# EVALUATION OF THE ANTIDEPRESSANT- LIKE ACTIVITY OF SENNA SINGUEANA (DEL.) LOCK, (FABACEAE) IN RODENTS

**BY MIKYAS BOGALE**

**A THESIS SUBMITTED TO THE DEPARTMENT OF PHARMACOLOGY AND CLINICAL PHARMACY, SCHOOL OF PHARMACY, COLLEGE OF HEALTH SCIENCES, ADDIS ABABA UNIVERSITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTER OF SCIENCE DEGREE IN PHARMACOLOGY**

**MARCH, 2021**

**ADDIS ABABA, ETHIOPIA**

**DEPARTMENT OF PHARMACOLOGY AND CLINICAL PHARMACY**

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# EVALUATION OF THE ANTIDEPRESSANT- LIKE ACTIVITY OF SENNA SINGUEANA (DEL.) LOCK, (FABACEAE) IN RODENTS

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## Abstract

Depression is a multifactorial, chronic, and life-threatening disease with high prevalence, disability, and societal cost. Currently, 20%–30% of patients treated with commonly used antidepressants do not achieve complete recovery and develop treatment-resistant depression. Drug resistance in addition to side effects and drug interactions are the major restrictions in the treatment of depression. Thus, there is an urgent need to identify new treatment options including herbal drugs. In this study, the essential oil extract of *Senna singueana* (Del.) (Fabaceae) was assessed for its antidepressant property using the tail suspension test (TST) and the forced swimming test (FST) models. To rule out the false-positive antidepressant-like activity of the oil, open field test (OFT) was used. The composition of essential oil was determined using gas chromatography–mass spectrometry (GC/MS) analysis.

All animals were randomly assigned to six different groups for each model. Group I received the vehicle (2% Tween 80) and served as a negative control. Group II received the standard drug imipramine (30 mg/kg) and served as a positive control. The test groups were from group III-VI and received increasing doses of the extract at 100 mg/kg, 200 mg/kg, 400 mg/kg, and 600mg/kg, respectively. In TST, 600 mg/kg showed a significant immobility time reduction (56.6%) as compared to 100 mg/kg, 200 mg/kg ($p<0.05$) and 2% Tween 80 ($p<0.01$) treated groups. In FST, on the other hand, 400 mg/kg (50.6%) and 600 mg/kg (61.8%) test doses exhibited a statistically significantly reduction in immobility time ($p< 0.01$) as compared to 2% Tween 80, 100 mg/kg and 200 mg/kg treated groups. It was also demonstrated that the essential oil extract of *Senna Singueana* didn't significantly alter the spontaneous locomotor activity of mice during the OFT test.

From GC-MS analysis a total of 33 compounds were identified and compounds like eugenol, linalool, n-hexadecanoic acid, octadecanoic acid and heneicosane, which previously reported to have antidepressant activity were found. In conclusion, this study indicated the essential oil extract of *Senna Singueana* may have potential therapeutic value for the management of depression.

**Key words:** Depression; *Senna Singueana*; Essential oil; Antidepressant-like activity

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# Contents

## Lists of Abbreviations and Acronyms

| | |
|---|---|
| 5-HT | 5-hydroxytryptamine (serotonin) |
| ACTH | Adrenocorticotropin hormone |
| AMPA | α-amino-3-hydroxy-4-isoxazoiepropionic acid receptor |
| ANOVA | Analysis of variance |
| AVP | Arginine vasopressin |
| BDNF | Brain derived neurotrophic factor |
| CBT | Cognitive behavioral therapy |
| CEN | Central executive network |
| CNS | Central nervous system |
| CREB | cAMP response element binding |
| CRF | Corticotrophin- releasing factor |
| CRH | Corticotrophin releasing hormone |
| DA | Dopamine |
| DG | Dental gyrus |
| DMDD | Disruptive mood dysregulation disorder |
| DMN | Default mode network |
| DRI | Dopamine reuptake inhibitors |
| DSM | Diagnostic and statistical manual for mental disorder |
| FA | Fraction anisotropy |
| FGF-2 | Fibroblast growth factor-2 |
| FST | Forced swimming test |
| GABA | Gamma aminobutyric acid |
| GC | Glucocorticoid |
| HPA | Hypothalamus-pituitary-adrenal |
| HTR2A | 5-Hydroxytryptamine (serotonin) receptor 2A |
| ICD | International classification of diseases |
| IFN | Interferon |
| IL | Interleukin |
| LD-50 | Lethal dose, 50% |
| MAO | Monoamine oxidase |

| | |
|---|---|
| MAOI | Monoamine oxidase inhibitors |
| MDD | Major depressive disorder |
| MRI | Magnetic resonance imaging |
| MSD | Mass selective detector |
| NASSA | Noradrenergic α2 -receptor antagonist with specific serotonergic receptors-2 and -3 antagonism |
| NDRI | Norepinephrine-dopamine reuptake inhibitor |
| NE | Norepinephrine |
| NF-κB | Nuclear factor kappa-light-chain-enhancer of activated B cells |
| NGF | Nerve growth factors |
| NMDA | N-methyl-D-aspartate |
| NRI | Norepinephrine reuptake inhibitors |
| NRISA | Norepinephrine reuptake inhibitor with serotonin receptors antagonism |
| NSAIDs | Nonsteroidal anti-inflammatory drugs |
| OECD | Organization for Economic cooperation and development |
| OFT | Open field test |
| OS | Oxidative stress |
| PD | Psychotic depression |
| PET | Positron emission tomography |
| PMDD | Premenstrual dysphoric disorder |
| PMS | Premenstrual syndrome |
| PVN | Paraventricular Nucleus |
| ROS | Reactive oxygen species |
| SAD | Seasonal Affective Disorder |
| SARI | Serotonin receptors antagonist with serotonin reuptake inhibition |
| SCN | Superchiasmatic Nucleus |
| SEM | Standard error of mean |
| SN | Salience network |
| SNRI | Serotonin-nor-epinephrine reuptake inhibitors |
| SPARI | Serotonin 5-HT1A auto receptor partial agonist with serotonin reuptake inhibition |

| | |
|---|---|
| SPSS | Statistical package for social sciences |
| SSRI | Selective serotonin reuptake inhibitors |
| TCA | Tricyclic antidepressants |
| TNF | Tumor necrosis factor |
| TPH | Tryptophan hydroxylase |
| TST | Tail suspension test |
| WHO | World Health Organization |
| YLD | Years Lived with Disability |

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# 1. Introduction

## 1.1 Background

Depression is a common mental disorder characterized by sadness, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, feelings of tiredness, and poor concentration (WHO, 2017). At a global level, around 322 million people are estimated to suffer from depression, equivalent to 4.4% of the world's population with a lifetime prevalence of 15–18%. It is more common among females (5.1%) than males (3.6%) and prevalence is higher in the older age groups (Andreescu & Reynolds, 2011; WHO, 2017). According to the Global Burden of Disease report, depression is a leading cause of disability worldwide and is a major contributor to the overall global burden of disease. The number of incident cases of depression worldwide increased from 172 million in 1990 to 258 million in 2017, representing an increase of 49.8% (Metrics, 2018; Liu et al., 2019). Poverty, unemployment, life events just like the death of a beloved or a relationship break-up, physical illness, and problems caused by alcohol and drug use, increase the risk of becoming depressed (WHO, 2017).

In sub-Saharan Africa, neuropsychiatric disorders account for nearly 10% of the total burden of disease (Duerkop, 2009). In Ethiopia, mental illness is the leading key to non-communicable disorders in terms of burden. Among every five persons, one will be affected by mental disorders at some period of his or her life. In the top ten most burdensome conditions, schizophrenia and depression are listed, out-ranking HIV/AIDS (FMOH, 2012; Sathiyasusuman, 2011). By the year 2016 depression alone accounted for 6.2 percent of total years lived with disabilities (YLD), ranking fourth out of all causes of YLD (Hanlon et al., 2019). From 2005 to 2016, the burden of depression is estimated to have increased by 39.6% (Hanlon et al., 2019). According to researches, the prevalence of depression in Ethiopia ranged between 2.2% to 9.1% (Hailemariam et al., 2012; FMOH, 2018).

## 1.2 Types of depression

There are different types of depressive disorders having a substantial heterogeneity in clinical symptoms, etiology, time of onset, course, severity, and treatment response. As a result of this heterogeneity there is a predictable lack of consistent genetic and neurobiological findings validating depression as a separate or even clear diagnostic entity (Harald & Gordon, 2012; Rantala et al., 2017). Here are listed some of the common types of depression;

**Major depressive disorder (MDD) -** is a debilitating disease that's characterized by at least one distinct depressive episode lasting a minimum of two weeks and involving clear-cut changes in mood, interests, and pleasure, changes in cognition, and vegetative symptoms (Otte et al., 2016; Vahia, 2013). The symptoms should cause clinically vital distress or impairment in social, occupational, or different vital areas of functioning. The episode isn't as a result of the physiological effects of a substance, or to a different medical condition. The incidence of the episode isn't better explained by psychotic disorders and the individual has never had a manic episode or a hypomanic episode (Otte et al., 2016).

**Persistent depressive disorder** (**Dysthymia)** - is a recurrent depressive disorder with vital functional impairment and no clearly demarcated episodes. In adult's, diagnosis is when individuals have not been free of their depressive symptoms for longer than 2 months over a 2-year period and have not experienced an episode of major depression or mania. Whereas in children and adolescents, the criteria span a 1-year period. Common co-morbid conditions include major depression, anxiety, personality, somatoform, and substance abuse disorders (Melrose, 2017).

**Bipolar disorder (Manic Depression) –** is a serious and chronic psychiatric illness that causes unusual shifts in mood, energy, activity levels, concentration, and the capacity to carry out day-to-day tasks. The disorder is characterized by alternating episodes of mania or hypomania and depression or mixtures of manic and depressive features. According to DSM-V, symptoms must be present for at least 1 week for a diagnosis of a manic episode, or 2 weeks for a diagnosis of a depressive episode. Generally, there are three types of bipolar disorder

**Bipolar I Disorder—** defined by manic episodes with a range of manifestations, including overconfidence, grandiosity, talkativeness, extreme disinhibition, irritability, decreased need for sleep, and highly elevated mood. Incidences of depression with mixed features (having depressive symptoms and manic symptoms at the same time) are also possible.

**Bipolar II Disorder—** unlike bipolar I disorder which is characterized by a full-blown manic episode, this disorder is defined by a pattern of depressive episodes and hypomanic episodes.

**Cyclothymic Disorder (also called Cyclothymia) -** defined by recurring depressive and hypomanic states lasting for at least 2 years (1 year in children and adolescents) that do not

meet the diagnostic threshold for a major affective episode (Carvalho & Firth, 2020; Angst et al., 2020).

**Seasonal affective disorder (SAD) -** also known as "winter depression" is a recurrent major depressive disorder with a seasonal pattern usually beginning in the fall and continuing into winter months. In diagnosis, it is important to carefully determine the time of onset and offset of previous depressive episodes and to ensure that patients have full remission in summer. A supportive clinical characteristic of SAD is a positive mood response to increased light exposure and to winter travel to more southerly latitudes. Patients with dysthymia and chronic MDD may experience winter worsening of their symptoms, but they can be differentiated from those with SAD because they are still symptomatic in the summer (Lam, 2007; Melrose, 2015).

**Psychotic Depression (PD) -** is a mental disorder characterized by the presence of delusions and/ or hallucinations in addition to depression. Apart from the psychotic features it also involves a psychomotor disturbance (either agitation or retardation), rumination, insomnia, cognitive dysfunction, and perplexity more often than non-PD. Furthermore, compared to non-PD, PD is associated with increased long-term psychosocial impairment, increased rates of relapse, and higher levels of mortality, possibly due to increased risk of suicide (Dinesen & Rothschild, 2012).

**Situational Depression (Reactive Depression or currently known as Adjustment Disorder) –** is a disorder defined by emotional or behavioral symptoms occurring within 3 months (DSM-IV) or 1 month (ICD-10) of exposure to an identifiable stressor. The symptoms must be clinically significant and/or there is major impairment in social or occupational functioning. The symptoms are not due to another axis I disorder (or bereavement in DSM-IV) and should resolve within 6 months once the stressor or its consequences are removed (Casey & Bailey, 2011; Baumeister et al., 2009)

**Disruptive Mood Dysregulation Disorder (DMDD) -** is a disorder characterized by the presentation of persistent irritability and frequent episodes of extreme behavioral decontrol or temper outbursts in children's up to 12 years of age. This symptom occurs on average, three or more times per week. Between the outbursts, the child will display a persistently irritable or angry mood, most of the day and nearly every day, that is observable by parents, teachers, or peers. For DMDD diagnosis the above symptoms must be present in at least two settings (at home, at school, or with peers) for 12 or more months, and symptoms must be severe in at least one of these settings (Zaky, 2015).

**Depression types unique to women**

**Perinatal depression.** This type of depression includes major and minor depressive episodes that occur during pregnancy (prenatally) and/or in the first 12 months after delivery (postpartum). Unlike the "baby blues," which occur in up to 80% of new mothers within several days of delivery because of regulatory biochemical changes, where the symptoms are usually brief and last no longer than 10 days, perinatal depression lasts more than 14 days and impairs a woman's quality of life (Niel & Payne, 2020).

**Premenstrual dysphoric disorder** (**PMDD).** This type of depression is a severe form of premenstrual syndrome (PMS) which usually begin shortly after ovulation and end once menstruation starts. It contains a vast array of psychological symptoms which include anger, irritability, depression, and internal tension (Duko et al., 2020).

