Evaluation of the antidepressant-like activity of solvent fractions of *Hypericum revolutum* (Hypericaceae) in rodent models of depression

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This is to certify that the thesis prepared by Seife Demissie entitled "Evaluation of the antidepressant-like activity of the solvent fractions of *Hypericum revolutum* (Hypericaceae) in rodent models of depression" and submitted in partial fulfillment of the requirements for the degree of master of science in pharmacology complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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ABSTRACT

Evaluation of the antidepressant-like activity of the solvent fractions of *Hypericum revolutum* in rodent models of depression

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Addis Ababa University, 2017

Various species of the genus *Hypericum* have been used for different purposes. A previous study showed that 80% methanol extract of *Hypericum revolutum* was active in animal models of depression. In the current study, the antidepressant-like activity of the various fractions of the plant at doses of 100, 200 and 400mg/kg were investigated using different depression paradigms. Furthermore, phytochemical screening and thin layer chromatography (TLC) investigation were made. Antidepressant-like activity was detected in the butanol and chloroform fractions of this species in the forced swim test (FST) and tail suspension test (TST) being both fractions with more profound effect at the middle and higher doses induced a significant reduction of the immobility time, producing no effects on spontaneous motor activity when assessed in open field test (OFT). However, the 100mg/kg of butanol fraction was unable to replicate the effect seen in TST in FST. In conclusion, the present study results together with phytochemical and TLC-data indicated that *Hypericum revolutum* possess antidepressant-like effects in mice/rats. Moreover, the different phytoconstituents, such as anthraquinones, flavonoids, alkaloids, saponins and tannins or combined derivatives, could be responsible at least in part for the antidepressant-like effect observed for this species.

Key words: *Hypericum revolutum*, antidepressant-like activity, solvent fractions, forced swimming test, tail suspension test, open field test.

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ABBREVIATIONS AND ACRONYMS

5- HT	5-hydroxytryptamine (Serotonin)
AAU	Addis Ababa University
AF	Aqueous Fraction
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
APA	American Psychiatric Association
BDNF	Brain Derived Neurotrophic Factor
BF	Butanol Fraction
$_{C}$AMP	Cyclic Adenosine Monophosphate
CF	Chloroform Fraction
CNS	Central Nervous System
CREB	Cyclic-AMP Response Element Binding Protein
DA	Dopamine
DNRI	Dopamine Norepinephrine Reuptake Inhibitor
DSM- V	Diagnostic and Statistical Manual of Mental disorders, 5th edition
EPHI	Ethiopian Public Health Institute
EPHARM	Ethiopian Pharmaceuticals Manufacturing
FST	Forced Swim Test
GABA	Gamma Aminobutyric Acid
HPA	Hypothalamic Pituitary Adrenal

H. revolutum	*Hypericum revolutum*
MAOI	Monoamine Oxidase Inhibitor
MDD	Major Depressive Disorder
NA	Noradrenaline
NAc	Nucleus Accumbens
NaSSA	Noradrenergic and Specific Serotonergic Antidepressant
NMDA	N-methyl-D-aspartate
OECD	Organization for Economic Cooperation & Development guideline
OFT	Open Field Test
$_P$CREB	Phosphorylated CREB
PDD	Persistent depressive disorder
P.O.	Per Oral (by mouth)
SJW	St John's Wort
SNRI	Serotonin Norepinephrine Reuptake Inhibitor
SPSS	Statistical Package for Social Sciences
SSRI	Selective Serotonin Reuptake Inhibitor
TCA	Tricyclic Antidepressant
TLC	Thin Layer Chromatography
TRD	Treatment Resistant Depression
TST	Tail Suspension Test
WHO	World Health Organization

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1. INTRODUCTION

1.1 Overview of depression

Mental disorders impose an enormous disease burden on societies throughout the world. According to World Health Organization (WHO), about 450 million people suffer from a mental or behavioural disorder (WHO, 2004; Cockerham, 2016). This amounts to 12.3% of the global burden of disease and predicted to rise up to 15% by 2020 (Reynolds, 2003).

Disorders of mood, or affect, have been described since the 4th century BC. Despite this early acknowledgement, their etiology is still a source of debate. There is a growing knowledge on depression. Recently, far from being a disease with purely psychological manifestations, depression is now known as a complex disorder involving the whole body. The diagnosis of depression is based on a heterogeneous set of symptoms (Gronli, 2006; Bhat *et al.*, 2016). Depression alone affects more than 350 million persons worldwide (WHO, 2013) and is the single largest contributor to years lived with disability globally and projected to be the second leading cause of disability, after heart disease by 2020 (Reddy, 2010; Feigin *et al.*, 2016) and will move to the first place by 2030 (WHO, 2012a).

Depression is characterized by depressed mood, loss of interest or pleasure in most activities, feelings of guilt or low self-worth, disturbed sleep or appetite, feelings of tiredness, poor concentration, psychomotor retardation or agitation, recurrent thoughts of death or suicide (Marcus *et al*., 2012; APA, 2013). It is normal to feel down once in a while, but if the sadness persists, become and substantially impair an individual's ability to function at work or school or cope with daily life, is characterized by depression. It could affect anyone, regardless of age, sex, social status, race or culture (APA, 2010; Patel, 2017).

Moreover, depending on the number and severity of symptoms, a depressive episode can be categorized as mild, moderate, or severe (APA, 2013). An individual with a mild depressive episode will have some difficulties in continuing with ordinary work and social activities but will probably not cease to function completely. On the other hand, it is very unlikely that the individual with severe depressive episode will be able to continue with social, work, or domestic activities, except to a very limited extent. As for moderate depression, the individual would normally have more than five symptoms that are needed to make the diagnosis of depression. Moderate episodes have a severity that is intermediate between mild and severe depression (WHO, 2008).

At its most severe, depression can lead to a suicidal attempt. Almost 1 million lives are lost yearly due to suicide (WHO, 2012). This number makes it be the second leading cause of death in the age range of 15-29 years (WHO, 2016; Patel, 2017). Yet, depression can be prevented and treated.

A better understanding of what depression is, and how it can be prevented and treated, will help to reduce the stigma associated with it, this further leads to more people seeking help that can be reliably diagnosed and treated by not specialists as part of primary health care. Specialist care is needed for a small proportion of individuals with complicated depression or those who do not respond to first-line treatments (Abadiano *et al*., 2015; Shimmy, 2016).

1.2 Types of depression

There are many different types of depression, of which some are caused by events of life and the rest caused by chemical changes in the brain. According to the recent edition of Diagnostic and Statistical Manual of Mental disorders of the American Psychiatric Association (DSM-V), some forms of depression are slightly different, or they may develop under unique circumstances, (APA, 2013; Jorm *et al*., 2013).

One form of depression is a major depressive disorder (MDD) or clinical depression. It is a common but serious mood disorder. It causes severe symptoms that affect how someone feels, thinks, and handle daily activities, such as sleeping, eating or working. Diagnosis of depression requires the existence of symptoms for at least two weeks. There are also other different types of depression including the new additions, persistent depressive disorder (PDD)**,** perinatal depression, psychotic depression, melancholic depression, seasonal affective disorder, bipolar disorder, disruptive mood dysregulation disorder, premenstrual dysphoric disorder, situational depression and atypical depression (Paykel, 2008; Rapkin and Lewis, 2013; Parker, 2014). The difference among them focuses on illness duration and etiology (APA, 2013).

1.3 Prevalence and risk factor for depression

According to the national institutes of health, factors that increase the risk of depression include genetic or other biological factors, interpersonal factors, and certain psychological and personality characteristics. While depression is the leading cause of disability for both males and females, the burden of depression is twice for females than males and for middle-aged more often than younger people (WHO, 2008). It is believed that the increased chance of depression in women may be related to changes in hormone levels that occur throughout a woman's life (Akhter, 2016). These changes are evident during puberty, pregnancy, and menopause, as well as after giving birth or experiencing a miscarriage. In fact, depression is the leading cause of disease burden for women in both high-income and low- and middle-income countries (WHO, 2008).

Although, prevalence varies, from a low of 2.6% among males in the Western Pacific Region to 5.9% among females in the African Region. Besides, prevalence rates vary by age, peaking in older adulthood (above 7.5% among females aged 55-74 years, and above 5.5% among males). Depression also occurs in children and adolescents below the age of 15 years but at a lower level than older age groups (WHO, 2017). The lifetime prevalence of depression is reported to be 16.9 and the 12-month prevalence is 10 in the US (WHO, 2012).

Although there were not sufficient nationwide survey conducted in Ethiopia to determine the prevalence of depression, a survey done by WHO in collaboration with Jimma University indicated that the prevalence of depression in Ethiopia was 9.1% (Hailemariam *et al.*, 2012). On the other hand, the prevalence of depression in Ethiopia was reported to be 6.5% according to the Ethiopian Federal Ministry of Health report of 2012 (Bitew, 2014).

Research in developing countries suggests that maternal depression may be a risk factor for poor growth in young children (Rahman *et al.*, 2008). Maternal mental health in low-income countries may have a substantial influence on child growth, with the effects of depression affecting not only this generation but also the next (WHO, 2012). The potential risk factors of depression also include chronic illness like HIV/AIDS (Ciesla and Roberts, 2001), the habit of substance abuse, stigma, partner violence, migration and parental violence (Bitew, 2014). Other factors that could increase the risk include: family history of mood disorders, history of mood disorders in early reproductive years, loss of a parent before age 10, loss of social support system or the threat of such a loss, ongoing psychological and social stress, such as loss of a job, relationship stress, separation or divorce, physical or sexual abuse as a child, and use of some medicines (Hammen, 2016).

1.4 Etiology and Pathophysiology of depression

The causes of depression are likely multifactorial. Although, the specific pathogenic cause is unknown, it is widely accepted that its etiology, course and long-term prognosis are influenced by genetic, neurobiological, psychological and social/environmental factors (England and Sim, 2009; Wittenborn *et al*., 2016). This disorder is characterized by wide a range of symptoms that reflect alterations in cognitive, psychomotor and emotional processes (Nemeroff and Vale, 2005; Ashwani and Preeti., 2012). Its chronicity is indicated by periods of remissions and recurrences, although the course of depression varies widely from person-to-person (O'Connor *et al.*, 2016). While adverse life events increase the likelihood of depression, genetic factors may predispose persons to be affected by environmental factors, such as life events, to a greater or lesser degree (Wittenborn *et al*., 2016).

According to the cognitive model of depression, depressed persons have characteristic "depressogenic" ways of acquiring and processing of information from their environment, which implies that the way individuals interpret their experiences directly, influences the development of depression (Yoshimura *et al*., 2013). Other factors come into play as well in psychological models of depression, such as social skills, pleasant activities, and other life skills such as problem-solving (Lewinsohn *et al*., 1996; Tsuang *et al*., 2004; Disner *et al*., 2011).

The neuronal circuits of brain areas that regulate mood are frontal cortex, ventral hippocampus, nucleus accumbens (NAc), amygdala, hypothalamus, ventral tegmental area, dorsal raphe nuclei and locus coeruleus by projection to other parts of the brain (Berton and Nestler, 2006). Evidence shows that imaging and postmortem studies show shrinkage of the hippocampus and prefrontal cortex of depressed patients, with loss of neurons (Gold *et al.,* 2015). However, the precise mechanism that triggers clinical depression is not known yet.

