Evaluation of the Antidepressant- like Activity of the Crude Extract and Solvent Fractions of *Rosa abyssinica* Lindley (Rosaceae) Using Rodent Models of Depression

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A Thesis Submitted to the Department of Pharmacology and Clinical Pharmacy

Presented in Partial Fulfillment of the Requirements for the Degree of Master of Science in Pharmacology

Addis Ababa University
Addis Ababa, Ethiopia
June, 2014

Addis Ababa University
College of Health Sciences

School of Pharmacy

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ABSTRACT

Depression is a disorder often manifested with symptoms at the psychological, behavioral and physiological levels. Herbal medicine holds a valuable place in the treatment of depression. It is also a reasonable alternative for developing novel drugs. Accordingly, 80% methanol extract of the fruits of *Rosa abyssinica* Lindley (Rosaceae) and its solvent fractions were assessed for their antidepressant-like effect using despair based models of depression including tail suspension test (TST) and forced swim test (FST). The effect of the crude extract on the locomotor activity was also assessed using the open field test (OFT).

Animals were randomly assigned to five groups, (n=8). Negative control group received 2% Tween 80, whereas positive control group received imipramine (30 mg/kg). The test groups received 100 mg/kg, 200 mg/kg and 400 mg/kg of the crude extract or the solvent fractions of *Rosa abyssinica*. The crude extract at the doses of 200 mg/kg and 400 mg/kg significantly reduced the time of immobility ($p<0.01$) in the TST and FST. The aqueous fraction at 200 mg/kg displayed a significant reduction (38%, $p<0.01$) in time of immobility in TST which was superior to the effect of imipramine. The methanol fraction displayed a significant reduction in the duration of immobility (33.93%, $p< 0.01$) only at 200 mg/kg. Interestingly the ethyl acetate fraction was devoid of activity. No significant change in locomotor activity was detected in all the doses of the crude extract and imipramine in OFT. These results suggest that this plant holds a potential value for the management of depression.

Keywords: *Rosa abyssinica* Lindley (Rosaceae), Depression, Antidepressant, crude extract, solvent fractions

Acknowledgements

First and for most, I would like to thank Addis Ababa University for funding this research and for providing laboratory supplies throughout the study period. I would love to give my deepest gratitude to my advisor Dr. Ephrem Engidawork, who has not only guided me with his most valuable remarks during the course of the research but also sharpened my awareness on the entire field of pharmacology. I would also like to thank my advisor Dr. Workineh Shibeshi for his much needed support in all aspects of the study and for keeping me on track. I would also like to appreciate Dr. Kaleab Asres for his unreserved assistance throughout the research.

Dearest to my heart, my mother, I can't utter how grateful I am for your incessant prayers and for believing in me. My father, thank you for being my icon of strength and audacity. I always run out of words to describe how thankful I am to my one and only sister for being there for me and offering a shoulder to lean on and my two amazing brothers for being my armors.

It would not have been possible without the constant help and co-operation of W/ro Fantu Assefa, Ato Molla Wale, Ato Haile Meskel and the entire staff of the Department of Pharmacology and Clinical Pharmacy.

Special thanks to my friends Helen Geremew, Wintana Amare, Abinet Tekle and Nebiyou Yirga for their relentless support for the success of the study.

Lord, my savior, I praise your name for every blessing you have sent my way. It's your light that led me from the ruts of the unknown to the high places of hope. I owe everything to you!

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List of Acronyms

5-HT	5- Hydroxy Tryptamine/ Serotonin
5HTT	Serotonin Transporter
ACE	Angiotensin Converting Enzyme
ANOVA	Analysis of Variance
AIDS	Acquired Immune Deficiency Syndrome
AVP	Arginine Vasopressin
BDNF	Brain Derived Neurotrophic Factor
BPD	Bipolar Disorder
CNS	Central Nervous System
CREB	Cyclic AMP Response element Binding protein
CRF	Corticotrophin Releasing Factor
CSF	Cerebrospinal Fluid
D2	Dopamine Receptors Type 2
DA	Dopamine
DD	Dysthymic Disorder
DSM-V	Diagnostic and Statistical Manual of Mental disorders, 5^{th} edition
ECT	Electro Convulsive Therapy
FST	Forced Swim Test
GABA	Gama Amino Butyric Acid
HIV	Human Immunodeficiency Virus
HPA	Hypothalamic Pituitary Axis
IFN	Interferon
IL	Interleukin

LC	Locus Ceruleus
MAOIs	Monoamine Oxdase Inhibitors
MAPK	Mitogen - Activated Protein Kinase
MDD	Major Depressive Disorder
NAc	Nucleus Accumbens
NADPH	Reduced Nicotinamide Adenine Dinucleotide Phosphate
NE	Norepinephrine
NK1	Neurokinin 1
ONS	Oxidative and Nitrosative Stress
OECD	Organization for Economic Cooperation and Development guideline
OFT	Open Field Test
PPD	Post Partum Depression
REM	Rapid Eye Movement
ROS	Reactive Oxygen Species
rTMS	Repetitive/ Transcranial Magnetic Stimulation
SAD	Seasonal Affective Disorder
SNPs	Single Nucleotide Polymorphisms
SNRI	Selective Norepinephrine Reuptake Inhibitors
SOD	Super Oxide Dismutase
SP	Substance P
SSRI	Selective Serotonin Reuptake Inhibitors
TCA	Tricyclic Antidepressants
TH	Tyrosine Hydroxylase

TLC	Thin Layer Chromatography
TNF	Tumor Necrosis Factor
TPH	Tryptophan Hydroxylase
TRH	Tyrotropin Releasing Hormone
TSH	Thyroid Stimulating Hormone
TST	Tail Suspension Test
VNS	Vagus Nerve Stimulation
VTA	Ventral Tegmental Area
WHO	World Health Organization
α_2 R	Alpha 2 Receptor

1. Introduction

1.1. Overview of depression

Depression is a chronic mental disorder that causes changes in mood, thoughts, behavior and physical health. It's a common but serious disease that can take away a person's ability to enjoy life and cause decline in capacity to undertake even the simplest daily tasks. Other than its chronic nature, symptoms associated with this mental disorder are often recurring and life threatening. According to the World Health Organization (WHO) unipolar depression is one of the leading causes of disability- adjusted life year (DALY) and approximately 350 people worldwide are said to suffer from this mental disorder. (WHO 2012; WHO Global Health Estimates, 2013)

As described in the Diagnostic and Statistical Manual of Mental Disorders, 5^{th} edition (DSM- V, 2013), the hallmark of major depressive disorder (MDD) is the occurrence of depressed mood (dysphoria) and loss of interest in activities that were rather pleasurable in the past (anhedonia) for a duration of at least two weeks. These symptoms must also be accompanied by at least four of the following manifestations such as changes in appetite or weight, sleep patterns, altered psychomotor activity, feelings of worthlessness or guilt, difficulty concentrating or making decisions and recurrent thoughts of death or suicidal ideation.

Even though there are plenty of drugs developed for the management of depression, one of the challenges in dealing with this disease is that a significant portion of the patients taking antidepressants fail to attain full remission. Some patients also develop treatment resistant depression in which the patients fail to respond to the available drugs or other therapeutic approaches. (Al-Harbi, 2012).

1.1.1. Types of depression

Depression is a heterogeneous disorder often mistaken for a single clinical mental illness. There are indeed diverse forms of depression that can either be mild or extremely severe conditions like psychotic depression in which the patients show symptoms such as hallucinations and delusions. Diagnosis of this disorder is complicated because of the co-occurrence of many other mental conditions such as anxiety disorders, including panic

agoraphobia syndrome, severe phobias, generalized anxiety disorder, social anxiety disorder, post traumatic stress disorder (PTSD) and obsessive-compulsive disorder (OCD). This co-morbidity is commonly seen in elderly patients and is also associated with severity of somatic symptoms. (Devane *et al*., 2005). The different types of depression are reviewed below.

- MDD: patients with this type of depressive disorder typically show dysphoric mood and anhedonia accompanied by physical changes such as weight loss or gain, increased or decreased appetite, alteration in sleep pattern and sustained fatigue. Disturbances in cognitive and executive functions are also manifested by lack of concentration and coherent thinking as well as morbid preoccupation by thoughts of death and suicide. Majority of these symptoms normally are present nearly every day and result in significant distress and impaired social life and occupational performance (DSM-V, 2013; Rajput *et al*., 2011).

- Dysthymic disorder: also known as persistent depressive disorder. Patients display depressed mood or sadness that persists for the majority of the duration of the day for a minimum of two years in adults and one year in children and adolescents. Majority of the patients do not meet the full criteria for MDD as there is interruption by short periods of remission. However, there are instances where patients meet full criteria in which they are diagnosed with MDD. (Benazzi, 2006; Sansone *et al*., 2005).

- Melancholic depression: there is an almost absolute lack of ability to experience pleasure. Psychomotor retardation and early morning worsening of mood is also apparent in this subset of patients. This type of depression is seen more commonly in the elderly, in patients with more severe forms of depression and psychotic depression. (Benazzi, 2006).

- Seasonal affective disorder (SAD): is a type of depression described as recurring annually during fall or early winter. This ‘winter blues’ or SAD is characterized by low mood, feelings of guilt and worthlessness and increased irritability, symptoms shared with other depressive disorders. Additionally patients show a

significant increase in appetite and craving for foods high in carbohydrates which result in weight gain (DSM-V, 2013; Baghai *et al*., 2008).

