EVALUATION OF SOME NEUROPHARMACOLOGICAL EFFECTS OF THE METHANOLIC LEAF EXTRACT OF *LOPHIRA LANCEOLATA* (*TIEGH EX KEAY*) IN MICE.

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DECLARATION

I hereby declare that the research work reported in this dissertationwas carried out by me under the supervision of Dr Musa Aliyu of the Department of Pharmacology, Faculty of Clinical Sciences. Bayero University, Kano.Nigeria.The work of other researchers is acknowledged and referred to accordingly. I solemnly declare that no part of this thesis has been submitted elsewhere for the award of any degree.

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CERTIFICATION

This is to certify that the dissertation entitled ‘’EVALUATION OF SOME NEUROPHARMACOLOGICAL EFFECTS OF THE METHANOLIC LEAF EXTRACT OF *LOPHIRA LANCEOLATA* (*TIEGH EX KEAY*) IN MICE’’ by Aisha AbdullahiAbubakar was carried out under my supervision and meets the regulation governing the award of the degree of MASTER OF SCIENCE (PHARMACOLOGY) of Bayero University, Kano, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to God Almighty for the blessing's showered upon me and for making everything a reality.

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ABSTRACT

Lophiralanceolata is a medicinal plant that has a variety of ethnomedicinal applications among which are sedation and anxiolysis. The present study is aimed at screening the methanolic leaf extract of the plant for possible neuropharmacological effects (sedative, anxiolytic and antidepressant effects).Standard protocols were employed to determine the phytochemical constituents of the methanolic leaf extract and the intraperitoneal median lethal dose (LD_{50}) was determined using Lorke's method. The extract was evaluated for sedative and anxiolytic actions in mice using diazepam induced sleeping time, hole board, beam walk assay, elevated staircase and elevated plus maze tests. The extract was also evaluated for possible antidepressant properties using tail suspension and forced swim tests in mice.The preliminary phytochemical screening of the methanolic leaf extract indicates the presence of flavonoids, tannins,phlabotannins,saponins, glycosides, triterpenes, alkaloids and reducing sugars. Steroids were absent.The intraperitoneal median lethal dose (LD_{50}) of the methanolic leaf extract in mice was calculated to be 1,131.9 mg/kg body weight. The methanolic leaf extract of *Lophiralanceolata*did not show significant effects on both onset and duration of sleep in the diazepam induced sleeping time test at the doses tested (87.5, 175 and 350 mg/kg body weight). However, there was a significant increase ($P \leq 0.05$) in the number of head dips in the hole board test. The extract at the tested doses did not show any significant effect on the number of foot slips and the duration of walk to the goal box in the beam walk assay, however there was a significant decrease in the number of stairs climbed as well as rearing in the elevated staircase test. It further did not show any significant effect on the number of entry and time spent in the open arm of the elevated plus maze test. The extract showed a significant decrease on the activity counts (immobility time) in the tail suspension test with no significant effect on immobility,

swimming and climbing times in the forced swim test.The above findings suggest that the methanolic leaf extract of *Lophiralanceolata* contains phytochemical constituents that possess pharmacological properties that could be usefully employed in the management of anxiety and depressive disorders.

CHAPTER ONE

1 INTRODUCTION

All activities of the body are controlled by the nervous system which is divided into two distinct parts: Central nervous system and the peripheral nervous system.

The Central Nervous System (CNS) comprises of the brain and spinal cord and is formed by neurons and some supporting cells called neuroglia. The structures of the brain and spinal cord are arranged in two layers namely gray matter and white matter. Gray matter is made up of nerve cell bodies and the proximal parts of the nerve fibers arising from the cell body. White matter is formed by the remaining parts of the nerve fibers. The brain and spinal cord are surrounded by three layers comprising of an outer duramater, middle arachnoid mater and an inner piamater. The space between arachnoid mater and piamater is termed the subarachnoid space; the space is filled with fluid called the cerebrospinal fluid onto which both the brain and the spinal cord are suspended (Bleakley and Sie, 2012).

A neurotransmitter is a chemical substance that acts as a mediator for the transmission of nerve impulses from one neuron to another through a synapse. Many substances of different chemical nature are identified as neurotransmitters, and depending upon their chemical nature they are divided into three groups:

1. Amino Acids: Neurotransmitters here are involved in fast synaptic transmission and are inhibitory or excitatory in action. Gamma amino butyric acid (GABA),Glycine, Glutamate (glutamic acid) and Aspartate (aspartic acid) belong to this group (Bleakley and Sie,2012).

2. Amines: These aremodified amino acids which are involved in slow synaptic transmission;they are also inhibitory or excitatory in action. Examples are noradrenaline, adrenaline, dopamine, serotonin and histamine.

3. Others: Some neurotransmitters do not fit into any of these categories; one of such substance is acetylcholine which is formed from choline and acetyl coenzyme A in the presence of an enzyme choline acetyltransferase. Another substance in this category is the soluble gas nitric oxide (NO)(Bleakley and Sie, 2012 and Rang *et al*., 2003a).

Depending on the function they perform, neurotransmitters can be classified into two types namely:

1. Excitatory neurotransmitter: An excitatory neurotransmitter is a chemical substance that is responsible for the conduction of impulse from presynaptic neuron to postsynaptic neuron. The neurotransmitter released from the presynaptic neuron does not cause development of action potential at the postsynaptic neuron rather it causes a slight change in the resting membrane potential that is slight depolarization by the opening of sodium channels in the postsynaptic membrane and influx of sodium ion from extracellular fluid (ECF).The slight depolarization is termed excitatory postsynaptic potential (EPSP). The EPSP in turn causes the development of action potential. Common excitatory neurotransmitters are acetylcholine and noradrenaline.

2. Inhibitory neurotransmitter: This is a chemical substance that inhibits conduction of impulse fromthe presynaptic neuron to the postsynaptic neuron. When it is released from the presynaptic axon terminal due to the arrival of action potential, it causes the opening of potassium channels in the postsynaptic membrane and efflux of potassium ions which leads to hyperpolarization termed the inhibitory postsynaptic potential (IPSP). When IPSP is developed action potential is not generated in the

postsynaptic neuron.Common inhibitory neurotransmitters are gamma aminobutyric acid (GABA) and glycine(Bleakley and Sie, 2012 and Rang *et al.,* 2003a).

A neurotransmitter is produced in the cell body of the neuron and transported through the axon terminal where it is stored in small packets called vesicles, under the influence of a stimulus the neurotransmitter is released into the synaptic cleft where it binds to specific receptors on the surface of the postsynaptic cell. The receptors are G-protein, protein kinase or ligand gated receptors.After its action the neurotransmitter is inactivated via four main mechanisms: It diffuses out of synaptic cleft to the area where it has no action, it is destroyed or disintegrated by specific enzymes, it is engulfed and removed by astrocytes (macrophages), it is removed by means of reuptake into the axon terminal.

Central nervous system (CNS) diseases can affect the spinal cord, the brain or both. The CNS is vulnerable to various disorders; it can be damaged by: trauma,infections, degeneration, structural defects, tumors, blood flow disruption and autoimmune disorders (Nervous System Problem, 2015).

Disorders of the nervous system may involve the following: vascular disorders example stroke, transient ischemic attack, subarachnoid hemorrhage, subdural hemorrhage, hematoma and extradural hemorrhage.Infections such as meningitis, encephalitis, poliomyelitis and epidural abscess.Structural disorders like brain and spinal cord injury, Bell's palsy, cervical spondylitis, carpal tunnel syndrome, brain or spinal cord tumors, peripheral neuropathy and Guillain-Barre syndrome (Nervous System Problem, 2015; John Hopkin's Medicine, ND).

Functional disorders such as headache, epilepsy, dizziness andneuralgia. Degenerative disorders such as Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis (ALS), Huntington's chorea and Alzheimer's disease (Nervous System Problem, 2015).

The different signs and symptoms of central nervous system disorders include: Persistent or sudden onset of headache, loss of feeling or tingling, weakness or loss of muscle strength, sudden loss of sight or vision, memory loss, impaired mental ability, lack of co-ordination, muscle rigidity, tremors and seizures, back pain that radiates to feet, toes, or other parts of the body, muscle wasting and slurred speech (Tripathi, 2006b; Rang*et al.,* 2003a)

Classification of drugs that affects the CNS.

The classes of drugs that affect CNS are:-

1. Stimulants: these speed up the activities of the CNS including the brain, resulting most often into a more alert and energetic feeling,examples are amphetamine, cocaine, pseudoephedrine, nicotine and caffeine.

2. Depressants (relaxants): these tend to slow down the activity of the CNS which often results in feeling of less pain, more relaxed and sleepy. The term depressant is used to describe the effect on CNS and not mood. CNS depressants most likely result in feelings of euphoria and not depression especially in moderate use, examples include alcohols, major tranquilizers, benzodiazepines (e.g diazepam, temazepam), opioids(heroin, morphine),volatile substances (glue, petrol, paint etc)(Tripathi,2006a).

3. Hallucinogens: these have the ability to change sensory perceptions by distorting the messages carried in the CNS. Hallucinogens alter one's perception and states of consciousness, example LSD, psilocybin (magic mushroom) and mescaline (peyote cactus).

4. Others: these include psycho-active drugs that do not neatly fit into one of these other categories; examples are antidepressants e g sertraline and mood stabilizers e g lithium, cannabis, volatile substances e t c.

Majority of the world population depends on traditional medicine such as herbs for treatment of a wide range of ailments. It is estimated that 25% of modern medicines are made from plants first used traditionally (WHO, 2000; Ezeonwumelu*etal*., 2012). The use of traditional medicine is rapidly growing and most people have gone ethno due to accessibility and affordability. Plants used in traditional medicine are relatively safe, but some may have undesirable adverse effects which may be due to over dosage or certain factors and these may lead to toxicity and death (Okigbo*et al.*, 2009).

Several plants have been studied for their neuropharmacological effects amongst which are *Cissuscornifolia*) *(*Musa *et al.,*2008; Yaro *et al*., 2009)., *Paullina pinnata (*Aliyu *et al.,2010).,Hypericumperforatum*(John's wort) (Jorge *et al*., 1994).,*Passifloraincarmata*(passion flower) (Morris *et al.*, 2003).,*Valerian officinalis* (Valerian) (Hibbeln*et al*.,1998).,*Humulus*lupulus (Hops) (Castellanos*et al.,* 2000), *Matricariarecutita* (Chamomile) (Bynum *et al.,* 1994).,*Oenotherabiennis(*evening primrose),*Panax ginseng*(ginseng) (Lake, 2000)., *Piper methystium* (Kava) (Martindale, 1995).,*Levendullaofficinalis* (lavender) (Mindell and Handell, 1981).,*Mellisaofficinalis* (lemon balm) (Molina-Hernandez *et al*., 2004) and *Scutellarialaterifolia*(Skullcap)(Thakur and Rana,2013).

The plant *Lophiralanceolata* belongs to the order Malpighiales, Family Ochnaceae and Genus Lophira.Ochnaceae is the major group of angiosperms (flowering Plants), which is made up of approximately 35 genera and about 600 species widely represented in the tropics.Ochna has nearly 90 species consisting mainly of tropical trees and shrubs, members usually have alternate simple leaves with closely parallel lateral veins and obvious stipules (Do- Nascimento*et al.,* 2008).

Lophiralanceolata is a multipurpose tree 8-12m tall straight or twisted with alternate leaves, clustered at the end of short straight branches, glabrous, bright and blade oblong lanceolate.The local names are Hausa: *namijin kadanya* or *namijin kade*, Yoruba*: Iponhom* and Igbo :*Okopia*.The leaves are narrowly

elongated, rounded at the apex,shiny grayish to pink when young and shiny green when mature, the leaves are very prominent in appearance. The stem bark is grayish,rough and broken into corky patches,flowers occur from October to Januaryand fruits are 0.1cm in diameter with one elongated seed. The plant have a wide range of uses in African traditional medicine where different parts of the plant (stem, root and leaves) are employed in treatment of abdominal pain, dysentery, diarrhea,headache, malaria and cardiovascular diseases, they are also used in epilepsy (Burkill, 1997)as anxiolytics and sedative (personal communication with herbalist) and for dermatological purposes where oil from the seeds is used to treat dermatitis, toothache, muscular tiredness and rubbing on the skin prevents dryness (Burkill, 1997). The young stem and roots are a good source of chewing stick and mouth washes (Burkill, 1997; Do-Nascimento*et al*., 2008).

Several phytochemical constituents have been isolated from the various parts of the plant such as flavonoids,alkaloids,oils,saponins,glycosides,anthraquinones, carbohydrates, acidic compounds, terpenoids and reducing sugars(Onyeto*et al.,* 2014; Audu*et al*., 2007; Burkill,1997). Studies on the various parts of species of both *L.alata*and *L.lanceolata* showed similar poly flavonoids however bioflavonoids such as *lanceolatin* A and B are useful chemotaxonomic markers in *lophira*and the ochnaceae family (Tih*et al*., 2003).

1.1 Statement of Research Problem

The complexity of daily life in modern society frequently leads to varying degree of anxiety and depression. Despite the known effectiveness of treatment for both depression and anxiety, majority of people in need do not access it due to lack of resources, lack of trained providers, social stigma and side effects of the medications (WHO, 2012).

Mood, depression and anxiety disorders have been found to be associated with chronic pain among medical patients in both developed and developing countries (Evans *et al*., 2005). A number of recent

studies have shown that depression predicts the onset of a number of clinical conditions including hypertension, coronary heart disease, cancer,neurological disorders,hypothyroidism as well as diabetes mellitus (Reus, 2008).Anxiety can also precipitate or aggravate cardiovascular and psychiatric disorders(Chatterjee *et al.,* 2011).These considerations implicate the search for new anxiolytic and antidepressant agents that have a fast onset of action.