In the early 20^{th} century the explanation of mental illness changed from a disease of the mind to brain dysfunction (Groli, 2006). Although many neurotransmitters and neurohormones have been linked to the pathophysiology of depression (e.g. norepinephrine, dopamine, thyroid hormones), studies have implicated disturbances in serotonin (5-HT, 5-hydroxytryptamine) system and Hypothalamic-Pituitary-Adrenal (HPA) axis, as well as interactions between the two systems (Hasler, G., 2010; Kharade *et al.,* 2010; Ashwani and Preeti., 2012). Animal models such as mice are critical in understanding cellular and molecular changes underlying depression. In addition, cell and molecular biology have proved useful to study the mechanisms of information processing, plasticity and neuronal survival involved in mood disorders (Groli, 2006; Yu *et al.*, 2011) and for development of better treatments.

1.4.1 Monoamines and depression

The main assumption of this classical hypothesis is that clinical depression is due to impairment of central monoaminergic function, a deficiency in the neurotransmission of 5-HT, norepinephrine (NA) and dopamine (DA) (Harvey, 1996; Lee *et al.,* 2010). There is growing evidence of a causal association between brain monoamines and affective disturbances. If brain monoamines are depleted by reserpine a significant proportion of mentally normal subjects suffer from a depression not distinguishable from severe endogenous depression (Sager and Torres, 2011; Ahmed *et al.,* 2014).

The monoamine concentrations may be altered as a result of disrupted synthesis, storage or release, or concentrations may be normal but postsynaptic receptors and/or sub-cellular messenger activity may be impaired (Nemeroff and Vale, 2005). Hence, the treatment of depression is supposed to increase the availability of the amines in the brain. Different mechanisms may increase the availability of brain monoamines. These include blocking the reuptake of the monoamine in the synapse, inhibiting the intraneuronal metabolism of the monoamine or blocking the presynaptic inhibitory auto- or heteroreceptors (Kharade *et al.,* 2010; Shalini, 2010). Monoamines affect a wide range of functions central in depression like sleep, vigilance, appetite, motivation, motor activity and reward and their imbalance may produce symptoms like aggression, euphoria and impulsiveness (Stevens and Price, 2016). Loss of interest or pleasure in activities that are normally pleasurable is one of the core symptoms of depression (McBrain, 2003). The brain dopaminergic system is also crucially involved in reward behavior and/or motivation, especially the mesolimbic projections to the NAc and prefrontal cortex (Ninković, 2006; Swamy, 2010). Moreover, a reduced plasma concentration of homovanilic acid (HVA), a dopamine metabolite, is found in depressed patients (Sher *et al.,* 2003).

Other brain chemicals and receptor may be involved in depression like neurokinins, gamma aminobutyric acid (GABA), glutamate, neuroactive steroids, opioids, cholecystokinin, histamine, nicotine and muscarinic (Bymaster and Felder, 2002; Oja and Saransaari, 2009). For example, several recent evidences suggested a single dose of ketamine, N-methyl-D-aspartate (NMDA) receptor antagonist, is effective in treating resistant depression with glutamate dysfunction (Murrough *et al*., 2013a; Murrough *et al*., 2013b). Thus there is no clear evidence for one transmitter being central to the etiology of depression (Tanti and Belzung, 2010). Therefore, the complex multifaceted nature of depression is made up of a variety of emotional, behavioral and cognitive elements (Lee *et al.,* 2010). It is possible that each of these components of the syndrome may involve different neurobiological substrates (Saveanu and Nemeroff, 2012).

1.4.2 Neuronal plasticity, information processing, neurogenesis and depression

One emerging hypothesis suggests that problems in information processing within specific neural networks might lead to depression without changing the chemical balance (Lindsay and Norman, 2013). Depression may arise when some neuronal systems do not exhibit appropriate and adaptive plasticity in response to external stimuli

(Chandrasekaran, 2013). Rodent studies have also shown altered neuronal plasticity in various brain regions including the hippocampus, prefrontal cortex, amygdala and NAs. This possibility is supported by the fact that most antidepressant drugs induce plastic changes in neuronal connectivity, which gradually leads to improvements in neuronal information processing and recovery of mood (Fisar and Hroudová, 2010; Kolb *et al.*, 2012).

Regulations of intracellular messenger cascades mediate the ability of neuronal systems to adapt in response to pharmacological and environmental stimuli. A broad division classifies the intracellular signal transduction pathways into two categories, those that are regulated by G-protein receptors coupled second messengers (e.g. cyclic adenosine monophosphate ($_{C}$AMP), Ca^{+2}, etc) and those that are regulated by receptors coupled directly or having a close interaction with protein tyrosin kinases. The first category is controlled by neurotransmitters (monoamines and neuropeptides), the second by cytokines and growth factors (including the neurotrophin family, e.g. brain-derived neurotrophic factor (BDNF)). Hence, regaining the plasticity within these molecular cascades is likely to contribute to the effects of the antidepressants (Tsai, 2007; Pilar-Cuéllar *et al.*, 2013).

BDNF is the most widespread growth factor in the brain, responsible, among other functions, of neuronal survival (Krishnan and Nestler, 2011). To activate transcription of BDNF a phosphorylation of calcium/cyclic-AMP response element binding protein (CREB) at its transcriptional regulatory residue Serine-133 is necessary (Banasr *et al.*, 2004, Gong *et al.*, 2014). Levels of serum BDNF are decreased in major depressive patients and are associated with vulnerability to develop mood disorders in healthy subjects (Kozisek *et al.*, 2008; Tanti and Belzung, 2010).

A common finding in animal studies is that, when administrated chronically, antidepressant increases CREB and its activated form, $_{\text{phosphorylated}}$-CREB ($_{P}$-CREB), in the hippocampus and cerebral cortex (Banasr *et al.*, 2004). In line with this finding, increased CREB levels have been reported in postmortem brains of antidepressant-treated subjects. Moreover, an increase in CREB and p-CREB is found in drug-free depressed suicide victims. In rats, both chronic antidepressant treatments and electroconvulsive therapy

(ECT) increase BDNF levels (Tanti and Belzung, 2010). Moreover, administration of BDNF displays antidepressant effects in two animal models of depression; the FST and the learned helplessness model (Krishnan and Nestler, 2011).

It has been also suggested that a reduced hippocampal cell number may be involved in the pathophysiology of depression and treatment with antidepressants has been shown to increase hippocampal neurogenesis (Eisch and Petrik, 2012). Clinically, there is evidence of reduced hippocampal volume in patients with MDD or other affective disorders (Hasler, 2010). A stress-induced decrease in dentate gyrus neurogenesis may be critically involved in the development of depressive episodes. Changes in neurogenesis do occur after acute stress; however, reduced cell proliferation in the dentate gyrus does not appear to be correlated to the development of learned helplessness (Malberg *et al*., 2000; Hanson *et al*., 2011).

1.4.3 Cytokines and depression

Inflammation also found to represent a common mechanism of disease that has been extended to include neuropsychiatric disorders such as major depression. Patients with MDD have been found to exhibit increased peripheral blood inflammatory biomarkers, including inflammatory cytokines, which have been shown to access the brain and interact with virtually every pathophysiologic domain known to be involved in depression (Hasler, 2010).

Indeed, activation of inflammatory pathways within the brain is believed to contribute to a confluence of decreased neurotrophic support and altered glutamate release/reuptake, as well as oxidative stress, leading to excitotoxicity and loss of glial elements, consistent with neuropathologic findings that characterize depressive disorders (Miller *et al.*, 2009). Further substantiating the link between inflammation and depression are data demonstrating that psychosocial stress, a well-known precipitant of mood disorders, is capable of stimulating inflammatory signaling molecules, including nuclear factor kappa B, in part, through activation of sympathetic nervous system outflow pathways (Krishnan and Nestler, 2011).

Interestingly, depressed patients with increased inflammatory biomarkers have been found to be more likely to exhibit treatment resistance, and in several studies, antidepressant therapy has been associated with decreased inflammatory responses (Miller *et al*., 2009).

Preliminary data from patients with inflammatory disorders, as well as medically healthy depressed patients, suggest that inhibiting proinflammatory cytokines or their signaling pathways may improve depressed mood and increase treatment response to conventional antidepressant medications (Raison *et al*., 2006). Translational implications of these findings include the unique opportunity to identify relevant patient populations and monitor therapeutic efficacy at the level of the immune system in addition to behavior (Miller *et al.*, 2009). Depressed individuals have high levels of cytokines and therefore induce depressive symptoms and HPA axis activation (Haapkoski *et al*., 2014).

1.4.4 Stress and depression

Depression is often associated with physiological changes characteristic of a normal stress response. Subjection of rodents for chronic stress shows some symptoms similar to depressed humans showing such as anxiety like behavior, less social interaction lack of interest, while not all human depression is triggered by stress. This model also shed light on the biology of depression (Abelaira *et al*., 2013). There has been a major focus on the role of the HPA axis as a marker of the stress response. Stress response activates the HPA axis and the release of glucocorticoids, which increases the heart rate, blood pressure, and metabolism (Hasler, 2010; Lee *et al.*, 2010). Recent data also have shown the HPA system might be involve in a remission of depression. Moreover, a consistent finding in depressed patients is hyperactivity and dysregulation of the HPA-axis, demonstrated by increased cortisol levels, enlargement of the pituitary and adrenal glands and decreased glucocorticoid receptor sensitivity (Mello *et al*., 2003). Also, the HPA axis is found to be a mediator of changes in brain monoamines, e.g. locus coeruleus noradrenaline and raphe serotonin (Gold *et al*., 2015).

Moreover, activation of HPA axis in depressed patient driven by hyperactivity of Corticotropin-releasing hormone **(**CRH) (Belmaker and Agam, 2008). Increased in CRH neuronal activity is also believed to mediate certain behavioral symptoms of depression such as sleep and appetite disturbances, reduced libido, and psychomotor changes (Arborelius *et al*., 1999). Animals with stressful situation show several changes that are reminiscent of depression, such as elevated levels of CRH and corticosterone (Krishnan and Nestler, 2011). Evidences have also shown that corticosteroid receptor function is impaired in many depressed patients (McQuade and Young, 2000). Antidepressants, both individually and in conjunction with anti-glucocorticoid agents, reverse a number of the HPA-axis abnormalities by enhancing their through corticosteroid receptors signaling (Arborelius *et al*., 1999; Gronli, 2006; Miller *et al*., 2009). Moreover, vasopressin a neuropeptide that reinforce CRH function and substance- P a neurokinin- 1 receptor have attracted attentions in the pathophysiology of depression (Jeon and Kim, 2016).

Therefore, different studies generally hypothesized that the brain areas, brain connectivity, neurotransmission, neurochemicals, neuroplasticity, immune-inflammation and HPA axis majorly involved in the pathophysiology of depression with multicausal disorder. Moreover, these were target areas in the understanding of pharmaceuticals development for the management of depression (Tanti and Belzung, 2010; Lang and Borgwardt, 2013; Shaikh *et al*., 2016; Yuksel, 2017).

