HAWASSA UNIVERSITY

COLLEGE OF MEDICINE AND HEALTH SCIENCES

SCHOOL OF PHARMACY

ANTIDEPRESSANT-LIKE ACTIVITY OF CRUDE LEAF EXTRACT AND SOLVENT FRACTION OF THE *Commelinna benghalensis* LINN IN THE RODENTS

TEGEGN HALALA (B. Pharm)

NOVEMBER, 2023

HAWASSA, ETHIOPIA

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A THESIS SUBMITTED TO THE DEPARTMENT OF PHARMACOLOGY, SCHOOL OF PHARMACY, COLLEGE OF MEDICINE AND HEALTH SCIENCES, SCHOOL OF GRADUATE STUDIES, HAWASSA UNIVERSITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PHARMACOLOGY

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NOVEMBER, 2023

HAWASSA, ETHIOPIA

HAWASSA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

ADVISORS' APPROVAL SHEET

(Submission sheet - 1)

This is to certify that the research thesis entitled "ANTIDEPRESSANT-LIKE ACTIVITY OF CRUDE LEAF EXTRACT AND SOLVENT FRACTION OF THE *C.benghalensis* LINN IN THE RODENTS" was submitted in partial fulfillment of the requirements for the degree of Master's with specialization in Pharmacology, the Graduate Program of the School of Pharmacy and has been carried out by Tegegn Halala, ID. No. 0010/13, under my supervision. Therefore, I recommend that the student fulfill the requirements and hence, submit the thesis to the department.

Dr. Serawit Deyno (PhD)	____________________	__________________
Name of Major Advisor	Signature	Date

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We, the undersigned members of the Board of Examiners of the final open defense by Tegegn Halala, have read and evaluated his thesis entitled "ANTIDEPRESSANT-LIKE ACTIVITY OF CRUDE LEAF EXTRACT AND SOLVENT FRACTION OF THE *C. benghalensis* LINN IN THE RODENTS" and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree of Master of Science in Pharmacology.

Name of Chair Person___________________________Signature______________Date___________

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Declaration statement

I hereby declare that this MSc thesis, with the title "ANTIDEPRESSANT-LIKE ACTIVITY OF CRUDE LEAF EXTRACT AND SOLVENT FRACTION OF THE *Commelinna benghalensis* LINN IN THE RODENTS," is my original work. I have followed all ethical and technical principles of scholarship in the preparation, data collection, data analysis, and compilation of the thesis. It has not been presented for the award of any academic degree, diploma, or certificate at any other university. All sources of materials that were used for this thesis have been acknowledged through citations.

Tegegn Halala (B. Pharm)	____________________	____________________
Name of Principal Investigator	Signature	Date

ACKNOWLEDGMENT

I would like to express my deepest gratitude to my research advisor, Dr.Serwait Deyno (Ph.D.), for his unwavering support, unreserved encouragement and provision of constructive comments and guidance during thesis preparation. Furthermore, I would like to express my gratitude to the School of Pharmacy, College of Medicine and Health Sciences, Hawassa University, for giving me this golden educational opportunity. I also thank the Department of Pharmacy at Wolaita Sodo University College of Medical and Health Science for allowing me to use their pharmacology animal house for research. Special thanks to my friends Mr. Dejene Hayilu (M.Sc. in Pharmacology), Mr.Hizkel Engiso (M.Sc. in Clinical Pharmacy) and Abdulkerim Mustefa (MPH) for their relentless encouragement and support. Lastly, I extend my heartfelt thanks to my parents for their wholehearted support, blessings, and inspiration to conquer all difficulties.

CONTENTS

ABBREVIATIONS AND ACRONYMS

CBAF	Commelina Benghalensis Aqueous Fraction
CBEA	Commelina Benghalensis Ethyl Acetate
CBHE	Commelina Benghalensis Hexane
CRH	Corticotrophin-Releasing Hormone
DSM-V	Diagnostic and Statistical Manual of Mental disorders
DW	Distilled water
FST	Forced swimming test
GABA	Gama Amino Butyric Acid
GC	Glucocorticoid
HIV	Human Immunodeficiency Virus
HPA	Hypothalamic-Pituitary-Adrenal axis
ICD-10	The Tenth International Classification of Diseases
OECD	Organization of Economic Corporation and Development
OFT	Open field test
SEM	Standard error of mean
SPSS	Statistical package for social science
TST	Tail Suspension Test
WHO	World Health Organization

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ABSTRACT

Background: An ethno-botanical study has shown that *Commelina benghalensis* has been used in the treatment of depression, but its efficacy and safety have not yet been established. Therefore, this study aimed to investigate the antidepressant-like activity of *C. benghalensis* crude leaf extract and the solvent fraction in rodents.

Method: The antidepressant-like activity of the crude leaf extract and solvent fraction of *C. benghalensis* was evaluated using the depression models of Forced Swim Test (TST), Forced Swim Test (FST), and Open Field Test (OFT). Animals were randomly assigned to five groups. Group I received the vehicle (10 ml/kg), and Group II received the standard drug of fluoxetine (20 mg/kg). The test group from III to V received 100 mg/kg, 200 mg/kg, and 400 mg/kg of the crude extract or solvent fractions of *C. benghalensis*. The data was statistically analyzed using ANOVA, followed by a post-hoc Tukey test. The results were considered significant at $P<0.05$, 95% and P<, 0.001, 99% confidence interval.

Result: The crude leaf extract of *C. benghalensis*, at 100 mg/kg doses, did not reduce immobility time in both the TST and FST models. However, at doses of 200 and 400 mg/kg ($p<0.05$, $p<0.001$), immobility time was significantly reduced. In TST mice treated with hexane and ethyl acetate fraction at doses of 200 and 400 mg/kg ($p<0.05$, $p<0.001$), there was a significant reduction in immobility time compared to the control group. The aqueous fraction at doses of 100, 200 ($p<0.05$), and 400 mg/kg ($p<0.001$) shows a significant reduction in immobility time compared to the control groups. The hexane and ethyl acetate fractions significantly reduced immobility duration in FST at 400 mg/kg compared to the control. Rats treated with aqueous fraction at doses of 200 mg/kg ($p<0.05$) and 400 mg/kg ($p<0.001$) showed a significant decrease in immobility duration compared to the control group.

Conclusion: The study found significant antidepressant-like activity in *C. benghalensis* crude leaf extract and solvent fraction. As no impact on locomotor activity was observed in the OFT model, the possible mechanism could not be non-specific motor stimulation. Further study on the isolation and identification of bioactive compounds responsible for the antidepressant activity of the extract should be done.

Keywords: Antidepressant, *C. benghalensis*, crude extract, depression, solvent fraction

1. INTRODUCTION

1.1 Background

Depression is one of the most common psychiatric disorders, affecting both mental and physical health and greatly influencing healthcare resources and costs (Arnaud *et al.*, 2021). Recent studies revealed that depression is one of the top five most often diagnosed conditions worldwide and is predicted to be the second-leading cause of disability by the year 2020 (Setorki, 2020). Major depression affects 4.7% of people globally (Fathinezhad *et al*., 2019). This indicates that one person experiences depression out of every 20 (Hoque *et al.*, 2021).

Among non-communicable diseases, mental disorders play a major role in causing premature mortality (Everall, 19). In 2016, depression was estimated to account for 14.3% of all deaths worldwide. Approximately eight million deaths occur each year, meaning that death is attributable to mental disorders (Vigo *et al.,* 2016).

