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COMPARATIVE ASSESSMENT OF *CITRUS LIMETTA* WITH IMIPRAMINE AND DIAZEPAM FOR DEPRESSION AND ANXIETY

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ABSTRACT
This study examines the antidepressant and anxiolytic effects of the ethanolic extract of Citrus limetta (EELC) in Swiss albino mice. The extract was obtained through the Soxhlet extraction technique and given orally at doses of 100, 200, and 300 mg/kg for a period of 14 days. The study was divided into different groups: a control group that received saline, a standard antidepressant group treated with imipramine (15 mg/kg), and a standard anxiolytic group treated with diazepam (1 mg/kg). To evaluate antidepressant activity, the forced swim test (FST) and tail suspension test (TST) were used. Meanwhile, the light–dark box, head dip, and marble burying tests were conducted to assess the anxiolytic effects. The findings revealed that oral administration of EELC led to a dose-dependent decrease in immobility time during both the FST and TST, with higher doses showing effects similar to imipramine. In the anxiety models, animals treated with EELC spent more time in the light compartment, performed a greater number of head dips, and showed a marked reduction in marble burying behavior, indicating significant anxiolytic activity. The results highlight Citrus limetta's potential as a natural alternative for the treatment of mood and anxiety disorders by showing that its ethanolic extract has both antidepressant and anxiolytic properties in mice.

Keywords: *Citrus limetta,* Depression,Anxiety, CNS depressant,Immobility.

1. Introduction
Depression and anxiety are among the most prevalent neuropsychiatric disorders, affecting more than 280 million people worldwide and ranking as leading contributors to global disability (World Health Organization, 2021). Although conventional pharmacological treatments—such as tricyclic antidepressants like imipramine and benzodiazepines like diazepam—are effective, they are often accompanied by drawbacks including sedation, dependency, and reduced long-term efficacy (Malhi & Mann, 2018; Baldwin et al., 2013). These limitations have sparked growing interest in plant-based therapies, which may offer safer alternatives with potential antidepressant and anxiolytic benefits.
Sweet lime (Citrus limetta, Rutaceae) is a commonly consumed fruit that also holds an important place in traditional medicine. Phytochemical investigations reveal that C. limetta is rich in flavonoids,

phenolic acids, vitamin C, and essential oils, all of which possess notable antioxidant and anti-inflammatory properties (Sharma et al., 2020; Kaur et al., 2014). Given that oxidative stress and neuroinflammation play key roles in the development of anxiety and depression, these bioactive compounds make C. limetta a particularly promising candidate for therapeutic exploration (Liu et al., 2017; Maes et al., 2011).

Rodent behavioral models are widely used to investigate the neuro-psychopharmacological effects of natural compounds. Among these, the Forced Swim Test (FST) and Tail Suspension Test (TST) are well-established for assessing antidepressant activity, where a reduction in immobility time is considered indicative of antidepressant-like effects (Porsolt et al., 1977; Cryan et al., 2005). For anxiety-related behaviors, the Marble Burying Test serves as a reliable model, as compounds like diazepam are known to decrease the number of marbles buried (Njung'e & Handley, 1991). Similarly, the Light–Dark Box and Head Dip (Hole-board) Test provide further insight into anxiolytic potential by examining exploratory behavior and responses to risk (Crawley & Goodwin, 1980; Takeda et al., 1998). Preliminary findings indicate that *Citrus limetta* may possess antidepressant-like properties. Singh et al. (2012) reported that the methanolic extract of *C. limetta* leaves significantly reduced immobility time in both the FST and TST, potentially through serotonergic modulation or antioxidant activity. Nevertheless, comprehensive investigations into the dose-dependent effects of the ethanolic extract of *C. limetta*, particularly in comparison with standard antidepressant (imipramine) and anxiolytic (diazepam) controls across a range of behavioral models, are still scarce.

This study aims to investigate the antidepressant and anxiolytic potential of the ethanolic extract derived from *Citrus limetta* juice in mice. Depression and anxiety are among the most prevalent neuropsychiatric disorders, often linked to altered neurotransmitter function, oxidative stress, and diminished neuroprotection. While conventional drugs such as imipramine and diazepam remain widely used, their long-term application is limited by side effects and safety concerns, highlighting the need for safer, plant-based alternatives. To address this, the present research evaluates the effects of *C. limetta* extract on depression- and anxiety-related behaviors using validated animal models, including the Forced Swim Test, Tail Suspension Test, Marble Burying Test, Light–Dark Box Test, and Head Dip Test. Imipramine and diazepam are employed as standard reference drugs for comparison. Ultimately, this work seeks to broaden the understanding of the bioactive compounds in *C. limetta* and their neurobehavioral effects, supporting the potential role of natural products as complementary or alternative strategies for managing mood and anxiety disorders..