1.5 Management of depression

The management of depression so far, starting from serendipitous discovery of the first effective antidepressant; imipramine, used the principles of pathophysiology explained above (Rouge, 2012). Moreover, the ever increasing knowledge of pathophysiological mechanisms of depression has led to the synthesis of other drugs. Surprisingly few variables have been established as either general or specific correlates of treatment response (Gideons *et al*., 2014; Hollon *et al*., 2014). Depression can be treated by pharmacological agents and non-pharmacological methods, and some of them are summarized below.

1.5.1 Pharmacological management of depression

There are different types of medications used in depression treatment conventionally. A large number of clinical drug trials have documented this to be a variety of heterocyclic antidepressants, monoamine oxidase inhibitors (MAOIs) and the new generation including the selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), dopamine-norepinephrine reuptake inhibitors (DNRIs), serotonin modulators as well noradrenergic and specific serotonergic antidepressant (NaSSA). Most of these antidepressants rapidly increase the amount of neurotransmitters serotonin and/or norepinephrine in the synapse. However, improvements' in the symptoms in patients usually do not occur immediately after starting treatment.

- ***Tricyclic and Tetracyclic Antidepressants***

The use of these antidepressants to treat depression began after the discovery of imipramine subsequently followed by amitriptyline, amoxapine, clomipramine, desipramine, doxepin, maprotiline, nortriptyline, protriptyline, trimipramine. Until SSRIs were introduced, these cyclic compounds were first-line treatment for depression (Mac Gillivray *et al*., 2003). These groups of antidepressants play major role by inhibiting presynaptic neurotransmitter reuptake of monoamines. Additionally, they block muscarinic, histamine and alpha-adrenergic receptors thus contrary to earlier expectations cause many side effects. This effect even considered more than the antidepressant activity for use (Nash and Nutt, 2007).

- ***Monoamine Oxidase Inhibitors***

Since the serendipitous discovery of iproniazide (a derivative of antibiotic isoniazide) in 1952, these classes of agents were found to be a potent antidepressant (Entzeroth, and Ratty, 2017). Isocarbaxozide, phenelzine, selegline and tranylcypromine are some of other agents and were also the first class in clinical use. However, MAOIs have been limited to be the first-line antidepressants due to food restrictions, drug-drug interactions and their side effect profile (Ramachandraih *et al*., 2011; Thomas *et al*., 2015).

- ***Selective Serotonin Reuptake Inhibitors***

SSRIs help to alleviate symptoms of depression by blocking reuptake of serotonin in the brain. SSRIs include citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine, sertraline (Pirraglia *et al*., 2003). Although SSRIs are relatively safe, there are concerns regarding their use with adverse effects occurring in up to 75% of subjects (Kauffman, 2009).

- ***Serotonin-norepinephrine Reuptake Inhibitors***

SNRIs appear to treat depression by blocking presynaptic serotonin and norepinephrine transporter proteins thus inhibits reuptake of these neurotransmitters and leads to increase stimulation of postsynaptic receptors. SNRIs include: desvenlafaxine, duloxetine, milnacipran, and venlafaxine. These medicines indicated for depressed patients with poor responses or intolerable side effects during first line treatment with SSRIs (Celikyurt *et al*., 2012).

- ***Dopamine-norepinephrine Reuptake Inhibitor***

Bupropion the only available DNRI acts *via* dual inhibition of norepinephrine and dopamine reuptake and is devoid of clinically significant serotonergic effects or direct effects on postsynaptic receptors. Thus, they are advantageous for the absence of side effects, such as sexual dysfunction, weight gain, and sedation. Moreover, study demonstrated that bupropion was found to increase dopamine neurotransmission in both the NAs and the prefrontal cortex (Stahl *et al*., 2004; Nash and Nutt, 2007).

- ***Serotonin Modulators***

Serotonin modulators antagonize postsynaptic serotonin receptors and inhibit reuptake of postsynaptic serotonin to varying degrees. Their effects upon norepinephrine reuptake are minimal. Drugs under this classification include: nefazodone, trazodone and vilazodone. They can be used for the initial treatment of MDD as well as treatment resistant depression (TRD) (Micallef-Trigona, 2014).

- ***Noradrenergic and specific serotonergic antidepressants***

NaSSAs act by antagonizing the adrenergic receptor and certain serotonin receptors such as 5-HT2A and 5-HT2C in some case also antagonize 5-HT3, 5-HT6, and/or 5-HT7. Thus due to their blockage of certain serotonin receptors incidence of unwanted side effects associated with SSRIs are prevented. NaSSAs are classified as tetracyclic antidepressants based on their chemical structures and include aptazapine, esmirtazapine, mianserin, mirtazapine and setiptiline/teciptiline (Nash and Nutt, 2007; Dekeyne *et al*., 2012).

- ***Other antidepressants***

In combination with the above categorical class of antidepressants, antipsychotics are also effective for treatment of depression. The efficacies of atypical antipsychotics for depression without combination are hypothesized to be effective because of their serotonergic, noradrenergic and dopaminergic effects (Zhou *et al*., 2015).

Virtually every known antidepressants have response rate which is not greater than 67% among depressed patients respond to any given medication and 33% failed to respond (Stahl, 2013). For choice of the medication, several factors need to be considered like predominant symptoms, previous experience, preference of the patient, as well as side effects of the drugs. Recent practice guidelines for treatment of depressive disorder emphasize the importance of symptom severity in determining the need for antidepressant medications (Saltiel and Silvershein, 2015).

Moreover, pharmacotherapy for depression should consist of acute therapy up until the best clinical response or remission is achieved (usually 6-12 weeks) and continuation of therapy for a further six months to prevent relapse. Sometimes, maintenance therapy will continue for months or years in case of recurrent or persistent disorders (Joffe, 1991; Stahl, 2003). Rates of relapse because of pharmacogenomics and other factors are about twice high when antidepressant medication is discontinued, even though early improvement is achieved (Dowrick and Frances, 2013). Thus adherence for medication is important.

1.5.2 Non-pharmacological management of depression

Non-pharmacological approaches such as somatic and psychotherapy can also help to manage depression symptoms. Somatic therapy which is device-based approach involves in activating or touching the brain directly with electricity, magnets or implants to treat depression (Insel and Gogtay, 2014). It is rapidly evolving and valuable options for patients who have failed numerous other treatments. This approach has been found in the metabolism of neurotransmitters and in the activation of different brain regions (Tanti and Belzung, 2010). *Electroconvulsive therapy* is the best studied brain stimulation therapy and has the longest history of use (Micallef-Trigona, 2014; Yuksel, 2017). Other stimulation therapies are newer, and in some cases still experimental methods. These include: *repetitive transcranial magnetic stimulation* (Masleniko et al., 2011; Aleman, 2013; Banerjee et al., 2017), *deep brain stimulation* (Mayberg et al., 2005; Hauptman et al., 2008; Kennedy et al., 2011) and *Vagus nerve stimulation* (Rush et al., 2005; Zobel et al., 2005; Nemeroff et al., 2006). To date, these somatic therapies for TRD used based on the acuteness of illness, the likelihood of response, costs and associated risks; for example, both *deep brain stimulation* and *Vagus nerve stimulation* are the options requiring surgical approach (Cusin and Dougherty, 2012). Moreover, memory loss and the need for repeated treatments to maintain efficacy preclude the use of all as a long-term treatment option for their drawback on mood regulation. Therefore, their uses for depression remain controversial.

There are also other effective non-pharmacological strategies have been shown to reduce depressive symptoms and improve quality of life. These include established options such as cognitive behavioral therapy (CBT) and interpersonal psychotherapy, as well as a number of lesser-known approaches for which supporting evidence is emerging (Jorm et al., 2013; Sarris et al., 2014). Including, e-Mental health tools, physical exercise, relaxation techniques, mindfulness-based (CBT), behavioral activation, interpersonal counseling, positive mood induction, exposure to bright light and tryptophan-rich diets (Bowden et al., 2012).

1.6 Traditional medicine in the management of depression

In the traditional systems of medicine, many plants and formulations have been used to treat depression for thousands of years. Therefore, herbal therapies should be considered as alternative/complementary medicines. The search for novel pharmacotherapy from medicinal plants for psychiatric illnesses has progressed significantly (Wasnik *et al.*, 2015) and thus revealed the pharmacological effectiveness of different plant species in a variety of animal models (Zhang, 2004).

Herbal remedies for human brain disorders is much preferred over synthetic drugs because of various side effects of synthetic drugs ranging from sleep disorders to withdrawal syndromes. Herbal treatment not only improves patient compliance but also enhance the bioavailability of many drugs. Active constituents extracted from specific parts of various plant origins have proved to be beneficial. Some formulations have drawn attention that in depth clinical trials should be conducted to prove the benefits of a patient (Ahangar *et al.*, 2011; Sandhya, 2010).

Medicinal plants most widely investigated for treatment of 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). Some of the claimed plants in Ethiopian traditional medicine for the treatment of depressions are: *Withania somnifera, Carissa edulis* (Moravec *et al*., 2014), *Asparagus leptocladodius* (Lulekal *et al*., 2008).

The only herbal remedy that has been shown beyond reasonable doubt to be effective as a treatment for mild to moderate depression is *Hypericum perforatum* (St John's wort) (SJW) (Edzard E., 2007; Szafranski, 2014) although it has been reported to disturb drug metabolizing system (Markowitz *et al.*, 2003). Therefore, the search for essential plants that could have effective antidepressant activity without side effects is important.

1.7 The Experimental plant

1.7.1 The Genus Hypericum

The genus name, Hypericum, is a combination of the Greek words hyper (above) and eikon (picture). Hypericum is a genus of 490 species of flowering plants in the family Hypericaceae. Hypericum includes SJW (*Hypericum perforatum L*.), an economically important medicinal crop plant with anti-viral, anti-oxidant, anti-microbial, anti-inflammatory, anti-depressive and anti-cancer properties (Linde *et al*., 1996). Many species are cultivated as ornamentals. Six species occur in Southern Africa. All members of the genus are commonly called *SJW*.

1.7.2 *Hypericum revolutum*

This is multi-stemmed shrub or small tree with fast-growing and evergreen plant. It grows up to 3 m high and spreads about the same width. This evergreen plant's leaves release a curry- like smell when crushed and after rain. The fresh, green foliage and bright yellow flowers are reasons to have this delightful plant in garden. Stems have reddish brown, scaly bark and drooping branches. The elliptical leaves are rolled under along the margins, 8–25 mm long, opposite and spaced closely and crowded at the ends of branches. The bright green leaves often have black gland dots and are soft textured. Flowers are single and bright yellow, up to 50 mm in diameter. Flowering time of the plant is in summer and autumn. The fruits are reddish brown capsules enclosing the seeds (Plowes and Drummond, 1990; Hundera *et al.*, 2007).

H. revolutum is found naturally in cool and damp areas, growing along stream banks and at the edges of forests. It is widespread, but occurs mainly in Eastern Regions such as the Eastern Cape, Gauteng, KwaZulu-Natal, Mpumalanga and Swaziland, extending West wards along Cape coastal area to near Riversdale in the Western Cape. It also occurs in tropical Africa and some Indian Ocean islands (Dressler *et al.*, 2014).

The wood of this plant is used as timber (local construction) and for building material in some African countries. It is said to be nature's firebreak, as it does not burn well and protects forests, has a role in soil conservation (Kewessa *et al*., 2015).