- Post partum depression (PPD): describes a heterogeneous group of depressive symptoms that affects mothers. These symptoms may surface before or after giving birth (Chaudron, 2003). Half of the "postpartum" episodes begin before the time of delivery. Thus, are referred to collectively as "peripartum" episodes. According to DSM-V (2013) mood swings and anxiety symptoms during pregnancy, as well as the "baby blues" increase the risk for a postpartum major depressive episode.

- Psychotic depression: is a type of depressive disorder which is very severe and accompanied by psychotic symptoms (Shatzberg, 2003; Swartz and Shorter, 2007). It is commonly seen as a combination of psychosis and depression that is not separable into either of the two. Symptoms include psychotic features such as hallucinations or delusions. Other than its severity psychotic depression is associated with prolonged course, poor response to available drugs and higher relapse rate (Baghai *et al*., 2008; Vythilingam, 2003).

1.1.2. Epidemiology of Depression

Depression is a major contributor to the global burden of disease and affects people in all communities across the world and 450 million people suffer from some type of mental or behavioral disorder (Murugan *et al*., 2011). The lifetime prevalence for major depression is reported to be as high as 14-17 % and the one-year prevalence is 4-8%. The lifetime prevalence rates of MDD among women are 10-25%, and for men 5-12 % (Garriock, 2006; WHO, 2012).

Almost 10% of the total burden of disease in sub-Saharan Africa is attributed to neuropsychiatric disorders. (Tomlinson *et al*., 2009). The lifetime prevalence of minor depressive disorder in Ethiopia was reported to be 2.2% (Fekadu *et al*., 2007). Other studies conducted in Ethiopia showed the one-year prevalence of depression to be 4.4% among women (Deyessa, 2010).The prevalence of depressive episodes was reported to be

9.1%. The major risk factors for these episodes were age, marital status, number of diagnosed chronic non communicable diseases and alcohol consumption (Hailemariam *et al.*, 2012). Depression contributes about 6.5% of the burden of diseases in Ethiopia which is even higher compared to major infectious diseases such as Human Immunodeficiency Virus (HIV) infection (Abdulahi *et al*., 2001). Moreover, major depression and bipolar disorder were associated with three-fold increased risk of premature mortality as compared to the general population (Fekadu, 2010).

The prevalence of depression in children is relatively low (<1% in most studies), but increases considerably all the way through adolescence with a one-year prevalence of 4–5% in mid to late adolescence. Depression in fact is a major risk factor for suicide observed in adolescents; it's one of the leading causes of death in this age group Depression also leads to serious social and educational impairments and associated with an increased rate of smoking, substance abuse and obesity (Chaudron, 2003; El Refaey and Amri, 2011; Thapar *et al*., 2012).

1.2. Pathophysiology of Depression

Even though there are numerous studies attempting to shed light on the pathphysiology of depression, it still remains elusive. This is in fact the major reason for the slow paced drug development against this disease. There are diverse theories on the pathogenesis of depression most based on measurement of indirect markers, post-mortem studies and neuro-imaging techniques. For decades, depression pharmacotherapy and a resultant explanation for the underlying pathology, focused on the brain monoamine neurotransmitters level following the serendipitous discovery of imipramine and iproniazid as antidepressants (Grønli, 2006; Krishnan and Nestler, 2008).

A) Neural circuitry of depression

Various structural and functional studies report abnormalities in the areas of the brain that are responsible for the regulation of mood, reward response and executive functions. Post-mortem and neuro-imaging studies have reported morphological changes indicated by reductions in grey-matter volume and glial density in the prefrontal cortex and the hippocampus, regions that have received the most attention in animal research on

depression. The decline in hippocampal function, which is believed to have an inhibitory effect on the hypothalamic-pituitary- adrenal (HPA) axis, could potentially be responsible for the hypercortisolemia seen in depression (Krishnan and Nestler, 2008; Nestler and Carlezon, 2006).

The mesolimbic dopamine system that consists of the nucleus accumbens (NAc) and the ventral tegmental area (VTA) also are believed to play a role in the pathogenesis of depression. These brain regions mediate the reward response to pleasurable stimuli such as food, sex and even drugs. Therefore, a peculiar lack of pleasure in depressed patients can possibly be explained as a dysfunction in this brain reward circuit (Nestler and Carlezon, 2006).Other studies have also shown a decrease in Locus ceruleus (LC) neuron density in some depressed and suicide population compared with controls (Ressler and Nemeroff, 1999).

B) Stress response circuits

Chronic stress and hyperactivity of the HPA axis (causing chronic hypercortisolemia) have been hypothesized to play a prominent role in the incidence of depression and even in recurrence after complete remission. Structural brain abnormalities have been documented in patients with elevated levels of corticosteroids. One of the brain structures affected is the amygdala, area of the brain involved in mainly regulating emotional reactivity and to some degree stress response. (Bouhuys *et al*., 2006; Brown *et al*., 2008) Another brain region shown to decrease in size with chronic administration of corticosteroids is the hippocampus, area of the brain that is believed to exert an inhibitory signal to the HPA axis. (Rot *et al*., 2009)

There is still a lack of complete understanding on how behavioral stress causes depression. However, chronic stress has been shown to alter the expression of genes regulating antioxidant systems, such as superoxide dismutases (SODs), catalase, glutathione peroxidase, glutathione reductase and NADPH oxidase. Moreover, animal studies uncovered that treatment with glucocorticoids cause elevation in the level reactive oxygen species (ROS) both *in vitro* and in the brains of animals, while also down-regulating various antioxidant enzyme and inducing depression-like behavior (Gold and Chrousos, 2002; Seo *et al*., 2012; Swaab *et al*., 2005).

C) Genetic vulnerability and environmental interaction

There is now a compelling argument that in order for depression to surface there needs to be a complex gene-environmental interaction that alters an individual response to stressful life situations. No single gene polymophism seems responsible for causing depression, it has been suggested that genetic factors make certain individuals susceptible to depression by increasing their vulnerability to stressful environmental factors. (Lee *et al.*, 2010).

A genetic polymotphism that has been perhaps a center of attention for years is the allelic variation in the promoter region of the gene encoding the serotonin transporter (5- HTT). The promoter region of 5- HTT gene (5-HTTLPR) contains a functional polymorphism resulting in a long (L)/short(S) variant in the promoter region upstream of the transcription starting site. The short allele of 5-HTT has a low-activity and has been shown to put carriers at a greater risk of developing depression in response to stressful life events. This allele has also been related with poorer outcomes after antidepressant pharmacological and non-pharmacological treatments (Chiavetto *et al*., 2008; Karg *et al*., 2011).

The rate-limiting enzyme in serotonin biosynthesis, tryptophan hydroxylase (TPH), is encoded by two distinct genes *Tphl* and *Tph2* and has been proposed to play a role in pathogenesis of depressive disorders and suicide. Single nucleotide polymorphisms (SNPs) on *Tph2* gene have been linked with increased incidence of MDD and completed suicide attempts. Also, *Tph l* gene, which is dominantly expressed in the pineal gland, is thought to influence suicidal risk by disrupting the synthesis of melatonin a hormone responsible for regulation of circadian rhythm resulting in an increase in suicidal risk. (Stefulj *et al*., 2006; Zupanc *et al*., 2013).

A functional polymorphism, producing a valine to methionine substitution at the codon 66 (Val66Met) in the pro-BDNF region, has been identified in the BDNF gene, exhibiting a detrimental effect on intracellular trafficking and activity-dependent secretion and influencing hippocampal function, episodic memory and brain morphology. Healthy individuals with the BDNF Met variant display a low emotional stability and

smaller hippocampus volume. Studies also suggest a complex interaction exists between the polymorphisms in genes encoding BDNF and 5- HTT to bring about a depressed phenotype (Chiavetto *et al*., 2008; Krishnan and Nestler, 2008).

D) The biogenic monoamine theory

The monoamine hypothesis of depression came into the picture after the serendipitous discovery of the first antidepressant drugs that were otherwise developed for other medical conditions. These clinical observations have contributed greatly to the understanding of the pathophysiological changes that take place in the brains of depressed individuals. The drugs were proposed to increase the amount of monoamine neurotransmitters in the brain either by blocking a monoamine degrading enzyme monoamine oxidase inhibitor (MAOI) or by blocking the reuptake of the neurotransmitters into the presynaptic neuron (Nutt, 2006).

i) The Serotonin hypothesis

Serotonin is a monoamine neurotransmitter with a wide range distribution throughout the central nervous system. It is involved in physiologic activities such as pain sensation, appetite regulation, aggression and mood. Dysfunction in serotonergic system has been implicated in mood and anxiety disorders. The basis for this hypothesis is the fact that the first antidepressant drugs worked by reviving the diminished monoamine activity in the brain. And later SSRIs alone were found to be sufficient to treat symptoms of depression effectively. This fact further strengthened the involvement of 5-HT in the pathogenesis of the disease (Cowen, 2008; Saldanha *et al*., 2009).