2. Materials and Methods

To verify the authenticity of the plant material, fresh Citrus limetta (mosambi) fruits were procured from a nearby market and verified by a certified botanist. After carefully washing the fruits with distilled water, they were peeled and their juice extracted for additional processing. The ethanolic extract of C. limetta fruit juice was made by ethanol extraction of the freshly extracted juice. It was then concentrated under low pressure and kept in an airtight container at 4 °C until it was needed for additional experimental research.

Preparation of Extract

The Fresh fruits of *Citrus limetta* were collected, thoroughly washed, and peeled before juice extraction. The juice was filtered to remove pulp and suspended particles, then subjected to ethanol extraction using a Soxhlet apparatus. Ethanol was employed as the extraction solvent, and the process continued until the siphon tube ran clear, indicating completion of extraction. The resulting extract was concentrated under reduced pressure using a rotary evaporator, yielding the crude ethanolic extract of *C. limetta* juice. The extract was stored in airtight containers at 4 °C until further experimental use

Animals

Twenty-five healthy adult albino mice, with weights ranging from 18 to 25 g, were utilized for the study. The animals were kept in standard laboratory settings with a 12-hour light/dark schedule,

regulated temperature (22 ± 2 °C), and relative humidity (55 ± 10%). They received a standard pellet diet and unrestricted access to water. The mice were randomly assigned to five groups, with each group containing five animals (n = 5). All experimental methods were performed in full compliance with the ethical standards set by the institution for the treatment and use of laboratory animals.

Acute Toxicity Studies

In accordance with OECD Guideline 423, the acute oral toxicity of the ethanolic extract of Citrus limetta fruit juice was evaluated in mice. Healthy adult mice weighing between 18 and 25 g of either sex were chosen at random, fasted for the entire night prior to dosing, and given unlimited access to water. To look for any indications of toxicity or death, the extract was taken orally in progressively higher dosages up to 2000 mg/kg body weight. Following dosing, the animals were closely watched for the first four hours, then every 24 hours, and finally every day for 14 days. Parameters such as alterations in skin, fur, eyes, mucous membranes, general behavior, tremors, drooling, seizures, lethargy, sleep patterns, and mortality were systematically observed. No major behavioral issues or deaths were observed even at the maximum tested dose, indicating that the ethanolic extract of Citrus limetta is safe up to 2000 mg/kg of body weight. Consequently, one-tenth of this dosage was chosen as a safe threshold for future pharmacological assessments in the current investigation.

Induction of Depression and Anxiety in Mice

Instead of chemical induction, validated behavioral paradigms were employed to assess depression- and anxiety-like behaviors in mice. Anxiolytic activity was evaluated using the Marble Burying Test, Light–Dark Box Test, and Head Dip Test, while depressive-like behaviors were assessed through the Forced Swim Test (FST) and Tail Suspension Test (TST). These models are well-established for screening potential anxiolytic and antidepressant agents in rodents. To minimize stress-related artifacts, all animals were acclimatized to the laboratory environment prior to testing. Behavioral parameters, including immobility, swimming, struggling, exploratory activity, and time spent in light and dark zones, were carefully recorded as indicators of depression- and anxiety-related responses.

Experimental Design

The animals were randomly divided into five groups (n = 5 per group):

- Group I (Control): Received vehicle only.
- Group II (Standard – Imipramine): Administered imipramine at a dose of 15 mg/kg body weight (for antidepressant evaluation).
- Group III (Standard – Diazepam): Administered diazepam at a dose of 2 mg/kg body weight (for anxiolytic evaluation).
- Group IV (C. limetta 100 mg/kg): Administered ethanolic extract of *Citrus limetta* fruit juice at a dose of 100 mg/kg body weight.
- Group V (C. limetta 200 mg/kg): Administered ethanolic extract at a dose of 200 mg/kg body weight.
- Group VI (C. limetta 300 mg/kg): Administered ethanolic extract at a dose of 300 mg/kg body weight.

