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ASSESSING THE ACUTE TOXICITY AND PSYCHOTROPIC PROPERTIES OF THE AQUEOUS EXTRACT FROM *LANNEA VELUTINA* A. RICH.

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Abstract:
People in Burkina Faso commonly use a great range of therapeutic plants as medicine for various health conditions, including neuropsychiatric disorders. Very few toxicological and psychotropic studies, however, are available on these plants, especially Lannea velutina. Hence, the current study was designed to determine the acute toxicity, anxiolytic, and antidepressant properties of the aqueous leaf extract derived from Lannea velutina A. Rich. in NMRI mice. The sample was macerated and then lyophilized. Its acute toxicity was measured using a threshold dose of 5000 mg/kg following OECD Guideline 423. Behavioral studies were conducted using the maze and open-field tests to assess anxiolytic activity and the caudal suspension test to examine antidepressant activity. Doses of 125, 250, and 500 mg/kg were chosen for every psychotropic test. Findings on acute toxicity revealed that the LD_{50} of the aqueous extract from Lannea velutinea leaves is higher than 5000 mg/kg body weight. Additionally, anxiolytic and antidepressant activity was detected at a dose of 125 mg/kg, although this became more significant at doses of 250 and 500 mg/kg. The time spent in immobility by treated mice declined by 76, 62, and 68 seconds, respectively, for doses of 125, 250 and 500 mg/kg as compared to the negative control (154 seconds), thereby confirming that the extract displays meaningful antidepressant activity. These outcomes imply that the extract's toxicity is low, with observable anxiolytic and antidepressant impacts on NMRI mice. Supplementary studies, including repeated-dose toxicity testing and research into the extract's operating mechanisms, are deemed essential.

Keywords: *Lannea velutina, acute toxicity, anxiolytic, antidepressant*

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INTRODUCTION:

The bioactive properties of medicinal plants provide significant applications in both traditional and modern medicine. As reported by the World Health Organization, they account for more than 80 percent of therapeutic treatments in sub-Saharan Africa due to their accessibility and availability to local populations.[1] Several species in Burkina Faso, belonging mainly to the Anacardiaceae family, serve as food and medicines for treating various disorders, ranging from neuropsychiatric conditions to chronic pain, insomnia, and anxiety.[2] One of these species, *Lannea velutina* A. Rich. (the same family with Anacardiaceae), deserves special mention by virtue of its frequent application by several ethnic groups in Burkina Faso.[3] Various parts of this plant, including the roots, bark, and leaves, undergo preparation as aqueous extracts and then administered orally, via inhalation, or in the form of baths to treat headaches, muscle spasms, agitation, and neuropathic pain.[4]

In Africa, people commonly use traditional medicines, drawing mainly on empirical knowledge inherited from one generation to the next within communities.[4,5] Based on observation, experience, and cultural traditions, this knowledge informs traditional healing practitioners and herbalists in their selection of medicinal plants as well as in methods of preparation and administration.[4,5] In the absence of stringent regulatory frameworks and systematic pharmacological validation, however, dosages typically are approximate and variable, varying according to the practitioner, pathology being treated, and the patient's specific clinical characteristics.[6,7] These non-standardized practices can result in discrepancies in therapeutic efficacy and safety, with risks of underdosing or overdosing.[6,7] To date, there have been relatively few rigorous scientific studies on *Lannea velutina* evaluating its toxicological and psychotropic properties. Hence, this study aims to assess the acute toxicity and psychotropic effects of aqueous extracts derived from *Lannea velutina* A. Rich when administered to NMRI laboratory mice.

1. Material and Methods

1.1. Plant Material

Dried *Lannea velutina* leaves were used as plant material. They were harvested in May 2026 and came from the Gonsé Protected Forest (rural municipality of Saaba) in the Central Region of Burkina Faso. They were botanically validated by a botanist from the Ziniaré University Center, with the geographic information for the collection site recorded at N 12°25.409' and W 001°18.962'.

1.2. Animal Subjects

Seven to eight-week-old NMRI strain mice were used in this study. To assess acute toxicity, female mice were preferred given their increased sensitivity to toxins as compared to males. However, when testing for psychotropic effects (anxiolytic and antidepressant effects), both sexes were used. The animals were allowed to acclimate for 7 days before the experiments began. They were housed in cages with free access to water and food. The experiments were conducted in accordance with the ethical guidelines for animal.