Subset of depressed patients have been reported to have a lowered level of 5-hydroxyindoleacetic acid (5-HIAA) a metabolite of 5-HT in the cerebrospinal fluid (CSF), which has been related to aggressive behavior and increased suicidal intent and impulsivity. The plasma level of the amino acid precursor (tryptophan) of 5- HT decreased and depressive symptoms can be induced in patients who are susceptible to depression by depleting this amino acid. Moreover, positron emission tomography (PET) imaging studies have reported a decrease in density of $5\text{-}HT_{1A}$ receptor subtype on depressed patients in different regions of the brain. There is also a decreased availability of 5-HTT in midbrain and brainstem regions. But this seretonergic dysfunction associated

in depression is debated whether it is an etiologic factor or increases susceptibility (Drevets *et al*., 2007; Jans *et al*., 2007).

ii) The cathecholamine hypothesis

The cathecholamine hypothesis of depression emerged in the 1960s after the observation that reserpine, an antihypertensive drug depletes central and peripheral amine storage in the nervous system, induced depression. However, there are no consistent findings on the alteration in the levels of NE metabolites in the CSF of depressed individuals. In subsequent years, the "supersensitivity hypothesis" was proposed which links depression to supersensitive presynaptic α_2-R which is also supported by an increased density of these receptor types in post mortum studies, leading to an impaired NE activity (Nutt, 2006; Ressler and Nemeroff, 1999).

Additionally, some symptoms of depression including anhedonia and psychomotor retardation are better explained by a derangement in the brain DA systems. These systems include the substantia nigra -basal ganglia motor system and the reward circuitry involving the NAc and VTA. There is a diminished DA activity in the NAc specifically which corresponds to the inability to experience pleasure which is one of the hallmarks of depression. The concentration of the dopamine metabolite homovanillic acid (HVA) in CSF is reported to be lower in depressed patients as well (Brunswick *et al*., 2003; Martinot *et al*., 2001; Treadway and Zald, 2011).

E) Inflammation and depression

The claim that depression is an inflammatory disorder is gaining popularity nowadays. This is supported by the fact that many pro-inflammatory marker levels are reported to be elevated in depressed patients. Examples of these markers are C-reactive protein (CRP), interleukin (IL)-6, IL-1 and tumor necrosis factor alpha (TNF-α).In fact depressive like behaviors can be induced in the laboratory by administration of (IFN)-α, a powerful inflammatory cytokine, that has also been shown to produce depression like symptoms in patients taking it for the treatment of hepatitis C. (Dowlati *et al*., 2010; Vogelzangs *et al*., 2012).

An increase in reactive oxygen and nitrogen species generation and damage by oxidative and nitrosative stress (ONS), including lipid peroxidation, damage to deoxyribonucleic acid (DNA) and proteins is also seen. Even though a complete understanding of the mechanisms involved remains obscure, an increase in pro- inflammatory cytokines results in a lack of neuronal plasticity and eventual neurodegeneration. Also pro-inflammatory cytokines can interfere with the activity of growth factors which results in reduced neurogenesis as the immune changes can damage glial cells and neurons (Audet and Anisman,2013; Maes *et al*., 2011).

F) Neurothrophism hypothesis

Significant atrophy of certain prefrontal cortex areas and hippocampus observed in depression as well as decreased levels of nerve growth factors (NGF) such as BDNF has led to the neurotophism hypothesis. BDNF is an important molecular regulator of neuronal development and plasticity. It increases survival of neurons, stimulates the growth of dendrites and increases the spine density and also involved in maturation of excitatory synapses, processes that are important in learning and adaptation process which seems to be deficient in depression. (Chiavetto *et al*., 2008; Groves, 2007: Sirianni *et al*., 2010)

The expression of BDNF is believed to be halted by chronic stress and normal level of this growth factor is attained after a successful treatment with antidepressants. This is consistent with the fact that antidepressants take at least 2-3 weeks to elicit their actions, possibly through causing a longer lasting neuroadaptive changes in the brain rather than a simple increase in the level of neurotransmitters. This neuroadaptive change includes the process of neurogeneis, a phenomenon recently revealed to also occur in specific areas such as the subventricular and subgranular zones of the dentate gyrus giving rise to neurons in the hippocampus. This process includes cell division, migration and differentiation medicated by NGFs (Angelucci *et al*., 2005; Heldt *et al*., 2007; Molendijk *et al*., 2011)

Vascular endothelial growth factor (VEGF) is another NGF that promotes proliferation of neuronal cells in some brain regions like the hippocampus. It achieves this by activating intracellular signaling cascades that involvese mitogen-activated protein kinase (MAPK)

pathway. This signaling pathway has also been postulated to underlie the late antidepressant response of currently available drugs. This is achieved through the activity of gene transcription inducer cyclic AMP response element Binding protein (CREB) which is activated by MAPK resulting in stabilization of synaptic plasticity (Huuhka, 2009; Varia and Alder 2009).

G) Neuropeptides and depression

There is increasing evidence that this neuropeptides are involved in the modulation of stress- related behaviors and mood by acting on neurokinin type-1 receptors (NK-1). Substance P (SP) is one of these neuropeptides known for its wide spread distribution in the brain and its co-localization with 5-HT and NE neurons (Brain and Cox, 2006). Elevated CSF SP concentrations have been reported in depressed patients and patients with PTSD after exposure to a stressful stimulus. Additionally, central administration of SP has been shown to induce stress response. This is supported by the antidepressant activity of NK-1 antagonists. (Herpfer and Lieb 2005; Holtzheimer and Nemeroff, 2008).

H) Hormones and depression

i. Thyroid hormones

Thyroid hormones (TH) imbalances are implicated in the pathophysiology of neurodegenerative and psychiatric conditions. These hormones are very essential for brain development, maturation and have been shown to promote neurogenesis, in particular, in the hippocampus, (Pedrazuela *et al*., 2006). Hypothyroidism has been linked to depressive -like behavior in that it impaired hippocampal neurogenesis which resolved with hormone replacement. Animal studies also reveled that thyroid hormone causes an increase in seretonergic neurotransimiion which supports the fact that TH supplementation has been beneficial in management of refractory cases of depression (Alkemade, 2003; Bauer *et al*., 2002).

ii. Estrogen involvement

Increased female susceptibility to depression mostly overlaps periods of low estrogen levels in the menstrual cycle, postpartum and after the onset of menopause. Animal studies indicate mood enhancing actions of estrogen as well as synergy with

monoaminergic drugs. Estogen enhances mood by increasing the rate of degradation of MAO and intraneuronal 5- HT transport, causing an overall increase in 5-HT availability in the synapse. In addition serotonergic neurotransmission, estrogen also is believed to have modulatory effect on hippocampal neurogenesis, BDNF signaling, and HPA axis function (Douma *et al*., 2005; Krishnan and Nestler, 2010).

iii) Vasopressin and depression

Arginine vasopressin (AVP) is a hypothalamic hormone that influences some key symptoms pertinent to major depressive disorder. Its level is reported to be elevated in patients suffering from this mental disorder (West *et al*., 2004). AVP has been linked to play a role in the regulation of stress response, one of the prominent features of depression, in that it synergizes with CRF at the level of the pituitary to influence the release of ACTH. (De Winter *et al*., 2003). Elevated AVP concentrations were also associated with psychomotor retardation in patients with major depressive disorder (Londen *et al*., 1998)

I) Implications of the circadian rhythm in depression

Melatonin, a hormone secreted by the pineal gland, in a circadian fashion, regulates the rhythm of various biological parameters like body temperature, cortisol secretion, and sleep cycles by acting on receptors in the suprachiasmatic nucleus(SCN) of the hypothalamus (Verster, 2009). Delayed circadian rhythm in patients with depression has been linked to diminished level of melatonergic signaling in the brain. Patients may manifest with delayed onset of sleep, difficulty in maintaining sleep and early morning awakening. This has given way to the discovery of new antidepressant agent, agomelatin, which acts on melatonin and serotonin receptors on the SCN. Disruption of circadian rhythm has also been proposed to make individuals susceptible to depression. (Coutu *et al*., 2007; Girish *et al*., 2010; Urretavizcaya and Soria, 2009).

1.3. Management of depression

An array of treatment options has been developed to combat depression over the decades. The various approaches include pharmacotherapy, psychotherapy and somatic therapy often employed for treatment resistant depression (Holtzheime and Nemeroff, 2008).

1.3.1. Pharmacotherapy

The first antidepressants were discovered by serendipity, following incisive clinical observations that iproniazid, a drug developed for the treatment of tuberculosis, showed mood elevating effects. Just as well, imipramine, an alleged antipsychotic drug showed antidepressant activity. These observations not only led the way for subsequent studies to develop the first groups of antidepressant drugs MAO inhibitors and TCAs, but also have contributed immensely to the pathophysiological understanding of depression as we know it today (Jacobsen *et al*., 2012; Yildiz *et al*., 2002).

Majority of the available antidepressant drugs work by modulating the brain monoamine neurotransmission. The primary mechanism of these drugs is increasing the overall synaptic concentration of monoamines (serotonin, norepinephrine and dopamine).They achieve this either by blocking their reuptake into the presynaptic neuron by binding to the respective neurotransmitter transporter or through inhibition of the monoamine degrading enzyme MAO reversibly or irreversibly. (Holtzheimer and Nemeroff, 2008) Certain antidepressants also act on presynaptic or postsynaptic neurotransmitter receptors to alter the neurotransmission. There also atypical antidepressant drugs that are emerging in the market. This list includes antipsychotics, NK-1 antagonist, GR antagonists and melatonergic drugs (Brain and Cox, 2006; Kasper and Hamon 2009; Šagud *et al*., 2011).