In 2017, the WHO estimated that more than 300 million people worldwide suffered from depression, representing 4.4% of the world population. This figure includes children, adolescents under the age of 15, men, women, the poor and the rich (WHO, 2017). Although it affects both sexes, it is more common in women (5.1%) than in men (3.6%). Its prevalence varied according to the age of the patient. However, the highest peak of depression is seen in patients aged 80 years and older, as a result of physical dysfunction, loss of personal control and low life expectancy (Charlson *et al*., 2019; *Donaldson et al., 2021*; Memirie *et al.*, 2022).

Over the past year, plenty of antidepressant drugs have been developed for the treatment of depression; however, clinical studies of this drug have shown disease recurrence: only 30% of patients respond adequately to the existing drugs and the rest do not achieve complete recovery (Setorki, 2020), drug interaction and worsened adverse effects (Gabriel & Sharma, 2017; Zwiebel & Viguera, 2022). Despite the availability of effective antidepressants that improve clinical and productivity, only 50–60% of patients receive adequate treatment and contraindications for certain diseases become serious challenges for existing drugs (E. Wilson & Lader, 2015). Therefore, the World Health Organization (WHO) urged researchers to investigate plant-derived products as less expensive, comprehensive medical care, especially in developing countries (Kato *et al*., 2021).

Ethiopians have a long history of treating a wide range of illnesses with plant products. This most valuable herbal remedy has been passed down through the generations via oral tradition. Around 80% of the population, herbal therapy as an alternative therapeutic option in developing countries (Ajayi et al., 2016; Tesfahuneygn & Gebreegziabher, 2019; WHO Regional Office for Africa, 2010). Herbal remedies derived from plants are potential sources and alternatives for developing synthetic antidepressants (Kassaye *et al*., 2007). Several experimental studies have shown that herbal medicines have antidepressant activities, including *Melissa officinalis* (Emamghoreishi & Talebianpour, 2009), *Lavandula angustifolia* (Kageyama *et al.*, 2012), *Cinnamomum zeylanicum* (Aryanezhad *et al.*, 2021), *Cinnamomum verum* (Moselm-Zadeh *et al.*, 2019), *Echium amoneum* (Sadeghi *et al.*, 2023), and the root bark of *Carissa spinarum* (Ali & Engidawork, 2022).

Commelina benghalensis is highly valued in Ethiopian traditional medicine and is applied to a wide range of various ailments such as headache, constipation, leprosy, snake bite, jaundice, mouth thrush, epilepsy and psychosis, laxative, anti-inflammatory, cancer and depression (Gebrekiristos, 2012; Batool *et al*., 2020; Tesfaye *et al*., 2020). Commelina diffuse in the genus was confirmed to have antidepressant activity (Kansagara & Pandya, 2019; Sultana *et al*., 2018). Moreover, the methanolic crude leaf extract of *C. benghalensis* showed significant decreases in the duration of immobility in two animal models of antidepressant activity: forced swimming and tail suspension tests (Chakrabarty *et al*., 2016). Therefore, this study aimed to provide scientific justification for the antidepressant-like activity of the crude leaf extract in an additional model and solvent fraction of the extract.

1.2 Statement of the problem

Globally, the prevalence and affection for depression disorders have increased over time. Several studies showed depression is now the fourth leading cause of disability worldwide, followed by lower respiratory infections, perinatal conditions and HIV/AIDS (Santomauro *et al*., 2021). According to the Global Burden of Disease report, depression contributes significantly to the overall global burden of disease. In 2017, there were 258 million incident cases of depression recorded worldwide, an increase of 49.8% from 172 million in 1990 (James *et al.*, 2018; Liu *et al., 2020).* According to a 2022 WHO report, depression affects more than 322 million people worldwide, or 4.4% of the total population, with a lifetime prevalence of 15–18%. Patients with untreated depression have a nearly 20% lifetime chance of suicide.

Over 700,000 people die due to suicide every year. About 2/3 of people who commit suicide experience depression at the time of their deaths. Approximately 7 out of every 100 men and 1 out of every 100 women who have been diagnosed with depression in their lives would commit suicide. Today, suicide is the fourth leading cause of death in 15–29-year-olds (American Association for Suicidology, 2009; N & W, 2016; Ahmed *et al*., 2017; WHO, 2022).

Around 1 billion people worldwide suffer from a mental disease. Anxiety and depression, two of the most common mental disorders, produce lost productivity that costs the global economy $1 trillion each year (J. Wilson, 2015). Depression also affects the global economy negatively. Moreover, it is associated with an enormous economic burden, mainly due to impaired work performance and partly from the widespread underuse and poor quality of effective and tolerable treatment. According to the World Economic Forum report, the global economy would lose about $2.5 trillion annually due to poor mental health and decreased productivity; by 2030, that amount is expected to rise to $6 trillion (Arnaud *et al*., 2021). So, depression has significant impacts on healthcare resource utilization and the total cost of care.

Nearly 10% of all diseases in sub-Saharan Africa are caused by neuropsychiatric disorders (Tomlinson *et al*., 2009). In Ethiopia, non-communicable disorders are the most burdensome when it comes to mental illness. Among every five persons, one will be affected by mental disorders throughout life. Schizophrenia and depression are ranked as two of the ten most troublesome conditions (FMOH, 2018; Memirie *et al*., 2022). According to a study, the prevalence of depression in Ethiopia ranges from 2.2% to 9.1% (Hailemariam *et al*., 2012).

Many of the conventional antidepressant drugs currently used are not only expensive but also have low efficacy (Pigott *et al.*, 2010), develop tolerance (long-term use) (Kato *et al*., 2021), and substantial side effects, such as headaches, addiction, sexual dysfunction, and seizures, remain of great concern (Gabriel & Sharma, 2017). Management of depression is a complicated and expensive program; thus, researching the traditional use of medicinal plants helps to solve the problem (Peng *et al.*, 2022).

1.3 Significance of study

Due to recurrent episodes of depression, worsening side effects, low efficacy, and tolerance to current medications, the quest for new antidepressant drugs is desperately needed. Efforts are being made all over the world to discover agents that can promote the healing of depression and thereby reduce the cost of hospitalization and health burden (Sun *et al*., 2022).

Due to the presence of natural sources and the long history of medicinal plant use by humans, herbal remedies have the potential to be an effective alternative to currently used modern synthetic antidepressant drugs (Sc & D, 2008). Several studied medicinal plants can induce proopiomelanocortin, decrease pro-inflammatory cytokines, increase antioxidant properties in the brain, and exert neuroprotective properties in depression patients (Psicologia, 2022). Flavonoids, lignans, phenolic acids, coumarins, diterpene alkaloids, terpenes, saponins, amines, quercetin derivatives, eugenol, hyperforin, riparian derivatives, and ginsenosides are among the compounds in plants that are responsible for elevating happiness and reducing depression symptoms (Batool *et al*., 2020; Singh *et al.*, 2023).

C. benghalensis is one of the medicinal herbs used for depression, but the solvent fraction of the extract has not been proven to be effective as an antidepressant. Thus, investigating the plant-derived molecule as an effective, safe, and affordable antidepressant agent by testing its antidepressant activity in different animal models is crucial. Therefore, the current study was conducted to evaluate the antidepressant activities of crude leaf extract with three depression models and solvent fractions of extract in rodents. Thus, the findings of this study serve as additional input for further exploration of novel antidepressant drugs.