1.3. Chemicals and Pharmaceuticals

The following substances were used:

- Diazepam (10-mg tablet) and fluoxetine (20-mg capsule) served as positive controls for anxiolytic and antidepressant activity, respectively
- 70% ethanol was also used for extraction and cleaning of equipment after each rat was tested.

1.4. Acute Toxicity Assessment

The acute toxicity assessment was performed following OECD Guideline 423.[8] The dose of 5000 mg/kg was selected based on previous work by Kaboré et al.[9], which reported an LD_{50} higher than 2000 mg/kg. According to the OECD, when a 5000 mg/kg test is conducted, the study uses only three animals. Accordingly, the first mouse was administered the dose and showed no signs of severe toxicity after 14 days of observation. This prompted the continuation of the test with the other two (02) mice paired with the first, for a total of three (03) mice, as specified by the OECD. The subjects were observed for 30 minutes, 1 hour, 2 hours, 4 hours, 16 hours, 24 hours, and daily for 14 days in order to check for any signs of toxicity, such as tremors, convulsions, salivation, diarrhea, changes in skin and fur, somatomotor activity, and mortality.

1.5. Psychotropic effects assessment

The behavioral models and doses used are based on the work conducted by Somé et al.[10] , using NMRI mice under the same experimental conditions, where the distilled water, the reference molecules and the aqueous extract were administered orally.

1.5.1-Anxiolytic effect: The Elevated Plus Maze (EPM) test

The Elevated Plus Maze (EPM) is a behavioral model validated for assessing anxiolytic activity in rodents.[11] It consists in four arms forming a cross (two closed arms and two open arms) elevated approximately 50 cm above the floor.[12]

The time spent in the maze is 5 minutes, and the interval between drug administration and the test is 1 hour. After weighing, the mice were divided into five (05) groups as follows:

- Group 1: negative control group given distilled water;

• Group 2: positive control group received 1 mg/kg of diazepam;
• Groups 3, 4, and 5 were administered 125 mg/kg, 250 mg/kg, and 500 mg/kg of body weight of the extract, respectively.

Each mouse was positioned in the center of the maze, facing the closed arm, where it was given complete freedom to explore. The maze was cleaned with 70% ethyl alcohol before each test to remove any odors from waste that might affect the results. A mouse's entry is defined as the entry of all four feet, while exit is defined similarly for both closed and open arms.[13] Accordingly, a monitoring camera was mounted on a wooden pole 2 meters above the ground and overlooking the maze so that the entire maze could be recorded. This camera is connected to a computer to enable detailed viewing and interpretation of the videos. The recorded parameters include entrance count and time spent in the open arms.[14] A significant increase in these parameters compared to the negative control group is interpreted as an anxiolytic effect; whereas a decrease indicates an anxiogenic or sedative effect.[14] The completion of the experiment is contingent upon computing the mean for each group in order to compare the different groups.

1.5.2 Exploratory Activity: Open Field Test

As described by Hall[15], the Open Field Test has been used to assess exploratory behavior and anxiety-related emotional responses in rodents. The experimental arena is square and divided into 64 small squares, with 48 in the peripheral zones and 16 in the central zone. There are two parts: one containing the squares that rest on the floor, and the other, approximately 40 cm high, placed above the first so that all squares are visible. The experimental duration in the equipment is 5 minutes, and the interval between administration and testing is 1 hour. The mice were divided into five (05) groups:

• a negative control group that received distilled water;
• a positive control group that received 1 mg/kg of diazepam (reference compound);
• a group that received a dose of 125 mg/kg body weight of the extract;
• a group that received a dose of 250 mg/kg body weight;
• a final group that received a dose of 500 mg/kg body weight

Each mouse was positioned in the center of the arena and observed for 5 minutes. Between each pair (02) of mice, the arena was thoroughly cleaned with 70% alcohol to eliminate olfactory traces. A longer time spent in the central zone is interpreted as an anxiolytic effect. Upon viewing the recorded videos, the time spent in the central zone was computed for analysis and interpretation.

