ANTIDEPRESSANT EFFECT AND THE POSSIBLE MECHANISM(S) OF ACTION OF SECONDARY METABOLITES FROM TRICHILIA MONADELPHA IN MURINE MODELS

BY

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DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY

JULY, 2016
DECLARATION

DECLARATION BY THE CANDIDATE

I hereby declare that this is the product of my own research undertaken under supervision and has neither been presented in whole nor in part for another degree elsewhere. I am solely responsible for any residual flaws in the work.

Signature……………………… Date……/……/………………

Mensah Jeffrey Amoako (10281708)

DECLARATION BY SUPERVISORS

We hereby declare that the principal work and presentation of the thesis were supervised by us in accordance with guidelines on supervision of thesis laid down by the University of Ghana.

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Signature……………………… Date……/……/………………

(Dr. Isaac J. Asiedu-Gyekye)

Co-supervisor

Signature……………………… Date……/……/………………

(Dr. Kennedy E. Kukuia)
ABSTRACT

Background: Current conventional antidepressant drugs have delayed onset of action, significant adverse effects, are costly and about 30-40% of patients are non-responsive, underscoring the need for alternative therapies for depression. Presently, no known scientific evidence exists to support the efficacy of *Trichilia monadelpha* in depression treatment, though used in Ghana in managing psychosis, epilepsy and mood disorders.

Aim: This study investigated the antidepressant effect of secondary metabolites extracted from the stem bark of *T. monadelpha* and the possible mechanism(s) of action of the most efficacious metabolite in murine models of depression.

Method: Powdered stem bark of *T. monadelpha* (4 kg) was sequentially extracted with petroleum ether, ethyl acetate and 70% (v/v) ethanol and the extracts screened for phytochemical constituents. A preliminary investigation using the Irwin test was conducted before the antidepressant effect of the three extracts were investigated using the forced swimming test (FST) and tail suspension test (TST). In a separate experiment, antidepressant effect of the extracted secondary metabolites [alkaloids (ALK), flavonoids (FLV), saponins (SAP), terpenoids (TER), tannins (TAN)] from the most efficacious extract was evaluated. Furthermore, the most efficacious metabolite was assessed for rapid-onset of antidepressant effect using the open space swim test and its mechanism(s) of action were investigated.

Results: The LD<sub>50</sub> of all the three extracts was above 3000 mg kg<sup>-1</sup> in mice. All three extracts (30-300 mg kg<sup>-1</sup> p.o) showed dose dependent antidepressant activity in both FST and TST (*P* < 0.001) with the hydroethanolic extract (HEE) showing the highest efficacy (*E<sub>max</sub> = 80.55). The FLV, ALK and SAP (dose for the metabolites: 30-300 mg kg<sup>-1</sup> p.o) extracted from HEE showed significant antidepressant effect (*P* < 0.001) in the FST and TST with the ALK being the most efficacious (*E<sub>max</sub> = 76.40). The ALK (30-300 mg kg<sup>-1</sup>
p.o) exhibited rapid-onset of antidepressant effect on day two of treatment and sustained it throughout the period of drug treatment in the open space swim test. Pre-treatment with para-chlorophenylalanine (a tryptophan hydroxylase inhibitor; 200 mg kg\(^{-1}\) i.p) reversed the antidepressant effect of the ALK (30-300 mg kg\(^{-1}\) p.o) and cyproheptadine (a 5-HT\(_2\) receptor antagonist; 80 mg kg\(^{-1}\), i.p) diminished its antidepressant effect, suggesting possible allosteric enhancement of serotoninergic activity. Similarly, pre-treatment with \(\alpha\)-methylldopa (200 mg kg\(^{-1}\), i.p) and/or reserpine (1 mg kg\(^{-1}\) s.c) abolished the antidepressant effect of the ALK indicating enhancement of noradrenergic activity. Pre-treatment with yohimbine (a selective \(\alpha_2\)-receptor antagonist; 3 mg kg\(^{-1}\), p.o) potentiated the antidepressant effect of the ALK, suggesting inhibitory effect on \(\alpha_2\)-receptors. Pre-treatment with prazosin (a selective \(\alpha_1\)-receptor antagonist; 3 mg kg\(^{-1}\), p.o) failed to reverse the effect of the ALK. Pre-treatment with D-serine (glycine/NMDA receptor agonist; 600 mg kg\(^{-1}\), i.p) reversed the antidepressant effect of the ALK while D-cycloserine (glycine/NMDA receptor partial agonist) potentiated the anti-immobility effect of the ALK, suggesting inhibitory effect on glycine/NMDA receptor complex. Antidepressant effect of the ALK was attenuated by pre-treatment with L-arginine (NOS substrate; 750 mg kg\(^{-1}\), i.p), however, a synergistic effect was observed with the pre-treatment of L-NAME (a non-selective NOS inhibitor; 30 mg kg\(^{-1}\), i.p) and methylene blue (direct inhibitor of both NOS and sGC; 10 mg kg\(^{-1}\), i.p) implicating the involvement of L-arginine-NO-cGMP pathway. The ALK significantly increased curling score in TST suggestive of enhancement of opioidergic activity.

**Conclusion:** The study showed that all three extracts had potent antidepressant activity. The ALK extracted from HEE exhibited a rapid and sustained antidepressant effect and may act via an interplay of noradrenergic, serotonergic, glycine/NMDA receptor, L-arginine-NO-cGMP nitric oxide and opioidergic pathways.
DEDICATIONS

This work is dedicated to the Almighty God and to Department of Pharmacology and Toxicology, University of Ghana, School of Pharmacy especially, my M.Phil colleagues Dr. Mrs. Simstewa Nana Adjoa Agyapong and Miss Audrey Serwaa Bonsu.
ACKNOWLEDGEMENTS

My sincere gratitude goes to the Almighty God for giving me the grace and strength to go through this programme successfully.

I am also grateful to my family for the support and encouragement they gave me during the period of study. The emotional, psychological and moral support from my parents, sister and brother cannot be overlooked. May the good Lord richly bless you.

I also thank the Department of Pharmacology and Toxicology, School of Pharmacy, University of Ghana, for the training, exposure and experience they offered me during my postgraduate studies. I am exceptionally grateful to Dr. I. J. Asiedu-Gyekye and Dr. Kennedy E. Kukuia for their excellent supervision, patience and dedication towards my training in scientific research. To Dr. Mrs. Dorcas Osei-Sarfo, I say a very big thank you for guiding me through the extraction and isolation aspects of my laboratory work. I also want to thank all the teaching and non-teaching staff at the Department of Pharmacology and Toxicology and other departments.

Finally, I appreciate the assistance of Madam Doris of the Centre for Plant Medicinal Research –Mampong, for being there whenever I needed her. Thank you all very much. May the good Lord continue to bless you.
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<tr>
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<th>DEFINITION</th>
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<tbody>
<tr>
<td>AC-cAMP-CREB</td>
<td>Adenylate cyclase-cyclic adenosine monophosphate- cAMP response element binding protein</td>
</tr>
<tr>
<td>ADHD</td>
<td>Attention deficit hyperactivity disorder</td>
</tr>
<tr>
<td>ALK</td>
<td>Alkaloids</td>
</tr>
<tr>
<td>AMPA</td>
<td>Alpha-3-Hydroxy-5-Methoxy-4-Isoxazole Propionic Acid</td>
</tr>
<tr>
<td>ATO</td>
<td>Atomoxetine</td>
</tr>
<tr>
<td>BDNF</td>
<td>Brain derived neurotrophic factor</td>
</tr>
<tr>
<td>BUP</td>
<td>Bupropion</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CBT</td>
<td>Cognitive behaviour therapy</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
</tr>
<tr>
<td>cGMP</td>
<td>Cyclic guanosine monophosphate</td>
</tr>
<tr>
<td>CRH 1</td>
<td>Corticotrophin releasing hormone 1</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DMN</td>
<td>Default mode network</td>
</tr>
<tr>
<td>DSM - IV</td>
<td>Diagnostic and Statistical Manual for Mental Disorders</td>
</tr>
<tr>
<td>DMDD</td>
<td>Disruptive mood dysregulation disorder</td>
</tr>
<tr>
<td>DA</td>
<td>Dopamine</td>
</tr>
<tr>
<td>DAT</td>
<td>Dopamine transporter</td>
</tr>
<tr>
<td>ECT</td>
<td>Electroconvulsive therapy</td>
</tr>
<tr>
<td>EAE</td>
<td>Ethyl acetate extract</td>
</tr>
<tr>
<td>FLV</td>
<td>Flavonoids</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>FLX</td>
<td>Fluoxetine</td>
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<tr>
<td>FST</td>
<td>Forced swimming test</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma Amino Butyric Acid</td>
</tr>
<tr>
<td>GDNF</td>
<td>Glial cell-line derived neurotrophic factor (GDNF)</td>
</tr>
<tr>
<td>HEE</td>
<td>Hydroethanolic extract</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-Hydroxytryptamine</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal</td>
</tr>
<tr>
<td>ICD-10</td>
<td>International Statistical Classification of Diseases and Related Health Problems</td>
</tr>
<tr>
<td>ICR</td>
<td>Imprinting Control Region</td>
</tr>
<tr>
<td>IMI</td>
<td>Imipramine</td>
</tr>
<tr>
<td>i.p</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>IPT</td>
<td>Interpersonal psychotherapy</td>
</tr>
<tr>
<td>L-arg</td>
<td>L-arginine</td>
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<tr>
<td>LDL-C</td>
<td>Lipoprotein-cholesterol</td>
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<tr>
<td>L-NAME</td>
<td>NG-nitro-L-arginine methyl ester</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>mRNA</td>
<td>micro Ribonucleotide acid</td>
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<tr>
<td>MDD</td>
<td>Major depressive disorder</td>
</tr>
<tr>
<td>MAO</td>
<td>Monoamine oxidase</td>
</tr>
<tr>
<td>MAOIs</td>
<td>Monoamine oxidase inhibitors</td>
</tr>
<tr>
<td>MT</td>
<td>Melatonin</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric oxide synthase</td>
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<tr>
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<td>--------------</td>
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</tr>
<tr>
<td>NA</td>
<td>Noradrenaline</td>
</tr>
<tr>
<td>NET</td>
<td>Noradrenaline transporter</td>
</tr>
<tr>
<td>ODD</td>
<td>Oppositional defiant disorder</td>
</tr>
<tr>
<td>pCPA</td>
<td>Para-chlorophenylalanine</td>
</tr>
<tr>
<td>PEE</td>
<td>Petroleum ether extract</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>p.o</td>
<td>Per os</td>
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<tr>
<td>SAP</td>
<td>Saponins</td>
</tr>
<tr>
<td>s.c</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>sGC</td>
<td>Soluble guanylate cyclase</td>
</tr>
<tr>
<td>SNRI</td>
<td>Selective noradrenaline reuptake inhibitors</td>
</tr>
<tr>
<td>SNDRI</td>
<td>Serotonin–norepinephrine–dopamine reuptake inhibitors</td>
</tr>
<tr>
<td>SSRIs</td>
<td>Serotonin reuptake inhibitors</td>
</tr>
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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Over the years, traditional medicines have been widely used throughout the world in disease prevention and treatment. Traditional medicine is the totality of knowledge, skills, and practices based on the theories, beliefs, and experiences native to diverse cultures that are used to avert, diagnose, or treat physical and mental illnesses (Mahomoodally, 2013). It is often an underestimated form of health delivery and may be termed complementary medicine whilst often practiced on empirical basis (W.H.O., 2013). Nearly 80% of the global population relies on traditional medicine to cater for their health needs (Buvaneswari et al., 2011), whilst close to 70% of Africans depend on some form traditional systems of healthcare. The recent renaissance observed in the use of traditional medicines can be attributed to historical, socio-economic and cultural belief systems particularly in rural areas.

The most common traditional medicine in widespread practice across the Africa is the use of medicinal plants. Plants provide most of the effective and affordable medicines in traditional medicine and also remains the world’s chief source of drugs. Furthermore, medicinal plants have contributed immensely to the discovery of most pharmaceutically active compounds (Ngo et al., 2013) which are been used in the commercial production of some drugs. A growing number of herbal medicines had been studied and discovered to provide alternative therapies for depression, such as Ginseng (*Panax ginseng*) (Dang et al., 2009), St. John`s wort (*Hypericum perforatum*) (Kukuia et al., 2014; Linde et al., 2008), *Kalanchoe integra* (Kukuia et al., 2015) etc. These plants produce diverse range of bioactive compounds that possess pharmacological and
toxicological effects in man and animals but do not play essential role in the metabolism of the plants hence referred to as secondary metabolites (Mailoa et al., 2013). These compounds can be considered as products of biochemical “supplements” in the plant cells and not required for daily functioning of the plant. Secondary metabolites from time immemorial have served as sources of effective medical therapies and are continuously a prime source of many essential efficacious drugs (Baliah and Astalakshmi, 2014). Several biologically active natural product compounds are secondary metabolites with complex structures. Humans use these secondary metabolites such as alkaloids, saponins, natural phenols, terpenoids as medicines (Baliah and Astalakshmi, 2014).

Sufficient researches suggest that some secondary metabolites exhibit inhibitory effects on the central nervous system. Reports indicate that total saponins from ginseng (Dang et al., 2009), total alkaloids from *Semen Zizyphi Sponosae* (Sun et al., 2013), total alkaloids from *Cissampelos sympodialis* Eichler (Mendonça-Netto et al., 2008), etc. have been discovered to possess antidepressant effect. Recent reports view the extraction of secondary metabolites and their use as single chemical compounds as a better replacement and which have resulted in the substitution of plant extracts’ use (Mahomoodally, 2013).

Descriptions of depression can be found in the Bible and in some Shakespeare`s literature, but no formal definition existed until the third version of the American Psychiatric Association`s classification systems for mental disorders was published in 1989 (Dowrick and Frances, 2013). It is a common mental disorder characterized by low mood, loss of interest in pleasurable activities, reduced energy, low self-esteem, feeling of guilt, sleep disturbances and lack of concentration (W.H.O., 2012). The W.H.O has ranked depression the 4th leading cause of disability worldwide and projects that by 2020, it will be the second leading cause of disability (Kessler and Bromet, 2013). Although impairment of monoaminergic neurotransmission
especially serotonin and noradrenaline neurotransmission plays an essential role in the pathophysiology of the depression, recent studies shows that neurotrophic, genetic, psychological stress and endocrine factors play a key role in the central biology of the disease (Hasler, 2010). Depression is associated with significant morbidity and mortality due to physical disorders and suicide usually linked with the disease. Depressed individuals usually become a burden to society and their quality of life is greatly affected. These adversely contribute to financial loss to the society (Ford et al., 2010).

Notwithstanding the treatment advances, standard medical practices and orthodox medicines available for treatment of depression had faced diverse limitations (Yan et al., 2013). Existing pharmacotherapies at the moment are costly, related toxicity and efficacy of these medications renders some unreliable and others prohibitive for use (Kukuia et al., 2014). High risk of recurrence associated with the use of medications coupled with the relatively slow onset of action leaves a lot to be desired about the effective treatment of depression (Kukuia et al., 2015). A number of newer antidepressant medications such as Fetzima (Levomilnacipran) and Brintellix (Vortioxetine) have been approved in 2014; however, they possess some side effects which are similar to current medications on the market. These limitations observed in the use of orthodox medicines have contributed to the increased use of natural medicinal plants in the management of depression.

The recent global renaissance in the use of complementary and alternate medical systems especially medicinal herbs for the management of mood disorders such as depression accentuates the need for more research to ascertain the efficacy, safety and establish the standard criteria requires for the use of the therapies (Qureshi and Al-Bedah, 2013). Active search into the discovery of newer antidepressant medications but more effective, more sustainable and with
minimal side effects are still ongoing (Ferguson, 2001). Research into phytomedicine has been generating considerable amounts of new data on the chemical, pharmacological, and clinical aspects of various isolated promising bioactive compounds from medicinal plant sources (El-Alfy et al., 2012; Iovieno et al., 2011).

As investigation into the discovery of novel active compounds intensify, it is important that researchers develop the most promising plant therapies. Extensive investigations are required into the pharmacological activities of potentially active compounds when it comes to medicinal plants. This will go a long way to help in the development of newer medications, provide information on the usage of the drug such as therapeutic doses, contraindications, potential drug-drug interaction as well as best methods of extraction and synthesis. (Stark et al., 2013).

*Trichilia monadelpha* (family Meliaceae) is a tree that is commonly found in lowland, evergreen and semi-deciduous forest, and mostly along rivers and in other moist localities (Lemmens, 2008). It is locally known in Akan as “Otanduro”. Economically, the trunk wood of *Trichilia monadelpha* is used in making timber, building material, fuels etc. *T. monadelpha* has a number of medicinal uses including managing pain and inflammation, treatment of respiratory disorders, cardiovascular disorders etc. (Burkhill, 1997). Its stem bark decoction is also known to cause sedation and drowsiness (Abbiw, 1990).

*Trichilia monadelpha* has a wide ethnomedical benefits in the society and have been shown to possess central nervous system depressant effect (Abbiw, 1990). Thus taking into accounts the search for safer and effective antidepressants was the huge propelling factor for the research into the antidepressant effect of the secondary metabolites of *Trichilia monadelpha* in this study.
1.2 PROBLEM STATEMENT

There has been a resurgence of interest in neuropsychiatry research mainly depression due to the socio-economic burden it imposes on countries. Several hypotheses pertaining to depression and its treatment have been proposed. These hypotheses are based largely on the neuroplasticity, genetic, endocrinology, neurotransmitter, and psychoneuroimmunology etc. (Nemade et al., 2013). Given the pervasive symptoms of depression, the pathophysiology of the disorder and the mechanisms by which currently available treatments reverse its symptoms are not yet well understood.

Depression is associated with increased resource utilization. It is estimated that depression is responsible for $ 85 billion direct and indirect cost per annum (Hollenberg, 2014). An individual who suffers from depression can expect on average to make 20 percent less in wages during his lifetime than if he were not depressed. Major depression is the second leading medical cause of long-term disability and fourth leading cause of global burden of disease (Kessler and Bromet, 2013). In patients with other chronic diseases like cardiovascular disease, depression has been associated with increased morbidity and mortality, when compared to nondepressed patients (Chung et al., 2011; Junger et al., 2005).

Notwithstanding the treatment advances, conventional treatment of depression had been faced with diverse limitations. Most current pharmacotherapies are expensive, efficacy of these medications renders them unreliable and several side effects are widespread (Kukuia et al., 2014). There exist a high risk of disease recurrence associated with these medications and possess a relatively slow onset of action. Therefore, cost effective, rapid acting, sustained and better-tolerated antidepressants are required. A huge literature focusing on psychoactive herbal
Phytochemicals have been published (Kennedy and Wightman, 2011). These secondary metabolites may be important sources of novel antidepressant drugs and may be proven better in the management of stress and depression.

1.3 JUSTIFICATION

Addressing the burden of mental disorders mainly depression, presents enormous challenges at the clinical and public health levels, particularly in the context of limited resources. Over the last three decades a number of initiatives have been undertaken with the aim of improving the treatment of depression globally yet; it still remains one of the major causes of suicidal deaths and a leading medical cause of long-term disability.

Current medications for depression include the tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), selective noradrenergic reuptake inhibitors (SNRI), monoamine oxidase inhibitors (MAOIs) etc. Most of these drugs produce serious side effects and have slow onset of drug action. For example, TCA cause orthostatic hypotension and arrhythmias, MAOI’s causes sexual dysfunction and hypertension, SSRI’s also mediates gastrointestinal effects and weight gain (Katzung et al., 2012). An estimated 40% of patients suffer conditions refractory to currents medications whiles some of them are expensive and not easily affordable.

In most African countries including Ghana, herbal remedies are used for primary healthcare needs (Abu-Irmaileh and Afifi, 2003). These medicinal plants have proven to be essential sources of some potent bioactive compounds – one of the reasons why the W. H. O. is advocating for the incorporation of traditional medicines in primary healthcare of developing nations (Calixto, 2000; Abu-Irmaileh and Afifi, 2003). *Trichilia monadelpha*, is a common plant
used in traditional medicine in Ghana for treating a spectrum of diseases including schizophrenia, depression and epilepsy. Although anecdotal reports suggest herbalism and herbal remedies are been reported to be efficacious, there exists no scientific evidence of the antidepressant effectiveness of *T. monadelpha*. It is against this backdrop that the pharmacological activities of *Trichilia monadelpha* stem bark secondary metabolites in managing depression and its possible mechanism(s) of action as well as CNS effect should be scientifically validated using various animal models. In addition, secondary metabolites from medicinal plants have been shown to possess antidepressant effect (Yan *et al.*, 2013; Mendonça-Netto *et al.*, 2008). *Trichilia monadelpha*, with a high phytochemical content could be a likely candidate for the treatment of depression. This will go a long way to aid in our pursuit of more effective agents for the management of depression.

### 1.4 HYPOTHESIS

There have been some anecdotal reports of the antidepressant effect of the stem bark of *Trichilia monadelpha*. It is with this knowledge that I hypothesize that the secondary metabolite(s) present in the stem bark of *Trichilia monadelpha* should possess antidepressant activity.

### 1.5 AIM

This research seeks to determine the antidepressant effect of extracts and secondary metabolites from the stem bark *Trichilia monadelpha* and the possible mechanism(s) of action of the most efficacious secondary metabolite in murine models of depression.
1.6 SPECIFIC OBJECTIVES

1. To determine the antidepressant effect of the petroleum ether, ethyl acetate and hydroethanolic extracts of *T. monadelpha* using the forced swimming and tail suspension tests.

2. To determine the antidepressant effect of the extracted secondary metabolites from *Trichilia monadelpha*.

3. To determine whether the most efficacious secondary metabolite would exhibit a rapid and sustained antidepressant effect.

4. To determine possible mechanism(s) of action of the most efficacious secondary metabolite.
CHAPTER TWO

LITERATURE REVIEW

2.1 DEPRESSION

2.1.1 Background

Depression is a complex chronic recurring illness with an important global public health problem. It is a heterogeneous group of illness that vary in symptomatology and most likely in aetiology (Duman, 2010). The major depression syndrome is a disorder of mood implicating perturbation in emotional, cognitive, behavioural and somatic regulation. It is a disorder whereby an individual exhibits an insistent sadness with a significant mood change which appears to be disproportionate to any cause (Atindanbila and Abasimi, 2011).

Depressive illnesses are disorders of the brain. Brain-imaging technologies, such as magnetic resonance imaging (M.R.I.), have revealed that certain brain regions involved in mood appear different in people who have depression when compared to those of people without depression (N.I.H., 2011). Certain brain regions are known to contribute to various aspects of depression. The striatum and amygdala mediates anhedonia and decreased motivation that preponderates many patients whereas the hippocampus and neocortex are known to be implicated in the cognitive aspects of depression (Schlaepfer et al., 2008). The various brain regions function in a series of highly interacting parallel circuits, which serves to formulate a neural circuitry involved in depression (Nestler et al., 2002).

Diagnosis and treatment of depression is based on relatively subjective assessments of diverse symptoms. Currently, no effective methods to objectively assess severity, endophenotypes, or response to treatment exists (Schmidt et al., 2011). The severity, frequency of depressive
episodes, and duration of symptoms vary depending on the individual. Although depression is most frequently encountered during primary healthcare, it is also most commonly under-diagnosed. Studies done by the World Health Organization Psychological Problems in General Health Care reported that only 42% of patients with major depression were appropriately diagnosed by their primary care physician (Lesperance and Frasure-Smith, 2003). Identification and proper recognition of depressive symptoms is impaired by the fact that most depressed patients present with non-specific physical complaints, without spontaneously revealing the psychological nature of their condition (Kessler et al., 1999). When depression is appropriately diagnosed and treated, available treatment modalities especially pharmacotherapy is often provided in a manner inconsistent with current evidence (Segal et al., 2001).