1.4. LITERATURE REVIEW

1.3.1 Overview and types of depression

Depression is categorized into subgroups according to the DSM-V, 5th edition, and ICD-10. This classification is essential for selecting effective treatments and avoiding potential resistance. As a result, the presence of five or more symptoms that extend to two weeks of depression episodes is a prerequisite for diagnostic criteria. Depressed mood, loss of interest or pleasure, weight loss or gain, appetite disturbances (increase or decrease), sleep disturbances (insomnia or hypersomnia), psychomotor agitation or retardation, fatigability, feelings of guilt or worthlessness, loss of concentration and functioning impairment, and suicidal ideation are all symptoms of depression (Gruenberg *et al.*, 2008; Haddad, 2009; Saito *et al*., 2010). There are numerous types of depressive disorders, each characterized by a unique set of clinical symptoms, etiology, time of onset, course, severity, and treatment response (Medicaid Mental Health.Org, 2018; N & W, 2016). As a result, the classification of depression is based on various criteria.

Depending on the polarity of symptoms, depression can be categorized as either unipolar or bipolar (Ali & Engidawork, 2022). According to reviewed studies, unipolar depression is classified into two subtypes: major depressive disorder and dysthymic disorder. Whereas, bipolar disorders are classified into two subtypes: bipolar I depression (mania), and bipolar II depression (hypomania) (American Psychiatric Association, 2018). Furthermore, depression is divided into mild (with or without somatic symptoms), moderate (with or without somatic symptoms), severe (with or without psychotic symptoms), and psychotic depression (mood-congruent or mood-incongruent) (Hagen *et al*., 2021; N & W, 2016). Based on the time-related specifies, depressive episodes can be classified into catatonic, melancholic, atypical, and postpartum depression (Hasin *et al*., 2018; Nabeshima & Kim, 2013).

Numerous types of depression are also strongly related to the combination of both environmental and biological factors. This includes reproductive depression, such as premenstrual dysphoric disorder, postpartum depression and premenopausal depression, which arise as a result of hormonal fluctuations in the brain influencing neurotransmission (Remes *et al*., 2021; Van Den Bosch & Meyer-Lindenberg, 2019).

1.3.2 Pathophysiology of depression

Despite significant progress in neuroscience research over the last few decades, the underlying pathophysiology of depression has not been properly described, and there is no single known cause of depression. Rather, it is the product of a combination of genetic, biochemical, social, and psychological factors (Brigitta, 2002; de Menezes Galvão *et al.*, 2021). Several theories or hypotheses about the mechanisms of depression have been proposed over the years and reviewed as follows:

I. The stress-induced depression theory

This theory of depression is based on hypothalamic-pituitary-adrenal (HPA) axis dysfunctions observed in the majority of depressed patients (Dai & Smith, 2023; LeMoult, 2020). The activity of the HPA axis is mediated by the hypothalamic secretion of corticotrophin-releasing hormone (CRH) and arginine-vasopressin (AVP), which increases the anterior pituitary secretion of adrenocorticotropic hormone (ACTH). ACTH stimulates the release of glucocorticoids from the adrenal gland, resulting in an increase in GC levels in the blood and cerebrospinal fluid. These inhibit CRH and AVP release via GC receptors in the hippocampus via a negative feedback mechanism. According to this theory, prolonged stress causes the HPA axis's negative feedback to fail, resulting in a continuation of increased GC levels. This, in turn, leads to long-term brain alterations that lead to long-term memories of the stressful experience, resulting in stress-related mental disorders such as depression (Iob *et al.*, 2020; Weymouth, 2017).

II. GABA-glutamatergic hypothesis

GABA, the main inhibitory neurotransmitter, plays an important role in stress management in the brain, modulation of hippocampus neurogenesis and neuronal development (O'Gorman Tuura *et al.*, 2018). Changes in the GABAnergic system, such as decreased GABA levels in plasma and CSF, as well as the involvement of genetic variants in the GABA-A receptor subunit genes, have been identified in patients with depression. According to the glutamate hypothesis, there is sustained glutamate accumulation caused by changes in glutamate release, clearance, and metabolism in selected areas of cognitive-emotional behaviors and the mood-regulating portion of the brain, like the hippocampus, amygdala, and prefrontal cortex, resulting in structural and functional changes and impaired synaptic function (Onaolapo, 2021). Another study found that there is a causal connection between GABA and/or glutamate neurotransmitter dysfunction and depression, which results in cognitive impairment, extracellular glutamate accumulation, and cytotoxic damage to neurons and glia (Duman *et al.*, 2019; Sarawagi *et al.*, 2021).

III. Biogenic monoamine hypothesis

There are numerous noradrenergic, serotonergic, and dopaminergic neurons in the brain. Noradrenergic neurons travel from the brain stem to practically all brain locations, where norepinephrine (NE) influences prefrontal cortex function and working memory processing and regulates behavior and attention. NE is also involved in the formation of emotionally charged memories by regulating cognition, motivation, and intellect. Serotonin (5-hydroxytryptamine, or 5HT) is the most abundant neurotransmitter and plays an important role in modulating motor function, pain perception, and appetite in the central nervous system (Filatova *et al*., 2021; Stein, 2012).

This hypothesis states that changes in the monoaminergic neurotransmitters like serotonin (5-HT), norepinephrine (NE), and/or dopamine (DA) in the CNS play a significant role in the pathophysiology of depression (Jiang *et al.*, 2022). Clinical examinations of individuals treated for hypertension with reserpine, which induces depletion of presynaptic stores of norepinephrine, serotonin and dopamine, indicate depression symptoms (Khoodoruth *et al.*,2022). Second, some antidepressant-like effects seen with iproniazid, which increases monoamine concentrations, can be provided as evidence for this hypothesis (Van Der Walt & Keddy, 2021).

Generally, this hypothesis proposes that depression is caused by a lack of the brain monoaminergic transmitters NE, 5-HT, and DA and that antidepressants work by raising the availability of neurotransmitters, resulting in long-term adaptive changes in monoaminergic receptor sensitivity (Seife Demissie, 2017). However, this hypothesis failed to clarify the reason why prolonged administration (3–4 weeks) was required to achieve the desired therapeutic outcomes, despite the fact that monoamine levels increased substantially with antidepressant therapy. Likewise, up to 30% of patients with MDD are refractory to currently available antidepressants (Al-Harbi, 2012; N & W, 2016).

IV. Inflammation hypothesis

This hypothesis proposes that any disturbance in the immune system would have a significant impact on modifying the behavior linked with depression. Proinflammatory cytokines are involved in the pathophysiology of depressive disorders by altering white matter anatomy, brain global connectivity, and functional activation, as well as increasing the amount of pro-inflammatory biomarkers (Abbott, 2018). Indeed, tumor necrosis factor-alpha (TNF-), interleukins (IL-1, IL-6), and C-reactive protein (CRP) levels have been found to rise in depressed people (Giovannini *et al*., 2011). Microglia, which regulates inflammation, also plays an essential role in the development of numerous neurological and psychological disorders, including depression (Gałecki & Talarowska, 2018).

1.3.3 Management of depression

There are various therapeutic options for depression, including pharmacotherapy, psychotherapy, and somatic therapy, which are often employed for treatment-resistant depression.

I. Somatic therapy

Somatic therapy for depression is a device-based approach that consists of introducing transient electric or magnetic current onto the scalp or to anatomically deep brain structures. It has wide applicability for maintaining effects after successful remission and can be used as an add-on therapy (Cusin & Dougherty, 2012).

Electroconvulsive therapy (ECT) is the first effective somatic therapy utilized for the treatment of mental problems, and it is still widely used in clinical settings today. According to electrophysiological studies, one of the most important effects of ECT on brain serotonergic systems in rodent brains is the sensitization of postsynaptic serotonin (5-HT) 1A receptors, which leads to an increase in serotonergic transmission (Song *et al.*, 2015). It provides a link between chemical and electrical signal transmission in the brains and other hormone levels like testosterone stimulating hormone will rise (Stippl *et al.*, 2020).