As mentioned earlier, there is a time delay on the onset of the response after treatment with antidepressants. It is believed that long term neuronal adaptations may underlie the effects rather than the acute modulation of transporters or receptors that alter the neurotransmission. Repetitive activation of the neurons by these drugs is believed to result in changes such as synaptic plasticity, axonal sprouting, neurite extention, and promotion of cell survival cue brought about by complex cellular signal transduction mechanisms involving neurotrophins and various transcription factors (Yildiz *et al*., 2002)

1.3.2. Somatic therapy

Somatic therapy for depression is a device-based approach that consists of introducing transient electric or magnetic current onto the scalp or to anatomically deep brain structures. The use of this approach is favored in the management of depression refractory to the available drugs. It also has a wide applicability for maintenance of effect after successful remission as well as can be used as an add-on therapy. The various somatic treatments are believed to induce transient seizures that are responsible for the clinical effects. The mechanism of action is largely attributed to increasing the level of neurotransmitters and sensitization of post synaptic receptors through changing the neuronal firing in the regions involved. There is also the participation of growth factors and induction of long lasting neuronal adaptation.

ECT on neurotransmitters, receptors, and postreceptor signaling mechanisms in the brain, particularly those that are implicated in the mechanism of action of antidepressant drugs. The emphasis has been primarily on serotonergic, noradrenergic, and dopaminergic systems with some consideration of γ-aminobutyric acid (GABA)-ergic and more recently glutamatergic mechanisms. Electrophysiological studies suggest that an important effect of ECT on brain serotonergic systems in rodent brain is sensitization of postsynaptic serotonin (5-HT)1A receptors and a consequent increase in serotonergic transmission. offers relationship between chemical and electric transmission of signals in the brain Increase in hormonal levels such as TSH

i) Electroconvulsive therapy

Electroconvulsive therapy (ECT) is the first effective somatic therapy to be used for the treatment of mental disorders with a widespread clinical use even up to now. Basically a seizure is induced by applying an electric current with pulse width from 0.3 to 1msec, frequency from 20 to 120 Hz, duration of the stimulus 0.5-8 sec to the surface of the head. This procedure requires the patients to be properly anesthetized before the actual session to avoid any serious complications (Eitan and Lerer, 2006). ECT is believed to cause increased blood level of norepinephrine and causes sensitization of 5-HT_1A receptors. (Cusin and Dougherty, 2012; Huuhka, 2009).

ii) Transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) is another type of somatic treatment option for treatment resistant depression. TMS induces depolarization of cortical neurons by the use of magnetic current that passes through a metal coil applied to the scalp of the patient, making it non- invasive (Chiavetto *et al*., 2008; Cusin and Dougherty, 2012). TMS results in elevated levels of dopamine and serotonin .It also causes up regulation of β-adrenergic and 5HT receptors in the frontal cortex. There are also reports of subsensitivity of presynaptic serotonergic autoreceptors observed after receiving TMS (Eitan and Lerer, 2006).

iii) Vagus nerve Stimulation

Vagus nerve stimulation (VNS) is a minimally invasive procedure where an impulse generator device is implanted in the chest area of the patient attached to the left vagus nerve with lead wires. The clinical effects of VNS treatment do not surface soon after treatment therefore making it a less appealing choice for managing acute treatment resistant depression. The mechanism of action of VNS remains elusive (Cusin and Dougherty, 2012; Gotto and Rapaport, 2005)

1.3.3. Medicinal plants used to treat depression

Medicinal plants around the world have been used to treat disorders of the body and the mind since antiquity. Herbal medicine has been a reasonable alternative for the management of mental disorders such as anxiety, depression and dementia among plenty others (Klemens, 2006). Developing antidepressants from herbal sources seems to be reasonable approach due to their therapeutic efficacy and lower incidence of side effects (Rajput *et al*., 2011).*Hypericum perforatum* commonly known as St. John's wort is the only herbal antidepressant that has been approved for the clinical management of mild to moderate cases of depression. Hypericin and hyperforin are flavonoids present in hypericum that are claimed to be responsible for the antidepressant activity of the plant (Rojecky, 2004)

Medicinal plants most widely used to treatment depression around the world are *Hypericum perforatum, Centella asiatica, Rhodiola rosea, Pfaffia paniculata, Rauwolfia*

serpentina, *Rhododendron molle*, *Schizandra chin*, *Thea sinensis* , *Uncaria tome* , *Valeriana officinalis* and *Withania somnifera* (Dhingra and Sharma, 2006; Mamedov, 2005; Rajput *et al*., 2011).There is a long history of using plants for treating many diseases in Ethiopia. This herbal based therapy is most valued and has been passed from generation to generation by word of mouth. Herbal therapy still remains to be the first line treatment option for nearly 80% of the population. Plants such as *Justicia odora*, *Whitiana somnifera*, *Calpurnia aurea* and *Asparagus leptocladodius* have traditionally been used for the treatment of depression (Fisseha *et al.*, 2009; Getahun, 1976)

1.4. The Genus *Rosa*

The Rosaceae family consists of the most common ornamental plants in the world that have been known as the king of flowers. The genus *Rosa* which belongs to the Rosaceae family comprises approximately 190 shrub species distributed widely throughout the temperate and subtropical habitats of the northern hemisphere. *Rosa* is one of the major economically important genera of ornamental horticulture and the area under cultivation continues to expand. The rose, admired since antiquity for its beauty and fragrance has multiple uses: its cut flowers, landscape decoration, medicinal oils (attar of rose), for perfume as well as culinary use (rose water), and the rose hips (fruits) as a source of vitamin C (Bruneau *et al*., 2007; Dolati *et al*., 2011; Hummer and Janick, 2009).

1.4.1. *Rosa abyssinica*

Rosa abyssinica Lindley (Fig 1) also known as “kega” in Amharic, is an evergreen shrub having stems with strong, wide based and curvy prickles. It has white to pale yellow fragrant flowers compound in rather few-flowered long styles connected in a delicate column. The plant bears edible fruits that are small with seeds inside and are red to bright orange when ripe(Thulin M., 1993) For the first time, *Rosa abyssinica* was found in 1805 in Abyssinia by R. Salt but it was named in 1814 by R. Brown, who published only its name in the appendix to Salt’s “A voyage to Abyssinia”. Formally this rose was described by Lindley in 1820 in his “Rosarum Monographia” (Browicz and Zielirski, 1991).

The plant holds a high value in the Ethiopian folk medicine and is used for the treatment of many ailments. It is used for the treatment of rheumatic pain, hypertension, scabies, cough, glandular tuberculosis and diabetes. The edible fruits which are adored by children are good tapeworm and round worm expectorants as well as excellent source of vitamins .The root extract of the plant also possesses a genuine anti-nociceptive and anti-inflammatory activity (Getahun, 1976; Sewuye and Asres, 2008).Thirty-five components were identified in the essential oil extracted from the aerial parts of *Rosa abyssinica* including the flowers. The main components were γ-muurolene (13.0%) and caryophyllene oxide (26.6%) (Kheyrodin, 2009).

An ethnobiological study conducted in Tara Gedam and Amba remnant forests (South Gondar zone, Ethiopia) which focused on plants used in traditional medicine and as wild foods around the area reported that the whole fruits of *Rosa abyssinica* are consumed for the management of depression by the locals (Chekole, 2011). This study therefore attempts to scientifically justify the use of this plant as no prior studies have been carried out in this regard. Other species within the genus *Rosa* that are claimed to have antidepressant activity are *Rosa canina* and *Rosa damascena* which also have beneficial effects on stress and tension (Dolati *et al*., 2011).

A) **B)**

Fig 1: A photograph of *Rosa abyssinica* A) Aerial parts including flowers B) The fruits

Hypothesis- The crude and solvent fractions of the fruits of *Rosa abyssinica* have antidepressant- like activity causing a reduction in the duration of immobility in despair based rodent models of depression.

2. Objective

2.1. General Objective

- To establish the antidepressant- like activity of 80% methanol extract and solvent fractions of the fruits of *Rosa abyssinica* Lindley (Rosaceae) in animal models of depression.

2.2. Specific Objectives

- To determine the acute oral toxicity profile of the crude extract of the fruits of *Rosa abyssinica.*
- To assess the anti-immobility effect of the crude extract of fruits of *Rosa abyssinica* and solvent fractions using tail suspension test in mice.
- To determine the anti-immobility effect of the crude extract of the fruits of *Rosa abyssinica* in forced swim model of depression in rats.
- To evaluate the effect of the crude extract of *Rosa abyssinica* on locomotor activity using open field test in mice.
- To assess the profile of secondary metabolites of the extract using phytochemical screening tests and establish finger print of the extract with thin layer chromatography (TLC).

