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Antidepressant-Like Effects of *Lavandula angustifolia Mill* (Lamiaceae) Aqueous and Total Crude Extracts in Wistar Albino Rats

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Background: Depression continues to be a serious mental health problem among communities in Uganda, with limited access to mental healthcare services. Communities often use medicinal plants, such as *L. angustifolia*, in the management of depressive disorders with limited information on its effectiveness.

Objective: Study assessed antidepressant-like effects of stem-leaf aqueous and total crude extracts of *L. angustifolia* in depression-like induced behavior in Wistar albino rats.

Methods: An experimental laboratory study was conducted on 36 Wistar albino rats (18 males, 18 females). Group I received normal saline, Group II received 10 mg/kg bwt escitalopram, Group III received 200 mg/kg bwt, Group IV received 1000 mg/kg bwt aqueous extract and same doses of total crude extract were used for Group V and Group VI, respectively, using intragastric tube. Depression-like behavior in rats was induced by several manipulations of CUS for 1–5 weeks. Sucrose preference test (SPT) was used to confirm depressive-like behaviors. Antidepressant-like effects were determined by FST. Durations of immobility, swimming, and struggling were recorded. Data were analyzed using STATA version 13.

Results: In the chronic mild stress group, 19.2% preferred sucrose compared to 66.9% in the unstressed group ($p<0.05$). *L. angustifolia* extract (*LAE*) exhibited antidepressant-like effects in the rats in a completely dose dependent manner at aqueous doses of 200 mg/kg bwt and 1000 mg/kg bwt, respectively. In the FST, dose of 200 mg/kg bwt and 1000 mg/kg bwt of the extract showed a significant reduction in mean immobility time of 1.33±0.52 min and 1.83±1.17 min ($p<0.0001$) as compared to 1.00±0.00 min for escitalopram drug and 3.17±0.41 min of the normal saline control groups.

Conclusion: Aqueous extract of *L. angustifolia* at a dose of 200 and 1000 mg/Kg bwt reduced the duration of immobility and similar findings were observed on struggling and swimming. Findings have provided evidence on the use of *L. angustifolia* by local communities in the management of depressive-like behaviors in Uganda.

Keywords: antidepressant effects, chronic unpredictable stress, sucrose preference test, forced swimming test, lavender, aqueous extract, total crude extract

Introduction

Depressive disorders continue to be a serious mental health issue globally,[1–5] including in Uganda,[5] and often lead to suicide attempts and death.[3] Globally, approximately 970 million people live with different forms of mental disorder, with anxiety and depressive disorders being rampant.[2,3] Of these, approximately 280 million live with depressive disorders, including 23 million children and adolescents.[2,3] Among individuals with depression, 5.0% are adults, 5.7% are older than 60 years, 10% are pregnant women or those who have just delivered, 4% are men, and 6% are women,[1–3] . Depression is the fourth leading cause of death among those aged 15–29 years, with the majority (77.0%) occurring in low- and middle-income countries in 2019.[1–3]

In Uganda, depression is a common problem, and the country is ranked among the top six countries in Africa with a high burden of depression. According to a systematic review and meta-analysis ofthe prevalence of depression, the

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burden of depression was 30.2%, which was higher during the COVID-19 pandemic.[6] The burden of depression is reported to be higher among refugees (67.7%), war victims (36.0%), individuals living with HIV (28.2%), pregnant or postpartum women (26.9%), university students (26.9%), children and adolescents (23.6%), and caregivers of patients (18.5%).[6] In another study, by Kaggwa et al,[6] the prevalence of depression in Uganda among university students was reported to range between 4.0 and 80.7%, with past 2 weeks suicidal ideation reported to be 13.9%. Depression is a complex, multifactorial, heterogeneous, and often chronic mental disorder.[7] Depression causes severe social and economic burdens globally.[8] Depressive disorders occur as a result of imbalances in various neurotransmitters in the brain, especially biogenic amines, including dopamine, serotonin, and norepinephrine.[9] The dysfunction of serotonergic and neuroendocrinological pathways due to chronic stressful environment exposure can lead to development of depressive illness,[10] and various clinical studies have shown that, the hyperactivity levels of the hypothalamic pituitary-adrenal (HPA) axis raise the cortisol levels thereby promoting hypersecretion of corticotropin releasing factor (CRF), which is a major regulator of the hypothalamic pituitary adrenal (HPA) axis and so dysregulation in the (CRF) leads to the development of depression. Furthermore, disturbances and reduction of major neurotransmitters including serotonin, dopamine, and norepinephrine in the brain, especially in the amygdala, hippocampus, and dorsomedial thalamus, which are commonly involved in behavioral modulation, lead to the development of depressive illness in predisposed individuals.[11–13] Depressive mood disorders have been defined as decreased quality of life, decreased energy, loss of pleasure or interest, feelings of guilt, disturbed sleep, inability to concentrate, disturbed sleep, low self-worth, depressed mood and/or anhedonia, changes in appetite, fatigue, and suicide.[14] Depression is considered a major risk factor for many other diseases, including cardiovascular, metabolic, and neuropsychiatric disorders.[13,15] Likewise, depression impairs cognitive function, attention, and memory even after symptoms are in remission.[16,17]