2.1.2 Classification of depression

Classification of depression is covered by both the Diagnostic and Statistical Manual for Mental Disorders (DSM-IV) and the International Statistical Classification of Diseases and Related Health Problems (ICD-10) (N.C.B.I., 2010). Despite advanced research on the aetiology of depression including neurobiological, genetic and psychological studies, no reliable classificatory system has come out that links either to the underlying aetiology or has proven strongly predictive of the disease (Cole et al., 2008). The categories and specifiers for mood disorders continue to increase with each successive edition of the DSM. Depression is currently classified based on pragmatic definitions that have emerged and enshrined in the classification system of DSM-IV-TR and ICD-10 according to the severity (mild, moderate or severe), duration (acute or chronic) and the course of the disorder (Klein, 2008).
2.1.2.1. Severity

The classification of depression into severity groupings based on DSM–IV criteria is not a unitary dimension and it is useful to make a judgement of severity consisting, at least, of number of symptoms, severity of individual symptoms and functional impairment (N.C.B.I., 2010). Generally, symptom severity and degree of functional impairment correlate highly (Zimmerman et al., 2008). The various classes of depression according to its severity includes: sub-threshold depressive symptoms (fewer than five symptoms of depression), mild depression (few, if any, symptoms in excess of the five are required to make the diagnosis, and the symptoms result in only minor functional impairment. A patient with a mild episode may be capable of continuing with the majority of their activities), moderate depression (symptoms or functional impairment are between ‘mild’ and ‘severe’. A patient with a moderate episode may have difficulties continuing with their ordinary activities), severe depression (most symptoms, and the symptoms markedly interfere with functioning. Suicidal thoughts and acts are common, and a number of somatic symptoms are present and can occur with or without psychotic symptoms) (Dowrick and Frances, 2013).

2.1.2.2. Duration

The duration of a depressive episode can vary considerably among individuals. The classical definition of acute depression by DSM-IV is one in which a severe depressive episode lasts for a minimum of two weeks and not longer than two years and treatment studies report a median duration of about twenty weeks (Havard Medical School, 2009). Chronic pain is also defined as one in which severe depressive symptoms persist for two years or longer. Chronic depression causes more functional inefficiency, increases risk of suicide, and is more likely to be coupled with other psychiatric disorders (Klein, 2010). Patients with chronic depression are also more
likely than patients with acute depression to report childhood trauma and a family history of mood disorders (Kühnen et al., 2011). Reports indicate that patients with chronic depression who respond to treatment are likely to suffer a relapse within one to two years if they stop treatment and thus some type of maintenance therapy may be necessary (Torpey and Klein, 2008).

2.1.2.3 Course

This was not explicitly considered as a classificatory issue in the previous DSM guidelines. However, it has essential treatment consequences, particularly for the likelihood of relapse/recurrence. The course of depression is classified into two main dimensions: The number of lifetime depressive episodes and the interval between recent episodes. The number varies from a single/first episode to increasingly frequent recurrences. At least two months of full or partial remission is required to distinguish episodes (N.C.B.I., 2010). There has been no broad unanimity as to how long a period of remission should occur in order to be able to declare recovery (Furukawa et al., 2008). Therefore, in practice it can be difficult to differentiate between relapse and recurrence, particularly when people have mild residual symptoms.

Stage of episode refers to where an individual is in the course of their depression. It determines if the depression is worsening, static or improving and whether sub-threshold depressive symptoms may reflect partial remission from prior major depression (N.C.B.I., 2010).

2.1.3 Types of depression

Depression is still recognized as a distinct clinical entity, particularly in primary care. However, the subtyping of depression is considered critical for effective treatment. The current subtyping of depression is based on DSM-V criteria (American Psychiatric Association, 2013).
2.1.3.1. Bipolar depression

Bipolar disorder, also referred to as manic-depressive illness, is characterized by cycling mood changes from extreme mania to extreme depression. Useful for clinical practice, bipolar depression is classified into two distinct forms: bipolar I depression and bipolar II depression (Benazzi, 2006). The clinical feature of bipolar II depression is known to exhibit more atypical symptoms such as hypersomnia, overeating and more co-occurring hypomanic symptoms including psychomotor agitation (Hantouche and Akiskal, 2005). Bipolar I depression on the other hand is characterized by hypersomnia and psychomotor retardation (Mitchell and Malhi, 2004).

2.1.3.2 Dysthymic disorder

According to DSM-IV-TR, dysthyemic disorder is also referred to as neurotic depression or chronic depression. Its diagnostic criteria require depressed mood for at least 2 years. Clinical picture of dysthymic depression according to DSM-IV-TR is characterized by lowgrade, insomnia or hypersomnia, low energy, low self-esteem, poor concentration and persistent depression, causing clinically significant distress or impairment of functioning (Gilbert et al., 2011). Dysthymia often co-occurs with other mental disorders. In the DSM-5, dysthymia is substituted by persistent depressive disorder (Grohol, 2013) which includes both chronic major depressive disorder and the previous dysthymic disorder.

2.1.3.3. Disruptive mood regulation disorder

Disruptive mood regulation disorder is an affective disorder characterized by chronic, persistently irritable or angry mood with frequent temper outburst (American Psychiatric Association, 2013). Disruptive mood dysregulation disorder (D.M.D.D.) is common among
children presenting to paediatric mental health clinics. The clinical presentations of DMDD are similar to those of other childhood disorders, notably attention deficit hyperactivity disorder (ADHD), oppositional defiant disorder (ODD), and bipolar disorder in children (Weis, 2014). The onset of D.M.D.D. is usually before age 10. Little is known about the course of the disorder and whether the condition presents only in age-delimited manner (Margulies et al., 2012).

2.1.3.4 Major depressive disorder

According to DSM-V, major depression disorder is characterized by persistent low mood and the inability to anticipate happiness or pleasure with feeling of worthlessness, self-loathing and suicidal thoughts been very common (American Psychiatric Association, 2013). The diagnostic code for major depression is dependent on whether there exist a single or recurrent episode, current severity, presence of psychotic features, and remission of status. Types of M.D.D. which have recently been the focus of most research include: mixed depression, atypical depression, melancholic depression, seasonal depression.

2.1.3.4.1 Seasonal depression

Seasonal depression, according to DSM-IV-TR is not a clearly different disorder, but a specifier of the major depressive episode of bipolar disorders and depressive disorders (Benazzi, 2006). Seasonal depressions may be also subsyndromal. Symptoms of seasonal depression are often atypical ones such as hypersomnia and overeating, and depressions are usually mild to moderate. It is more common in women and in young age. Seasonal affective disorder is thought to be mainly caused by less natural light mainly during the winter month may be effectively treated with phototherapy (Magnusson and Partonen, 2005).
2.1.2.4.2 Melancholic depression

Melancholic depression is a DSM-IV-TR subtype of clinical depression that can be found in almost all mood disorders. It occurs as part of either a major depressive episode or bipolar disorder with the melancholic specifier (American Psychiatric Association, 2008) . Melancholic depression was the first form of depression studied extensively. The incidence of melancholic depression has been found to increase when the temperature and/or sunlight are low (Radua et al., 2010).

2.1.2.4.3 Atypical depression

Atypical depression is a form of M.D.D. which can be present in almost all mood disorders. It is the most common form of depression identified in outpatient departments in mental hospitals (Singh and Williams, 2006). Atypical depression was considered as a distinct diagnosis primarily because medication trials clearly showed patients responded better to monoamine oxidase inhibitors (MAOIs) compared to tricyclic antidepressants (Quitkin et al., 2003). According to DSM-IV, diagnosis of atypical depression requires mood reactivity. Reports also indicates depression with atypical features exhibit onset in childhood or adolescences (Stewart et al., 2005).

2.1.4 Epidemiology of depression

Worldwide, depression is a leading cause of disease burden of very high prevalence (Collins et al., 2011; Kupfer et al., 2012). However, precise statistical data on the prevalence and other essential correlates of depression for most countries are not known. Reports indicates that there exist wide variability in prevalence estimates, although some features of descriptive epidemiology such as age-of-onset, persistence are reasonably coherent (Kessler and Bromet,
Major depression disorder has meaningful proportions of lifetime cases starting in late adolescence, in early-middle adulthood and in late adulthood. The rate of diagnosed major depression increased proportionally with age from 2.8% in adults aged between 18-24 years to a peak of 4.6% for adults between 45-64 years. Thus, with the growing proportions of older people, the overall numbers of people with depressive symptoms are expected to rise (Nyirenda et al., 2013). Age-of-onset distributions suggest that depression is prevalent for the entire lifespan (Kessler et al., 2007).

Recent epidemiological surveys conducted globally showed a lifetime prevalence of depression within the range of 10% to 15% (Lépine and Briley, 2011). Reports from studies conducted reveals that prevalence levels of depression are higher in developed countries (28.1%) than in developing countries (19.8%) (Kessler and Bromet, 2013). Research findings reports female superiority in prevalence, incidence and morbidity risk of depression (Piccinelli and Wilkinson, 2000). Studies revealed that depression prevalence rates among students ranged from 10% to 85% and are relatively higher than those found in the general population (Ibrahim et al., 2013).

According to the W.H.O. (2011), more than 2 million Ghanaians experience moderate to mild mental disorders whereas 650,000 people suffer from severe mental illness (Asante and Andoh–Arthur, 2014). A survey conducted on Ghanaian adults reflects a high prevalence rate of 21% for moderate to severe psychological distress (Canavan et al., 2013). Depression is reported to be the commonest psychiatry illness of Akan rural women (Read and Doku, 2012). Research conducted by Atindanbila and Abasimi, (2011) stated that 16.1% of students in the University of Ghana showed signs of mild depression. A study among university students in Ghana revealed that 31.1% of students suffer from mild to moderate depression and 8.1% experience severe forms of depressive symptoms (Asante and Andoh–Arthur, 2014). However, these reported rates were
higher than those recorded in other West African university students. An estimated 80% of Ghanaians are suspected to be suffering from different forms of depression (Kintampo Medical Research Centre, 2012). The relatively high prevalence rate recorded among Ghanaian students reinforces the assertion made by the Kintampo Medical Research Centre.

2.1.5 Comorbidity of depression

In recognition of the full impact of depression, it is worth noting that depression mostly co-occurs with various medical, psychiatric as well as substance abuse disorders (Kupfer and Frank, 2003). In most cases, depression comorbid with other diseases. The association between depression and psychiatric disorders are more potent than that which exists between major depression and medical conditions (Melartin et al., 2002). The presence of both or all conditions is often unidentified and unfortunately, causes deleterious consequences for patients (Kupfer and Frank, 2003).

The impact of comorbid depression and anxiety is substantial. Report indicates that comorbid anxiety and depression may be responsible for as much as 2% to 4% of all medical disability worldwide and is associated with barriers to treatment and worse psychiatric outcomes (Aina and Susman, 2006). A strong association has been reported to exist between depression and pain, with at least one pain symptom being present in 65% of depressed patients, and about 5 to 85% of patients with pain will be suffering from depression (Bair et al., 2003). Studies show that 13% of patients with panic disorder suffer from depression and depressive disorder is known to precede the panic disorder in about 25% of these patients with concurrent depression (Hranov, 2007). An estimated 50–75% of patients diagnosed of anorexia nervosa or bulimia have a clinical history of major depression (N.I.M.H. Forum, 2001).
Substance abuse disorders have been shown to co-occur with depression at very significant levels. A study of 43,000 persons suffering from major depressive episodes where shown to have a lifetime incidence of substance abuse disorder comorbidities; alcohol abuse disorder (40.3%), nicotine dependence (30.0%), drug use disorder (17.2%) (Hasin et al., 2005).

Depression frequently co-occurs with diabetes and the course of depression in diabetic patients most often chronic and severe. Reports indicate that up to 80% of depressed diabetic patients may experience a relapse of depressive symptoms over a 5-year period (Katon, 2008). Comorbid psychiatric illnesses, particularly major depression have been found to be highly predictive of suicidal thoughts in HIV positive individuals. A recent study of HIV positive patients revealed that those with a history of suicide attempt showed significantly higher levels of depressive symptoms (Badiee et al., 2012). Depression in patients with coronary heart disease (CHD) is common. Patients with comorbid condition of myocardial infarction or acute coronary syndrome and depressive symptoms shows a point prevalence of 33% (Ziegelstein, 2001). There is a broad unanimity that an estimated 25% of cancer patients suffer from major depression (N.I.M.H. Forum, 2001).

2.2 THEORETICAL MODELS OF DEPRESSION

2.2.1 Biopsychosocial model

The biopsychosocial model states that biological, psychological and social factors (diathesis-stress) are all interconnected and essential with regard to promoting or causing depression (McLeod, 2015). It is known that the mind and the body are not independent and autonomous entities but rather are interlinked and interdependent (Nemade et al., 2007). The model therefore explains that there is no single cause of depression, however, these three key factors (biological,
psychological and social) have regularly played significant contributions pertaining to the onset of depressive symptoms in an overlapping fashion (MacDonald, 2015).

2.2.1.1 Biological factors

The various biological theories on depression mostly focus on diverse constituents of the overall pathophysiology of depression. A depressed patient is vulnerable to developing a wide variety of physical disorders, in the same way that a person suffering from a physical disorder is often more likely to be depressed (Nemade et al., 2007). This is most likely because a depressed patient is often significantly unbalanced with regard to hormonal, immunological, genetic and neurotransmitter system functioning (Nemade et al., 2007).

Studies show that genetic, hypothalamic-pituitary-adrenal axes, brain structure integrity, cytokine hypothesis, macrophage theory of depression, monoamine theory of depression among others play very crucial roles and act as key components of the body’s vital stress response system (Roy and Campbell, 2013). These distinct biological theories however, have a considerable overlap between them and function at the inter-face between psychosocial stress and the physiological changes associated with depression (Papageorgiou, 2010; Roy and Campbell, 2013).

2.2.1.2 Psychological factors

Psychological theories to some extent can be influenced by some biological vulnerabilities which affects biologically-based personality characteristics such as people’s innate temperament and by social factors such as stress which induces depressive symptoms (Garcia-Toro and Aguirre, 2007).
Several psychological theories have been proposed to mediate the aetiology of depression. Psychological models reported to make an individual vulnerable to depression include, negative cognitive schemas, poor self-esteem, pessimism and poor coping skills (McLeod, 2015). Others include negative childhood experiences, past trauma, judgment problems, and impaired emotional intelligence (the ability to perceive, understand, and express emotions) (McLeod, 2015).

2.2.1.3 Social factors

The sociological facets of depression are influenced by and also influence biological and psychological aspects of depression (Nemade et al., 2007). Reports indicate that stress, lifestyle factors, poor social skills, low social support, and sociocultural context are the main social and environmental risk models associated with the occurrence of depression (Keller et al., 2007; Roy and Campbell, 2013).

2.2.2 Psychodynamic model

Psychodynamic theory of depression was the prevalent model within clinical psychiatry during the early part of the 20th century. Historically, psychodynamic theories were widely critiqued for their lack of empiricism (Nemade et al., 2007). However, recent models of psychodynamic theories of depression have been derived and are therefore influencing a transition to the initial resistance on the existing psychodynamic theories in the scientific world.

Freud (1971) explained that depression is a phenomenon which is produced as a result of conflict between the conscious and unconscious parts of the mind. This then results in a state where one becomes unaware of his/her troubling motives, desires, and wishes but is negatively influenced by these conditions simultaneously (Freud, 1917). Freud’s ideas showed that early
loss can make an individual more susceptible to depression in later life. Children who lose their mother are more likely to develop depression in adult life (Bifulco et al., 2002). Wade (2011) also explained that psychodynamic theory of depression states that depression is a result of anger from imbalanced cognitions which usually results in self hatred. Children of over anxious or neurotic parents are more prone to experiencing exaggerated anxiety and/or depression feelings (Nemade et al., 2007; Wade, 2011).

A contemporary psychodynamic theory, known as object relations theory, is focused on how relationships are understood and mentally represented. According to this theory, moods, emotions and various aspects of a personality can only be properly apprehended against the backdrop of the relationships that one has experienced (Luyten and Blatt, 2013). The object relations theory describes depression as caused by difficulties in promoting representations of healthy relationships. Depression is an outcome of an existing strife to develop and maintain emotional relationships (desired objects) (Nemade et al., 2007).

Coyne's interpersonal theory of depression is a modern derivative of psychodynamic theory which states that depressed patients’ negative interpersonal behaviours influence their rejection by society (Nemade et al., 2007). Symptoms of depression begin to manifest and worsen due to the lack of reassurance, feeling of rejection and stigmatization (Joiner and Metalsky, 1995; Nemade et al., 2007).

### 2.2.3 Behavioural model

Behavioural theories of depression betone the influence of inadequate adaptation to a situation in the development and maintenance of depression. The model originated from the principles of learning and conditioning. The theories significantly emphasize the reactions individuals have to
new environment and how they develop adaptive or maladaptive coping strategies (Ainsworth, 2000). According to the behavioural theory, depression was described to occur when a person develops a narrow repertoire of passive behaviour and efficiently avoids aversive stimuli (Veale, 2008).

2.2.3.1  
*Behavioural Activation*

Behavioural activation is a development of activity scheduling and functional approach to depression which is a constituent of cognitive therapy (Veale, 2008). It focuses on two main arguments; people with depression use avoidance activities that maintain their depression and determine the origin of depressive episodes in the environment (Jacobson et al., 2001). Behavioural activation is centred on learning theory and contextual functionalism. Behavioural activation theories address biological factors that contribute to depression, but do not focus on an internal cause of depression such as thoughts, inner conflicts or serotonergic dysfunction (Veale, 2008).

2.2.3.2  
*Social Skills*

Lack of social skills and poor social interactions have been empirically proven to contribute to the development and maintenance of depression (Oltmanns and Emery, 2014). Studies show that social skills reflect the absence of social interaction acquiring behaviours shown off by the depressed individual (Everett-Haynes, 2010). The deficit in social conversation and exchange results in social isolation that facilitates the development of isolation and negative self-concept (Steger and Kashdan, 2009).
2.2.3.3 Reinforcement contingencies

Reinforcement contingencies theory states that depression is the outcome of lack of adequate environmental reward contingencies and punishment in healthy behaviours (Carvalho and Hopko, 2011). Cognitions and open behaviours that act as avoidant function are thought to be essential precursors to the diminished reward and positive reinforcement that predispose people to depression (Martell et al., 2001).

Learned helplessness is defined as a sense of having no control over outcomes, irrespective of your actions (Carlson, 2010). Learned helplessness theory illustrates that clinical depression and associated psychiatric conditions results from lack of control over a condition (Nolen, 2014).

2.3 BIOLOGICAL BASIS OF DEPRESSION

In addition to genetic pre-dispositions, various environmental factors are also well-established to contribute to the pathogenesis of depressive disorders.

2.3.1 Neurochemical factors

2.3.1.1 Monoamine hypothesis

The monoamine hypothesis has dominated study into the pathophysiology and pharmacotherapy of depression over the last decade. The monoamine hypothesis of depression proposes that depression is associated with the reduced levels of monoamines such as noradrenalin, serotonin and dopamine in the anterior cingulated cortex, raphe nuclei, ventral tegmentum and Brodmann area 25 regions of the brain (Amthor, 2014). The evidence of this hypothesis has predominantly
come to light from the apparent efficacy and success of antidepressant medications that increase the concentration of these neurotransmitters (Rodger, 2014).

Several agents are known to be implicated in such kinds of effects including the nonselective inhibitor of the 5-hydroxytryptamine (5-HT) and NA, drugs that inhibit monoamine oxidase, tricyclic antidepressants, enzymes involved in monoamine metabolism, and tranylcypromine. Earlier evidence reported that reserpine which cause depletion of monoamines by disrupting monoamine vesicles storage, could cause depression, although recent studies were unsuccessful in consistently replicating this findings (Duman, 2013). Iproniazid is known to nullify psychotic depression that a drug used to treat hypertension can educe (Potter, 2006). Several studies have also provided the evidence that serotonin and norepinephrine were responsible for sustained therapeutic effects to antidepressant drug, as reduction of monoamine levels resulted in relapse in patients being treated with selective 5-HT or NA reuptake inhibitor (Jacobsen et al., 2012). Depressed patients responsive to drugs that act via the 5-HT pathway such as fluoxetine frequently suffer relapse when given diets free of tryptophan, a serotonin synthesis precursor (Jacobsen et al., 2012; Yamada and Higuchi, 2002). However, relapse is less likely to occur in depressed patients responsive to NA antidepressants (Yamada and Higuchi, 2002).

The diversity of depression is known to have several origins, including genetic polymorphism. Scientific evidence suggests that polymorphisms of the gene for tryptophan hydroxylase are linked depression in some patients (Jacobsen et al., 2012). The low, short variant of the serotonin transporter gene are more vulnerable to developing depression, anxiety-related traits and suicidal behaviour in response to stress or trauma (Caspi and Hariri, 2010). Research supports the evidence that suicide and depressed patients often expressed reduced numbers of
NA (α2), 5-HT1A, 5-HT1B and 5-HT2A receptors and diminished binding affinity of 5-HT1A receptors following demonstration using post mortem studies as well as PET scanning analysis (Savitz et al., 2009).

Recent studies on depressed patients have discovered no significant modification in the function and levels of monoamines, with some novel antidepressant drugs known to act via other pathways and not directly via the monoaminergic pathway (Boerkamp and Wijnberger, 2011). This is supported by a study done by Duman (2012) which demonstrates that newer antidepressants acting via glutamate neurotransmission, particularly ketamine, exhibits rapid antidepressant effect in hard-to-treat depressed patients. Reports also suggest that pre-existing deficits in mood regulating neural circuits predispose vulnerable individuals to react abnormally to monoamine deficits in the pathophysiology of depression (Cowen et al., 2012). Therefore, monoamine functions are considered to be an essential contributor but not an absolute or exclusive factor in the pathophysiology of depression.

2.3.1.2 Modification in GABA and glutamate neurotransmission

A paradigm shift from a monoamine hypothesis of depression to functions of glutamate and GABAergic neurotransmission may represent a considerable advancement in the working hypothesis that motivates the search for new antidepressant drugs. Essentially, notwithstanding the availability of diverse classes of drugs with monoamine-based mechanisms of action, there remain a huge proportion of depressed patients who fail to achieve a sustained remission of depressive symptoms (Sanacora et al., 2012).

Clinical and preclinical evidence strongly associate GABAergic dysfunction in mood disorders including depression (Brambilla et al., 2003). A report by Duman (2012) showed that reduced
GABA detected by magnetic resonance spectroscopy was reversed by antidepressant treatments. Research suggests GABA receptor antagonism may serve as a basis for the development of novel antidepressants (Mombereau et al., 2004). Studies of the cerebral spinal fluid and with the use of proton magnetic resonance spectroscopy provided a direct evidence for in vivo reductions of GABA functional levels in cortical brain regions of depressed patients (Sanacora and Saricicek, 2007). Dorsal lateral PFC of depressed patients demonstrated a deficit in GABAergic interneurons during a post-mortem studies (Maciag and Hughes, 2009). Levinson and Fitzgerald (2010), in an imaging study, detected functional loss of GABA inhibition in the cortical regions of the brain. A post-mortem investigation revealed decreased levels of glutamic acid decarboxylase, a primary GABA synthesizing enzyme (Karolewicz and Maciag, 2010).