**Vascular depression-** In the late 1990s, investigators proposed the concept of "vascular depression" as a research subtype of late-life unipolar depression (Andreescu & Reynolds, 2011). This type of depression develops after ages 60–65 in the absence of a prior history of affective illness. Symptoms of low energy, lack of insight, anhedonia, deficits in self-initiation, psychomotor retardation, executive dysfunction (difficulty of task completion and discussion making), and additional cognitive deficiencies (reduced speed of processing and impairments in concentration and attention) have been reported by different researches (Aizenstein et al., 2016). The assessment for vascular depression ideally includes a review of vascular risk factors, history of vascular disease, and findings of deep white matter and periventricular hyperintensities as well as subcortical gray matter lesions as evidenced by magnetic resonance imaging (MRI) based studies (Potter et al., 2009; Taylor et al., 2019). Despite the large volume of research, vascular depression is not represented in DSM-5 classification and has different operational definitions in various classifications (Taylor et al., 2019).

## 1.3 Pathophysiology

Despite great progress in neuroscience research over the past few decades, the primary pathophysiology of depression has not been clearly defined and there's no single known cause of depression. Rather, it likely results from a mixture of genetic, biological, social, and psychological factors (Craddock, 2006). Over the past several theories or hypotheses have been proposed about the mechanisms of depression.

**Monoamine Hypothesis,** a hypothesis proposed in 1965, which has dominated the understanding of both the pathophysiology of depression and the action of pharmacological treatments for the last decades, and it has led to the creation of several generations of antidepressant agents (Massart et al., 2012).

Evidence for this hypothesis came from clinical observations of patients treated for hypertension with reserpine, which causes a depletion of presynaptic stores of norepinephrine (NE), 5-Hydroxytryptamine (5-HT), and dopamine (DA), showed a syndrome resembling depression (Freis, 2010). Further, the effect of imipramine-like drugs and monoamine oxidase inhibitors (MAOI), which are used in the treatment of depression, in increasing catecholamine at adrenergic receptor sites shows the functional deficiency of noradrenergic transmission in this disease (Cosci & Chouinard, 2019; Bondy, 2002). The hypothesis generally suggests a deficiency of the brain monoaminergic transmitter's NE, 5-HT, and/or DA will result in depression, and antidepressants act by increasing these transmitters' availability and by producing long-term adaptive changes in monoaminergic receptor sensitivity (Mongeau et al., 1997). However, the hypothesis fell to explain why prolonged administration (three to four weeks) required to obtain treatment effects, even though monoamine levels sharply increase upon antidepressant administration. Moreover, up to 30% of patients with MDD are refractory to currently used antidepressants. Therefore, factors beyond monoamine deficiency or imbalance are most probably implicated in the development of major depression (Massart et al., 2012; Al-Harbi, 2012).

**Neural Plasticity and Neurogenesis hypothesis**

This hypothesis of depression is based on the dysfunctions of the Hypothalamus-pituitary-adrenal (HPA) axis witnessed in the majority of depressed patients (Massart et al., 2012; Anacker et al., 2011). The activity of the HPA axis is governed by the secretion of corticotropin-releasing hormone (CRH) and arginine–vasopressin (AVP) from the hypothalamus, which promotes the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary. ACTH promotes the secretion of glucocorticoids (GC) from the adrenal gland causing an elevated level of GC in the blood and cerebrospinal fluid. These suppress the secretion of CRH and AVP via GC receptors in the hippocampus by the negative feedback mechanism. According to this hypothesis, chronic stress causes the failure of the negative feedback of the HPA axis, which leads to the continuation of the elevated levels of GC. This elevated GC decreases the volume of the hippocampus which plays an essential role in the pathophysiology of MDD (Boku et al., 2017). Due to these

mechanisms, two hypotheses have been proposed: the neuroplasticity and neurogenesis hypotheses. **The neuroplasticity hypothesis** suggests that chronic stress induces the atrophy of mature neurons in the hippocampus and these decrease the expression of Brain-derived neurotrophic factor (BDNF), which leads to negative morphological changes of neurons in the hippocampus. Antidepressants can recover stress-induced morphological changes by increasing the expression of BDNF (Karege et al., 2002). **The neurogenesis hypothesis** proposes that stress decreases the number of newborn neurons and neural precursor cells in the dentate gyrus (DG) of the hippocampus, which is reversed by antidepressants. Antidepressants recover the negative effects of GC on neural precursor cells indirectly via the neuron–noradrenaline– cAMP response element-binding (CREB) pathway and/or the astrocytes– fibroblast growth factor-2 (FGF2) pathway (Boku et al., 2017). Unlike the monoamine hypothesis, these hypotheses are able to explain the latency of response to antidepressants. However, contrary results have been reported on BDNF changes in response to stress and antidepressants (Groves, 2007).

**Neuroimmune and Cytokine Hypothesis**. According to this hypothesis, stress triggers the activation of the HPA axis and the inflammatory response system. Activation of the inflammatory response system produces an increase in pro-inflammatory cytokines which elevate Corticotrophin- releasing factor (CRF) production and interrupt negative cortisol feedback. These cytokines and excessive cortisol inhibit neurogenesis in the brain by interfering with the activity of growth factors. Moreover, pro-inflammatory cytokines promote shifts from tryptophan to kynurenine rather than serotonin affecting the serotonin pathway. As a result, the utilization rate of serotonin decreases leading to depression as stated in the monoamine hypothesis (Jeon & Kim, 2016). This hypothesis is supported by the fact that many pro-inflammatory marker levels are reported to be elevated in depressed patients. In fact, depressive-like behaviors can be induced in the laboratory by the administration of the cytokine, interferon (IFN)-α as seen in patients taking it for the treatment of hepatitis C or cancer, which can be treated by classical antidepressants (Raison et al., 2012). Furthermore, a population-based study has shown that both prior severe infections and autoimmune diseases increase the risk of subsequently developing MDD (Benros et al., 2013). Other studies using positron emission tomography (PET) imaging have also indicated neuroinflammation and microglial activation in the central nervous system (CNS) of patients with MDD (Setiawan et al., 2016). But despite these researches, it is not clear whether an increase in inflammation causes depression, or whether inflammation increases as a result of depression.

**Glutamatergic hypothesis-** Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system which also serves as a metabolic precursor to γ-aminobutyric acid (GABA) and as a component of various amino acid-based derivatives like the antioxidant glutathione (Niciu et al., 2015).

Recently, clinical and preclinical studies revealed that maladaptive changes in excitatory/inhibitory circuitry, particularly in glutamate homeostasis and neurotransmission, have a primary role in mood and anxiety disorders (Sanacora et al., 2013; Mccarthy et al., 2012). All these studies have provided the background for the formulation of a new hypothesis for the pathogenesis of depression: the glutamate hypothesis of depression. According to this hypothesis, there is a sustained glutamate accumulation, caused by alterations of glutamate release, clearance, and metabolism, in selected brain areas associated with cognitive-emotional behaviors and mood regulation (hippocampus, amygdala, and prefrontal cortex), which causes a structural/functional changes and impaired synaptic activity in these regions (Musazzi et al., 2012).

Evidence for this hypothesis comes from a number of clinical studies showing glutamate level increment in plasma and in postmortem samples of frontal cortex from patients with major depressive disorder (Hashimoto et al., 2007). Other studies using proton magnetic resonance spectroscopy, provided evidence of reduced glutamate metabolite levels in the frontal cortex and cingulate regions of depressed patients during depressive episodes (Yüksel & Öngür, 2011). Abnormal glutamate metabolite levels were also found to normalize with electroconvulsive therapy treatment, suggesting pathophysiological importance during active episodes of major depression (J. Zhang et al., 2014). The rapid and prolonged antidepressant action of ketamine (glutamate receptor antagonists) is further support for this hypothesis (Carlos A. Zarate et al., 2006).

***Inflammation and glutamate-*** Different studies have found glutamate and inflammatory mediator's cross-talk in depressive disorders (Mcnally et al., 2008). Inflammatory mediators like cytokines activate the kynurenine pathway and the two major end products of this pathway (Kynurenic acid and quinolinic acid (antagonist and agonist respectively)) bind to N-methyl-D-aspartate (NMDA) receptors. Plasma kynurenic acid levels are increased in MDD patients with a history of suicide attempts relative to patients without a history of suicide (Sublette et al., 2011). Generally, the glutamate involvement in depression and its link with inflammation appears to be a promising line of research and the mechanism behind awaits further translation in humans.

**The oxidative stress hypothesis-** According to this hypothesis, the imbalance between oxidative stress and the antioxidant defense system may be associated with the development of neuropsychiatric disorders, such as depression and anxiety. Evidence for this hypothesis came from study findings that correlated major depression and anxiety disorders with a lowered total antioxidant state and an activated oxidative stress pathway (Xu et al., 2014).

Oxidative stress is a major component in the signaling cascade involved in the activation of redox-sensitive transcription factors and pro-inflammatory gene expression which leads to an inflammatory response. In certain chronic disease states where oxidative stress is activated, there is increased activation of inflammation-related transcription factors, such as NF-KB and CREB, which begin to play a role in the processes of mood disorders (Xu et al., 2014; Kelley et al., 2013). Increasing evidence suggests that mitochondria are a prime target of oxidative damage, which induces further mitochondrial dysfunction and reactive oxygen species (ROS) generation, all of which may result in neuronal death. In humans, decreased mitochondrial ATP production rate and deletions of mtDNA have been observed in patients with MDD comorbid with somatic illnesses (Xu et al., 2014; Hayashi, and Tanuma, 2012).

Oxidative stress is also associated with BDNF reductions, as well as reductions in CREB and synapsin-I molecules that are involved in cellular plasticity cascades. Furthermore, excessive formation of ROS in the brain under disease states may lead to dysfunction of genes and proteins involved in neuroplasticity and neurogenesis, which result in neuronal apoptosis (Wu et al., 2004). Generally, clinical and preclinical studies on oxidative stress suggest that oxidative stress may induce a neuroinflammatory response, mitochondrial dysfunction, and neuroplastic deficits which may have a role in the pathogenesis of depression. Thus, understanding the functional relationship between oxidative stress and depression may help the discovery of novel targets for treatment.

**Circadian rhythms and depression-** Circadian rhythms allow synchronization of biological and behavioral processes to the external temporal environment. Endogenous circadian rhythms have a period of ~24 h which is controlled by molecular clockworks within the brain (suprachiasmatic nucleus (SCN) of the hypothalamus) and are reset on a daily basis to precisely 24 h through exposure to environmental signals or “zeitgebers” (Ii et al., 2020). Light is a major zeitgeber for SCN. Via the retinohypothalamic tract, a non-image-forming pathway, and indirectly via the intergeniculate leaflet of the lateral geniculate complex, light reaches the SCN neurons. The major output of the SCN is to the paraventricular nucleus (PVN) of the hypothalamus and via a

multisynaptic pathway, to the pineal gland where melatonin is secreted. Melatonin is a biochemical transducer of photoperiodic information to all cells in the body signaling the seasonal variations of day/night cycle length. The PVN is also the site of autonomic neurons, which communicates the time-of-day signal to different body organs, and corticotrophin-releasing factor secreting neurons, which are part of the HPA axis, endowed with diurnal rhythmicity. Therefore, the SCN signal is translated into hormonal and autonomic signals for peripheral organs mainly within the PVN (Monteleone & Maj, 2008). Disturbances to circadian rhythms have been found among patients with major depression. Exposure to light at night, shift work (working outside of the typical shifts) or jet lag (travels across multiple time zones) may perturb the circadian system. This disruptions of biological rhythms underlie hallmarks of the disease; specifically, alterations in sleep/wake states, social rhythms, hormone rhythms (reduced amplitude in melatonin and cortisol rhythms), and body temperature rhythms (reduced amplitude and increase in nocturnal body temperature) (Monteleone & Maj, 2008; Vadnie & Mcclung, 2017). Clinical studies demonstrate that the severity of MDD is associated with the degree of misalignment of circadian rhythms. Circadian disruption may not be the sole cause of MDD or generally mood disorders but it may elicit or exacerbate symptoms in individuals with a predisposition for mental health disorders (Vadnie & Mcclung, 2017; Ii et al., 2020).

**Structural and functional abnormalities in Depression-** Depression is related to structural and functional abnormalities in the limbic-cortico-striatal-pallido-thalamic tract which is connected to the hippocampus, amygdala, caudate nucleus, putamen, and the frontal cortex (Kaltenboeck & Harmer, 2018). Structurally, depressed subjects showed both grey matter and white matter abnormalities. The volume of gray matter in the brain is associated with many physiological senses and higher functions including muscle control, vision, hearing, memory, emotion, language, decision-making, and self-control. Whereas the white matter helps to transmit information efficiently and accurately between different gray matter areas of the central nervous system. Reduction in white matter can lead to impaired information delivery which may cause deficits in attention, declarative memory, executive function, and intelligence (Dai et al., 2019). Grey matter abnormalities associated with depression have been found in the hippocampus, prefrontal cortex, orbitofrontal cortex, anterior cingulate cortex, and basal ganglia structures. Abnormal white matter as described by lower fraction anisotropy (FA) value is also found in the superior frontal gyrus,

superior longitudinal fasciculus, and corpus callosum of depressed patients (Dai et al., 2019; Disabato et al., 2017; Kaltenboeck & Harmer, 2018).

Functional imaging studies of patients with depression have examined task-related and resting-state brain activity patterns by detecting fluctuations in blood oxygen levels. Abnormalities of cerebral blood flow are reported for the prefrontal cortex (orbitofrontal, dorsolateral and dorsomedial cortex), the subgenual anterior cingulate cortex, the amygdala, the thalamus, and basal ganglia structures (Disabato et al., 2017; Kaltenboeck & Harmer, 2018).

