Indonesian Journal of Global Health Research
Volume 8 Number 4, August 2026
e-ISSN 2715-1972; p-ISSN 2714-9749
https://jurnal2.globalhealthsciencegroup.com/index.php/IJGHR

ANTIDEPRESSANT POTENTIAL OF ETLINGERA CALOPHRYS IN MICE TAIL SUSPENSION TEST AND FORCED SWIM TEST

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ABSTRACT
Depression is a mood disorder characterized by feelings of sadness, loss of interest, and decreased activity, and is associated with neurotransmitter imbalance and oxidative stress. The use of conventional antidepressant drugs still has limitations so that alternatives from natural ingredients such as Etlingera calophrys are needed which are known to contain potential bioactive compounds. This study aims to determine the antidepressant potential of methanol extract of Etlingera calophrys rhizome in mice (Mus musculus) using the Tail Suspension Test (TST) and Forced Swim Test (FST) methods. This study used the Chronic Mild Stress (CMS) model in mice which were divided into several treatment groups, namely normal control, negative control, positive control (amitriptyline 25 mg/kgBW), and extracts with doses of 100, 200, and 300 mg/kgBW. The parameters observed were the duration of immobility in the TST and FST tests. Data were analyzed using One Way ANOVA followed by the Tukey HSD test. Phytochemical screening results showed the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids. In vivo tests showed that all doses of the extract were able to reduce the duration of immobility compared to the negative control, with a dose of 300 mg/kgBW providing the most significant effect and approaching the positive control ($p < 0.05$). The methanol extract of Etlingera calophrys rhizome has significant antidepressant activity, with a dose of 300 mg/kgBW being the most effective dose. These findings indicate that the methanolic extract of Etlingera calophrys rhizome exhibits significant antidepressant activity, which may be attributed to the synergistic effects of its bioactive compounds through modulation of monoaminergic neurotransmission and antioxidant mechanisms. In conclusion, Etlingera calophrys rhizome has promising potential as a natural antidepressant candidate, warranting further investigation at the molecular and clinical levels.

Keywords: antidepressant; etlingera calophrys; forced swim test; mice; phytochemicals; tail suspension test

How to cite (in APA style)
Berlian, N. K. A., Ruslin, R., Jabbar, A., Irnawati, I., Fristiohady, A., & Sahidin, S. (2026). Antidepressant Potential of Etlingera Calophrys in Mice Tail Suspension Test and Forced Swim Test. *Indonesian Journal of Global Health Research*, *8*(4), 839–846. https://doi.org/10.37287/ijghr.v8i4.1967.

INTRODUCTION

Depression is an emotional or mood disorder characterized by disappointment, hopelessness, guilt, and feelings of worthlessness, which can affect motivation to engage in daily activities. Several factors contribute to depression, including genetics, psychosocial factors, changes in appetite and weight, changes in sleep and activity patterns, feelings of guilt, impaired thinking and decision-making, and recurrent thoughts of death and suicide (Malhi & Mann, 2018).

Conventional antidepressant medications, such as selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs), have proven effective in some patients. However, these therapies still face several challenges, including a relatively slow onset of action, a high relapse rate, and side effects that can reduce patient compliance (Malhi & Mann, 2018; Cipriani et al., 2018). Therefore, it is necessary to explore new therapeutic alternatives, including those derived from natural resources, which have the potential to produce antidepressant drug candidates with faster mechanisms of action, minimal side effects, and greater efficacy.

Etlingera calophrys is a herb from the genus Etlingera in the Zingiberaceae family that is widely used in traditional medicine and includes various species with interesting pharmacological potential. This plant is traditionally used as a spice, vegetable, traditional medicine, and for various other purposes. In Southeast Sulawesi, Etlingera calophrys is commonly known locally as olae. Research on Etlingera calophrys also shows that this plant contains flavonoid and phenolic compounds known to have strong antioxidant activity, which indirectly plays a role in the antidepressant mechanism by reducing oxidative stress in the central nervous system. This antioxidant activity is supported by another study which reported that the fruit of Etlingera calophrys contains alkaloids, tannins, and phenolics with very high free radical scavenging capacity, which is relevant in protecting neurons from oxidative damage (Diah et al.,2025).