1.5.3 Antidepressant Effect: Tail Suspension Test (TST)

The Tail Suspension Test (TST) is a commonly used behavioral method for investigating the antidepressant activity of pharmacological substances.[16] The equipment is approximately 30 cm high and 15 cm deep, designed to restrict the animal's access to the external environment. The equipment contains a metal bar in the center to which the mouse is suspended after its tail has been secured with adhesive tape.
The mice were divided into 5 groups of 5 mice each:

• a negative control group received distilled water;
• a positive control group received 20 mg of fluoxetine;
• one group received a dose of 125 mg/kg body weight of the extract;
• one group received a dose of 250 mg/kg body weight;
• a final group received a dose of 500 mg/kg body weight.

Each mouse was hung by the tail using a non-invasive adhesive device placed approximately 1 cm from the caudal end, at a height of 60 cm above the floor. The total testing time was 5 min. A camera was installed in front of the mice to record their movements. If a significant reduction in the duration of immobility was observed in the treated groups compared to the negative control group, it meant that the extract had a potential antidepressant effect.[17]

2.3-Statistical Analysis

The data were entered into Excel and imported into GraphPad Prism for statistical analysis. Intergroup comparisons were performed using one-way ANOVA, followed by Dunnett's test to identify significant differences. The threshold for statistical relevance in our analysis was set at $p < 0.05$.

2. RESULTS:

2.1. Acute Toxicity

A single oral dose of 5000 mg/kg body weight of the aqueous extract from *Lannea velutina* administered to NMRI mice did not result in any signs of toxicity during the test period. Over the 14-day observation period, no mortality or changes in physical appearance were observed. These observations suggest that the LD_{50} of the aqueous extract of *L. velutina* is higher than 5000 mg/kg body weight.

2.2. Psychotropic Tests

2.2.1. Anxiolytic Tests

The purpose of this test is to study anxiety-related behavior in rodents. Preliminary results suggested that diazepam was efficacious compared to the negative control. These results reinforced our confidence in the quality of our reference compound.

2.2.1.1. Effects on the Number of Entries by Mice into the Open Arms of the Maze

Following observation of the recorded videos and counting of the number of entries into the open arms, a comparison was drawn between the various treatment groups and the negative control (Figure 1). An increase in the number of entries, with an average of 4 ± 0 entries in the Diazepam group, was observed compared to the negative control (1.8 ± 0.2 entries), indicating that diazepam increased the mice's exploration by encouraging them to enter the open arms more frequently, resulting in a drastic reduction in anxiety. Regarding the doses, a significant effect was also observed compared to the negative control. Specifically, the average number of entries was 2.8 ± 0.3, 3.8 ± 0.3, and 4.2 ± 0.3 for the 125, 250, and 500 mg/kg doses, respectively. These findings reveal that the 500 mg/kg dose of the extract was the most effective, with a mean value slightly surpassing that of Diazepam.