Behavioral assessments were conducted on the 1st and 14th days of treatment. Antidepressant activity was evaluated using the Forced Swim Test (FST) and Tail Suspension Test (TST), while anxiolytic activity was measured through the Marble Burying Test, Light–Dark Box Test, and Head Dip Test. For each animal, parameters such as immobility time, swimming, struggling, number of marbles buried, time spent in light and dark compartments, and frequency of head dips were carefully recorded.

Antidepressant Activity

Forced Swim Test

The FST was conducted in a plexiglass cylinder (46 cm × 20 cm) filled with water to a depth of 15 cm, maintained at 25 ± 1 °C. Mice received either imipramine (standard), extract, or control treatment

daily for 14 days, with the final dose administered 1 hour before testing. After a 1-hour acclimatization in the testing area, each mouse was placed in the water for 6 minutes. The first 2 minutes served as habituation, and immobility was recorded during the last 4 minutes using a stopwatch. Immobility was defined as the absence of limb movement except for minimal motions needed to keep balance and the head above water. Mobility time was calculated by subtracting immobility from 240 seconds. Animals were removed in the same order they were placed, dried with tissue paper to prevent hypothermia, and returned to their cages. Reduced immobility time was considered an indicator of antidepressant-like activity (Can et al., 2012; Kaur et al., 2019; Porsolt et al., 1978).

Tail Suspension Test:
First, the animals were transferred to the testing room and allowed to acclimatize for at least one hour. The experiments were conducted one hour after administration of the extract, standard drug, or control. Each mouse was individually suspended in a tail suspension apparatus made of plastic, measuring 55 cm in height, 60 cm in width, and 11.5 cm in depth. To prevent visual contact with other animals, each mouse was placed in a separate three-sided compartment. An aluminum hook was positioned at the top of the box, 50 cm above the floor, to suspend the animal by its tail, with approximately 1 cm of the tail secured using adhesive tape. A stopwatch was used to measure mobility and immobility behaviors during the 6-minute trial. All animals showed initial active movements during the first 2 minutes, followed by a 4-minute period during which immobility time was recorded. Mice were considered immobile when they remained passive and motionless.In this experiment, animals were pretreated for one hour with ethanolic extract of *Citrus limetta* (EECL) at doses of 100, 200, and 300 mg/kg. The control group received saline, while the standard group was administered Imipramine at 15 mg/kg. At the end of the test, adhesive tape was carefully removed from each animal's tail, and the mice were returned to their cages. After each use, the suspension apparatus was thoroughly cleaned with a sterilizing solution (Can et al., 2012; Kaur et al., 2019).

Anxiolytic Activity
Head Dip Method
The Head Dip Test is used to evaluate exploratory behavior and anxiety-like responses in rodents, based on their natural tendency to investigate holes in their environment. The apparatus, typically made of wood or Plexiglas, measures approximately 40 × 40 × 25 cm and features 8–10 evenly spaced holes (~3 cm in diameter) on the floor. Testing is conducted in a quiet, dimly lit room to minimize external disturbances (File & Wardill, 1975).
Each mouse is gently placed in the center of the apparatus and allowed to explore freely for 5 minutes. During this period, the number of head dips—defined as the mouse lowering its head into a hole so that its ears disappear below the surface—is recorded. Additional parameters, such as the duration of head dips and latency to the first head dip, may also be noted for detailed analysis. Between trials, the apparatus is cleaned with 70% ethanol to eliminate odor cues from previous animals. Increased head-dipping activity reflects reduced anxiety or enhanced exploratory behavior, whereas a decrease indicates heightened anxiety or reduced exploratory drive, potentially due to pharmacological or experimental interventions (Boissier & Simon, 1962).

Light And Dark Method
The Light–Dark Box Test is a widely used behavioral model for assessing anxiety-like behavior in mice, based on their natural conflict between avoidance of brightly lit areas and the tendency to explore novel environments. The apparatus consists of two connected compartments: one brightly illuminated and the other dark and enclosed. During testing, each mouse is placed in the light compartment and allowed to explore freely for 5 minutes. Key behavioral parameters recorded include time spent in the light area, number of transitions between compartments, and latency to enter the dark chamber. Anxiolytic agents typically increase both the time spent in the light compartment and the number of transitions, reflecting reduced anxiety. This test is sensitive to various pharmacological

interventions and is commonly used to screen central nervous system–active drugs and natural extracts (Crawley & Goodwin, 1980).