METHOD

This research was conducted at the Pharmaceutical Research Laboratory and Faculty of Pharmacy, Halu Oleo University, Kendari, Southeast Sulawesi. The tools used in this research include stirring rods, blenders, dark bottles, funnels, Erlenmeyer flasks, beakers, measuring cylinders, flasks, filter paper, volumetric flasks, ovens, droppers, tweezers, TLC plates, knives, rotary vacuum evaporators, horn spoons, test tubes, analytical balances, glass jars, and vials. The materials used in this research include aluminum foil, amitriptyline, distilled water, *Etlingera calophrys* rhizomes, ethyl acetate, filter paper, mice (Mus musculus), methanol, sodium-CMC, n-hexane, mouse feed, Dragendorff's reagent, Liebermann-Buchard's reagent, and magnesium powder.

Research Procedures

a. Plant Collection and Identification
   Etlingera calophrys rhizome samples were collected from Lakanaha Village, Wadaga District, West Muna Regency, Southeast Sulawesi, and then identified in the Laboratory of the Faculty of Pharmacy, Halu Oleo University to confirm species authenticity.
b. Sample Preparation
   The samples were washed (wet sorting), cut into small pieces, dried in the sun (dry sorting), then ground into powder and stored in a closed container protected from light.
c. Extraction
   Extraction was carried out using methanol as a solvent on 3 kg of the rhizome for 3 x 24 hours. The filtered filtrate was evaporated using a rotary vacuum evaporator to obtain a concentrated extract.

Preparation of the Test Preparation

The test preparation was prepared by weighing 0.5 grams of sodium carboxymethyl cellulose (Na CMC). Then, it was slowly dissolved in 10 mL of warm distilled water to dissolve all the Na CMC. The mixture was then transferred to a 100 mL volumetric flask and distilled water was added to the mark to obtain a 0.5% suspension.

Phytochemical Screening

a. Alkaloid
   E. Calophrys rhizome extract was added with 2 drops of Dragendorff's reagent. The presence of alkaloids is indicated by the formation of an orange, yellow, or brown precipitate (Harbone, 1998).
b. Tanin
   The rhizome extract from the *E. calophrys* plant was added with a few drops of 1% $FeCl_3$ reagent. A positive result was indicated by a color change from dark blue to greenish-black (Harbone, 1998).

c. Flavonoid
The rhizome extract from the *E. calophrys* plant was added with 2 mg of Mg powder and 3 drops of concentrated HCI. A positive test for flavonoids was indicated by the formation of a red, yellow, or orange color (Harbone, 1998).
d. Saponin
The rhizome extract from the *E. calophrys* plant was placed in a test tube and shaken vigorously. If the extract foamed, 1 drop of concentrated HCl was added. The extract was positive for saponins if foam formed 1-3 cm high and persisted for 15 minutes (Harbone, 1998).
e. Terpenoids
E.Calophrys rhizome extract was added with 3 drops of Liebermann-Buchhard reagent (glacial acetic acid and H^2SO^4(p)). A positive test for triterpenoids results in a red or purple color, and a positive test for steroids results in a green or blue color (Harbone, 1998).

Test Animal Selection Procedure
The experimental animals used in this study were mice (*Mus musculus*). These small mammals are often used in experiments because they have relatively uniform genetic traits, are easy to care for, reproduce quickly, and exhibit physiological responses quite similar to humans (Soewolo, 2010). Mice (*Mus musculus*) are small, white, and experience an estrus cycle every 4–5 days. They require a clean, dry, and quiet environment with a temperature of 18–19°C and a humidity of 30–70%. Adult female mice aged 35–60 days weigh 18–35 grams, have a lifespan of 1–3 years, and are ready to mate at eight weeks of age. The gestation period lasts approximately 19–20 days, with a litter size of 6–15 offspring weighing 0.5–1.5 grams (Soewolo, 2010).