Furthermore, network-level deficits may play an important pathophysiological role in depression as seen from different neuroimaging studies. Based on this conception two models of depression have been proposed; the "dual network model" and the "triple network model" (Figure 1). The dual network model of depression proposed by Mayberg (1997), consists of ventral and dorsal networks. The ventral network consisted of hyperactive brain regions and was hypothesized to mediate vegetative and somatic symptoms. Whereas, the dorsal network consisted of hypoactive brain regions and was thought to mediate the cognitive aspects of depression. In the triple network model of depression, proposed by Menon (2011), abnormal functional connectivity of three brain networks is closely related to various mental illnesses, including depression. The three networks include the central executive network (CEN), which is involved in higher-order cognitive processes, default mode network (DMN), which is primarily active at rest, and salience network (SN), which is essential for shifting attention to relevant stimuli. This hypothesis shifted the assumption of depression as a dysregulation between dorsal and ventral networks to the concept of dysregulation between and within large-scale brain networks.

Despite these findings, the relationship between abnormalities in brain structure and function in patients with depression still remains unclear and needs to be further explored in order to define diagnostic and therapeutic applications (Scheepens et al., 2020).

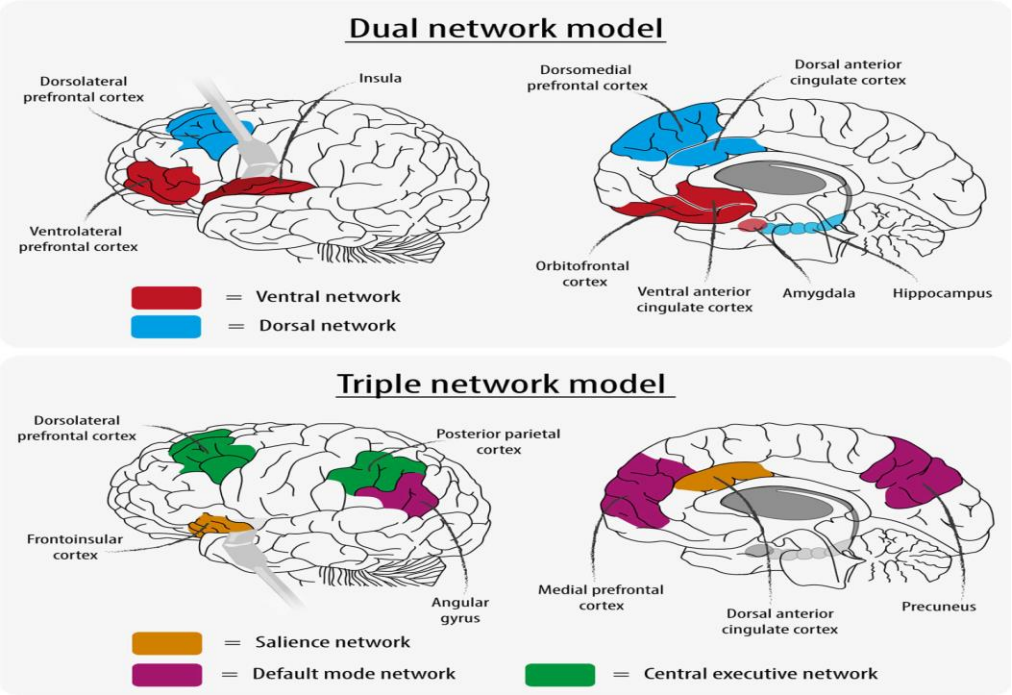


Figure 1. Network models (Scheepens et al., 2020).

**Genetic polymorphisms and depression**- Based on twin studies, first-degree relatives of patients with MDD have a threefold increased risk and heritability for this disorder (Geschwind et al., 2015). Some of the genes linked to the pathogenesis of depression found by this studies include- serotonin transporter (SLC6A4) gene, tyrosine hydroxylase (TH) genes, cAMP response element-binding 1 (CREB1) gene, piccolo presynaptic cytomatrix protein (PCLO) genes, 5-hydroxytryptamine (serotonin) receptor 2A (HTR2A) gene and brain-derived neurotrophic factors (BDNF) (Jeon & Kim, 2016).

**Other**s- some of the other abnormalities or dysfunctions that may be involved in the pathophysiology of depression include- reduced neurotransmission of gamma-aminobutyric acid (GABA), deficient neurosteroid synthesis, impaired endogenous opioid functions, acetylcholine imbalance, and thyroxin abnormality (Bymaster & Felder, 2002; Oja & Saransaari, 2009).

## 1.4 Management

Treatment plan for depression will depend on type and severity. Some people get psychotherapy others take antidepressants. Most patients are best treated with a combination of antidepressants and psychotherapy, modulated according to the course of their illness. If that's not enough, other

options like brain stimulation techniques such as electroconvulsive therapy or transcranial magnetic stimulation can be used. Exercise can help, too.

**Antidepressants**- The introduction of tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs) in the 1950s transformed the treatment of MDD. Ever since then, the search for more selective and possibly better-tolerated antidepressants has continued. This movement of rational drug development gave birth to selective serotonin reuptake inhibitors (SSRIs) and other newer-generation antidepressants (Mrcp, 2000).

Based on their mechanism of action, the currently available antidepressants can be classified into three groups.

- Antidepressants that block one or more of the reuptake transporters pumps and/or receptors for the three monoaminergic neurotransmitters, namely 5-HT, NE, and DA
- Antidepressants that inhibit the enzyme MAO and
- Antidepressants that work by blocking the NMDA-glutamatergic inceptors (Fasipe, 2019).

New generation antidepressants which have gained approval as treatments for MDD include Serotonin-norepinephrine reuptake inhibitors (SNRIs), Norepinephrine-dopamine reuptake inhibitors (NDRIs), Selective norepinephrine reuptake inhibitors (NRIs), Serotonin receptors antagonist with serotonin reuptake inhibition (SARI), Serotonin 5-HT1A autoreceptor partial agonist with serotonin reuptake inhibition (SPARI), Noradrenergic α2 -receptor antagonist with specific serotonergic receptors-2 and -3 antagonism (NASSA), Norepinephrine reuptake inhibitor with serotonin receptors antagonism (NRISA), Serotonin-norepinephrine reuptake inhibitor and serotonin receptors antagonism antidepressant with potent antipsychotic D2 receptor blockade/antagonism (SNRISA with potent antipsychotic D2 receptor blockade/antagonism), atypical antipsychotics that exhibit weak D2 receptor antagonism with potently strong 5-HT2A/2C receptor blockade, NMDA-glutamatergic ionoceptor blockers that exhibit a direct action on the excitatory glutamatergic neurotransmission system (Millan, 2006; Fasipe, 2019).

**Ketamine**- Studies have explored ketamine, an NMDA antagonist that has a long history in analgesia and anesthesiology, useful in treating treatment-resistant depression and acute suicidal ideation (DiazGranados et al., 2010). Ketamine found to have fast antidepressant effects within hours or a day and it was used to help protect patients from suicidal thinking or acute dysphoria when they're in the hospital. Unfortunately, its effects only last 7-10 days and there is substantial

heterogeneity in clinical response. Limitations in the clinical use of ketamine due to its poor safety profile (psychotomimetic effects, cognitive impairments, and tolerance) hinder a widespread application of this molecule in the treatment of depression. Further ketamine's underlying mechanisms of action are not entirely understood (Xu et al, 2015; M. W. Zhang & Roger Ho, 2016; Musazzi et al., 2012).

**Agomelatine-** is an agonist of melatonergic (MT1 and MT2) and antagonist of serotonergic (selective 5-HT2C) receptors. Its melatonergic effect results in resynchronizing the photo-sensitive circadian rhythms and 5-HT2C receptor inhibition results in the release of norepinephrine and dopamine and increasing their extracellular levels. Based on clinical studies agomelatine has been demonstrated to be an effective antidepressant in placebo-controlled trials and equivalent efficacy compared to SSRIs and venlafaxine. It has also good tolerability (does not affect sexual functioning or weight gain) which supports the low discontinuation rate of the medication. The only burden of taking agomelatine is the monitoring of liver enzymes since it can elevate hepatic enzyme levels (Plesničar, 2014; Pringle et al., 2015).

**Anti-inflammatory agents**- Several studies indicate that the adjunctive use of anti-inflammatory agents in patients with MDD improves depressive symptoms, in particular in treatment-resistant depression with an inflammatory profile as defined by a C-reactive protein. Recently several anti- Interleukin (Il)-1 β-targeted compounds have been developed, including antibodies and IL-1β receptor antagonists (Ceskova, 2018; Husain et al., 2017; Kappelmann et al., 2016).

**Opioids**- The clinical use of opioid agonists in the treatment of depression remains highly limited because of unresolved issues of abuse and dependence (Rosenthal et al., 2013). To overcome this limitation, a combination of a μ and κ opioid partial agonist, buprenorphine, and a mu-opioid antagonist, samidorphan, were developed. Based on recent studies the modulation of the opiate system may be a novel treatment approach for treatment-resistant depression (Maurizio Fava et al., 2015).

**Nutraceuticals-** Recently, several reviews showed co-administration of nutraceuticals (standardized pharmaceutical-grade nutrients) may provide an effective and safe approach to enhancing antidepressant effects, which may be a synergistic effect or providing a range of additional biological effects (Sarris et al., 2016).

**Neuromodulation techniques**- The current technology progress and emerging knowledge about the dysfunctional brain circuits underlying depression have led to the development of different

new neuromodulation techniques which usually used as add-on therapy. Some of these techniques include- transcranial magnetic stimulation, vagus nerve stimulation, and deep brain stimulation (Ceskova, 2018).

**Non-pharmacological treatments-** some of the non-pharmacological strategies that have been used to reduce depressive symptoms and improve quality of life include- cognitive-behavioral therapy (CBT), interpersonal psychotherapy, physical exercise, and other relaxation techniques (Morgan et al., 2019; Sarris et al., 2014).

**Herbal medicines**: from experimental studies, herbal medicines confirmed to have antidepressant activity include *Melissa officinalis, Lavandula angustifolia, Cinnamomum zeylanicum, Viola odorata, Echium amoneum,* St. John's wort *(hypericum oil), Valeriana officinalis, Aloysia triphylla, Citrus aurantium,* and *Salix aegyptica* (Bakhshaei, 2017).

Aromatic plants whose essential oils displayed some antidepressant-like effects on preclinical studies include: *Acorus tatarinowii Schott* (Acoraceae), *Asarum heterotropoides F. Schmidt* (Aristolochiaceae), *Citrus limon (L.) Osbeck* (Rutaceae), *Eugenia uniflora L.* (Myrtaceae), *Lavandula angustifolia Mill, Litsea glaucescens Kunth* (Lauraceae), *Mentha piperita L.* (Lamiaceae), *Perilla frutescens (L.) Britton* (Lamiaceae), *Rosmarinus officinalis L.* (Lamiaceae), *Salvia sclarea L., Schinus terebinthifolius Raddi* (Anacardiaceae), *Syzygium aromaticum (L.) Merr. & L.M. Perry* (Myrtaceae), *Toona ciliata Roem var. Yunnanensis* (C. DC.) *C.Y. Wu* (Meliaceae), *Valeriana wallichii DC.* (Caprifoliaceae) *and Zingiber officinale Roscoe* (Zingiberaceae) (Sousa et al., 2017).

## 1.5 The Experimental Plant

*Senna singueana* (Del.) (syn. *Cassia singueana*) Lock. (Fabaceae) (Figure 2) is a species of the drier tropical Africa regions and is often found in thickets, deciduous woodland, and savannah. In Ethiopia and Eritrea, it is a component of the mid and highland dry evergreen forests known by different local names including hambhambo (Tigrigna); bosko-bassha, bsbssha, gufa, gwefyya, qey'ntcet (Amharic); busha (Agew and Amharic) (Gebrelibanos et al., 2007). Throughout Africa, the plant is used traditionally to treat malaria, skin cancer, diabetes, stomach pains, leprosy, rheumatism, microbial and sexually transmitted infections, inflammation, and fever (Keter & Mutiso, 2012; Moshi & Mbwambo, 2002). In the rural area of south-eastern Sudan, the plant root decoction is used to treat constipation (Musa et al., 2011). In Nigeria, the acetone fraction of stem

bark has been reported to have antioxidant and anti-diabetic activities (Madubunyi & Ode, 2012; Auwal & Islam, 2014). In Kenya, the root bark showed antinociceptive activities in vivo (Kariuki et al., 2012). In Tanzania, the bark extract showed trypanocidal activities (Nibret et al., 2010). In Cameroon, its root decoction improves chronic stress-induced anxiety disorders (Véronique et al., 2018). In Northern Ethiopia, the leaves showed promising antimalarial activities alone or in a combination with chloroquine (Gebrelibanos et al., 2014). Furthermore, the leaves and bark extract also shown to scavenge free radicals and inhibit erythrocyte hemolysis (Gebrelibanos, 2012).

An ethnobotanical study of traditional medicinal plants conducted in and around Fiche district, central Ethiopia reported that the fresh leaf of *Senna singueana* mixed with *Rumex nervosus* flowers is used in the treatment of depression and evil eye by the locals using fumigation technique (Enyew et al., 2014). From previous study *Senna Alata* one of the species within the genus *Senna,* was claimed to have antidepressant activity (Pamulaparthi et al., 2016) and studies on preliminary phytochemical analysis of the leaves, seed, and root of *Senna singueana* discovered several classes of plant secondary metabolites including phenols, saponins, tannins, and anthraquinones as well as alkaloids, sterols, and terpenes (OJINNAKA, 2012; Babiaka et al., 2015; Farag et al., 2015) which may be responsible for possible antidepressant activity.