In Uganda, economic hardships, persistent poverty, unemployment, chronic diseases, poor mental healthcare services, and chronic stress have exacerbated this problem. Furthermore, the burden of depressive disorders is further exacerbated by limited mental healthcare services and lack of effective medicines. Antidepressant drugs are used in the treatment of depressive disorders, by enhancing the monoamine neurotransmitter levels including 5-hydroxytryptamine (5-HT), dopamine (DA), and norepinephrine (NE) in the brain. However, where they are accessible, adverse drug reactions associated with them, including anticholinergic effects, gastrointestinal effects such as nausea, vomiting and constipation, orthostatic hypotension, arrhythmias, weight gain, and sexual dysfunction remain a challenge and affects the medication adherence rate.[18] As a consequence of these challenges, affected people in various communities in Uganda seek alternative forms of treatment for depression, including spiritual and visiting elderly people in communities or herbalists who commonly utilize medicinal plants such as *Lavandula angustifolia* as supplementary and alternative medicines to manage depressive illnesses. *L. angustifolia* contains various phytochemicals including polyphenols, alkaloids, saponins, anthraquinones, flavonoids, tannins, sterols, and cardiac glycosides that may have antidepressant -like effects.[19–26]

In addition, *L. angustifolia* has been reported to contain essential oils including linalyl-acetate (30–55%), linalool (20–35%), beta-ocimene, cineol, camphor (0.5%), 1, 8 cineole (<1.8%), ß- ocimene, usually, both cis and trans isomers (6–16% total for both isomers), terpinen-4-ol (2–6%), sesquiterpene, caryophyllene oxide, tannin, rosmarinic acid derivatives, coumarin, and flavonoids; and some of these compounds may have effects on the brain neurotransmitters including serotonin and dopamine.[19–25,27] Previous studies on different medicinal plants have reported that various phytochemical compounds present in them have anxiolytic and antidepressant effects including alkaloids, flavonoids, phenolic acids, ligonanscinnamtes, terpenes, and saponins in different animal models.[8,19–25] Flavonoids in *L. angustifolia* have been reported to have an inhibitory effect on monoamine oxidases (MAO-A) enzymes that breakdown the dopamine, serotonin and norepinephrine neurotransmitters in the brain.[28] Furthermore, natural flavonoids compounds such as luteolin (2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-chromenone) may have effects on modulating GABA signaling in the brain and thus may play a role as antidepressant, antinociceptive, and anxiolytic-like effects.[29] Many medicinal plants are common herbs that have been used by various communities in the management of depressive illnesses,[30] particularly in low- and middle-income countries, including Uganda. Medicinal plants or herbs have long been a key component of the African traditional medicine (ATM) system, where they are key to managing various diseases or ill health, including mental health illnesses.[31–33] However, there is limited scientific information on the effectiveness of *L. angustifolia* in the management of depressive disorders in various communities in Uganda. Therefore, the present

study assessed the antidepressant-like effects of stem-leaf aqueous and crude extracts of *L. angustifolia* on depression-like induced behavior in Wistar albino rats. So, herein, the antidepressant-like effects of *LAE* were carried out to explore more about its use as herbal medicine by the communities in Uganda in the management of depression on the wistar albino rats models. Furthermore, the *L. angustifolia* extracts were utilized to compare their effects on the immobility period (minutes) using the forced swim test (FST) comparing it with escitalopram medicine commonly used in the management of depressive disorders.[25]

Materials and Methods

Study Design and Setting

An experimental laboratory study was conducted at the Department of Pharmacology and Therapeutics Laboratory at Makerere University College of Health Sciences (MakCHS) in Kampala, Uganda.

Collection and Identification of the Medicinal Plant

The plant's fresh stems and leaves were collected during the dry month of February 27/2021 from Kabarole rain forests in Kabarole District in Western Uganda at the Coordinates: 00 36N,30 18E (Latitude: 0.6000; Longitude: 30.3000) with the collection number of 01122la.j.0. The plant grows well in the sandy soils of the district. The plant samples were kept at the Pharmacology teaching laboratory. The plant was subjected to taxonomic authentication process. It was identified by Dr. Mary Namaganda, a botanist at Makerere University Herbarium, and the voucher specimen of the plant was was prepared at the Herbarium with a voucher accessory number MHU 51240.

Medicinal Plant Preparation and Processing

L. angustifolia (*Lavender*) stem leaves were dried in the shade at room temperature in the Pharmacology teaching laboratory for several days until a constant weight was attained. They were then pounded using a metallic motor and pestle to produce *L. angustifolia* powder.