1.2 Significance of the Study

The growing public interest in traditional medicine and its accessibility as a primary healthcare tool to majority of the population in developing countries necessitates the need to validate the folkloric claims of herbal medicines and or medicinal plants so that other alternatives could be found. Drugs prescribed for neuropsychiatric disorders have more side effects than they are efficacious, thus these considerations necessitate the search for new anxiolytic and antidepressant agents that have fast onset of action and present with less side effects.Neuropharmacologicalscreeningof *Lorphiralanceolata*extract has not been established scientifically to the best of our knowledge and this may provide a safer alternative and a justification for continued use of the plant traditionally.

1.3 Research Hypothesis

Methanolic leaf extract of*Lophira Ianceolata*contains bioactive constituents that are useful in the management of some neuropharmacological disorders.

1.4Aim

To evaluate themethanolic extract of the leaf of *Lopihralanceolata*for some neuropharmacological properties in mice

1.5Specific Objectives

i) To conduct preliminary phytochemical screening and determination of the median lethal dose (LD_{50})of themethanolic leaf extract of *Lophiralanceolata*.

ii) To study the anxiolytic, antidepressant and sedative activity of the of methanolic leaf extract of *Lophira lanceolata*.

1.6 Scope and Limitations

The scope will entail some behavioral studies to determinewhether the extract has sedative, anxiolytic as well as antidepressant properties using mice. The studies carried out were diazepam induced sleeping time test, hole board test, beam walk assay test,elevated staircase test, elevated plus maze test, tail suspension test and the forced swim test all in mice.

The limitations of the study include determination of the possible mechanism of action and toxicity studies of the extract.

CHAPTER TWO

2 LITERATURE REVIEW

Neuropharmacology is a branch of neuroscience involving the study of drugs that alter the nervous system and it's functioning, specifically within the brain (Bosari, 2015). The goal of neuropharmacology is to understand the basic functioning of impulses and signals within the brain in order to discover ways in which drugs can be used to treat neurological disorders and drug dependence.

There are basically two branches of neuropharmacology namely: Behavioral neuropharmacology which focuses on ways that drugs affects human mind (very useful in drug dependence and addiction) and molecular neuropharmacology which deals with the study of neurons, neurotransmitters and neuron receptors with the goal of developing new drugs that will treat neurological disorders such as anxiety, depression, psychosis and schizopherenia.

2.1 Anxiety

2.1.1 Definition and epidemiology anxiety

Anxiety, a feeling of apprehension or fear, combined with symptoms of increased sympathetic activity is a normal response to stress. A clinical problem arises only if the anxiety becomes severe or persistent and interferes with everyday performance (WHO, 2012). It can also be defined as a complex progressive behavioral alteration of the organism, which ultimately leads to a wide variety of central nervous system (CNS) disorders, if left untreated. In anxiety states responses to threatening stimuli comprising of defensive behavior, autonomic reflexes, arousal and alertness, corticosteroids secretion and negative emotions occur independent of external events but rather in an anticipatory manner. There is no clear cut distinction between "pathological" and a normal state of anxiety but it represents a point where it

interferes with normal productive activities (Thakur and Rana, 2013; Feyot *et al.,* 2012). Thus by age of 50 years, a quarter of the population are dissatisfied with their sleep, the proportion rising to 30-40 % (two-third of them women) among individuals over 65 years (Bleakley and Sie., 2012).

Anxiety is wide spread, with life-time prevalence rates ranging from 13.6 % to 28 % in Western countries (Thakur and Rana, 2013). Individuals aged between 10 and 25 years are at highest risk of developing anxiety, the prevalence is generally higher in developed countries than in the developing countries (Thakur and Rana, 2013). In any given year, about forty million adults are affected by anxiety disorder; it can also precipitate or aggravate cardiovascular and psychiatric disorders (Weissman *et al*., 1990).

2.1.2 Risk factors for anxiety

The risk factors for the development of an anxiety disorder include, genetic factors, external influences, such as nutrition, smoking, socioeconomic status and environmental conditions (Thakur and Rana, 2013). Females have a 2 fold chances of having anxiety than males. An ongoing stressful event or stress due to other illnesses like cancer. Young children and adolescents are more predisposed than adults, children who endured abuse or trauma or witnessed a traumatic event are at highest risk, although adult also develop post traumatic anxiety disorder.(CDC, 2013; WHO, 2012; Beyond blue, 2015).

Other risk factors include physical health problems like hormonal problems (overactive thyroid, diabetes), asthma and heart disease. Substance use, heavy or long term use of substances such as alcohol, cannabis, amphetamines or sedatives can cause the development of anxiety (Beyond blue, 2015).

Personality factors are also risk factors. Some researchers suggest that people with certain personality traits are more likely to develop anxiety, for example children who are perfectionists, easily flustered,

lack self-esteem or want to control everything sometimes develop anxiety during childhood or as adults (Bleakley and Sie., 2012).

2.1.3 Symptoms of anxiety

Generally the symptoms of anxiety include mental, emotional, physical, behavioral and social symptoms. Of the mental symptoms hyper-sensitivity, confusion, hyper-reactivity, hyper-vigilance, poor co-ordination and concentration and poor memory are notable. Some of the physical symptoms include headache, palpitation, muscles ache, joint pain and rapid breathing. Agitation, confusion, health problems, anger, fear about specific things and memories of general fear for self and loved ones, safety, health and financial security are some of the emotional symptoms of anxiety. Some of the behavioral symptoms are avoidance of performance, avoidance of a feared thing and excessive attention to control or details in order to prevent mistakes, indulging in promiscuity or reckless sex and other reckless behaviors such as excessive spending. Sweating is a physiological symptom (Thakur and Rana, 2013; CDC, 2013; BleaPkley and Sie., 2012).

2.1.4 Pathophysiology of anxiety.

Environmental influences occurring throughout the life span of an individual contributes immensely to the development of an anxiety disorder as this occurs the human body tries to maintain homeostasis. Anything in the environment that disturbs homeostasis is termed a stressor, a homeostatic balance is re-established by physiologic adaptations that occur in response to the stress response. In humans this stress response is under a cascade of hormonal events, including, the release of corticotrophin releasing factor (CRF) which stimulates the release of corticotrophin which subsequently leads to the release of the stress hormones from the adrenal cortex (glucocorticoids and adrenaline). However, the release of (CRF) is decreased via negative feedback to the hypothalamus by glucocorticoids; this stress response is

hardwired into the brain and is most often triggered when the survival of an organism is threatened. The response is not triggered only by physical challenge but also by the anticipation of a homeostatic challenge thus, when humans chronically believe that homeostatic challenge is about to occur they enter the realm of anxiety, neurosis and paranoia. The amygdala is the primary modulator of the response to fear/anxiety inducing stimuli. It registers the significance of a stressful stimuli and creates an emotional memory. It receives input from neurons in the cortex and also sensory inputs that bypasses the cortex which tend to be subconscious.

When activated the amygdala stimulates regions in the midbrain and brainstem, causing autonomicWhen activated the amygdala stimulates regions in the midbrain and brainstem, causing autonomic hyperactivity which correlates well with the physical symptoms of anxiety. Thus, the stress response involves activation of the hypothalamus-pituitary-adrenal axis. This axis is hyperactive in anxiety and depression (Thakur and Rana, 2013 and Bleakley and Sie., 2012).

2.1.5 Neurotransmitters involved in anxiety.

Neurotransmitters are chemicals located and released in the brain to allow an impulse from one nerve cell to pass to another. Approximately 50 neurotransmitters have been identified (Thakur and Rana, 2013). Some of the common neurotransmitters are acetylcholine, dopamine, norepinephrine, serotonin and gamma amino butyric acid (GABA). GABA, serotonin and dopamine are inhibitory while acetylcholine and norepinephrine are excitatory neurotransmitters. The neurotransmitters can directly or indirectly influence neurons in a specific portion of the brain, thereby affecting behavior (Jerald and Liebermann, 1997; Salgueiro*et al.,* 1997). Anxiety is believed to be as a result of chemical imbalances affecting the brain's limbic system which is the seat of human emotions (Health communities, 2015). Low or unbalanced levels of serotonin plays a role in anxiety and this can occur naturally/genetically; it

can also be created by the level of emotions,over production of thyroid hormone has also been linked to the development of severe anxiety and panic attacks. Low levels of GABA may have excitatory properties the put one on edge whereas higher levels have mood bobbling qualities thus GABA has been shown to have a strong association with the development of mood disorders. Noradrenaline is responsible for many of the symptoms of anxiety (Calm Clinic, 2015). Thus low levels, unbalanced levels or high levels of the neurotransmitters implicated in anxiety results in the different types of the disorder.

2.1.6 Types of anxiety disorders.

Anxiety disorders recognized clinically include:

I. Generalized anxiety disorders: This is an ongoing state of excessive anxiety lacking any clear reason or focus.

II. Panic disorders: These are attacks of overwhelming fear occurring in association with marked somatic symptoms such as chest pain, sweating, tachycardia, trembling and choking.

III. Phobias: This comprises of fears of specific things or situations like snakes, open spaces, flying, social interactions e t c.

IV. Post-traumatic stress disorder: This is anxiety triggered by insistent recall of past stressful experience (Thakur and Rana, 2013).

2.1.7 Treatment of anxiety.

Anxiolytic drugs do not cure anxiety but can provide useful short term symptomatic treatment. Before starting symptomatic treatment the primary cause of anxiety should be investigated and treated appropriately where possible. Most anxiety states are more effectively treated in the long term by non-pharmacological interventions including counseling, psychotherapy, behavioral and cognitive methods, relaxation and anxiety training techniques which is a learning process that enable the patient to develop improved stress-coping techniques but they tend to be time consuming, labor intensive and expensive. Antidepressants and psychological methods are more appropriate than anxiolytics in long term treatment for anxiety (Thakur and Rana, 2013).

The ideal anxiolytic should selectively damp down excess activity in the limbic and somatic arousal systems, it should not interfere with learning process and should not produce undue sedation, the onset of action should be rapid and it should be suitable for long term use. No available drug meets this requirement.

The different classes of drugs used in anxiety disorders are:

2.1.7.1*Benzodiazepines*: They are the most commonly prescribed drugs for anxiety. At low doses they possess potent anxiolytic effect with minimal sedation. Their major site of action is the limbic system which is mediated at GABA/benzodiazepine receptors resulting in the enhancement of inhibitory GABA activity. Evidence exist that shows a reduced number of benzodiazepine receptors in key areas of the brain that regulate anxiety response in patients with anxiety disorders (Roy-Byrne, 2005). The choice of an appropriate benzodiazepine depends largely on their pharmacokinetic properties and despite their drawbacks they are very valuable in the short term treatment of anxiety disorders. Examples include alprazolam, lorazepam, clonazepam, diazepam and oxazepam. The benzodiazepines have a rapid onset of action but impair cognitive processes along with undesirable effects if used for a long time. Most

antidepressants have a delayed onset of action but are useful andeffective in most types of anxiety, some of the antidepressants have potentially toxic effects and all are difficult to withdraw. β-blockers can alleviate some of the somatic symptoms of anxiety but have very little effects on subjective symptoms.

2.1.7.2*Antidepressants*: They are now the drugs of first choice in long term anxiety disorders (Lader, 2004).Trials have shown that they are as effective as benzodiazepines in generalized anxiety disorders and most likely more potent in panic disorders, agoraphobia and other forms of phobias, they are of value in anxiety/depression that occurs during benzodiazepine withdrawal or in anxiety associated with depression. They cause little tolerance to anxiolytic effects with little impairment of cognition. They act via inhibition of neurotransmitter reuptake at monoaminergic synapses, thus there is an increase in central serotonergic and noradrenergic activity which may cause further anxiety but this is followed after a time by down regulation of some adrenergic and serotonergic receptors in certain parts of the brain, which accounts for their delayed antidepressant and anxiolytic effects (Bleakley and Sie., 2012).

Their main drawback is their slow onset of action which may take 2-4 weeks and their initial exacerbation of anxiety symptoms or even panic attack with first dose, thus they should be combined with a benzodiazepine initially (2-4 weeks) and should be started at the low dose. They are also toxic in overdose and possess many adverse effects; they cause withdrawal effects if withdrawn abruptly so withdrawal should be gradual with tapering of dose over several weeks.

The different classes of antidepressant are:

2.1.7.2.1*Tricyclic antidepressants*.

This is one of the important classes of drugs employed in the management of anxiety disorders, this class of drugs acts by blocking reuptake of both adrenaline and serotonin at central synapses, they have sedative actions and are beneficial in anxiety states. Examples include Imipramine, clomipramine and amitriptyline. Their sedative action is independent of the anxiolytic effect and is manifested at low doses, early in the treatment this effect is very helpful in anxiety that occurs with insomnia for full anxiolytic effect will only be apparent after some weeks. They can cause dry mouth, blurred vision, constipation, and urinary retention due to their anticholinergic effect. Occasionally arrhythmias and heart block follow their use thus they are contraindicated in the presence of heart disease and should be avoided in the elderly (Thakur and Rana, 2013).

2.1.7.2.2*Selective serotonin reuptake inhibitors (SSRI's)*

These have fewer side effects and less toxic effects as compared to tricyclic antidepressants. They act selectively by inhibiting synaptic serotonin reuptake with little effect on the reuptake of noradrenaline, their main side effects are gastrointestinal and include diarrhea, vomiting, nausea, dyspepsia, abdominal pain, anorexia and weight loss. Examples include paroxetine, sertraline, fluoxetine e t c.

2.1.7.2.3*Serotonin and noradrenaline reuptake inhibitors (SNRIs)*

Valafaxine is licensed for generalized anxiety disorder; it is a non-selective reuptake inhibitor which is effective in non-depressed anxiety patients. It is an extended release preparation with mostly gastrointestinal side effects, drowsiness and sexual dysfunction.

2.1.7.2.4*Monoamine oxidase inhibitors (MAOI)*

The irreversible MAOI (e.g phenelzine) and the reversible MAOI (e.g meclobemide) are sometimes useful in phobic and panic disorders. They have a delayed onset of action with several adverse effects including food-drug interactions and withdrawal reactions thus their use is restricted to only patients that are refractory to other antidepressants.