Therefore, the fresh leaf of *Senna singueana* is selected for this study since it may have antidepressant property and the essential oil is used since volatile components may be involved in its action.

Figure 2. Senna singueana (Del.) Lock (Fabaceae) Leaves (Gebrelibanos et al., 2014).

## 1.6 Rationale for the study

According to studies, 20%–30% of patients treated with commonly used antidepressants do not achieve complete recovery and develop treatment-resistant depression. Nonadherence and early discontinuation of treatment are important factors that may significantly contribute to sub-optimal outcomes (Yau et al., 2014), which is mainly associated with adverse effects of the drugs (Hung, 2014). Studies have shown that up to 43% of patients with MDD may discontinue antidepressants due to treatment-emergent adverse effects (Bull et al., 2002). In addition to adverse effects, drug interactions and treatment refractoriness are major restrictions in the treatment of depression (Keller et al., 2002). Thus, there is an urgent need to identify new treatment options including herbal drugs.

Owing to their natural sources and history of human use, herbal remedies have the potential to provide effective alternatives to currently employed modern synthetic antidepressant drugs. Some of the herbal plants can induce happiness in patients with depression by exerting antioxidant properties in the brain, decreasing pro-inflammatory cytokines, increasing proopiomelanocortin, and exerting neuroprotective properties. Certain compounds in plants such as flavonoids, lignans, phenolic acids, coumarins, diterpene alkaloids, terpenes, saponins, amines, naringenin, quercetin derivatives, eugenol, piperine, berberine, hyperforin, riparian derivatives, and ginsenosides are responsible for enhancing happiness and decreasing symptoms of depression (Bahramsoltani et al., 2015).

This study attempted to provide scientific evidence related to the antidepressant properties of *S. singueana* essential oil extract since it can be a potential source of antidepressant-based therapies.

# 2. Objectives

## 2.1. General Objective

- To evaluate the antidepressant-like activity of essential oil extract of *S. singueana* in rodents.

## 2.2 Specific Objectives

- To assess the acute toxicity profile of the essential oil extract of the plant.
- To evaluate the antidepressant-like activity of the essential oil extract of the plant using forced swim test.

- To evaluate the antidepressant-like activity of the essential oil extract of the plant using a tail suspension test.
- To rule out false-positive antidepressant-like activity of the essential oil extract of the plant.
- To identify possible phytochemical constituents for the anti-depressant-like effect of the plant using GC-MS.

# 3. Materials and Methods

## 3.1 Materials

### 3.1.1 Chemicals and reagents

Imipramine (Remedica, Cyprus), 2% Tween 80 (BDH Chemicals Ltd, England), anhydrous sodium sulfate, distilled water (Ethiopian Pharmaceuticals Manufacturing (EPHARM)), Hexane (LOBA chemie pvt. Ltd., India) were purchased from the respective vendors and all chemicals used were of analytic grade.

### 3.1.2 Plant Material

The fresh leaf of *S. singueana* was collected from Fiche District found in North Shewa Zone of Oromia Regional State Ethiopia 86 km from the capital. Identification and authentication of the plant specimens were done by Ato Melaku Wondafrash, a taxonomist, a voucher specimen (collection no. HA 12008) was deposited at the National Herbarium, College of Natural and Computational Sciences, Addis Ababa University for future reference.

### 3.1.3 Experimental Animals

Healthy male albino Swiss mice (6-8 weeks, 20-30 g) as well as Sprague-Dawley rats (8-12 weeks, 200-250 g) bred at the animal house of the School of Pharmacy, Addis Ababa University or purchased from Ethiopian Public Health Institute were used for the experiment. Female rats were used for acute toxicity tests. The animals were housed in polypropylene cages (6 rats per cage) under standard environmental conditions and 12 h light/dark natural illumination cycle and were left for a week for the purpose of acclimatization. They were provided with a laboratory pellet diet and clean water. All procedures and techniques in the study were used in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (Press, 2011).

## 3.2 Methods

### 3.2.1 Plant extraction

The fresh leaves of *S. singueana* were used for the preparation of the extract. The fresh leaves were hydro distilled for 3 h using a Clevenger-type apparatus to obtain the essential oil. The oil was further extracted using hexane, dried with anhydrous sodium sulfate, concentrated, weighed, and stored in dark sealed vials at 4 ºC until further use. The yield was 0.1%.

### 3.2.2 Acute Toxicity Test

An acute toxicity test was performed according to the Organization for Economic Cooperation and Development (OECD) guideline 425. Fasted (overnight) female rats of 6-10 weeks old were used for this study. Following the period of fasting, the rats were weighed and the test substance was administered based on the weight of each rat. After the administration of the substance, food was withheld from the rats for a further 3-4 h. First, an observational study was performed to determine the starting dose. For this, a single female rat was given 2000 mg/kg of the extract as a single dose by oral gavage. Immediately after administration of the extract, the rat was kept under strict observation for any sign of toxicity for 24 h, with special attention during the first 4 h. Since death was not recorded within the first 24 h, another 4 female rats were given the same dose and were observed for onset, duration, and severity of toxic signs such as changes in behavior, physical appearance, motor, and feeding activities, and other signs of acute toxicity and mortality for the next 14 days (OECD 425, 2008).

### 3.2.3 Grouping and dosing

All animals were randomly assigned to six different groups for each model. Group I received the vehicle and served as a negative control. Group II received the standard drug imipramine (30 mg/kg) and served as a positive control (Moallem et al., 2007; Umadevi et al., 2011). The test groups were from group III-VI, which received increasing doses of the extract at 100 mg/kg, 200 mg/kg, 400 mg/kg, and 600 mg/kg respectively. The different doses of the extract and the standard drug were dissolved in 2% Tween 80 solution immediately prior to use and administered orally and the maximum volume administered was 10 ml/kg.

### 3.2.4 Gas chromatography–mass spectrometry (GC-MS) analysis

The analysis was performed by a GC/MS instrument (Agilent 7820A GC coupled with 5977E mass selective detector (MSD)) and Agilent Mass Hunter software (Agilent Technologies, Palo Alto, CA). The analyte was separated on a capillary column Agilent HP-5ms (30m×0.25mm I.d.,

0.25 µm film), which was inserted directly into the ion source of the MS system. The oven temperature was program with: initial temperature 60 $^0$C for 2 min, then 280 $^0$C, 10 $^0$C/min to 200 $^0$C for 0 min, and 3 $^0$C/min to 240 $^0$C for 0 min respectively. Splitless injection with helium (99.999%) as the carrier gas was used. Injection volume was 1µl of the oil. The electron impact ionization conditions were: ion energy 70eV, MS Source temperature 230 $^0$C and MS QUAD temperature 150 $^0$C. Compounds were identified using the NIST Mass Spectral Search Program (National Institute of Standards and Technology, Washington, DC). The relative amounts of individual components of the essential oil were expressed as percentages of the peak area relative to the total peak area.

### 3.2.5 Determination of antidepressant activity

The antidepressant activity was determined by the forced swim test (FST) and tail suspension test (TST). To rule out any nonspecific locomotor effect that the extract might possess, an open field test (OFT) was carried.

**A. Forced swim test**

FST is a widely used behavioral test described by Porsolt in 1977 for determining the antidepressant activity of a compound. The experiment was carried out in two sessions with 24 h interval. In the first session (pre-test), rats were placed into an acrylic cylinder (20 cm in diameter and height of 40 cm containing freshwater of 19 cm height which were maintained at 25°C (± 3°C)) for 15 min. Immobility was not scored in this session. The objective of this session was to acclimate the animals to the experimental situation as well as to persuade stable and high levels of immobility during the test session. Twenty-four hours after the pre-test session, the rats were once again exposed to the same conditions for 6 min (test session). Treatment was given 24 h, 4 h, and 60 min prior to the test. As used water is known to alter the behavioral pattern of the animals, water was changed for each after every session. The rat was judged immobile if it remained floating in the water, except for small movements used to keep its head above the water. Considered as mobile if quick movements of the forelimbs are observed such that the front paws break the surface of the water or if movement of forelimbs or hind limbs in a paddling fashion is observed. (Steru et al., 1985; Porsolt et al., 1977; Yankelevitch-yahav et al., 2015)

**B. Tail suspension test**

For TST the technique of Steru (1985) was followed. The test animals were allowed to acclimatize the laboratory conditions for 1-2 h. After 60 min of administration of the treatment as per the

respective grouping, male mice were hung upside down from a countertop of 50 cm of height using an adhesive tape which was placed approximately 1 cm from the tip of their tails. The total period of immobility was noted for 6 min. The animals were considered to be immobile if they do not show any movement, hung passively, and making only those movements necessary for respiration. Escaping behaviors like attempting to arrive at the suspension bar, powerful shakes of the body, and movement of the appendages similar to running are considered mobility. Slight movements that are bound to forelimbs without the addition of the hind limbs are not considered mobility. Furthermore, the pendulum-like swinging of the animal body because of the momentum picked up amid the earlier mobility sessions was not considered as mobility (Steru et al., 1985; Aslam, 2016). This procedure has advantages over the forced swim procedure because no hypothermia is induced and the animals, once removed from the experiment, resume normal spontaneous activity immediately. As a consequence, no special post-experimental treatment (rubbing down, maintenance in a warmed environment) is required, increasing experimenter comfort. Moreover, in contrast to the forced swim test, where the animals sometimes have to make small movements to maintain their heads above the water, immobility in the tail suspension test is easier to distinguish.

**C. Open field test**

In OFT**,** one of the rodent models of anxiety designed by Hall in 1934, after an hour of dosing the animals were individually placed in the center of the OFT apparatus (a simple cubic dark wooden box with the floor divided into 16 squares to make up the central and peripheral squares which were illuminated by a 60W bulb placed perpendicularly above it) or one of the four corners of the open field and were allowed to explore the apparatus for 5 min (Royce, 1977). The number of peripheral crossings (ambulation), activity in the center (central crossings), and total locomotion, which are the sum of the two, were recorded for 5 min.

### 3.2.6 Data analysis

The results of the study were expressed as mean ± standard error of the mean (S.E.M). Statistical package for social science (SPSS) version 20 was used to analyze the results. Statistically significant differences among groups were evaluated by analysis of variance (one-way ANOVA) followed by Tukey's multiple comparison test. The analysis was performed with a 95% confidence interval and probabilities less than 0.05 ($p<0.05$) were considered significant.

# 4. Results

## 4.1 Acute toxicity test

The acute toxicity test showed the non-toxic nature of the essential oil extract at a limit dose of 2000 mg/kg during the 14 days observation period, indicating that the LD 50 of the extract to be above 2000 mg/kg.

## 4.2 GC-MS analysis

From the GC-MS analysis, a total of 33 compounds were identified. The main compounds were (Carbonic acid, dihexyl ester), (4-Piperidinone, 1,3-dimethyl) and (Pyrrolidine, 1-(1-oxobutyl)) having a relative percentage content of 21.9, 5.9 and 5.7(w/w), respectively. The components, their molecular formula, molecular weight and percentage composition are summarized in Table 1.

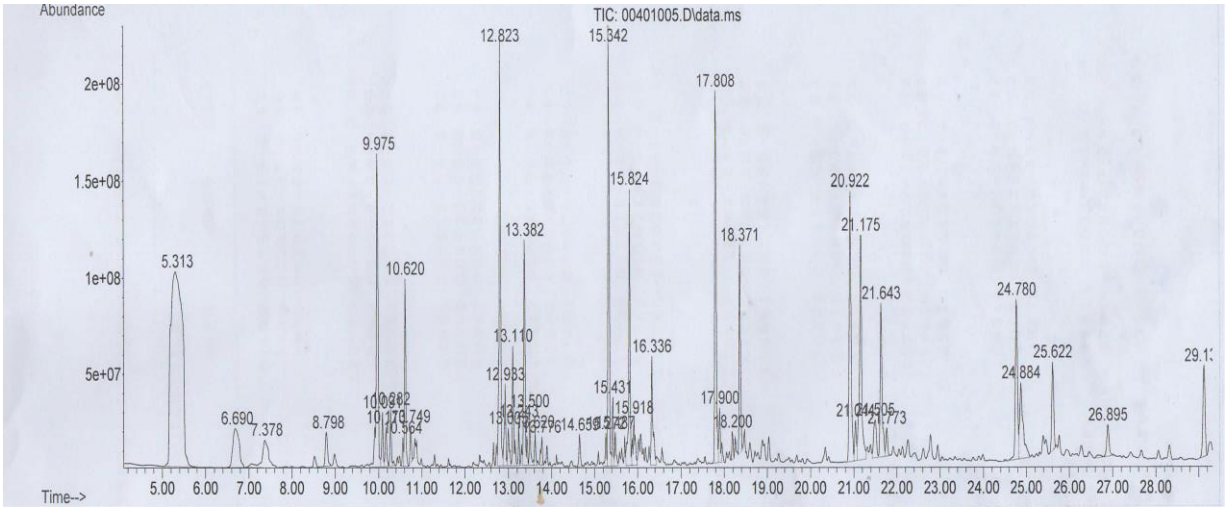


Fig. 3: GC/MS chromatogram of essential oil of *Senna singueana*

Table 1. Chemical composition of *Senna singueana* essential oil extract.