Marble Burying Method

The Marble Burying Test was used to assess anxiety- and compulsive-like behavior in mice. Each animal was placed individually in a clean plastic cage (40 × 28 × 15 cm) containing 5 cm of sawdust bedding. Twenty glass marbles (10 mm diameter) were evenly arranged in a 4 × 5 grid on the bedding surface. Mice were allowed to explore freely for 30 minutes, after which the number of marbles buried at least two-thirds was recorded as an index of anxiety-like or compulsive behavior. Testing was conducted in a quiet environment with controlled lighting and temperature, and the apparatus was cleaned between trials to remove olfactory cues. This test is widely used in psychopharmacology to evaluate the anxiolytic or antidepressant effects of compounds (Njung'e & Handley, 1991).

Statistical Analysis:

Behavioral data for each group (n = 5) were expressed as mean ± SEM. To assess differences among groups, one-way ANOVA was applied to the results from the Forced Swim Test, Tail Suspension Test, Open Field Test, Head Dip Test, Marble Burying Test, and Light–Dark Box Test. Dunnett's post hoc test was used to compare treatment groups with the control group. Statistical significance was set at $p < 0.05$. All analyses were performed using SPSS software (version 27) .

Results

The effects of the ethanolic extract of *Citrus limetta* (E.E.Cl)juice on antidepressant- and anxiolytic-like behaviors were assessed using the FST, TST, Marble Burying Test, Head Dip Test, and Light–Dark Box Test on Days 1 and 14. In the FST, the control group showed no notable change in immobility time (161.4 ± 2.41 on Day 1 vs. 165.2 ± 1.79 on Day 14). In contrast, the reference drug imipramine significantly reduced immobility from 160.0 ± 1.58 to 82.8 ± 1.79 ($P < 0.001$). Treatment with E.E.Cl at 100, 200, and 300 mg/kg produced a dose-dependent decrease in immobility, reaching 127.8 ± 1.92, 108.4 ± 1.95, and 93.2 ± 1.92, respectively, on Day 14, all statistically significant compared to control ($P < 0.01$).

Table 1: Effect of ethanolic extract of Citrus limetta juice on immobility time in the force swim test in mice.

Groups	Day 1	Day 14
Control	163.6 ± 2.30	165.2 ± 1.79
Standard	117.4 ± 1.82	82.8 ± 1.79
E.E. 100mg/kg	145.0 ± 1.58	127.8 ± 1.92
E.E 200mg/kg	133.8 ± 2.86	108.4 ± 1.95
E.E 300mg/kg	124.2 ± 1.92	93.2 ± 1.92

Values are expressed as mean ± SEM (n=5) one way ANOVA followed by Dunnett's test, $P<0.05$

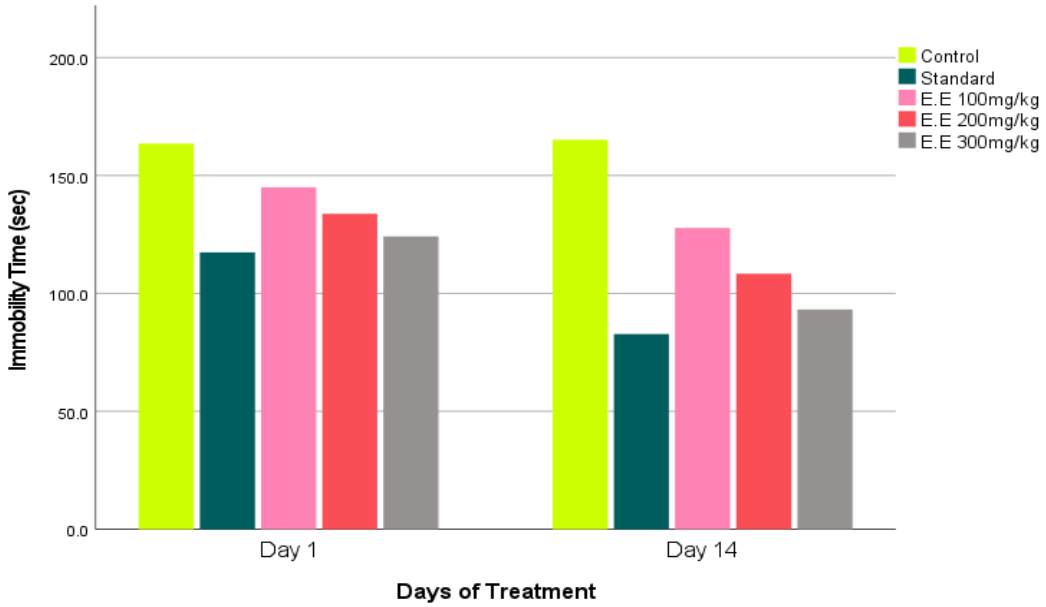

Figure 1: Effect of Citrus limetta juice extract on immobility time in forced swimming test in mice

Similar results were observed in the TST. The control group maintained high immobility (183.2 ± 1.92), whereas the standard drug significantly reduced it from 118.0 ± 1.58 to 97.0 ± 2.83 ($P < 0.001$). E.E. at 200 and 300 mg/kg also caused significant reductions (125.6 ± 2.70 and 99.0 ± 1.58, $P < 0.01$), indicating antidepressant-like effects.