2.1.7.3*Other anxiolytic drugs*

I. Buspirone: This possesses mixed agonist/antagonist actions at serotonergic receptors that are involved in anxiety. It anxiolytic effects are comparable to those of benzodiazepine but with delayed onset of action of up to 3 weeks. However, it does not have sedative/hypnotic, muscle relaxant or anticonvulsant effects. It does not produce withdrawal effect or dependence and it is only recommended for short time use (Zammit, 2009).

II. β-blockers: Propranolol is of value in acutely stressful situations or in panic attacks where the dominant symptoms are physical; it is used in small doses and does not produce hypotension.

It relieve somatic symptoms such as tremor and palpitations, it is used by actors to avert stage fright and by musicians to avert tremor at concerts. It should be withdrawn gradually if regularly used to avoid rebound tachycardia (Thakur and Rana, 2013).

Antipsychotic drugs example chlorpromazine and haloperidol have been used as anxiolytics but should be avoided because of the risk of inducing dyskinesia and other adverse effects (Lader, 2004).

The most effective long term treatment of anxiety is by psychological methods which can include self-help groups, counseling, behavioral and cognitive techniques, anxiety management training and psychotherapy. All these measures take time to be effective (Bleakley and Sie., 2012). Some selectively new drugs that were originally developed as anticonvulsants with GABAergic action appear to have

anxiolytic effects example gabapentin and pregabalin. These drugs were promising in preliminary trials in social anxiety, pain and generalized anxiety disorder (Ashton and Young, 2003).

2.2 Depression

2.2.1 Definition and epidemiology of depression

Depression is a common mental disorder that present with depressed mood, decreased energy, disturbed sleep or loss of interest or pleasure, feelings of guilt or low self-worth, and poor concentration (Marcus, *et al* ., 2014).

It often comes with symptoms of anxiety. These problems can become recurrent or chronic leading to the individual being unable to take care of his or her every day responsibilities. At its worse depression can lead to suicide (Marcus *et al.*, 2014*).*Depression is the leading cause of disability worldwide. Today, depression is estimated to affect 350million people (WHO, 2012). The world mental health survey conducted in 17 countries found that, on average about 1 in 20 people have been reported to have had an episode of depression in 2011 (WHO, 2012). Almost 1 million lives are lost yearly due to suicide, which translates to about 3000 suicide deaths every day (WHO, 2012).

2.2.2. Risk factors for depression

The risk factors associated with depression include but not restricted to the following:

i) Maternal depression this may be a risk factor for poor growth in children (Rahman *et al,* 2008).

ii) Young age (estimated average age of onset is in mid-twenties)

ii) Stress especially in individuals with genetic predisposition (Banerjee *et al*., 2013).

iv) Chronic diseases.

2.2.3 Etiology of depression

Like most psychiatric disorders the causes of affective disorders are unknown.

In depression, it is likely that genetic, hormonal, biochemical, environmental and social factors all have the same role in the development of the disorder (Banerjee, *et al.,* 2013).

a) Genetic Causes:-There is about 20 % incidence of an affective disorder in First–degree relatives of someone with severe depression; which is almost three times the risk for relatives in the control group (Pratt, 2012).Comparison of the risk of an affective disorder in children of both parents having an affective disorder is four times whereas for one parent having the disorder is double (Pratt, 2012). An evidence for a genetic factor has been found in twins, also genetic link was found in studies of children from parents with affective disorder.

b) Environmental Factors:-Environmental stresses can often be identified prior to an episode of mania or depression but a causal relationship has not been established. However, employment, higher socioeconomic status and the existence of a close and confiding relationship has been noted to offer some protection against the development of episode (Banerjee *et al.,* 2013; Pratt, 2012).

c) Biochemical Factors:- This postulates a deficiency of neurotransmitter amines in certain areas of the brain, this theory was developed to suggest that receptor sensitivity changes may be important. There is an also alternative proposition of a central role of acetylcholine arising from dysregulation of the cholinergic and nor-adrenergic neurotransmitter systems (Banerjee *et al.,* 2013; Pratt, 2012). Though, many neurotransmitters may be implicated, the theory focuses on an involvement of the neurotransmitters nor-adrenaline (norepinephrine) Serotonin (5- hydroxytryptamine) and dopamine.

d) Endocrine Factors:-The endocrine system particularly hypothalamic –pituitary-adrenal axis (HPA) and hypothalamic pituitary-thyroid (HPT) axis, is implic ated in the development of affective disorders. Changes in mood have been associated with some endocrine disorders namely hypothyroidism and Cushing syndrome. There is an increase level of cortisol in patients with depression which supports the fact that mood disorders may be linked to dysfunction within the hypothalamic – pituitary – adrenal axis. This is the basis for the dexamethasone suppression test in depression (Pratt, 2012; Marcus *et al*., 2014).

e) Physical illness and side effects of medications: Disorders of mood have been associated with several types of medication and a number of physical illnesses especially depression (Marcus *et al*., 2014). Examples of the drugs implicated in mood disorders are analgesics, antidepressants, antihypertensive, opiates withdrawal, benzodiazepine withdrawal etc. Examples of illnesses associated with mood disorders are thyroid disease, carcinoma, neurological disorders, diabetes, multiple sclerosis etc.

Guidelines suggest a step care approach to management of depression with an increasing evidence that antidepressant therapy is more likely to be effective in the more severe episodes (Nice, 2006). Depression may contribute to exacerbation of physical problems such as increased pain and worsening outcomes of cardiac disease (Nicholson *et al*., 2006). Also an increase in death cases has been found in patients with co-morbid depression.

2.2.4 Clinical manifestations of depression

The clinical manifestations include: Low mood, loss of interest or pleasure in normally enjoyable activities, low energy, anxiety, sleep disturbances, weight loss, loss of appetite, gastric problems,

nonspecific aches, sexual drive is often reduced, some lose interest in sex altogether (Marcus *et al*., 2014; Pratt, 2012).

2.2.5 Types of depressive syndromes

Depressive syndrome is of two distinct types namely:

i) Unipolar depression: This is a depression in which mood swings are always in the same direction. It is commonly non-familial (about 75 % of cases) and clearly associated with stressful life-events and accompanied by symptoms of anxiety and agitation, this type is sometimes referred to as reactive depression. Other patients (about 25 %) show a familial pattern, unrelated to stressful life events and with a somewhat different symptomatology is termed endogenous depression.

Depressive symptoms have both an emotional and a biological component. The emotional components comprises of misery, apathy, pessimism, low self-esteem, indecisiveness and loss of motivation (Pratt, 2012 and Rang *et al.,* 2003b).

ii) Bipolar affective disorder, in which depression alternates with mania, usually appears in early adult life and it is usually less common. Here there is an oscillating depression with mania over a period of a few weeks, there exist a strong familial tendency but no specific gene or genes have been identified either by genetic linkage studies of affected families or by comparison of affected and non-affected individuals.

The severity of the disorder is rated using various rating scales, two of the most commonly used rating scales are the Beck depression inventory and the Hamilton depression rating scale.

a. Beck depression inventory: this is a self-reporting scale looking at 21 depressive symptoms, the patient is asked to read a series of statements and mark on a scale of 1-4 how severe their symptoms are. The higher the score the more severely depressed a person may be.

b. Hamilton depression rating scale: this is what is employed by healthcare professionals at the end of an interview to rate the severity of the depression (Pratt, 2012).

2.2.6 Treatment of depression

Treatment generally involves two approaches namely: Non pharmacologic strategies as first line intervention especially in mild depression and use of different classes of antidepressants.

The aim of treatment is to relieve distress, to prevent harm and to be prophylactic. However, it is important to differentiate symptoms of the disorder from the premorbid personality. In moderate to severe depression, non-pharmacological therapies along with social, environmental and cultural influences are applicable.

Generally, accurate diagnosis, appropriate doses, adequate duration of therapy and involvement of the patient in the treatment regimen are the cornerstone of effective management of affective disorders.

The severity of depression varies from mild through moderate to severe depression, thus it is inappropriate for people with mild form of the disorder to be seen on specialist basis rather they should be treated by a primary healthcare team in the absence of a risk of serious self-harm.

2.2.6.1 *Tricyclic antidepressants*

Much support has been given to the so-called biochemical theory of depression in the understanding of the pharmacology of antidepressants. Although much data is available on the pharmacology of tricyclic antidepressants, it is still not clear how these drugs relieve the symptoms of depression.

The notion that depression is a simple lack of or imbalance of chemicals has little basis, in fact it merely provides a frame work usefully from which to discuss the benefits and harms of antidepressants. Originally it was thought that their primary effect is as a result of their ability to block the reuptake of noradrenaline (norepinephrine) and/or 5-HT following their release and action as neurotransmitters, these effects occur some weeks before the antidepressant effect indicating clearly there is more to it. Following chronic administration other biochemical changes occur particularly with pre- and post-synaptic receptor sensitivity. Reduction in pre-synaptic α_2-inhibitory receptor sensitivity occurs and this increases the production of noradrenaline (Pratt, 2012).

Other relevant effects are an increase in α_1 and β_1 receptor sensitivity thus it is now factthat these receptor changes in the cerebral cortex and hippocampus may be more relevant to antidepressant response than simple re-uptake inhibition.

A number of tricyclic antidepressants are available in current clinical use, their basic chemical structure is similar but there are differences between them. All tricyclic antidepressants block the reuptake of noradrenaline and 5-HT to a greater or lesser degree. In view of the risks associated with cardiac abnormalities an electrocardiogram (ECG) is advised prior to commencement of therapy with these drugs. Examples of tricyclic antidepressant are imipramine and amitriptyline which are both metabolized by demethylation to an active metabolite; both parent drugs and metabolites have long halve lives. Imipramine is less sedating than amitriptyline, thus can only be used where cardiac tolerability is assured and intentional overdose can be prevented. Clomipramine is one of the first antidepressants found to be a potent 5-HT reuptake inhibitor. Other examples are doxepin, lofepramine, nortriptyline, trimipramine etc.

2.2.6.2 *Monoamine oxidase inhibitors (MAOIs)*

MAOIs are drugs that inhibit the activities of monoamine oxidase enzymes. Two forms of the monoamine oxidase have been found to exist namely monoamine oxidase A and monoamine oxidase B.

Two basic types of MAOIs exist namely the traditional MAOIs which are non- selective and irreversible and selective reversible inhibitor monoamine oxidase type A (example meclobamide). Due to potential food drug interaction in clinical practice the traditional MAOIs are not widely prescribed except if patients are able to tolerate adequate doses particularly those with atypical symptom, they are reserved for use where first line SSRI antidepressants have failed. MAOIs interact with other drugs and tyramine containing foods examples cheese, dairy foods excetra resulting ultimately in hypertensive crisis thus it is important that patients are made aware of the dietary restrictions and potential serious drug interactions.

Although, the inhibitory effect of these drugs on monoamine oxidase is well understood as with other antidepressants it is still not clear exactly how the MAOIs exert their antidepressant effects.

The traditional MAOIs are all non-selective and inhibit both forms of the enzymes, they also have little anticholinergic side effects including dry mouth, constipation and urinary retention examples are phenelzine and tranylcypromine. Reversible inhibitors of monoamine oxidase include meclobamide with less propensity for tyramine rich food interaction but other drug interactions occur so caution should be exercised, it could be considered after a suitable wash-out period as an option if a first or second line SSRI is ineffective (Pratt, 2012)

2.2.6.3 *Selective serotonin reuptake inhibitors (SSRIs)*

These were developed in an attempt to reduce the problems associated with the tricyclic antidepressants. The SSRIs are better tolerated by most patients and are considerably less toxic in overdose, this means

that they should be considered first line choice for the pharmacological management of moderate to severe depression (NICE, 2009).

The degree of specificity for serotonin reuptake differs between the SSRIs but this does not correlate with clinical efficacy. If given in adequate doses and for an adequate period of time all the drugs in this class appears to be equally effective examples include fluvoxamine, fluoxetine, paroxetine, sertraline and citalopram. They possess reduced propensity for interactions with drugs metabolized by the cytochrome $P_{450}2D_6$ iso-enzyme which is an advantage.

2.2.6.4 ***Other antidepressant drugs***

I.Trazodone: Clinically it is thought to operate as a serotonin agonist but *in- vitro* it appears to operate as a mixed serotonin agonist/antagonist. This is a safer drug than the tricyclics following overdoses but causes pronounced sedative and hypertensive effects in some patients. Priapism has also been noted as a rare but distressing side effect, which is probably due to its potent α-receptor blocking properties (Pratt, 2012; NICE, 2009).

II. Mianserin: It is one of the first antidepressants to demonstrate an improved toxicity profile following overdose. It has few anti- muscarinic side effects compared to the tricyclic's but there is the need for a monthly blood monitoring during the first 3 months of treatment due to high reported incidence of blood dyscrasias particularly in the elderly.

III Venlafaxine: is the first reported in a new class of antidepressants, the serotonin-noradrenaline reuptake inhibitors (SNRI's). It is developed in order to improve efficacy over the standard agents. They prevent the reuptake of both serotonin and noradrenaline, a mechanism shared with the tricyclics. It was hoped to have comparable efficacy to tricyclics without any anti-muscarinic effects. However due to highlighted poor tolerability and increased risk of toxicity compared to SSRI's it is not recommended as

first-line treatment for moderate to severe depression according to guidelines for depression (NICE, 2009).

IV Duloxetine: it is also an SNRI, it weakly inhibits dopamine reuptake and may be less well tolerated than the SSRI's. It is considered second-line treatment option considering its relative benefit/tolerability profile.

V Reboxetine: It is a specific noradrenergic reuptake inhibitor (NARI) with response rates similar to other antidepressants. Patient's experiencing problems with serotonergic side effects may benefit from it.