There is substantial evidence which implicates glutamate alteration or dysregulation in the pathophysiology of depression. Several studies have suggested the presence of increased functional levels of glutamate as well as decreased plasma glutamine/glutamate ratios in the plasma of depressed patients compared to healthy individuals (Küçükibrahimoğlu et al., 2009). A post-mortem investigation of the frontal cortex demonstrated a significant increase in tissue glutamate levels in patients with major depressive and bipolar disorders (Hashimoto et al., 2007). Sanacora et al., (2012) reported that altered platelet glutamate uptake recorded in depressed patients could contribute to the pathophysiology of the disease since platelets possess significantly high affinity to glutamate uptake system and are to express the same glutamate transporters in the brain. The need for functional balance glutamate signalling is highlighted by therapeutic efficacy of novel antidepressant which act via a transient burst of glutamate neurotransmission (Duman, 2012). Scientific evidence showed that significantly higher
glutamate metabolite levels were reported in the frontal cortex of subjects experiencing post-stroke depression and late life depression (Wang et al., 2012).

### 2.3.1.3 Nitric oxide and NMDA receptor complex

Nitric oxide (NO) has been shown to play an essential role in the nervous system including processes being associated to major psychiatric disorders (Knott and Bossy-Wetzel, 2009). Lee et al., (2006) demonstrated that samples obtained from depressed suicide attempters had elevated levels of NO metabolites. Reduced number of nitric oxide synthase (the enzyme responsible for the synthesis of NO) containing neurons in the hypothalamus (Bernstein et al., 2005) and hippocampus (Oliviera et al., 2008) was recorded in depressed patients. Studies on human genetic association have consistently linked NO signalling and bipolar disorder (Reis et al., 2006). Several preclinical investigations have shown that impairment of nitric oxide synthase activity produces antidepressant-like effects in various animal models (Wegener and Volke, 2010).

It has been shown that N-methyl-D-aspartate (NMDA) receptor complex is strongly linked to the pathogenesis of depression (Mantovani et al., 2003b). Several recent clinical and preclinical studies have shown that NMDA receptor antagonists such as ketamine or Ro25-6981 binding at N2B specific subunit, exert an antidepressant-like effect and also counteracts depressive-like behaviours in chronically stressed rodents (Li et al., 2010).

### 2.3.2 Neuroplasticity and neurotrophic factors

Substantial evidence indicates that disruption of neuroplasticity in the brain region is strongly associated with depression, including reductions of hippocampal neurogenesis and cortical
synaptogenesis (Hayley and Litteljohn, 2013). Deteriorated trophic maintenance stemming from stressor-induced reductions of growth factors, mainly brain derived neurotrophic factor (BDNF) is likely to influence such aberrant neuroplasticity. In this respect, significant substantiation has supported the contention that protracted disturbances of neuroplasticity is implicated in the pathophysiology of depression (Sacher et al., 2012).

Essentially, reduced hippocampal volume has been observed in patients diagnosed with major depression (Colla et al., 2007). Recent reports have focused on the possibility that continued alterations of neuroplasticity could be responsible for the faulty communication between anterior cingulate cortex, hippocampal, and amygdaloid regions, therefore resulting in the disordered processing of emotionally salient information observed in depressed individuals (Carballedo et al., 2011). According to Scheidegger et al., (2012) ketamine treatment targets the default mode network (D.M.N.) within the cingulate and prefrontal cortices to help “re-wire” faulty circuitry and reduce DMN metabolic activity. Changes in glial cell density, the composition and complexity of dendritic arbors have been reported to contribute to the brain volumetric changes in depression (Gittins and Harrison, 2011). Other findings have also revealed decrease in cortical and/or hippocampal astrocytes in depressed patients (Liu et al., 2011). It is essential to note that in addition to reductions of neuroplasticity, increased neuroplasticity in some case may also contribute to depressive symptomology and relapse (Hayley and Litteljohn, 2013).

Scientific evidence reports that platelet and serum BDNF protein concentrations are diminished in depressed patients, with a positive correlation between the levels of the growth factor and symptom severity (Yoshida et al., 2012). Reduced BDNF mRNA expression was recorded in the leukocytes of depressed individuals (Cattaneo et al., 2010). Several clinically beneficial therapies, including selective serotonin reuptake inhibitors (SSRIs), tricyclics, and
electroconvulsive therapy (ECT), were reported to boost hippocampal BDNF expression and increase neurogenesis in animal models (Voleti and Duman, 2012).

Irrespective of the fact that BDNF has been the centre of focus about the role growth factors in depression, emerging and recent evidence indicates that other growth factors, particularly glial cell-line derived neurotrophic factor (GDNF) (a member of the transforming growth factor beta family) is also likely to be associated with the pathogenesis of depression (Tsenga et al., 2013). A study by Diniz et al., (2012) recorded reduced levels of GDNF protein and mRNA in the blood of depressed patients. However, effective antidepressant therapies including SSRIs and ECT caused a return to normal levels (Zhang et al., 2009). Other neurotrophic/growth factors that have been associated with depression include vascular endothelial growth factor, fibroblast growth factor 2 and insulin-like growth factor 1 (Duman, 2012).

Stressors have been implicated in the inhibitory actions on dendritic branching (Son et al., 2012), suggesting the likelihood that grey matter shrinkage may partly be associated to the considerable stress experienced by depressed individuals (Hayley and Litteljohn, 2013). Moreover, studies on depressed human subjects appear to substantiate the animal data on the effect of neurotrophic factors in stress states (Zhang et al., 2015). In this regard, neuroplasticity and neurotrophic factors continue to be passionately researched into and continue to yield new and novel potential treatment targets of major depressive disorder (Duman and Li, 2012; Voleti and Duman, 2012).

2.3.3 Neuroendocrine factors

Considerable research findings have been documented over the few decades regarding the role of hormones in the pathophysiology depression (Hatzinger, 2000). Several clinical and preclinical studies have provided substantial evidence that abnormalities in the hypothalamic-pituitary-
adrenal (HPA) axis is implicated in depression (Gotlib et al., 2008). Research showed that an estimated 73% of depressed patients had increased cortisol levels compared to non-depressed individuals (Stetler and Miller, 2011). Preliminary evidence suggests that corticotropin releasing hormone 1 (CRH1) receptor antagonists decrease symptoms of depression (Holsboer and Ising, 2008). Scientific findings shows that the elevation of both endogenous cortisol and exogenous glucocorticoids are implicated in cognitive deficits and affective disorders such as major depression (Markopoulou et al., 2009). Research suggests that some antidepressants exert their pharmacological action via enhancement of type II glucocorticoids receptor function with a subsequent restoration of HPA axis via negative feedback (Barden, 2004). Thyroid hormone dysregulation has been reported in depressed patients (Engum et al., 2002). Therapeutic effect of standard antidepressant medications have been shown to be intensified when they are used concurrently with thyroid hormones in the treatment of depression (Engum et al., 2002). Several research findings have also linked dysregulation of steroidal sex hormones to the pathophysiology of depression (Almeida et al., 2004). Postpartum and postmenopausal periods which are usually characterized by estrogen deficiencies have been implicated in the aetiology of depression in women (Kessler, 2003). Similarly, acute testosterone deficiency has been reported to play a role in depressive symptoms observed in men (Kessler, 2003).

2.4 ANTIDEPRESSANT DRUGS

2.4.1 History

Earlier in time to the introduction of the first clinical antidepressants, the therapeutic tools in use in treating mood disorders were tremendously limited (Lopez-Munoz and Alamo, 2009). During the early periods of the 20th century, insulin comas, electrical shock therapy or the famed “sleep cures” had a widespread use in the treatment depressive symptoms (Lopez-Munoz and Alamo,
The few chemical products at hand to physicians were non-specific ones, including malonic nitrite, succinic dinitrite and lactic acid (Lopez-Munoz and Alamo, 2009).

The fields of psychiatry and psychopharmacology witnessed an absolute transformation in the 1950s with the introduction of clinical psychoactive drugs used today. Reports indicates that serendipity played an essential role in the finding of the majority of the psychotropic medications (Judd, 1998).

From 1957-1980, the first generation antidepressant drugs which includes tricyclic antidepressants such as imipramine and maprotiline which act via 5-HT and NA reuptake inhibition with blocking action on diverse receptors were discovered (Kuhn, 1958). The irreversible MAO inhibitors including phenelzine and iproniazid were also introduced within the periods of 1960-1965 (López-Muñoz et al., 2007). Drugs which act as antagonist of α2 autoreceptors such as mianserine also came into the existence within 1970-1980 (Schulz and Remschmidt, 2001). Moclobemide and other drugs that acts as reversible MAO inhibitors were introduced from 1980-1995 (Lopez-Munoz and Alamo, 2009).

The second generation of antidepressant drugs such as fluoxetine, paroxetine and setraline acting via selective 5-HT inhibition were also introduced between 1980-1990 (Domino, 1999). Within the same time period, selective dopamine reuptake inhibitors such as bupropion were discovered (Lopez-Munoz and Alamo, 2009).

Between 1975 and 2000, a third generation of antidepressant medications generally called atypical antidepressants were also introduced. These include mirtazapine,(a drug which acts as an antagonist of α2 auto- and hetero-receptors and 5HT2 and 5-HT3 receptors) reboxetine, (selective NA reuptake inhibitors) and venlafaxine, (a NA and 5-HT reuptake inhibitor) (Lopez-
Munoz and Alamo, 2009). Over the last decade, a number of clinical studies have shown that ketamine, acting via non-competitive NMDA receptor antagonism exhibits antidepressant effects (Hillhouse and Porter, 2015).

2.4.2 Antidepressant drugs and their mechanisms of action

Antidepressant effects are produced by drugs that inhibit monoamine oxidase action, inhibit monoamine reuptake, stimulate and/or modulate monoamine, antagonize monoamines at their receptors sites and antagonism at the $\alpha_2$ auto – and hetero – receptors (Hughes et al., 2005; Lopez-Munoz and Alamo, 2009). In addition, blockade of the glycone site of the NMDA receptor, agonism of melatonin receptor, and inhibition of L-arginine-NO-cGMP pathway can also protect against the occurrence of depressed state in animal models (Adongo et al., 2015; Laudon and Frydman-Maron, 2014). In principle, antidepressant drugs act by targeting one or a combination of these systems (Yildiz et al., 2002).

2.4.2.1 Monoamine oxidase inhibitors

Monoamine oxidase inhibitors (MAOI) are chemicals that inhibit the conversion or metabolism of monoamines into inactive products via the impairment of the activity of the monoamine oxidase enzyme family (Lehne, 2004). Thus, they increase the concentration of monoamine transmitters; 5-HT, NA, and DA. Two subtypes of MAO have been discovered and are located in both neuronal cells like the brain and non-neuronal cells such as liver and intestines within the body (Lovatt, 2011). Therefore the functional levels of neurotransmitters are potentiated by inhibiting MOA and thus relieving the symptoms of depression.

The classical MAOIs such as tranylcypromine, phenelzine and isocarboxazid are irreversible and nonselective. Following their oxidation to reactive intermediates by MAO, these "suicide"
substrates interacts irreversibly to inactivate the flavin prosthetic group of the MAO enzyme (Krishnan, 2007). This group of nonselective and irreversible MOAIs in addition, also inhibit the biotransformation of dietary tyramine leading to symptoms of ‘the cheese effect’ often associated with the usage of these drugs (Brent et al., 2005). This adverse effect resulted in the MAOIs becoming a last choice in the pharmacological management of depression (Lovatt, 2011). Several reversible, short-acting, selective MAO-A inhibitors such as moclobemide, had been developed. Studies indicates that the ‘cheese effect’ is less likely to occur with this group of drugs due to their limited and reversible duration of action (Yamada and Yasuhara, 2004).

2.4.2.2 Monoamine receptor antagonists

5-HT receptor antagonists have been shown to accelerate and perhaps augment the clinical effects of most antidepressants and as well alleviate and prevent several adverse effects associated with the use of other antidepressant medications (Artigas et al., 1996). Several reports also indicates that a combination of SSRI drugs treatment with the mixed 5-HT\textsubscript{1A}/\beta-adrenoceptor antagonist pindolol, caused a marked decline in the latency of the antidepressant response and additionally intensified the clinical response in previously untreated patients with depression (Whale et al., 2010). Scientific research suggests that co-administration of the selective 5-HT\textsubscript{1A} antagonist WAY-100635 and the 5-HT\textsubscript{1B} receptor antagonist SB-224289 acts additively to potentiate the neurochemical actions of the SSRI, fluoxetine (Artigas, 2013). This co-administration could elevate serotonin and therefore potentially be an effective strategy to treat depression (Artigas, 2013). Nefazodone and trazodone are both 5-HT\textsubscript{2} receptor blockers, however, unlike SSRIs, they do not produce some of the side effects that SSRIs may cause such as the short-term increase in anxiety or insomnia, akathisia, and sexual dysfunction (Yildiz et al., 2002).
Mirtazapine is a noradrenergic and specific serotonergic antidepressant which possesses a dual mechanism of action that increases the concentration of 5-HT and noradrenalin in the synaptic cleft (Stahl, 1997). It is known to block noradrenaline α2-autoreceptors and noradrenaline α2-heteroreceptors in addition to possessing a selective blockade effect on 5-HT1A receptors (Yildiz et al., 2002). Etoperidone exhibits its antidepressant effect by acting primarily as an antagonist of 5-HT2A, α1-adrenergic, 5-HT1A, and α2-adrenergic receptors has very weak affinity and hence blockade effect on D2 receptors (Index Nominum, 2000).

2.4.2.3 Monoamine reuptake inhibitors

Serotonin–norepinephrine–dopamine reuptake inhibitors (SNDRI), popularly referred to as triple reuptake inhibitors (TRI), consist of a class of drugs that concomitantly acts as reuptake inhibitors for the monoamine neurotransmitters serotonin (5-HT), noradrenaline (NA) and dopamine (DA) (Shao et al., 2011). These effects are mediated by blocking the action of the serotonin transporter (SERT), noradrenaline transporter (NET), and dopamine transporter (DAT). This results in elevated functional levels of extracellular concentrations of these monoamine neurotransmitters and, therefore, an increase in their neurotransmission (Marks et al., 2008). Amitifadine, is an antidepressant drug with a typical serotonin–norepinephrine–dopamine reuptake inhibition effect (Skolnick et al., 2003). GSK1360707F is a novel and potent selective triple reuptake inhibitor structurally related to amitifadine which is still under clinical trials and has shown to be effective in the treatment of major depressive disorder (Comley et al., 2013).

Serotonin noradrenalin reuptake inhibitors (SNRIs) are a class of antidepressant that selectively bind to 5-HT and NA transporters to block their reuptake from the synaptic clefts (Stahl et al., 2005). SNRIs possess a “dual mode of action” by inhibiting the action of 5-HT transporters
(SERT) and NA transporters (NAT) in a way similar to tricyclic antidepressants (TCAs), but differ from TCAs in that SNRIs exhibit lesser affinity for other receptors. Studies have shown that SNRIs also contribute to the elevation of functional levels of dopamine in the prefrontal cortex via NAT inhibition (Ipek et al., 2012). Most SNRIs show varying selectivity to the reuptake of both 5-HT and NA. Milnacipran inhibits serotonin and noradrenalin reuptake with equal affinity, duloxetine shows a 10-fold greater selectivity for 5-HT reuptake inhibition while venlafaxine exhibits a 30-fold greater selectivity for 5-HT (Celikyurt et al., 2012). Desvenlafaxine, a synthetic form of the isolated major active metabolite of venlafaxine, is approximately 10 times more effective at blocking 5-HT uptake than NA uptake (Septien-Velez et al., 2007; Stahl et al., 2005).

The first Tricyclic antidepressant (TCA), imipramine was originally derived from the antipsychotic drug phenothiazine (López-Muñoz and Alamo, 2009). TCAs typically block the reuptake of serotonin or norepinephrine. In addition to their effects on amine uptake, most TCAs interact with several other types of neurotransmitter receptor, including alpha adrenergic (α1) receptor blockade, muscarinic acetylcholine receptors, histamine receptors and 5-HT receptors. The outcome of TCAs interaction with most of these receptors do not contribute to the antidepressant effect but are responsible for the various adverse effects associated with the use of TCAs. A modest overdose of TCA has been reported to be lethal due to the narrow gap between their ability to inhibit the biogenic amine uptake pumps and the blocking effect of fast sodium channels (Preskorn, 2010). Tricyclic antidepressants also can desensitize D2 dopamine autoreceptors through uncertain mechanisms and with uncertain behavioural contributions (Brown and Bottomley, 1990). TCAs with secondary-amine side chains or the N-demethylated (nor) metabolites of agents with tertiary-amine moieties such as amoxapine, desipramine,
maprotiline, and nortriptyline are moderately selective inhibitors of norepinephrine transport. However, most tertiary-amine TCAs also block the reuptake of 5-HT. Among the tricyclic antidepressants, trimipramine has been reported to possess exceptional features in that, it lacks conspicuous blockade effects at monoamine transport, and its clinical actions remain elusive (Cohen, 2003).

The selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed class of antidepressants. These drugs inhibit the neuronal uptake of serotonin both immediately or chronologically by blocking the SERT, thus increasing its synaptic concentration (Moltzen and Bang-Andersen, 2006). Citalopram and fluoxetine have been shown to be racemic mixtures whereas sertraline and paroxetine are also two distinct enantiomers. Escitalopram is the (S)-enantiomer of citalopram. Studies have shown that elevated synaptic serotonin level stimulates a large number of postsynaptic serotonin receptor types. Stimulation of 5-HT₃ receptors is suspected to mediate the common side effects associated with the use of SSRIs, such as gastrointestinal effects (nausea and vomiting) and delayed or impaired orgasm. Moreover, stimulation of 5-HT₂C receptors have been reported to contribute to the agitation or restlessness characteristic of SSRIs (Azmitia et al., 1995). Thus novel SSRIs under discovery have been reported to exhibit auxiliary interactions with other receptors, most notably additional antagonist activity at 5-HT receptors located pre-synaptically as well as post-synaptically (Moltzen and Bang-Andersen, 2006).

Selective noradrenalin reuptake inhibitors selectively and significantly block NA reuptake (Frazer, 2000). Recent clinical evidence shows that NA neurotransmission is augmented in depressed patients treated with NA reuptake inhibitors (Miller et al., 1996). The therapeutic effects of NA reuptake inhibitors such as reboxetine and mazindol are reversed by catecholamine
depletion in depressed patients, indicating that euthymia is sustained by elevation of NA neurotransmission (Miller et al., 1996).

2.4.2.4 Modulators and/or stimulants of monoamines

Antidepressant drugs that acts as modulators and/or stimulators of monoamines mainly elevates serotonergic, noradrenergic, dopaminergic, and at times cholinergic, histaminergic and glutamatergic neurotransmission in brain structures (Sancheza et al., 2014). They are also known to augment SSRI effects on extracellular 5-HT functions in the rodent brain (Blier and Rueter, 1999). One of such drug is vortioxetine, a novel antidepressant which act as a 5-HT$_{1B}$ receptor partial agonist, 5-HT$_{1A}$ receptor agonist and serotonin (5-HT) transporter (SERT) inhibitor as well as a 5-HT$_{1D}$ receptor antagonist, a 5-HT$_{3}$, and a 5-HT$_{7}$ receptor antagonist (Guilloux et al., 2013; Westricha et al., 2015). Vilazodone is also another drug which act as a serotonin reuptake inhibitor and 5-HT$_{1A}$ receptor partial agonist (Wang et al., 2013).

2.4.2.5 NMDA receptor antagonists

Glutamate plays an important role as an excitatory neurotransmitter in diverse physiological functions, and its release results in activation of NMDA receptors, an underlying cause of depression (Sanacora and Schatzberg, 2015). Several studies with NMDA receptor antagonists of mGluR1 and mGluR5 receptors have been shown to possess antidepressant activity in a variety of animal models (Lemke and Williams, 2012). NMDA antagonists block the Ca$^{2+}$ channels within NMDA receptors and thus inhibit the influx of excessive Ca$^{2+}$ which mostly characterized the pathophysiology of depression (Ates-Alagoz and Adebloye, 2013). The antidepressant therapeutic effects of NMDA antagonists have been reported to be mediated by
increased AMPA-to-NMDA glutamate receptor throughput in critical neural circuits (Lemke and Williams, 2012).

Ketamine, a non-competitive NMDA antagonist is known to produce rapid, robust and persistent antidepressant effects clinically (Owen, 2012). Several antidepressant compounds under clinical trial including CGP 37849 and CGP 40116, a noncompetitive, non-subunit selective NMDA receptor antagonist MK-801 have been reported to effectively block NMDA receptor activity (Autry et al., 2011; Lima-Ojeda et al., 2013). The NR2B selective antagonist RO-25-6981 (Adongo et al., 2015; Lima-Ojeda et al., 2013) has consistently been shown to exhibit antidepressant-like properties in rodent models.

2.4.2.6 Melatonin receptor agonists

Melatonin agonists are analogs of melatonin. Melatonin is the primary neurohormone produced by the pineal gland in the brain responsible for the regulation of the body’s circadian rhythm (Lemke and Williams, 2012). An antidepressant effect of melatonin receptor agonist markedly differs from other classes of antidepressant drugs: its primary molecular targets in vivo are the melatonin MT$_1$ and MT$_2$ receptors, where it acts as a potent agonist. The antidepressant potential of agomelatine is as a result of a synergistic or a complementary action on MT receptors as well as its antagonistic effect on 5-HT$_2C$ receptors (Bourin and Prica, 2009). Studies show that agomelatine has no affinity for histaminergic, dopaminergic, cholinergic benzodiazepine, α-adrenergic or β-adrenergic receptors. Activation of MT$_1$ and MT$_2$ receptors by agomelatine trigger the release of melatonin causing the induction of sleep, and rezynchronous of the irregular sleep/wake circadian rhythm of depressed individuals by delaying the sleep phase rhythm (Lemke and Williams, 2012). Through the antagonism at 5-HT$_2C$ receptors, agomelatine stimulate the release of DA and NA in the frontal cortex of the brain which may contribute to its
antidepressant efficacy (Dawoodi et al., 2012). Other melatonin receptor agonists approved for use in humans include ramelteon and tasimelteon.

### 2.4.2.7 L-arginine-NO-cGMP pathway

L-arginine-nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) is an essential signalling pathway known to be implicated in the pathogenesis of depression (Mantovani et al., 2003a). Nitric oxide is synthesized from L-arginine by nitric oxide synthase (NOS) and has been reported to play a role in neurotransmission, learning, perception of pain, aggression, synaptic plasticity and depression (Esplugues, 2002). Several scientific evidences have shown that decrease of NO levels within the hippocampus stimulates antidepressant-like effects (Joca and Guimaraes, 2006). A number of physiological effects of NO are facilitated via its association with the heme iron of soluble guanylate cyclase leading to enzyme activation and subsequent increase in cGMP (Kaster et al., 2005). NOS inhibitors such as L-NNA (L-NG-nitroarginine), L–NMMA (L-NG-monomethylarginine) and NG-propyl-L-arginine have been shown to be effective in enhancing the antidepressant effect of various drugs in animal models (Wegener and Volke, 2010). MK-801(dizocilpine) has been reported to exhibit its antidepressant effect via the involvement of NO signalling pathway in mice (Dhir and Kulkarni, 2008).

