Zongo, F., Ribuot ,C., Boumendjel, A., & Guissou, I. (2013). Botany, traditional uses, phytochemistry and pharmacology of *Waltheria indica* L. (syn. *Waltheria americana*): a review. *Journal of Ethnopharmacology*. 148(1), 14–26.

APPENDICES

APPENDIX I : ETHICS CLEARANCE

OFFICE OF THE DIRECTOR OF GRADUATE STUDIES AND RESEARCH

UNIVERSITY OF EASTERN AFRICA, BARATON

P. O. Box 2500-30100, Eldoret, Kenya, East Africa

November 14, 2016

Gracelyn Portia Anthony Doss
Department of Biological Sciences
University of Eldoret

Dear Gracelyn,

Re: ETHICS CLEARANCE FOR RESEARCH PROPOSAL (REC: UEAB/2/11/2016)

Your research proposal entitled ***"Culcasia falcifolia Ethanolic Extract Efficacy and Safety in the Treatment of Chemical Inducing Epilepsy in Laboratory Mice"*** was discussed by the Research Ethics Committee (REC) of the University and your request for ethics clearance was granted approval.

This approval is for one year effective November 14, 2016 until November 14, 2017. For any extension beyond this time period, you will need to apply to this committee one month prior to expiry date. Note that you will need a clearance from the study site before you start gathering your data.

We wish you success in your research.

Sincerely yours,

Dr. Jackie K. Obey
Chairperson, Research Ethics Committee

University of Eastern Africa, Baraton
15 NOV 2016
P. O. Box 2500-30100, ELDORET
Research Ethics Committee

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APPENDIX II : SIMILARITY REPORT

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