Serial Extraction of Medicinal Plant

Five hundred grams (500 g) of *L. angustifolia* (lavender) powder were weighed using an electrical weighing scale (Mettler PJ 3000, Mettler-Toledo GmbH, Ockerweg, Germany), and serially extracted. First, it was soaked in 2.0 liters of 96% diethyl ether for three days with intermittent shaking to allow maximum extraction. Diethyl ether is a non-polar solvent and was used to extract hydrophobic phytochemical compounds in the plant and was used in the preparation of the total crude extract. The resultant mixture was filtered using Whatman® number 1 filter paper (Cat No.1001 125, White International Ltd, England), and the filtrate was stored at room temperature. The residue was air-dried for an hour and then soaked in 1.5 liters of methanol solvent and the process was repeated like for the diethyl ether. The methanol solvent was used to extract most of the polar compounds in the plant, but there are some which are not extracted and hence the use of the aqueous as asolvent. Finally, for aqueous extraction, 1.0 liter of distilled water was added (extract hydrophilic compounds in the plant) with a few mL of 99% pure ethanol to prevent fungal attack. Diethyl ether and methanol filtrates were concentrated using a rotary evaporator (CH-8230, BUCHI Rotavapor R-205 Labortechnik AG, Germany) at 50°C. Total crude extracts were obtained by mixing uniform amounts of diethyl ether, methanol, and aqueous extracts. The dry aqueous and total crude extracts used in the experiments were stored at 4°C until the experimental procedures were performed.

Preparation of Stock Solutions of Extracts and Control Drugs for the Experimental Studies

Stock solutions of both the aqueous and total crude extracts were prepared by dissolving 5.0 g of the extract in a few drops of 10% dimethyl sulfoxide (DMSO) to increase the extract solubility or reduce the hydrophobicity of the extracts. These were then topped up to 5.0 mL of distilled water to produce concentrations of 1000 mg/mL stock solutions, which were used to produce doses of 200 mg/mL of each of the total crude and aqueous extracts used in the experiments. The

acute toxicity study was carried out prior, and showed no toxicity of the *L. angustifolia* extracts up to the limited dose of 5000 mg/Kg bwt which would have been used to calculate the dose levels. Therefore in the present study, a low dose (200 mg/Kg) and high dose (1000 mg/Kg) as described by Ochola et al were used, and these doses have been used in previous studies.[33–35]

Escitalopram (First Pharmacy, Kampala, Uganda), at a dose of 10 mg/kg was used as a positive control. Escitalopram (10 mg, 10 tablets) was dissolved in 10 mL of water to produce a 10 mg/kg dose, which was administered to the animals. Different doses were administered orally using intragastric tubes (mg/kg bwt) to each animal in the different experimental groups. Normal saline (0.9%) was used as a negative control and 1.0 mL was administered orally to each rat in the control group. These doses were selected basing on the fact that in the acute toxicity, LD50 was below the limit dose and it was based on previous studies.[36]

Experimental Animal Selection and Consent to Participate

A total of 36 (18 males, 18 females) Wistar albino rats weighing 120–140 g and aged 6–8 weeks were purchased from Makerere University College of Veterinary Medicine, Animal Resources and Biosecurity (MAKCoVAB) animal house. They were kept separately in wooden cages (34 cm × 47 cm × 18 cm) with soft-wood peals as bedding. The animals were kept separate until the end of the experiment since the ovarian cycle of the female animals was not known. The cages were kept in the Pharmacology Teaching Laboratory of the Department of Pharmacology and Therapeutics, MAKCHS. The animals were housed under standard environmental conditions: a temperature of 20–24°C, light/dark cycle of 12/12 h, and a relative humidity of 65%. The animals had free access to water and commercial pellets purchased from Ngano Miller, Kampala, Uganda. They were acclimatized for at least one week before commencing the experiments. All experiments were performed in accordance with the Organization for Economic Cooperation and Development (OECD) guidelines 425[37] and in accordance with the accepted principles for laboratory animal use and care.[38] The rats were subjected to several manipulations to induce depression-like behavior, such as chronic unpredictable stress (CUS). Depression-like behavior in rats was measured using the forced swim test testing, whereby rats were forced to swim individually in a glass jar of 15 cm height containing fresh water.[39–41]

Group Treatment of Experimental Animals for Anti-Depressant Activity

A total of 36 Wistar albino rats of either sex weighing–140 g, aged 6–8 weeks, and disease-free were randomly selected from their separated cages and divided into six equal groups of the same sex, each with six animals as the minimum number recommended by international guidelines to obtain statistical differences (Table 1). This was based on OECD guideline 407, which recommends that a minimum of 6–10 animals per group should be used in experiments (Table 1).[38,42] Six groups, each comprising six animals (n=6, same sex), were used. Group I (negative control) received normal saline, Group II (positive control) received 10 mg/kg body weight of escitalopram, Group III received 200 mg/kg bwt of aqueous extract, Group IV received 1000 mg/kg bwt aqueous extract, Group V received 200 mg/kg bwt of total crude extract, and Group VI received 1000 mg/kg bwt of total crude extract (Table 1). Drug treatment was administered 60 min prior to behavioral testing, and food was withdrawn from the rats at 10 p.m.; however, they were allowed to consume tap water. These doses were selected based on the previous study by Enegide et al[36]