### 2.4.2.8 Opioid receptor agonists

Central opioid systems are also known to influence the pathophysiology of depression (Berrocoso et al., 2013). Endogenous opioid peptides co-expressed in brain areas have been reported to be implicated in affective disorders and in the action of antidepressant drugs via three receptor subtypes (mu, delta and kappa). Moreover, antidepressants which act via increase the inhibition of the reuptake of monoamines lead to the enhancement of the opioid pathway
The role of opioid as recognized antidepressants is buttressed by the clinical effectiveness of \( \mu \)-opioid receptor agonists such as tramadol, oxycodone, oxymorphone, etc., in the treatment of refractory depression (Shapira et al., 2001; Hegadoren et al., 2009).

### 2.5 NON-PHARMACOLOGICAL TREATMENTS OF DEPRESSION

#### 2.5.1 Psychotherapy

It is satisfactorily recognized that psychological interventions are effective in the treatment of depression (Cuijpers et al., 2014). Even though psychiatric medication is the often prescribed therapy for depression (Carson, 2000), psychotherapy may be useful, either alone or in combination with pharmacological intervention. However, combining psychotherapy and antidepressant medications may prevent a "slight advantage" (Cuijpers et al., 2014).

Cognitive behaviour therapy (CBT) is the most examined form of psychotherapy considered to be effective. In this therapy, patients are taught to study a set of cognitive and behavioural skills, which they utilize on their own. A recent investigation by Roth and Fonagy (2006) indicates that CBT is equally effective in treating patients with moderate to severe depression. A study reported that CBT and fluoxetine outperformed treatment with only fluoxetine (March et al., 2004).

Interpersonal psychotherapy (IPT) emphasize on the social and interpersonal sparks that may mediate depression. Substantial evidence has shown that (IPT) is an effective treatment for depression. IPT undertakes a well designed course with a set number of weekly sessions (usually 12) similar to CBT. However, the focus is on relationships with others. IPT is essential to
improve interpersonal skills, build a more effective communication and reduce stress (Weissman et al., 2000).

Introduced by Martell et al., (2001) behavioural activation therapy emphasize on the improvement of activity scheduling. This form of therapy inspire patients to approach activities that they are staying away from and focus on analyzing the function of cognitive processes that serve as a form of avoidance (Martell et al., 2001). Patients are therefore repositioned on their ambitions and valued directions in life. A core merit of behavioural activation over customary cognitive–behavioural therapy for depression is that it makes it easier to train staff in it and is applied to both in-patient and out-patient settings (Veale, 2007).

2.5.2 Electroconvulsive therapy

Electroconvulsive therapy (ECT) is a standard psychiatric treatment in which spasm are electrically stimulated in patients to provide relief from psychiatric illnesses (Rudorfer et al., 2003). An informed consent is always required when ECT is to be used which is usually considered as a last line of intervention for major depressive disorder (Beloucif, 2013). Reports have shown that a cycle of ECT is effective in 50% of patients with treatment-resistant major depressive disorder (Dierckx et al., 2012), however, studies have also revealed that about half of patients who respond, relapse with twelve months (Jelovac, 2013). The most common effects on the brain are confusion and memory loss (American Psychiatric Association, 2001). Nonetheless, ECT is regarded as one of the least harmful treatment options available for severely depressed pregnant women (Pompili, 2014). Drug therapy is usually continued after ECT, yet, some patients receive maintenance ECT (Dierckx et al., 2012).
2.5.3 Light therapy

Light therapy or heliotherapy comprises of exposure to daylight or to specific and precise wavelengths of light using polychromatic polarized light, lasers, dichroic lamps, full-spectrum light or light-emitting diodes. The rays strike the retina of the eye. Light therapy is used to treat circadian rhythm disorders and seasonal affective disorder. A meta-analysis of bright light therapy revealed a significant decrease in the severity of depression symptom (Tuunainen et al., 2004).

2.6 THERAPEUTIC BENEFITS OF SOME PLANT SECONDARY METABOLITES

2.6.1 Background

The utilization of several medicinal herbs has a protracted history. Since ancient times, medicinal plants are being used especially in oriental countries. However, the introduction of synthetic compounds and antibiotics in early 20th century influenced the regression in the usage of medicinal herbs and diminished interest in proving the scientific bases to their pharmacological effects (Cowan, 1999). Interestingly, the hostile adverse effects associated with usage of antibiotics and several other synthetic compounds have reignited interest in the disciplines of phyto-chemistry, phyto-pharmacology and phyto-medicine (Makkar et al., 2009).

The past decade has witnessed a remarkable renaissance in the interest and use of medicinal plant products due to their rich phytochemical constituents. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant (Briskin, 2000). Several studies report that the therapeutic actions of medicinal plants are inimitable to peculiar plant species and these are coherent with the view that combination of secondary metabolites in a particular plant are usually taxonomically unique (Wink, 1999).
Polyphenols are naturally occurring secondary metabolites of plants. The most studied classes are the flavonoids, tannins, stilbenes, and lignans. A number of studies have demonstrated that polyphenols have cardio-protective effects (García-Lafuente et al., 2009), anticancer potentials (Yang et al., 2001), neuro-protective effects (Letenneur et al., 2007), anti-aging (Pandey and Rizvi, 2009), and anti-diabetic effects (Rizvi and Zaid, 2005) among others.

Triterpene saponins collectively known as ginsenosides are the major phytochemicals present in ginseng and have been reported to heavily contribute to its vast health benefits which include reduction of levels of cholesterol, low-density lipoprotein-cholesterol (LDL-C), serum triglycerides, and atherogenic indices (Song et al., 2012). According to a study by Tsai et al., (2010) soy saponins may be effective in preventing colon cancer by affecting cell morphology, cell proliferation enzymes, and cell growth. Research findings indicate that soybean saponins possess not only a significant antimutagenic activity but also a strong inhibitory action against carcinogen-induced DNA damages (Jun et al., 2002). Platyconic acid, a saponin from Platycodi radix, improves glucose homeostasis by enhancing insulin sensitivity in vitro and in vivo (Kwon et al., 2012).

Alkaloids are known to create extreme physiological action in humans and are commonly used as therapeutic agents. Several alkaloidal drugs have been identified to stimulate the central nervous system either as depressants (e.g.: morphine) or stimulants (e.g.: caffeine). Other known alkaloids have an effect on the autonomic nervous system and are been used as anticholinergics (e.g.: atropine), ganglioplegics (e.g.: nicotine) sympathomimetics (e.g. ephedrine), sympatholytics (e.g.: yohimbine), and parasympathomimetics (e.g.: pilocarpine). Some local anaesthetics such as cocaine and other alkaloidal agents are used to treat fibrillation, (e.g. quinidine) as analgesics, (e.g.: codeine and morphine) anti-tumor agents, (e.g.: vinblastine) anti-
malarials, (e.g.: quinine) antibacterials, (e.g.: berberine) and amoebicides (e.g.: emetine) (Roberts and Wink, 1998).

Phyto sterols and stanols have been recognized to lower serum cholesterol level via the reduction of cholesterol from the gut (Law, 2000). A study conducted by Gylling et al., (1997) showed that plant sterol or stanols added to a daily portion of margarine decreases serum levels of low-density-lipoprotein cholesterol in the aged causes a reduction in the risk of heart disease of about 25%.

2.6.2 Antidepressant properties of secondary metabolites of some medicinal plants

Discovery of novel sources of natural products for depression treatment will continue to be an essential field of research. Despite the great advances in our understanding of depression, not much is known about the antidepressant mechanisms of many plant phytochemicals. However, some advantages of plant secondary metabolites with regards to the discovery of antidepressants have been reported and the antidepressant activity of plant phytochemical in particular has attracted tremendous attention in recent years (Gong et al., 2014).

The total flavonoids of Hypericum perforatum, also known as St. John’s wort used to treat depression have shorten the motionless time of FST mice in a dose-dependent manner (Shi and Wang, 2006). Studies by Nöldner and Schötz, (2002) revealed that the antidepressant activity of quercetin-3-O-glucuronide exhibits the same efficacy as imipramine. The extract of total flavonoids (XBXT-2) from Xiao Bu Xin Tang showed antidepressant potential in mice and a prolong intake of XBXT-2 helped despairing mice to recover their behaviour and increase the adrenaline content in their serum (An et al., 2008). Han et al., (2007) showed that apigenin, luteolin (flavone), and quercetin (flavonol) could regulate the activity of MAO activity with
their IC_{50} values at 6.5, 22.6 and 31.6 μmol/L respectively (Han et al., 2007). (Wang et al., 2007) found that flavanones showed a potential structure for antidepressant activity. High-density Hypericum monogynum flavonoids may exert its antidepressant properties by building up the levels of 5-HT, DA and NA in both hypophysis cerebri and brainstern (Zhen et al., 2012). The antidepressant effect of general flavonoids of cotton seed is known to be mediated by increasing the neurological function of 5-HT in the brain and improving the nutrient level and plasticity by enhancing the access function of AC-cAMP-CREB and its associated neurotrophic factor with 5-HT receptors (Y. F. Li et al., 2006).

Work done by (Martínez-Vázquez et al., 2012) revealed that alkaloid extract (1,2-dimethoxy-5, 6,6a,7-tetrahydro-4H-dibenzoquinoline-3,8,9,10-tetraol, anonaine, liriodenine, and normuciferine) from the aerial parts of Annona cherimola produces an antidepressant-like action from a generalized increase in monoaminergic turnover. The total tertiary alkaloids extract of Cissampelos sympodialis Eichler (Menispermaceae) possess a significant antidepressant effect in two animal models of depression; forced swim test and reserpine test in mice (Mendonça-Netto et al., 2008). A study undertaken to investigate the antidepressant potential of total alkaloids isolated from Semen Zizyphi Sponosae (SZS) showed that total alkaloids of SZS significantly reduced immobility time compared with the control group in the TST and FST in mice and remarkably inhibited the decrease of hypothermia in reserpine-induced-hypothermia (Qiao et al., 2011). Experimental data reported that diterpene alkaloids of Aconitum baicalensis exhibit antidepressant effects possibly via the modulation of sensitivity to serotonin (Nesterova et al., 2011).

Several literatures had reported numerous preclinical outcomes supporting the role of saponins as natural cure for depression and warranted their inclusion in antidepressant drug discovery
programs (Abbas et al., 2015). Saponins have been demonstrated to modulate various neurochemical pathways attributed to the onset of depression (Chen et al., 2014). Scientific evidence revealed that saponins possesses neuroprotective effect via the inhibition of apoptosis and intraneuronal calcium dynamics (Xiang et al., 2011). The orthodox antidepressants together with saponins have been reported to reduce oxidative stress (Xu et al., 2010). Total saponins from Areca catechu nut (Abbas et al., 2015), Asparagus racemosus (Singh et al., 2009), Bupleurum falcatum (Sun et al., 2012), Panax ginseng (Dang et al., 2009), Panax notoginseng (Xiang et al., 2011) and Trichopus zeylanicus (Rishikesh et al., 2012) have been shown to exhibit antidepressant potentials in rodents using various behavioural paradigms. Moreover, numerous bioactive saponins including bacopasides (bacopaside I, bacopaside II and bacopasaponsin C) derived from Bacopa monnieri (Liu et al., 2013; Rauf et al., 2013), ginsenosides Rb3 (P. notoginseng) (Cui et al., 2012) and Yuanzhi-1 (a triterpenoid saponin isolated from dried root of Polygala tenuifolia) had demonstrated potent antidepressant activity in rodents using various animal models of depression (Jin et al., 2014).

Cannabis terpenoids such as linalool, citronellol, and α-terpinen have been shown to possess significant antidepressant effect by causing a reduction in the duration of immobility in mice (Mander and Lui, 2010). The effective anxiolytic and antidepressant potential of Valerian officinalis L. extracts have long been attributed to the presence of a wide range of monoterpenes and sesquiterpenes (Kennedy and Wightman, 2011).
2.7 ANIMAL MODELS OF DEPRESSION

2.7.1 Acute Models

2.7.1.1 Forced Swimming Test (FST)

The forced swimming test, a behavioural model originally described by Porsolt et al., (1977) has been designed to be “a primary screening test for antidepressant” (Petit-Demouliere et al., 2005). This to a large extent is as a result of its strong predictive validity, good reliability, some face validity, poor construct validity and its capacity to meet the high through-put demands of the pharmaceutical industry (Rupniak, 2003). The assumption of a state of immobility when rodents are introduced into an inexorable cylinder of water, is thought to cease struggling and remain floating motionless in the water making only movements necessary to keep its head above water. The FST models a very precise cluster of stress-induced behaviours and is exquisitely sensitive to monoaminergic manipulations (Holmes, 2003). In an effort to enhance the sensitivity of the traditional FST in rodents, a modified forced swimming test has been developed. These alterations enabled investigators to distinguish specific behavioural components of active behaviours, namely; climbing and swimming (Cryan et al., 2002; Lucki, 1997). The major advancement of the modified FST over its traditional counterpart is that demonstrates that catecholaminergic agents diminish immobility with a parallel increase in climbing behaviour, whereas serotonin related drugs causes decline in immobility but increase swimming behaviour (Rénéric et al., 2001).

2.7.1.2 Tail Suspension Test (TST)

The tail suspension test is a rodent behavioural test suitable for the assessment of potential broad spectrum antidepressant drugs, and evaluation of other alteration that is expected to affect
depression related behaviours (Can et al., 2012). Mice are suspended by their tails and after initial escape-oriented movement, develop an immobile posture in such an inescapable stressful situation. The stressful situation is associated with the haemodynamic stress of being hung by their tail (Thierry et al., 1986). Acute antidepressant treatment decreases immobility scores. TST is a valuable tool in drug discovery for high-throughput screening of prospective antidepressant and to evaluate the effects of environmental, neurobiological, and genetic manipulations linked with depression (Crowley et al., 2005).

2.7.2 Chronic Model

2.7.2.1 Open space swim test

The open space swim test model originally developed by Sun and Alkon, (2003) was based on the use of continual sessions of the forced swim test (FST) where rodents gradually build up a generalized impasse behaviour of low distance swum and long duration of immobility. This behaviour stabilizes with the repetition of swimming sessions over periods of weeks (Stone and Lin, 2011). The model exhibits strong predictive, face and constructs validity in that it is sensitive to chronic antidepressants but not receptive to anxiolytics or antipsychotics (Stone et al., 2008). The model is known to employs a wider swimming area than the traditional FST and thus can be useful in studying interactions of depressive behaviour with behavioural flexibility and perseverance. The model represents an inescapable stress accompanied by changes in neural activity and brain cell proliferation that are characteristic of depression, but less effective in producing anhedonia (Stone and Lin, 2011).
2.8 **TRICHILIA MONADELPHA**

![Trichilia monadelpha plant](image)

**Figure 2.1** *Trichilia monadelpha* plant

Botanical name: *Trichilia monadelpha* (Thonn.) J.J. de Wilde  
Family: Meliaceae  
Local names: Twi: *Tanduro* (Ghana), Nzema: *Tenuba* (Ghana), Yoruba: *ako rere* (Nigeria)  
Commercial status: Class IV

### 2.8.1 Plant morphology

*Trichilia monadelpha* is a small evergreen, fast growing hairless tree with a large open spreading crown. It can grow to about 20-60 feet tall; bole straight and cylindrical, usually low-branching, up to 40–60 cm in diameter and lacks buttresses. The plant stem is woody, asymmetrically twisted and with a characteristic knobby twigs. The stem bark appears pale greenish with its
surface showing some slight exudates of whitish latex with a pale brown slashes. The leaves are compound, imparipinnate, opposite sporadically curled about 50 cm long with 3-7 pairs of leaflets. Each leaflet is borne on a petiole which is about 4-13 cm long. It has a dense, terminal inflorescence with an axillary panicle 12-21 cm long. The flowers which are unisexual appear tubular in shape and greenish-yellow in colour. Its fruits are obovoid to globose in shape, pendulous, and dotted with glands that are attractive to insects (Abbiw, 1990; Lemmens, 2008).

2.8.2 Geographical profiling

*Trichilia monadelpha* is an African tropical understorey tree that dwells in lowland semi-deciduous secondary jungle and often found along river banks and in other moist areas. It is found from Guinea to DR Congo, and south to north Angola. In Ghana, it is located throughout the Brong-Ahafo and Ashanti Regions (Lemmens, 2008).

2.8.3 Traditional uses

*Trichilia monadelpha* is being used traditionally for managing diverse medical conditions whereas other parts of the tree is also utilised for several domestic and agricultural purposes.

2.8.3.1 Medicinal

In Ghana and Nigeria, the stem is chewed for treating cough (Burkhill, 1997; Burkill, 1985). A bark decoction is also taken to soothe cough (Lemmens, 2008). A decoction of its stem bark is known to possess some sedative effects and occasionally used as an alcoholic beverage and stimulant (Burkill, 1985). Herbal preparation from the root is used for treating diarrhoea, dysentery, gout, ulcers and other gastrointestinal complaints (Busia, 2007; Lemmens, 2008). The roots and stem barks can be utilised as genital arousal and usually considered as aphrodisiac (Lemmens, 2008). Decoctions of the leaf is used to manage heart troubles and palpitations.
The stem bark of the plant can be milled into powder and applied on skin infections, wounds, etc. The stem bark decoctions can be taken to treat syphilis and gonorrhoea (Abbiw, 1990).

2.8.3.2 Non-medicinal

The tree is beneficial for soil improvement and soil fortification. A reddish brown dye obtained from the bark can be used for dyeing cloth and hides. The woof from the trunk is useful in producing timber, construction materials, designing wooden musical instruments, firewood etc. The wood is moderately durable and resist water thus it is been used in making canoes and boats. The seed oil is also used in cooking (Burkill, 1985; Lemmens, 2008).

2.8.4 Plant photochemistry

Busia, (2007) reported that chemical constituents identified in *T. monadelpha* in Ghana include tannins (protocatechuic acid), reducing sugars, and limonoids (dregeanin, heudelobin, etc). Alkaloids and tannins have been identified from the stem bark (Lemmens, 2008). However, there exists little information on scientific investigation on these phytochemical constituents of *T. monadelpha*. 
CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY DESIGN

The study design was experimental.

3.2 PLANT ACQUISITION AND EXTRACTION

3.2.1 Plant acquisition and authentication

The stem bark (together with other plant parts) of *T. monadelpha* was collected from Bomaa, Brong-Ahafo Region, Ghana (7°05′06″N, 2° 10′01.66″W) and authenticated at the Ghana Herbarium, Department of Botany, University of Ghana.

3.2.2 Preparation of stem bark extracts

The stem bark was chopped into pieces, air dried for fourteen days and pulverized into fine powder. An initial mass of 4 kg of the powdered plant bark was serially extracted with petroleum ether, ethyl acetate and 70% ethanol over a 72-hour period using cold maceration technique. The weight of the residue after air-drying was determined before the subsequent extraction. The resulting extracts were concentrated under reduced pressure at 40-60°C to a dark brown syrupy mass in a rotary evaporator. They were further dried using a water-bath, and stored. The percentage yield of each crude extract was then calculated.

The research was conducted in the Animal Experimentation Unit of the School of Biomedical and Allied Health Sciences and the Department of Pharmacology and Toxicology, University of Ghana. All behavioural studies were performed in the light cycle between 7:30 a.m and 2:30 p.m with experimentally naive I.C.R. mice.
This study was approved by the College of Health Sciences Ethical and Protocol Review Committee, University of Ghana and was assigned a protocol identification number: MS-Et/M.3-P31/2015-2016.

3.3 QUALITATIVE PHYTOCHEMICAL ANALYSIS

The extracts were screened for the presence of phytochemical constituents such as alkaloids, glycosides, tannins, sterols, flavonoids, terpenoids and saponins as described by Trease and Evans (1989).

3.3.1 Test for tannins
An amount 0.2 g of each extract was boiled with 25 ml of water for 5 minutes, cooled and filtered. The volume was then adjusted to 25 ml. To 1 ml aliquot of each extract was added 10 ml of water and 2 drops of 1% ferric chloride for the appearance of a blue-black or green precipitate.

3.3.2 Test for glycosides
An amount of 0.2 g of each extract was warmed with 5 ml dilute H₂SO₄ on a water bath for 2 minutes. The mixture was cooled, filtered and 4 drops of 20 % NaOH was added to each filtrate. A volume of 1ml of Fehling’s A and B solutions were added to each filtrate, warmed and observed for a red-brown precipitate.

3.3.3 Test for saponins
An amount of 0.2 g of each extract was shaken vigorously with about 10 ml of water in a stoppered test tube and observed for the presence of a persistent froth.

3.3.4 Test for alkaloids
An amount 0.2 g of each extract was boiled with 10 ml of dilute HCl for 5 minutes. The supernatant liquid was filtered into another test tube and 1ml of the filtrate taken, into which 3
drops of Dragendorff’s reagent (potassium bismuth iodide solution) was added. The mixture was shaken and observed for the appearance of an orange spot precipitate.

3.3.5 Test for flavonoids

A volume of 10 ml of 98% ethanol was added to 0.2 g of each extract. A small amount of zinc metal was added to the resulting extracts followed by drop wise addition of concentrated HCl. They were examined for the appearance of colours ranging from orange to red (flavones), orange to crimson (flavonols), crimson to magenta (flavonones).

3.3.6 Test for sterols

An amount of 0.2 g of each extract was added to 2 ml of chloroform and filtered. A volume of 2 ml of acetic anhydride was added to 1 ml of the filtrate after which few drops of concentrated H$_2$SO$_4$ were carefully added along the sides of the test tube. A violet to blue colouration was an indication of the presence of sterols.

3.3.7 Test for terpenoids

An amount of 0.2 g of each extract was added to 2 ml of chloroform in a test tube followed by addition of 1 ml of concentrated H$_2$SO$_4$. A reddish-brown colouration at interface shows the presence of terpenoids.

3.4 ANIMAL HUSBANDARY

Nine hundred and seventy (970) ICR mice (20 – 25 g) were obtained from the Nogochi Memorial Institute for Medical Research, University of Ghana and kept at the Animal Experimentation Unit of the Department of Microbiology, School of Biomedical and Allied Health Science, University of Ghana. The animals were housed in cages with wood shavings as bedding and fed with standard mice chaw, given water and maintained under the required laboratory conditions. All animals used in this study were handled according to the Guide for the
Care and Use of Laboratory Animals (N.R.C., 1996). During the study, mice were re-used after a time-lag of at least three months.

3.5 PRIMARY OBSERVATION TEST

The behavioural and neuroactive effects of the extracts were first evaluated according to standardized observation grid similar to that described by Irwin, (1968). ICR mice were randomly divided into various groups (n=7) and kept in the experimental environment for 7 days to acclimatize. Animals were then fasted overnight, but had access to water ad libitum, and then treated orally with the extracts at various doses or vehicles. The mice were observed at 0, 15, 30, 60, 120 and 180 min, up to 24 hours after treatment for general changes in behaviour and physiological function as well as mortality. The animals were then assessed for behaviours related to neurotoxicity, central nervous system (CNS) stimulation and depression. Effects on autonomic functions were also noted. This test was to ensure that the right doses of extract were selected.

3.6 ACUTE ANTIDEPRESSANT MODELS

3.6.1 Tail suspension test (TST)

The TST was carried out as previously described by Steru et al., (1985). Mice were allowed to acclimatize to the room for 3.5-4 hours before the test. Ten groups of mice (n=7) were treated with the extracts, fluoxetine and imipramine or vehicle. One hour after oral administration of the test drugs, mice were then suspended by the tail individually from a horizontal bar (distance from floor is 30 cm) using adhesive tape (distance from tip of tail is 1 cm). Duration of immobility, defined as the absence of all movements except those required for respiration, curling, swinging and pedalling behaviour was recorded by an observer for 5 min from video recordings of the test
with the aid of public domain software JWatcher Version 1.0. Decline in immobility score was an index for antidepressant effect; increase in curling behaviour was suggestive of opioiergic activity.