VI Mirtazepine: It is a noradrenergic and specific serotonergic antidepressant (NaSSA). It enhances both noradrenergic and 5-HT_1 serotonergic transmissions. Specific 5-HT_1 neurotransmission is achieved as the drug acts as 5-HT_2 and 5-HT_3 antagonist, this receptor specific reactions may explain the reduction in nausea and sexual dysfunction compared to the SSRI.

VII Agomelatine: This is structurally similar to melatonin; it is thought to act via Melatonin MT_1 and MT_2 as an agonist and antagonist at $5HT_2C$ (Kasper and Hamon, 2009).

It has no effect on monoamine uptake system, as liver dysfunctions have been reported liver function test prior to commencement of therapy is required and at intervals during treatment (Pratt, 2012).

There is increasing evidence that patients with more severe episodes of depression respond well to antidepressant medications than placebo as compared to less severe episodes (Fournier *et al*., 2010). Some evidence also suggests that sertraline and escitalopram may have a more favorable risk/benefit profile than other antidepressants (Cipriani *et al*., 2009). But the magnitude of the difference is not enough to classify them as agents of first choice in all forms of disorder; some generalizations could

help individualize choice of an antidepressant such as males having a better tolerance to migraine than females.

The SSRIs are more tolerated and less toxic in over dose than the tricyclic antidepressants and patients may prefer one drug over the other based solely on the previous experience of benefit or side effects. In summary the main differences between different antidepressants is in cost, side effect profile and toxicity in overdose (Cipriani *et al*., 2009).

In most studies of antidepressants a strong response to placebo has been found thus tolerability is an important factor that determines the choice of drug, this is because patients that experience side effects are likely to discontinue use of the drugs. In order to achieve full response on antidepressant therapy they should be taken in adequate doses for 4-6 weeks and up to 12 weeks in older people (Pratt, 2012).

In patients with a single episode of depression, treatment should be continued for 6 months at the same dose at which remission occurs before attempting withdrawal. However, with patients experiencing multiple depressive episodes the treatment should be for longer period about 2 years. The withdrawal of an antidepressant medication should be carried out gradually so as to avoid withdrawal symptoms that could be experienced by the patients; these symptoms include gastrointestinal symptoms, headache, sweating, giddiness, shaking and insomnia. Also extrapyramidal effects can be experienced with SSRIs antidepressants (Pratt, 2012; Tripathi, 2006a).

Therefore, after a successful treatment, antidepressants should be reduced gradually over a period of 4 weeks; this period can be increased if the treatment is for an extended period and if the patient experienced a problem. Patients on MAOI may experience psychomotor agitation following discontinuation, the long half- life of fluoxetine makes it easier for the drug to be discontinued without

need for tapering and then patients should be warned that there is a risk with abrupt withdrawal of medications.

Some patients respond better to a particular antidepressant which leads to the widely held view that previous response to treatment is a strong indicator for use in future episodes. In addition to all these side effects, contra indication, toxicity in overdose, patients preference and clinician familiarity are other considerations to be made.

Cipriani *et al*., (2009) suggested that setraline and escitalopram should be considered first choice antidepressants for majority of patients but it is still unclear whether it will be translated in to clinical routine practice. The severity of the disorder, patient preference and previous experience should also be considered as they affect outcome.

In clinical practice, the identification of patient at high risk of suicide is difficult and all patients with severe depression should be considered at risk of self-harm and the quantities of medications supplied to them should be carefully monitored.

2.3 Insomnia

2.3.1Definition and epidemiology of insomnia

Insomnia refers to difficulty in failing asleep or staying asleep or lack of refreshment from sleep (Bleakley and Sie., 2012; Pigeon, 2010). It is a term used to describe the presence of disturbed sleep so

that it is characterized by long sleep latency, frequent nocturnal awakenings, or prolonged periods of wakefulness during the sleep period or even frequent transient arousal (Thomas, 2007).

Estimated prevalence depends on the criteria used to define insomnia. The Diagnostic and Statistical Manual of Mental Disorders fourth edition (DSM-IV) gave a prevalence of 6% and it is the most stringent diagnostic criteria(Thomas,2007; American Psychiatric Association, 2000).

2.3.2 Risk factors for insomnia

The risk factors identified by the state of the Science Conference in June 2005 are:

Age and gender (demographic risk factors), increased prevalence in women where there is increased prevalence with menses and menopause.Comorbid medical conditions especially in elderly, working nights and rotating shifts as well as chronic illnesses are an important risk factor for insomnia.Approximately 75-90% of people with insomnia have an increase for comorbid medical conditions such as psychiatric disorders (Thomas, 2007; Pigeon, 2010).

About 40% of all insomnia patients have a coexisting psychiatric conditions and among the psychiatric conditions, depression is the most common.Insomnia is associated with substantial impairments in an individual's quality of life (Pigeon, 2010; Thomas, 2007).

2.3.3 Pathophysiology of insomnia

Insomnia reflects disturbance of arousal and / or sleep system in the brain. These systems are interrelated and their activity determines the degree and type of alertness during wakefulness and the depth and quality of sleep (Ritu and Karl, 2010).

The arousal system is maintained by at least 3 interconnected systems: a general arousal system, an emotional arousal system and an endocrine/autonomic arousal system. The general arousal system is mediated by the midbrain reticular formation, thalamic nuclei and basal forebrain bundle; it serves to link the cerebral cortex with in coming sensory stimuli and provides a tonic influence on cortical reactivity or alertness. Hyper arousal as seen in insomnia is caused by the excessive activity in this system due to internal or external stresses (Bleakley and Sie, 2012; Pigeon, 2010).

The emotional aspects of arousal such as fear and anxiety are contributed by the limbic system which also serves to focus attention on selected aspects of the environment. These arousal systemsactivate somatic responses to arousal such as increased muscle tone, increased sympathetic activity and increased output of anterior and posterior pituitary hormone. Several neurotransmitters have been particularly implicated in the arousal systems.

Acetylcholine is the main neurotransmitter in the arousal system but there is increasing evidence that heightened emotional arousal is associated with increased noradrenergic and serotonergic activity; such that drugs that antagonize such activity have anxiolytic effects. The inhibitory neurotransmitter gamma amino butyric acid (GABA) exerts an inhibitory control on other transmitter pathways and increased GABA activity may have a protective effect against excessive stress reactions.At the other end of the arousal spectrum ,the phenomenon of sleep is actively induced and maintained by neural mechanisms in several brain areas including the lower brainstem pons and parts of the limbic system. These systems

have reciprocal inhibitory connections with the arousal systems so that activation of sleep systems at the same time inhibits waking and vice versa.

In normal sleep there are two distinct levels of consciousness which are promoted from separate neural centers; these are orthodox and paradoxical sleep. About 75% of sleep time is the orthodox sleep and it is divided into four stages (1-4) which merge into each other forming a continuum of decreasing cortical and behavioral arousal, stages 3 and 4 represent the deepest level of sleep termed slow wave sleep and is associated with increasing amounts of high voltage delta slow waves shown on electroencephalograph (EEG).Paradoxical sleep(rapid eye movement sleep REMS) normally takes 25% of sleeping time and it is characterized differently. EEG shows unsynchronized fast activity similar to that found in the alert conscious state and the eye shows rapid jerky movements. Peripheral autonomic activityis increased during REMS and there is an increased output of catecholamines and free fatty acids. In REMS sleep vivid dreams and nightmares most often occur (Bleakley and Sie., 2012; Edewor-Kuponiyi, 2013).

2.3.4 Types of insomnia

There are basically three types of insomnia

1. Transient insomnia: here insomnia could result from changes in routine such as change in time zone, overnight travel, alterations in shift work or a temporary admission in hospital. In this situation a hypnotic with a rapid onset of action, medium duration of action and fewer residual effects could be used on one or two occasions.

2. Short term insomnia: usually from temporary environmental stress. Here a hypnotic may be employed at a low dose for 1-2 weeks only or preferably on alternate nights or one in three nights.

3. Chronic insomnia: this is usually secondary to other conditions whether organic or psychological at which treatment should initially be aimed. However in selected cases a hypnotic may be helpful when prescribed at the minimal effective dose and administered intermittently or temporary (not more than 2-3 weeks). It might be necessary to occasionally repeat the short intermittent dose at an interval of a few months (Tripathi, 2006b; Pigeon, 2010; Thomas, 2007).

2.3.5 Treatment of insomnia

Insomnia is treated pharmacologically and non-pharmacologically or a combination of both. Relaxation, sleep restriction, stimulus control and sleep hygiene are known behavioral therapies for insomnia (Edewor-Kuponiyi, 2013).Before starting symptomatic medication, the primary cause of insomnia should be investigated and appropriately treated where possible. Sleep hygiene,counselling and psychological methods are more appropriate than hypnotics as long term treatment for patients with insomnia (Edewor-Kuponiyi, 2013).

A sedative is a drug that subdues excitement and calms the subjectwithout inducing sleep but drowsiness may be produced, it is also a decreased responsiveness to any level of stimulation and associated with some decrease in motor activity and ideation example benzodiazepines whereas a hypnotic is a drug that induces and / or maintains sleep, similar to normalarousable sleep example barbiturates (Tripathi, 2006b).

Both hypnotics and sedatives are more or less general CNS depressants. An ideal hypnotic should gently suppress the brain arousal systems at the same time activating systems that promote deep and satisfying sleep, thus allowing a return to normal sleep. It should have a rapid onset of action,moderate duration of action and should not have any hangover effects, cause any dependence or tolerance after prolong use or prone to withdrawal symptoms when stopped. It should not depress respiration and

should be safe for use in the elderly patients. Unfortunately no such hypnotic exists; the different classes of hypnotics are as follows:

(a) Benzodiazepines: these are the most prescribed sedatives and there are quite a number of them, they differ only slightly based on their clinical effects but considerably based on their rate of elimination and in potency that is at equivalent dose. Most of them are well absorbed and rapidly penetrate the brain thus producing their effects within 30minutes of oral administration. Their rate of elimination varies considerably with elimination half-lives between 6-100 hours. They undergo hepatic metabolism via oxidation or conjugation and some form a pharmacologically active metabolite with longer half-lives. Their oxidation is decreased in the elderly, patients with hepatic impairment and in the presence of some drugs like alcohol (Bleakley and Sie., 2012; Pigeon, 2010).Benzodiazepines act selectively on gamma amino butyric acid A ($GABA_A$) receptors which mediates fast synaptic transmission throughout the central nervous system, they enhance the response to GABA by facilitating the opening of GABA-activated chloride channels and act specifically to a regulatory site of the receptor, distinct from the GABA binding site and act allosterically to increase the affinity of GABA for the receptor (Rudolph *et al*., 2001; Rang *et al.,* 2003b; Sharma *et al*., 2012).

A single channel recording shows an increase in the frequency of channel opening by a given concentration of GABA,with no change in conductance and mean opening time which is consistent with an effect on GABA binding rather than on the channel gating mechanism.$GABA_A$ receptor has different subtypes distributed in the brain, the benzodiazepines binds to three or more subtypes and it appears that combination with α_2 containing subtypes mediates their anxiolytic effects,α_1 containing subtypes mediates their sedative and amnesic effects and $\alpha_1$as well as α_2 andα_5 their anticonvulsant effects (Rudolph *et al.,* 2001; Rang *et al.,* 2003b).Their pharmacological effects and uses include: reduction of

anxiety and aggression, sedation and induction of sleep, reduction of muscle tone and coordination,anticonvulsant effects and retrograde amnesia.

Their unwanted effects include, toxic effects resulting from overdose, unwanted effects occurring during normal therapeutic use as well as tolerance and dependence. Benzodiazepines are relatively safe in over dosage and this is because they cause prolonged sleep in overdose without serious depression of respiration or cardiovascular functions. This is an advantage as compared to other anxiolytic/hypnotic especially in cases of suicide attempt (Tripathi., 2006b; Bleakley and Sie., 2012; Sharma *et al*., 2012; Rang *et al.,* 2003b). However in the presence of other CNS depressants particularly alcohol they can cause severe respiratory and even life threatening respiratory depression. Another advantage is the availability of an antagonist flumazenil which can be used in acute overdose to counteract their effects.

Their main side effects are drowsiness, confusion, amnesia and impaired coordination which affect manual skills such as driving performance.They also enhance the depressant effects of other drugs in a more than additive way (Foyet*et al*.,2012; Thakur and Rana, 2013).

Tolerance (a gradual escalation of dose needed to produce the required effects) occurs with all benzodiazepines as does dependence which is their main draw back (Rang *et al.,* 2003b). The physical and psychological withdrawal symptoms make it difficult for patients to give up taking benzodiazepines but addiction (that is severe psychological dependence which outlasts the physical withdrawal syndrome) which occur with many drugs of abuse,is not a major problem (Sharma *et al.,* 2012;Rang *et al*., 2003b).

Regular use of benzodiazepines in pregnancy is contraindicated since the drugs are concentrated in fetal tissues where hepatic metabolism is minimal; they can cause neonatal depression, hypotonia and feeding difficulties if given inlate pregnancy. Infants exposed in utero to regular hypnotic maternal doses may

develop withdrawal symptoms 2-3weeks after birth (irritability, crying andmuscles twitches).They enter breast milk as such long acting benzodiazepines are contraindicated in lactation but short to medium acting ones appear to be safe(Bleakley and Sie., 2012).

(b) Non benzodiazepines (Z- drugs):

I.Zopiclone : Acycloyrrolone, is a non- benzodiazepine that binds to benzodiazepine receptors but it is said to be more selective for the α_1-subtype. It has hypnotic effects similar to those of benzodiazepines with the same potential for adverse effects including tolerance, dependence and abstinence effects on withdrawal. Some psychiatric reactions have also been documented shortly after the first dose, including hallucinations, behavioral disturbances and nightmares. It hasless alteration of sleep stages as compared to benzodiazepines, with no other particular advantage over them (Gottesmann, 2002; Bleakley and Sie., 2012).