| PK | RT | LRI | Peak area (%) | Compound name | Molecular formula | Molecular weight (g/mol) | Nature of the compound |
|---|---|---|---|---|---|---|---|
| 1 | 5.3129 | 91 | 21.9151 | Carbonic acid, dihexyl ester | $C_{13}H_{26}O_3$ | 230.34 | Ester |
| 2 | 6.6898 | 1054 | 2.3894 | 1-Iodo-2-methylnonane | $C_{10}H_{21}I$ | 268.18 | Iodo compound |
| 3 | 7.3778 | 1099 | 1.7994 | Decane, 3,7-dimethyl- | $C_{12}H_{26}$ | 170.33 | Alkane |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 4 | 8.7985 | 893 | 0.7356 | Linalool | $C_{10}H_{18}O$ | 154.25 | Monoterpene |
| 5 | 9.9752 | 875 | 5.5749 | Octane, 4-methyl- | $C_9H_{20}$ | 128.25 | Alkane |
| 6 | 10.0914 | 883 | 0.734 | Heptadecane, 8-methyl- | $C_{18}H_{38}$ | 254.5 | Alkane |
| 7 | 10.1733 | 889 | 0.5872 | Pentadecane | $C_{15}H_{32}$ | 212.42 | Alkane |
| 8 | 10.2823 | 896 | 0.6915 | Heptadecane | $C_{17}H_{36}$ | 240.471 | Alkane |
| 9 | 10.5639 | 817 | 0.2849 | Dodecane, 1-iodo- | $C_{12}H_{25}I$ | 296.23 | Iodo compound |
| 10 | 10.6205 | 821 | 2.2381 | Sulfurous acid, hexyl octyl ester | $C_{14}H_{30}O_3S$ | 278.45 | Ester |
| 11 | 10.7486 | 830 | 0.4809 | Pentadecane, 7-methyl- | $C_{16}H_{34}$ | 226.44 | Alkane |
| 12 | 12.8232 | 887 | 5.9213 | 4-Piperidinone, 1,3-dimethyl- | $C_7H_{13}NO$ | 127.18 | Aminoketone |
| 13 | 12.9335 | 896 | 1.0905 | 2-Bromo dodecane | $C_{12}H_{25}Br$ | 249.23 | Alkane |
| 14 | 13.0054 | 901 | 0.5767 | Hexadecane | $C_{16}H_{34}$ | 226.41 | Alkane |
| 15 | 13.1101 | 910 | 1.51 | Eugenol | $C_{10}H_{12}O_2$ | 164.2 | Phenol |
| 16 | 13.2431 | 921 | 0.3946 | Tridecane, 1-iodo- | $C_{13}H_{27}I$ | 310.26 | Iodo compound |
| 17 | 13.3815 | 933 | 3.2972 | Disulfide, di-tert-dodecyl | $C_{24}H_50S_2$ | 402.79 | Dialkyldisulfides |
| 18 | 13.4995 | 943 | 0.6195 | Hexadecane | $C_{16}H_{34}$ | 226.41 | Alkane |
| 19 | 13.6197 | 953 | 0.3731 | Heneicosane | $C_{21}H_{44}$ | 296.57 | Alkane |
| 20 | 13.7759 | 966 | 0.4276 | Heptadecane | $C_{17}H_{36}$ | 240.471 | Alkane |
| 21 | 14.6585 | 841 | 0.4366 | 1-Anthracenamine | $C_{14}H_{11}N$ | 193.24 | Organic compound |
| 22 | 15.272 | 895 | 0.3906 | Heptadecane | $C_{17}H_{36}$ | 240.471 | Alkane |
| 23 | 15.342 | 1301 | 5.4217 | Caprolactam | $C_6H_{11}NO$ | 113.16 | Cyclic amide |
| 24 | 15.4314 | 1309 | 0.751 | Heptacosane | $C_{27}H_{56}$ | 380.73 | Alkane |
| 25 | 15.4867 | 1314 | 0.4636 | Hexadecane | $C_{16}H_{34}$ | 226.41 | Alkane |
| 26 | 15.8239 | 1345 | 3.4378 | Oxalic acid, bis(6-ethyloct-3-yl) ester | $C_{22}H_{42}O_4$ | 370.6 | Ester |
| 27 | 15.9183 | 1353 | 0.9978 | Tridecane, 1-iodo- | $C_{13}H_{27}I$ | 310.26 | Iodo compound |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 28 | 16.336 | 1391 | 1.6985 | Diethyl Phthalate | $C_{12}H_{14}O_4$ | 222.24 | Phthalate, plasticizer |
| 29 | 17.8084 | 1312 | 5.6877 | Pyrrolidine, 1-(1-oxobutyl)- | $C_8H_{15}NO$ | 141.21 | Amine |
| 30 | 17.8998 | 1319 | 0.6855 | 2-Bromo dodecane | $C_{12}H_{25}Br$ | 249.23 | Bromic alkane |
| 31 | 18.1998 | 1342 | 0.3343 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | $C_{26}H_{54}$ | 366.71 | Alkane |
| 32 | 18.3707 | 1354 | 2.7913 | Oxalic acid, bis(6-ethyloct-3-yl) ester | $C_{22}H_{42}O_4$ | 370.6 | Ester |
| 33 | 20.9224 | 1324 | 5.0653 | Oxalic acid, bis(6-ethyloct-3-yl) ester | $C_{22}H_{42}O_4$ | 370.6 | Ester |
| 34 | 21.044 | 1331 | 0.6549 | Heneicosane | $C_{21}H_{44}$ | 296.57 | Alkane |
| 35 | 21.1745 | 1339 | 4.9915 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256.4 | Fatty acid (palmitic acid) |
| 36 | 21.5046 | 1358 | 1.0544 | Dibutyl phthalate | $C_{16}H_{22}O_4$ | 278.34 | Plasticizer |
| 37 | 21.6431 | 1366 | 2.8498 | Docosane | $C_{22}H_{46}$ | 310.60 | Alkane |
| 38 | 21.7731 | 1374 | 0.4461 | Octacosane | $C_{28}H_{58}$ | 394.77 | Alkane |
| 39 | 24.7801 | 1588 | 3.3848 | Hexadecane, 2-methyl- | $C_{17}H_{36}$ | 240.5 | Alkane |
| 40 | 24.8844 | 1588 | 2.5232 | Octadecanoic acid | $C_{18}H_{36}O_2$ | 284.48 | Fatty acid (Stearic acid) |
| 41 | 25.6225 | 1585 | 1.5791 | Heptacosane | $C_{27}H_{56}$ | 380.7 | Alkane |
| 42 | 26.8955 | 1579 | 0.8475 | 9-Octadecenamide, (Z)- | $C_{18}H_{35}NO$ | 281.47 | Fatty amides |
| 43 | 29.1303 | 1569 | 1.8656 | Hexadecane, 2-methyl- | $C_{17}H_{36}$ | 240.5 | Alkane |

PK= peak area; RT= retention time (min); LRI= linear retention index

## 4.3 Effect of the extract in the tail suspension test

As shown in Table 2 the 100 mg/kg, 200 mg/kg, and 400 mg/kg essential oil extract doses of *S. singueana* did not show any significant difference in the reduction of immobility in comparison

with the control. However, the 600 mg/kg extract dose showed a significant immobility time reduction (56.6%) as compared to 100 mg/kg, 200 mg/kg ($p<0.05$) and control group ($p<0.01$). Furthermore, the standard drug imipramine produced a significantly lower immobility time (70.0%, $p<0.01$) than all doses of the essential oil except 600mg/kg.

Table 2: Effect of the essential oil extract of *Senna singueana* on the duration of immobility in mice tail suspension test.

| Treatment groups | Duration of immobility (sec) | Reduction in time of immobility (%) |
|---|---|---|
| Control | 125.17 ± 13.67 | - |
| Imipramine 30 mg/kg | 37.50 ± 13.69 a** c** d** e** | 70.04 |
| SS 100 mg/kg | 106.50 ± 12.28 | 14.92 |
| SS 200 mg/kg | 110.67 ± 11.78 | 11.58 |
| SS 400 mg/kg | 109.00 ± 9.26 | 12.92 |
| SS 600 mg/kg | 54.33 ± 10.79 a** c* d* e* | 56.59 |

Values represent mean ± S.E.M (n= 6); $^{a}$ compared to control; $^{b}$ compared to standard; $^{c}$ compared to 100mg/kg; $^{d}$ compared to 200mg/kg; e compared to 400mg/kg *p< 0.05, **p< 0.01; Control received 2% Tween 80 in distilled water; SS= *Senna Singueana* essential oil extract

## 4.4 Effect of the extract in the forced swim test

The essential oil extract of *Senna singueana* showed a decrease in immobility time in the FST model in a dose-dependent manner (Table 3). The extract at the dose of 100 mg/kg (4.3%) and 200 mg/kg (10%) failed to show a marked decrease in immobility compared with the control group. Whereas the 400 mg/kg (50.6%) and 600 mg/kg (61.8%) test doses were able to exhibit a statistically significantly reduction in immobility time ($p< 0.01$) as compared to control, 100 mg/kg and 200 mg/kg treated groups. Imipramine treated group showed a significant immobility time reduction (70.04%, $p<0.01$) as compared to 100 mg/kg, 200 mg/kg, and control group.

Table 3: Effect of the essential oil extract of *Senna singueana* on the duration of immobility rat forced swim test.

| Treatment groups | Duration of immobility (sec) | Reduction in time of immobility (%) |
|---|---|---|
| Control | 191.50 ± 12.88 | - |
| Imipramine 30 mg/kg | 86.83 ± 7.64 a** c** d** | 54.66 |

| | | |
|---|---|---|
| SS 100 mg/kg | 183.33 ± 13.10 | 4.27 |
| SS 200 mg/kg | 172.33 ± 10.23 | 10.01 |
| SS 400 mg/kg | 94.50 ± 9.94 a** c** d** | 50.65 |
| SS 600 mg/kg | 73.17 ± 11.97 a** c** d** | 61.79 |

Values represent mean ± S.E.M (n= 6); $^{a}$ compared to control; $^{b}$ compared to standard; $^{c}$ compared to 100mg/kg; $^{d}$ compared to 200mg/kg; *p< 0.05, **p< 0.01; Control received 2% Tween 80 in distilled water; SS= *Senna Singueana* essential oil extract

## 4.5 Effect of the extract in open field test

In order to rule out whether changes in immobility were associated with changes in motor activity, the animals treated with the extract were tested for activity in an open field. As indicated in Table 4, the doses of the extract that were able to display antidepressant-like responses in TST and FST did not show any significant change in locomotion.

Table 4: Effect of the essential oil extract of *Senna singueana* on the duration of immobility in mice open field test

| Treatment group | Number of crossings | | |
|---|---|---|---|
| | Peripheral | Central | Total |
| Control | 61.33 ± 10.34 | 8.33 ± 2.25 | 69.67 ± 11.73 |
| Imipramine 30 mg/kg | 64.67 ± 9.81 | 10.67 ± 3.20 | 75.33 ± 12.62 |
| SS 100 mg/kg | 75.33 ± 5.82 | 5.83 ± 0.91 | 81.17 ± 6.12 |
| SS 200 mg/kg | 78.50 ± 4.03 | 6.33 ± 2.08 | 84.83 ± 5.26 |
| SS 400 mg/kg | 50.17 ± 6.36 | 4.17 ±1.45 | 54.33 ± 7.67 |
| SS 600 mg/kg | 56.67 ± 6.71 | 6.50 ± 1.73 | 63.17 ± 7.59 |

Values represent mean ± S.E.M (n= 6); Control received 2% Tween 80 in distilled water; SS= *Senna Singueana* essential oil extract

# 5. Discussion

Depression is a multifactorial, chronic, and life-threatening disease with high prevalence, disability, and societal cost. Hence, it is very essential to address these problems and find effective remedies. Although a number of synthetic drugs are being used as a standard treatment for

clinically depressed patients, all are associated with some limitations (Jawaid et al., 2015). So, there is a crucial need for alternative medications for these disorders. Medicinal plants may be effective alternatives and the research of their effects in the treatment of depression has progressed significantly since the past decade (Zhang, 2004).

Among medicinal plants, aromatic plants may be an alternative option. These plants produce essential oils which are a complex mixture of volatile compounds. According to works in the literature, essential oils of medicinal plants and their components are endowed with interesting biological activities and have therapeutic potential (Djilani & Dicko, 2014; Sousa et al., 2017). Various essential oils are known to have broad-spectrum antimicrobial (Burt, 2004), antiviral (Garozzo et al., 2009), and antifungal activities (SRIDHAR et al., 2003) and may be useful as natural remedies. Moreover, due to the lipophilic character of volatile compounds and their small size, essential oils can be absorbed from the food matrix or as pure products and cross the blood-brain barrier easily which helps them to have a CNS effect as a stimulant, relaxant, and/or as an antidepressant. This property may be due to one of the compounds or due to the entire mixture (Djilani & Dicko, 2014). Several studies have shown that the essential oils could be used as a complementary and alternative therapy for patients with depression and secondary depressive symptoms (Yim et al., 2009) including anxiety disorders (Lee et al., 2011).

In the present study, the antidepressant effect of the essential oil of *S. singueana* was assessed using TST and FST models. These models of depression are the most common models of depression that measure the duration of immobility when rodents are exposed to an inescapable situation. Although these tests lack sufficient face and construct validity to be considered models of depression, they have good predictive validity and allow rapid and economical detection of substances with potential antidepressant-like activity (Castagn et al., 2011).