Another form of somatic treatment option for treatment-resistant depression is transcranial magnetic stimulation (TMS). It causes depolarization of cortical neurons by passing a magnetic current through a metal coil placed on the patient's scalp, making it non-invasive. Finally, this results in a rise in dopamine and serotonin levels. Additionally, it also increases the activity of adrenergic and 5-HT receptors in the frontal brain. There have also been reports that TMS reduces the sensitivity of presynaptic serotonergic autoreceptors (Khoodoruth *et al*., 2022).

II. Pharmacotherapy

The majority of current antidepressant drugs act by altering monoamine neurotransmission in the brain. These drugs work by increasing the whole synaptic concentration of monoamines (serotonin, norepinephrine, and dopamine) (Moncrieff *et al.,* 2022). They perform this by either reducing their reuptake into the presynaptic neuron by binding to the appropriate neurotransmitter transporter or by reversibly or irreversibly inhibiting the monoamine degradation enzyme (Edinoff *et al.,* 2022).Certain antidepressants also change neurotransmission by acting on presynaptic or postsynaptic neurotransmitter receptors (Taylor *et al.,* 2005). Atypical antidepressant medications exist as well.

1.3.4 Medicinal plants with reported anti-depressant activities

Medicinal herbs have been historically used for centuries by local practitioners and traditional healers to prevent, diagnose, maintain, and improve physical ailments and mental disorders. The WHO estimates that approximately 3.5 billion people who live in developing nations rely on these medicinal herbs regularly (Fitzgerald *et al*., 2019; Petrovska, 2012). There are around 45,000 plant species in Africa, with 5,000 having medicinal uses and being utilized traditionally. Furthermore, due to the vast diversity of floral content in Ethiopia, where there are nearly 6500–7000 different species, 800 of which have medicinal properties used to treat approximately 300 different disorders (Admasu Moges and Yohanis Moges, 2021; Ajayi *et al.*, 2016).

Plant-derived products play an important role in the development of synthetic drugs. Several medicinal plants were identified as having antidepressant activity and working by repairing the brain's disrupted neurotransmitters or associated systems, such as the 5-HT system (*Hypericum perforatum*) (Mennini & Gobbi, 2004), the glutamate system (*Siphocampylus verticillatus*) (Sharma, 2017), the DA system *(Rosmarinus officinalis)* (Emamghoreishi & Talebianpour, 2009), the NE system *(Valeriana wallichii)* (Müller *et al*., 2012), and the HPA axis *(Ptychopetalum olacoides)* (Singla *et al.,* 2022). Other experimental studies also report that medicinal plants that have antidepressant activity can induce happiness in depressed patients by reducing pro-inflammatory cytokines, increasing proopiomelanocortin, and promoting neuroprotectione. Alkaloids, terpenes, saponins, flavonoids, lignans, and phenolic acids are among the compounds that contribute to these effects (Ali & Engidawork, 2022).

1.3.5 Overview of experimental plant

I .Commelinna benghalensis Linn

Commelina benghalensis L. belongs to the Commelinaceae family and the genus Commelina. The family Commelinaceae, commonly known as spiderwort, consists of 41 genera and 650 species globally. The name *Commelina* was derived from the Dutch botanist Jan Commelijn (1629–1692), who identified around 170 or more species occurring in the world's tropical regions. This genus contains approximately 250 species and it is the most numerous within the family Commelinaceae (Kansagara & Pandya, 2019). *Commelina* is the largest genus of Commelinaceae in Africa, with over 100 species. At least 65 species are found in East Africa, most commonly in Kenya, Uganda, Tanzania, and Ethiopia (Gebrekiristos, 2012).

In Ethiopia and Eritrea, the family is represented by nine genera and 19 species have been recognized, including two endemic Ethiopian species. Among these two are species of Commelina (*Commelina benghalensis*, *Commelina africana*, *Commelina diffusa*, and *Commelina subulata*) and two Tradescantia species (*T. fluminensis* and *T. zebrina*). The genus *Commelina* is a perennial or annual herb having fibrous or tuberous roots. It has creeping, ascending, or upright stems that are branching, with cleistogamous flower-bearing underground stolons.

Commelina benghalensis has ascending, 0.9–2.5 m-long upright or creeping stems that branch and root at the nodes. The leaves are oval or elliptical with a thin base that narrows into a petiole. Flowers consist of three lavender-blue petals, with the flower being smaller than the two laterals, and they are sometimes white. It grows at altitudes ranging from 400 to 2500 m.a.s.l. and grows in forests, woodland, stream banks, waste grounds, rocky hillsides, gardens, and under bushes with partial shade.

In Ethiopia, it is well distributed in most floristic regions of the country, including Wollo, Gojam, Shewa, Wellega, Illubabor, Kefa, Gamo Gofa, Harerge, and Sidama (Heidi Busse, 2013).

It has different Wohaankkur in Amharic (Tesfaye et al., 2020) and Laalunxe in Sidamigna (Tamene, 2020).

Fig. 1: A photograph of *Commelina benghalensis* captured by Tegegn Halala

II. Reported ethno-pharmacological activity

In Ethiopia, *C. benghalensis* leaves and stems are used to treat ophthalmia, infertility in women, snake bites, leprosy, laxatives, anti-inflammatory drugs, depression, sore throats, and burns, and the liquid contained in the flowering spath is used to treat eye complaints (Gebrekiristos, 2012; Heidi Busse, 2013).

Furthermore, several pharmacological studies have confirmed that most of the claims explored show that the study plants have promising activities such as antibacterial activity (Fitoquimica & Actividad, 2010), sedative and anxiolytic activity (Islam *et al*., 2021), analgesic and anti-inflammatory activity (Hossain *et al*., 2014), fertility-inducing property (Oloro, 2023), antioxidant and anti-cancer activity (Batool et al., 2020), wound healing activity (Kansagara & Pandya, 2019), antimalarial activity (Tadege *et al*., 2022), anti-viral activity (Taint *et al*., 2013) and anthelminthic activity (Tewelde, 2021).

III. Phytochemical constituents of an experimental plant

C. *benghalensis* was qualitatively screened and yielded various compounds such as noctacosanol, n-triacontanol, compesterol, stigmasterol, and hydrocyanic acid (Batool *et al*.,2020). A phytochemical screening study claimed that plants contained bioactive compounds such as alkaloids, cardiac glycosides, saponins, phlobatannins, carbohydrates volatile oils, resins, balsams, flavonoids, tannins, anthraquinones, total phenols, and terpenoids (Ghosh et al., 2019; Of & Benghalensis, 2017; Tesfaye *et al.*, 2020). The presence of this secondary metabolite exhibits several activities. For example, the presence of flavonoids suggests that the plant may have anti-inflammatory, antibacterial, anti-allergic, antioxidant, or anti-cancer properties and analgesic activity (Gebrekiristos, 2012; Moselm-Zadeh *et al*., 2019). Thus, the presence of these secondary metabolite plants was implicated in producing anti-depressant activity.

2 OBJECTIVE

2.1 General objective

To evaluate the antidepressant-like activity of *C. benghalensis* of crude leaf extract and solvent fraction using a depression model in rodents.