II. Zolpidem: Is an imidazopyridine that binds preferentially to the α_1 benzodiazepine receptor subunit thought to mediate hypnotic effects; it has a good hypnotic effect with weak anticonvulsant and myorelaxant property. It has a short elimination half-life of about 2hours thus hangover effects are rare but rebound effect may occur in the later part of the night that will cause early morning waking and day time anxiety. Similarly high dose predisposes to psychotic episodes, tolerance and withdrawal effects (Bleakley and Sie., 2012).

III. Zaleplon: is a pyrazolopyrimidinewhich binds selectively to the α_1 benzodiazepine receptor. It is a very effective hypnotic with a very short elimination half-life of 1hour,residual effects on psychomotor and cognitive function after 5hours are minimal with little evidence of tolerance or withdrawal effects and appears to be suitable for use in the elderly(Doble*et al*., 2004)

All Z-drugs are recommended for short term use only (2-3weeks) and they are more expensive than benzodiazepines. Hypnotics generally are contraindicated in patients with acute pulmonary insufficiency, significant respiratory depression, obstructive sleep apnoea or severe hepatic impairment. However, in patients with chronic pain or terminal conditions, suitable analgesics including non-steroidal anti-inflammatory agents or opiates, sometimes combined with neuroleptics provide satisfactory sedation in such patients.The possibility of drug dependence is a less significant issue and regular use of hypnotics with a medium duration of action should not be denied if they provide symptomatic relief of insomnia (Bleakley and Sie., 2012).

(c) Anti-histamines:act via histamine H_1 receptor. Examples include promethazine, diphenhydramine etc.They are sometimes used as sleeping pills for wakeful children(Rang *et al*., 2003b; Edewor-Kuponiyi, 2013; Thakur and Rana, 2013).

(d) Barbiturates: They are another class of hypnotics that are now largely obsolete due to their side effects, tendency towards tolerance and dependence and also due to their ability for drug interactions.

2.4 Traditional Medicine

Traditional medicine as defined by world health organization (WHO) is the sum total of all knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures whether explicable or inexplicable used in the maintenance of health as well as in the prevention, diagnosisand treatment of physical, social and mental illness which may rely exclusivel n past experiences or observations handed down from generation to generation whether verbally or in writing (WHO, 1999).

A medicinal plant is any plant which, in one or more of its organs contains substances that can be used for therapeutic purpose or as a precursor for the synthesis of useful drugs. Herbal medicines on the other

hand are finished and labelled medicinal products that contain as active ingredient aerial or under-ground part of identified and proven plant materials or a combination thereof whether in crude form or as a plant preparations. They include plant juices, gums, fatty oils and essential oils (WHO, 1978).

The use of medicinal and aromatic plants has always been in existence throughout history. We are now witnessing a different approach to their utilization (Abdelmigid, 2013). Eighty percent of the population in low and middle income countries rely on traditional medicines (TM) as part of their primary healthcare need whereas 65% of the population in high income countries utilize complementary alternative medicine (CAM) (Abdelmigid, 2013). WHO in 2002developed its WHO traditional medicine strategy 2002-2005 to provide technical guidance to proper use of traditional medicine/complimentary alternative medicine(TM/CAM) (WHO, 2002). It has four main objectives: policy, safety, quality and efficacy.

Traditional medicine is perceived by public as relatively low risk.They possess beneficial effects but also carry some risks. These potential risks are more recognized as their use increases (Abdelmigid, 2013).

Despite their use for many centuries only relatively small number of plants species have been studied for possible medicinal applications (Jordan *et al.,* 2010; Foyet *et al*., 2012).

Safety and efficacy data are available for even a smaller number of plants, their extracts, active ingredients and preparations containing the plants (Jordan *et al.*, 2010).

Various plants are being used in complementary and alternative medicines for the management of anxiety, these include volatile oils isolated from grapefruit (*Citrus paradise*), lemon (*Citrus limon*), bergamot (*Citrus bergamia*), lime *(Citrus aurantifolia*), mandarin(*Citrus nobilis*) and orange (*Citrus aurantium*) (McGowan, 1998).Skull cap (*Scutellarialaterifolia*) has been demonstrated to have sedative

and anticonvulsant effects, italso lessen alcohol withdrawal syndrome (Martindale, 1995).*Lavendullaofficinalis* (lavender) showed promising results as a CNS depressant, anticonvulsant, sedative and in treating restlessness (Mindell andHandell, 1981).

Jorge *et al*., (1994) studied the antidepressant, anxiolytic and sedative effects of *Hypericumperforatum* (St John's wort*). Panax ginseng* (Ginseng) increase overall body's resistance to day stress and anxiety(Lake,2000). Also studied for their tranquilizing effects on nervous system, anxiolytic andsedative potentials are*Passifloraincarnata* (passion flower)(Morris*et al*.,2003),*Valerian officinalis* (Valerian)(Hibbeln,1998), *Humuluslupulus*(Hops) (Castellanos *et al.,*2000), *Oenatherabiennis* (Evening primrose)(Hibbeln,1998)*Matricariarecutita* (Chamomile)(Byrum,1994),*Piper methystium* (Kava) (Martindale, 1995) and Pinax ginseng (Ginseng) (Lake, 2000).Hypnotic or sleep promoting effects of lavender, lavender oil and linalool have been shown both in animals and human beings (Buchbauer, 2002). Local anesthetic effects of linalool were demonstrated. Lavender flowers are used against states of restlessness, uneasiness, nervousness, difficulties in falling asleep and anxiety. Lavender tea is taken as a sedative and to promote sleep (Schultz *et al.,* 1997). Lavender bath relieves stress of the day and brings about calmness and relaxation. Lavender pillows are recommended for difficulties in falling asleep and for relaxation. Lavender oil is sedative, anti-stress, relaxant and sleep promoter (Buchbauer, 2002a). Studies concerning the influence of essential oils and aromachemicals on the nervous system have been carried out. These studies have provided proof of aromatherapy and more significantly to the efficacy of essential oils or their components in conventional therapy (Buchbauer, 2002a). Lavender oil and its components linalool and linalyl acetate have been shown to have sedative effects on both animals and human beings in a dose dependent manner. In studies using Electroencephalographs (EEC) on the use of inhaled compounds α-wave dominancy refers to a relaxed state and β-wave dominancy to stimulation. Lavender oil, sandalwood oil and apple aroma induced sedation as evidenced by α-wave

dominancy, while jasmine odour increased β-wave activity hence induced stimulation. In an experiment with healthy volunteers R-(-)-, S-(+) and racemic linalool were tested. R-(-) - linalool found in lavender oil and the racemic mixture both showed sedative activity. S-(+)-linalool which is obtained from coriander oil, however, showed the reverse (Sugawara *et al*., 1998).Lavender oil positively affected mood by inducing a less depressed mood, and more relaxed feeling.

Afrormosialaxiflora, *Chenopodiumambrosiodes*, *Microglossapyrifolia* and *Mimosa pudica* are plants used in traditional medicine in Cameroon to treat insomnia, epilepsy, anxiety and agitation. They were evaluated for anxiolytic activity in mice, all the four plants showed anxiolytic activity (Bum *et al*., 2011).From literatures it is known that most herbal medicines that benefit anxiety disorders had effects on the GABA system (Sarris, 2007).The mechanism of the reported action indicate the induction of ionic channel transmission blocking voltage gates or altering membrane structures (Sarris and Kavanagh, 2009).GABA transaminase or glutamic acid decarboxylase inhibition has also been reported (Awad*et al*., 2007). In some cases, the herbal anxiolyticaction was attributed to binding with benzodiazepine receptor site for example the α- subunit (Spinella, 2001).

The increased GABA neurotransmission that subsequently followed had a calming effect on stimulatory pathways, which ultimately provide a psychologically calming effect (Baldwin and Polkinghorn, 2005).

It has been postulated that secondary metabolites may be responsible for the observed pharmacological actions. Flavonoids have been demonstrated to possess a mild sedative effect and a clear anxiolytic activity (Edewor-kuponiyi, 2013). Saponins and flavonoids have been reported to be responsible for sedative activity as well as inhibition of spontaneous motor activity in mice (Won *et al.,* 1980; Dubios*et al.,*1986; Viswanatha*et al.,* 2006; Musa *etal.*, 2006; Yaro *et al*., 2009). Sharma *et al*., (2012) in a systematic updated review of scientifically tested plants used for anxiety reported active compounds

responsible for anxiolytic actions to include a variety of secondary metabolites such as flavonoids, terpanoids, alkaloids and phenols; with terpanoids forming the majority of the reported purified natural anxiolytic compounds (> 42%) and flavonoids (nine compounds) forming the second major group. Other secondary metabolites such as alkaloids (five compounds), phenols (four compounds) and other derivatives were less reported.

Earlier reports on the chemical constituents of plants and their pharmacology suggest that plants containing flavonoids, tannins and saponins possess activity against many CNS disorders (Bhattacharya and Satyan, 1997).

2.4.1 The plant *Lophira lanceolata*(Description and geographical location).

Lophiralanceolata is a species of the genera lophira that belongs to the family Ochnaceae.It is a multipurpose tree. Flowers occur from October to January and fruits are 0.1cm in diameter with one elongated seed and fruiting period is between February to April.The fresh fruit consists of 37% pericarp and 63% kernel, and the whole contains 15.85% fatty substances, of the latter the kernel hold 27%. Composition varies with age. They arenutrious with carbohydrates 31%, proteins 18% and oil 48%. The fatty portion is a creamy yellow semi-solid substance generally known as meni. It is free of smell and is much valued even to preference over shea oil by certain people, it is used for cooking, as hair oil, body embrocation and for soap making. Its composition is a mixture of acids: palmitic 40%, linoleic 30%, behenic 12%, oleic 11% and traces of others.

The plant is widely distributed in the Sudano-guinean savannah zone from Senegal through the African Central Republic and Northernmost DR Congo to Uganda. It also occurs in Cameroon, Zaire, Benin ,Gambia, Ghana ,Nigeria Togo and Mali. The plant is also referred to by a number of names which include *banyun* (Senegal), *fulapulaar* or *manayini* (Gambia),in Nigeria it is termed karehigori (fula

Fulfulde), *koduba* (Nupe=male shea) and*karehigori*(Kanuri)(Burkill, 1997; Abdullahi *et al.,* 2003 and Leandrea*et al*., 2013).

However, the species exist in the dryer savannah zone with its counterpart in the tropical rain forest *Lophiraalata*, in particular there has been difficulty in distinguishing between their timbers and their uses are mainly similar subject to limitation of size and irregularity of *lanceolata* andthe enormouslylarger statue of *L. alata*. Medicinal uses seem to be largely interchangeable *mutatis mutandis* according to availability (Burkill, 1997).

Plate 1: *Lophiralanceolata* Tree in it's natural habitat

2.4.2 Ethnomedicinal uses

The plant has a wide range of uses in African traditional medicine; the decoction of the fresh leaves is administered orally against headaches, dysentery, diarrhea, dermatosis, toothache and muscular tiredness (Burkill, 1997).

In Mali powdered roots mixed with flour are used to treat constipation while it's decoction is used to cure chronic wounds.A concoction prepared from the roots is drunk by women against menstrual pain, intestinal problems and Malaria.The bark of the roots and trunk is used against pulmonary diseases. The bark is used to treat fevers and gastrointestinal problems and in Southern Nigeria the root bark is a remedy for yellow fever (Burkill, 2000).

Young stem and sometimes roots are used as chewing stick while an infusion of the bark is used as mouthwash in Guinea, Mali and Nigeria (Onyeto*et al.,* 2014; Leandrea*et al.,* 2013 and Burkill, 1997).Powdered leaves and stem bark decoction is use in epilepsy, as an anxiolytic and sedative (personal communication with a herbalist BabanIya of Rimi market Kano Nigeria).

2.4.3 Phytochemical screening

Preliminary phytochemical screening of themethanolic leaf extract revealed the presence of flavonoids, alkaloids, oils, saponins, glycosides, carbohydrates, acidic compounds, terpenoids and reducing sugars (Onyeto*et al*.,2014; Audu *et al*., 2007).

Phytochemical analysis of the bark has shown the presence of several flavonoids with some ibacterial and antiviral activity, they include a group of related bioflavonoids called lophirones A-J, the bioflavonoids isombami-chalcone and the tetraflavonoidslanceochalcone(Lohlum*et al.,*2010; Van der Vossen and Mkamilo, 2007).

The wood contains the nitrile glycoside esters lanceolin A and B while the leaves contains lanceolatin A and B and in addition to the benzoyl glycoside lanceoside A and prenylatedisoflavonoidslanceolone. The seeds were found to contain all amino acids except tryptophan.Mineral content include sodium, potassium, calcium, magnesium, zinc, iron and phosphorus (Lohlum *et al.,* 2010).

2.4.4 Previous works on extracts of *Lophiralanceolata*

A study conducted byPengyeub*et al.*,(1994) on the stem bark of*Lophiralanceolata*showed different types of flavonoids some of which possess antibacterial and antiviral activities. The leaves when studied showed the presence of minor bioflavonoids constituents as lanceolata A and B which were isolated and characterized. Aqueous extract of the stem bark studied in rats showed an increase in sperm count (Etuk and Muhammad, 2009).

Oral intake of the aqueous stem bark extract was found to be relatively safe with an associated increase in body weight and some hematological parameters with no acute toxicity (Etuk and Muhammad, 2010).

However repeated administration of high doses of the extract may result in testicular damage which can lead to infertility (Etuk and Muhammad,2010). Also Betulinic acid was isolated from the leaves (Ali*et al*., 2011).

Antimicrobial properties of the aqueous leaf extract was studied by Ali*et al*., (2011).The hypotensive effects of aqueous decoction of the leaves was studied on arterial blood pressure andelectrocardiogram in anesthetized rabbits(Leandrae*et al*., 2013) It was also shown to contain benzamide at a concentration of 0.05% which possess anti-depressant activity (Burkill,1997).