Table 2: Effect of ethanolic extract of Citrus limetta juice on immobility time in the tail suspension test in mice.

Group	**Day 1**	**Day 14**
Control	183.2 ± 1.92	183.2 ± 1.92
Standard	118.0 ± 1.58	97.0 ± 2.83**
E.E 100 mg/kg	160.4 ± 1.82	144.8 ± 1.92*
E.E 200 mg/kg	139.8 ± 2.28	125.6 ± 2.70
E.E 300 mg/kg	130.4 ± 1.82	99.0 ± 1.58*

Values are expressed as mean ± SEM (n=5) one way ANOVA followed by Dunnett's test, $P<0.05$

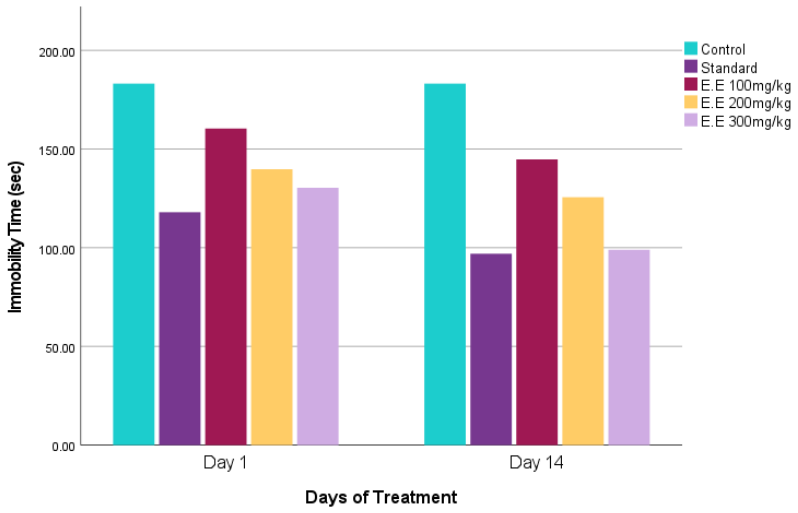

Figure 2: Effect of Citrus limetta juice extract on immobility time in tail suspension test in mice

In the Marble Burying Test, the control group buried 15.2 ± 0.84 marbles on Day 14, while diazepam markedly reduced this number to 4.0 ± 0.71 ($P < 0.001$). E.E.Cl demonstrated dose-dependent anxiolytic activity, decreasing marble burying to 9.8 ± 0.84, 7.2 ± 0.84, and 5.2 ± 0.84 at 100, 200, and 300 mg/kg, respectively ($P < 0.01$). In the Head Dip Test, control mice performed 10.8 ± 0.84 dips, while diazepam significantly enhanced exploratory behavior to 18.6 ± 0.84 ($P < 0.001$). E.E. at 100, 200, and 300 mg/kg also increased head-dipping behavior in a dose-dependent manner, confirming its anxiolytic potential. In the head dip test, the control group showed 10.8 ± 0.84 dips on Day 14, whereas diazepam significantly increased exploratory activity to 18.6 ± 0.84 ($P<0.001$). E.E.Cl at 100, 200, and 300 mg/kg increased head dips to 13.8 ± 0.55, 15.8 ± 0.84, and 17.4 ± 0.63, respectively ($P<0.05$ to $P<0.001$). In the light–dark test, the control group spent 235.8 ± 1.30 seconds in the dark, whereas diazepam significantly reduced this time to 217.6 ± 1.14 ($P<0.01$). E.E.Cl at 200 and 300 mg/kg also significantly reduced dark phase time to 223.6 ± 1.14 and 219.6 ± 1.14, respectively ($P<0.05$ to $P<0.01$), supporting anxiolytic activity. Overall, one-way ANOVA at a 0.05 level of significance confirmed that *Citrus limetta* extract exhibited dose-dependent antidepressant and anxiolytic effects comparable to a standard drug.