Male mice and rats were used in this study because the primary female sex hormone estrogen may affect the serotonergic system in the brain which could lead to increased vulnerability to stressors, different coping strategies, and differentiated response to antidepressants in females (Dalla et al., 2009; Kornstein et al., 2010).

From this study, 100 and 200 mg/kg dose of the plant extract did not show any reduction in immobility time in both TST and FST models suggesting that they are sub-threshold doses. Whereas 400 and 600 mg/kg doses showed antidepressant activity in FST and only the 600 mg/kg dose showed antidepressant activity in TST by decreasing the immobility time suggesting a

relatively high dose of the extract would be needed to produce an antidepressant-like effect. As seen from the result of this study, there are variations in the ability to reduce immobility observed by the same doses in the two models. For example, the 400 mg/kg dose showed a significant percentage of reduction in FST but not in TST. Such discrepancies may stem from the difference in sensitivity of the tests because of differences in underlying mechanisms of inducing immobility as well as in interstrain and interspecies variations as stated from previous studies (El-Alfy et al., 2010; Chatterjee et al., 2012). The majority of clinically used antidepressants decrease the duration of immobility in both tests. TCAs, MAOI, atypical antidepressants, functional NMDA antagonists, and AMPA receptor potentiators are included in this category. However, antidepressants like SSRIs, which are active in TST, fail to consistently reduce immobility in FST at pharmacologically relevant doses. In contrast, other ‘‘atypical’’ agents such as rolipram and levoprotiline reduce immobility in FST but have been reported to be inactive in TST. This provides evidence that the neural circuitry mediating behavior in these tests is not identical and results of one test may not be replicated in the other (Castagn et al., 2011; Bai et al., 2001).

In order to rule out any possible non-specific motor stimulation, the OFT model was used. This test is used to assess the general locomotion activity of the plant-based on the total number of square crossings. Unlike psychostimulant drugs, antidepressants do not increase locomotor activity. Therefore, the outcome of this test is important to rule out any nonspecific activity of this plant (Stanford, 2007). Accordingly, the essential oil extract of *S. singueana* didn’t significantly alter the spontaneous locomotor activity of mice during the OFT, showing that, at the doses tested, the antidepressant-like activity of the extract is not likely to be a false positive as a result of psychostimulant action.

In the present study, the essential oil extract of *S. singueana* was analyzed using GC-MS and a total of 33 compounds were identified. Among this compounds, eugenol and linalool are the most studied isolated compounds from essential oils with antidepressant-like activity (Irie et al., 2004; Irie, 2012; Guzmán-Gutiérrez et al., 2012). Other studies reported the antidepressant and antioxidant activities of n-hexadecanoic acid, linolenic acid and octadecanoic acid, the most common saturated fatty acids found in plants (Surana & Wagh, 2018; Ravikumar & Jeyam, 2019). Compounds like Heneicosane have also reported to have good antidepressant activity (Surana & Wagh, 2018). Therefore, antidepressant activity of *Senna Singueana* essential oil extract may be associated with these phytoconstituents.

## 6. Conclusion

This study provides evidence that the essential oil extract of *Senna singueana* displayed antidepressant-like effect in established animal models of depression as demonstrated by a reduction in immobility time and compounds like eugenol, linalool, n-hexadecanoic acid, octadecanoic acid and heneicosane may be involved in this action.

## 7. Recommendations

- Chronic and other models of depression should be carried out to support its antidepressant effect.
- Pharmacological and neurobiological tests should be performed in order to explain their possible mechanism of action.
- Further sub-acute and chronic toxicities studies should be carried out in order to assess its long-term effects.
- Higher doses should be assessed for probable psychostimulant effects and
- The composite extract of *Senna singueana* and *Rumex nervousus* should be assessed for further antidepressant activity.

## 8. References


Aizenstein, H. J., Baskys, A., Boldrini, M., Butters, M. A., Diniz, B. S., Jaiswal, M. K., Jellinger, K. A., Kruglov, L. S., Meshandin, I. A., Mijajlovic, M. D., Niklewski, G., & Pospos, S. (2016). Vascular depression consensus report – a critical update. *BMC Medicine*, *14*(161), 1–16. https://doi.org/10.1186/s12916-016-0720-5

Al-Harbi, K. S. (2012). Treatment-resistant depression : therapeutic trends , challenges , and future directions. *Patient Preference and Adherence*, *6*, 369–388.

Anacker, C., Zunszain, P. A., Carvalho, L. A., & Pariante, C. M. (2011). The glucocorticoid receptor : Pivot of depression and of antidepressant treatment ? *Psychoneuroendocrinology*, *36*(3), 415–425. https://doi.org/10.1016/j.psyneuen.2010.03.007

Andreescu, C., & Reynolds, C. F. (2011). L a t e - l i f e De p res s i o n : Tre a t m e n t a n d P ro m i s i n g N e w D i rec t i o n s f o r R e s e a rc h a n d Clinical Practice. *Psychiatric Clinics of NA*, *34*(2), 335–355. https://doi.org/10.1016/j.psc.2011.02.005

Angst, J., Gross, V. A., & Rössler, W. (2020). Bipolar disorders in ICD - 11 : current status and

strengths. *International Journal of Bipolar Disorders*, *8*(3), 4–8. https://doi.org/10.1186/s40345-019-0165-9

Aslam, M. (2016). Tail suspension test to evaluate the antidepressant activity of experimental drugs. *Bangladesh J Pharmacol.*, *11*, 292–294. https://doi.org/10.3329/bjp.v11i2.26517

Auwal, M., & Islam, M. S. (2014). Anti-diabetic effects of the acetone fraction of Senna singueana stem bark in a type 2 diabetes rat model. *Journal of Ethnopharmacology*, *153*(2), 392–399. https://doi.org/10.1016/j.jep.2014.02.042

Babiaka, S. B., Ntie-kang, F., Ndingkokhar, B., Mbah, J. A., Sippl, W., & Yong, J. N. (2015). The chemistry and bioactivity of Southern African fl ora II : fl avonoids , quinones and minor compound classes. *RSC Advances*, *5*, 57704–57720. https://doi.org/10.1039/C5RA05524E

Bahramsoltani, R., Farzaei, M. H., Farahani, M. S., & Rahimi, R. (2015). Phytochemical constituents as future antidepressants : a comprehensive review. *Rev. Neurosci.* https://doi.org/10.1515/revneuro-2015-0009

Bai, F., Li, X., Clay, M., Lindstrom, T., & Skolnick, P. (2001). Intra- and interstrain differences in models of "' behavioral despair ."' *Pharmacology, Biochemistry and Behavior*, *70*, 187–192.

Bakhshaei, S. (2017). Phyto-Pharmacological Effect of Nine Medicinal Plants as a Traditional Treatment on Depression. *Journal of Applied Pharmacy*, *9*(3), 1–5. https://doi.org/10.21065/1920-4159.1000244

Baumeister, H., Maercker, A., & Casey, P. (2009). Adjustment Disorder with Depressed Mood A critique of its DSM-IV and ICD-10 conceptualization and recommendations for the future. *Psychopathology*, *March*, 1–25. https://doi.org/10.5167/uzh-31723

Benros, M. E., Waltoft, B. L., Nordentoft, M., Østergaard, S. D., Eaton, W. W., Krogh, J., & Preben B. Mortensen. (2013). Autoimmune Diseases and Severe Infections as Risk Factors for Mood Disorders. *JAMA PSYCHIATRY*, 1–9. https://doi.org/10.1001/jamapsychiatry.2013.1111

Boku, S., Nakagawa, S., Toda, H., & Hishimoto, A. (2017). Neural basis of major depressive disorder: Beyond monoamine hypothesis. *Psychiatry and Clinical Neurosciences*, *72*, 3–12. https://doi.org/10.1111/pcn.12604

Bondy, B. (2002). Pathophysiology of depression and mechanisms of treatment. *Dialogues in*

*Clinical Neuroscience*, *4*(1), 7–20.

Bull, S. A., Hunkeler, E. M., Lee, J. Y., Rowland, C. R., Williamson, T. E., Schwab, J. R., & Hurt, S. W. (2002). Discontinuing or Switching Selective Serotonin-Reuptake METHODS : RESULTS : *The Annals of Pharmacotherapy*, *36*, 578–584.

Burt, S. (2004). Essential oils : their antibacterial properties and potential applications in foods — a review. *International Journal of Food Microbiology*, *94*, 223–253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

Bymaster, F. P., & Felder, C. C. (2002). Role of the cholinergic muscarinic system in bipolar disorder and related mechanism of action of antipsychotic agents. *Molecular Psychiatry*, *7*, 57–63. https://doi.org/10.1038/sj/mp/4001019

Carlos A. Zarate, J., Singh, J. B., Carlson, P. J., Brutsche, N. E., Ameli, R., Luckenbaugh, D. A., Charney, D. S., Manji, H. K., & Context: (2006). A Randomized Trial of an N-methyl-D-aspartate Antagonist in Treatment-Resistant Major Depression. *Arch Gen Psychiatry*, *63*, 856–864.

Carvalho, A. ., & Firth, J. (2020). Bipolar Disorder. *The New Engl and Journal of Medicine*, *383*, 58–66. https://doi.org/10.1056/NEJMra1906193

Casey, P., & Bailey, S. (2011). Adjustment disorders : the state of the art. *World Psychiatry*, *10*(22), 11–18.

Castagn, V., Moser, P., Roux, S., & Porsolt, R. D. (2011). Rodent Models of Depression : Forced Swim and Tail Suspension Behavioral Despair Tests in Rats and Mice. *Current Protocols in Neuroscience*, *55*(8), 1–14. https://doi.org/10.1002/0471142301.ns0810as55

Ceskova, E. (2018). Novel treatment options in depression and psychosis. *Neuropsychiatric Disease and Treatment*, *14*, 741–747.

Chatterjee, M., Jaiswal, M., & Palit, G. (2012). Comparative Evaluation of Forced Swim Test and Tail Suspension Test as Models of Negative Symptom of Schizophrenia in Rodents. *ISRN Psychiatry*, 1–5. https://doi.org/10.5402/2012/595141

Cosci, F., & Chouinard, G. (2019). The Monoamine Hypothesis of Depression Revisited : Could It Mechanistically Novel Antidepressant Strategies ? In *Neurobiology of Depression*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-813333-0.00007-X

Craddock, N. (2006). Genetics of mood disorders. *Psychiatry*, *5*(5), 170–174. https://doi.org/10.1383/psyt.2006.5.5.170

Dai, L., Zhou, H., Xu, X., & Zuo, Z. (2019). Brain structural and functional changes in patients with major depressive disorder : a literature review. *PeerJ*, *7*, 1–17. https://doi.org/10.7717/peerj.8170

Dalla, C., Pitychoutis, P. M., Kokras, N., & Papadopoulou-daifoti, Z. (2009). Sex Differences in Animal Models of Depression and Antidepressant Response. *Basic & Clinical Pharmacology & Toxicology*, *106*, 226–233. https://doi.org/10.1111/j.1742-7843.2009.00516.x

DiazGranados, N., Ibrahim, L., Brutsche, N., Ameli, R., Henter, I. D., Luckenbaugh, D. A., And, R. M.-V., & Jr, C. A. Z. (2010). Rapid Resolution of Suicidal Ideation after a Single Infusion of an NMDA Antagonist in Patients with Treatment-Resistant Major Depressive Disorder. *J Clin Psychiatry*, *71*(12), 1605–1611. https://doi.org/10.4088/JCP.09m05327blu.Rapid

Dinesen, S., & Rothschild, A. J. (2012). Considerations on the ICD-11. *Psychother Psychosom*, *81*, 135–144. https://doi.org/10.1159/000334487

Disabato, B., Bauer, I. E., Soares, J. C., Sheline, Y., & Matter, G. (2017). Neural Structure and Organization of Mood Pathology. *Oxford Handbooks Online*, *October 2018*, 1–21. https://doi.org/10.1093/oxfordhb/9780199973965.013.19

Djilani, A., & Dicko, A. (2014). The Therapeutic Benefits of Essential Oils. *Nutrition, Well-Being and Health*, *May*, 155–178. https://doi.org/10.5772/25344

Duerkop. (2009). The epidemiology of major depression in South Africa: Results from the South African Stress and Health study. *S Afr Med J.*, *99*(1), 367–373. https://doi.org/10.1038/jid.2014.371

Duko, B., Mekuriaw, B., Molla, A., & Ayano, G. (2020). The prevalence of premenstrual dysphoric disorder among adolescents in Ethiopia : a systematic review and meta-analysis. *Rish Journal of Medical Science*.

El-Alfy, A. T., Ivey, K., Robinson, K., Ahmed, S., Radwan, M., Slade, D., Khan, I., ElSohly, M., & Ross, S. (2010). Antidepressant-like effect of Δ9 tetrahydrocannabinol and other cannabinoids isolated from Cannabis sativa L. *Pharmacology, Biochemistry and Behavior*, *95*, 434–442.

Enyew, A., Asfaw, Z., Kelbessa, E., & Nagappan, R. (2014). Ethnobotanical Study of Traditional Medicinal Plants in and Around Fiche Ethnobotanical Study of Traditional

Medicinal Plants in and Around Fiche District , Central Ethiopia. *Current Research Journal of Biological Sciences*, *6*(4), 154–167.

Farag, M. A., Porzel, A., Mahrous, E. A., El-massry, M. M., & Wessjohann, L. A. (2015). Integrated comparative metabolite profiling via MS and NMR techniques for Senna drug quality control analysis. *Anal Bioanal Chem*. https://doi.org/10.1007/s00216-014-8432-1

Fasipe, O. J. (2019). The emergence of new antidepressants for clinical use : Agomelatine paradox versus other novel agents. *IBRO Reports*, *6*(January), 95–110. https://doi.org/10.1016/j.ibror.2019.01.001

FMOH. (2012). *Federal Democratic Republic of Ethiopia Ministry of Health National Hygiene and Sanitation Strategy 2012/13 - 2015/16*.