3. Materials and Methods

3.1. Chemicals

Imipramine (Torrent Pharmaceuticals, India), absolute methanol (Cheshire, UK), Tween 80 (BDH chemical reagents, England), petroleum ether (Carlo Erba reagents, France), ethyl acetate (Fluka, Germany), chloroform (BDH chemical reagents, England), ethyl alcohol (Changshu Yangyuan chemicals, China), distilled water, dilute ammonia, acetic anhydride, Dragendrof's reagent, 10% ethanolic ferric chloride, concentrated sulfuric acid, glacial acetic acid, 5% ethanolic ferric chloride, 1% $KFe(CN)_6$, 10% sodium nitrate, 2% lead acetate, 1% ferric chloride and 1% aqueous hydrochloric acid were purchased from reliable sources and were of analytical grade.

3.2. Plant Material

The fruits of *Rosa abyssinica* were collected from Debre Tsigie; a town in northern Shewa, located 89 km from the capital. The plant material was identified by Ato Melaku Wondafrash, a taxonomist, and a voucher specimen (# 001) was deposited at the National Herbarium for future reference.

3.3. Experimental Animals

Animals used for this study were male albino Swiss mice (6 –8 weeks, 20–30 g) as well as Sprague-Dawley rats (8-12 weeks, 200-250 g). A total of 245 animals were used for this study. Female rats were used for acute toxicity test and male rats were used for activity testing. The animals used for this study were either bred at the animal house facility of School of Pharmacy or purchased from Ethiopian Health and Nutrition research Institute. The animals were housed in groups of 8 and were allowed to acclimatize to laboratory conditions for a minimum of 5 days before the time of experimentation. All animals had free access to standard animal feed and clean water and were maintained on a 12/12 hours of light/dark cycle. The ambient temperature was 22 ±3 °C. All animals used in the study were cared for and treated humanely throughout the study period following international guidelines (OECD, 2001).

3.4. Extraction Procedure

3.4.1. Crude extract

The fruits of *Rosa abyssinica* (1000g) were washed, wiped dry and crushed manually using mortar and pestle. The crude extract was obtained by maceration of the plant material with 80% methanol for 24 h at room temperature, and this was repeated for three cycles with fresh solvent. The extract solution was then filtered, first by using cotton gauze and later by suction filtration apparatus (Oakton, U.S.A) using Whatman filter paper (No. 1). Rotavapor (Büchi, Switzerland) was used to dry the methanol in the sample under reduced pressure at a temperature of 40^{o}c and the remaining water was freeze dried by the use of a lyophilizer (Delvac, India). The extract obtained had a distinctive translucent amber color and a thick and sticky honey-like consistency. The percentage yield for the crude extract was calculated to be 15.8% w/w.

3.4.2 Fractionation

The crude extract of the fruits of *Rosa abyssinica* (30 g) was defatted with petroleum ether using a soxhlet apparatus. Then the remaining marc was allowed to dry overnight and fractionated with the same apparatus using different solvents of increasing polarity namely ethyl acetate and methanol (100%) to obtain the corresponding fractions. The marc left was then macerated with distilled water to obtain the aqueous fraction. Each fraction then was dried in an oven at 40^{o}c and was stored in a refrigerator until further use. The percentage yield for the aqueous, methanol and ethyl acetate fractions was 25.7%, 32.4 % and 6.6% respectively.

3.5. Acute Toxicity Test

Acute toxicity study was carried out using the limit test dose of 2000 mg/kg as has been described by Organization for Economic Cooperation and Development guideline (OECD, 2001). A total of five female rats were fasted for 4 h and were administered with the limit dose 2000 mg/kg of the crude extract of *Rosa abyssinica*. The animals were then observed individually for mortality and overt signs of toxicity during the first 30 min, periodically during the first 24 h, with special attention given during the first 4h and daily afterward for a total of 14 days. Mortality and any change in behaviors

such as alertness, motor activity, breathing, restlessness, diarrhea, convulsions and coma were observed carefully.

3.6. Grouping and Dosing of Animals

There were five groups of animals for each model .All animals were randomly assigned to different groups. Group I received the vehicle (2% Tween 80 in distilled water) and served as negative control. Group II received the standard drug imipramine (30 mg/kg) and served as positive control. The test groups were group III-V, which received increasing doses of the extract or the solvent fractions at 100 mg/kg, 200 mg/kg and 400 mg/kg, respectively. The different doses of the extract, the solvent fractions and the standard drug were dissolved in 2% Tween 80 solution immediately prior to use and administered orally one hour before the experiment sessions and maximum volume administered was 10 ml/kg.

Doses of the extract were selected based on the outcome of the acute toxicity test. As per OECD (2001) guideline $1/10^{th}$ of the limit dose 2000 mg/kg was taken as a mid dose (200 mg/kg) after which half and double of the mid dose were selected as minimum and maximum doses (100 mg/kg and 400 mg/kg respectively) for the study. The dose of the standard drug imipramine (30 mg/Kg) was set based on prior antidepressant screening studies (Moallem *et al*., 2007; Murugan *et al*., 2011; Porsolt *et al*., 1977; Selvi *et al*., 2012).

3.7. Antidepressant activity tests

3.7.1. Tail Suspension Test

The tail suspension test (TST) is based on the fact that animals subjected to the short-term, inescapable stress of being suspended by their tail, will develop an immobile posture. This protocol is conceptually related to the forced swim test (FST) (Steru *et al*., 1985; Castagné *et al*., 2010). After 60 min of administration of the treatment as per the respective grouping, male mice were hung upside down from a counter top of 50 cm of height using an adhesive tape placed approximately1cm from the tip of their tails. The mice were judged to be immobile when they hanged motionless, making those movements necessary for respiration only. The duration of immobility was recorded for

6 min using a stopwatch and a video record of the entire session was documented for blind recording to avoid bias (Rojecky *et al*., 2004).

3.7.2. Forced Swim Test

Male rats were forced to swim individually in a transparent glass container (20cm in diameter and height of 40 cm) containing fresh water of 19 cm height which was maintained at 25^0C (± 3^oC). There were two sessions; in the first session (pre- test) the untreated male rats were allowed to swim in the container for 15 min each without any behavioral recording. This was done in order to check the fitness level of each animal and to obtain a stable immobility time profile (Velmurugan *et al*., 2013). In the second session (24 h after the pre-test), the rats were allowed to swim for 5 min following treatment (60min, 4h and 24h before the test) and the total duration of immobility was recorded using a stop watch The test was recorded with a video camera placed horizontally to the glass container (Muhammad *et al.,* 2011; UmadevI *et al.,* 2011).

The rats were judged to be immobile when they remained floating without struggling, and making movements necessary only to keep their heads above the water. On the contrary, swimming was defined active escape or struggling movements such as head dipping, paddling with all four legs, circling the tank, and clambering at the walls. Fresh water was replaced after each rat. Following the sessions, each rat was dried with a towel and placed in a rodent heater (UGO basile, Italy) before it was returned to its cage (Batool *et al*, 2011; Porsolt *et al*., 1977).

3.7.3. Open Field Test

Open field test (OFT) was carried out in order to rule out any non-specific locomotor effect the extract might possess. Mice were administered with the same doses that produced anti-immobility effects in the TST and FST to assess their effect on locomotor activity using the open-field paradigm. Briefly, after an hour of dosing, the animals were individually placed in the centre of the OFT apparatus, which is a simple cubic dark wooden box (68cm x 68cm x 45 cm) with the floor divided into 16 squares to make up the central and peripheral squares (Machado *et al*.,2008). The OFT apparatus was illuminated by a 60W bulb placed perpendicularly above it. Number of peripheral

crossings (ambulation), activity in the centre (central crossings) and total locomotion, which is the sum of the two, were recorded for 5 min. The surface of the apparatus was cleaned with alcohol and cotton swab after each mouse to avoid potential cues. Caution was taken not to make any sudden movement and sound noise was reduced as much as possible. Video recording of the whole session was taken.

3.8. Preliminary phytochemical Screening and TLC analysis

3.8.1. Phytochemical Screening

The 80% methanol crude extract of *Rosa abyssinica* and its solvent fractions were used to run various preliminary phytochemical screening tests. This was done to verify the presence or absence of certain secondary metabolites following standard procedures as described in Trease and Evans (1989).

Test for polyphenols

To 2 ml of filtered solutions of aqueous and 80% methanol extracts of the plant material, three drops of a mixture (prepared immediately before the reaction) of 1 ml 1% $FeCl_3$ and 1 ml 1% $KFe(CN)_6$ were added separately and the formation of a green blue color was inspected.

Test for Saponins

Froth formation: 0.5 g of a hydro-alcoholic extract was dissolved in 10 ml of distilled water in a test tube. The test tube was stoppered and shaken vigorously for 30 sec and allowed to stand in a vertical position and observed over 30 min. The formation of "honey comb" froth that persisted for half an hour indicates the presence of saponins.

Chemical test: To 2 ml of the aqueous solution of the 80% methanol extract, 1 ml of 10% solution of sodium nitrate and 3 drops of concentrated H_2SO_4 were added and the formation of a bloody red color was inspected.

Test for steroids (Salkowski test)

Hundred milligrams of the 80% methanol crude extract were dissolved in 2 ml of chloroform to which concentrated H_2SO_4 was added carefully to form a lower layer. The

formation of a rose color which changes to violet and bright blue or reddish brown color at the interface was inspected.