In Cameroon, the plant is traditionally used to treat several ailments including malaria, skin infections, venereal diseases, gastrointestinal disorders, tumours, infertility and epilepsies (Zofou *et al*., 2011). Moreover, Rwandan used this plant for treatment of cough and respiratory disease (Cos *et al*., 2002).

The Greeks used plants of this genus to decorate religious images in order to ward off evil spirits, especially around midsummer's eve (St. John's Eve) (Nürk, 2011)**.** Similarly, in Ethiopia, this plant is traditionally used for Irreecha (Oromo's thanks giving day).

The vernacular names of *H. revolutum* include: *Curry bush* (comes from its distinctive smell), *Giant SJW* (English), *Ikinyamucus* (Rwanda), *Awidi* (Agewgna), *Amija*, *Avetia* (Wollo) (Amharic), *Edera*, *Garamba*, *Hendi*, *Hinnie Ule foni*, *Muka foni*, *Gorgora* (Afan Oromo) (Bekele, 2007; Hundera *et al.*, 2007).

H. revolutum have also been reported in the literature that the leaves as well as aerial parts (Shiu and Gibbons, 2009), were used for treatment of stomach-ache (Bussmann *et al*., 2011) and for its anti-bacterial effect (Gibbons *et al*., 2002; Adugna *et al*., 2014; Andualem *et al.*, 2014; Akgöz, 2015). As it was reported by Kassu *et al.* (1999) the stick is also used for a toothbrush for oral hygiene. Moreover, as-medicinal agent (powdered dry leaves and stems, oily extracts), bee forage (Kewessa *et al*., 2015), reports also indicated that its bark is implicated as anti-malarial agent (Zofou *et al.*, 2011). A study done in Rwanda reported that the leaves have shown antimicrobial, antifungal and antiviral activities (Cos *et al*., 2002).

Moreover, a recent pharmacological study showed the anti-depressant-like activity of 80% methanol crude extract of the plant in animal models of depression (Ejigu and Engidawork, 2014). Therefor the present study was undertaken to evaluate antidepressant-like effects of solvent fractions of *H. revolutum* using FST and TST, in rodents.

Figure 1: Pictures of aerial parts of *H. revolutum*

1.8 Rationale of the study

Statistically, the number of vulnerability for depression is increasing. This results in a reduction of living quality and functional situation of patients, increase in the fatigue levels and impairments in daily life activities in working capacity and social interactions among depressed. In addition, there are still challenges in access of modern medicine, cultural acceptability, cost affordability, availability and sustainability of anti-depressants, especially in developing countries. On the other hand, the clinical application and the efficacy of these drugs quite limited, and the treatment with some synthetic antidepressant drugs has to be paused owing to certain unbearable side-effects (Chen *et al*., 2004; Kima *et al*., 2005; Mesfin *et al*., 2013; Teklay *et al*., 2013). For example, the use of antidepressant like tricyclic anti-depressants (TCA) can cause memory impairment, hypertensive crisis (Ramachandraih *et al*., 2011; Eizadi -Mood *et al.*, 2015; Shaikh *et al*., 2016), and sexual dysfunction is associated with SSRI'S (Hardman *et al.*, 2001), TCA toxicity of combination TCA with SSRI, serotonin syndrome of MAOI combining with SSRI (Thomas *et al*., 2015; Entzeroth, and Ratty, 2017).

Moreover, the treatment of depression with currently available pharmacological agents is only successfully achieved in about 65-70% of cases (Rowshan, 2014). The limitations of this approach includes a long time lag for a therapeutic response (weeks to months) and low response rates (only one-third respond to the first drug prescribed, and up to two-thirds after multiple trials, often taking months to years) (Holtzheimer and Nemeroff, 2006; Duman and Voleti, 2012; Shaikh *et al*., 2016).

On top of that, even if pharmacotherapy is effective in more than half of depressed patients, between 20% and 30% of patients suffering from depression may have TRD (Philip *et al*., 2010; Cusin and Dougherty, 2012). Although the newer antidepressant drugs have clinically important differences in efficacy and tolerability, most drugs development focus remains on moderations of the same monoamine targets (Ashewani and Preeti, 2012).

Thus there is a continuous need to identify newer natural antidepressants that could act more quickly, more specifically and more effective than currently available treatments (Astin, 1998). Various plants and their extracts have been reported to possess antidepressant-like activity. Moreover, they are widely used across the globe due to their wide applicability and therapeutic efficacy coupled with least side effects; this, in turn, has accelerated the scientific research regarding the antidepressant activity (Arya and Verma, 2012). Despite the widespread uses of *H. revolutum*, no scientific work is reported in the literature regarding the solvent fractions of aerial parts of *H. revolutum* against depression like states.

2. OBJECTIVES

2.1 General objective

⇛ To evaluate the antidepressant-like activities of solvent fractions of the aerial parts of *Hypericum revolutum* in rodents.

2.2 Specific objectives

⇛ To perform acute oral toxicity test of solvent fractions of *Hypericum revolutum*

⇛ To assess the anti-immobility effect of the solvent fractions of *Hypericum revolutum* using tail suspension test in mice.

⇛ To determine the anti-immobility effect of the solvent fractions of *Hypericum revolutum* using forced swim model of depression in rats.

⇛ To evaluate the anti-depressant like effect of the solvent fractions of *Hypericum revolutum* on locomotors activity using open field test in mice.

⇛ To assess the secondary metabolites profile of fractions using phytochemical screening tests and establish finger print of the extract.

3. MATERIALS AND METHODS

3.1 Drugs, Chemicals and reagents

Drugs and chemicals used in the present study include: Imipramine (Torrent Pharmaceuticals, India), Distilled water [Ethiopian Pharmaceuticals Manufacturing (EPHARM)], Tween 80 (BDH Chemicals Ltd, England), Absolute Methanol (Carlo Erba reagents, France), Chloroform and butanol (BDH, Poole, England), Petroleum ether (Carlo Erba reagents, France), were purchased from reliable local sources. Glacial acetic acid, H_2SO_4, Ammonia, HCl, Acetic anhydride, $FeCl_3$, ethyl acetate, Mayer's and Dragendorff's reagents were obtained from the Department of Pharmaceutical chemistry and Pharmacognosy, AAU. All solvents used for the extraction process are of laboratory grade.

3.2 Plant material

The aerial parts of *H. revolutum* were collected during the flowering period from Menagesha (50 km West of Addis Ababa), in October 2015. A taxonomist identified the plants and a voucher specimen (voucher number SD/003) was deposited at the National Herbarium, College of Natural and Computational Sciences, AAU for future reference.

3.3 Experimental animals

Male and female Swiss Albino mice (6-9 weeks of age, 20–32 g of weight) and male Wistar rats (8-11 weeks, 180-220 g) bred at the animal house of School of Pharmacy, Addis Ababa University and purchased from Ethiopian Public Health Institute (EPHI) were used for the experiments. The animals were housed in groups with free access to laboratory pellets and tap water and maintained under standard conditions (12/12 h light-dark cycle). They were acclimatized for a week to the laboratory condition before the commencement of the experiment and used only once throughout the study. All experiments were carried out in the afternoon (4-10 PM, Test room lighting, room temperature and noise level were kept constant for all mice/rats used in the study) (Mitchell *et al.*, 2015). All animals used in the study were cared for and treated humanely according to the Principles of Laboratory Animal Care (OECD, 2008) and ethical clearance was obtained from an Ethical review board of school of pharmacy, AAU.

3.4 Preparation of crude extract

The aerial parts of *H. revolutum* were thoroughly washed with tap water to remove dirt and soil and then sliced to smaller pieces and dried at room temperature in the shade light for three weeks. The shade dried whole plant sample (1000 g) was milled in the form of a coarse powder. The powdered sample was defatted by maceration at room temperature using petroleum ether for 6 h shaking thoroughly (Abdelgadir *et al*., 2011). The defatted marc was taken and spread as a bed on a clean paper and dried for 15-30 min so as to completely evaporate petroleum ether from the plant surface (Solanki and Nagori, 2012).

Extraction was carried out by maceration technique using 80% methanol as a solvent. Accordingly, 900 g of the dried powder was weighed using electronic digital balance and then divided into three portions. The divided powder placed in three different Erlenmeyer flask (300 g each for ease of extraction) and was soaked in a closed flask with 200 ml of the solvent for each extract for a period of 3 days with continuous agitation using shaker (Bibby scientific limited stone Staffo Reshire, UK), at room temperature. The resultant solution was combined and filtered through double layered muslin cloth followed by Whatman (No.1) filter paper.

The residue was re-macerated twice using the same solvent to exhaustively extract the plant material. The extract was then concentrated using a rotary evaporator (Buchi Rota-vapor R-200, Switzerland) under reduced pressure at 40°C. The concentrated filtrate was frozen in a refrigerator overnight and then freeze dried in a lyophilizer (Operon, Korea vacuum limited, Korea). Finally, percentage yield of the crude extract was calculated and found to be 16.6 % (w/w). The dried extract was used for the commencement of the pilot study and further fractionation.

3.5 Solvent fractionation

The 80% methanol crude extract of *H. revolutum* showed antidepressant activity as it was observed from the current pilot study as well as in a previous study done by Ejigu and Engidawork (2014). The extract was then fractionated using solvents of differing polarity.

Accordingly, a total of 100 g of 80% methanol crude extract of *H. revolutum* was suspended in 250 ml of distilled water using a separatory funnel. The suspended hydro-alcoholic extract was partitioned with 250 ml chloroform and repeated until the chloroform layer became clear. The filtrate was concentrated in a rotary evaporator (Buchi Rota vapor, Switzerland at 80 rpm and 40^0C) to obtain the chloroform fraction (CF). The yield obtained was 6.6%. The aqueous residue was further partitioned with 250 ml butanol. The butanol filtrate was concentrated similarly as CF and the yield was 40%.

Finally, the remaining aqueous residue was kept in the deep freezer overnight and then freeze dried with a lyophilizer and a total of 40 grams (40%) of aqueous fraction (AF) was obtained. All fractions were kept in tightly closed containers in a refrigerator at -20^0C until used.

3.6 Acute toxicity test

Acute toxicity test was performed according to the Organization for Economic Cooperation and Development (OECD, 425) (OECD, 2008) guideline. Female mice were used for the toxicity study. Initially, three female mice were fasted food but not water for overnight and were administered with 2000 mg/kg of each solvent fraction as a single dose by oral gavage separately. Then the mice were observed for any signs of toxicity with in the first 24 h (continuously for 4 h with 30 min interval) for changes in skin and fur, somatomotor activity and behavioral pattern, salivation and diarrhea, tremor and convulsions, lethargy and paralysis, food and water intake and mortality. No sign of toxicity was observed and thus another 12 female mice were recruited and fasted for overnight with food but not water. Thereafter, they were given the same dose and were observed for any sign of toxicity or death in the next 14 days.

3.7 Grouping and dosing of animals

There were five groups of animals comprising of six animals each for the respective model. All animals were randomly assigned to different groups. Group I received vehicle (2% Tween 80 or distilled water) and served as negative control. Group II received the standard drug Imipramine (64 mg/kg) and served as positive control (Castagné *et al.*, 2011). The test groups were group III-V, which received different doses of 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 either in 2% Tween 80 or distilled water immediately prior to use and administered orally one hour before the experiment using oral gavage. The maximum volume administered was 10 ml/kg (Castagné *et al.*, 2011).