CHAPTER THREE

3MATERIALSAND METHODS

3.1Collection and Identification of Plant material

The leaves of *Lophiralanceolata* were collected in November, 2014 at Kajewa Village of Takai Local Government Area, Kano State Nigeria. The plant was identified and authenticated in the herberium of the Biological Sciences Department,Bayero University, Kano byMalamBahauddeenSai'd Adam by comparing with a voucher specimen number Bukhan 0300.

3.2 Experimental Animals.

Swiss albino mice of both sexes weighing 16-37g were obtained from the Department of Pharmacology, Faculty of Clinical Sciences, Bayero University, Kano. The animals were allowed free access to standard feed and water *ad libitum*. They were kept in clean cages with saw dust as beddings which were replaced every three days. The study was conducted in accordance with National Institute of Health Revised Guidelines for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, 1996).

3.3Chemicals and Drugs.

The following drugs and chemicals were used methanol(Sigma-Aldreich), normal saline, distilled water, diazepam injection (Roche), Tabs imipramine (Tofranil), Fluoxetine (Flutex) capsules.The chemicals used for the phytochemical screening were obtained from the Department of Biochemistry,Bayero University, Kano. All reagents are of analytical grade.

3.4 Materials and Equipment's.

The following materials and equipments were used for the study digital weighing balance, motar and pestle, syringes (2ml,1ml,5ml),masking tape, marker, duster, hand gloves, sample bottles, stop watch,

video recorder, filter paper, funnel, conical flask, beaker, measuring cylinder, thermometer, laboratory record book, beam walk apparatus, hole board, elevated staircase apparatus, elevated plus maze apparatus, desiccator, tail suspension apparatus and forced swim apparatus.

3.5 Preparation of Plant Extract.

The leaves of *Lophiralanceolata* were collected and air dried under shade to constant weight and then powdered using mortar and pestle. About 700g of the powdered material was cold macerated and extracted to exhausion using a mixture of methanol (70%) 3.5L and water (30%) 1.5Las solvent with occasionalstirring. It was then filtered using no 1 Whatman filter paper. The solvent was evaporated on a water bath at a regulated temperature of 40°C. It was further dried in a dessicator and the resultant dried extract was weighed and the percentage yield determined. It was preserved in an air tight container ready for future use.

3.6 Phytochemical Screening.

The phytochemical screening was carried out on the dried extract to detect the presence of alkaloids, saponins, tannins, flavonoids, triterpanoids, steroids, reducing sugars, phlobatannins and cardiac glycosides using standard protocol (Trease and Evan's, 2002; Brain and Turner, 1975 and Abba *et al*., 2009).

3.6.1 Test for saponins (Frothing test).

Five hundred milligram (500 mg) of the extract was dissolved in 10ml of distilledwater, it was then shaken vigorously for 30 seconds and was allowed to stand for 30minutes. The appearance of froth that lasts for more than 15minutes indicates the presence of saponins (Brain and Turner,1975).

3.6.2 Test for alkaloids (Dragendoff's test).

To 0.5g of the extract and 20ml dilute hydrochloric acid was added in a conical flask. This was heated on a water bath and then filtered. The filtrate was made alkaline with diluteammonia solution and then extracted with chloroform ($3 \times 10cm^3$).The combined $CHCl_3$ extracts was concentrated and treated with equal volume of 1% HCl,2ml of Dragendoff's, Mayer's and Wagner's reagents were added. The occurrence of an orange-red and cream precipitate indicates the presence of alkaloids(Trease and Evan's,2002).

3.6.3 Test for tannins.

Five hundred milligram (500 mg) of extract was dissolved in 10ml of distilled water and the filtered. A few drops of ferric chloride were added to the filterate.The formation of a blue-black precipitate indicates the presence of hydrolysable tannins and green precipitate indicates the presence of condensed tannins(Trease and Evan's, 2002).

3.6.4 Test for phlobatannins.

Five hundred milligram (500mg) of the aqueous portion of the plant extract and 1% of aqueous hydrochloric acid was added and boiled. The formation of a red precipitate indicates the presence of phlobatannins.

3.6.5 Test for steroids and triterpenes (Liebermann-Burchard's test).

Five hundred milligram (500 mg) of the extract was dissolved in 5ml of chloroform and filtered. To the filtrate 5ml of acetic anhydride was added, then 1ml of concentrated sulphuric acid was added down the side of the tube and the color change was observed immediately or later. Appearance of red, pink or purple colour indicates presence of triterpenes while blue or blue-green indicates steroids (Trease and Evan's, 2002).

3.6.6 Test for flavonoids (Shinoda test).

Five hundred milligram (500 mg) of the extract was dissolved in 5ml of 50% methanol and warmed on a steam bath. Three pieces of metallic magnesium ribbons and 5 drops of concentrated hydrochloric acid were then added. A red or orange colour indicates the presence of flavonoids (Abba *et al.*, 2009).

3.6.7 Test for cardiac glycosides (Keller-Kiliani's test).`

To 5ml of the extract, 3ml of glacial acetic acid was added with one drop of ferric chloride. The mixture was kept for 1minute and then transferred to a test tube. 1.5ml of sulphuric acid was added with a pipette such that it runs down the wall of the tube and forms a separate layer at the bottom. On standing, a brown colour at the interface indicates the presence of deoxy sugars and a pale green in the upper layer indicates the presence of asteroid nucleus (Brain and Turner, 1975).

3.6.8 Test for reducing sugars.

One milliliter each of Fehling's solutions I and II were added to 2ml of the aqueous solution of the extract. The mixture was then heated in a boiling water bath for about 5 minutes. The production of a brick red precipitate indicates the presence of reducing sugars.

3.7 Acute Toxicity Studies.

The LD_{50} was determined using Lorkes method (1983).According to this method the procedure was divided into two phases. In phase one nine mice were divided into three groups of three miceeach and administered the extract at doses of 10,100 and 1000 mg/kg body weight intraperitonially. They were observed for signs and symptoms of toxicity including death for 4hours then for the rest of24 hours.In the second phase specific doses were administered based on the result of the first phase. The LD_{50} was calculated as the geometric mean of the smallest lethal dose and the highest non-lethal dose of the extract.

3.8 Neuropharmacological Studies.

3.8.1 Diazepam induced sleeping time in mice

The method of Rakotonirina*et al.,*(2001)was used for this study. The sleep potentiation time of the extract was assayed in 24 mice that were randomly grouped into four with six mice per test group.GroupI-III received the extract at doses of 87.5, 175 and350 mg/kg *(ip*) respectively, while group IV received normal saline at a dose of 10ml/kg and served as control. Thirty minutes after administration, mice in all the groupsreceived diazepam (DZ) at a dose of 25mg/kg body weight. The onset and duration of sleep were determined for each animal, loss of righting reflex was considered as the criterion for sleep (Rolland *et al*., 1991) while the interval between the loss and recovery of righting reflex was regarded as the duration of sleep (Fujimori,1965).

3.8.2Hole board test in mice.

The hole board experiment is the measure of exploratory behavior in animals. The experiment was conducted using wooden apparatus of dimension 40×40 cm with 16 evenly spaced holes (Perez *et al*., 1998)**.**Mice were divided into five groups of six each. Groups I and V received normal saline at a dose of 10 ml/kg and diazepam at 2 mg/kg body weight intraperitonially respectively. Group II-IV received 87.5, 175 and 350 mg/kg body weight of the extract. Thirtyminutes post treatment the mice weresingly placed on the board and the number of head dips into the holes during a 5 minutes period was recorded.

3.8.3 Beam walk assay (motor co-ordination) in mice.

The beam was made of wood 8mm in diameter, 60cm long and elevated 30cm above the bench by metal supports. The mice were trained to walk from a start platform along a ruler (80cm long, 3cm wide) elevated 30cm above the bench by metal support to a goal box. Three trials were performed for each mouse. The mice that have successfully walked along the ruler were randomly divided into 5 groups of six mice each.Group I received normal saline at a dose of 10ml/kg body weight whereas those in groups II-IV received 87.5, 175 and 350 mg/kg of the extract(*ip*) respectively and lastly group V received diazepam 2mg/kg body weight(*ip)*. Thirty minutes post treatment, each mouse was placed at one end and allowed to walk to the goal box, those that fell were returned to the position they fell from within an allowable period of 60seconds. The number of foot slips was recorded (Stanley *et al*., 2005).

3.9Anxiolytic Studies.

3.9.1 Elevated staircase test.

The staircase test was carried out in accordance with the method of Simiand*et al*.,(1984).It consist of placing an experimentally naïve mouse in an enclose staircase with five steps (2.5×10×7.5cm).The apparatus was 45cm in length, with one end 12cm and the other 25cm in height. Five groups of six mice each were used for the experiment, the first three groups were treated with 87.5, 175 and 350 mg/kg body weight doses of the extract (*ip)* and the fourth and fifth groups received diazepam 0.25mg/kg *ip* and normal saline10ml/kg respectively. After 30minutes of extract administration each mouse was placed individually on the floor of the box with its back to the staircase and then the behavior was videotaped, the number of stairs climbed and rearing was counted for 3minutes.A step is considered climbed only if the mouse placed all the four paws on the steps. Rearing was recorded when the mouse rose on its hind legs either on the steps or against the wall to sniff the air. The number of steps descended was not counted.

3.9.2 Elevated plus maze(EPM)test in mice.

The elevated plus (EPM) consists of two open arms(35×5cm)crossed with two closed arms(35×5×20cm).The arms are connected together with a central square of 5×5 cm. The apparatus was elevated to a height of 25 cm in a dimly illuminated room. Mice in groups I-III were treated with 87.5, 175 and 350 mg/kg body weight doses of the extract and then the remaining two groups (IV and V) were treated with diazepam (0.5mg/kg,*ip*) and normal saline 10ml/kg thirty minutes prior to being placed individually at the center of the EPM, facing the closed arm. The time spent in both the open and close arms were recorded over a period of 5minutes.The number of entries into the open and close arms were

also counted during the test. An entry was defined as having all four paws within the arm (Pellow *et al*., 1985; Hogg, 1996;Adeyemi*et al*., 2006).

3.10Antidepressant Study.

3.10.1 Tail suspension test in mice

The study was carried out as described by Steru*et al*.,(1985). Adult mice of either sex were randomly selected and divided into five groups of six mice each. Groups I, II and III received 87.5, 175 and 350 mg/kg body weight doses of the extract (I.P*)* whereas mice in the 2 control groups (IV and V) received imipramine (2mg/kg,I.P) and normal saline (10 ml/kg) respectively. After thirty minutes each mouse was suspended by the tail on the edge of a shelve 58cm above a table top and the duration of their immobility recorded during a 6 minutes period, after discarding activity in the first 2minutes,during which the animal will try to escape. A mouse was considered immobile only when it hung passively and remained motionless.

3.10.2 Forced swim test.

Mice of either sex were individually forced to swim in an open cylindrical container (diameter 12cm, height 20 cm),containing 15 cm of water at 25± 1°C. The mice were trained 24hours prior to the test period each for a swimming duration of 10minutes. These mice were randomly grouped into six groups of six mice each. The first group served as control and received normal saline at 10ml/kg whereas groups II-IVreceived 87.5, 175 and 350 mg/kg body weight doses of the extract(I.P) and lastly the fifth and sixth groups received the standard drugs imipramine and fluoxetine at 2mg/kg and 20mg/kg respectively (Porsolt*et al*.,1978).

The total duration of immobility was recorded during the last 4 minutes of the 6 minutes test period. Each mouse was judged to be immobile if it ceases struggling and remains floating motionless in the water, making only movementsnecessary to keep its head above water. A decrease in the duration of immobility shows an antidepressant like effect (Berton and Nestler, 2006).

3.11 Statistical Analysis

The results were presented as mean ±SEM.Statistical analysis of variance was done using one way ANOVA and Student t-test, values of $P \leq 0.05$ wereconsidered significant.

CHAPTER FOUR

4 RESULTS

4.1 Phytochemical Screening.

The phytochemical analyses of the methanolic leaf extract of *Lophiralanceolata* gave positive results for saponins, tannins, flavonoids, alkaloids, cardiac glycosides,phlabotannins,triterpenes and reducing sugars whereas negative result was obtained for steroids (Table 1).

Table 1: Phytochemical Constituents of Methanolic Leaf Extract of *Lophiralanceolata.*

Constituents	Inference
Phlabotannins	+
Saponins	+
Tannins	+
Flavonoids	+
Steroids	–
Alkaloids	+
Cardiac glycosides	+
Reducing sugars	+
Triterpenes	+

Key:

+	Present
–	Absent

4.2 Percentage Yield

The percentage yield was determined to be 18.07%

4.3 Acute Toxicity Studies.

The intraperitonial median lethal dose (LD_{50}) value of the methanolic leaf extract of *Lophiralanceolata* in mice was calculated to be 1,131.9 mg/kg body weight.

4.4 Behavioral Studies

4.5.1 Effect of methanolic leaf extract of *Lophiralanceolata* on diazepam induced sleeping time inmice.

The methanolic leaf extract of *Lophiralanceolata* did not affect the onset of sleep significantly as compared to the control. The time of onset increased from 2.8 ± 1.2 minutes in the control groupto23.7 ± 5.7, 12.7 ± 4.4 and 11.2 ± 5.7 minutes for the extract at 87.5, 175 and 350 mg/kg body weight respectively (Fig 1). The duration of sleep was also not affected significantly as compared to the control group. The duration of sleep in the control group was 211.0 ± 69.5 minutes but increased insignificantly to 227.0 ± 104, 277.7 ± 82.6 and 279.3 ± 76.3 minutes with the extract at doses of 87.5, 175 and 350 mg/kg body weight (Table 2) $P > 0.05$.

Table 2: Effect of Methanolic Leaf Extract of *Lophira lanceolata* on Diazepam Induced Sleeping Time in Mice.