### 3.6.2 Forced swimming test (FST)

The FST was based on that described by Porsolt et al., (1977). Mice were divided into 10 groups of 7 animals each and received the vehicle (water), extracts, or reference drugs; fluoxetine and imipramine. One hour after the oral administration of the test drugs, mice were gently dropped individually into transparent cylindrical polyethylene tanks (25 cm high, 10 cm internal diameter) containing water (25 to 28 °C) up to a level of 20 cm and allowed to swim for 6 min. Each session was recorded by a video camera suspended approximately 100 cm above the cylinders. The mean immobility score (when the mouse is floating upright and makes only small movements to keep its head above the water) swimming (active horizontal movements) and climbing (active vertical movements) scores during the 6 min test was scored, with the aid of public domain software JWatcher Version 1.0 (University of California, Los Angeles, USA and Macquarie University, Sydney, Australia. Available at http://www.jwatcher.ucla.edu/). A reduction in immobility score was an indication of antidepressant effect. An increase in climbing score without commensurate change in swimming behaviour was suggestive of adrenergic mechanisms while an increase in swimming score without change in climbing suggested serotoninergic interactions.
3.7 EXTRACTION OF SOME SECONDARY METABOLITES PRESENT IN THE (HYDROETHANOLIC) MOST EFFICACIOUS EXTRACT

3.7.1 Saponins (Nahapetian and Bassiri, 1975)

A mass of 50 g of hydroethanolic extract was dispersed in 500 ml of 20% ethanol. The suspension was then heated over a hot water bath for 4 hours with continuous stirring at about 55 °C. The mixture was filtered and the residue re-extracted with another 500 ml of 20% ethanol. The combined extracts were then reduced to 40 ml over water bath at about 90 °C. The concentrate was then transferred into a 500 ml separating funnel and a volume of 50 ml diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer discarded. The purification process was repeated. Sixty (60) ml of n- butanol was added to the remaining fraction. The resulting solution was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample were dried in the oven into a constant weight.

3.7.2 Alkaloids (Obadoni and Ochuko, 2001; Harbone, 1973)

A mass of 50 g of the hydroethanolic extract was weighed into a beaker. A volume of 1000 ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 hours. The solution was filtered and the resulting filtrate was concentrated on a water bath to about one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise until the formation of a precipitate was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloids, which was dried and weighed. Qualitative phytochemical analysis was subsequently
done on the isolated alkaloids extract to test for the presence/absence of the other secondary metabolites.

**3.7.3 Flavonoids (Bohm and Kocipai-Abyazan, 1994)**

A mass of 50 g of the hydroethanolic extract was twice extracted with 500 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight. Qualitative phytochemical analysis was subsequently done on the isolated flavonoids extract to test for the presence/absence of the other secondary metabolites.

**3.7.4 Terpenoids (Ferguson, 1956)**

A mass of 50 g of the hydroethanolic extract was soaked in ethanol for 24 hours. The resulting solution was filtered; the filtrate was extracted with petroleum ether. The ether extract was treated as total terpenoids. Qualitative phytochemical analysis was subsequently done on the isolated terpenoids extract to test for the presence/absence of the other secondary metabolites.

**3.7.5 Tannins (Strumeyer and Malin, 1975)**

A mass of 50 g of crude hydroethanolic was weighed into a flask and 500 ml of 80% acetone (v/v) was added. The resulting solution was placed in a water bath at 70°C and carefully shaken for 15 minutes. After cooling, the supernatant was decanted carefully. The extraction was repeated twice and the supernatants were combined and concentrated using a rotary evaporator at 40 °C to obtain the crude phenolic fraction. The crude phenolic extract (2.5 g) was dissolved in
20 ml of ethanol and applied on a column packed with 40 g of Sephadex LH-20 gel. Ethanol was used as first eluent, to allow the removal of lower molecular weight phenolic compounds. Then 50% acetone in water (v/v) was used to elute tannins.

The ethanol and 50% acetone fractions were applied on pre-coated TLC plates by using capillary tubes and developed in a TLC chamber using ethanol: acetone (1:1) mobile phase. The developed TLC plates were air dried and observed under ultra violet light UV at both 254 nm and 366 nm. The TLC plates were later sprayed with anisaldehyde reagent and heated for about 1 minute for the development of colour in separated spots.

3.8 ACUTE ANTIDEPRESSANT EFFECT OF EXTRACTED SECONDARY METABOLITES FROM THE HYDROETHANOLIC EXTRACT

3.8.1 Tail suspension test

The procedure used was similar to that describes in section 3.8.1.

3.8.2 Forced swimming test

The procedure used was similar to that describes in section 3.8.2
3.9 CHRONIC ANTIDEPRESSANT EFFECT OF THE (TOTAL ALKALOIDS) MOST EFFICACIOUS SECONDARY METABOLITE

3.9.1 Open space swim test

The method described by Stone and Lin (2011) with some slight modifications was used. Swimming was carried out in rat tub cages (28×26×41 cm, w×h×l) filled with 13 cm high tepid tap water (32–34 °C). Mice were swum individually for 15 min/day on 4 consecutive days in order to induce a depressive state characterized by the decrease in mobility and distance travelled by the mice. Drug treatment started from day 5, through days 7, 10, 14, 18. All swim sessions were videotaped from above. No special procedures were used to dry or warm the animals as they rapidly dried themselves with no observable episodes of shivering. The distance swum was rated as the number of quadrants of the tub entered and duration of immobility from the total time the animal was observed to float, which is defined as drifting with the tail fully extended and no motion observed in the tail or limbs.

3.10 POSSIBLE MECHANISMS OF ACTION OF ISOLATED ALKALOID

Based on recent literature of the mechanism of action of antidepressants and the pathophysiology of depression, the involvement of noradrenergic, serotonergic, Glycine/NMDA receptor complex and nitric oxide pathways in the antidepressant effect of the secondary metabolite were investigated.

3.10.1 Involvement of noradrenergic systems

Mice were pre-treated with reserpine and/or α-methyldopa (α-MD) in order to investigate the possible role of noradrenergic system in the antidepressant activity of the alkaloids (ALK
30, 100 and 300 mg kg\(^{-1}\) \textit{p.o.}). The doses of α-MD and reserpine were chosen based on work done by others (Woode \textit{et al.}, 2010; Kukuia \textit{et al.}, 2014). To deplete newly synthesized pools of noradrenaline (NA) and dopamine (DA), mice were treated with a single dose of α-MD (400 mg kg\(^{-1}\), i.p.) 3.5 hours before behavioural testing. To deplete vesicular pools of NA and DA, mice were treated with a single dose of reserpine (1 mg kg\(^{-1}\), s.c.) 24 hours before behavioural testing. In an effort to deplete both the vesicular and cytosolic pools of NA and DA, mice were pre-treated with a combination of reserpine (1 mg kg\(^{-1}\), s.c., 24 hours before behavioural testing) and α-MD (200 mg kg\(^{-1}\), i.p., 3.5 hours before behavioural testing), respectively. The tail suspension and modified forced swimming tests were used.

In a separate experiment, the effects of a selective α\(_1\) receptor antagonist (prazosin, 3 mg kg\(^{-1}\) \textit{p.o.}) on the antidepressant actions of the ALK (30, 100 and 300 mg kg\(^{-1}\), \textit{p.o.}), imipramine (10 mg kg\(^{-1}\), \textit{p.o.}) and atomoxetine (1 mg kg\(^{-1}\), \textit{p.o.}) Mice were grouped into twelve (12) groups (\(n=7\)). Groups 1 - 6 were pre-treated with prazosin (3 mg kg\(^{-1}\), \textit{p.o.}) thirty (30) minutes before the ALK (30, 100 and 300 mg kg\(^{-1}\), \textit{p.o.}), atomoxetine (1 mg kg\(^{-1}\), \textit{p.o.}), imipramine (10 mg kg\(^{-1}\), \textit{p.o.}) or saline treatment on the day of experiment. Group 7 received saline only, group 8-10 received the ALK (30, 100 and 300 mg kg\(^{-1}\) \textit{p.o.}) respectively, group 11 received atomoxetine (1 mg kg\(^{-1}\), \textit{p.o.}) and group 12 received imipramine (10 mg kg\(^{-1}\), \textit{p.o.}). The tail suspension and modified forced swimming tests were used to assess the immobility time.

In another experiment, the effects of a selective α\(_2\) receptor antagonist (yohimbine, 3 mg kg\(^{-1}\) \textit{p.o.}) on the antidepressant actions of the ALK (30, 100 and 300 mg kg\(^{-1}\), \textit{p.o.}), imipramine (10 mg kg\(^{-1}\), \textit{p.o.}) and atomoxetine (1 mg kg\(^{-1}\), \textit{p.o.}) Mice were grouped into twelve (12) groups (\(n=7\)). Groups 1 - 6 were pre-treated with yohimbine (3 mg kg\(^{-1}\), \textit{p.o.}) thirty (30) minutes before the ALK (30, 100 and 300 mg kg\(^{-1}\), \textit{p.o.}), atomoxetine (1 mg kg\(^{-1}\), \textit{p.o.}), imipramine (10 mg kg\(^{-1}\),
p.o.) or saline treatment on the day of experiment. Group 7 received saline only, group 8-10 received the ALK (30, 100 and 300 mg kg\(^{-1}\) p.o.) respectively, group 11 received atomoxetine (1 mg kg\(^{-1}\), p.o.) and group 12 received imipramine (10 mg kg\(^{-1}\), p.o.). The tail suspension and modified forced swimming tests were used to assess the immobility time.

### 3.10.2 Involvement of serotoninergic systems

Mice were put into twenty (20) groups, n=7 pCPA (200 mg kg\(^{-1}\), i.p.) was administered once daily for 3 consecutive days to 10 groups of animals. On the fourth day, group 1 received saline, groups 2-4 received the ALK (30, 100 and 300 mg kg\(^{-1}\) p.o.); groups 5-7 received fluoxetine (3, 10 and 30 mg kg\(^{-1}\), p.o.), groups 8-10 received imipramine (3, 10 and 30 mg kg\(^{-1}\), p.o.). The remaining 10 groups received which did not undergo pre-treatment, received the ALK (30, 100 and 300 mg kg\(^{-1}\), p.o.), fluoxetine (3, 10 and 30 mg kg\(^{-1}\), p.o.), imipramine (3, 10 and 30 mg kg\(^{-1}\), p.o.) or saline on the day of experiment. The tail suspension and modified forced swimming tests were used.

In a separate experiment, the effects 5-HT\(_2\) receptor antagonist (Cyproheptadine, 8 mg kg\(^{-1}\) p.o.) on the antidepressant actions of the ALK (30, 100 and 300 mg kg\(^{-1}\), p.o.), imipramine (10 mg kg\(^{-1}\), p.o.) and fluoxetine (10 mg kg\(^{-1}\), p.o.). Mice were grouped into twelve (12) groups (n=7). Groups 1 - 6 were pretreated with cyproheptadine (8 mg kg\(^{-1}\), p.o.) thirty (30) minutes before the ALK (30, 100 and 300 mg kg\(^{-1}\), p.o.), fluoxetine (10 mg kg\(^{-1}\), p.o.), imipramine (10 mg kg\(^{-1}\), p.o.) or saline treatment on the day of experiment. Group 7 received saline only, group 8-10 received the ALK (30, 100 and 300 mg kg\(^{-1}\) p.o.) respectively, group 11 received fluoxetine (10 mg kg\(^{-1}\), p.o.) and group 12 received imipramine (10 mg kg\(^{-1}\), p.o.). The tail suspension and modified forced swimming tests were used to assess the immobility time.
3.10.3 Involvement of glycine/NMDA receptor complex

Mice were initially divided into groups, A and B. Each group was further subdivided into 10 groups each (n=7). Each group of mice from group A was pre-treated with D-cycloserine (2.5 mg kg\(^{-1}\), i.p.) and 30 min after the first three groups received an oral dose of the ALK (30, 100 and 300 mg kg\(^{-1}\) p.o) with the next three groups receiving fluoxetine (3, 10 and 30 mg kg\(^{-1}\) p.o) and the last three groups receiving imipramine (3, 10 and 30 mg kg\(^{-1}\), p.o). The tenth group received only D-cycloserine. Ten groups of mice from group B received D-serine (600 mg kg\(^{-1}\) i.p) pretreatment and 30 min after, the first three groups received an oral dose of the ALK (30, 100 and 300 mg kg\(^{-1}\) p.o) with the next three groups receiving fluoxetine (3, 10 and 30 mg kg\(^{-1}\), p.o) and last three groups receiving imipramine (3, 10 and 30 mg kg\(^{-1}\), p.o). The tenth group from group B received only D-serine. The forced swimming and tail suspension tests were used as described above to investigate the antidepressant mechanism.

3.10.4 Involvement of L-arginine-NO-cGMP Pathway

The possible participation of L-arginine-NO-cGMP system in the antidepressant effect of the ALK was investigated. Mice [(n=7) in each group] were pre-treated with a sub-effective dose of L-arginine (750 mg kg\(^{-1}\), i.p., a precursor of nitric oxide (NO) or saline 15 min before the ALK (30, 100 and 300 mg kg\(^{-1}\) p.o.) administration and assessed 45 min later for immobility time in the TST and FST.

In separate experiments, mice [(n=7) in each group] were pre-treated with L-NAME (30 mg kg\(^{-1}\), i.p., a non-selective nitric oxide synthase (NOS) inhibitor) or saline 15 min before ALK (30, 100 and 300 mg kg\(^{-1}\) p.o.) administration and assessed 45 min later for immobility time in the TST and FST.
In separate experiments, mice [(n=7) in each group] were pretreated with methylene blue (10 mg kg\(^{-1}\), i.p., an inhibitor of NOS and an inhibitor of soluble guanylate cyclase(sGC) or saline 15 min before ALK (30, 100 and 300 mg kg\(^{-1}\) p.o.) administration and assessed 45 min later for immobility time in the TST and FST.

In another experiment, the possible role of cyclic guanosine monophosphate (cGMP) in the antidepressant action of ALK was investigated. Mice received an injection of sildenafil (5 mg kg\(^{-1}\), i.p., a phosphodiesterase 5 inhibitor) or saline 15 min before ALK (30, 100 and 300 mg kg\(^{-1}\) p.o.) administration and assessed 45 min later for immobility time in the TST and FST.

### 3.11. STATISTICAL ANALYSIS

GraphPad Prism for windows version 4.03 (GraphPad Software, San Diego, CA, USA) was used for all data analysis. P<0.05 was considered statistically significant. Differences in means was analysed by one-way ANOVA followed by Newman-Kuel’s post hoc test. Doses for 50% of the maximal effect (ED\(_{50}\)) for each drug were determined using an iterative computer least square method, with the following nonlinear regression (three-parameter logistic) equation:

\[
Y = a + \frac{(b - a)}{(1 + 10^{(\text{Log ED}_{50} - X)})}
\]

Where, X is the logarithm of dose and Y is the response. Y starts at a (the bottom) and goes to B (the top) with a sigmoid shape.
4.1 QUANTITATIVE AND QUALITATIVE ANALYSIS OF CRUDE EXTRACTS

4.1.1 Yield of crude extracts

The hydroethanolic extract (HEE) gave the highest yield followed by the ethyl acetate extract (EAE) and the petroleum ether extract (PEE) producing the lowest yield (Table 4.1).

Table 4.1: Percentage yield of crude HEE, EAE and PEE from the stem bark of *Trichilia monadelpha*

<table>
<thead>
<tr>
<th>Crude extract</th>
<th>Initial weight (g)</th>
<th>Final weight (g)</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEE</td>
<td>4000</td>
<td>19.6</td>
<td>0.49</td>
</tr>
<tr>
<td>EAE</td>
<td>3790</td>
<td>22.74</td>
<td>0.60</td>
</tr>
<tr>
<td>HEE</td>
<td>3640</td>
<td>266.22</td>
<td>7.31</td>
</tr>
</tbody>
</table>
4.1.2 Phytochemical test

Preliminary phytochemical screening indicated the presence of alkaloids, flavonoids, glycosides, saponins, sterols, tannins, and terpenoids in HEE. EAE had alkaloids, glycosides, tannins, sterols and terpenoids while PEE showed the presence of alkaloids, sterols, and terpenoids (Table 4.2).

**Table 4.2: Preliminary phytochemical screening crude HAE, EAE and PEE from the stem bark of *Trichilia monadelpha***

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Inference</th>
<th>HEE</th>
<th>EAE</th>
<th>PEE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Saponins</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Tannins</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Sterols</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
</tbody>
</table>
4.2 IRWIN TEST

No signs of toxic effects were manifested during the 24 hour observation period for all the three extracts except for sedation for the doses from 30 – 3000 mg kg\(^{-1}\). There were signs of analgesic effects, frequent urination and defecation in all three extracts. No death was also recorded after 24 hours for all the administered doses in all the three extracts (Table 4.3).

**Table 4.3: Observations in the acute toxicity test after oral administration of PEE, EAE and HEE of T. monadelpha in mice**

<table>
<thead>
<tr>
<th></th>
<th>PEE</th>
<th>EAE</th>
<th>HEE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>Mortality</td>
<td>Latency</td>
<td>Observed drug</td>
</tr>
<tr>
<td>(mg/kg)</td>
<td>D/T (min)</td>
<td>(min)</td>
<td>effects</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>-</td>
<td>30</td>
<td>Analgesia, urination, defecation</td>
</tr>
<tr>
<td>100</td>
<td>-</td>
<td>15</td>
<td>Analgesia, urination, defecation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sedation analgesia, urination, defecation</td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>300</td>
<td>-</td>
<td>15</td>
<td>300</td>
</tr>
<tr>
<td>1000</td>
<td>-</td>
<td>15</td>
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</tr>
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<td>-</td>
<td>15</td>
<td>3000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedation, analgesia, urination, defecation</td>
<td>Sedation, analgesia, urination, defecation</td>
<td>Sedation, analgesia, urination, defecation</td>
<td>Sedation, analgesia, urination, defecation</td>
</tr>
</tbody>
</table>

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4.3 ANTIDEPRESSANT EFFECTS OF CRUDE EXTRACTS

4.3.1 Tail suspension test

4.3.1.1 Immobility score

All three extracts, (PEE, EAE and HEE) significantly decreased immobility score ($F_{9,60} = 55.11 \ P < 0.0001; F_{9,60} = 66.02 \ P < 0.0001; F_{9,60} = 34.83 \ P < 0.0001$ respectively). In TST, the order of antidepressant efficacy calculated from the log-dose response curve (Figure 4.4) with regards to immobility was fluoxetine > imipramine > HEE > EAE > PEE ($E_{\text{max}} = 90.00, \ ED_{50} = 1.58 \pm 0.2; E_{\text{max}} = 78.01, \ ED_{50} = 4.32 \pm 0.3; E_{\text{max}} = 75.44, \ ED_{50} = 26.64 \pm 0.6; E_{\text{max}} = 61.51, \ ED_{50} = 66.83 \pm 0.6; E_{\text{max}} = 42.43, \ ED_{50} = 196.10 \pm 1.2$ respectively). HEE was the most potent and efficacious in reducing immobility score in TST (Table 4.4).

Figure 4.1: Effects of the extracts, (a) PEE (30 – 300 mg/kg), (b) EAE (30 -300 mg/kg) and (c) HEE (30 - 300 mg/kg), fluoxetine (3 -30 mg/kg) and imipramine (3 -30 mg/kg) on immobility score in TST. Data are represented as group Means ± SEM. Significantly different from vehicle: *** $P < 0.0001$ (One-way ANOVA followed by Newman-Kuel’s test)
4.3.1.1 Swinging score

All three extracts, (PEE, EAE and HEE) exhibited a significant increase in swinging \( (F_{9,60} = 57.83 \, P<0.0001; \, F_{9,60} = 44.30 \, P <0.0001; \, F_{9,60} = 16.15 \, P <0.0001 \) respectively) scores.

![Bar chart showing the effects of extracts on swinging score](image)

Figure 4.2: Effects of the extracts, (a) PEE (30 – 300 mg/kg), (b) EAE (30 - 300 mg/kg) and (c) HEE (30 - 300 mg/kg), fluoxetine (3 -30 mg/kg) and imipramine (3 -30 mg/kg) on swinging scores in TST. Data are represented as group Means ± SEM. Significantly different from vehicle: *** \( P < 0.0001 \); ** \( P < 0.001 \) (One-way ANOVA followed by Newman-Kuef’s test)
4.3.1.1 Curling score

All three extracts, (PEE, EAE and HEE) generally exhibited a significant increase in curling score in a dose-dependent manner ($F_{9,60} = 8.236 \ P<0.0001$; $F_{9,60} = 44.85 \ P<0.0001$; $F_{9,60} = 12.59 \ P<0.0001$ respectively).

Figure 4.3: Effects of the extracts, (a) PEE (30 – 300 mg/kg), (b) EAE (30 -300 mg/kg) and (c) HEE (30 - 300 mg/kg), fluoxetine (3 -30 mg/kg) and imipramine (3 -30 mg/kg) on curling scores in TST. Data are represented as group Means ± SEM. Significantly different from vehicle: *** $P$ < 0.0001 (One-way ANOVA followed by Newman-Kuel’s test)
Figure 4.4: Log dose-response curve of the extracts, PEE (30 – 300 mg/kg), EAE (30 -300 mg/kg) and HEE (30 - 300 mg/kg), fluoxetine (3 -30 mg/kg) and imipramine (3 -30 mg/kg) showing a % decrease in immobility score in TST in mice. Each point is the mean ± S.E.M of 7 mice.

4.3.2 Forced Swimming Test

4.3.2.1. Immobility score

All three extracts, (PEE, EAE and HEE) significantly decreased immobility score \( F_{9,60} = 87.33 \quad P<0.0001; \quad F_{9,60} = 95.33 \quad P<0.0001; \quad F_{9,60} = 81.73 \quad P<0.0001 \) respectively) in a dose dependent manner. In FST, the order of antidepressant efficacy calculated from the dose response curve (Figure 4.8) with regards to immobility was fluoxetine > HEE > imipramine > EAE > PEE (\( E_{\text{max}} = 81.79, \quad \text{ED}_{50} = 2.88 \pm 0.2; \quad E_{\text{max}} = 80.55, \quad \text{ED}_{50} = 10.86 \pm 1.0; \quad E_{\text{max}} = 66.74, \quad \text{ED}_{50} = 35.92 \pm 0.6; \quad E_{\text{max}} = 59.34, \quad \text{ED}_{50} = 108.70 \pm 0.6; \quad E_{\text{max}} = 54.40, \quad \text{ED}_{50} = 132.20 \pm 6.0 \) respectively). From the calculated \( \text{ED}_{50} \) and \( E_{\text{max}} \) values, HEE was the most potent and efficacious among all the test extracts but less potent than fluoxetine and imipramine in reducing immobility score in FST (Table 4.4).
Figure 4.5: Effects of the extracts, (a) PEE (30 – 300 mg/kg), (b) EAE (30 -300 mg/kg) and (c) HEE (30 - 300 mg/kg), fluoxetine (3 -30 mg/kg) and imipramine (3 -30 mg/kg) on immobility score in FST. Data are represented as group Means ± SEM. *** P < 0.0001; compared to vehicle-treated group (One-way ANOVA followed by Newman-Keuls test)
4.3.2.1 Swimming score

All three extracts, (PEE, EAE and HEE) showed a significant increase in swimming ($F_{9,60} = 82.04 \ P<0.0001$; $F_{9,60} = 108.1 \ P <0.0001$; $F_{9,60} = 55.06 \ P <0.0001$ respectively) scores.