Doses of the fractions were selected based on the outcome of the acute toxicity test as well as from a previous study done by Ejigu and Engidawork (2014).

3.8 Behavioral tests for antidepressant activity

3.8.1 Forced swim test

The method described by Porsolt *et al.* (1977) with slight modification was used in this study. In this model, male rats were forced to swim in a restricted space from which they could not escape. The apparatus consisted of clear plexiglass cylinder (40 cm height x 20 cm diameter) filled to 15cm depth with water (25°C). Two sessions were conducted: an initial 15 min training session (pre-test session) without any behavioral recording in order to check the fitness of each animal followed by a 5 min test session 24 h later. During the test session, the immobility time, swimming and climbing times were observed. The total duration of immobility was measured during the 5 min test. Upon removal from the water, the rats were towel dried, allowed to dry for 15 min in a heated enclosure (32°C) after each test and finally returned to their cage (Umadevi *et al.*, 2011).

Each rat was judged to be immobile when it ceased struggling and remained floating (motionless) in the water, including movements to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant like effect. The vehicle or fractions were administered three times (1, 4 and 24 h) before a test session (Beijamini *et al.,* 2011, Castagné *et al.*, 2011).

3.8.2 Tail suspension test

The total duration of immobility induced by tail suspension was measured according to the method described by Steru *et al.* (1985). Briefly, male mice were suspended 70 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Individual mice were subjected to test 1 h after drug administration. Immobility time was registered during a 6 min period (Machado *et al*., 2013). The animal was considered immobile when it did not show any movement of the body except for those required for respiration and hanged passively (Kothari *et al.*, 2010; Can *et al*., 2012).

The percentage reduction in the duration of immobility was calculated by dividing 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.

3.8.3 Open-field test

To assess the possible effect of the solvent fractions of *H. revolutum* on locomotor activity, mice were evaluated in the open-field paradigm, as previously described by Machado *et al*. (2013). Mice were individually placed in a wooden box (68 x 68 x 45 cm) with the floor divided into 16 squares of equal fields to make up the central and peripheral squares. Number of crossings (number of squares crossed by the animal with the four paws centrally, peripherally (ambulation) and total locomotion which is the sum of the two) were used to evaluate locomotor activity. These parameters were registered in a 5 min period.

In all the behavioral tests video camera was used to record the activities for later counting and behavioral analysis. A stopwatch was used for counting each activity. Moreover, after each activity, the materials were carefully cleaned with sterilized wipes to prevent behavioral changes of the animals (Castagné *et al.*, 2011; Machado *et al*., 2013).

3.9 Phytochemical screening

Both extract and solvent fractions of aerial parts of *H. revolutum* were screened for the presence of different chemical constituents in the plant, following standard procedures described by Trease and Evans (1989), Jones and Kinghorn (2006) to relate the antidepressant activity of the plant with the presence or absence of these constituents.

Test for alkaloids

The formation of a yellowish orange precipitate when one ml of the test solution was treated with a few drops of Dragendorff's reagent or the production of a creamy or white precipitate when the sample solution is mixed with a few drops of Mayer's reagent indicates that the test is positive. Both tests were used for detection of alkaloids.

Saponin test

To 0.25 g of the crude extract, 5 ml of distilled water was added in a test tube. Then, the solution was shaken vigorously and observed for a stable persistent froth. Formation of froth indicates the presence of saponins. For butanol and the aqueous fraction: 0.5 g of the fraction was dissolved in 10 ml of distilled water in a test tube. The test tube was stopped and shaken vigorously for 30 seconds and allowed to stand in a vertical position and observed over 30 min. Formation of "honey comb" froth over the surface of liquid and persistence after 30 min indicates the presence of saponins. For chloroform fraction: The fraction was diluted with an appropriate solvent and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 minutes. One cm layer of foam indicates the presence of saponins.

Test for flavonoids

Both crude extract and fractions were dissolved in a mixture of an appropriate solvent. To 2 ml of the crude extract and fractions solution, three to five drops of 2 % lead acetate solution were added. Then, it was observed whether it develops yellow or orange color which indicates the presence of flavonoids.

Test for terpenoids

Five ml of extracts and fractions dissolved in distilled water was mixed in 2 ml of chloroform, and 3 ml concentrated H_2SO4 was carefully added to form a layer. A reddish brown coloration of the interface was formed to show a positive result for the presence of terpenoids.

Test for steroids

A red color produced in the lower chloroform layer when 2 ml of extract and each fraction were dissolved in 2 ml of chloroform and 2 ml concentrated sulfuric acid indicates the presence of steroids. Another test was performed by mixing extract and each fraction with 2ml of chloroform. Then 2ml of each of concentrated H2SO4 and acetic acid was poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

Test for tannins

About 2 ml of the extract was stirred with 2 ml of distilled water and few drops of Iron (III) Chloride/ Ferric Chloride (FeCl3) solution was added. The formation of greenish precipitates indicates the presence of tannins.

Test for cardiac glycosides

Two ml of each extract was dissolved in 2 ml of glacial acetic acid containing one drop of Iron (III) Chloride/ Ferric Chloride (FeCl3) solution. The mixture was then poured into a test tube containing 1 ml of concentrated H2SO4. A brown ring at the interphase indicates the presence of a deoxy sugar, characteristics of cardenolides.

Test for anthraquinones

One-half gram of the extract and fractions were boiled with 10 ml of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for color changes.

3.10 Thin layer chromatography

Pre-coated plastic silica sheets (Macherey-Nagel Gmbh & Co, Germany) of 0.2 mm thick were used to run analytical TLC. A small amount of butanol and chloroform fractions were separately dissolved in 99.8% methanol and a 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 polar) 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 3/4th 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 a wavelength of 254 nm and 366 nm. The TLC was done to provide a finger print of the respective solvent fractions by providing a rough number of components and nature in the fraction indicated by the bands on the plate (Alebiosu and Yusuf, 2015).

3.11 Statistical analysis

Comparisons between experimental and control groups were performed by SPSS windows version 20 using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. All the results observed from the experiment were expressed as mean ± S.E.M and a P value < 0.05 was considered to be statistically significant. Linear regression was also used where appropriate.

4. RESULTS

4.1 Acute toxicity test

Following administration of the solvent fractions, mice used in the acute toxicity study were observed for the first four hours continuously in 30 min interval during the study and for the next 14 days to see if any of the fractions of the plant have toxicity. No visible signs of toxicity and gross behavioral changes and mortality were observed within 24 hours as well as the following 14 days in solvent fractions treated animals. The LD_{50} of the three solvent fractions of the plant was thus found to be above 2000 mg/kg.

4.2 Effect of the solvent fractions in mice tail suspension test

As it is indicated in Table 1, comparison of the AF doses did not show difference with control in reduction of immobility. However, the standard displayed the highest percentage reduction (65.28%) in the duration of immobility in comparison with the control or AF treated groups.

Table 1: Effects of aqueous fraction of *Hypericum revolutum* on the duration of immobility in mice tail suspension test.

Solvent fraction	Animal (treatment) group, *p.o.*	Duration of immobility (sec)	% reduction in time of immobility
Controls	CON	156.00 ± 4.033	-
	IMI 64 mg/kg	54.17 ± 4.757	65.28
Aqueous fraction	AF 100 mg/kg	157.17 ± 7.657	-0.75
	AF 200 mg/kg	147.67 ± 5.789	5.34
	AF 400 mg/kg	151.17 ± 6.828	3.10

Data are expressed as mean ± SEM; n=6 per group; CON= control (received distilled water), IMI= imipramine, AF= aqueous fraction, *p.o.*= per oral, (- sign indicates increase in time of immobility), sec= seconds

Table 2: Effect of butanol and chloroforms fractions of *Hypericum revolutum* on the duration of immobility in mice tail suspension test.

Solvent fractions	Animal (treatment) group, *p.o.*	Duration of immobility (sec)	% reduction in time of immobility
Controls	CON	155.67 ± 4.573	-
	IMI 64 mg/kg	60.50 ± 3.677[a3c3d3e1f3g3]	61.14
Butanol fraction	BF 100 mg/kg	123.33 ± 6.697[a3]	20.77
	BF 200 mg/kg	107.33 ± 7.079[a3]	31.05
	BF 400 mg/kg	84.67 ± 3.955[a3c3d1f3]	45.61
Chloroform fraction	CF 100 mg/kg	124.33 ± 2.333[a1]	20.13
	CF 200 mg/kg	101 ± 1.461[a3c1f1]	35.12
	CF 400 mg/kg	68.5 ± 3.603[a3c3d3f3g3]	56.00

Values are expressed as mean ± SEM; n=6 per group; [a]: against control, [c]: against BF 100mg/kg; [d]: against BF 200mg/kg; [e]: against BF 400mg/kg; [f]: against CF 100mg/kg; [g]: against CF 200mg/kg; [1]: $p<0.05$; [2]: $p<0.01$; [3]: $p<0.001$, CON= Control (received 2% Tween 80 in distilled water), IMI= imipramine, BF= butanol fraction, CF= chloroform fraction, *p.o.*= per oral, sec= seconds

Unlike the AF, a notable effect was observed with the butanol and chloroform fractions, particularly with increasing dose (Table 2). Accordingly, all doses of the fraction dose-dependently decreased the immobility time compared to the vehicle-treated animals ($p<0.001$ for all and $p<0.05$ for CF100). Comparison with the standard revealed that the standard produced a significantly lower immobility time than all doses of the BF as well as lower (100 mg/kg) and moderate (200 mg/kg) doses of both fractions. However, immobility time was comparable between the higher dose (400 mg/kg) of the CF and the standard.

On the other hand, no significant differences were observed between the moderate (200 mg/kg) dose of BF and the lower (100 mg/kg) doses of both fractions. However, there was a significant difference between BF 100 mg/kg with the higher (400 mg/kg) doses of both fractions ($p<0.001$) which as well had shown remarkable significance with CF 200mg/kg ($p<0.05$). Moreover, a significant difference was observed between the moderate (200 mg/kg) dose of BF and higher (400 mg/kg) doses of both fractions ($p<0.05$ for BF and $p<0.001$ for CF). But when comparison had been made between CF 200 mg/kg and CF 400 mg/kg the significant difference was $p<0.001$.

The percentage reduction in time of immobility among the BFs were 20, 31 and 45 for 100, 200 and 400 mg/kg respectively and the effect was in dose dependent manner (R^2= 0.9917, Figure 2). Moreover, the percentage reduction in time of immobility among CFs for 100, 200 and 400 mg/kg of doses had shown 20, 35 and 56 respectively. Similarly, the effect was found to increase in dose dependent manner (R^2= 0.9909, Figure 2).

4.3 Effect of the solvent fractions in rat forced swim test

As indicated in Table 3, the AF once again did not produce a detectable effect in FST compared to controls in the reduction of immobility at all dose levels. However, the standard treated group had shown the highest (56.18) percentage reduction in time of immobility.

Table 3: Results of an aqueous solvent fraction of *Hypericum revolutum* in the forced swimming test in rats.