Treatment Dose(mg/kg)	Onset of Sleep (mins)	Duration sleep (mins)
Normal saline 10ml /kg	2.83 ± 1.2	211.0 ± 69.5
Extract 87.5	23.7 ± 5.6	227 ± 104
Extract 175	12.7 ± 4.4	277.7 ± 82.6
Extract 350	11.2 ± 5.7	279.3 ± 76.3

n = 6, Data presented as means ± SEM, $P > 0.05$ compared to control, one way ANOVA followed by student's t-test.

4.5.2. Effect of methanolic leaf extract of *Lophiralanceolata* on hole board test (exploratory behavior) in mice.

*Lophira lanceolata*methanolic leaf extract at the doses of 175 and 350 mg/kg exhibited an increase in the number of head dips in the mice. There was a significant ($P < 0.05$) increase at the highest dose of 350 mg/kg similarly diazepam significantly increased the number of head dips in this study (Fig. 1) ($p < 0.05$).

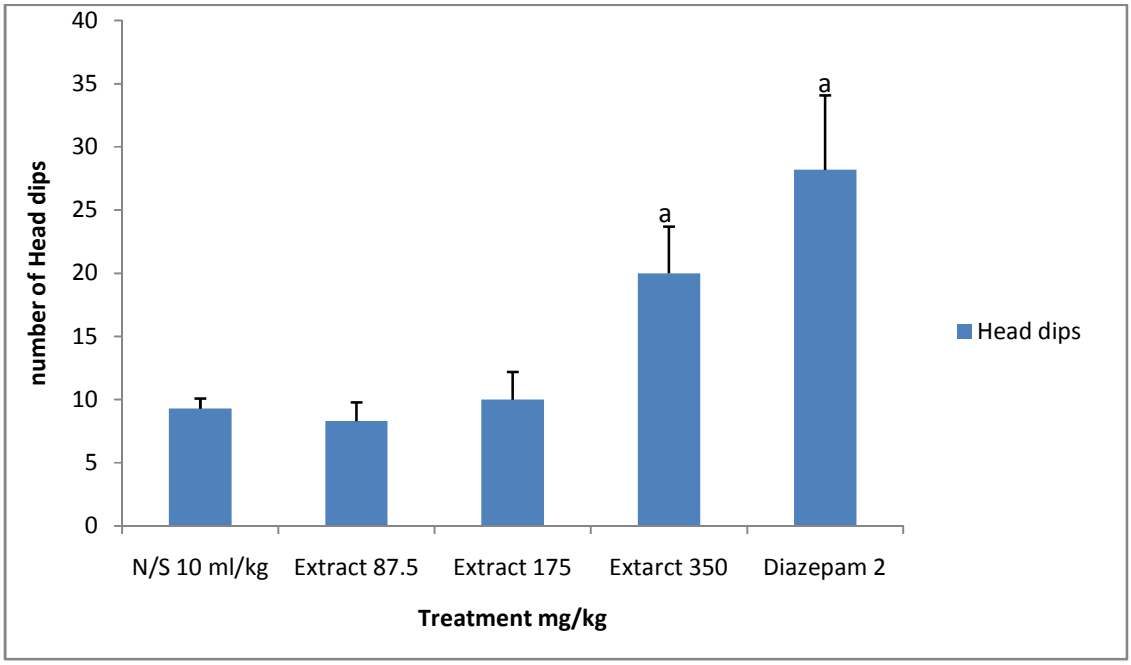

Fig. 1 Effect of Methanolic Leaf Extract of *Lophira lanceolata* on Hole Board Test in Mice

n = 6. Data presented as means ± SEM, a = $p< 0.05$ as compared to the control, one way ANOVA followed by student's t-test.

4.5.3 Effect of methanolic leaf extract of *Lophiralanceolata* on motor co-ordination (beam walk assay) in mice.

The methanolic extract of *Lophiralanceolata* showed no significanteffect in the number of foot slips and the duration of beam walk as compared to the control at the doses tested. However, the extract exhibited a non-significant increase in the number of foot slips in a dose dependent manner (Table 3). Diazepam was observed to show a significant ($P < 0.05$) increasein number of foot slips (Table 3).

Table 3: Effect of Methanolic Leaf Extract of *Lophiralanceolata* on Motor Co-ordination in Mice (Beam Walk).

Treatment (mg/kg)	No. of Foot Slips	Duration of beam walk (sec)
Normal Saline 10ml / kg	3.0 ± 0.8	27.8 ± 5.5
Extract 87.5	3.0 ± 0.7	31.0 ± 8.0
Extract 175	4.8 ± 0.8	26.0 ± 4.3
Extract 350	5.2 ± 1.1	42.2 ± 10.7
Diazepam 2	8.3 ± 1.4 [a]	26.0 ± 7.0

n = 6. Data presented as means ± SEM, a = P 0.05, as compared to control,one way ANOVA followed by student's t-test.

4.5.4Effect of methanolic leaf extract of *Lophiralanceolata* on the elevated staircase test in mice.

The methanolic extract of *Lophiralanceolata* showed a significant (P< 0.005) dose dependent decrease in the number of stairs climbed and P< 0.0001 decrease in the number of rearing compared to the control group (Table 4). There was no significant change in the mice treated with diazepam (0.25 mg/kg) on the stair climbing parameter. The extract at all the doses tested showed a more potent anxiolytic effect than diazepam (Table 4).

Table 4: Effect of Methanolic Leaf Extract of *Lophiralanceolata* on the Elevated Staircase Test in Mice.

Treatment (mg/kg)	No of Stairs climbed (counts / 3mins)	No of Rearing (Counts/ 3mins)
Normal saline 10ml / kg	11.2 ± 2.5	10.8 ± 2.1
Extract 87.5	4.7 ± 1.7[a]	1.5 ± 0.9[b]
Extract 175	3.0 ± 1.4[a]	1.5 ± 0.7[b]
Extract 350	1.3 ± 0.9[a]	1.0 ± 0.5[b]
Diazepam 0.25	9.0 ± 2.0	5.3 ± 1.4[a]

n= 6. Data presented as means ± SEM, a = P< 0.005, b = P< 0.0001, as compared to control, one way ANOVA followed by student's t- test.

4.5.5 Effect of the methanolic leaf extract of *Lophiralanceolata* on the elevated plus maze in mice.

The methanolic leaf extract of *Lophiralanceolata* at the tested doses did not show any significant effect on both number of entry and time spent in the open arm of the elevated plus maze (Table 5). But as expected, diazepam significantly affected the number of entry as well as the time spent in the open arm ($P< 0.05$) (Table 5).

Table 5: Effect of Methanolic Leaf Extract of *Lophiralanceolata* on Elevated Plus Maze in Mice.

Treatment (mg/kg)	No of Closed arm Entry	Time spent in the enclosed arm(Sec)	No of open arm entry	Time spent in the opened arm (Sec)
Normal Saline 10ml/kg	7.5 ± 0.6	299.2 ± 0.8	0.2 ± 0.2	0.8 ± 0.8
Extract 40	6.7 ± 1.7	298 ± 1.5	0.3 ± 0.2	1.8 ± 1.5
Extract 87.5	4.8 ± 2.5	297.7 ± 2.3	0.2 ± 0.2	2.3 ± 2.3
Extract 175	6.0 ± 2.4	286.7 ± 13.3	0.2 ± 0.2	13.3 ± 13.3
Extract 350	8.8 ± 1.4	300 ± 0.0	0.0 0.0	0.8 ± 0.8
Diazepam 0.5	8.0 ± 2.2	256.7 ± 16.5	0.8 ± 0.3[a]	43.3 ± 16.1[a]

n = 6. Data presented as means ± SEM, a = $P < 0.05$ as compared to control, one way ANOVA followed by student's t – test

4.6 Antidepressant studies

4.6.1 Effect of methanolic leaf extract of *Lophiralanceolata* on tail suspension test in mice.

There was a significant decrease in the activity counts (immobility time) at the doses of 87.5 and 350 mg/kg body weight ($P< 0.0001$ and $P< 0.05$) respectivelyas compared to the control group. Imipramine at a dose of 2 mg/kg significantly reduced immobility time as compared to the control group ($P < 0.0001$) (Table 6)

Table 6: Effect of Methanolic LeafExtract of *Lophiralanceolata* in Tail Suspension Test in Mice.

Treatment (mg/kg)	Immobility Time (sec)
Normal saline 10ml/kg	140.7 ± 4.91
Extract 87.5	51.0 ± 4.09 [a]
Extract 175	125.2 ± 8.2
Extract 350	116.8 ± 8.1[b]
Imipramine 2	16.0 ± 4.0[a]

n = 6. Data presented as means ± SEM, a = $P < 0.0001$, b = $P< 0.05$ as compared to the control, one wayANOVA followed bystudent's t- test.

4.6.2 Effect of methanolic leaf extract of *Lophiralanceolata* in forced swim test in mice

The extract at all the doses tested 87.5, 175 and 350 mg/kg body weight did not show any significant effect as compared to the control on immobility, swimming and climbing parameters. Similarly Fluoxetine did not show any significant effect on immobility, swimming and climbing as compared to the control. However, imipramine showed a significant decrease in immobility ($P< 0.05$).

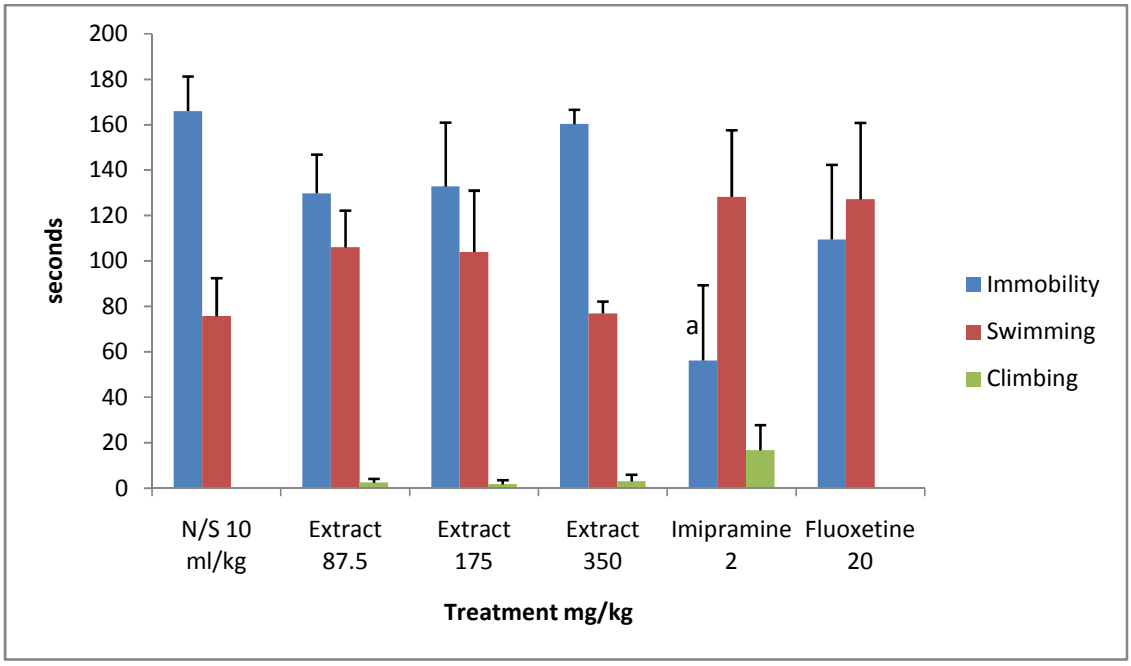

Fig 2: Effect of Methanolic Leaf Extract of *Lophira lanceolata* on Forced Swim Test in Mice.
n = 6, values are presented as means ± SEM, a = $P< 0.05$ as compared to control, one way ANOVA followed by student's t-test.

4.7DISCUSSION`

Plants are a rich source of ecologically developed secondary metabolites which are potential remedies for different ailments of the plant. And it is believed that these same constituents termed phytochemical compounds (which can be contained in any part of the plant example leaf, bark, root e.t.c) can initiate the same curative actions in humans.

The preliminary phytochemical screening of *Lophiralanceolata*methanolic leaf extract revealed the presence of alkaloids, saponins, tannins, flavonoids, glycosides, reducing sugars, phlabotannins and triterpenes whereas steroids were found to be absent. These secondary metabolites may be responsible for the observed pharmacological actions. Flavonoids have been demonstrated to possess a mild sedative effect and a clear anxiolytic activity (Edewor-kuponiyi, 2013). Saponins and flavonoids have been reported to be responsible for sedative activity as well as inhibition of spontaneous motor activity in mice (Won *et al.,* 1980; Dubios*et al.,* 1986; Viswanatha*et al.,* 2006; Musa *etal.*, 2006; Yaro *et al*., 2009). Earlier reports on the chemical constituents of plants and their pharmacology suggest that plants containing flavonoids, tannins and saponins possess activity against many CNS disorders (Bhattacharya and Satyan, 1997). Studies reported by Onyeto *et al*, 2014; Audu *etal,* 2007; Burkill, 1997 shows the presence of steroids which was found to be absent in this study this could be due the differences in the type of solvent employed in the extraction process and the different geographical locations of the plant.

A dose estimate for acute toxicity is the LD_{50}. It is used to assess toxicity in the target tissue; it thus gives a relative idea of how toxic a substance is. According to a scale proposed by Lorke, (1983), substances can be classified as very toxic (LD_{50}< 1.0 mg/kg), toxic (upto 10 mg/kg), less toxic (up to 100 mg/kg), slightly toxic (1000 mg/kg) or non- toxic (LD_{50}> 5000 mg/kg). The effects thatwere observed during acute toxicity studies of the methanolic leaf extract of *Lophira laceolata* were

hypoactivity and hind limbs distension . The intra-peritoneal median LD_{50}value for the methanolic leaf extract of *Lophiralanceolata* in mice was found to be 1,131.9 mg/kg. This shows that the extract was slightly toxic by this route.