Figure 4.6: Effects of the extracts, (a) PEE (30 – 300 mg/kg), (b) EAE (30 -300 mg/kg) and (c) HEE (30 - 300 mg/kg), fluoxetine (3 -30 mg/kg) and imipramine (3 -30 mg/kg) on swimming scores in FST. Data are represented as group Means ± SEM. *** $P < 0.0001$; compared to vehicle-treated group (One-way ANOVA followed by Newman-Kuef’s test)
4.3.2.2 Climbing score

All three extracts, (PEE, EAE and HEE) exhibited a significant increase in climbing ($F_{9,60} = 10.71 \ P < 0.0001$; $F_{9,60} = 18.14 \ P < 0.0001$; $F_{9,60} = 14.47 \ P < 0.0001$respectively) scores.

![Climbing score](image.png)

Figure 4.7: Effects of the extracts, (a) PEE (30 - 300 mg/kg), (b) EAE (30 -300 mg/kg) and (c) HEE (30 - 300 mg/kg), fluoxetine (3 -30 mg/kg) and imipramine (3 -30 mg/kg) on climbing scores in FST. Data are represented as group Means ± SEM. *** $P < 0.0001$; ** $P < 0.001$; compared to vehicle-treated group (One-way ANOVA followed by Newman-KueI’s test)
Figure 4.8: Log dose-response curve of the extracts, PEE (30 – 300 mg/kg), EAE (30 -300 mg/kg) and HEE (30 - 300 mg/kg), fluoxetine (3 -30 mg/kg) and imipramine (3 -30 mg/kg) showing a % decrease in immobility score in FST in mice. Each point is the mean ± S.E.M of 7 mice.

Table 4.4: ED\textsubscript{50} and E\textsubscript{max} values of test drugs used in tail suspension and forced swim tests

<table>
<thead>
<tr>
<th>TEST</th>
<th>DRUG</th>
<th>DURATION OF IMMOBILITY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ED\textsubscript{50}</td>
</tr>
<tr>
<td>TST</td>
<td>FLX</td>
<td>1.58 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>IMI</td>
<td>4.32 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>PEE</td>
<td>196.1 ± 12.2</td>
</tr>
<tr>
<td></td>
<td>EAE</td>
<td>66.83 ± 5.6</td>
</tr>
<tr>
<td></td>
<td>HEE</td>
<td>26.64 ± 3.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FST</td>
<td>FLX</td>
<td>2.88 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>IMI</td>
<td>10.86 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>PEE</td>
<td>132.20 ± 14.2</td>
</tr>
<tr>
<td></td>
<td>EAE</td>
<td>108.70 ± 10.6</td>
</tr>
<tr>
<td></td>
<td>HEE</td>
<td>35.92 ± 2.0</td>
</tr>
</tbody>
</table>
4.4 ANALYSIS OF EXTRACTED SECONDARY METABOLITES FROM THE HYDROETHANOLIC EXTRACT

4.4.1 Yield of isolated secondary metabolites

Alkaloids, terpenoids, flavonoids, saponins and tannins were extracted from HEE (Table 4.5.)

Table 4.5: Phytochemical screening and yield of secondary metabolites isolated from crude HEE from the stem bark of *Trichilia monadelpha*

<table>
<thead>
<tr>
<th>Secondary metabolites</th>
<th>Final weight (g)</th>
<th>% Yield</th>
<th>Phytochemical Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponins</td>
<td>4.05</td>
<td>8.05</td>
<td>Saponins present</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Terpenoids present</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Steroids present</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Glycosides present</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>1.52</td>
<td>3.03</td>
<td>Flavonoids present</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Glycosides present</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>2.11</td>
<td>4.20</td>
<td>Alkaloids present</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>0.31</td>
<td>0.62</td>
<td>Terpenoids present</td>
</tr>
<tr>
<td>Tannins</td>
<td>0.76</td>
<td>1.52</td>
<td>Tannins present</td>
</tr>
</tbody>
</table>

4.5 ACUTE ANTIDEPRESSANT EFFECTS OF SECONDARY METABOLITES EXTRACTED FROM THE (HYDROETHANOLONIC) MOST EFFICACIOUS EXTRACT

4.5.1 Tail suspension test

Three secondary metabolites, SAP, FLV and ALK significantly decreased immobility score 
\(F_{9,60} = 82.40 \ P< 0.0001; \ F_{9,60} = 154.2 \ P<0.0001; \ F_{9,60} = 100.6 \ P<0.0001\) respectively. In TST, the order of antidepressant efficacy calculated from the dose response curve (Figure 4.12) with regards to immobility score was fluoxetine > imipramine > ALK > SAP > FLV (\(E_{\text{max}} = 96.0, \ ED_{50} = 1.86 \pm 0.9; \ E_{\text{max}} = 95.4, \ ED_{50} = 4.58 \pm 1.2; \ E_{\text{max}} = 71.1, \ ED_{50} = 122.70 \pm 5.4; \ E_{\text{max}} = 55.3, \ ED_{50} = 227.20 \pm 6.9; \ E_{\text{max}} = 20.7, \ ED_{50} = 912.80 \pm 9.2\) respectively). From the calculated ED\(_{50}\) and E\(_{\text{max}}\) values, the ALK was the most potent and efficacious among all the test drugs in reducing immobility score in TST (Table 4.6)
Figure 4.9: Effects of the secondary metabolites, (a) FLV (30 – 300 mg/kg), (b) TAN (30 -300 mg/kg), (c) ALK (30 - 300 mg/kg), (d) TER (30- 300 mg/kg) and (e) SAP (30 -300 mg/kg), fluoxetine (3 -30 mg/kg) and imipramine (3 -30 mg/kg) on immobility score in TST. Data are represented as group Means ± SEM. Significantly different from vehicle: *** P < 0.0001 (One-way ANOVA followed by Newman-Kuel’s test)
4.5.1.1 Swinging score

FLV and ALK demonstrated a significant increase in swinging scores ($F_{9,60} = 78.70 \ P<0.0001$; $F_{9,60} = 50.64$, respectively).

Figure 4.10: Effects of the secondary metabolites, (a) FLV (30 – 300 mg/kg), (b) TAN (30 -300 mg/kg), (c) ALK (30 - 300 mg/kg), (d) TER (30- 300 mg/kg) and (e) SAP (30 -300 mg/kg), fluoxetine (3 -30 mg/kg) and imipramine (3 -30 mg/kg) on swinging scores in TST. Data are represented as group Means ± SEM. Significantly different from vehicle: *** $P < 0.0001$ (One-way ANOVA followed by Newman-Keul’s test).
4.5.1.2 Curling score

SAP and ALK displayed a significant increase in curling scores ($F_{9,60} = 17.58 \ P<0.0001$; $F_{9,60} = 9.655 \ P <0.0001$ respectively).

Figure 4.11: Effects of the secondary metabolites, (a) FLV (30 – 300 mg/kg), (b) TAN (30 -300 mg/kg), (c) ALK (30 - 300 mg/kg), (d) TER (30- 300 mg/kg) and (e) SAP (30 -300 mg/kg), fluoxetine (3 -30 mg/kg) and imipramine (3 -30 mg/kg) on curling scores in TST. Data are represented as group Means ± SEM. Significantly different from vehicle: *** $P < 0.0001$ (One-way ANOVA followed by Newman-Kuel’s test)
4.5.2 Forced Swimming Test

4.5.2.1 Immobility score

All five secondary metabolites (FLV, TAN, ALK, TER and SAP) significantly decreased immobility score ($F_{9,60} = 101.7 \ P<0.001; F_{9,60} = 164.4 \ P<0.001; F_{9,60} = 91.43 \ P<0.001; F_{9,60} = 82.12 \ P<0.001; F_{9,60} = 72.80 \ P<0.001$ respectively). In FST, the order of antidepressant efficacy calculated from the dose response curve (Figure 4.16) with regards to immobility score was fluoxetine > imipramine > ALK > SAP > FLV > TER > TAN ($E_{max} = 98.3, ED_{50} = 2.21 \pm 1.2; E_{max} = 98.0, ED_{50} = 6.80 \pm 1.8; E_{max} = 76.4, ED_{50} = 83.15 \pm 6.3; E_{max} = 66.1, ED_{50} = 141.10 \pm 7.4; E_{max} = 40.5, ED_{50} = 376.70 \pm 9.2; E_{max} = 40.3, ED_{50} = 408.80 \pm 10.3; E_{max} = 24.8, ED_{50} = 1348.00 \pm 9.7$ respectively). From the calculated ED$_{50}$ and E$_{max}$ values, ALK was the most potent and efficacious among all the test drugs in reducing immobility score in FST (Table 4.6).
Figure 4.13: Effects of the secondary metabolites, (a) FLV (30 – 300 mg/kg), (b) TAN (30 -300 mg/kg), (c) ALK (30 - 300 mg/kg), (d) TER (30- 300 mg/kg) and (e) SAP (30 -300 mg/kg), fluoxetine (3 -30 mg/kg) and imipramine (3 -30 mg/kg) on immobility score in FST. Data are represented as group Means ± SEM. Significantly different from vehicle: *** $P < 0.0001$ (One-way ANOVA followed by Newman-Kuels test).
4.5.2.1 Swimming score

All five secondary metabolites (FLV, TAN, ALK, TER and SAP) showed a significant increase in swimming ($F_{9,60} = 58.08 \ P < 0.0001; \ F_{9,60} = 93.05 \ P < 0.0001; \ F_{9,60} = 69.09 \ P < 0.0001; \ F_{9,60} = 89.25 \ P < 0.0001; \ F_{9,60} = 42.95 \ P < 0.0001$ respectively) score.

Figure 4.14: Effects of the secondary metabolites, (a) FLV (30–300 mg/kg), (b) TAN (30–300 mg/kg), (c) ALK (30–300 mg/kg), (d) TER (30–300 mg/kg) and (e) SAP (30–300 mg/kg), fluoxetine (3–30 mg/kg) and imipramine (3–30 mg/kg) on swimming scores in FST. Data are represented as group Means ± SEM. Significantly different from vehicle: *** $P < 0.0001$; ** $P < 0.001$; * $P < 0.01$ (One-way ANOVA followed by Newman-Kuel’s test)
4.5.2.1 Climbing score

Only SAP, TER and ALK showed a significant increase in climbing scores ($F_{9,60} = 10.52 \ P<0.0001$; $F_{9,60} = 7.907 \ P<0.0001$; $F_{9,60} = 12.78 \ P<0.0001$ respectively).

Figure 4.15: Effects of the secondary metabolites, (a) FLV (30 – 300 mg/kg), (b) TAN (30 -300 mg/kg), (c) ALK (30 - 300 mg/kg), (d) TER (30- 300 mg/kg) and (e) SAP (30 -300 mg/kg), fluoxetine (3 -30 mg/kg) and imipramine (3 -30 mg/kg) on climbing scores in FST. Data are represented as group Means ± SEM. Significantly different from vehicle: *** $P < 0.0001$; ** $P < 0.001$; * $P < 0.01$ (One-way ANOVA followed by Newman-Kuel’s test)
Figure 4.16: Log dose-response curve of the secondary metabolites, FLV (30 – 300 mg/kg), TAN (30 -300 mg/kg), ALK (30 - 300 mg/kg), TER (30- 300 mg/kg) and SAP (30 -300 mg/kg), fluoxetine (3 -30 mg/kg) and imipramine (3 -30 mg/kg) showing a % decrease in immobility score in FST in mice. Each point is the mean ± S.E.M of 7 mice.

Table 4.6: $ED_{50}$ and $E_{max}$ values of secondary metabolites and drugs used in tail suspension and forced swimming tests

<table>
<thead>
<tr>
<th>TEST</th>
<th>DRUG</th>
<th>DURATION OF IMMOBILITY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$ED_{50}$</td>
</tr>
<tr>
<td>TST</td>
<td>FLX</td>
<td>1.86 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>IMI</td>
<td>4.58 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>FLV</td>
<td>912.80 ± 19.2</td>
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<tr>
<td></td>
<td>ALK</td>
<td>122.70 ± 11.4</td>
</tr>
<tr>
<td></td>
<td>SAP</td>
<td>227.20 ± 113.9</td>
</tr>
<tr>
<td>FST</td>
<td>FLX</td>
<td>2.21 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>IMI</td>
<td>6.80 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>FLV</td>
<td>376.70 ± 14.2</td>
</tr>
<tr>
<td></td>
<td>TAN</td>
<td>1348.00 ± 23.7</td>
</tr>
<tr>
<td></td>
<td>ALK</td>
<td>83.15 ± 6.3</td>
</tr>
<tr>
<td></td>
<td>TER</td>
<td>408.80 ± 14.8</td>
</tr>
<tr>
<td></td>
<td>SAP</td>
<td>141.10 ± 12.4</td>
</tr>
</tbody>
</table>
4.6 CHRONIC ANTIDEPRESSANT EFFECT OF THE (ALKALOIDS)

MOST EFFICACIOUS SECONDARY METABOLITE

4.6.1 The open space swim test

4.6.1.1 Open space swim test of the ALK

The extracted total alkaloids exhibited significant antidepressant potentials in mice when taken through the open space swim test. From the time course curve, ALK caused a significant decline in immobility after day two of treatment and this was maintained up to the fourteenth day of treatment ($F_{3,68} = 21.76$ $P<0.0001$) (Figure 4.17a). Disparately, the ALK increased the number of quadrants entered (distance travelled) by the mice ($F_{3,68} = 14.46$ $P<0.0001$) (Figure 4.17b). ALK was less more potent and efficacious than FLX and BUP in reducing immobility time which is a more reliable index for mice. Similarly, ALK was also the least efficacious and potent with regards to distance travelled (Figure 4.20 and Table 4.7).

Figure 4.17 Effects of ALK (30 - 300 mg kg$^{-1}$) treatment on the (a) duration of immobility and the (b) distance travelled in the open space swim test. Significantly different from control: *$P<0.05$, **$P<0.001$, ***$P<0.001$ by Newman Keuls’ test.
4.6.1.2 Open space swim test of FLX

Fluoxetine showed a decrease in immobility time after day 6 of treatment (Figure 4.18a) but similar effect as ALK with regards to distance travelled (Figure 4).

Figure 4.18 Effects of FLX (3 - 30 mg kg\(^{-1}\)) treatment on the (a) duration of immobility and the (b) distance travelled in the open space swim test. Significantly different from control: *P<0.05, **P<0.001, ***P<0.001 by Newman Keuls' test.

4.6.1.2 Open space swim test of BUP

Bupropion showed a decrease in immobility time after day 6 of treatment (Figure 4.19a) but similar effect as ALK with regards to distance travelled (Figure 4.19b).

Figure 4.19 Effects of BUP (3 - 30 mgkg\(^{-1}\)) treatment on the (a) duration of immobility and the (b) distance travelled in the open space swim test. Significantly different from control: *P<0.05, **P<0.001, ***P<0.001 by Newman Keuls' test.
Figure 4.20 Log dose-response curves showing the effect of ALK (30 - 300 mg kg⁻¹), FLX (3 - 30 mg kg⁻¹) and BUP (3 - 30 mg kg⁻¹) on (a) % decrease in immobility time and (b) % increase in distance travelled in the open space swim test in mice. Each point is the mean ± S.E.M. of 10 animals.

Table 4.7 ED₅₀ and Eₘₐₓ values of drugs used in the open space swim test

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ALK</th>
<th>FLX</th>
<th>BUP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED₅₀</td>
<td>Eₘₐₓ</td>
<td>ED₅₀</td>
</tr>
<tr>
<td>Immobility time</td>
<td>11.61±2.2</td>
<td>89.7</td>
<td>1.71±0.6</td>
</tr>
<tr>
<td>Distance travelled</td>
<td>58.4±5.1</td>
<td>83.9</td>
<td>26.9±6.3</td>
</tr>
</tbody>
</table>
4.7 POSSIBLE MECHANISMS OF ACTION OF EXTRACTED TOTAL ALKALOIDS

4.7.1 INVOLVEMENT OF NORADRENERGIC SYSTEMS

4.7.1.1 α-methyldopa pre-treatment

Results from figure 4.21 indicates that pre-treatment of mice with α-methyldopa (400 mg kg⁻¹, i.p.) alone reversed the antidepressant effect of ALK (30, 100 and 300 mg kg⁻¹) and IMI (3, 10 and 30 mg kg⁻¹) but not FLX (3, 10 and 30 mg kg⁻¹) in both (a) TST and (b) FST but α-methyldopa alone did not alter immobility score ($F _{19,120} = 80.50$, $P<0.0001$; TST; $F _{19,120} = 60.83$, $P<0.0001$; FST).

![Bar graph showing immobility scores for untreated and α-MD-treated groups for ALK, FLX, and IMI at different doses.]

Figure 4.21: Effects of pre-treatment of mice with α-MD (400 mg kg⁻¹, i.p.) alone on immobility scores of ALK (30, 100 and 300 mg kg⁻¹ p.o.), FLX (3, 10 and 30 mg kg⁻¹ p.o.) and IMI (3, 10 and 30 mg kg⁻¹ p.o.) in (a) TST and (b) FST. Data are represented as group Means ± SEM of 7 animals. Significantly different from vehicle: ***$P<0.0001$; **$P<0.01$; *$P<0.05$ (One-way ANOVA followed by Newman-Kuel's test). ◯◯ ◯ $P<0.01$; ◯ ◯ $P<0.05$; significant difference between treatment and dose (Two Way ANOVA with Bonferonni post hoc test).
4.7.1.2 Reserpine pre-treatment

Pre-treatment with reserpine (1 mg kg\(^{-1}\), s.c.) alone also significantly blocked the reduction in immobility exhibited by ALK (30, 100 and 300 mg kg\(^{-1}\)) and IMI (3, 10 and 30 mg kg\(^{-1}\)) but not FLX (3, 10 and 30 mg kg\(^{-1}\)) in both (c) TST and (d) FST but administration of reserpine alone did not show any difference when compared to the control \((F_{19,120} = 59.92, P<0.0001;\) TST; \(F_{19,120} = 64.89, P<0.0001;\) FST).

![Graph showing immobility scores](image)

Figure 4.22: Effects of pre-treatment of mice with reserpine (1 mg kg\(^{-1}\), s.c.) on immobility scores of ALK (30, 100 and 300 mg kg\(^{-1}\) p.o.), FLX (3, 10 and 30 mg kg\(^{-1}\) p.o.) and IMI (3, 10 and 30 mg kg\(^{-1}\) p.o.) in (c) TST and (d) FST. Data are represented as group Means ± SEM of 7 animals. Significantly different from vehicle: ***\(P<0.0001; \)**\(P<0.001; \)*\(P<0.01\) (One-way ANOVA followed by Newman-Kuel’s test). ◊◊◊ \(P<0.001; \) ◊◊ \(P<0.01; \) ◊ \(P<0.05;\) significant difference between treatment and dose (Two Way ANOVA with Bonferonni post hoc test).
### 4.7.1.3 Pre-treatment with α-methyldopa/reserpine

Simultaneous administration of reserpine (1 mg kg\(^{-1}\), s.c.) and α-MD (200 mg kg\(^{-1}\), i.p.) obliterated the antidepressant activity of ALK (30, 100 and 300 mg kg\(^{-1}\)) and IMI (3, 10 and 30 mg kg\(^{-1}\)) but not FLX (3, 10 and 30 mg kg\(^{-1}\)) in (f) FST but partially reversed the antidepressant effect of FLX (30 mg kg\(^{-1}\)) in (e) TST \((F_{19,120} = 62.29, \ P<0.0001;\ TST; \ F_{19,120} = 60.89, \ P <0.0001;\ FST)\).

Figure 4.23: Effects of pre-treatment of mice with a combination of α-MD (200 mg kg\(^{-1}\), i.p) and reserpine (1 mg kg\(^{-1}\), s.c.) on immobility scores of ALK (30, 100 and 300 mg kg\(^{-1}\) \(p.o\)), FLX (3, 10 and 30 mg kg\(^{-1}\) \(p.o\)) and IMI (3, 10 and 30 mg kg\(^{-1}\) \(p.o\)) in (e) TST and (f) FST. Data are represented as group Means ± SEM of 7 animals. Significantly different from vehicle: *** \(P < 0.0001\); ** \(P < 0.001\); * \(P < 0.01\) (One-way ANOVA followed by Newman-Kuel’s test). ◯◯◯ \(P < 0.001\); ◯◯ \(P < 0.01\); ◯ \(P < 0.05\); significant difference between treatment and dose (Two Way ANOVA with Bonferonni post hoc test).
4.7.1.3.1 \( \alpha \)-methyldopa/reserpine pre-treatment on swimming and climbing score in FST

ALK, FLX and IMI increased swimming score but simultaneous reserpine (1 mg kg\(^{-1}\), s.c.) and \( \alpha \) MD (200 mg kg\(^{-1}\) i.p.) pre-treatment caused a significant reversal in the swimming scores of ALK, and IMI, but not FLX \( F_{19,120} = 59.95; P<0.0001 \) (Figure 4.21g). Administration of ALK, FLX and IMI caused an increase in climbing score but was revered in ALK (300 mg kg\(^{-1}\)) and IMI (3 and 30 mg kg\(^{-1}\)) with simultaneous reserpine (1 mg kg\(^{-1}\), s.c.) and \( \alpha \)-MD (200 mg kg\(^{-1}\), i.p.) pre-treatment \( F_{19,120} = 7.546; P<0.0001 \) (Figure 4.21h).

![Graph showing the effects of \( \alpha \)-MD (200 mg kg\(^{-1}\), i.p.) and reserpine (1 mg kg\(^{-1}\), s.c.) on swimming (g) and climbing scores (h) of ALK (30, 100 and 300 mg kg\(^{-1}\) p.o.), FLX (3, 10 and 30 mg kg\(^{-1}\) p.o.) and IMI (3, 10 and 30 mg kg\(^{-1}\) p.o.) in FST. Data are represented as group Means ± SEM of 7 animals. Significantly different from vehicle: *** \( P<0.0001 \); ** \( P<0.001 \); * \( P<0.01 \) (One-way ANOVA followed by Newman-Keuls test). \( \diamond \diamond \diamond \) \( P<0.001 \); \( \diamond \diamond \) \( P<0.01 \); \( \diamond \) \( P<0.05 \); significant difference between treatment and dose (Two Way ANOVA with Bonferroni post hoc test).](image-url)
4.7.1.4 Involvement of $\alpha_1$ receptor

In attempt to investigate the involvement of $\alpha_1$ and $\alpha_2$ receptor stimulation in the antidepressant effects of ALK (Figure 4.25), mice pre-treated with prazosin (3 mg kg$^{-1}$ p.o.), a specific $\alpha_1$ receptor antagonist did not alter the reduction in immobility of ALK (30, 100 and 300 mg kg$^{-1}$) and IMI (10 mg kg$^{-1}$) but blocked the antidepressant potentials of ATO (1 mg kg$^{-1}$) in both (a) TST and (b) FST ($F_{11.72} = 42.13, P<0.0001$: TST; $F_{11.72} = 46.01, P<0.0001$: FST).