Solvent fraction	Animal (treatment) group, *p.o.*	Duration of immobility (sec)	% reduction in time of immobility
Controls	CON	148.33 ± 6.009	-
	IMI 64 mg/kg	65.00 ± 3.152	56.18
Aqueous fraction	AF 100 mg/kg	154.00 ± 8.266	-3.82
	AF 200 mg/kg	166.33 ± 3.528	-12.14
	AF 400 mg/kg	158.50 ± 4.897	-6.86

Values are expressed as mean ± SEM; n=6 per group, CON= control (received distilled water), IMI= imipramine, AF= aqueous fraction, *p.o.*= per oral, (- sign indicates increase in time of immobility), sec= seconds

Table 4: Antidepressant-like effect of solvent fractions of *Hypericum revolutum* in the forced swimming test in rats.

Solvent fractions	Animal (treatment) group, *p.o.*	Duration of immobility (sec)	% reduction in time of immobility
Controls	CON	155.17 ± 7.195	-
	IMI 64 mg/kg	61.67 ± 4.208^{a3c3d3f3g3}	60.26
Butanol fraction	BF 100 mg/kg	135.00 ± 4.091	13.00
	BF 200 mg/kg	96.67 ± 4.410^{a3f3}	37.70
	BF 400 mg/kg	63.33 ± 2.376^{a3d3f3g3}	59.19
Chloroform fraction	CF 100 mg/kg	130.33 ± 2.319^{a2}	16.01
	CF 200 mg/kg	99.67 ± 3.106^{a3f2}	35.77
	CF 400 mg/kg	71.83 ± 6.030^{a3d2f3g2}	53.71

Values are expressed as mean ± SEM; n=6 per group; ^{a}: against control, ^{c}: against BF 100mg/kg; ^{d}: against BF 200mg/kg; ^{f}: against CF 100mg/kg; ^{g}: against CF 200mg/kg; ^{1}: p<0.05; ^{2}: p<0.01; ^{3}: p<0.001, CON= Control (received 2% Tween 80 in distilled water), IMI= imipramine, BF= butanol fraction, CF= chloroform fraction, *p.o.*= per oral, sec= seconds

As it is demonstrated in Table 4, the moderate (200 mg/kg) and higher (400 mg/kg) doses of both fraction exhibited a statistically significant reduction in time of immobility ($p<0.001$) compared to the control group. Moreover, the lower (100 mg/kg) dose of CF showed a significant difference in time of immobility ($p<0.05$). On the contrary, the lower (100 mg/kg) dose of BF didn't show a statistically significant difference with all study groups in this model, unlike the TST.

Comparison with the standard revealed that the standard produced a significantly lower immobility time than the lower (100 mg/kg) and moderate (200 mg/kg) doses of both fractions. However, immobility time was comparable between the higher (400 mg/kg) doses of both fractions and the standard.

The higher (400 mg/kg) dose of BF had shown significant difference with the lower (100 mg/kg) and moderate (200 mg/kg) doses of CF ($p<0.001$). Moreover, the higher (400 mg/kg) doses of both fractions exhibited a significant difference (59, $p<0.001$ for BF and 54, $p<0.01$ for CF) in comparisons with BF 200 mg/kg. When comparison had made among BFs the % reduction in immobility by the 100, 200, and 400mg/kg with respective values were 13, 37 and 59, and the effects were dose dependent manner ($R^2 = 0.9479$, Figure 3).

The CF 100 mg/kg had significant difference with CF 200 mg/kg ($p<0.01$) and 400 mg/kg ($p<0.001$) and when we compared CF 200 mg/kg with CF 400 mg/kg there was significant difference ($p<0.001$). Like the BF, the maximum percentage reduction by 100, 200 and 400mg/kg of CF was observed at 400mg/kg, with respective values of 16, 35, and 53, and the effect was found to increase dose dependently indeed ($R^2 = 0.9532$, Figure 3).

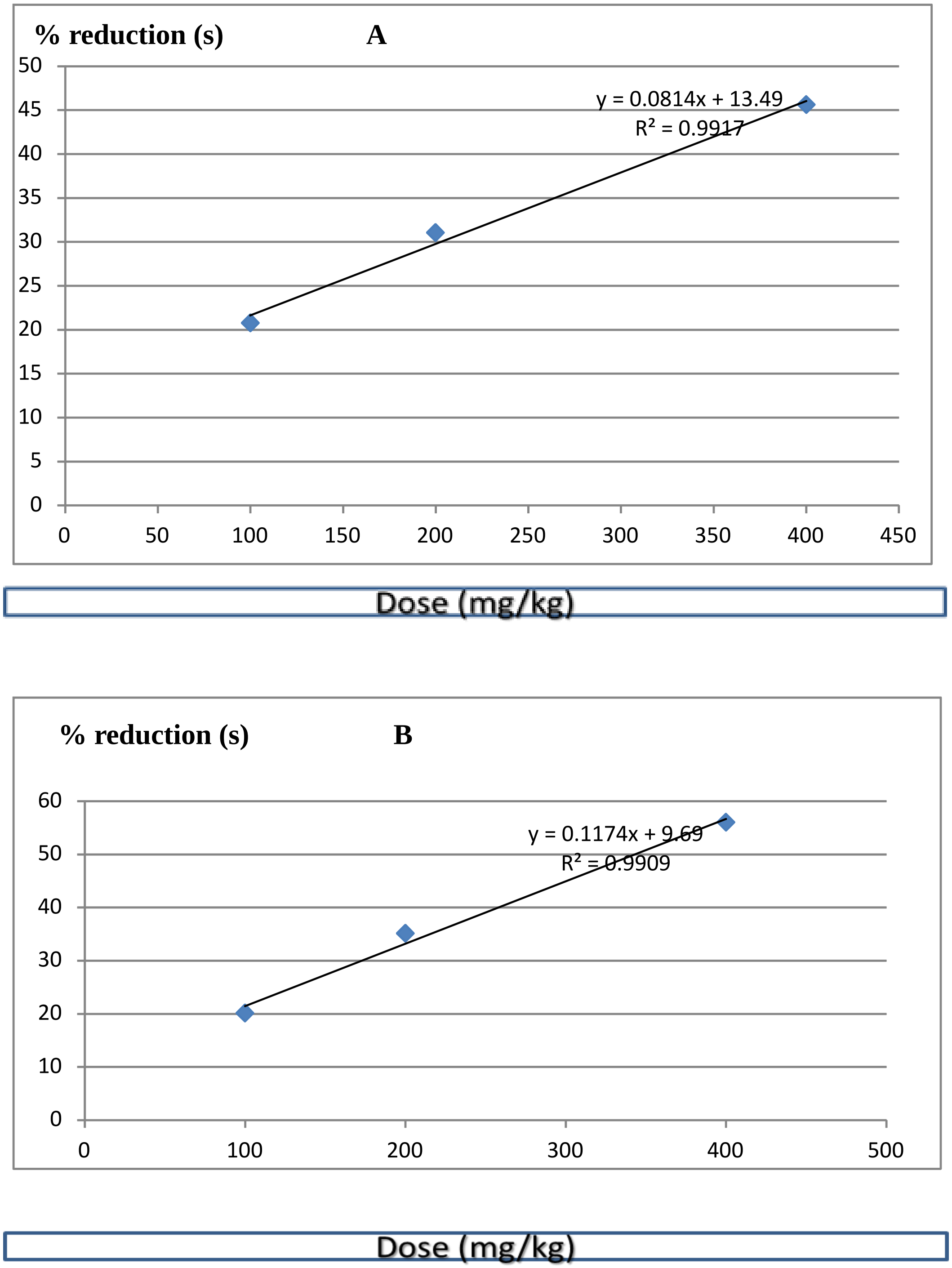
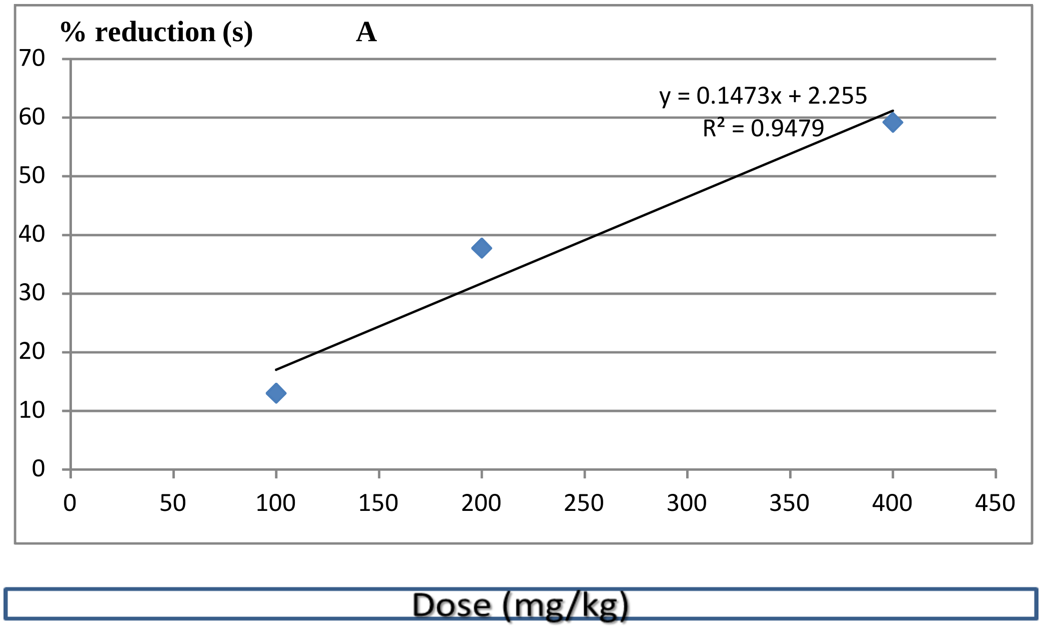


Figure 2: Linear chart showing the effect of the dose at (100, 200 and 400mg/kg) on % reduction in time of immobility in the Tail Suspension Test for butanol fraction (A) and Chloroform fraction (B).