2.2 Specific objective

- ✓ To assess the acute toxicity profile of the *C. benghalensis* Linn extract of the plant.
- ✓ To determine the anti-immobility effect of the crude leaf extract and solvent fractions of *C. benghalensis* leaves using a tail suspension test in mice
- ✓ To determine the anti-immobility effect of the crude leaf extract and solvent fractions of the *C. benghalensis* leaves in a forced swimming model of depression in rats
- ✓ To evaluate the effect of the crude leaf extract of *C. benghalensis* on locomotor activity using an open-field test in mice

3 MATERIALS AND METHODS

3.1 Material

3.1.1 Chemicals, drugs and regents

Fluoxetine (ZAFA Pharmaceutical, India), absolute methanol (Cheshire, UK), ethyl acetate (Fluka, Germany), chloroform (BDH Chemical Reagents, England), hexane (LOBA Chemie Pvt. Ltd., India), and distilled water (Ethiopian Pharmaceuticals Manufacturing (EPHARM)). All drugs and reagents purchased were of analytic grade.

3.1.2 Instruments and Apparatus

Sensitive digital weighing balance (Shinko Denshi Co., Ltd., Vietnam), rotary evaporator (Lab Scale, REV100-P, China), deep freezer, water bath, mortar and pestle, an oven, rectangular open field apparatus, transparent glass, conical flask, beaker, buchner funnel, adhesive plaster, gauze, elastic bandage, glove, Whatman filter paper No. 1, permanent marker, transparent polythene sheet, stopwatch timer and thermometer were used.

3.1.3 Experimental Setting

The plant extraction was conducted at Hawassa University, College of Medicine and Health Science School of Pharmacy, in the clinical and molecular pharmacology laboratory. The animal experiment was conducted at Wolaita Sodo University's College of Medicine and Health Science Department of Pharmacy in the pharmacology laboratory.

3.1.4 Plant material

C. benghalensis Linn (Laalunxe in Sidamigna) was collected from the Wondo Genet Botanical Garden and found in the western direction of Sidama National Regional State, Ethiopia, away 25 km from Hawassa City in March 2023 G.C. The specific species of experimental plant was identified with the help of herbalist Tegenu Mekuria (Wondogenet National Herbarium Department). Then, the fresh aerial part of the plant was sent to a taxonomist, and an authenticated voucher specimen (TH001) was given and deposited at the Wondo Genet National Herbarium, College of Forestry and Natural Resources, Hawassa University, for future reference.

3.1.5 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 in the animal house at the Department of Pharmacy, College of Medicine and Health Science, Wolaita Sodo University, were used for the experiments. Female rats were used for acute toxicity tests. The animals were housed in polypropylene cages (5 rats per cage) under standard environmental conditions and a 12-hour light/dark natural illumination cycle and were acclimatized to laboratory conditions for a minimum of 5 days before the time of experimentation. They were provided with a laboratory pellet diet and clean water. All procedures and techniques in the study were used following the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (Bayne, 1996).

3.2 Methods

3.2.1 Preparation of crude extract

The leaves of *C. benghalensis* Linn were collected, cleaned, washed under running tap water to remove the surface pollutants, and air-dried under shade at room temperature. Thereafter, the dried leaves were coarsely powdered using a mortar and pestle. One thousand grams of dried and powdered leaves of the extract were soaked in 8000 mL of methanol and macerated for 72 hours at room temperature. The procedure was repeated for three cycles with fresh solvent to increase the yield. Then, the extract solution was filtered, first by using cotton gauze and later by suction filtration apparatus using Whatman filter paper No. 1. A rotary evaporator was used to dry the methanol in the sample under reduced pressure at a temperature of 40 °C and the remaining water was dried in the oven. The final yield of the extract was stored in the refrigerator until it was used for the experiment.

3.2.2 Solvent fractionation

One hundred twenty grams of crude extract was subjected to successive extractions using a separator funnel with hexane, ethyl acetate, and water using different solvents of increasing polarity. One hundred twenty grams of the dried crude extract of the plant were suspended in 720 mL of distilled water and slightly shaken to mix it completely with the solvent. The mixture was transferred into a separator funnel. Then, an equal volume of hexane was added to it. The new mixture was shaken gently and allowed to settle until it formed two layers, and then the hexane fraction was collected.

The same volume of hexane was added to repeat the procedure twice, as described above. Then, to the remaining aqueous portion in the separating funnel, an equal volume of ethyl acetate was added, followed by vigorous shaking as described for the preceding solvent fractionation. The upper layer was ethyl acetate, which was separated from the aqueous portion, and the procedure was repeated twice. The filtrates of the hexane and ethyl acetate fractions were concentrated by a rotary evaporator at 50 rpm and 40°C to obtain the hexane and ethyl acetate fractions. The remaining aqueous residue was frozen in a deep freezer overnight and then freeze-dried using the oven to obtain an aqueous fraction. All fractions were stored in screw-cap vials in a refrigerator at −4 °C until use.

3.2.3 Grouping and dosing of experimental animals

All animals were randomly assigned to five different groups for each model. Group I received distilled water as a vehicle and served as a negative control; Group II received the standard drug of fluoxetine (20 mg/kg) and served as a positive control. The test groups from group III-V received increasing doses of the extract at 100, 200, and 400 mg/kg, respectively. Before being administered, different doses of the crude extract, solvent fractions, and standard drug were dissolved in distilled water and given orally one hour prior to the experiment sessions. The maximum amount that was administered was 10 ml/kg (Ali & Engidawork, 2022).

3.3 Acute toxicity test

According to the Organization for Economic Cooperation and Development (OECD) guidelines, an acute toxicity study was conducted using a limit test dose of 2000 mg/kg (OCDE, 2001). A total of five female rats were fasted for 4 hours, and the animals received the extracts sequentially, with each mouse receiving a single dose of 2000 mg/kg of extract. The animals were observed individually for mortality and overt signs of toxicity during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily afterward for a total of 14 days. Mortality as well as any behavioral changes, including coma, convulsions, restlessness, diarrhea, abnormal breathing, abnormal motor activity, and alertness, were closely monitored.

3.4 Antidepressant activity test

3.4.1 Tail suspension test (TST)

The tail suspension test was performed according to the technique of Steru (1985). Animals were transferred from the housing colony to the pharmacology laboratory in their individual cages and allowed for two hours to acclimate to the new environment. After 60 minutes of acclimatization, the distilled water, standard drug, crude extract, and solvent fraction were administered to each mouse according to group and dose. Each mouse was individually suspended upside-down by its tail on a countertop that was 35–50 cm above the floor using adhesive tape placed 1 cm from the tip of the tail. A digital video camera (Sony video camera WDS-140-China) was used to record the mouse movement. The length of immobility was scored and calculated over the entire duration of the test for 6 minutes (counting in seconds) using stopwatch software called XNote Timer. Finally, a mouse was considered to be immobile if it was hanging passively and motionlessly with no body movement (Costa-nunes et al., 2015).

3.4.2 Forced swimming test (FST)

The FST is a widely used behavioral test described by Porsolt in 1977 to determine the antidepressant activity of a compound. Male rats were forced to swim alone in a clear glass container (20 cm in diameter and 40 cm in height) containing 19 cm (height) of freshwater at 25°C. The experiment was performed in two sessions, 24 hours apart. In the first session of the pre-test, rats were allowed to swim freely for 15 minutes without a recording camera. This activity was carried out to check each rat's degree of fitness and produce a stable immobility time profile. In the second session (after 24 hours of pre-test), rats were forced to swim for 5 minutes following treatment (24 hours, 4 hours, and 1 hour before the test). A stopwatch was used to record every second of immobility, and a video camera was placed horizontally near the glass container to record the action of the mouse after the test. Floating and the lack of any struggle behaviors other than those required to keep the head above water to breathe were taken as immobile behaviors, suggesting that there was some kind of adaptive reaction to the stressful conditions in the water (Kedzierska & Wach, 2016; N & W, 2016, Surana & Wagh, 2018). Finally, at every round, the rat was removed and dried with a clean towel to maintain body temperature and avoid hypothermia, and returned to the cage.