Table 1 Group Treatment for Antidepressant-Like Effects Study

Group Treatment	Dose (mg/kg bwt)	No of Animals per Group
I. Normal saline	2mL	6
II. Escitalopram	10	6
III. Aqueous leaf extract (AQ200)	200	6
IV. Aqueous leaf extract (AQ1000)	1000	6
V. Total crude leaf extract (TC200)	200	6
VI. Total crude leaf extract (TC1000)	1000	6
		Total number of rats=36

Induction of Depression-Like Behavior Using Chronic Unpredictable Stress (CUS)

Depression was induced using standard methods and procedures.[39–41] Depression-like behavior in rats was induced by subjecting them to several manipulations of chronic unpredictable stress (CUS) for 1–5 weeks. Rats were first housed in groups of six, placed in a cage tilted at an angle of 45° for 3 h, and then deprived of food (18 h) but allowed access to water. After the rats were deprived of water (18 h) they were allowed to eat food. After 24 h, the rats were exposed to an empty water bottle for 3 h.[39–41] They were then placed in a cage with wet bedding (with 300 mL of water at room temperature spilled in the bedding) for an 8-h night cycle and then exposed to continuous light (24 h). Thereafter, the rats were placed in a hot environment provided by a bulb connected with wires to the socket, to provide a temperature of approximately 40°C. The presence of depression-like behavior was confirmed using the sucrose preference test of Zeldetz et al.[40] The time for each step was modified according to how quickly the animals developed depression-like behavior to prevent them from suffering.

Sucrose Preference Test (SPT)

The inability of the animals to feel pleasure (anhedonia) or a sense of distaste when drinking sucrose solution (sweetened water) was evaluated using an SPT.[39–41] About 24.0 g of sucrose was dissolved in 1200 mL distilled water to obtain a 2% sucrose solution. Two bottles, one containing 200 mL 2% sucrose solution and the other 200 mL distilled water were placed in each treatment group cage. For the two-bottle sucrose preference test, all stressed and control rats were trained to drink a 2% sucrose solution during one-hour-long sessions for five days (from 10.00 am to 11.00 am) each day. The positions of the sucrose and distilled water bottles were changed during each training session to avoid any preference for one side. One week after the last training session, the chronic mild stress (CMS) period was started. At the end of each week of CMS, the rats were deprived of food and water for 20 h and SPT was assessed using standard methods and procedures.[40] The results of the SPT were calculated using the following formula: percentage (%) preference = [(mL of sucrose consumed/(total fluid intake = mL of sucrose consumed + mL of normal saline consumed) ×100] of the same treatment group.

Behavioral Testing Procedure

Forced Swim Test (FST)

Experiments were performed according to standard methods and procedures.[39–41] Despair behavior/helplessness is correlated with immobility exhibited by rats when placed in a swimming chamber, and this was classified as induced depression. The FST is a standard laboratory model for testing despair behavior in rats, which is considered equivalent to depression in humans.

Depression-like behavior was identified by forcing the animals to swim individually in a glass jar containing fresh water (15 cm height and maintained at 25°C). The rats were divided into six groups and pretreated with *L. angustifolia* extract (200 mg, 1000 mg, and total crude extracts of 200 mg and 1000 mg/kg, P.O.), 1 mL normal saline and escitalopram (10 mg/kg bwt, P. O). The 200 and 1000 mg/kg bwt doses were selected because, in the acute toxicity test, there was no death up to the limit of 5000 mg/kg bwt; therefore, previous studies recommended using a lower dose of 200 mg/kg bwt and a higher dose of 1000 mg/kg bwt,[36,38] They were then placed individually in polypropylene cylinders (height 25 cm, diameter 10 cm) containing 15 cm^3 of water at the beginning, maintained at 25°C, which was checked with a thermometer, and the depth of water was added according to the size of the rat; thus, it could not touch the bottom of the container with its hind legs. Each cylinder was labeled to ensure the ability to correctly identify the test subjects and to provide blinding to their treatment. The video camera, which was placed in front of the cylinder, was turned on, and the rat was placed in a water cylinder for 6 min. The camera was turned off, and the rat was removed from the container and placed in a transient drying cage with a heat lamp at a temperature of 40°C above it and a heat pad under it. The recording device was placed at least 30 cm above the cylinder using public domain software (J. Watcher TM, version 1.0; University of California, Los Angeles, USA, and Macquarie University, Sydney, Australia). Behavioral assessment was performed during the 6-min test period, according to standard methods and procedures.[39–41] Three different behaviors were rated: (1) immobility, defined as when rats remained passively floating in the water; this was

measured in time (minutes); (2) swimming, measured when rats were made active to swimming motions, more than necessary, and maintained their head above water and measured in time (minutes); and (3) climbing or struggling, measured in minutes when the rats made active movements in and out of the water with their forepaws, usually directed against the walls, and also measured in time (minutes). The duration of immobility, swimming, and climbing or struggling periods was recorded after administering the drugs to the groups.