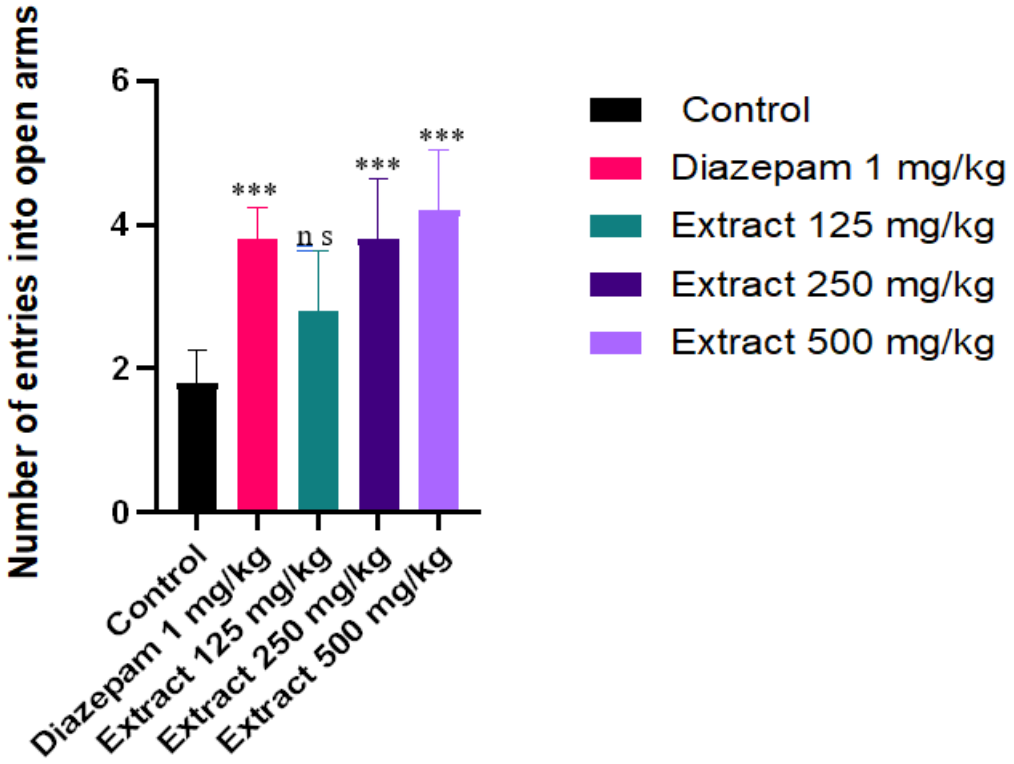

Figure 1: Effect of the aqueous extract of *Lannea velutina* on the number of entries into the open arms (n=5; ns = not significant; ***p<0.001).

2.2.1.2. Effect on time spent in the open arms of the maze

At a dose of 125 mg/kg, a significant difference ($p < 0.05$) was noted compared to mice that received distilled water. Compared to the negative control, the extract at doses of 250 and 500 mg/kg resulted in a highly significant increase ($p < 0.001$) in the time spent in the open arms (Figure 2). Diazepam also resulted in a marked reduction in anxiety by increasing the time spent in the open arms (from 13 ± 2.08 to 45 ± 1.2 s). All three (03) doses tested thus showed a reduction in anxiety compared to the negative control, with effects similar to those of Diazepam at a dose of 250 mg/kg.

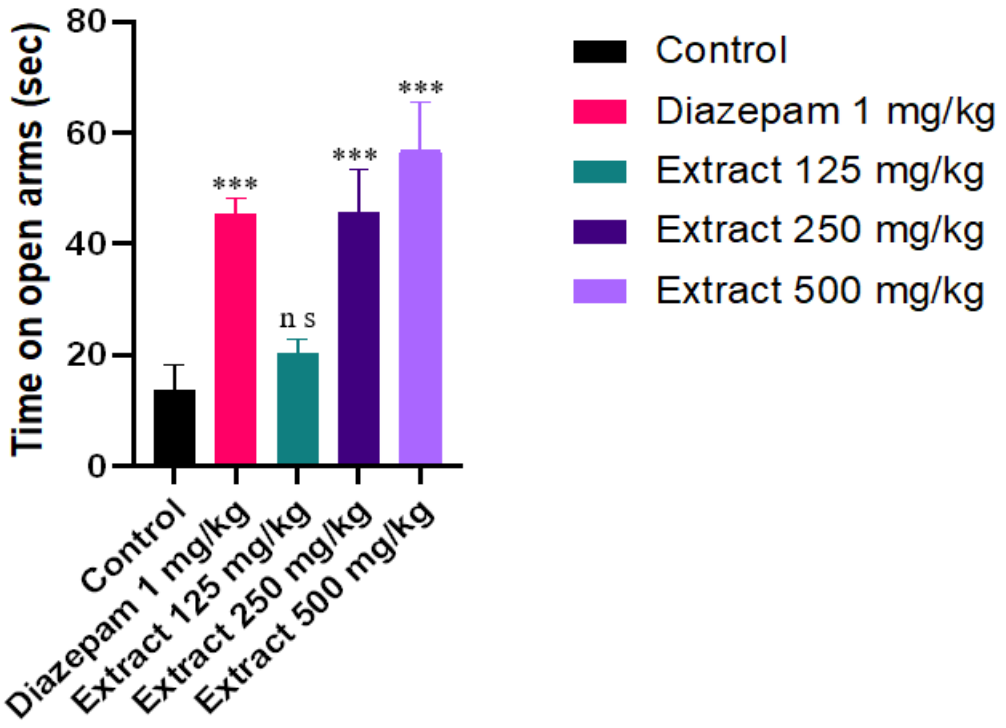

Figure 2: Effects of the aqueous extract of *Lannea velutina* on the time mice spent in the open arms of the maze (n=5; ns = not significant; ***p<0.001).