3.4.3 Open field test (OFT)

The open field test (OFT) measures the overall locomotor activity to rule out a false-positive effect. The open field apparatus is a rectangular or cubic dark box (68 cm x 68 cm x 45 cm) with a surface divided into 16 squares to make up the central and peripheral squares. A 60-watt bulb positioned perpendicularly above the OFT apparatus provided illumination. The vehicle, standard drug, crude extract, and solvent fraction were administered one hour before the test. The mouse was peripherally placed in the OFT and followed many peripheral crossings (ambulation) and central crossings (central activity), and total locomotion was recorded as the sum of the peripheral squares in 5-minute video recordings. The surface of the apparatus was cleaned with alcohol and cotton swabs after each mouse to avoid potential cues. Care was taken to avoid sudden movements, and noise was reduced as much as possible (Fekadu *et al*., 2016; Engidawork *et al*., 2022).

3.5 Statistical data analysis

The results of the experiments were analyzed using Statistical Package for the Social Sciences (SPSS) Windows version 25 using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Results were expressed as mean ± standard error of the mean (SEM), and the significance level was set at a p-value of less than 0.05.

4 RESULTS

The percentage yield for the plant material was calculated using the following formula:

$$\text{Percent yield} = \frac{\text{weight of the extract}}{\text{weight of the plant material}} \times 100$$

=75g/310gx100

=24% yield was obtained

4.1 Acute toxicity test

An acute oral toxicity test performed in female rats indicated that the crude aqueous root extract of *C. benghalensis* did not cause any mortality up to a dose of 2000 mg/kg. In addition, no signs of toxicity related to behavioral, autonomic, neurologic or physical profiles were observed after treatment with the extracts during the follow-up period.

4.2 Effect of the crude leaf extract in the tail suspension test

As shown in Table 1, the crude leaf extract of *C. benghalensis* at a dose of 100 mg/kg showed an 8.8% reduction in the duration of immobility, which was insignificant as compared to the negative control group. On the other hand, the crude leaf extract of *C. benghalensis* at doses of 200 and 400 mg/kg exhibited a significant reduction in immobility (p<0.001). In addition, the extract at a dose of 200 and 400 mg/kg showed a significant immobility time reduction (15.05 and 42.9%, respectively) as compared to 100 mg/kg of the extract (p<0.001).

Table 1: Anti-depressant-like effect of the crude leaf extract of *C. benghalensis* on the duration of immobility in mice using the tail suspension test

Treatment groups	Duration for immobility (sec)	Percent reduction in time of immobility
DW 10mg/kg	170 $\pm$ 8.62	-
Fluoxetine 20mg/kg	94.20 $\pm$ 2.74$^{12\ a}$	44.4

CB 100mg/kg	155.4 ± 8.26	8.8
CB 200mg/kg	114.4 ± 4.85$^{12\ a}$	15.05
CB 400mg/kg	97 ± 3.17$^{12\ a}$	42.9

Values are expressed as mean ± SEM for each group (N= 5). The results are compared [1]against control, [2]against CB-100.[a]<0.001. Control = distilled water, standard= Fluoxetine CB= *Commelina benghalensis*

4.3 Effect of the solvent fractions in mice tail suspension test

As indicated in Table 2, mice treated with the hexane and ethyl acetate fractions of the extract at a dose of 100 mg/kg did not show a significant reduction in the duration of immobility compared to the controls. However, pretreatment with the hexane and ethyl acetate fractions of the extract at doses of 200 and 400 mg/kg displayed a significant ($p<0.05$, $p<0.001$, respectively) reduction in the duration of immobility in comparison with the control groups. Pretreatment with the aqueous fraction of the extract showed a significant reduction in the duration of immobility at the doses of 100, 200 mg/kg ($p<0.05$), and 400 mg/kg ($p<0.001$) compared to the control groups. The standard drug fluoxetine at a dose of 20 mg/kg exhibited a significant reduction in the duration of immobility as compared to the negative control groups and the ethyl acetate fraction of the extract at a dose of 100 mg/kg.

Table 2: Antidepressant-like effect of hexane, ethyl acetate and an aqueous fraction of the leaf extract of *C. benghalensis* in mice using a tail suspension test

Treatment groups	Duration for immobility (sec)	Percent reduction in time of Immobility (%)
DW 10mg/kg	188.3 ± 13.48	-
Fluoxetine 20mg/kg	96 ± 4.7^{1c2a}	50.4
CBHE 100mg/kg	145.3 ± 6.17	22.8
CBHE 200mg/kg	122 ± 7.23^{1a}	35.2
CBHE 400mg/kg	102.6 ± 3.48^{1c}	45.5
CBEA 100mg/kg	153.6 ± 14.3	18.4
CBEA 200mg/kg	116 ± 7.0^{1b}	38.3
CBEA 400mg/kg	101.3 ± 6.48^{1c}	46.2

CBAF 100mg/kg	134 ± 16.19[1a]	28.8
CBAF 200mg/kg	127.6 ± 12.9[1a]	32.2
CBAF 400mg/kg	102 ± 13.07[1c]	45.8

Values are expressed as mean ± SEM (N=5) [1]against control, [2]against CBEA 100mg/kg; [a]$p<0.05$; [b]$p<0.01$; [c]$p<0.001$. DW=Distilled water, CBHE=*Commelina benghalensis* fractionated by hexane, CBEA=*Commelina benghalensis* fractionated by ethyl acetate, CBAF=*Commelina benghalensis* fractionated by an aqueous

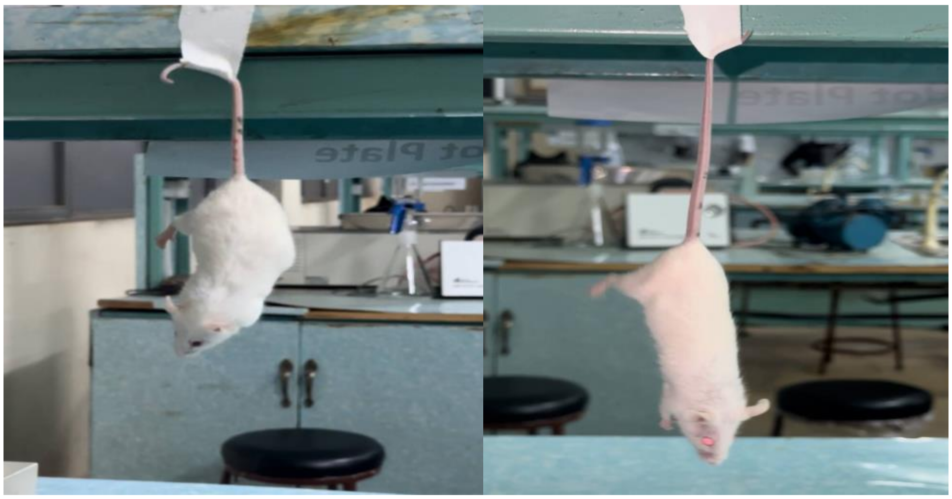

Fig. 2: Effect of the solvent fractions on the duration of immobility in mice during the tail suspension test

4.4 Effect of the crude leaf extract using a forced swim test in rats

The crude leaf extract of *C. benghalensis* at a dose of 100 mg/kg (12.8%) failed to show a marked decrease in immobility compared with the control group (Table 3). Mice pretreated with crude leaf extract of *C. benghalensis* at doses of 200 mg/kg (21.9%) and 400 mg/kg (44.8%) were able to exhibit a statistically significant reduction in immobility time ($p<0.05$, $p<0.001$) as compared to the control groups. In addition, pretreatment with a crude leaf extract of *C. benghalensis* at a dose of 400 mg/kg showed a significant immobility time reduction (70.04%, $p<0.01$) as compared to the crude extract at a dose of 100 mg/kg (Table 3).