Table 3: Effect of ethanolic extract of Citrus limetta juice in the Marble Burying Test in mice.

Group	**Day 1**	**Day 14**
Control	14.8 ± 0.84	15.2 ± 0.84
Standard	7.2 ± 0.84	4.0 ± 0.71*
E.E 100 mg/kg	11.8 ± 0.84	9.8 ± 0.84
E.E 200 mg/kg	10.4 ± 0.89	7.2 ± 0.84*
E.E 300 mg/kg	8.8 ± 0.84	5.2 ± 0.84*

Values are expressed as mean ± SEM (n=5), one-way ANOVA followed by Dunnett's test, $P<0.05$.

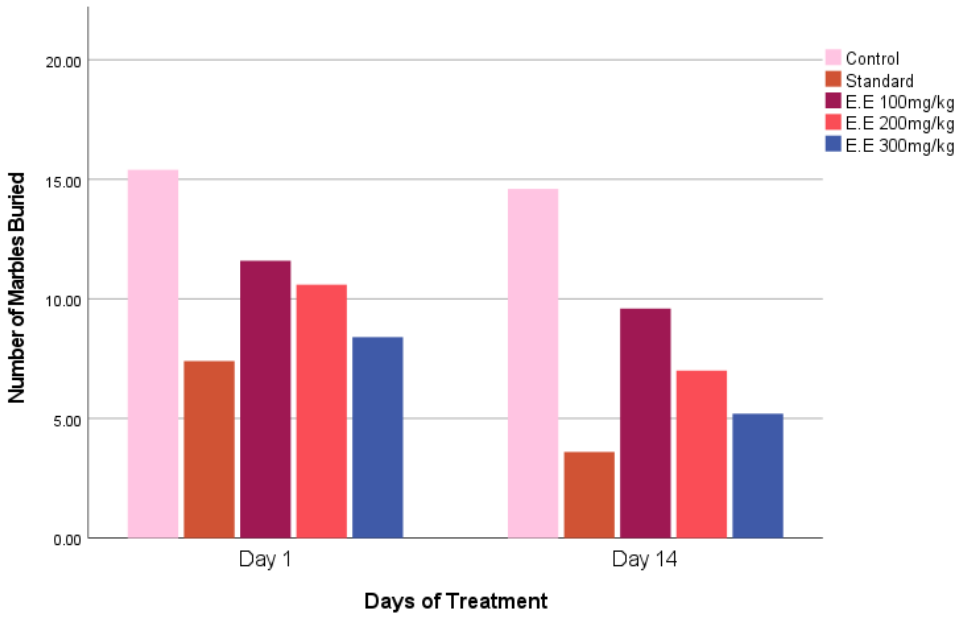

Figure 3: Effect of Citrus limetta juice extract on immobility time in marble burying test in mice.

In the Head Dip Test, control mice performed 10.8 ± 0.84 dips, while diazepam significantly enhanced exploratory behavior to 18.6 ± 0.84 ($P < 0.001$). E.E. at 100, 200, and 300 mg/kg also increased head-dipping behavior in a dose-dependent manner, confirming its anxiolytic potential.

Table 4: Effect of ethanolic extract of Citrus limetta juice on immobility time in the head dip test in mice

Groups	Day 1	Day 14
Control	9.2 ± 1.14	10.8 ± 0.84
Standard	10.6 ± 0.84	18.6 ± 0.84*
E.E. 100mg/kg	10.2 ± 1.14	13.8 ± 0.55*
E.E 200mg/kg	9.8 ± 0.84	15.8 ± 0.84
E.E 300mg/kg	8.8 ± 0.84	17.4 ± 0.63*

Values are expressed as mean ± SEM (n=5), one-way ANOVA followed by Dunnett's test, P<0.05 .