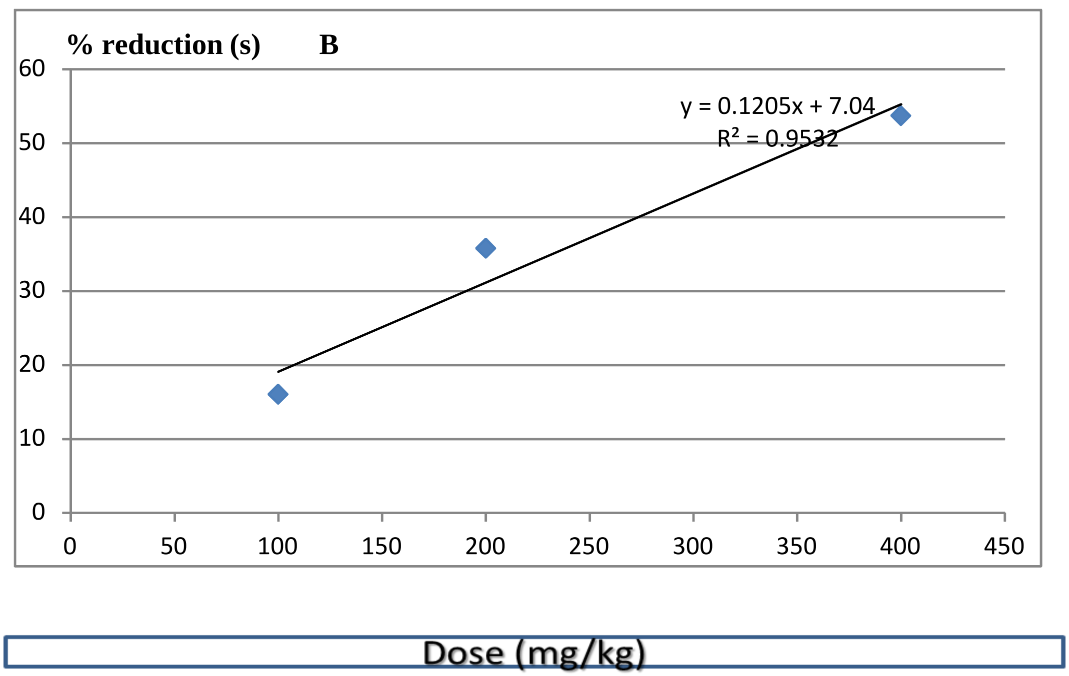


Figure 3: Linear chart showing the effect of dose at (100, 200 and 400mg/kg) on % reduction in time of immobility in the Forced Swim Test for butanol fraction (A) and Chloroform fraction (B)

4.4 Effect of the solvent fractions on locomotion in the open field test

In order to analyze whether changes in immobility were associated with changes in motor activity or not, a measurement of locomotor activity was performed. As presented in Table 5, there was no apparent difference in the number of crossings among all the groups.

Table 5: Results of an open field test in mice administered with solvent fractions of *Hypericum revolutum.*

Solvent fraction	Treatment group, *p.o.*	Number of crossings			%change in total locomotion
		Peripheral squares	Central squares	Total squares	
Controls	CON[1]	63.67 ± 4.232	4.83 ± .601	68.50 ± 3.793	-
	IMI 64 mg/kg	60.00 ± 2.309	4.50 ± .764	64.50 ± 2.377	5.84
Aqueous fraction	AF 100 mg/kg	63.50 ± 4.056	4.00 ± .816	67.50 ± 4.072	1.46
	AF 200 mg/kg	56.33 ± 1.856	4.67 ± .558	61.00 ± 1.633	10.95
	AF 400 mg/kg	57.83 ± 1.493	3.33 ± .211	61.33 ± 1.333	11.69
Controls	CON[2]	63.83 ± 4.175	4.83 ± .601	68.67 ± 3.955	-
	IMI 64 mg/kg	61.17 ± 2.845	3.00 ± .365	64.17 ± 2.664	6.55
Butanol fraction	BF 100 mg/kg	62.50 ± .764	6.00 ± .577	68.83 ± 1.327	-0.23
	BF 200 mg/kg	59.67 ± 1.282	6.00 ± .577	65.67 ± 1.174	4.37
	BF 400 mg/kg	60.17 ± 1.662	6.00 ± .577	66.17 ± 1.641	3.64
Controls	CON[2]	63.83 ± 4.175	4.83 ± .601	68.67 ± 3.955	-
	IMI 64 mg/kg	61.17 ± 2.845	3.00 ± .365	64.17 ± 2.664	6.55
Chloroform fraction	CF 100 mg/kg	63.17 ± 2.023	6.17 ± .601	69.33 ± 2.108	-0.96
	CF 200 mg/kg	63.00 ± 1.966	4.50 ± .428	67.67 ± 1.801	1.46
	CF 400 mg/kg	56.83 ± 2.257	4.83 ± .792	61.67 ± 2.789	10.19

Values are expressed as mean ± SEM, n=6 per group, CON= control ([1]: received distilled water and [2]: received 2% Tween 80 in distilled water), IMI= imipramine, AF= aqueous fraction, BF= butanol fraction, CF= chloroform fraction, *p.o.*= per oral, (- sign indicates increase in total locomotion).

4.5 Phytochemical analysis

Preliminary phytochemical screening for secondary metabolites was carried out on the crude 80% methanol extract, and solvent fractions of *H. revolatum*. The result revealed a differential distribution of secondary metabolites in the solvent fractions as shown in Table 6.

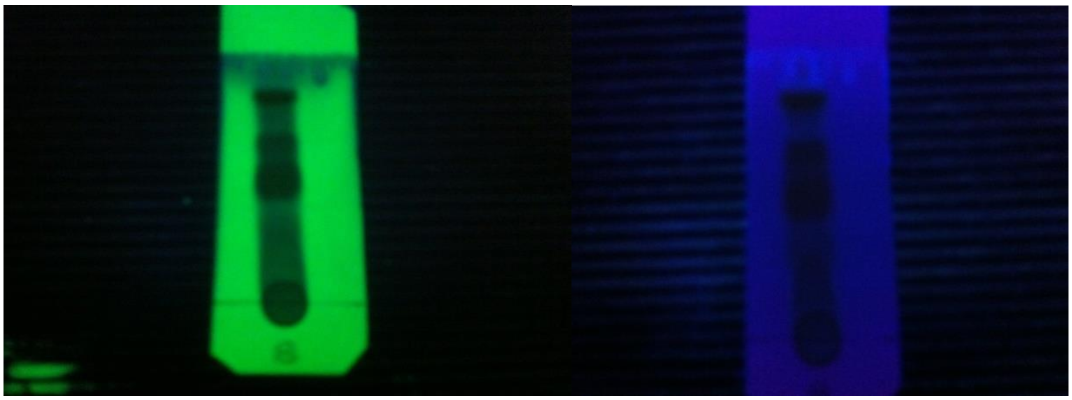
Table 6: Secondary metabolites detected in 80% methanol crude extract and solvent fractions of the aerial parts of *H. revolutum.*

Secondary metabolites tested	Crude extract	Solvent fractions		
		Aqueous fraction	Butanol fraction	Chloroform fraction
Alkaloids	+	+	+	+
Anthraquinones	+	-	+	+
Cardiac glycosides	+	+	+	-
Flavonoids	+	-	+	-
Saponins	+	+	+	+
Steroids	-	-	-	-
Tannins	+	+	+	-
Terpenoids	-	-	-	-

KEY: + indicates presence, - indicates absence

4.6 Thin layer chromatography finger print

TLC analysis was performed using various combinations of solvent systems. The solvent system containing a mixture of chloroform and methanol (4:1) produced a better separation of the constituents. The analysis revealed the presence of at least four compounds at 254 nm and 366 nm wavelength (Figure 4).



A B

Figure 4: Thin Layer Chromatography profile of butanol fraction of *Hypericum revolutum* at 254 nm (A) and 366 nm (B)

5. DISCUSSION

Natural products have been used as food and for medicinal purposes for centuries where research interest has focused on various species that possess antidepressant-like properties for the management of depression in humans. Various species of the genus *Hypericum* was proposed for such purposes. In the present study, aqueous, butanol and chloroform fractions of *Hypericum revolutum* at doses of 100, 200 and 400 mg/kg were investigated for exhibiting antidepressant-like activity in the two behavioral models, the FST and TST in rats and mice respectively.

Male mice and rats were used for antidepressant-like activity tests in this study. The rationale for using male sex in anti-depressive models is due to the fact that estrogen, the primary female sex hormone, on the brain from inception to death may affect the serotonergic system which could lead to increased vulnerability to stressors, different coping strategies and differentiated response to antidepressants in females by a line of evidences (Dalla *et al.,* 2010; Kornstein *et al., 2010*). This also correlated with models rely on innate social behavior among pairs or groups of male rodents allowing for the formation of stable dominant relationships (Krishnan and Nestler, 2011). The preference of female in toxicity is based on the recommendation of OEC, as the species of choice for toxicity screening studies. Moreover, the increased susceptibility of female rodents to toxicity offers a more accurate insight into the toxic nature of compounds to be tested (OECD, 2008).

The FST and TST are widely used for the screening of antidepressant activity. These tests are quite sensitive and relatively specific to all major classes of antidepressant drugs including TCAs, MAOIs, atypical agents, functional NMDA antagonists and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors potentiators (Porsolt *et al*., 1977; Steru *et al*., 1985; Belzung, 2014). However, SSRIs fail to consistently reduce immobility in the FST at pharmacological dose unlike TST (Bai *et al*., 2001). Otherwise, the FST is a good screening tool with good reliability and predictive validity (Rupniak, 2003; Petit-Demouliere *et al.,* 2005). This test involves the scoring of active (swimming and climbing) or passive (immobility) behavior when rodents are forced to swim in a cylinder, in which absence of escape and reduction in passive behavior is interpreted as an antidepressant-like effect of the manipulation (Slattery and Cryan, 2012). The model's induced state of immobility in animals is similar to human depression and is amenable to be reversed by antidepressant drugs. Moreover, these animal models are based on the despair or helpless behavior to an inescapable and confined space in animals with similar *prima facie* (Castagné *et al.*, 2011; Can *et al.*, 2012). In this study, both BF and CF fractions of *H. revolatum* produced a significant decrease in the duration of immobility in the animals in both tests as compared to the vehicle control groups, as the treatment of rodents with the BF and CF by oral route significantly reduced the immobility time in the models, as compared to a control group, without accompanying changes in locomotion in the OFT. However, AF was devoid of activity.

Although, the higher (400 mg/kg) and medium (200 mg/kg) doses of BF had shown significant immobility reduction, the 100mg/kg of BF (Table 4) was unable to replicate the effect seen in TST in FST. Moreover, comparisons with the standard revealed that the reduction in time of immobility produced by the higher (400 mg/kg) dose of CF was comparable with the standard in TST but unable for FST. On the contrary in FST, the higher (400 mg/kg) doses of both fractions were comparable with the standard. Moreover, the increments observed in CF in both models were relatively consistent for all doses, unlike BF. It can be inferred from this, the order of efficacy in reduction of immobility time from this study was found to be in order of CF>BF. Such disparity in order of effectiveness of the fractions in the reduction of immobility time could be due to: i) any confounds induced by stressful hypothermic exposure in the FST producing shock of being dropped in water and different underlying mechanisms of inducing immobility (Chatterjee *et al*., 2012). ii) the differential partitioning of phytochemicals into the fractions and the associated difference in mechanism of action of the secondary metabolites (table 6). iii) sub-threshold dose in base line immobility in the FST. Moreover, it has been reported that TST is less stressful and has higher pharmacological sensitivity than FST because the animal will remain immobile longer in the TST than the FST (Can *et al.*, 2012) with characteristic advantage of this procedure providing a complementary approach to the behavioral screening of antidepressant (Castagné *et al.*, 2011; Porsolt *et al*., 2001) in this study.

Furthermore, different studies of Hypericum species have shown that the CF have better activities in the reduction of immobility in the process of sub-fractions (Sánchez-Mateo *et al.,* 2005; Sánchez-Mateo *et al.,* 2007; Sánchez-Mateo *et al.,* 2009). Moreover, *H. revolutum* appears to be in the same range of potency as other Hypericum species in the FST and TST, since the effective dose of other Hypericum species extracts has shown at a higher dose (Sánchez-Mateo *et al.,* 2005). In line to this, there is a fundamental inter-strain and inter-species difference of response in the FST and TST (Petit-Demouliere *et al.,* 2005). In light of the above information, it is also important to remind various differences in their sensitivity and performance of the behavioral effects of these fractions were different strains that might contribute to a selective clinical response in some and resistance to treatment in others (López-Rubalcava and Lucki, 2000; Can *et al.,* 2012). Therefore, results of one test may not necessarily be replicated in the other.