Table 3: Anti-depressant effect of the crude leaf extract of *C. benghalensis* on the duration of immobility in mice using a forced swim test

Treatment groups	Duration for immobility (sec)	Percent reduction in time of immobility (%)

DW 10mg/kg	175 ± 13.1	-
Fluoxetine 20mg/kg	93.8 ± 3.15[1 b2]	46.4
CB 100mg/kg	152.6 ± 10.4	12.8
C B 200mg/kg	136.6 ± 6.71[1 a]	21.9
CB 400mg/kg	96.6 ± 4.4[1 b2 b]	44.8

Values are expressed as mean ± SEM for each group (N= 5). The results are compared [1]against control, [2]against CB-100 [a]<0.05 [b]<0.001. Control = distilled water, standard= Fluoxetine CB= *Commelina benghalensis*

4.5 Effect of the solvent fractions using the forced swim test in rats

As shown in Table 4, rats pretreated with the hexane and ethyl acetate fractions of the extract at doses of 100 and 200 mg/kg did not show a significant reduction in immobility time compared to the control group. However, the duration of immobility appeared to be significantly reduced with the hexane and ethyl acetate fractions at a dose of 400 mg/kg ($p<0.001$) compared to the control groups. Rats treated with the aqueous fraction of the extract at a dose of 100 mg/kg did not show a significant reduction in the duration of immobility compared to controls. The pretreatment with the aqueous fraction showed a significant reduction in immobility time at doses of 200 mg/kg ($p<0.05$) and 400 mg/kg ($p<0.001$) compared to the control groups.

Table 4: Antidepressant-like effect of hexane, ethyl acetate and aqueous fraction of the leaf extract of *C. benghalensis* in mice using a forced swim test

Treatment groups	Duration for immobility (sec)	Percent reduction in time of immobility (%)
DW 10mg/kg	182±18.17	-
Fluoxetine 20mg/kg	94±4.35[1b]	48.35
CBHE 100mg/kg	150.6±15.67	17.5
CBHE 200mg/kg	129.3±6.69	28.9
CBHE 400mg/kg	97.3±7.31[1b]	46.5
CBEA 100mg/kg	147.3±20.73	19.06

CBEA 200mg/kg	136.6±4.48	24.9
CBEA 400mg/kg	94.7±7.3^{1b}	48.02
CBAF 100mg/kg	135.3±8.68	25.6
CBAF 200mg/kg	124±4.04^{1a}	31.8
CBAF 400mg/kg	94.6±7.96^{1b}	48.02

Values are expressed as mean ± SEM (N=5) ^{1}against control $^{a}p<0.05$; $^{b}p<0.001$. DW=Distilled water, CBHE=*Commelina benghalensis* fractionated by hexane, CBEA=*Commelina benghalensis* fractionated by ethyl acetate, CBAF=*Commelina benghalensis* fractionated by an aqueous

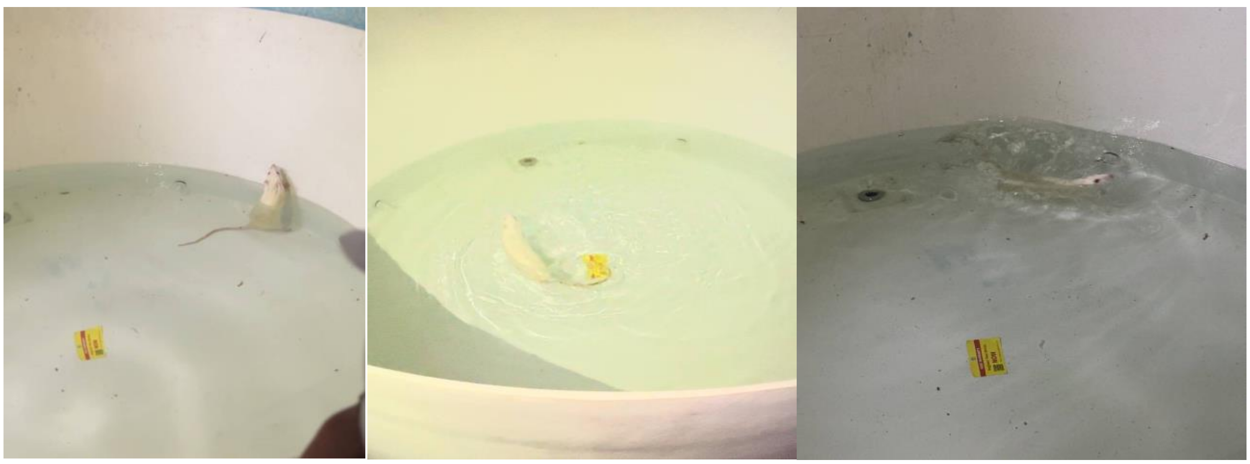

Fig. 2: Effect of the solvent fractions on the duration of immobility in rats through the forced swimming test

4.6 Effect of the extract using an open field test in mice

To exclude the possibility that changes in immobility were associated with changes in motor activity; the extract-treated animals were tested for activity in the open field. As shown in Table 5, the doses of extract that were able to show antidepressant-like responses in the TST and FST did not show any significant change in locomotion.

Table 5: Antidepressant-like effect of the crude leaf extract of *C. benghalensis* on the duration of immobility in the mice using an open-field test

	Number of squares crossed

Treatment groups	Peripheral	Central	Total
DW 10mg/kg	61.4 + 3.14	22.4 + 2.58	83.8 + 2.7
Fluoxetine 20mg/kg	57.8 + 2.74	14 + 0.83	71.8 + 2.78
CB 100mg/kg	62.2 + 3.39	19 + 1.41	81.2 + 4.06
CB 200mg/kg	60.4 + 1.88	21.8 + 1.77	82.2 + 2.35
CB 400mg/kg	57 + 2.82	19.2 +1.06	76.2 + 3.68

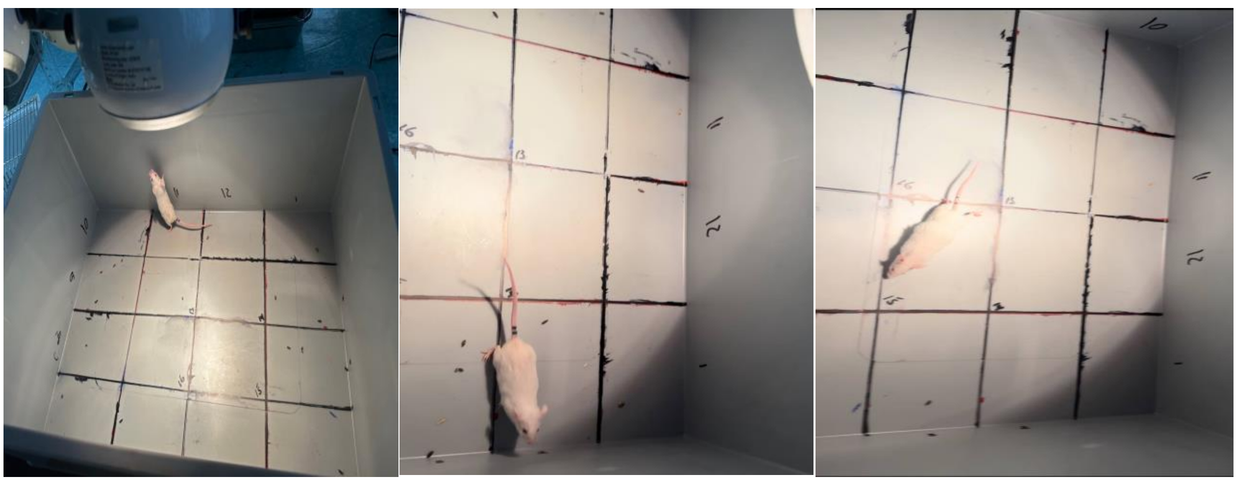

Fig.2: Effect of the crude extract to rule out false positive in mice using an open field test