3.1.3.3. Anxiolytic effects of the aqueous extract in the open-field test

In this experiment, the time spent in the center of the arena was recorded. In comparison with the negative control, a statistically significant ($p < 0.001$) increase in time spent in the center was observed across the various administered doses, thereby confirming anxiolytic activity. Among the five (05) treated groups, the Diazepam -treated group had the highest mean, at 40.6 ± 2 seconds, followed by the group that received a dose of 500 mg/kg, at 34 ± 1.2 seconds (Figure 3).

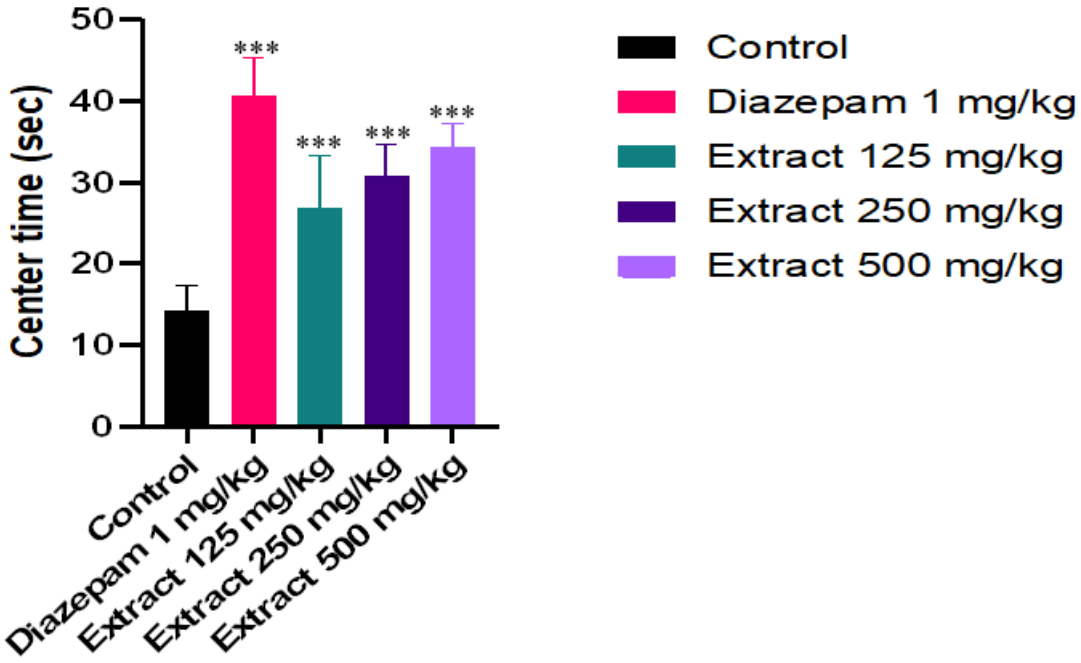

Figure 3: Effects of the aqueous extract of *Lannea velutina* on the time spent in the center of the arena

(n=5; ***p<0.001)

2.2.2. Antidepressant Effects

The aqueous extract resulted in a highly significant ($p < 0.001$) decrease in the immobility time of mice treated with fluoxetine at various doses compared to the negative control (Figure 4). Fluoxetine significantly decreased the immobility time, reducing it from 154 to 65.2 ± 7.5 seconds compared to the negative control. The 250 and 500 mg/kg doses produced comparable effects to those of fluoxetine.