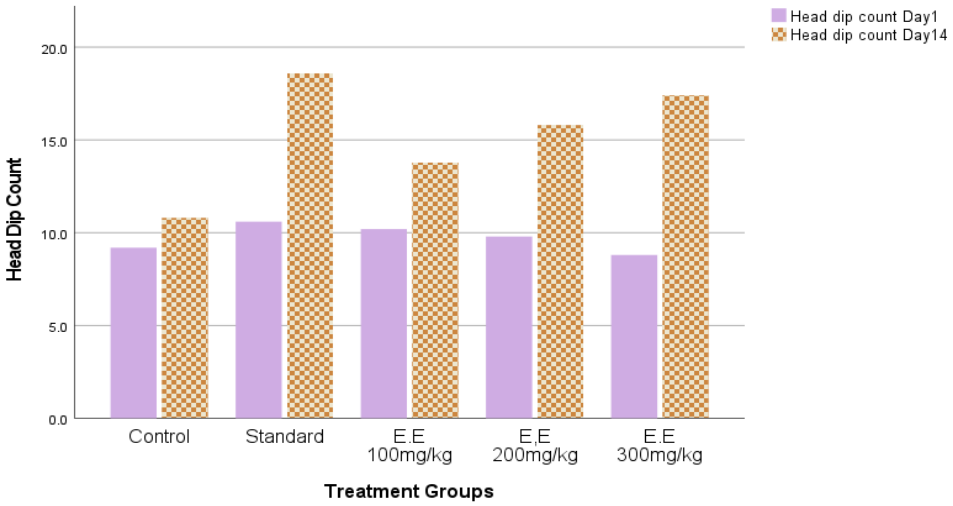

Figure 4: Effect of ethanolic extract of Citrus limetta juice on immobility time in the head dip test in mice

Control mice allocated 64.2 ± 2.14 seconds in the light section on Day 14. The treatment with Diazepam notably raised this to 142.6 ± 2.08 seconds (P < 0.001). E.E. at doses of 100, 200, and 300 mg/kg resulted in dose-dependent increases in time allocated to the light area, achieving 98.4 ± 1.92, 121.6 ± 2.10, and 136.8 ± 1.95 seconds, respectively (P < 0.01). Furthermore, E.E. treatment raised the frequency of transitions among compartments, suggesting improved exploratory behavior and decreased anxiety.

Table 5: Effect of ethanolic extract of Citrus limetta juice on time spent in the light compartment in the light/dark test in mice

Group	Day 1 (Light)	Day 14 (Light)
Control	62.4 ± 1.67	64.2 ± 1.30
Standard Drug	68.8 ± 1.30	82.4 ± 1.14 **
E.E 100 mg/kg	64.6 ± 1.14	72.6 ± 1.14 *
E.E 200 mg/kg	63.6 ± 1.14	76.4 ± 1.14 *
E.E 300 mg/kg	61.8 ± 1.30	80.4 ± 1.14 *

Values are expressed as mean ± SEM (n=5), one-way ANOVA followed by Dunnett's test, P<0.05.

Table 6::Effect of ethanolic extract of Citrus limetta juice on time spent in the dark compartment in the light/dark test in mice

Group	Day 1 – Dark	Day 14 – Dark
Control	237.6 ± 1.67	235.8 ± 1.30
Standard	231.2 ± 1.30	217.6 ± 1.14**
E.E 100 mg/kg	235.4 ± 1.14	227.4 ± 1.14
E.E 200 mg/kg	236.4 ± 1.14	223.6 ± 1.14*
E.E 300 mg/kg	238.2 ± 1.30	219.6 ± 1.14*

Values are expressed as mean ± SEM (n=5), one-way ANOVA followed by Dunnett's test, P<0.05 .

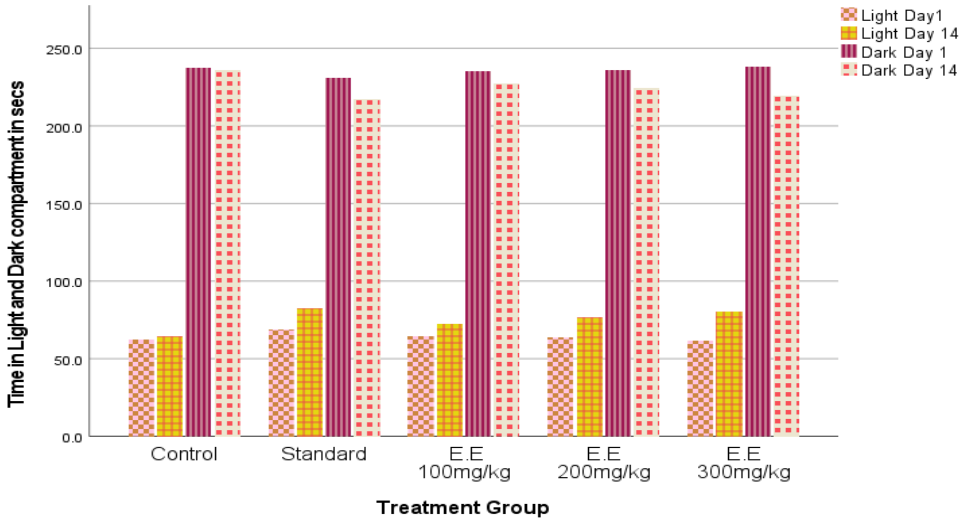

Figure 5:Effect of ethanolic extract of Citrus limetta juice on time spent in the light and dark compartment in the light/dark test in mice