Test for alkaloids

Two grams of the crude extract was treated in a test tube with 10 ml of 1% HCl for 30 min in a water bath and then filtered through cotton into a test tube. Small portion of the solution was transferred into two test tubes and to one of the test tubes, five drops of Dragendorff's reagent and to the other one five drops of Mayer's reagent was added. The formation of yellowish orange precipitate (Dragendroff's reagent) or whitish opalescence (Mayer's reagent) was inspected.

Test for free anthraquinones

Hundred milligrams of the 80% methanol extract was shaken vigorously with 10 ml of benzene and the extract was filtered. The filtrate was treated with 5 ml of 10% ammonia solution and was shaken. The formation of pink, violet or red colour in the ammonia phase was considered as positive for free anthraquinones.

Test for flavonoids

To 2 ml of the 80% methanol extract in methanol, five drops of 2% lead acetate solution were added and the development of yellow or orange color was inspected.

Test for tannins

Two milliliters of water diluted sample was treated with 3 drops of 10% ferric chloride and formation of bluish-black color indicates presence of tannins.

Test for terpenoids

Five milliliters solution of the extract was mixed with 2 ml of chloroform and concentrated sulphuric acid of 3 ml was added to form layer. The appearance of a reddish brown color at the interface indicates the presence terpenoids.

Test for coumarins

To 100 mg of the 80% methanol extract dissolved in 5 ml of ethanol, 2 ml of 10% ammonia were added and the occurrence of an intensive fluorescence under UV light was

inspected. Comparison was made by taking another 5 ml of the extract in ethanol as a reference.

3.8.2. TLC analysis

Pre-coated plastic silica sheets (Macherey-Nagel Gmbh & Co, Germany) 0.2 mm thick, were used to run analytical TLC. 500mg of the crude extract was dissolved in 2 ml 80%methanol and couple of drops were loaded using a thin capillary glass rod at 1 cm from the bottom of the plate. The solvent systems used were hexane: ethyl acetate: in a ratio of 9:1(non-polar), hexane: ethyl acetate in the ratio of 1:1 (medium polarity) and chloroform: methanol in the ratio of 4:1 (polar). The chambers (for each solvent system) were allowed to saturate for at least 10 min before the TLC plates were placed. After the solvent front reached $3/4^{th}$ of the TLC height, the plates were removed from the chamber and allowed to dry. The plates were then observed in a UV cabinet at different wavelengths. This TLC was done to provide a finger print of the crude extract by providing a rough number of components and nature in the extract indicated by the bands on the plate.

3.9. Data Analysis

The data obtained from the experiment was evaluated by SPSS windows version 16.0 using one-way analysis of variance (ANOVA) followed by Tukey's post-Hoc analysis. All the results observed from the experiment were expressed as mean ± S.E.M and level of significance was set at $p<0.05$. The percentage reduction in the duration of immobility was calculated by divining the mean duration of immobility for the respective treatment group by the mean duration of immobility for the control group and multiplied by one hundred.

4. Results

4.1. Acute toxicity study

The acute toxicity study revealed the non-toxic nature of the 80% methanolic fruit extracts of *Rosa abyssinica* at a limit dose of 2000 mg/kg. This finding suggests the LD_{50} of the extract to be above 2000 mg/kg as no signs of overt toxicity and mortality were observed in the extract-treated animals. Moreover, the extract did not produce significant changes in behaviors such as alertness, restlessness, breathing, diarrhea, convulsions and coma during the observation period of two weeks.

4.2. Effect of the crude extract in mice tail suspension test

As shown in Table 1, at the dose of 100 mg/kg, the crude extract showed 12.8% reduction in the duration of immobility which was insignificant as compared to the control group. On the other hand, at doses of 200 mg/kg (24.7%, $p< 0.01$) and 400 mg/kg (37.1%, $p< 0.01$) the extract exhibited a significant reduction in immobility.

Table 1: Effect of 80% methanol fruit extract of *Rosa abyssinica* on duration of immobility in mice tail suspension test.

Treatment groups	Duration of immobility(s)	Percent reduction in time of immobility
Control	160.4±7.1	-
Imipramine 30 mg/kg	91.4±4.7 $^{a**, c**}$	43.0
RA 100 mg/kg	139.9±7.1^{b**}	12.8
RA 200 mg/kg	120.6±7.5 $^{a**, b*}$	24.7
RA 400 mg/kg	100.9±5.1 $^{a**, c**}$	37.1

Values represent mean ± S.E.M (n= 8); ^{a} compared to control; ^{b} compared to standard; ^{c} compared to to 100mg/kg; *p< 0.05, **p< 0.01; Control received 2% Tween 80 in distilled water; RA= *Rosa abyssinica* crude extract

The highest effect of the extract was observed at the dose of 400 mg/kg, which had a significant difference with the group receiving 100mg/kg of the extract ($p< 0.01$). The

standard drug imipramine exhibited the highest percentage reduction in immobility compared to the other groups (43.0%). However, no statistically significant difference was observed between the effect produced by imipramine and 400 mg/kg of the extract.

4.3. Effect of the crude extract in rat forced swim test

The anti-immobility effects demonstrated by different doses of 80% methanolic extract of *Rosa abyssinica* in the rat FST are given in Table 2. The extract at the dose of 100mg/kg failed to show a marked decrease in immobility (9.71%) compared with the control group. The test dose of 200 mg/kg, on the other hand, was able to exhibit a significantly higher percentage reduction in immobility time at (33.6%, $p< 0.01$) with regard to the vehicle treated group. A similar but greater reduction in duration of immobility was also observed at 400 mg/kg (35.6%, $p< 0.01$). Imipramine displayed the highest percentage reduction (36.7%) in the duration of immobility in comparison with the vehicle or extract treated groups. However, significant difference was noted only when compared to controls and 100 mg/kg dose ($p< 0.01$ in both cases).

Table 2: Effect of 80% methanol extract of fruit of *Rosa abyssinica* in rat forced swim test.

Treatment group	**Duration of immobility(s)**	**Percent reduction in time of immobility**
Control	151.9±11.18	-
Impiramine 30 mg/kg	96.1±9.45 $^{a**, c**}$	36.7
RA 100 mg/kg	137.1±4.3 ^{b*}	9.7
RA 200 mg/kg	100.9±5.9 $^{a**, c*}$	33.6
RA 400 mg/kg	97.9± 5.8 $^{a**, c**}$	35.6

Values represent mean ± SEM (n= 8); ^{a} compared to control; ^{b} compared to standard; ^{c} compared to 100 mg/kg; *p<0.05, **P< 0.01; Control received 2% Tween 80 in distilled water; RA= *Rosa abyssinica* crude extract

4.4. Effect of the crude extract on locomotion in the open field test

The effect of treatment with 80% methanol crude extract of *Rosa abyssinica* on the locomotor behavior of mice in the OFT is presented in Table 3. The outcome of the test revealed that the doses of the extract that were able to display antidepressant-like response, did not exhibit significant change in locomotion. The total square crossing recorded after administering doses 200 mg/kg (4.7%) and 400 mg/kg (16.1%) though not significant, were higher than all the other groups. The plant at 100 mg/kg displayed an insignificant decrease of 8.4 % in total locomotion. The groups receiving imipramine and the vehicle had comparable total locomotion. On the other hand, central crossings for the extract treated animals seemed to be highest for the lowest dose (100mg/kg), though the difference was not statistically significant compared to the control.

Table 3: Effect of 80% methanol extract of *Rosa abyssinica* on locomotion and exploratory behaviors in mice open field test

Treatment group	Number of crossings			Percent change in total locomotion
	Peripheral squares	Central squares	Total squares	
Control	60.2 ± 10.8	7.2 ±2.3	67.3 ± 12.7	-
Imipramine 30 mg/kg	60.7 ±10.0	5.5 ± 1.3	66.2 ± 11.0	- 1.7
RA 100 mg/kg	59.5 ±6.9	6.3 ± 2.9	61.7 ± 9.7	-8.4
RA 200 mg/kg	67.5 ± 10.8	3.0 ± 0.7	70.5 ± 11.2	4.7
RA 400 mg/kg	74.5 ± 8.3	3.7 ± 0.8	78.2 ± 8.8	16.1

Values represent mean ± SEM (n=8); (- sign indicates decrease in total locomotion); Controls received 2% Tween 80 dissolved in distilled water; RA= *Rosa abyssinica* crude extract

4.5. Effect of the solvent fractions in mice tail suspension test

The antidepressant-like activity of the aqueous, methanol and ethyl acetate fractions of the crude extract of *Rosa abyssinica* was evaluated using the mice tail suspension test (Table 4). The different test doses of the aqueous fraction exhibited a considerable antidepressant-like effect. At 100 mg/kg, duration of immobility was decreased by 19.8% (Fig 2) compared to the control group, which failed to reach statistical significance. Both 200 mg/kg and 400 mg/kg doses significantly reduced duration of immobility by 38% ($p< 0.01$) and 30% ($p< 0.05$), respectively. This effect was comparable to that produced by the standard drug and the effect of 200 mg/kg seemed to be even better than imipiramine (36.7%).

The methanol fraction at the lowest test dose (100 mg/kg), slightly decreased immobility time (24.9%), which was found to be insignificant. However, at 200 mg/kg, there was a marked decrease in the duration of immobility with a percentage reduction of 31% ($p<0.05$) relative to control. This effect was also comparable to the effect to that observed with the standard drug imipramine which exhibited a 33.9% reduction ($p< 0.01$) compared to the control group. Surprisingly, the highest dose of 400 mg/kg, the methanol fraction displayed the least effect (10.1%) in reduction of immobility, which was not significantly different to that noted with control groups (Fig 2).