5. DISCUSSION

This investigation revealed that the leaf extracts of *C. benghalensis*, both in the crude and solvent fractions, had a significant antidepressant-like effect (Chatterjee *et al.*, 2012). The decrease in immobility time in the validated TST and FST-based depression models serves as evidence for this. On the other hand, the OFT showed that the plant has no discernible influence on locomotor activity, indicating that non-specific motor stimulation cannot be the cause of the observed antidepressant-like effect (Id *et al*., 2023). Both in TST and FST models, the crude leaf extract of *C. benghalensis* at a dose of 100 mg/kg did not reduce the immobility period, indicating that these are sub-threshold doses. In contrast, the crude leaf extract of *C. Benghalensis* significantly decreased immobility time in both the TST and FST models at doses of 200 and 400 mg/kg (Abelaira & Re, 2013; Hsu *et al*., 2012).

As can be seen from the results of this study, there are minor differences in the ability to reduce immobility observed between the two doses in the two models. For instance, the 400 mg/kg dose showed a relatively higher percentage reduction in immobility in FST but not in TST. These disparities might result from variations in the underlying mechanisms of immobility induction, variations in inter-strain and inter-species, and variations in test sensitivity, as shown in earlier studies (Li *et a*l., 2020; Mannan *et al*., 2015).

In the TST models, pre-treatment of mice with hexane and ethyl acetate fractions at a dose of 100 mg/kg did not show significant antidepressant-like activity. This suggests that 100 mg/kg is a sub-therapeutic dose for this effect. However, the aqueous fraction of the extract revealed significant antidepressant-like activity at a dose of 100 mg/kg. Pretreatment with the aqueous, ethyl acetate and hexane fractions of the extract showed a significant reduction in the duration of immobility at a dose of 200 mg/kg compared to the control groups. A further increase in the dose of the solvent fraction of the *C. benghalensis* extract resulted in the highest level of antidepressant-like activity. Accordingly, a significant reduction in immobility was seen at doses of 400 mg/kg in mice pretreated with aqueous, hexane, and ethyl acetate fractions.

In the FST models, pre-treatment of rats with aqueous, hexane, and ethyl acetate fractions at a dose of 100 mg/kg did not show significant antidepressant-like activity. This suggests that 100 mg/kg is a sub-therapeutic dose for this effect. Pretreatment with the aqueous fraction of the extract showed a significant reduction in the duration of immobility at the dose of 200 mg/kg compared to the control groups. However, the hexane and ethyl acetate fractions of the extract did not reveal significant antidepressant-like activity at the dose of 200 mg/kg. A further increase in the dose of the solvent fraction of the *C. benghalensis* extract resulted in the highest level of antidepressant-like activity. Accordingly, a significant reduction in immobility was seen at doses of 400 mg/kg in rats pretreated with aqueous, hexane and ethyl acetate fractions. Moreover, the aqueous fraction was the most active fraction amongst all fractions at all doses, and 400 mg/kg could be taken as the most effective dose for reducing immobility, which is comparable with the standard drug.

FST and TST are similar in their operating principles, and in most cases, the data from these two models converge. The activity of the extract in both models observed in the present study thus strengthens the possibility that *C. benghalensis* might be a viable option for the treatment of major depressive disorder. The variability in response to different antidepressants in these models, however, suggests that there may be potentially different substrates and neurochemical pathways mediating performance in these tests. These issues may underlie the observed behavioral differences between the two tests in the current study.

The antidepressant-like activity of the crude and solvent fractions of the extract observed in the present study might be attributed to the presence of phytoconstituents. Previous studies have reported that *C. benghalensis* contained phytoconstituents like alkaloids, flavonoids, coumarins, triterpenoids, steroids, resins, carbohydrates, phenols, tannins, amino acids, quinones, oils and fats, saponins, salicylic acid, chlorogenic acid, 8-hydroxyquinoline, caffeic acid, quinol, resorcinol, catechol, anthocyanin, beta-amyrin, lutein, zeaxanthin, violaxanthin, carotenoids, neutraceuticals such as vitamin C, proteins, calcium, iron, and wax (Ghosh *et al*., 2019; Kansagara & Pandya, 2019; Pradesh *et al*., 2014; Gebrekiristos, 2012).

Many scientific studies have reported that phytoconstituents found in the plant extract are responsible for antidepressant-like activity. Accordingly, in this study, the antidepressant-like activity of the crude and solvent fractions of the leaf extract of *C. benghalensis* might be due to the presence of flavonoids. Flavonoids such as quercetin and kaempferol are reported to possess antidepressant-like activity. By blocking monoamine oxidase A and catechol-O-methyltransferase, preventing monoamines from being absorbed and so raising their levels, and interacting with free radicals, flavonoids have been shown to shorten the period of immobility in an animal model of depression (El-hamid & Bous, 2019; Gowthami, 2021; Tesfaye *et al*., 2020).

The presence of tannins might be responsible for the antidepressant-like activity of both the crude and solvent fractions of the *C. benghalensis* leaf extract. By concurrently altering the HPA axis, monoaminergic responses, and oxidative processes during persistent mild stress, tannins have been demonstrated to reduce mice's immobility.

In addition, it’s possible that the antidepressant-like properties of the *C. benghalensis* leaf extract's solvent and crude fractions stem from the presence of alkaloids. Alkaloids have been shown to have antidepressant-like activity by reducing immobility behavior in different depression models. Through increased monoaminergic turnover, interaction with the serotonergic system, stimulation of the HPA axis through the serotonergic system, and enhancement of p-CREB/CREB and BDNF in the frontal cortex and hippocampus, alkaloids have been demonstrated to shorten the duration of immobility in rodents (Hsu *et al*., 2012; Sun *et al*., 2022).

Moreover, the antidepressant-like activity of the crude and solvent fractions of the extract might be due to the presence of saponins in this extract. Studies have revealed a connection between saponins and the elimination of excess free radicals, the control of monoamine neurotransmitters, and the reduction of MAO (Ali, 2022).

To rule out any possible non-specific motor activation, the OFT model was used. This test assessed the overall locomotor activity of the plant by counting the number of square crossings. Unlike psycho stimulant drugs, antidepressants do not improve motor function. Therefore, it is imperative to ascertain the test result to exclude any nonspecific action of this plant. Consequently, the *C. benghalensis* extract didn't considerably change the mice's spontaneous locomotor activity during the OFT, demonstrating that at the studied doses.

6. CONCLUSION AND RECOMMENDATION

The results of this investigation indicate that there is a considerable antidepressant-like effect in both crude and solvent fraction extracts of *C. benghalensis* leaves. This is demonstrated by the reduction in immobility time in validated TST and FST-based depression models. Furthermore, the OFT shows that the plant has no discernible impact on locomotor activity, indicating that non-specific motor stimulation is not the source of the observed antidepressant-like action.

As the current study demonstrated noteworthy antidepressant activity, it warrants further study. These studies include an evaluation of the long-term safety, sub-acute and chronic toxicity, effectiveness of chronic and alternative depression models, and potential modes of action.

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