Figure 4.25: Effect of pre-treatment of mice with prazosin (3 mg kg$^{-1}$ p.o., a selective $\alpha_1$-receptor antagonist) on ALK (30, 100 and 300 mg kg$^{-1}$ p.o.), ATO (1 mg kg$^{-1}$, p.o.) and IMI (10 mg kg$^{-1}$, p.o.) induced reduction in immobility time in the (a) TST and (b) FST. Each column represents the mean ± SEM (n=7) Significantly different from vehicle: *** $P<0.0001$; ** $P<0.001$; * $P<0.01$ (One-way ANOVA followed by Newman-Kuel’s test). $\Diamond\Diamond\Diamond P<0.001; \Diamond\Diamond P<0.01; \Diamond P<0.05$; significant difference between treatment and dose (Two Way ANOVA with Bonferonni post hoc test).
4.7.1.5 Involvement of α₂ receptor

Pre-treatment of mice with yohimbine (3 mg kg⁻¹ p.o.) a specific α₂ receptor antagonist significantly potentiated the antidepressant effect of ALK (30, 100 and 300 mg kg⁻¹) and ATO (1 mg kg⁻¹) in (c) TST and (d) FST (F₁₁,₇₂ = 28.22, P<0.0001; TST; F₁₁,₇₂ = 40.71, P<0.0001; FST) (figure 4.26).

![Figure 4.26: Effect of pre-treatment of mice with yohimbine (3 mg kg⁻¹, p.o., α₂-receptor antagonist) on ALK (30, 100 and 300 mg kg⁻¹, p.o.), ATO (1 mg kg⁻¹, p.o.) and IMI (10 mg kg⁻¹, p.o.) induced reduction in immobility time in the (c) TST and (d) FST. Each column represents the mean ± SEM (n=7) Significantly different from vehicle: *** P<0.0001; ** P<0.001; * P<0.01 (One-way ANOVA followed by Newman-Kuels test). ◊◊◊◊ P<0.001; ◊◊◊ P<0.01; ◊◊ P<0.05; significant difference between treatment and dose (Two Way ANOVA with Bonferoni post hoc test)](image-url)
4.7.2 INVOLVEMENT OF SEROTONINERGIC SYSTEMS

4.7.2.1 pCPA pre-treatment

Pre-treatment of mice with pCPA (200 mg kg\(^{-1}\), i.p) abolished the antidepressant effect of ALK (30, 100 and 300 mg kg\(^{-1}\), p.o.), FLX (3, 10 and 30 mg kg\(^{-1}\), p.o.) and IMI (3, 10 and 30 mg kg\(^{-1}\), p.o.) in the (a) TST and (b) FST. The mean counts for immobility (\(F_{19,120} = 118.7; P<0.0001\)) (Figure 4.27a; TST) and (\(F_{19,120} = 81.45; P<0.0001\)) (Figure 4.27b; FST) in the ALK and FLX treated groups after pCPA treatment did not show any difference when compared with the control.

![Immobility score graph](image)

Figure 4.27: Effect of pre-treatment of mice with pCPA (200 mg kg\(^{-1}\), i.p) on ALK (30, 100 and 300 mg kg\(^{-1}\), p.o.), FLX (3, 10, and 30 mg kg\(^{-1}\), p.o.) and IMI (3, 10 and 30 mg kg\(^{-1}\), p.o.) immobility score in the (a) TST and (b) FST. Each column represents the mean ± SEM (n=7) Significantly different from vehicle: *** \(P<0.0001\); ** \(P<0.01\); * \(P<0.05\) (One-way ANOVA followed by Newman-Kuel’s test). ♠♠♠ \(P<0.001\); ♠♠ \(P<0.01\); ♠ \(P<0.05\); significant difference between treatment and dose (Two Way ANOVA with Bonferonni post hoc test).
4.7.2.1.1 pCPA pre-treatment on swimming and climbing scores in FST

ALK, FLX and IMI increased swimming score which was reversed by pCPA treatment ($F_{19,120}=54.23; P<0.0001$) (Figure 4.28c). Administration of ALK (30, 100 and 300 mg kg

and IMI (30, 10, 30 mg kg

) and IMI (3, 10, 30 mg kg

) caused an increase in climbing score but was reversed in ALK (300 mg kg

) and IMI (30 mg kg

) with pCPA treatment ($F_{19,120}=8.607; P<0.0001$) (Figure4.28d).

Figure 4.28: Effect of pre-treatment of mice with pCPA (200 mgkg

, i.p) on ALK (30, 100 and 300 mg kg

, p.o.), FLX (3, 10 and 30 mg kg

, p.o.) and IMI (3, 10 and 30 mg kg

, p.o.) swimming score (c) and climbing score (d) in FST. Each column represents the mean ±SEM (n=7) Significantly different from vehicle: *** $P<0.0001$; ** $P<0.001$; * $P<0.01$(One-way ANOVA followed by Newman-Kue1’s test). ◊◊◊ $P<0.0001$; ◊◊ $P<0.01$; ◊ $P<0.05$; significant difference between treatment and dose (Two Way ANOVA with Bonferroni post hoc test).
4.7.2.2. Involvement of 5-HT₂ receptor

In an attempt to investigate the involvement of 5HT₂ receptor activation in the antidepressant effects of ALK (Figure 4.29), mice were pre-treated with cyproheptadine (8 mg kg⁻¹ p.o.), a specific 5-HT₂ receptor antagonist. It was observed that pre-treatment with cyproheptadine significantly reversed the antidepressant of ALK (30, 100 and 300 mg kg⁻¹), FLX (10 mg kg⁻¹) and IMI (10 mg kg⁻¹) both (a) TST and (b) FST (F₁₁.72 = 92.24, P<0.0001; TST; F₁₁.72 = 46.18, P <0.0001; FST).

Figure 4.29: Effect of pre-treatment of mice with cyproheptadine (8 mg kg⁻¹, p.o., a selective 5-HT₂ receptor antagonist on ALK (30, 100 and 300 mg kg⁻¹, p.o.), FLX (10 mg kg⁻¹, p.o.) and IMI (10 mg kg⁻¹, p.o.) immobility score in the (a) TST and (b) FST. Each column represents the mean ± SEM (n=7) Significantly different from vehicle: *** P<0.0001; ** P<0.001; * P<0.01 (One-way ANOVA followed by Newman-Kuels test). ❀❀ P<0.001; ❀ P<0.01; ❀ P<0.05: significant difference between treatment and dose (Two Way ANOVA with Bonferroni post hoc test).
4.7.3 INVOLVEMENT OF GLYCINE /NMDA RECEPTOR COMPLEX

4.73.1 D-serine pre-treatment

Figure 4.30 shows the effect of combined administration of D-serine (600 mg kg$^{-1}$, i.p., a full agonist on glycine/NMDA receptor) on ALK, FLX and IMI on TST and FST. The antidepressant effect of ALK (30, 100 and 300 mg kg$^{-1}$, p.o.), FLX (3, 10 and 30 mg kg$^{-1}$, p.o.) and IMI (3, 10 and 30 mg kg$^{-1}$, p.o.) was significantly reversed by the pre-treatment of D-serine (600 mg kg$^{-1}$, i.p) in both (a) TST ($F_{19,120} = 67.26$, $P<0.0001$) and (b) FST ($F_{19,120} = 29.37$, $P<0.0001$).

![Graph showing immobility scores](image)

Figure 4.30: Effect of pre-treatment of mice with D-serine (600 mg kg$^{-1}$, i.p., a full agonist on glycine/NMDA receptor) on ALK (30, 100 and 300 mg kg$^{-1}$, p.o.), FLX (3, 10, and 30 mg kg$^{-1}$, p.o.) and IMI (3, 10 and 30 mg kg$^{-1}$, p.o.) immobility score in the (a) TST and (b) FST. Each column represents the mean ± SEM (n=7) Significantly different from vehicle: *** $P<0.0001$; ** $P<0.001$; * $P<0.01$ (One-way ANOVA followed by Newman-Kue1’s test). ⋆⋆⋆ $P<0.001$; ⋆⋆ $P<0.01$; ⋆ $P<0.05$; significant difference between treatment and dose (Two Way ANOVA with Bonferroni post hoc test).
4.7.3.1.1. D-serine pre-treatment on swimming and climbing scores in FST

ALK, FLX and IMI all caused increase in swimming score and this was observed to be reduced by D-serine in ALK and IMI but unaffected in IMI. Climbing scores were increased by ALK (30, 100 and 300 mg kg\(^{-1}\), p.o.), FLX (10 mg kg\(^{-1}\), p.o.) and IMI (3, 10 and 30 mg kg\(^{-1}\), p.o.). Pre-treatment of D-serine further increased climbing score in ALK (300 mg kg\(^{-1}\), p.o.) and FLX (3, 10 and 30 mg kg\(^{-1}\), p.o.) but rather caused a reduction in that of IMI (3, 10 and 30 mg kg\(^{-1}\), p.o.).

![Graph showing the effect of D-serine pre-treatment on swimming and climbing scores in FST](image-url)

Figure 4.31: Effect of pre-treatment of mice with D-serine on ALK (30, 100 and 300 mg kg\(^{-1}\), p.o.), FLX (3, 10, and 30 mg kg\(^{-1}\), p.o.) and IMI (3, 10 and 30 mg kg\(^{-1}\), p.o.) swimming (e) and climbing (g) scores. Each column represents the mean ± SEM (n=7) Significantly different from vehicle: *** P <0.0001; ** P <0.001; * P < 0.01 (One-way ANOVA followed by Newman-Kuels test). ◇◇◇ P <0.001; ◇◇ P < 0.01; ◇ P < 0.05; significant difference between treatment and dose (Two Way ANOVA with Bonferonni post hoc test)
4.7.3.2 D-cycloserine pre-treatment

Figure 4.32 shows the effect of D-cycloserine on ALK, FLX and IMI in TST and FST. Pre-treatment of D-cycloserine (2.5 mg kg$^{-1}$, i.p) caused a further decline in immobility score in ALK (30, 100 and 300 mg kg$^{-1}$, p.o.), thus potentiating its observed antidepressant effect but not in FLX (3, 10 and 30 mg kg$^{-1}$, p.o.) and IMI (3, 10 and 30 mg kg$^{-1}$, p.o.) in both (c) TST ($F_{19,120} = 38.86, P<0.0001$) and (d) FST ($F_{19,120} = 35.46, P<0.0001$).

![Graph showing immobility score comparison between untreated and D-cycloserine treated groups for TST and FST.](image-url)

Figure 4.32: Effect of pre-treatment of mice with D-cycloserine (2.5 mg kg$^{-1}$, i.p., a partial agonist on glycine/NMDA receptor on ALK (30, 100 and 300 mg kg$^{-1}$, p.o.), FLX (3, 10, and 30 mg kg$^{-1}$, p.o.) and IMI (3, 10 and 30 mg kg$^{-1}$, p.o.) immobility score in the (c) TST and (d) FST. Each column represents the mean ± SEM (n=7) Significantly different from vehicle: *** $P < 0.0001$; ** $P < 0.001$; * $P < 0.01$ (One-way ANOVA followed by Newman-Kuel’s test). ◊◊◊ $P < 0.001$; ◊◊ $P < 0.01$; ◊ $P < 0.05$; significant difference between treatment and dose (Two Way ANOVA with Bonferonni post hoc test)
4.7.3.2.1 D-cycloserine pre-treatment on swimming and climbing scores in FST

Swimming behaviour was rather further enhanced by D-cycloserine pre-treatment in ALK (30, 100 and 300 mg kg\(^{-1}\), p.o.). However, climbing score was unaffected in ALK (30, 100 and 300 mg kg\(^{-1}\), p.o.) and FLX (3, 10 and 30 mg kg\(^{-1}\), p.o.) but was further increased in IMI (3, 10 and 30 mg kg\(^{-1}\), p.o.) with D-cycloserine pre-treatment.

Figure 4.33: Effect of pre-treatment of mice with D-cycloserine on ALK (30, 100 and 300 mg kg\(^{-1}\), p.o.), FLX (3, 10, and 30 mg kg\(^{-1}\), p.o.) and IMI (3, 10 and 30 mg kg\(^{-1}\), p.o.) swimming (f) and climbing (h) score in FST. Each column represents the mean ± SEM (n=7) Significantly different from vehicle: *** \( P < 0.001 \); ** \( P < 0.01 \); * \( P < 0.05 \) (One-way ANOVA followed by Newman-Kuels’s test). ◇◇◇ \( P < 0.001 \); ◇◇ \( P < 0.01 \); ◇ \( P < 0.05 \); significant difference between treatment and dose (Two Way ANOVA with Bonferonni post hoc test)
4.7.4 INVOLVEMENT OF L-ARGININE-NO-cGMP PATHWAY

Figure 4.34 - 4.37 depicts the involvement of nitric oxide pathway in the antidepressant potential of ALK (30, 100 and 300 mg kg$^{-1}$, p.o.) in both TST and FST.

4.7.4.1. Pre-treatment with L-arginine

Administration of L-arginine (750 mg kg$^{-1}$, i.p., a precursor of nitric oxide) had no anti-immobility effects on mice in the (a) TST and (b) FST compared with saline (vehicle) treated group. Pre-treatment with L-arginine did not significantly abolished the antidepressant effect of ALK (30, 100 and 300 mg kg$^{-1}$) ($F_{7,48} = 17.04, P < 0.0001$) and FST (b) ($F_{7,48} = 42.48, P < 0.0001$).

Figure 4.34: Effects of pre-treatment of mice with L-arginine (750 mg kg$^{-1}$, i.p., a precursor of nitric oxide) on ALK (30, 100 and 300 mg kg$^{-1}$, p.o.) induced reduction in immobility time in the (a) TST and (b) FST. Each column represents the mean ± SEM (n=7). Significantly different from vehicle: *** $P < 0.0001$; ** $P < 0.001$; * $P < 0.01$ (One-way ANOVA followed by Newman-Kuel’s test). ◊◊◊ $P < 0.001$; ◊◊ $P < 0.01$; ◊ $P < 0.05$; significant difference between treatment and dose (Two Way ANOVA with Bonferonni post hoc test).
4.7.4.2 Pre-treatment with L-NAME

L-NAME (30 mg kg\(^{-1}\), i.p., a non-selective NOS inhibitor) when administered alone did not affect immobility score in comparison with the saline treated group only in (c) TST, however, L-NAME exhibited a significant antidepressant effect in the (d) FST. Pre-treatment of L-NAME caused an enhancement in the antidepressant effect of ALK (30, 100 and 300 mg kg\(^{-1}\)) by causing a further reduction in immobility scores in both (c) TST (\(F_{7,48} = 42.89.04, P<0.0001\)) and (d) FST (\(F_{7,48} = 59.88, P<0.0001\)).

Figure 4.35: Effects of pre-treatment of mice with L-NAME (30 mg kg\(^{-1}\), i.p., a non-selective nitric oxide synthase inhibitor) on ALK (30, 100 and 300 mg kg\(^{-1}\), p.o.) induced reduction in immobility time in the (c) TST and (d) FST. Each column represents the mean ± SEM (n=7). Significantly different from vehicle: *** \(P < 0.0001\); ** \(P < 0.001\); * \(P < 0.01\) (One-way ANOVA followed by Newman-Kuels test). ◇◇◇ \(P < 0.001\); ◇◇ \(P < 0.01\); ◇ \(P < 0.05\); significant difference between treatment and dose (Two Way ANOVA with Bonferonni post hoc test).
**4.7.4.3 Pre-treatment with methylene blue**

Pre-treatment of methylene blue (10 mg kg$^{-1}$, i.p., an inhibitor of NO synthase and an inhibitor of sGC) caused an enhancement in the antidepressant effect of ALK (30, 100 and 300 mg kg$^{-1}$) by causing a further reduction in immobility scores in both (e) TST ($F_{7,48} = 45.09, P < 0.0001$) and (f) FST ($F_{7,48} = 298.1, P < 0.0001$).

![Bar chart](image)

Figure 4.36: Effects of pre-treatment of mice with methylene blue (10 mg kg$^{-1}$, i.p., an inhibitor of NO synthase and an inhibitor of sGC) on ALK (30, 100 and 300 mg kg$^{-1}$, p.o.) induced reduction in immobility time in the (e) TST and (f) FST. Each column represents the mean ± SEM (n=7). Significantly different from vehicle: *** $P < 0.0001$; ** $P < 0.001$; * $P < 0.01$ (One-way ANOVA followed by Newman-Kuels's test). ΔΔΔ $P < 0.001$; ΔΔ $P < 0.01$; Δ $P < 0.05$; significant difference between treatment and dose (Two Way ANOVA with Bonferroni post hoc test).
4.7.4.3. Pre-treatment with Sildenafil

Pre-treatment of sildenafil (5mg kg⁻¹, i.p., a phosphodiesterase-5 inhibitor) did not have any significant effect on the antidepressant activity of ALK (30, 100 and 300 mg kg⁻¹) in both (g) TST and (h) FST.

Figure 4.37: Effects of pre-treatment of mice sildenafil (5mg kg⁻¹, i.p., a phosphodiesterase-5 inhibitor) on ALK (30, 100 and 300 mg kg⁻¹, p.o.) induced reduction in immobility time in the (g) TST and (h) FST. Each column represents the mean ± SEM (n=7). Significantly different from vehicle: *** P < 0.0001; ** P < 0.001; * P < 0.01 (One-way ANOVA followed by Newman-Kuel’s test). ◊◊ P < 0.001; ◊ P < 0.01; ◊ P < 0.05; significant difference between treatment and dose (Two Way ANOVA with Bonferroni post hoc test)
CHAPTER FIVE

5.1 DISCUSSION

Depression is a multifarious disorder in which several underlying presentations may share a collective phenomenology but have diverse etiologies. Clinical studies indicate that a considerable percentage of patients who seek medication for major depression have a chronic-recurrent course of the disorder (Hardeveld et al., 2010). Moreover, depressed patients often have several associated physical disorders, suicidal tendencies and the prolonged improvement of symptomatology as seen with most current antidepressants may cause an upsurge in mortality if drugs with rapid onset, continued effect and multiple therapeutic targets that better addresses the heterogeneous nature of the disorder are not sought (Lucas, 2008). Additionally, discovery of active bioactive compounds in herbal medicinal preparations may turn out to be useful in clarifying the pharmacological activity and possible mechanisms of action for potential drug discovery (Tseng et al., 2007). It was based on this assertion that the antidepressant effect of secondary metabolites isolated from the stem bark extract of *Trichilia monadelpha* was ascertained in this study and to further evaluate the possible mechanisms of action of the most potent and efficacious phytochemical.

Preliminary phytochemical screening of the stem bark extracts of *Trichilia monadelpha* in this present study revealed the presence of alkaloids, flavonoids, glycosides, saponins, sterols, tannins, and terpenoids in hydroethanolic extract. HEE, a more polar solvent was used and this was expected to extract polar constituents from the stem bark of the plant. Ethyl acetate extract, a slightly polar solvent had alkaloids, glycosides, tannins, sterols and terpenoids while the petroleum ether extract showed the presence of alkaloids, sterols, and terpenoids possibly due to its non-polar properties as a solvent therefore isolated the non-polar components from the plant’s stem bark. This observation was harmonious with similar studies done by Ben et al., (2013).
Several scientific reports have indicated that the respective pharmacological and therapeutic potentials of medicinal plants are due to the presence of active biological compounds most of which are secondary metabolites (Maganha et al., 2010; Rogerio et al., 2010). Thus the presence of such a wide collection of phytochemicals in the stem bark may imply widespread phyto-pharmacological effects as reported by its multipurpose uses in African traditional medicine. Saponins are the naturally occurring strong foam-forming glycosides (Quante et al., 2010). Scientific evidence revealed that saponins possess neuroprotective effect via the inhibition of apoptosis and intraneuronal calcium dynamics, antidepressant effects, anticancer, anti-inflammatory, antifungal, moluscidal, spermicidal etc. (Xiang et al., 2011). Alkaloids are reported to be considered as one of the most effective and therapeutically essential plant substance (Makkar et al., 2007). They possess diverse clinical properties including antidepressant effects, agents for CNS and analgesia, inhibition of muscle spasms, anti-inflammatory and antineoplastic effects (Nesterova et al., 2011; Yang et al., 2006). Terpenoids isolated from Valerian officinalis are known to possess effective anxiolytic and antidepressant effects, anticancer properties among others (Kennedy and Wightman, 2011). Phenolic compounds have been reported to possess multiple biological effects including antidepressant activity (St. John’s wort), neuroprotective, anticancer, anti-inflammatory as well as enhancing endothelial functioning (Shi and Wang, 2006; Chao et al., 2009). Sterols are cholesterol-like compounds and are known for their antimicrobial, anti-diabetic, anticancer, ability to reduce cholesterol level and boost immune function, (Breytenbach et al., 2001; Katan et al., 2003). Although the individuals potent bioactive compounds are yet be isolated, characterized and tested for their respective pharmacological properties, the occurrence of these phytochemicals may validate the traditional use of T. monadelpha in managing most psychiatric conditions including depression.
In the Irwin test, all three extracts (HEE, EAE and PEE) exhibited sedative effects at higher doses without impairing motor coordination and respiration function in mice. This is indicative of possible CNS depressant effect. In addition, no mortality was also recorded after 24 hours for all the administered doses in all the three extracts suggesting that the minimum lethal dose for our test extracts in mice may be above 3000 mg kg\(^{-1}\). The Irwin test is used as a safety approach for detecting untoward effects of a new compound on general behaviour, physiological function and for evaluating its acute neurotoxicity (Roux et al., 2005; Lynch et al., 2011). All three extracts showed analgesic effects confirming the traditional usage of the plant in managing the pain (Woode et al., 2012).

In this study, oral administration of HEE, EAE and PEE demonstrated potent antidepressant activity in both the forced swimming and tail suspension tests which are two most widely used pharmacological models for assessing antidepressant activity. In both models, all the extracts produced a dose-related decline in immobility scores by increasing specific active behavioural components like swimming and climbing in the FST, and curling and swinging in the TST respectively.

Mice develop immobility when they are in a stressful condition without the opportunity for escape. The immobile behaviour is known to reflect either a failure to endure in an escape-directed behaviour after persistence stress or the occurrence of passive behaviour that extricate the animal from active forms of coping with stressful stimuli (Cryan and Lucki, 2000). Decline in immobility is used as the principal index for the antidepressant effect of test substances in these models (Cryan et al., 2005). Several antidepressants in clinical use selectively induces reduction of behavioural immobility in TST and FST (Borsini and Meli, 1988; Petit-Demouliere et al., 2005). Thus HEE, EAE and PEE were able to cause a reduction in immobility behaviour which is suggestive of an antidepressant effect.
Recent modifications of the traditional FST and TST demonstrated that specific behavioural components of active behaviours differentiate neurochemically unique antidepressants (Cryan et al., 2002; Lucki, 1997). In the modified FST, drugs that cause an upsurge in the swimming score without significantly altering the climbing behaviour are purported to be sensitive to the serotonergic pathway, while drugs with selective effects on catecholamine neurotransmission increase the climbing score without affecting swimming score (Page et al., 1999; Réneric et al., 2001). In this study, HEE, EAE and PEE exhibited significant increase in swimming and climbing scores thus suggesting that all three extracts might be acting possibly via serotonergic and catecholaminergic pathway. The unique active behaviours observed by antidepressants have been reported to be superimposable on combinations of serotonergic and noradrenergic compounds (Slattery and Cryan, 2012). In the TST, all three extracts demonstrated significant increase in swinging and curling behaviours. Antidepressants that inhibit noradrenaline and/or serotonin re-uptake cause a diminution in immobility of mice by increasing their swinging behaviour but with no effect on their curling behaviour. However, curling behaviour is reported to be indicative of possible enhancement of opioidergic activity (Berrocoso et al., 2013). Therefore, the observed antidepressant activity of HEE, EAE and PEE may partly be dependent on its effect on opioidergic systems. Antidepressants which act via increase in the inhibition of reuptake of monoamines have been reported to mediate the enhancement of the opioid pathway (Berrocoso et al., 2009; Jutkiewicz and Roques, 2012). The role of opioids as recognized antidepressants is buttressed by the clinical effectiveness of μ-opioid receptor agonists such as tramadol, oxycodone, oxymorphone, etc., in the treatment of refractory depression (Shapira et al., 2001; Hegadoren et al., 2009; Berrocoso et al., 2013). Berrocoso et al. (2009) further demonstrated that blocking opioid receptors with naloxone inhibited the
antidepressant-like effect of codeine and μ-opioid receptor gene knockout mice exhibited decreased normal curling behaviour in TST.