3. Discussion

The present research examined the antidepressant and anxiolytic effects of ethanolic extract from Citrus limetta juice at doses of 100, 200, and 300 mg/kg in mice through various established behavioral models. The findings consistently showed dose-dependent impacts in both anxiety- and depression-related models, indicating that C. limetta has considerable psychotropic effects. Antidepressant-like effects were evaluated using the Forced Swim Test (FST) and Tail Suspension Test (TST), both acknowledged as dependable techniques for assessing depressive behavior in rodents. In these models, lack of movement is seen as a behavioral sign of hopelessness, and decreases in immobility duration suggest antidepressant effects. The administration of C. limetta extract notably decreased immobility duration for all doses tested, with the most pronounced effect seen at 300 mg/kg. This indicates that the extract improves stress-management strategies and promotes proactive behavioral reactions, akin to the effects seen with the conventional antidepressant, imipramine. The reduction in immobility based on dose suggests that bioactive compounds in the extract, likely flavonoids or limonoids, might influence central neurotransmitter systems, like serotonergic and noradrenergic pathways, which play a vital role in regulating mood.

Anxiolytic effects were evaluated using the Marble Burying Test, Light–Dark Box Test, and Head Dip Test. In the Marble Burying Test, a reduction in the number of buried marbles indicates decreased compulsive or anxiety-driven behavior. Treatment with *C. limetta* extract led to a significant dose-dependent decrease in marble burying, mirroring the anxiolytic effects of diazepam. Similarly, in the Light–Dark Box Test, the extract increased the time spent in the illuminated compartment, demonstrating reduced aversion and anxiety. The Head Dip Test further corroborated these findings,

as mice treated with the extract showed increased exploratory head-dipping behavior, reflecting decreased anxiety levels. Collectively, these results indicate that *C. limetta* exerts anxiolytic activity, likely by influencing GABAergic neurotransmission or modulating the balance between excitatory and inhibitory signals in the central nervous system .

In all behavioral assessments, the extract's effects were dose-dependent, with the 300 mg/kg dose showing the most significant antidepressant and anxiolytic effects. Although the effect size was slightly less than that of the conventional medications imipramine and diazepam, the extract resulted in notable behavioral alterations without any evident toxicity. This implies that C. limetta might provide a safer option or supplementary treatment for mood and anxiety disorders, especially in groups looking for plant-based solutions. The effects seen can be linked to the bioactive phytochemicals present in C. limetta, including flavonoids, limonoids, and vitamin C. Flavonoids are recognized for their ability to influence monoaminergic neurotransmission and boost neurotrophic factors, whereas limonoids demonstrate neuroprotective and anxiolytic properties in preclinical studies. The combined effects of these compounds might account for the shared antidepressant and anxiolytic effects observed in this research. The extract was well tolerated and produced dose-dependent behavioral improvements, suggesting its potential as a safe, natural alternative or adjunct therapy for depression and anxiety disorders. Further research is warranted to isolate active compounds, explore molecular mechanisms, and evaluate long-term efficacy and safety.

4. Conclusion

The findings of this study indicate that the ethanolic extract of *Citrus limetta* juice has notable antidepressant and anxiolytic effects in mice. Across multiple behavioral tests—such as the Forced Swim Test, Tail Suspension Test, Marble Burying Test, Light–Dark Box, and Head Dip Test—the extract significantly reduced depressive-like behavior, as reflected by decreased immobility time, and alleviated anxiety-like behavior, as seen by increased exploration and reduced compulsive actions. The effects were dose-dependent, with higher doses producing more pronounced improvements, and showed activity comparable to the standard drugs imipramine and diazepam. These results suggest that the bioactive compounds present in *C. limetta* may modulate neurotransmitter systems and enhance neuroprotection, contributing to its mood-regulating effects. Overall, the study supports the potential of *C. limetta* as a natural therapeutic option for managing depression and anxiety. However, further research is needed to isolate the active constituents, understand the precise mechanisms of action, and assess long-term safety and efficacy in both preclinical and clinical settings.

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