FMOH. (2018). *Addressing the Impact of Noncommunicable Diseases and Injuries in Ethiopia* (Issue November).

Freis, E. D. (2010). Mental depression in hypertensive patients treated for long period with large doses of reserpine. *The New England Journa of Medicine*, *251*(25), 1006–1008.

Garozzo, A., Timpanaro, R., Bisignano, B., Furneri, P. M., Bisignano, G., & Castro, A. (2009). In vitro antiviral activity of Melaleuca alternifolia essential oil. *The Society for Applied Microbiology*, *49*, 806–808. https://doi.org/10.1111/j.1472-765X.2009.02740.x

Gebrelibanos, M. (2012). In vitro Erythrocyte Haemolysis Inhibition Properties of Senna singueana Extracts. *Momona Ethiopian Journal of Science (MEJS)*, *4*(2), 16–28.

Gebrelibanos, M., Asres, K., & Veeresham, C. (2007). In Vitro Radical Scavenging Activity of the Leaf and Bark Extracts of Senna In Vitro Radical Scavenging Activity of the Leaf and Bark Extracts of Senna singueana ( Del ). Lock. *Ethiopian Pharmaceutical Journal*, *25*(January), 77–84. https://doi.org/10.4314/epj.v25i2.35121

Gebrelibanos, M., Gebremedhin, G., Sintayehu, B., Desta, H., & Belay, S. (2014). Evaluation of Senna singueana leaf extract as an alternative or adjuvant therapy for malaria. *Journal of Traditional and Complementary Medicine*, *2014*, 1–6. https://doi.org/10.1016/j.jtcme.2014.11.014

Geschwind, D. H., Flint, J., Angeles, L., & Angeles, L. (2015). Genetics and genomics of psychiatric disease. *Science*, *349*(6255), 1489–1494. https://doi.org/10.1126/science.aaa8954.Genetics

Groves, J. O. (2007). Is it time to reassess the BDNF hypothesis of depression? *Molecular*

*Psychiatry*, *12*(12), 1079–1088. https://doi.org/10.1038/sj.mp.4002075

Guzmán-Gutiérrez, S. L., Gómez-Cansino, R., García-Zebadúa, J. C., Jiménez-Pérez, N. C., & Reyes-Chilpa, R. (2012). Antidepressant activity of Litsea glaucescens essential oil: Identification of β-pinene and linalool as active principles. *Journal of Ethnopharmacology*, *143*(2), 673–679. https://doi.org/10.1016/j.jep.2012.07.026

Hailemariam, S., Tessema, F., Asefa, M., Tadesse, H., & Tenkolu, G. (2012). The prevalence of depression and associated factors in Ethiopia : findings from the National Health Survey. *International Journal of Mental Health Systems*, *6*(23), 1–11.

Hanlon, C., Alem, A., Lund, C., Hailemariam, D., Assefa, E., & Giorgis, T. W. (2019). Moving towards universal health coverage for mental disorders in Ethiopia. *International Journal of Mental Health Systems*, *13*(11), 1–16. https://doi.org/10.1186/s13033-019-0268-9

Harald, B., & Gordon, P. (2012). Meta-review of depressive subtyping models. *Journal of Affective Disorders*, *139*(2), 126–140. https://doi.org/10.1016/j.jad.2011.07.015

Hashimoto, K., Sawa, A., & Iyo, M. (2007). Increased Levels of Glutamate in Brains from Patients. *BIOL PSYCHIATRY*, *62*, 1310–1316. https://doi.org/10.1016/j.biopsych.2007.03.017

Hayashi, M., And, R. M., & Tanuma, N. (2012). OXIDATIVE STRESS IN DEVELOPMENTAL BRAIN DISORDERS. *Neurodegenerative Diseases*, 278–290.

Hung, C. (2014). Factors predicting adherence to antidepressant treatment. *Curr Opin Psychiatry*, *27*(5), 344–349. https://doi.org/10.1097/YCO.0000000000000086

Husain, M. I., Strawbridge, R., Stokes, P. R. A., & Young, A. H. (2017). Anti-inflammatory treatments for mood disorders : Systematic review and meta-analysis. *Journal of Psychopharmacology*, *00*(0), 1–12. https://doi.org/10.1177/0269881117725711

Ii, W. H. W., Walton, J. C., Devries, A. C., & Nelson, R. J. (2020). Circadian rhythm disruption and mental health. *Translational Psychiatry*, *10*(28), 1–13. https://doi.org/10.1038/s41398-020-0694-0

Irie, Y. (2012). Effects of Eugenol on the Central Nervous System: Its Possible Application to Treatment of Alzheimers Disease, Depression, and Parkinsons Disease. *Current Bioactive Compounds*, *2*(1), 57–66. https://doi.org/10.2174/157340721060201005 7

Irie, Y., Itokazu, N., Anjiki, N., Ishige, A., Watanabe, K., & Keung, W. M. (2004). Eugenol exhibits antidepressant-like activity in mice and induces expression of metallothionein-III in

the hippocampus. *Brain Research*, *1011*(2), 243–246. https://doi.org/10.1016/j.brainres.2004.03.040

Jawaid, T., Imam, S. A., & Kamal, M. (2015). ANTIDEPRESSANT ACTIVITY OF METHANOLIC EXTRACT OF VERBENA OFFICINALIS LINN . PLANT IN MICE. *Asian J Pharm Clin Res*, *8*(4), 308–310.

Jeon, S. W., & Kim, Y. K. (2016). Molecular neurobiology and promising new treatment in depression. *International Journal of Molecular Sciences*, *17*(381), 1–17. https://doi.org/10.3390/ijms17030381

Kaltenboeck, A., & Harmer, C. (2018). The neuroscience of depressive disorders : A brief review of the past and some considerations about the future. *Brain and Neuroscience Advances*, *2*, 1–6. https://doi.org/10.1177/2398212818799269

Kappelmann, N., Lewis, G., Dantzer, R., Jones, P. B., & Khandaker, G. M. (2016). Antidepressant activity of anti-cytokine treatment : a systematic review and meta-analysis of clinical trials of chronic in fl ammatory conditions. *Molecular Psychiatry*, *00*(April), 1–9. https://doi.org/10.1038/mp.2016.167

Karege, F., Perret, G., Bondolf, G., Schwald, M., Bertschy, G., & Aubry, J. (2002). Decreased serum brain-derived neurotrophic factor levels in major depressed patients. *Psychiatry Research*, *109*, 143–148.

Kariuki, H. N., Kanui, T. I., Yenesew, A., Mbugua, P. M., & Patel, N. B. (2012). Antinocieptive activity of the root extracts of Rhus natalensis Kraus and Senna singueana. *Phytopharmacology*, *2*(2), 312–317.

Keller, M. B., Hirschfeld, R. M. A., Demyttenaere, K., & Baldwin, D. S. (2002). Optimizing outcomes in depression : focus on antidepressant compliance. *International Clinical Psychopharmacology*, *17*(6), 265–271.

Kelley, K. W., O'Connor, J. C., Lawson, M. A., Dantzer, R., Sandra L. Rodriguez-Zas, A., & McCusker, R. H. (2013). Aging Leads to Prolonged Duration of Inflammation-Induced Depression-Like Behavior Caused by Bacillus Calmette-Guérin. *Brain Behav Immun.*, *32*(217), 63–69. https://doi.org/10.1016/j.bbi.2013.02.003.Aging

Keter, L. K., & Mutiso, P. C. (2012). Ethnobotanical studies of medicinal plants used by Traditional Health Practitioners in the management of diabetes in Lower Eastern Province , Kenya. *Journal of Ethnopharmacology*, *139*(1), 74–80.

https://doi.org/10.1016/j.jep.2011.10.014

Kornstein, S. G., Young, E. A., Harvey, A. T., Wisniewski, S. R., Barkin, J. L., Thase, M. E., Trivedi, M. H., Nierenberg, A. A., & Rush, A. J. (2010). The influence of menopause status and postmenopausal use of hormone therapy on presentation of major depression in women. *The Journal OfThe North American Menopause Society*, *17*(4), 828–839. https://doi.org/10.1097/gme.0b013e3181d770a8

Lam, R. W. (2007). Seasonal Affective Disorder : *Annals of Clinical Psychiatry*, *19*(4), 239–246. https://doi.org/10.1080/10401230701653476

Lee, Y.-L., Wu, Y., Tsang, H. W. H., Leung, A. Y., & W.M. Cheung. (2011). A Systematic Review on the Anxiolytic Effects of Aromatherapy in People with Anxiety Symptoms. *THE JOURNAL OF ALTERNATIVE AND COMPLEMENTARY MEDICINE*, *17*(2), 101–108. https://doi.org/10.1089/acm.2009.0277

Liu, Q., He, H., Yang, J., Feng, X., Zhao, F., & Lyu, J. (2019). Changes in the global burden of depression from 1990 to 2017 : Findings from the Global Burden of Disease study. *Journal of Psychiatric Research*, *08*(002), 0–1. https://doi.org/10.1016/j.jpsychires.2019.08.002

Madubunyi, I. I., & Ode, O. J. (2012). In vitro and in vivo antioxidant potential of the methanolic extract of Cassia singueana Delile ( Fabaceae ) Lock leaves. *Comp Clin Pathol*, *21*, 1565–1569. https://doi.org/10.1007/s00580-011-1328-y

Massart, R., Mongeau, R., Lanfumey, L., & Psychiatrie, C. De. (2012). Beyond the monoaminergic hypothesis : neuroplasticity and epigenetic changes in a transgenic mouse model of depression. *Philosophical Transactions of The Royal Society*, *367*, 2485–2494. https://doi.org/10.1098/rstb.2012.0212

Maurizio Fava, Memisoglu, A., Thase, M. E., Bodkin, J. A., Trivedi, M. H., Somer, M. de, Du, Y., Leigh-Pemberton, R., DiPetrillo, L., Silverman, B., & Ehrich, E. (2015). Opioid Modulation With Buprenorphine / Samidorphan as Adjunctive Treatment for Inadequate Response to Antidepressants : A Randomized Double-Blind. *AJP in Advance*, *17*, 1–10. https://doi.org/10.1176/appi.ajp.2015.15070921

Mayberg, S. (1997). Limbic-Cortical Dysregulation: A Proposed Model of Depression. *Journal of Neuropsychiatry*, *9*(3), 471–481.

Mccarthy, D. J., Alexander, R., Smith, M. A., Pathak, S., Kanes, S., Lee, C., & Sanacora, G. (2012). Glutamate-based depression GBD. *Medical Hypotheses*, *78*(5), 675–681.

https://doi.org/10.1016/j.mehy.2012.02.009

Mcnally, L., Bhagwagar, Z., & Hannestad, J. (2008). Inflammation, Glutamate, and Glia in Depression: A Literature Review. *CNS Spectr*, *13*(6), 501–510.

Melrose, S. (2015). Seasonal Affective Disorder : An Overview of Assessment and Treatment Approaches. *Depression Research and Treatment*, *2015*, 1–6.

Melrose, S. (2017). Persistent Depressive Disorder or Dysthymia : An Overview of Assessment and Treatment Approaches. *Open Journal of Depression*, *6*, 1–13. https://doi.org/10.4236/ojd.2017.61001

Menon, V. (2011). Large-scale brain networks and psychopathology : a unifying triple network model. *Trends in Cognitive Sciences*, *15*(10), 483–506. https://doi.org/10.1016/j.tics.2011.08.003

Metrics, G. H. (2018). Global , regional , and national incidence , prevalence , and years lived with disability for 354 diseases and injuries for 195 countries and territories , 1990 – 2017 : a systematic analysis for the Global Burden of Disease Study 2017. *Lancet*, *392*, 1789–858. https://doi.org/10.1016/S0140-6736(18)32279-7

Millan, M. J. (2006). Multi-target strategies for the improved treatment of depressive states: Conceptual foundations and neuronal substrates, drug discovery and therapeutic application. *Pharmacology and Therapeutics*, *110*(2), 135–370. https://doi.org/10.1016/j.pharmthera.2005.11.006

Moallem, S. A., Hosseinzadeh, H., & Ghoncheh, F. (2007). Evaluation of Antidepressant Effects of Aerial Parts of Echium vulgare on Mice. *IJBMS*, *10*(3), 189–196.

Mongeau, R., Blier, P., & Montigny, C. De. (1997). The serotonergic and noradrenergic systems of the hippocampus : their interactions and the effects of antidepressant treatments. *Brain Research Reviews*, *23*, 145–195.

Monteleone, P., & Maj, M. (2008). The circadian basis of mood disorders : Recent developments and treatment implications. *European Neuropsychopharmacology*, *18*, 701–711. https://doi.org/10.1016/j.euroneuro.2008.06.007

Morgan, A., Reavley, N., Jorm, A., Bassilios, B., Hopwood, M., Allen, N., & Purcell, R. (2019). A guide to what works for depression; 3rd Edition. *Beyond Blue:*, *3rd Editio*(Melbourne).

Moshi, M. J., & Mbwambo, Z. H. (2002). Experience of Tanzanian Traditional Healers in the Management of Non-insulin Dependent Diabetes Mellitus Experience of Tanzanian

Traditional Healers in the Management of Non-insulin Dependent Diabetes Mellitus. *Pharmaceutical Biology*, *40*(7), 552–560. https://doi.org/10.1076/phbi.40.7.552.14691

Mrcp, I. M. A. (2000). Selective serotonin reuptake inhibitors versus tricyclic antidepressants : a meta-analysis of efficacy and tolerability. *Journal of Affective Disorders*, *58*, 19–36.