Study Variables

- **Immobility**: Rats remained floating passively in the water, measured in time (minutes).
- **Climbing**: Rats were judged to be climbing when they made active movements in and out of the water with their forepaws, usually directed against the walls, measured in time (minutes).
- **Swimming**: Time spent swimming with forelimbs or hind limbs in a paddling fashion and more than necessary to maintain their head above water, measured in time (minutes).

Independent Variables

- Sucrose preference
- Forced swim test

Data Collection and Management

A database was created in a Microsoft Excel 2013 spreadsheet, and double entry of the data was conducted to minimize entry errors. Data were entered, cleaned, and exported to STATA version 13 for statistical analysis.

Statistical Data Analysis

Data was expressed as mean ± standard error of the mean (SEM) for each experimental group/treatment. Statistical analysis was performed using STATA version 13, and one-way analysis of variance (ANOVA) for dose-dependent comparison, as well as treatment comparison was used to determine significant differences between the means of the treatment groups followed by Bonferroni's multiple comparison post-hoc test between the control groups and different groups. The p-values, F-values and degree of freedom for multiple comparison between the control groups and the different treated groups were noted. Values of $p < 0.05$ was considered significant. Statistical significance was set at a 95% confidence interval.

Quality Control

All chemicals and reagents used in the experiment were of analytical grade and were checked to ensure that they did not expire before conduction of the experiment. Inbred rats of similar age groups in the same environment and on the same diet were used to reduce differences in metabolism.

The equipment used was well calibrated. Standard methods and procedures were followed, and the minimum number of animals in the study of six animals per group (cage) was adhered to. The animals used in this study were healthy, and the experiments were performed in replicates.

Ethical Consideration and Research Ethics

The study was approved by the School of Biomedical Sciences, Research and Ethics Committee (SBS-REC) and Institutional Review Board (IRB), clearance number SBS-2021-27, at Makerere University College of Health Sciences and by the School of Veterinary Medicine and Animal Resources (SVAR) Institutional Animal Care and Use Committee (SVR IACUC), clearance number SVR IACUC/79/2021, at the Makerere University College of Veterinary Medicine, Animal Resources, and Biosecurity. Experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals guide (eighth edition). Of the principles of the 3Rs (Replacement - *avoiding or replacing the use of animals in areas where they otherwise would have been used*; Reduction – *minimizing the number of animals used consistent with scientific*; and Refinement – *minimizing the pain, suffering, distress or lasting harm that research animals might experience*) in the humane animal research, two of them were taken into consideration: refinement and reduction in number of animals (rats) and fewer animals (6) per group were used since any number less

than 6 would not result in a statistically significant difference.[43] The five freedoms (5Fs) – from hunger, discomfort, pain and injury, fear and distress (by ensuring conditions and treatment that avoid mental suffering), and to express normal behavior – were also considered.[44] However, the study utilized the already established methods and procedures for studying antidepressant-like effects in animal models.

Results

Antidepressant-Like Effects of the Two Different Dose Extracts of *L.angustifolia* on Physical Manipulation-Induced Depression-Like Behavior in Wistar Albino Rats

A physical manipulation method using CUS was used to induce depression-like behavior and despair in Wistar albino rats. The SPT was used to confirm the occurrence of depression-like behavior. The swim test was performed to assess the antidepressant-like effects.

Effect of CUS and SPT on Depression-Like Behavior in Wistar Albino Rats

As the main index for evaluating anhedonia, the sucrose consumption of each group of rats was measured on day zero (baseline), day one and day two, three, four, and five post-chronic unpredictable stress. The percentages of sucrose and distilled water intake by each group during a two (2) day experiment were recorded and calculated. After a 5-day procedure, there was a significant reduction of sucrose preference in CMS rats compared to non-CMS rats. The percentage of sucrose preference in chronic mild stress group was 19.2% (24 mL sucrose consumed and 101 mL of normal saline consumed) by the same group treatment compared to 66.9% (98 mL of normal sa;line consumed and 198 mL of sucrose consumed of the same group treatment) of the unstressed groups ($p<0.05$). Rats that had diminished amount of consumed sucrose solution and sucrose intake (19.2%) were regarded as anhedonic ($p<0.05$) and rats that consumed much of the sucrose (66.9%) were regarded as non-anhedonic ($p<0.05$).