The ethyl acetate fraction appeared to be the least effective among the fractions. All doses of the fraction failed to exhibit a significant reduction in the duration of immobility compared to controls, although they tended to produce a varying degree of reduction, with the highest effect noted with 100 mg/kg. This time the effect produced with the standard drug (35.9%) was significantly greater than that of 200 mg/kg ($p<0.05$) and 400 mg/kg ($p<0.05$).

Table **4:** Effect of the solvent fractions of the fruits of *Rosa abyssinica* on duration of immobility in mice tail suspension test.

Solvent fractions	**Animal Group**	**Duration of immobility**
Aqueous fraction	CON	151.0±10.6
	IMP 30 mg/kg	97.8±7.6 [a**]
	AF 100 mg/kg	121.1±8.4
	AF 200 mg/kg	93.8±10.9 [a**]
	AF 400 mg/kg	113.8±5.6 [a*]
Methanol fraction	CON	144.4±9.6
	IMP 30 mg/kg	95.4±6.9[a**]
	MF 100 mg/kg	108.4±16.2
	MF 200 mg/kg	99.6± 7.3 [a*]
	MF 400 mg/kg	129.9±3.4
Ethyl acetate fraction	CON	141.3±7.1
	IMP 30 mg/kg	90.5±5.9[a*]
	EAF 100 mg/kg	114.4±14.1
	EAF 200 mg/kg	140.1± 15.9 [b*]
	EAF 400 mg/kg	144.0±6.9 [b*]

Values represent mean ± SEM (n= 8); [a] compared to control; [b] compared to standard;; *p<0.05, **p< 0.01; (- sign indicates increase in time of immobility); CON= control (received 2% Tween 80 in distilled water); (IMP= imipramine, AF= aqueous fraction, MF= methanol fraction and EAF= ethyl acetate fraction)

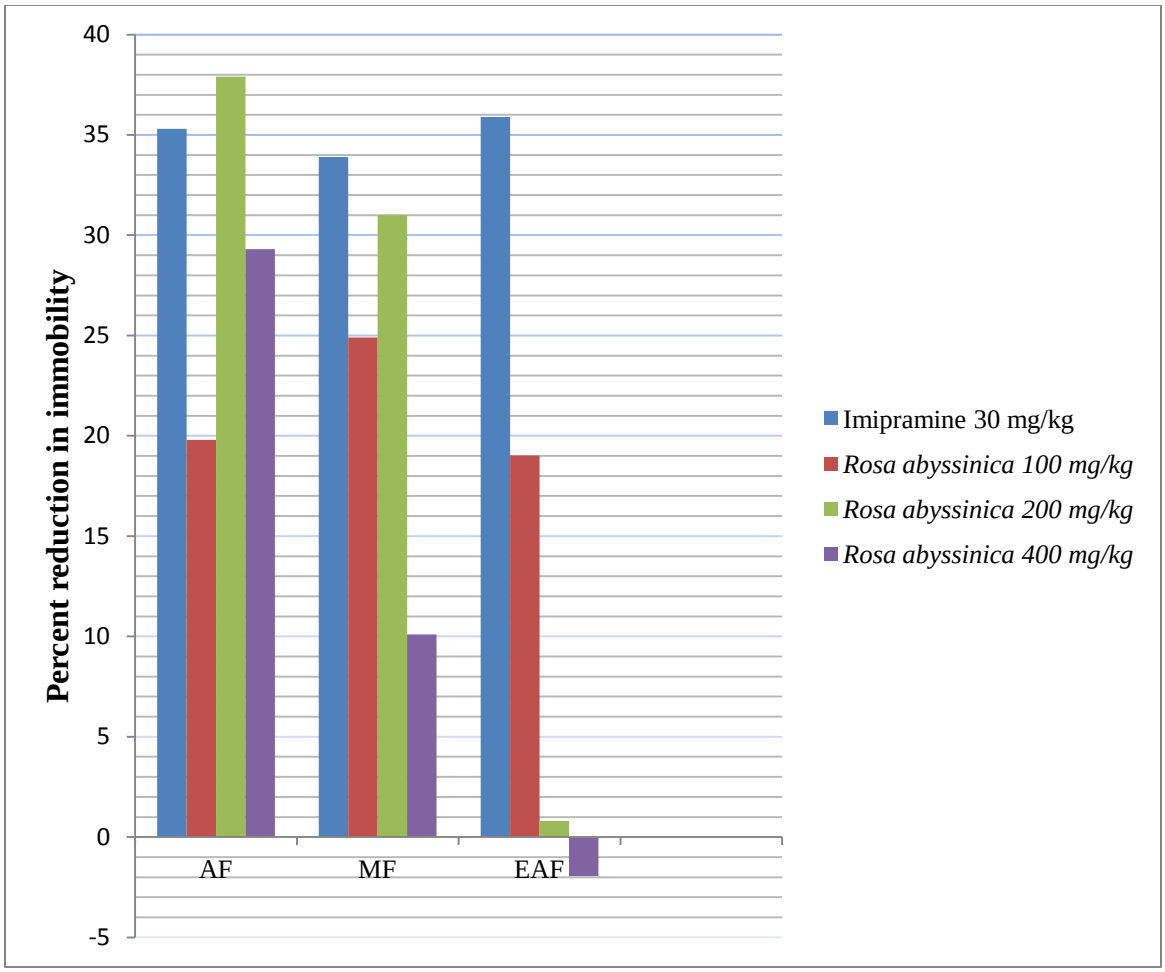

Fig 2. Effect of the solvent fractions on the percentage reduction in immobility time in mice tail suspension test (AF= Aqueous fraction; MF= Methanol fraction; EAF= Ethyl acetate fraction)

4.6. Preliminary phytochemical screening

The various preliminary phytochemical screening tests carried out indicate the presence of certain secondary metabolites in the fruits of *Rosa abyssinica* (Table 5). Both the crude extract and fractions appeared to have by and large a similar profile of secondary metabolites. Constituents such as alkaloids, saponins and terpenoids were absent. In contrast, polyphenols and tannins were detected in both the crude extract and its fractions.

Polyphenols are phytoconstituents with a wide distribution in the plant kingdom. Their basic structure consists of aromatic rings with varying number of hydroxyl groups

attached to it. There are various classes of polyphenolic compounds based the number of phenol rings and functional groups (D'Archivio *et al.*, 2007).The polyohenolic compounds detected in the fruits of *Rosa abyssinica* include flavonoids and tannins. Flavonoids have a wide distribution in plants and more than 4000 types have being identified so far. They are valued for their appreciable anti-inflammatory and CNS action (Jäger and Saaby , 2011). Tannins are complex plant products known for their astringent properties and have been used to tan animal hides. Tannins isolated from certain plants have been shown to possesss antidepressant activities (Pemminati *et al*., 2010).

Table 5: Secondary metabolites detected in 80% methanol crude extract and solvent fractions of the fruits of *Rosa abyssinica*.

Secondary metabolites tested	**Crude extract of *Rosa abyssinica***	**Solvent fractions**		
		Aqueous fraction	**Methanol fraction**	**Ethyl acetate fraction**
Alkaloids	-	-	-	-
Saponins	-	-	-	-
Polyphenolic compounds	+	+	+	+
Terpenoids	-	-	-	-
Tannins	+	+	+	+
Flavonoids	+	-	+	+
Steroidal compounds	+	-	-	+
Coumarins	-	-	-	-
Free anthraquinones	-	-	-	-

Key: + indicates presence, – indicates absence

4.7. TLC fingerprint

TLC analysis was performed using various combinations of solvent systems. The solvent system containing a mixture of hexane and ethyl acetate produced a better separation of the constituents. The analysis revealed the presence of at least four compounds (Fig 3).

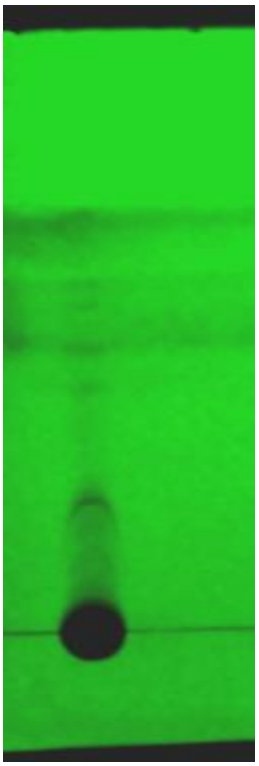

Fig 3: Thin layer chromatography fingerprint of 80% methanol extract of the fruits of *Rosa abyssinica*: plate developed by a solvent system containing hexane: ethyl acetate (1:1) and is observed under UV-light at a short wavelength of 254nm.

5. Discussion

Over the years many drugs have been introduced in the market for the management of depression although they are associated with disabling side effects and questionable efficacy. This fact calls for the development of antidepressants derived from herbal sources with a better rate of success and ability to achieve full remission and minimal incidence of side effects (Rajput, 2011). The present study attempted to evaluate the antidepressant-like activity of *Rosa abyssinica*, a plant claimed to be used for the management of depression in the Ethiopian folk medicine (Chekole, 2011).