Musa, M. S., Abdelrasool, F. E., Elsheikh, E. A., Ahmed, L. A. M. N., Mahmoud, A. L. E., & Yagi, S. M. (2011). Ethnobotanical study of medicinal plants in the Blue Nile State , South-eastern Sudan. *J. Med. Plant. Res.*, *5*(17), 4287–4297.

Musazzi, L., Treccani, G., & Popoli, M. (2012). Glutamate hypothesis of depression and its consequences for antidepressant treatments. *Expert Review of Neurotherapeutics*, *April 2014*, 1–5. https://doi.org/10.1586/ern.12.96

Nibret, E., Ashour, M. L., Rubanza, C. D., & Wink, M. (2010). Screening of Some Tanzanian Medicinal Plants for their Trypanocidal and Cytotoxic Activities. *Phytother. Res*, *24*(October 2009), 945–947. https://doi.org/10.1002/ptr

Niciu, M. J., Ionescu, D. F., Erica M. Richards, A., & Jr., C. A. Z. (2015). Glutamate and its receptors in the pathophysiology and treatment of major depressive disorder. *J Neural Transm*, *121*(8), 907–924. https://doi.org/10.1007/s00702-013-1130-x.Glutamate

Niel, M. S. Van, & Payne, J. L. (2020). Perinatal depression : A review. *CLEVELAND CLINIC JOURNAL OF MEDICINE*, *87*(5), 273–277. https://doi.org/10.3949/ccjm.87a.19054

OECD 425. (2008). *OECD Guideline for Testing of Chemicals: Acute Oral Toxicity- Up- and Down Procedure, Organization for Economic Co-operation and Development (OECD 425)*.

Oja, S. S., & Saransaari, P. (2009). NEUROTRANSMITTERS AND MODULATORS. *PHYSIOLOGY AND MAINTENANCE*, *V*, 1–10.

OJINNAKA, C. M. (2012). *African Herbal Medicine : The Chemical Constituents and Phytotherapeutic Applications of West African Senna (Syn. Cassia) Species* (Issue December 2012).

Otte, C., Gold, S. M., Penninx, B. W., Pariante, C. M., Etkin, A., Fava, M., Mohr, D. C., & Schatzberg, A. F. (2016). Major depressive disorder. *Nature Publishing Group*, *2*(September), 1–21. https://doi.org/10.1038/nrdp.2016.65

Pamulaparthi, A., Prathap, V. R., Banala, M., & Nanna, R. S. (2016). EXPERIMENTAL EVALUATION OF ANTIDEPRESSANT AND ANTIANXIETY ACTIVITIES OF AQUEOUS LEAF EXTRACTS OF SENNA ALATA ( L .) ROXB . USING IN VITRO

ANIMAL MODELS. *Int J Curr Pharm Res*, *8*(4), 60–63.

Plesničar, B. K. (2014). Efficacy and tolerability of agomelatine in the treatment of depression. *Patient Preference and Adherence*, *8*, 603–612.

Porsolt, R. D., Pichon, M. LE, & Jalfre, M. (1977). Depression, a new animal model sensitive to antidepressant treatments. *Nature*, *266*, 730–732.

Potter et al. (2009). Neuropsychological correlates of magnetic resonance imaging- defined subcortical ischemic depression. *Int J Geriatr Psychiatry*, *24*(3), 219–225. https://doi.org/10.1002/gps.2093.Neuropsychological

Press, T. national academies. (2011). *Guide for the care and use of laboratory animals. Eighth Edition*.

Pringle, A., Bogdanovskaya, M., Waskett, P., Zacharia, S., Cowen, P. J., & Harmer, C. J. (2015). Does melatonin treatment change emotional processing ? Implications for understanding the antidepressant mechanism of agomelatine. *Journal of Psychopharmacology*, 1–4. https://doi.org/10.1177/0269881115592341

Raison, C. L., Capuron, L., & Miller, A. H. (2012). Cytokines sing the blues: inflammation and the pathogenesis of depression Charles. *Trends Immunol.*, *27*(1), 24–31. https://doi.org/10.1016/j.it.2005.11.006.Cytokines

Rantala, M. J., Luoto, S., Krams, I., & Karlsson, H. (2017). Depression subtyping based on evolutionary psychiatry : Proximate mechanisms and ultimate functions. *Brain , Behavior , and Immunity*, 1–15. https://doi.org/10.1016/j.bbi.2017.10.012

Ravikumar, P., & Jeyam, M. (2019). Antidepressant activity and HPTLC fingerprinting of stearic acid in different days of wheat seedlings. *Grain & Oil Science and Technology*, *2*(1), 6–10. https://doi.org/10.1016/j.gaost.2019.04.002

Rosenthal, R. N., Ling, W., Casadonte, P., Vocci, F., Bailey, G. L., Kampman, K., Patkar, A., Chavoustie, S., Blasey, C., Stacey Sigmon, A., & Beebe, K. L. (2013). Buprenorphine Implants for Treatment of Opioid Dependence: Randomized Comparison to Placebo and Sublingual Buprenorphine/Naloxone. *Addiction*, *108*(12), 2141–2149. https://doi.org/10.1111/add.12315.Buprenorphine

Royce, J. R. (1977). On the Construct Validity of Open-Field Measures. *Psychological Bulletin*, *84*(6), 1098–1106.

Sanacora, G., Giulia Treccani, & Popoli, M. (2013). Towards a glutamate hypothesis of

depression. *Neuropharmacology*, *62*(1), 63–77. https://doi.org/10.1016/j.neuropharm.2011.07.036.Towards

Sarris, J., Murphy, J., Mischoulon, D., & Papakostas, G. I. (2016). Adjunctive Nutraceuticals for Depression : A Systematic Review and Meta-Analyses. *AJP in Advance*, 1–13. https://doi.org/10.1176/appi.ajp.2016.15091228

Sarris, J., Neil, A. O., Coulson, C. E., Schweitzer, I., & Berk, M. (2014). Lifestyle medicine for depression. *BMC Psychiatry*, *14*(1), 1–13. https://doi.org/10.1186/1471-244X-14-107

Sathiyasusuman, A. (2011). Mental health services in Ethiopia: Emerging public health issue. *Public Health*, *125*(10), 714–716. https://doi.org/10.1016/j.puhe.2011.06.014

Scheepens, D. S., Waarde, J. A. Van, Lok, A., & Vries, G. De. (2020). The Link Between Structural and Functional Brain Abnormalities in Depression : A Systematic Review of Multimodal Neuroimaging Studies. *Frontiers in Psychiatry*, *11*(June), 1–10. https://doi.org/10.3389/fpsyt.2020.00485

Setiawan, E., Wilson, A. A., Romina Mizrahi, M., M., P., Rusjan, Miler, L., Rajkowska, G., Suridjan, I., Kennedy, J. L., Rekkas, P. V., Sylvain Houle, A., & Meyer, J. H. (2016). Increased Translocator Protein Distribution Volume, A Marker of Neuroinflammation, in the Brain During Major Depressive Episodes. *JAMA Psychiatry*, *72*(3), 268–275. https://doi.org/10.1001/jamapsychiatry.2014.2427.Increased

Sousa, D. P. de, Silva, R. H. N., And, E. F. da S., & Gavioli, E. C. (2017). Essential Oils and Their Constituents : An Alternative. *Molecules*, *22*(1290), 1–21. https://doi.org/10.3390/molecules22081290

SRIDHAR, S. R., RAJAGOPAL, R. V., RAJAVEL, R., MASILAMANI, S., & NARASIMHAN, S. (2003). Antifungal Activity of Some Essential Oils. *J. Agric. Food Chem*, *51*(14), 7596–7599.

Stanford, S. C. (2007). The Open Field Test: reinventing the wheel. *Journal of Psychopharmacology*, *21*(134), 28–30. https://doi.org/10.1177/0269881107073199

Steru, L., Chermat, R., Thierry, B., & Simon, P. (1985). The tail suspension test: A new method for screening antidepressants in mice. *Psychopharmacology*, *85*, 367–370.

Sublette, M. E., Galfalvy, H. C., Fuchs, D., Lapidus, M., Grunebaum, M. F., & Oquendo, M. A. (2011). Plasma Kynurenine Levels are Elevated in Suicide Attempters with Major Depressive Disorder. *Brain Behav Immun.*, *25*(6), 1272–1278.

https://doi.org/10.1016/j.bbi.2011.05.002.Plasma

Surana, A. R., & Wagh, R. D. (2018). Phytochemical analysis and antidepressant activity of Ixora coccinea extracts in experimental models of depression in mice. *Turkish Journal of Pharmaceutical Sciences*, *15*(2), 130–135. https://doi.org/10.4274/tjps.14622

Taylor, W. D., Schultz, S. K., Panaite, V., David, C., Haley, J. A., Hospital, V., & Sciences, B. (2019). Perspectives on the Management of Vascular Depression. *Am J Psychiatry*, *175*(12), 1169–1175. https://doi.org/10.1176/appi.ajp.2018.18050568.Perspectives

Umadevi, P., Murugan, S., S, J. S., & Subakanmani, S. (2011). EVALUATION OF ANTIDEPRESSANT LIKE ACTIVITY OF CUCURBITA PEPO SEED EXTRACTS IN RATS. *Int J Curr Pharm Res*, *3*(1), 108–113.

Vadnie, C. A., & Mcclung, C. A. (2017). Circadian Rhythm Disturbances in Mood Disorders : Insights into the Role of the Suprachiasmatic Nucleus. *Neural Plasticity*, *2017*, 1–28.

Vahia, V. N. (2013). Diagnostic and statistical manual of mental disorders 5: A quick glance. *Indian Journal of Psychiatry*, *55*(3), 220–223. https://doi.org/10.4103/0019-5545.117131

Véronique, M., Pierre, J., Omam, O., Bougolla, D. P., Fleur, M., Okomolo, C., & Elisabeth, N. B. (2018). Anxiolytic Effects of Senna singueana in Mice after Exposure to Chronic Restraint-Stress. *International Journal of Brain and Cognitive Sciences*, *7*(2), 36–41. https://doi.org/10.5923/j.ijbcs.20180702.02

WHO. (2017). *Depression and other common mental disorders* (Vol. 2, Issue 1).

Wu, A., Ying, Z., & Gomez-pinilla, F. (2004). The interplay between oxidative stress and brain-derived neurotrophic factor modulates the outcome of a saturated fat diet on synaptic plasticity and cognition. *European Journal of Neuroscience*, *19*(December 2003), 1699–1707. https://doi.org/10.1111/j.1460-9568.2004.03246.x

Xu, Y. et al. (2015). Effects of low-dose and very low-dose dose ketamine among patients with major depression : a systematic review and meta-analysis Ying. *International Journal of Neuropsychopharmacology*, *29*(9), 2341–2386. https://doi.org/10.1093/rfs/hhw031

Xu, Y., Wang, C., Klabnik, J. J., & Donnell, J. M. O. (2014). Novel Therapeutic Targets in Depression and Anxiety : Antioxidants as a Candidate Treatment. *Current Neuropharmacology*, *12*(2), 108–119.

Yankelevitch-yahav, R., Franko, M., Huly, A., & Doron, R. (2015). The Forced Swim Test as a Model of Depressive-like Behavior. *J. Vis. Exp.*, *March*, 1–7. https://doi.org/10.3791/52587

Yau, W., Chan, M., Wing, Y., Lam, H., Lin, W., Lam, S., & Lee, C. (2014). Noncontinuous use of antidepressant in adults with major depressive disorders – a retrospective cohort study. *Brain and Behavior*, *4*(3), 390–397. https://doi.org/10.1002/brb3.224

Yim, V. W. C., Sc, M., Ng, A. K. Y., Sc, M., Tsang, H. W. H., Ph, D., & Leung, A. Y. (2009). A Review on the Effects of Aromatherapy for Patients with Depressive Symptoms. *THE JOURNAL OF ALTERNATIVE AND COMPLEMENTARY MEDICINE*, *15*(2), 187–195. https://doi.org/10.1089/acm.2008.0333

Yüksel, C., & Öngür, D. (2011). Magnetic Resonance Spectroscopy Studies of Glutamate-Related Abnormalities in Mood Disorders. *Biol Psychiatry*, *68*(9), 785–794. https://doi.org/10.1016/j.biopsych.2010.06.016.Magnetic

Zaky, E. A. (2015). Disruptive Mood Dysregulation Disorder ( DMDD ). *Clinical Depression*, *1*(1), 1–2. https://doi.org/10.4172/cdp.1000e102

Zhang, J., Narr, K. L., Woods, R. P., Phillips, O. R., And, J. R. A., & Espinoza, R. T. (2014). Glutamate normalization with ECT treatment response in major depression. *Mol Psychiatry*, *18*(3), 268–270. https://doi.org/10.1038/mp.2012.46.Glutamate

Zhang, M. W., & Roger Ho. (2016). Critical Appraisal of Existing Ketamine Trials: Existing Limitations and Limited Applicability for Treatment. *American Journal of Psychiatry*, *173*(4), 429–431. https://doi.org/10.1176/appi.ajp.2015.15121545

Zhang, Z. (2004). Therapeutic effects of herbal extracts and constituents in animal models of psychiatric disorders. *Life Sciences*, *75*, 1659–1699. https://doi.org/10.1016/j.lfs.2004.04.014