In this study, one could also notice that there were inconsistencies in the percentage reduction observed at the same dose in the two models. For example, reductions produced for BF at different doses in TST were different for corresponding values in FST, similarly for CF as indicated in this study (Table 2 and 4). This could also be explained as one of reasoning in the difference of immobility reduction for the two models (López-Rubalcava and Lucki). On top of that pharmacokinetic and pharmacodynamic difference might accounts on part for the variability among species and across ages (Bai *et al.,* 2001; Mitchell *et al.,* 2013).

The doses used in this study, linear regression analysis showed that the BF and CF have dose-dependent antidepressant-like activity with the more profound effect seen at the middle and higher doses. Where both fractions exhibited a percentage of reduction in time of immobility (R^2= 0.9917 for BF and 0.9909 for CF in TST and R^2= 0.9479 for BF and 0.9532 for CF in FST) comparing dose-response relationship, thus immobility periods significantly decreased in a dose-dependent manner as compared to control in both FST and TST. Since there is a clear correlation between antidepressant-like effect in potency and ability to reduce immobility time which in line with other studies (Butterweck, 2003; Husain *et al*., 2011). However, the sensitivity and the pattern of dose-response for the fractions were different between the TST and FST as well correlated for model preference could be justified by observing the linear pattern of activity. For example, both BF and CF in TST had shown a linear pattern of activity (Figure 2), suggesting that maximal response may be achieved at minimum effective doses while in FST different pattern of dose-response produced by the three doses showed relatively a nonlinear relationship with responses (Figure 3) requiring adequate time and frequent early-dose adjustment (Bai *et* al., 2001; Saleem *et al.,* 2011).

In these behavioral tests, false-positive results are occasionally obtained for agents that stimulate locomotor activity according to report studies have shown (Batool *et al*., 2011; Machado *et al*., 2013). Therefore, determining whether *H. revolutum* have excitatory or inhibitory actions on the CNS was important by using OFT for the study. In this study, *H. revolutum* had shown no effect on the spontaneous locomotor activity of mice. The results which were devoid at lower doses of the models could not affect the parameters significantly. This finding indicates that *H. revolutum* had no excitatory or inhibitory action on the CNS in effective doses. Therefore, it eliminates the probability of false-positive results in the TST and FST. This finding suggests that the reduction of immobility time elicited by *H. revolutum* treatment in both tests were less likely to be due to a psychomotor-stimulant effect but rather an antidepressant-like effect of the plant (Table 5).

Herbal medicines are usually complex mixtures of chemicals. The combined actions of these chemicals may contribute to the overall effects of the medicines (Butterweck, 2003). With regard to the components responsible for the antidepressant activity, from the phytochemical analysis, both the butanol and the chloroform fractions derived from the methanol extract of H. *revolutum* showed a significant activity, in which several different constituent might involve in the pharmacological effects of this plant. Phytochemical analysis of the present study revealed that aerial parts contained alkaloids, anthraquinones and saponins in both fractions. Moreover, tannins and flavonoids were detected in BF.

Flavonoids, which are a group of polyphenolic compounds, recently; as studies have shown, have attracted increasing attention for its antidepressant and multiple pharmacological activities (Butterweck *et al*., 2000; Rochaa *et al*., 2007; Gong *et al.*, 2014). Flavonoids by inhibiting monoamine oxidase A and catechol-O-methyltransferase enzyme (Patocka, 2003), inhibiting the uptake of monoamines and thus increasing the level of monoamines (Shewale *et al.,* 2012; Okokon *et al.,* 2016; Dubey *et al.,* 2017), acting on free radicals (Sharma and Agarwal, 2011) were found to reduce the time of immobility in animals model of depression. Moreover, flavonoids from *Hypericum perforatum* and other findings have been also demonstrated antidepressant effects in both FST and TST (Butterweck et al., 2000, 2003a; Nöldner and Schötz, 2002; Zhang, 2004; Sakakibara *et al*., 2006, Rowshan, 2014), it is possible that at least part of the antidepressant activity showed in this study and the inconsistencies observed by the BF unlike the other fractions might be for the presence of flavonoids although the analysis is believed to be a primary one and similar activities could be observed in non-flavonoid extracts (Hurley *et al.*, 2014).

The secondary metabolite; tannins, which also detected in the BF of the plant on its part might contribute to the differences in activities and discrepancies observed in the tests. For example, tannins from *Terminalia Catappa* leaf extract *via* simultaneous alterations in HPA axis, monoaminergic responses, BDNF-CREB system and oxidative processes during chronic mild stress has shown decreasing immobility of mice (Chandrasekhar *et al*., 2017).

Alkaloids rich plants produced a reduction of immobility time *via* increasing in monoaminergic turnover (Hsu *et al.,* 2012; Martínez-Vázquez *et al*., 2012; Antkiewicz-Michaluk *et al.,* 2013), interaction with serotonergic system (Hamid *et al*., 2017; Kumar *et al.,* 2017), activation of HPA axis through serotonergic system (Cassani *et al*., 2014), enhancing p-CREB/CREB and BDNF in the frontal cortex and hippocampus (Liu *et al*., 2012) thus in this study alkaloid might be responsible for the activities of the two fractions.

Moreover, the presence of saponins in this study might be responsible for the activities observed in both fractions. As studies showed saponins can associated with the MAO inhibition, the modulation of monoamine neurotransmitter and antioxidative enzymes, and the elimination of excessive free radicals (Huang *et al.,* 2012; Mannan *et al.,* 2015; Liang *et al*., 2016), cytoprotective (Zhu *et al.,* 2006). Saponins also at least in part could play, by the inhibition of MAO-A and reversed the swim stress -induced increases in serum corticosterone levels thus decrease immobility (Liao *et al.,* 2013).

Natural anthraquinones are distinguished by a large structural variety, a wide range of biological activity and low toxicity. Anthraquinones unlike other phytoconstituents in this study only detected in BF and CF but not in the AF. Besides, some anthraquinones and its derivatives present in medicinal plants used as antioxidant and memory enhancing activity. Emodin, derivatives of anthraquinones was shown to inhibit MAO B in a dose-dependent (Kong *et al*., 2004). Moreover, Decosterd et al. (1989) were isolated active principle of hyperforin- an anthraquinone-derived from the root bark of *H. revolutum* which might signify activities observed in reduction of immobility in this study by large through uptake inhibition of monoamines, GABA and Glutamate (Chatterjee *et al.*, 1998). Moreover, the main wealth secondary metabolites biologically active are naphthodianthrones (hypericin, pseudo-hypericin, proto-hypericin, and proto pseudo-hypericin), phenylpropanes, flavonol derivatives (hyperoside and rutin), biflavones, proanthocyanidins, phloroglucinols (hyperforin, adhyperforin, hyperfirin, and adhyperfirin), different amino acids and essential oil constituents (Chatterjee *et al.,* 1998; Alali and Tawaha, 2009). Being, the most actives are naphthodianthrones, the flavonoids and the phloroglucinols (Tian et al., 2014).

Nevertheless, due to the exhaustive extraction method used in this study, the selective solubility of the phytochemicals was probably responsible for conferring a wide spectrum of biological activities attributed to the aerial of *H. revolutum* suggesting the importance of the solvent as a decisive factor (Roopalatha and Nair, 2013). For example, as depicted in Table 6, the quantities of secondary metabolites being the crude extract and BF contained many followed by AF and then CF. Moreover, trace amounts might have remained in some fractions to be detected. Consequently, the phytochemical constituents that could be used as antidepressant were either absent or present in an undetectable amount in the others. Studies showed that the antidepressant effects of Hypericum *specious* extracts may be due to a combination of different biological constituents rather than any single compound (Rowshan, 2014). This also explains why the differences in activities and discrepancies in the reduction of immobility observed in the two fractions.

It has been also shown that different polyphenols can increase the synaptic plasticity of hippocampal neurogenesis and promote hippocampal long-term potentiation (Lau *et al.*, 2005; Van Praag, 2009; Stringer *et al*., 2015). Moreover, it has been verified that polyphenols can enhance learning and memory and reduce the risk of developing age-related neurodegenerative diseases, possibly *via* a decrease in reactive oxygen species production and inflammation in models of ageing (Zhang *et al*., 2014). Besides different polyphenolic compounds have been observed to have antidepressant-like effects in rodents and human. Therefore, the phytochemicals present in single or combination in the sample could act as potential antidepressant agents by involving monoamine neurotransmitters-based, HPA axis-based, and neurotrophic factors, or neurogenesis based (Wang and Xu, 2012, Rowshan, 2014). However, further investigation is necessary to determine the exact phytoconstituents and mechanism of action that are responsible for the biological activities observed in antidepressant-like effect in this study.

TLC-data also revealed the presence of at least four compounds in *H. revolutum* which were detected with UV light at 254 nm and 366 nm wavelength for BF. However, clear spots were not observed in CF like that of BF which might associate with the absence of glycosides. Moreover, the result of the UV analysis of the fractions gave absorption peaks at higher wave length probably because of the principle of absorption maxima. The formation of absorption bands in the UV region and in the visible region depend on the presence of the glycosides which is in accordance with the TLC analysis in this study (Shewale *et al.*, 2012). Moreover, it could be inferred that the compounds present in the fractions have chromophores and hence, absorption takes place to allow transition (Alebiosu and Yusuf, 2015). The portion of the yield for CF, on the other hand, could worthwhile in the reasoning of the difference. Similarly, the concentration of the different biological components might not be enough to have the required effect because of selective solubility. At this point, it is interesting to notice that even though the starting material was 80% methanol for this study suggesting the solvent as a decisive factor is important (Roopalatha and Nair, 2013). Clearly indicative for deciding the starting materials and somewhat reason out the underline inconsistencies observed in the reduction of immobility between CF and BF apart from the effect of models. Moreover, compounds those are semi-polar to non-polar probably responsible for antidepressant-like effect and use of less polar solvents as starting material for crude extraction such as petroleum-ether might be important (Decosterd[*]*et al.*, 1989).

6. CONCLUSION

From the results of this study, it can be concluded that the aerial parts of *H. revolutum* possesses a significant antidepressant-like activity and exhibited safe in animal models. This is indicated by a statistically significant decrease in the duration of immobility in established behavioral despair based models of depression. The antidepressant-like effect of the solvent fractions of the plant as observed in the TST and FST induced a significant reduction of the immobility with CF being the most active fraction followed by BF and no effect with AF. Moreover, the 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. In addition, the confirmatory of phytochemical and TLC analysis gives remarkable evidence of plant reliance in containing different secondary metabolites with antidepressant-like effect.

7. RECOMMENDATIONS

Despite the fact that the *H. revolutum* used in the study was considered safe, there is a need to advance the current status. Therefore:

- ⇛ Further studies using other models of depression should be carried out, including a chronic model of depression.
- ⇛ Further pharmacological and neurobiological tests should be performed in order to elucidate the mechanism of action involved.
- ⇛ Further sub-acute and chronic toxicities studies should be done in order to assess the long-term effect of each fraction.
- ⇛ 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 solvent fractions.
- ⇛ To undertake further investigation by using different solvent as starting material with less polarity.

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