5.1. Behavioral despair based tests

Rodent models of depression namely FST in rats and TST in mice, were used to assess the antidepressant-like activity of the experimental plant. The immobility displayed by rodents when subjected to aversive stimuli such as being suspended by the tail or forced to swim in a confined cylinder is thought to reflect a state of despair or lowered mood (Krishnan, 2011; Parra *et al*., 1999; Pemminati *et al*., 2010). The duration of immobility has been shown to be reduced by treatment with antidepressant drugs and a significant correlation was found between the clinical efficacy of antidepressant drugs and their potency in both models (Sharma, *et al*., 2009; Steru *el al*., 1985).

Male animas were used to screen the potential antidepressant-like activity of the plant. This is based on previous findings that female rats or mice are less susceptible to behavioral suppression in the models of depression compared to their male counter parts due to a higher arousal level even at the basal state (Palanzi, 2001).Moreover, the OECD recommends rats as the species of choice to do toxicity screening studies. And the increased susceptibility of female rats to toxicity offers a more accurate insight into the toxic nature of compounds to be tested (OECD, 2001).

TST was used as the model of choice for assessing the antidepressant- like activity of the solvent fractions for various reasons. First, the “immersion in water” in FST, which is necessary to produce the "behavioral despair", induces hypothermia in animals causing a behavioral change that can affect the result. Second, the ease of identifying immobility in TST as compared to FST makes TST a more suitable model. Third, TST is claimed to be

more sensitive to lower doses of drug and provides a clearer dose-effect relationship. Finally, the highest percent inhibition in immobility in this study was seen in the TST as compared to the FST (Steru *et al*., 1985).

After administering the test dose of 100 mg/kg, the crude as well as all the solvent fractions failed to show a significant antidepressant-like activity in the TST. This indicates that 100 mg/kg is a sub-therapeutic dose for this effect. The crude extract as well as the aqueous and methanol fractions exhibited a reduction in the time of immobility at the dose of 200 mg/kg. However, the effect of the aqueous fraction at this dose was relatively better than imipramine, while the effects of the crude extract and the methanol fraction were not. Moreover, the aqueous fraction was the most effective amongst all fractions, and 200 mg/kg could be taken as the maximum effective dose.

Increasing the dose further produced the highest effect with the crude extract but this trend was not observed in the fractions. In the aqueous fraction increasing dose produced a significant reduction, albeit to a smaller extent compared to 200 mg/kg. By contrast, no apparent change in immobility was observed with 400 mg/kg of the methanol fraction compared to controls. In the ethyl acetate fraction, one could somehow see a similar trend, even more, there was an increase in immobility at 400 mg/kg though the change failed to reach statistical significance.

These observations could possibly be explained in a variety of ways as follows: i) The doses of the fractions might not be in the linear portion of the dose-response curve, as there was a prior increase in activity followed by a subsequent drop. Alternatively, the fractions might contain constituents, whose concentration increased with dose and possibly interfere with the activity and this influence might be relieved in the crude extract. ii) Many flavonoids and neuroactive steroids have been reported to be ligands (like benzodiazepines) for the $GABA_A$ receptors in the CNS. It is established, that GABA agonists such as diazepam respond to the TST by increasing time of immobility (Sajid *et al.*, 2013; Steru *et al*., 1985). Thus, lack of effect by the ethyl acetate fraction could be attributed to the presence of steroids and flavonoids in this fraction. Indeed, the absence of both secondary metabolites in the aqueous fraction could probably be the major reason why this fraction was endowed with the highest activity. Another note one should make

at this juncture is that the methanol fraction did contain flavonoids but not steroidal compounds. However, this fraction displayed activity at 200 mg/kg, which was lost at 400 mg/kg. This might indicate that steroids could play the major inhibitory role than flavonoids, and a positive role, if any, played by flavonoids is overcome with increasing concentration. iii) It is interesting to note that the antidepressant-like activity of the solvent fractions in the TST decreased with decreasing polarity, possibly indicating to the fact that polar constituents are responsible for the observed effect. iv)The absence of waning of effect with dose in the crude extract reiterates once again the importance of interaction amongst the different constituents that either create synergy or overcome interferences.

In FST, the crude extract displayed a notable reduction in the duration of immobility in comparison to the negative control animals, which is suggestive of a considerable antidepressant-like activity. At 100 mg/kg the extract didn't show a significant reduction in the duration of immobility probably due to a sub-threshold concentration of the neuroactive phytochemicals as observed in the TST. Nevertheless, significant reduction in immobility was seen at the doses of 200 mg/kg and 400 mg/kg, which were comparable to the commercially available TCA imipramine. This indicates an increase in the concentration of the active principles or in the ability to neutralize the effect of inhibitors with increasing dose of the extract.

FST and TST are similar in their working principle and more often than not the data from these two models is converging (Cryan *et al.,* 2003). The activity of the extract in both models observed in the present study thus strengthens the possibility that *Rosa abyssinica* indeed is a potential candidate for the treatment of major depressive disorders. The variability in the response of different antidepressants in these models, however, indicates potentially different substrates and neurochemical pathways mediating performance in these tests (Chatterjee et al., 2011; Cryan and Mombereau, 2004). These issues possibly underlie the observed behavioral differences between the two tests in the current study. The highest percent reduction in time of immobility was seen in the TST than FST, possibly due to the fact that any possible confounds induced by stressful hypothermic exposure in the FST are not present in TST.

Even though at this point it's a bit farfetched to decide which phytoconstituents are responsible for the antidepressant-like activity of *Rosa abyssinica*, it is probably reasonable to attribute the antidepressant-like activity partially to the presence of polyphenolic compounds such as tannins in the extract. Previous studies indicated that tannic acid has been shown to possess a non selective inhibitory effect on MAO, causing a general elevation of monoaminergic neurotransmission in the brain (Dar *et al*., 1998; Pemminati *et al*., 2010; Shekar *et al*., 2012). Other polyphenolic compounds such as rosmarinic acid and its metabolite have also been reported to have antidepressant activity (Emamghoreishi and Talebianpour, 2009). Another possible mechanism of action is the attenuation of oxidative stress, by the polyphenols and tannins present in *Rosa abyssinica.* Exact mechanisms underlying the antidepressant- like activity of the extracts remain to be seen.

5.2 Effect on locomotor activity in the OFT

OFT is one of the rodent models of anxiety and was designed by Hall in 1934 (Bourin *et al*., 2007). This test is also used to assess locomotor activity based on total number of square crossings. It is known that antidepressants do not increase the locomotor activity of mice but instead cause a slight decrease in motor activity and can cause loss of muscle tone. Psychostimulant drugs such as caffeine and amphetamine may give a false positive result in TST. Therefore, the outcome of this test is important to rule out any non-specific activity of this plant (Machado *et al*., 2008; Porsolt *et al.*, 1977).

In this study, it was demonstrated that the crude extract of *Rosa abyssinica* didn't significantly alter the spontaneous locomotor activity of mice during the OFT. This in indicative that, at the doses tested, the antidepressant-like activity of *Rosa abyssicia* is unlikely to be a false positive as a result of psychostimulant action. But it is noteworthy that, although it was insignificant, there was an increase in the total squares crossed with increasing dose of the extract. This might be a crucial indicator that at higher dose the plant might possess a psychostimulant activity.

5.3. Limitations of the study

The major limitation of this study arises from the very nature of the antidepressant screening tests. Even though the FST and TST both have excellent predictive validity in screening for a potential monoamine based antidepressant, they lack face validity and have poor construct validity which are characters of a good animal model. They do not possess the ability to mimic the true disease process and antidepressant response which usually takes at least 2-3 weeks before any resolution of symptoms is observed. The other limitation is the absence of a mechanistic approach to measure neurochemical changes that occur with drug administration which would have been helpful to elucidate the mechanism of action involved. Activity recording was carried out manually which can also be a limiting factor as it can be source of bias. And lastly, scarcity of resources has also restricted the scope of the study.

6. Conclusion

Based on the findings obtained from this study, it is safe to infer that the crude extract of the fruits of *Rosa abyssinica* possesses a significant antidepressant-like activity. This is indicated by the decrease in the duration of immobility in established behavioral despair based models of depression. The antidepressant-like effect of the aqueous and methanol fractions of the extract as observed in the TST is also remarkable whereas the ethyl acetate fraction is devoid of antidepressant-like activity, indicating that the polar constituents are possibly the ones responsible for the activity. The outcome of OFT indicates that the plant has no significant effect on locomotor activity suggesting that the antidepressant- like activity observed is not caused by a non-specific motor stimulation. Hence, further studies on this plant should be pursued in order to reap the best possible therapeutic benefits.

7. Recommendations

- The outcome of this study supports the potential use of *Rosa abyssinica* in the management of depressive disorders. Thus, further studies using other models of depression should be carried out, including chronic model of depression.
- Further pharmacological and neurobiological tests should be performed in order to elucidate the mechanism of action involved.
- The fractions of the crude 80% methanolic extract should be tested using other models of depression other than the TST.
- Additional confirmatory screening tests are required to verify the phytoconstituents responsible for the observed antidepressant effect displayed by the plant.
- It is also worth to undertake further research in isolating the various phytochemicals since it may provide compounds which may have great potential for management of depression.
- The probable psychostimulant effect of the plant should be assessed at higher doses of the extract.

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