Mansur *et al*., (1971) stated that reduced excitability of the central nervous system is typified by a decrease in spontaneous motor activity; this could lead to sedation (Ozturk *et al*., 1996). Pentobarbitone induced sleeping time potentiation is the test usually employed to elucidate the hypnotic and sedative properties of drugs. CNS stimulants and analeptics shortened pentobarbitone sleeping time whereas neuroleptics as well as antidepressants also prolong pentobarbitone sleeping time (Vogel and Vogel, 1997). However these compounds can promote pharmacokinetic changes due to interaction with the cytochrome P_{450} complex, an activity that can promote an enhancement of the central depressant effect of barbiturates and consequently produce false positive results to compounds devoid of this property (Duarte, *et al.,* 2007). In this study a modified method that utilizes diazepam instead of pentobarbitone as described by Rakotonirina *et al*., (2001) was employed.Diazepam a benzodiazepine act selectively on gamma amino butyric acid A ($GABA_A$) receptors which mediates fast synaptic transmission throughout the central nervous system. It enhances the response to GABA by facilitating the opening of GABA-activated chloride channels and act specifically to a regulatory site of the receptor, distinct from the GABA binding site and act allosterically to increase the affinity of GABA for the receptor (Rudolph *et al*., 2001; Rang *et al.,* 2003b; Sharma *et al*., 2012). However, substances or extracts that potentiate diazepam induced sleeping time are thought to act via the same mechanism. In this study the methanolic leaf extract of *Lophira lanceolata*did not significantly affect the onset of sleep and the duration of sleep in mice at the doses tested. Thus it can be inferred that the extract at the doses tested does not possess sedative activity.

The hole board experiment is ameasure of exploratory behavior in animals. Head dipping into a hole is a typical behavior of the mice indicating a certain degree of curiosity (File and Wardil, 1975). An agent that decreases this parameter reveals a sedative property (File and Pellow, 1985).The neuropharmacological activity was demonstrated by the significant increase ($P < 0.05$) compared with the control in the exploratory behavior in head dip test treated with extract (350 mg/kg body weight) showing no sedative activity at the doses tested but an anxiolytic activity. This is in line with an earlier work reported by (Archana, 2013; Roy *et al.,* 2015) where the anxiolytic state was shown as an increase in the number nose poking behavior. Thus, the methanolic leaf extract of *Lophira lanceolata* at the doses tested did not show any sedative activity but an anxiolytic property as typified by increase in the nose poking behavior.

The methanolic leaf extract of *Lophiralanceolata* did not show any observable effect on motor co-ordination in the beam walk assay test when compared to the control in the protocol studied, suggesting that the insignificant decreased locomotor action observed may not be exerted through peripheral neuromuscular blockade or centrally mediated impairment of motor function (Adzu*et al.,* 2002). Stanley *et al*., (2005) reported that the number of foot slips made by the mice is a measure of motor co-ordination deficit which was also found to be a sensitive measure at determining benzodiazepine induced motor co-ordination deficit.

Now, there are impressive array of natural products that are known to influence the functions of inotropic receptors for GABA, the major inhibitoryneurotransmitter in the brain (Johntson*et al.,* 2006). These substances exert their anxiolytic action by allosterically enhancing the actions of GABA at $GABA_A$ receptor via benzodiazepine–binding site (Richter *et al.,* 2012). The GABAergic system and serotoninergic neurotransmission are involved in anxiety. In addition, selective serotonin reuptake inhibitors are effective in anxiety disorders and are known to have strong antidepressant effects (Foyet *et*

al., 2012). The elevated staircase test is a simple and efficient procedure forscreening of anxiolytic agents. It combines stair climbing which is an index of locomotor and exploratory activity and rearing which is an index of anxiety (Simiand *et al.,* 1984). Diazepam induced anxiolytic effect in the elevated staircase test is shown by decreasing the number of rearing without affecting the number of stairs climbed (Simiand *et al.,* 1984). Also there was no observable effect on the locomotor activity of the control mice at diazepam doses of 0.25 and 0.5 mg/kg as reported by Takeda *et al.,* (2002).

The methanolic leaf extract of *Lophiralanceolata* significantly and dose dependently decreased the number of stairs climbed ($P < 0.05$) and rearing ($p < 0.0001$), diazepam significantly decreased the number of rearing without any effect on number of stairs climbed. However, it is important to stress here that non-benzodiazepine psychotrophic agents such as tricyclic antidepressants, antipsychotics and buspirone can induce suppression of both rearing and climbing behavior but a dissociation of the two can be elicited by agents with agonistic activity at the $GABA_A$/benzodiazepine receptor/chloride ion channel complex (Simiand *et al.,* 1984).Milman*et al.,* (2006) and Bellavite *et al.,* (2009) reported that a significant reduction in the number of stairs ascended indicates a suppression of locomotor activity, which can be interpreted as sedative rather than anxiolytic activity. However, since the decrease in number of stairs climbed is significantly dose dependent it could be suggested that at lower doses the extract acts as an anxiolytic whereas at higher doses as sedative (greater than the doses used in this experiment since the highest dose utilized here is 350 mg/kg which did not show any significant effect in the diazepam induced sleeping time. Thus indicating that the methanolic leaf extract of *Lophira lanceolata* at the doses tested possess anxiolytic activity and could possess sedative activity at doses higher than the ones in this protocol.

The elevated plus maze is a well-accepted, experimental animal model typically used to test the effectiveness of anxiolytic drugs (Pellow*et al*., 1985; Hogg, 1996). Rodents on elevated plus maze tend

to avoid the open arms and prefer to stay in the enclosed arm. An entry is defined as having all the four paws within the arm (Adeyemi*et al.,* 2006). Drugs that increase the open arms exploration are considered anxiolytics and the reverse holds true for anxiogenic compounds (Handley and McBlane, 1993). As expected diazepam increased the activity in the open arms of the elevated plus maze apparatus confirming its anxiolytic actions. The methanolic leaf extract of *Lophiralanceolata* did not produce any significant difference in the open arms exploration indicating a lack of anxiolytic-like action. The reason for this is presently unknown. However, data in the literature demonstrated that drugs that alter general motor activity may give false-positive/negative results in the plus maze test (Treit and Fundytus, 1988). A major problem of anxiolytic compounds is that their anxiolytic activity cannot be easily separated from sedation (Costa and Guidotti, 1996; Atack, 2003). *Lophiralanceolata*methanolic leaf extract has been found to contain alkaloids, flavonoids and saponins whose anxiolytic effects have been reported suchas alkaloids (Martinez-Vazquez *et al.,* 2012), flavonoids (Li *et al*., 2011) and saponins (Wei *et al.,* 2007).

The essential requirement for any antidepressant screening test is prediction about antidepressant activity, with other characteristics such as robustness, cheapness, reliability and ease of use. Based on these requirements two behavioral despair models in mice were selected namely the tail suspension test (TST) and the forced swim test (FST)(Potdar and Kibile, 2011).

The tail suspension paradigm has gained considerable acceptance as a reliable screening method for depression (Steru*et al.,* 1985). The immobility displayed by rodents when subjected to unavoidable and inescapable stress has been suggested to reflect behavioral despair which may reflect depressive disorders in humans. Clinically effective antidepressants reduce the immobility time displayed by rodents after active and unsuccessful attempt to escape when suspended by the tail. The test is quite sensitive to major antidepressant drugs including tricyclic antidepressants, selective serotonin reuptake

inhibitors, monoamine oxidase inhibitors and the atypical antidepressants (Porsolt*et al.,* 1978). The principle of this test is that mice suspended upside down leads to characteristics behavior immobility which resembles human depression (Potdar and Kibile, 2011). The methanolic leaf extract of *Lophiralanceolata*showed a significant reduction in immobility time as compared to the negative control at the lowest and highest dose (87.5 and 350 mg/kg) body weight. It is however noteworthy that lack of dose-dependent effect could be explained based on the biological variability and secondary metabolites interaction present in *Lophiralanceolata*immobility time in the tail suspension test. The possible explanation for this might be due partly to the difference in the concentration of the constituents of*Lophiralancoelata*. It is possible that each chemical constituents of the extract exhibited the biological activity influencing the neurobehavior involving depression activity (Onasanwo *et al.,*2010).

The forced swim test (FST) is the most widely used pharmacological model for assessing antidepressant activity (Cryan *et al.,* 2002). The development of immobility when the rodents are placed in an inescapable cylinder of water reflects the cessation of persistent escape-directed behavior (Ulak *et al.,* 2008). The following behaviors were recorded during the last 4 minutes namely immobility typified by floating in water without swimming, swimming which is active movements of extremities and circling in the container and lastly climbing which is active movements of forelimbs on the container wall (Potdar and Kibile, 2011). The methanolic leaf extract of *Lophiralanceolata* at the doses tested showed a non- significant effect on immobility, swimming and climbing as compared to the control. However, imipramine showed a significant reduction in immobility time as compared to the negative control and fluoxetine showed a non-significant effect as compared to the negative control which may be due to the fact that pharmacotherapy of depression typically requires chronic drug treatment to obtain a full response in terms of antidepressant effect. It is therefore critical to perform repeated treatments in the FST model (Hellion-Ibarrola *et al.,* 2008).

The results of this study generally showed active anxiolytic effects which might have been elicited centrally without sedation and without peripheral neuromuscular blockade typified by lack of motor co-ordination deficit. There was an antidepressant activity shown as reduced immobility time (characteristics behavior immobility which resembles human depression). Anxiolytic effects of alkaloids (Martinez-Vazquez et al., 2012), flavonoids (Li et al., 2011) and saponins (Wei et al., 2007) have been earlier reported and they were found to be present phytochemically in the leaf extract of *Lophira lanceolata* this could be responsible for the observed activity. Thus the extract can be employed in mixed anxiety and depressive disorders.

CHAPTER FIVE

5 SUMMARY, CONCLUSION AND RECOMMENTIONS

5.1 Summary

The methanolic leaf extract of *Lophiralanceolata* was found to contain alkaloids, flavonoids, saponins, tannins, phlabotannins, triterpenes, glycosides and reducing sugars. It'sintraperitonialLD_{50}in mice was calculated to be 1,131.9 mg/kg body weight. The extract did not significantly affect the onset and duration of sleep in the diazepam induced sleeping test in mice but showed a significant increase in the number of head dips at a dose of 350 mg/kg body weight. The extract at the doses tested did not show any significant effect on the beam walk assay indicating it does not cause motor co-ordination deficit. On the elevated stair case test there was a dose dependent significant decrease on the number of stairs ascended and rearings with no significant effect in the elevated plus maze experiment. Further, investigations on possible antidepressant activity of the extract showed a significant non-dose dependent effect on the tail suspension test and a non- significant effect in the forced swim test.

5.2 Conclusion

The above findings proved that the methanolic leaf extract of *Lophiralanceolata* possessanxiolytic as well as antidepressant activity at the doses tested which could be due to the several phytochemical constituents therein and could be employed in the management of mixed anxiety and depressive disorders.

5.3 Recommendations

I. Further studies should be carried out on other solvents (non-polar) such as petroleum ether and hexane.

II. The bioactive constituents responsible for the pharmacological activities should be isolated and characterized.

III. The toxicity profile (sub-chronic and chronic) should be studied since anxiety and depressive disorders require long term therapy.

IV. Further studies should be carried out to ascertain the mechanistic anxiolytic and antidepressant actions of the plant.

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APPENDIX I

Figure 1: Effect of Methanolic Leaf Extract of *Lophira lanceolata* on Hole Board (Exploratory Behaviour) in Mice.

Treatment Doses (mg/kg)	No. of Head dips
Normal saline 10ml / kg	9.3± 0.8
Extract 87.5	8.3 ± 1.5
Extract 175	10.0 ± 2.2
Extract 350	20.0 ± 3.7[a]
Diazepam 2	28.2 ± 5.9[a]

n = 6. Data presented as means ± SEM, a = $P < 0.05$, as compared to control, one way ANOVA followed by student's t-test.

APPENDIX II

Figure 2 : Effect of Methanolic Leaf Extract of *Lophira lanceolata* on Forced Swim Test in Mice

Treatment Doses mg/kg	Immobility time(sec)	Swimming time (sec)	Climbing Time (sec)
Normal saline 10ml/kg	166.0 ± 15.2	75.7 ± 16.8	0.0 ± 0.0
Extract 87.5	129.8 ± 17.1	106.0 ± 16.2	2.5 ± 1.6
Extract 175	132.8 ± 28.2	104.0 ± 27.0	1.8 ± 1.8
Extract 350	160.3 ± 6.3	77.0 ± 5.2	3.0 ± 3.0
Imipramine 2	56.2 ± 33.2[a]	128.2 ± 29.3	16.7 ± 11.1
Fluoxetine	109.5 ± 32.8	127.2 ± 33.6	0.0 ± 0.0

n = 6, Data presented as means ± SEM, a = $P < 0.05$ as compared to control, one way ANOVA followed by student t-test .

ABBREVIATIONS

5-Hydroxytryptamine	5-HT
Amyotrophic lateral sclerosis	ALS
Central Nervous system	CNS
Centre for disease control	CDC
Complementary alternative medicine	CAM
Corticotrophin releasing factor	CRF
Diagnostic and Statistical Manual of Mental Disorders Fourth Edition	DSM-IV
Diazepam	DZ
Electrocardiogram	ECG
Electroencephalogragh	EEG
Elevated plus maze	EPM
Excitatory postsynaptic potential	EPSP
Extracellular fluid	ECF
Forced swim test	FST
Gamma amino butyric acid	GABA
Hypothalamic-pituitary-adrenal axis	HPA
Hypothalamic-pituitary-thyroid axis	HPT
Inhibitory postsynaptic potential	IPSP
International criteria for diseases and other related disorders revision 10	ICD 10
Melatonin	MT
Monoamine oxidase inhibitors	MAOI

Nitric OxideNO		
Rapid eye movement sleep		REMS
Tail suspension test	TST	
Traditional medicine	TM	
Tricyclic antidepressant		TCA
World Health Organization		WHO
Milligram per kilogramme		mg/kg
Millilitre per kilogramme		ml/kg
Grammes		g
Milligrams		mg