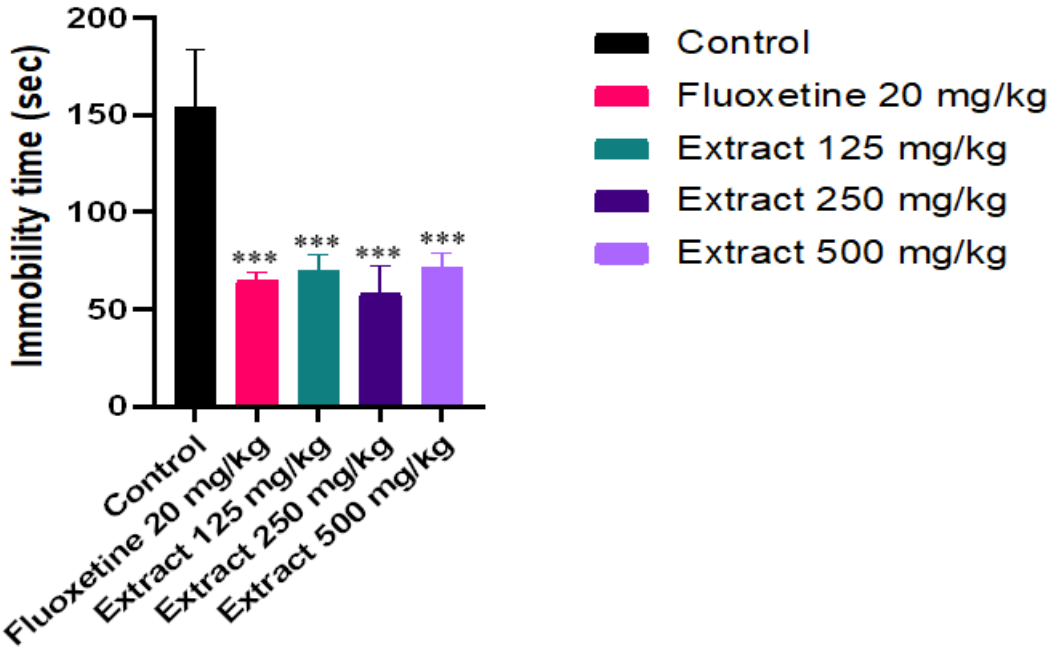

Figure 4: Effect of the aqueous extract of *Lannea vetutina* on the reduction of immobility time (n=5; ***p<0.001)

DISCUSSION:

The aim of this research was to determine the toxicological properties and psychotropic effects of the aqueous extract obtained from *L. velutina* in NMRI mice. Following administration of a single dose of 5000 mg/kg, neither toxicity nor mortality was observed during the two (2) weeks of treatment. Our findings are consistent with those reported by Kaboré et al.[9], who demonstrated that the LD_{50} of the aqueous extract of *L. velutina* bark is higher than 2000 mg/kg body weight. Likewise, Ouattara et al.[18] found a LD_{50} higher than 2000 mg/kg in NMRI mice treated with a methanolic extract of *L. coromandelica* leaves, a related species to *L. vetutina*. This suggests that the aqueous leaf extract from *L. vetutina* can be classified as Category 5 (low toxicity) in accordance with the OECD's Globally Harmonized System (GHS).[8]

The aqueous leaf extract from *L. vetutina* demonstrated anxiolytic and antidepressant effects compared to negative controls. Maze tests showed that all three (03) doses (125, 250, and 500 mg/kg) led to increased exploration of the open arms by the mice, as evidenced by both the number of entries and the time spent there. Similar effects were observed in the open-field test, with an increase in the time spent in the center. These results are consistent with those of Islam et al.[19], who observed an increase in the time spent in the open arms of the maze following administration of a methanolic extract of *L. coromandelica* leaves at doses of 100, 150, and 200 mg/kg in mice. The maze system capitalizes on the tension between the animal's natural tendency to explore its environment and its inborn instinct to avoid open and elevated spaces, which are perceived as dangerous.[20]

The antidepressant activity of the extract was assessed and a reduction in immobility time was observed at the various doses compared to the negative control group. These results support those of Adongo et al.[21], who reported a reduction in immobility time with the hydroethanolic extract of *Pseudospondia microcarpa*. They concluded that the reduction in immobility time was dose-dependent, in line with our findings at doses of 125, 250, and 500 mg/kg.

CONCLUSION:

Results from our study established the LD_{50} of *Lannea velutina* to be greater than 5000 mg/kg, thereby demonstrating the extract's safety in NMRI mice. The doses selected for evaluating psychotropic effects also showed that the aqueous extract of the plant possesses anxiolytic activity in

the maze and open-field tests, as evidenced by increased exploration of the open arms, and antidepressant activity in the caudal suspension test, indicated by a reduction in immobility time. These findings provide preliminary evidence regarding the toxicity and psychotropic activities (anxiolytic and antidepressant) of the extract. Further phytochemical studies are needed to identify the secondary metabolites responsible for the species' biological activities. The action mechanisms can also be explored to identify the processes by which the extract reduces anxiety or depression, and repeated-dose toxicity studies should be conducted.

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