The decline in sucrose consumption in animals subjected to CMS reflects the inability to feel pleasure. The findings on the duration of immobility were reduced by 200 mg/Kg bwt (AQ200) dose with 1.33±0.52 mins and 1000 mg/Kg bwt (AQ1000) dose with 1.83±1.17 min aqueous extract comparable to the Escitalopram control drug with 1.00±0.00 min, while in addition the AQ200 also reduced the struggling time (2.83±0.98 min) and the swimming time (4.50±1.22 min) which were comparable to the control drug (Table 2 and Figure 1). The results showed that rats which received doses (1000 mg/kg bwt and 200 mg/kg bwt) of *L. angustifolia* aqueous extract orally had significant ($p<0.005$) reduction in immobility time (minutes) when compared to the control groups; Escitalopram (10 mg/kg bwt). However, while animals that received doses (1000 mg/kg bwt and 200 mg/kg bwt) total crude extract of *L. angustifolia* had a statistically significant ($p<0.05$) increase in mobility time when compared to the control groups ($p<0.05$). On struggling time, the results showed animals that received aqueous doses (1000 mg/kg, 200 mg/kg bwt) of *L. angustifolia* extract, had no

Table 2 Effects of Two Dose Treatment of *L. angustifolia* Aqueous and Total Crude Extracts on Duration of Immobility, Struggling, and Swimming Among Depressant-Like Induced Wistar Albino Rats

Drug/Extract	Dose (mg/kg bwt)	Mean Duration of Activity ±SEM (Minutes)		
		Immobility	Struggling	Swimming
TC1000	1000.0	4.17±0.75	7.83±1,47	7.00±0.89
TC200	200.0	2.83±0.41	6.67±0.52	6.17±0.75
AQ1000	1000.0	1.83±1.17	3.00±1.26	5.17±1.83
AQ200	200.0	1.33±0.52	2.83±0.98	4.50±1.22
Escitalopram	10.0	1.00±0.00	0.00±0.00	2.17±0.41
Normal saline	2 mL	3.17±0.41	3.67±0.52	4.33±0.52
ANOVA P-value		***p<0.0001***	***p<0.0001***	***p<0.0001***

Note: Bold values indicates the ANOVA p values showing statistical significance between treatment groups and within groups.

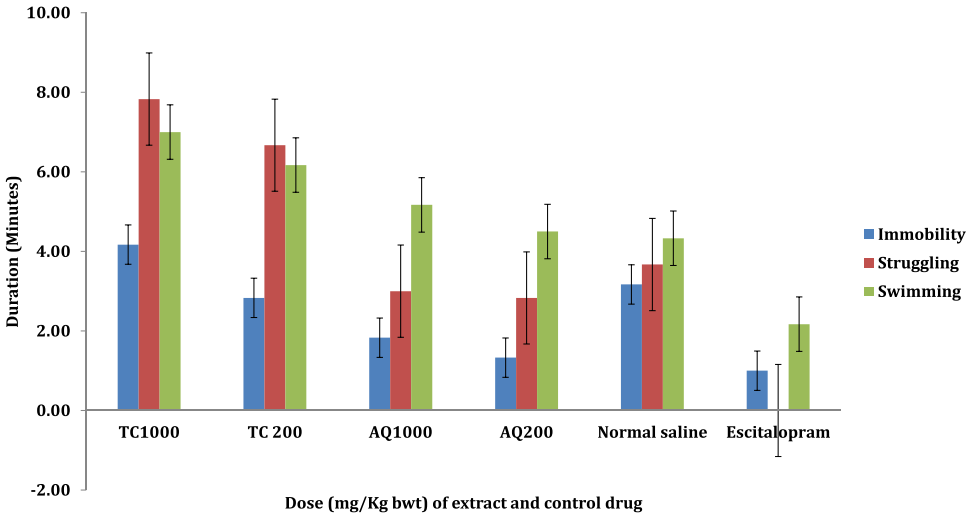

Figure 1 Effects of the extracts on duration of immobility, struggling and swimming among depressant-like behavior induced Wistar albino rats. An experimental laboratory based study was conducted on six treatment groups (TC1000, TC200, AQ1000, AQ200), 10.0 mg escitalopram and 2 mL normal saline using forced swim test (FST) behavioral method. Statistical analysis was performed using STATA version 13 and ANOVA was used to compare means between six groups and then Bonferroni's multiple comparison post-hoc test was used for binary comparisons of the different groups. Mean duration of immobility, struggling and swimming were statistically significant for all the six treatment groups ($p<0.0001$). TC1000 – 1000 mg/Kg bwt total crude extract, TC200 – 200 mg/Kg bwt total crude extract, AQ1000 – 1000 mg/Kg bwt aqueous extract, AQ200 – 200 mg/Kg bwt aqueous extract. Blue bar with an error bar represents mean duration of immobility, red bar with an errot bar for mean duration of struggling and green bar with an error bar for mean duration of swimming.

significant ($p<0.05$) difference in struggling time (minutes) when compared to the control groups. However, animals that received doses (1000 mg/kg, 200 mg/kg) total crude extract of *L. angustifolia* extracts, on the other hand, had a statistically significant increase in struggling time as compared to the control groups ($p<0.05$). On the other hand, findings showed that animals that received oral doses (1000 mg/kg, 200 mg/kg) aqueous extract of *L. angustifolia*, had statistically significant ($p<0.05$) difference in swimming time (minutes) when compared to the control groups. While animals that received total crude doses (1000 mg/kg, 200 mg/kg) extracts of *L. angustifolia* had statistically significant ($p<0.05$) increase in swimming time (minutes) as compared to control groups (Figure 1). The Post-hoc comparison test between different group treatments using the Bonferroni correction showed statistically significant difference in some group treatments when compared to the control drug ($p<0.0001$) (Table 3).