The results presented shows that HEE was the most potent and efficacious followed by EAE with the PEE having the least potency and efficacy in reducing immobility score in both TST and FST. This observation could be due, partly to the active bioactive metabolites present in each of the extracts (Briskin, 2000) and could possibly be attributed to the different mechanisms by which each extract could be acting to elicit its pharmacological effect.

Determination of active major biochemical constituents are generally recommended for standardization and quality control of plant crude products and related investigations as this may prove essential in elucidation pharmacological activity for potential drug development (Chao et al., 2009; Tseng et al., 2007). Based on the results in this study, alkaloids, saponins, flavonoids, terpenoids and tannins were extracted from the HEE (the most potent and efficacious crude extract) and their antidepressant activity was investigated. Interestingly, role of plant secondary metabolites in the discovery of antidepressants have attracted tremendous attention in recent years (Gong et al., 2014).

Qualitative test of the extracted saponins from the HEE revealed the presence of steroids, terpenoids and glycosides indicating that the saponins present are steroidal glycosides and triterpenoidal glycosides. Saponins occur as glycosides whose aglycone is either triterpenoid or steroidal structures. Their ability to produce detergent or soap-like characteristic in aqueous solution is due to the combination of lipophilic sugars at their ends (Kabera et al., 2014). Saponins are naturally occurring plant glycosides with significant medicinal benefits such anti-inflammatory, antispasmodic, aphrodisiac, antidepressant effects.

Flavonoids are a group of more than 4000 phenolic compounds consisting of a central three-ring structure that occur in plant. In nature, flavonoids can occur either in the free or
conjugated forms, and often in plants they are mainly present as glycosides with a sugar moiety or more sugar moieties linked through a hydroxide group or through carbon-carbon bonds (Sisa et al., 2010) and hence the detection of glycosides in its qualitative screening. They are usually classified according to their aglycone chemical structure, into flavols, flavones, flavonones, anthocyanidins, isoflavones, catechins and chalcones (Hernández et al., 2009) Alkaloids present the largest single group of secondary metabolites that contain basic nitrogen atoms. They are synthesized by secondary metabolism of primary metabolites, usually amino acids (e.g: phenylalanine, tyrosine, tryptophan etc) and are normally classified according to their heterocyclic ring system they possess (Woolley, 2001). Alkaloids have proven to possess useful medicinal properties that improve the health of mankind. Morphine and cocaine are some useful isolated alkaloidal compounds which act on the nervous system (Charbogne et al., 2014).

Terpenoids constitute a large family of phytoconstituents of little functional and structural common ground. They are polymeric isoprene derivatives and synthesized from acetate via the mevalonic acid pathway. Compounds of terpenoids are lipophilic, volatilize easily, have strong odours and flavours. Traditionally, plant-based terpenoids have been used by humans in the food, pharmaceutical, and chemical industries (Breitmaier, 2008; Kabera et al., 2014).

Tannins are natural, water-soluble, polyphenolic compounds with molecular weight ranging from 500 to 4,000, usually classified into 2 classes: hydrolysable tannins and condensed tannins. Hydrolysable tannins are mostly found combined with polysaccharide, proteins and in some cases alkaloids whereas the condensed tannins are not conjugated to any sugar moiety (Huang et al., 2007).

The results from the present study indicate that ALK, SAP, FLV, TER and TAN have antidepressant effects in FST, a widely used animal model with a high predictive validity for
investigating the antidepressant effect of test compounds. All five metabolites significantly decreased immobility scores and as well showed increase in swimming score demonstrating possible activation of serotonergic system in their respective mechanism of action (Cryan et al., 2002). However, only SAP, TER and ALK showed a significant increase in climbing behaviour revealing them as potential catecholaminergic agents (Cryan et al., 2002; Rénéric et al., 2001). Therefore, we hypothesized that SAP, TER and ALK might be eliciting their observed antidepressant effect via both serotonergic and catecholaminergic pathways. Dissimilarly, results obtained from the TST in the study indicate that only three metabolites ALK, FLV and SAP significantly reduced immobility score. The non-uniform nature of the results implies that both of these models might follow different pathophysiological mechanisms in inducing immobility. Chatterjee et al., (2012) reported that although these models are similar in face behavioural symptoms, pharmacological evaluation indicates that dopamine functioning is a necessity for performance of mice in the FST, whereas both serotonergic and dopaminergic systems are involved in TST model. FLV and ALK showed a significant increase in swinging scores whereas SAP and ALK induced diminution in immobility by causing increase in curling behaviour in TST. It is therefore likely that their observed antidepressant effect is partly due to its effect on opioidergic systems since curling behaviour is reported to be indicative of opioidergic activity (Berrocoso et al., 2013; Kukuia et al., 2015). Comparing the efficacy of HEE to that of the individual active phytoconstituents evaluated, it can be deduced that the antidepressant efficacy observed in the HEE can be attributed to the synergistic effect produced by the individual active phytoconstituents and possibly via varying mechanisms of action (Briskin, 2000).

The results presented shows that the ALK was the most potent and efficacious secondary in reducing immobility score in both TST and FST. Thus in our search for pure, most efficacious and potent bioactive compound responsible for the antidepressant effect observed
in *Trichilia monadelpha*, alkaloids isolated from the HEE was considered to be an ideal phytoconstituent to warrant further investigations into its pharmacological roles in treating depression.

ALK exhibited a rapid and sustained antidepressant effect in the chronically depressed mice. The metabolite caused a decline in immobility significantly at the second day of treatment and increased distanced travelled at the sixth day of treatment, the primary indices of antidepressant effect, in the open space swim test (Sun and Alkon, 2003; Stone and Lin, 2011). Depression is induced either over a period of weeks and/or require exposure to traumatic stress and depressed subjects only respond prolonged chronic treatment with antidepressants but not to acute or subacute treatment (Stone and Lin, 2011). Most current depression pharmacotherapies exhibit delay in symptom improvement, aside from the untoward side effects and lack of efficacy against refractory depression (Kukuia et al., 2016). Antidepressant effect in this model with respect to the duration of immobility which is deemed the more consistent index in mice (Stone et al., 2008; Stone and Lin, 2011) started on the second day of drug treatment whiles a delay in the onset of action was observed in fluoxetine and bupropion treated mice. Although depression is conventionally described as a state of neurotransmitter imbalance and currently used antidepressants such as SSRIs, TCAs and bupropion have been designed to achieve these effects more potently and selectively via blockade of transporters (Andrade and Rao, 2010). These effects can be detected immediately after drug administration, however, the therapeutic effect of some of these antidepressant treatments requires a number of weeks to become clinically important (Frazer and Benmansour, 2002; Harmer et al., 2009; Samuels and Hen, 2011). Several reports have indicated a complex array of downstream neuropharmacological changes, such as changes in signal transduction, down regulation of neurotransmitter receptors, desensitization of autoreceptors (5HT<sub>1A</sub> and 5HT<sub>1B/D</sub>) and transporters, increases in
hippocampal neurogenesis and mobilization of neurotrophins that develop after chronic
treatment with some of these may be implicated in their clinical efficacy and the duration
taken for these processes to take place may play a role to the time lag in their clinical
onset of action (Duman and Monteggia, 2006; Harmer et al., 2009; Manji et al., 2003;
Tardito et al., 2006). Scientific research findings have revealed that 5-HT2C antagonists are
putative fast-onset antidepressants, which act through enhancement of mesocortical
dopaminergic signalling (Opal et al., 2014). In addition, potential faster acting
antidepressants known to respond to chronic treatment only include serotonin receptor-4
agonists (Lucas et al., 2007; Mendez-David et al., 2014). Antidepressants that possess
antagonist effect on 5-HT1A somatodendritic autoreceptors have been reported to exhibit
accelerated onset of action (Levinstein and Samuels, 2014). The inhibitory effect of the 5-
HT1A autoreceptors is one of the reasons for the delay in onset of action of
antidepressants affecting 5-HT systems (Moret, 2005). This has been the basis for
adjunctive therapies with 5HT1A receptor partial agonists buspirone or pindolol and SSRIs
(Keks et al., 2007). Recently antidepressants that activate melatonin receptors have been
reported to demonstrate faster onset of action in animal models (Alamo et al., 2014).

Recent studies have found that ketamine, a NMDA receptor antagonist, elicits rapid and
protracted antidepressant effects in animals exposed to chronic stress (Levinstein and
Samuels, 2014). It can therefore be hypothesized that the ALK which may have revealed to
produce an enhancement in monoaminergic neurotransmission in the TST and FST might as
well possibly be acting on other systems and/or receptors thus demonstrating a rapid onset of
behavioural effect in the open space swim test.

The detailed specification of the neural mechanisms underlying the effects of potential
antidepressants can characterized novel candidates implicated in the pathophysiology of
depression and the search for possible new targets for therapeutic intervention (Cryan and
Lucki, 2000). The present study investigated the role of noradrenaline and dopamine in the acute behavioural effects of the ALK in the modified FST and TST by using drugs that impede with their neurotransmitter biosynthesis and/or release. Pre-treatment with α-methyldopa, a competitive inhibitor of the enzyme DOPA decarboxylase that inhibits the conversion of L-DOPA to dopamine and hence inhibiting the biosynthesis of catecholamines (Chhabra, 2016) abolished the antidepressant effects of the ALK and imipramine but failed to attenuate the behavioural effects of fluoxetine. This suggests that the ALK may affect the biosynthesis of noradrenaline and/or dopamine. Moreover, dopamine beta-hydroxylase converts α-methyldopa to α-methylnorepinephrine which is an agonist of pre-synaptic CNS α2-adrenergic receptors. Activation of these receptors in the brainstem leads to inhibition of vesicular release of noradrenaline (Chhabra, 2016). Thus results may also suggest that ALK may also be involved in the vesicular release of NA probably via blockade of presynaptic α2-adrenergic receptors. In addition, pre-treatment of mice with reserpine alone reversed the decline in immobility induced by the ALK and IMI whiles that of fluoxetine was not significantly affected in both TST and FST. Reserpine irreversibly blocks the vesicular monoamine transporter 2 (VMAT-2) which is located primarily within the CNS and is responsible for transporting free intracellular monoamines into secretory vesicles (Ji et al., 2007; Metzger et al., 2002; Wimalasena, 2011). Thus the ability of reserpine pre-treatment to attenuate the reduction in immobility of ALK may suggest that the depletion of vesicular pools of monoamines may affect the action of the ALK since reserpine treatment leads to depletion of vesicular monoamine stores (Fukui et al., 2007). The ability of reserpine pre-treatment to reverse the antidepressant effects of the extract and imipramine but not fluoxetine however, seem to suggest that reserpine does not affect vesicular storage of 5-HT to the same extent as that of noradrenaline. Wimalasena, (2011) reported that reserpine has a lower inhibitory affinity for VMAT 2 – mediated 5HT transport into secretory vesicles. This
report is coherent with the results obtained by Woode et al., (2010) showing that reserpine at the dose used produced a 93 and 95% diminution of cortical noradrenaline and dopamine content respectively, and a 78% depletion of 5-HT levels. To inhibit both synthesis and deplete vesicular storage of noradrenaline and dopamine, mice were pre-treated with α-methyldopa and reserpine. The results demonstrated that a combined treatment of reserpine and α-methyldopa significantly abolished the antidepressant effect of the ALK and IMI but not FLX similar that obtained when reserpine and α-methyldopa were administered alone. Additionally the increase in both climbing and swimming behaviours observed in the ALK and IMI treated mice were reversed in both ALK and IMI but these behaviours were not affected in FLX after pre-treatment with both reserpine and α-methyldopa. Thus, the combination had only a reasonable effect on NA and DA but failed to have any significant affect 5-HT neurotransmission. These results demonstrate that the antidepressant effect of ALK may be dependent on noradrenergic neurotransmission.

The pharmacological role of some specific adrenergic receptors in the antidepressant activity of ALK was investigated. Results of the study shows that the anti-immobility effects of the ALK was not affected by prazosin (α₁-receptor antagonist) but the antidepressant effect of atomoxetine (selective noradrenaline re-uptake inhibitor) was inhibited by prazosin pre-treatment. Stone et al., (2003) reported that activation of brain α₁-adrenoceptors are instrumental in behavioural activation, particularly sensitive to stress and its desensitization or blockade has been implicated in depressive illness. The inability of prazosin to reverse the antidepressant effect of the ALK may suggest that the ALK may not be acting via activating α₁-adrenoceptors. The study also revealed that yohimbine (α₁-receptor antagonist) potentiated the anti-immobility effect of ALK thus a demonstration of potential noradrenaline toxicity. Studies have shown that major depression is accompanied by increased α₂-adrenergic receptors in the hippocampus and cerebral cortex (Hieble, 2007). Presynaptic α₂-adrenergic
receptors are inhibitory autoreceptors that regulate NA release and therefore their antagonism increases noradrenaline release (O'Donnell, 2009). Similarly, if presynaptic α2-adrenergic receptors are essential for the antidepressant-like effect of a drug, blockade of the receptor would be expected to potentiate rather than block its effects on behaviour, however, the opposite is observed if postsynaptic α2-adrenergic receptors are involved (Zhang et al., 2009). Studies using post-mortem specimens from depressed suicide victims shows an increase in α2A-receptors in the prefrontal cortex which suggest that α2-adrenergic antagonists may produce antidepressant effect (Zhang et al., 2009). The present results suggest that blockade of pre-synaptic, α2-adrenergic receptors may play a major role in the mediation of antidepressant effects of the ALK. These results demonstrate that the antidepressant effect of ALK may partly be dependent on noradrenergic neurotransmission.

In the present study, the pre-treatment of pCPA, a selective inhibitor of the rate-limiting enzyme in the biosynthesis of serotonin, tryptophan hydroxylase, was the approach used to deplete endogenous brain serotonin levels without affecting noradrenergic and dopaminergic synthesis (O'Leary et al., 2007). This treatment was able to abolish the antidepressant effect of ALK, FLX and IMI in the TST and FST, indicating that its observed antidepressant activity is dependent on the enhancement of serotonergic neurotransmission. The reversal of the antidepressant activity of fluoxetine in pCPA treated mice is coherent with reports that fluoxetine demonstrate its acute behavioural effects via the selective blockade of the serotonin transporter thus increasing extracellular serotonin in the synaptic cleft (Colla et al., 2012). ALK, imipramine and fluoxetine increased swimming score which was reversed with pCPA pre-treatment, further augmenting their action on the serotonergic system (Chau et al., 2011). In the present study, the anti-immobility effect elicited by ALK in TST and FST was blocked by the pre-treatment of mice with cyproheptadine (a 5-HT2 receptor antagonist). Several clinical studies have shown that antidepressant with affinity for 5-HT2A
receptors augment the clinical response to SSRIs in treatment-resistant patients (Shelton et al., 2001) recent study indicates its involvement in SSRI-mediated actions, showing also antidepressant-like properties of selective 5-HT$_{2B}$ receptor agonists (Diaz et al., 2012). Desensitization of 5-HT$_{2C}$ receptors is also reported following chronic SSRI treatment. Preclinical data show that 5-HT$_{2C}$ antagonism augments the neurochemical and behavioural effects of SSRIs (Cremers et al., 2007). This further confirms the role of the 5-HT system in the mechanism of the antidepressant activity of ALK.

Several studies have support the role of the glutamatergic system in the pathophysiology of affective disorders as well as the efficacy of glutamatergic compounds such as functional glycine/NMDA receptor antagonists as antidepressant agents (Zarate et al., 2013). These agents have been reported to demonstrate a relatively lower side effect profiles compared to the competitive and non-competitive NMDA antagonists (Poleszak et al., 2011). This study demonstrated that the pre- treatment of mice with D-cycloserine (a partial agonist of glycine/NMDA receptors) further potentiated the anti-immobility effect of ALK in both the TST and FST suggesting that ALK may be acting as an antagonist on the glycine/NMDA receptor complex. In contrast, pre-treatment of D-serine (a full agonist on glycine/NMDA receptors) blocked the anti-immobility effect of ALK, FLX and IMI indicating that activation of the glycine/NMDA receptor complex causes a diminution of antidepressant activity of both serotonin and noradrenaline-based compounds (Wlaz’ et al., 2011). Preclinical and clinical observations have shown that monoaminergic-based antidepressants decrease NMDA binding, expression and function (Skolnick, 1999). Several reports have stated that the interaction between NMDA receptor and serotonergic neurotransmission is more palpable than NMDA receptor and noradrenergic pathway and thus interaction between glutamate and serotonin may be important for the control of many brain activities (Poleszak et al., 2011; Szewczyk et al., 2009). The increase in swimming behaviour demonstrated by ALK saw a
further rise with D-cycloserine pre-treatment but a reversal effect was exhibited with D-serine pre-treatment. Climbing scores of the ALK treated mice were not affected by both D-serine and D-cycloserine pre-treatment. This result further supports the theory that the interaction between NMDA receptor and serotoninergic neurotransmission is more palpable than NMDA receptor and noradrenergic pathway.

The glycine/NMDA receptor antagonistic effect of the ALK in addition to its enhancement in monoaminergic neurotransmission strongly supports the rapid onset of behavioural effect demonstrated in the open space swim test. Recent studies demonstrate that a NMDA receptor antagonist ketamine, could address the limited efficacy and time lag for therapeutic response to typical antidepressants (Duman and Li, 2012). Chronic physical and social stress impairs neurogenesis, while chronic antidepressant treatments including serotonin-selective reuptake inhibitors (SSRIs) and norepinephrine-selective reuptake inhibitors (NSRIs) enhance neurogenesis (Sahay and Hen, 2007). Effective chronic antidepressant treatments are associated with an improvement of hippocampal neurogenesis, upsurge in neurotrophin levels, increase of synaptic function and a resultant rise in hippocampal function which can be quantified as neuronal response or behaviour (Monteggia et al., 2007). Thus the effectiveness and the sustained antidepressant effect of ALK may be partly due to effect on synaptogenesis and neurotrophic factors.

The L-arginine-NO-cGMP pathway has been associated with the pathophysiology of depression (Kaster et al., 2005). Nitric oxide plays an essential role in the CNS and pharmacological modulation of the NO pathway may be seen as a novel therapeutic approach for the effective management of depression (Zomkowski et al., 2012). The involvement of nitric oxide signalling pathway has been demonstrated in the antidepressant activity of venlafaxine, bupropion and berberine chloride (an alkaloid obtained from Berberis aristata) (Dhir and Kulkarni, 2007).
In this study, it was shown that the reduction of immobility time elicited by ALK in the FST and TST was attenuated by pre-treatment of mice with L-arginine (NOS substrate). Previous studies have demonstrated that the antidepressant-like effects of paroxetine was blocked by pre-treatment with L-arginine (Ghasemi et al., 2009) and that of putative antidepressant ascorbic acid (Moretti et al., 2011). This result may suggest that the antidepressant effect of ALK may be dependent on the inhibition of NO synthesis. Furthermore, a potentiated antidepressant effect was observed with the pre-treatment of L-NAME (a non-selective NOS inhibitor) in ALK treated mice. Several studies have exhibited that NOS inhibitors elicit antidepressant activity in animal models predictive of antidepressant effect (Mutlu et al., 2009). In addition, pre-treatment of mice with methylene blue (direct inhibitor of both NOS and sGC) produced a synergistic antidepressant effect with ALK. This result also suggests that the anti-immobility effect of ALK may as well be partly mediated by the inhibition of NOS and/or sGC activity. Similar to our results, a research demonstrated that methylene blue potentiated the antidepressant-like effect of venlafaxine in the FST (Dhir and Kulkarni, 2007). Heiberg et al. (2002) demonstrated that excessive cGMP levels may generate some depressive symptoms and decreasing its levels may show antidepressant-like actions. Phosphodiesterase enzyme is responsible for the degradation of cGMP. Therefore inhibiting the enzymatic activity of phosphodiesterase may cause an upsurge in the levels of cGMP and thus produce depressive-like conditions (Dhir and Kulkarni, 2007). In this study, the anti-immobility effect of ALK was not affected by the pre-treatment with sildenafil (a selective phosphodiesterase-5 inhibitor). This indicates that ALK may probably not exert its antidepressant by directly decreasing cGMP levels but via nitric oxide synthesis inhibition.

It has been reported that L-arginine blocks the antidepressant effects of the classical TCA, imipramine (Harkin et al., 2003). This observation has led to hypotheses regarding the potential contribution of serotonergic/noradrenergic mechanisms in the observed
antidepressant-like effects of the NOS inhibitors. In addition, NO can inactivate tryptophan hydroxylase, the rate limiting enzyme in the synthesis of serotonin (Kuhn and Arthur, 1997) and also ineffective doses of NOS inhibitors potentiate antidepressant effect of TCAs, SSRIs but not SNRIs in the FST (Harkin et al., 2003). Therefore, the possible mediation of the nitric oxide system in the observed antidepressant activity of the ALK could be attributed to its interaction with the serotoninergic system. These results further reinforces the assumption in this study that the ALK may have demonstrated its antidepressant effect partly via inhibition of NO synthesis and thus points to a significant role of nitrergic system in the mechanism of action of antidepressant agents (Wegener and Volke, 2010).
Figure 5.1: A schematic representation of the proposed mechanism of action of the ALK extracted from HEE of the stem bark of *T. monadelpha*. The interaction of the ALK with the serotonergic-noradrenergic-nitric oxide-glycine/NMDA-opioidergic pathways leads to a cascade of signal transduction mechanisms which are responsible for the observed antidepressant effect.

5.2 CONCLUSION

The research provides scientific evidence that the hydroethanolic, ethyl acetate and petroleum ether extracts from the stem bark of *Trichilia monadelpha* demonstrate antidepressant effect in murine models. The hydroethanolic extract was found to be the most efficacious.

Total alkaloids, saponins, flavonoids, tannins and terpenoids extracted from the hydroethanolic extract exhibited antidepressant potentials in various acute murine models. Total alkaloids were
found to be the most efficacious. Total alkaloids demonstrated a rapid and sustained antidepressant effect in the open space swim test.

The observed antidepressant effect of the ALK may be attributed to its possible complex interaction with several systems: noradrenergic, serotonergic, glycine/NMDA receptor, L-arginine-NO-cGMP, opioidergic pathway. Specifically, blockade of $\alpha_2$-adrenergic receptors, enhancement of 5-HT$_2$ receptors, antagonism of glycine/NMDA receptors and inhibitory effect on nitric oxide synthesis (NOS) and soluble guanylate cyclase (sGC) may explain the observed behavioural effects.

5.3 RECOMMENDATION

- The active pure compound(s) responsible for the rapid and sustained antidepressant effect of the crude alkaloids should be isolated and characterized.
- Chronic toxicity on the alkaloids should be evaluated to establish its safety.
- Other models of depression should be used to assess/confirm the antidepressant properties of the alkaloid and investigate other possible mechanism(s) of action.
- Some of the secondary metabolites should be combined and their antidepressant efficacy and onset of antidepressant effect evaluated.

5.4 LIMITATION

- The study did not incorporate the determination of the onset of antidepressant action of the crude hydroethanolic extract in the open space swim test.
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