Discussion

The present study investigated the antidepressant-like effects and safety of aqueous and total crude leaf extracts of *L. angustifolia* in depression-like behavior induced in Wistar albino rats.

Table 3 Post-Hoc Comparison Using the Bonferroni Analysis for the Different Treatment Groups Dosed with Different Doses of the Extracts

Treatment Group	Mean Immobility Comparisons Between Different Treatment Groups df (5,30), F(20.79)	p_{bonf}
Escitalopram	AQ1000	0.019
	AQ200	<0.0001
TC1000	AQ1000	<0.0001
TC200	TC1000	0.019
AQ200	TC1000	<0.0001
	TC200	0.006
Normal videosaline	TC1000	<0.0001
	TC200	<0.0001
	Escitalopram	<0.0001

(*Continued*)

Table 3 (Continued).

Treatment Group	Mean Immobility Comparisons Between Different Treatment Groups df (5,30), F(20.79)	p_{bonf}
	Struggling, df (5,30), f(55.06)	
Escitalopram	TC1000	<0.0001
	TC200	<0.0001
Normal saline	TC1000	<0.0001
	TC200	<0.0001
	AQ1000	<0.0001
	AQ200	<0.0001
	Escitalopram	<0.0001
AQ1000	TC1000	<0.0001
	TC200	<0.0001
AQ200	TC1000	<0.0001
	TC200	<0.0001
	Swimming, df (5,30), F (15.16)	
Escitalopram	TC1000	0.002
AQ200	TC1000	0.004
Normal saline	TC1000	<0.0001
	TC200	<0.0001
	AQ1000	<0.0001
	AQ200	0.009
	Escitalopram	0.019

Effects of Chronic Unpredictable Stress (CUS) on Depression-Like Behavior Induced in Wistar Albino Rats on Sucrose Preference

The chronic unpredictable stress (CUS) was used to induce depression-like behavior in Wistar albino rats, and this was confirmed by sucrose preference test. The findings of CUS in Wistar albino rats showed that all the animals in the treatment groups were able to develop depression-like behavior. The occurrence of depression-like behavior was confirmed by the use of SPT. The findings showed that, rats that were subjected to chronic unpredictable stress had low sucrose intake ($p<0.05$) as compared to non-CMS rats. The percentage of sucrose preference in chronic mild stress group was 19.2% compared to 66.9% of the unstressed groups. Rats that had diminished the amount of consumed sucrose solution and sucrose intake (19.2%) were regarded as anhedonic and rats that consumed much of the sucrose (66.9%) were regarded as non-anhedonic. The decline in sucrose consumption in animals subjected to CMS reflects the inability to feel pleasure when compared to the controls. The findings were in agreement with the study done by Williams et al,[45] which indicated that a decreased consumption of sucrose solution after CMS or chronic social deficit exposure was a sign of anhedonia which is one of the signs of depression.[46]

Effects of Two Dose Treatment of L. angustifolia Aqueous and Total Crude Extracts on Depression-Like Behavior in Wistar Albino Rats During the Forced Swim Test Testing

In the present study, the antidepressant-like effects of *L. angustifolia* extracts was measured in rats on FST testing. The FST is one of the behavioral tests for evaluating the medicines and or herbals for their antidepressant-like effects.[47,48] The immobility in rats is reflected when rats attain the state of despair (lowered mood), rats surrendered the expectations of escaping away from the limited space describing to the depression in humans.[49] According to Abelaira et al,[50] antidepressant medicines have the effects on reducing immobility period in animal models.[50] In the present study, the *L. angustifolia* aqueous extracts administered to the rats at lower and higher doses significantly degraded the immobility time during the FST testing compared to the controls in a U-shaped dose-dependent manner. At the same dose, the aqueous extracts showed a significant decrease in immobility time (minutes) when compared to

same dose of total crude extracts indicating that the aqueous extracts of *L. angustifolia* showed more antidepressant-like effects just like the control group compared to the total crude extracts.[47] In this study, antidepressant-like effects of *LAE* in rats were demonstrated after acute treatment (60 mins prior the experiment). On the other hand, the efficacy of antidepressant drug like escitalopram in humans is evident after chronic treatment for 2–3 weeks, though its effective in the FST at doses of 10 mg/kg bwt. Thus, *LAE* at low doses of 200 mg/kg bwt after an acute treatment in FST, is more effective just like escitalopram and this might be a possible reason of *L. angustifolia* use in humans in the management of depression-like symptoms. Therefore, these findings suggest that *L. angustifolia* aqueous extracts have antidepressant-like effects in low and higher doses. The findings are in line with Kageyama et al,[47] Hritcu et al[51] and Seol et al[52] findings that reported that inhalation of dried aerial parts of *L. angustifolia Mill* essential oil vapor in treated rats, for controlled time period (60 minutes) prior to performing behavioral testing, significantly reduced the immobility time in FST.[51,52] Furthermore, studies have reported that *L. angustifolia* exhibits some positive therapeutic effects on depressed patients, by most importantly reducing the mean depression score.[53,54] Thus, the findings of the present study support the documented data about antidepressant-like properties of *L. angustifolia* extracts. More so, *L. angustifolia* has been reported to contain different phytochemicals including polyphenols, alkaloids, saponins, anthraquinones, tannins, flavonoids, tannins, sterols, and cardiac glycosides,[19,26,55,56] as well as essential oils including linalyl-acetate (30–55%), linalool (20–35%), beta-ocimene, cineol, camphor (0.5%), 1, 8 cineole (<1.8%), ß- ocimene, usually both cis and trans isomers (6–16% total for both isomers), terpinen-4-ol (2–6%), sesquiterpene, caryophyllene oxide, tannin, rosmarinic acid derivatives, coumarin and flavonoids;[20–25] and some of these compounds have been reported to have effects on the brain neurotransmitters such as the serotonin, dopamine and norepinephrine[19,27] and these neurotransmitters are implicated in the pathophysiology of depression in humans. Previous studies on different medicinal plants have reported that various phytochemical compounds present in them with anxiolytic and antidepressant-like effects including alkaloids, flavonoids, phenolic acids, ligonanscinnamtes, terpenes and saponins in different animal models.[19–25,55–57] Flavonoids found in *L. augustifolia* have been reported to have an inhibitory effect on monoamine oxidases (MAO-A) enzymes,[28] and this could be one of the mechanism by which its extracts in the present study were able to exhibit the anti-depressant like effects in the Wistar albino rats. Furthermore, natural flavonoids compounds such as luteolin (2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-chromenone) has been reported to modulate GABA signaling in the brain and hence useful as antidepressant, antinociceptive, and anxiolytic-like effects.[19,29] Furthermore, it has been reported that FST stress significantly increases the MAO-A activity which decreases the neurotransmitter levels in the rat's brain thus leading to depressive-like behaviors.[58] Thus, in the present study,*LAE* may have caused a decrease in the MAO-A activity in the rat's brain and this could possibly be due to the bioactive compounds like lanoline, polyphenols, alkaloids, saponins, and flavonoids that have been reported to be present in *LAE* extract and this is in harmony with previous studies.[19–25,59] According to Porsolt[60] study on the FST testing, the brain neurotransmitters levels such as glutamate, serotonin, and dopamine are the major elements interceding the immobility period reduction.[60] Therefore, data provided in this present study proved that the FST stress grossly degraded the neurotransmitter levels in the rat's brain and in line with the above study findings. The observed antidepressant-like effects in the present study show that the presence of polyphenols, alkaloids, saponins, and flavonoids in different extracts of *L. angustifolia* could possibly be responsible for the antidepressant-like effects of *L. angustifolia* extracts in the Wistar albino rats and hence it could be the possible reason why different communities in Uganda use *LA* in the management of depressive-like disorders. The major limitation of the study was that some animals fell sick, and they were treated with capsules of tetracycline 250 mg/kg bwt and they all recovered well. However, this caused some delay in the experiment from progressing as it was intended.

Conclusions

The findings have revealed that *L. angustifolia* extract exhibits antidepressant-like effects in the Wistar albino rats in a dose dependent manner. Besides, the 200 mg/kg bwt of the extract was found to have the highest effect on immobility, an important parameter for depressive-like behaviors in Wistar albino rats. The extract may have inhibited the activity of MAO-A in vivo in the rat's brain in a dose-dependent manner. Therefore, the results indicated that the antidepressant-like effects of *L. angustifolia* extracts in rat's immobility testing could be correlated to the inhibition of MAO activity in the

rat's brain, particularly MAO-A activity and regulation of the central neurochemical axis and HPA in response to FST testing induced stress. Therefore, this work recommends the use of *L. angustifolia* extracts as a botanical supplement for the management of depression-like symptoms by local communities since it is also used as a spice in tea.

Data Sharing Statement

All data materials are available and can be accessed.

Consent for Publication

All authors have consented to publication of the manuscript.

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Author Contributions

Regarding the work, all authors made a significant contribution to the work reported, right from the conception, study design, execution, acquisition of data, analysis and interpretation, critically reviewed the article, gave final approval of the version to be published, agreed on the journal to which the article is to be submitted, and also agreed to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest regarding the publication of this paper.

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