Treatment of Mice
This study used the Chronic Mild Stress (CMS) method as a model for inducing depression in mice. This method involves alternating exposure to various mild stressors over two weeks with the goal of inducing a chronic stress state that mimics the symptoms of depression in humans. During the stress exposure period, the mice were exposed to situations that could affect their physiological and psychological state, such as changing light-dark cycles, predator sounds, and cage shaking. This is expected to induce behavioral changes reflecting depression, such as decreased appetite, weight loss, and increased passive behavior (Liu et al., 2015; Sun et al., 2013).

In-Vivo Pharmacology Testing
Tail Suspension Test (TST)
Antidepressant activity testing was conducted using the Tail Suspension Test (TST) to assess the passive behavioral responses of mice to stressful conditions after treatment. Prior to testing, each mouse was given the test extract orally, and observations were made 60 minutes after administration to ensure optimal absorption of the active ingredient. The mice's tails were suspended using a special adhesive at a distance of approximately 1 cm from the tip of the tail, with the body hanging freely from a horizontal bar approximately 60 cm above the table surface. Observations were conducted for 6 minutes, consisting of 2 minutes of adaptation and 4 minutes of effective observation. The main parameter measured was immobility time, the length of time the mouse did not actively move or climb to escape from the suspended position. A decrease in immobility time indicates increased antidepressant activity, as more active mice attempting to resist stressful situations indicate better psychomotor performance (Steru *et al*., 1985).

Forced Swimming Test (FST)
The Forced Swimming Test (FST) evaluates the behavior of test animals in dealing with the stress of forced swimming. Prior to the test, mice are given an oral test extract, and observations are made 60 minutes after administration to ensure the active ingredient reaches its

pharmacological effect.

In this test, each mouse is placed individually in a transparent cylinder filled with room temperature water (±25°C) and at a depth sufficient to prevent the animal from touching the bottom or jumping out. The total test duration is 6 minutes, with the first 2 minutes for adaptation and the final 4 minutes for effective observation. Parameters observed include immobility time and climbing time. Mice are considered immobilized if they only move their heads above the water surface. Mice are considered mobile if they actively swim and climb (Buccafusco, 2009).

Data Analysis

The research data were statistically analyzed using SPSS (Statistical Package for the Social Sciences) software. One-Way Analysis of Variance (ANOVA) was used to test for differences between treatment groups. After data processing and significant results ($p < 0.05$) were obtained, the analysis was continued with the Tukey HSD (Honestly Significant Difference) test to identify more specific differences between treatment groups.

RESULT

Phytochemical screening is the process of determining the class of compounds contained in a sample. Chemical compound screening is carried out using the tube method. The purpose of phytochemical screening is to provide an overview of the class of compounds contained in the plant being studied, such as flavonoids, terpenoids/triterpenoids, alkaloids, steroids, saponins, and tannins (Simaremare, 2014; Gultom and Hartika, 2019). The results of the phytochemical screening were as follows :

Table 1.
phytochemical screening of *Etlingera calophrys* Rhizome Extract

Phytochemical Screening	Result	Description
Alkaloid	(+)	Precipitate forms
Tanin	(+)	Golden yellow
Flavanoids	(+)	Yellow
Saponin	(+)	Presence of foam
Terpenoids	(+)	Greenish yellow

Table 2.
Mean Duration of Immobility TST and FST

Treatment	Immobility Time (Seconds) (Average ± SD)	
	TST	FST
Normal Control	0,1 ± 0,6	0,00 ± 0,00
Negative Control	258,57 ± 5,73	184,86 ± 249,07
Positive Control	11,45 ± 4,28	0,87 ± 2,5
E. Calophrys Dose 100 mg/KgBW	50,19 ± 7,15	5,60 ± 13,5
E. Calophrys Dose 200 mg/KgBW	33,31 ± 9,72	8,86 ± 13,9
E. Calophrys Dose 300 mg/KgBW	21,31 ± 10,28	1,66 ± 0,09

Statistical analysis using a one-way ANOVA showed a significant difference between treatment groups in the duration of immobility in mice ($p < 0.05$). A post-hoc test showed that the 300 mg/kgBW extract group showed a significant difference compared to the negative control and exhibited effects comparable to those of the positive control group.

DISCUSSION

The results of phytochemical screening showed that alkaloids were detected positively indicating their ability to modulate neurotransmitters such as serotonin and dopamine in the central nervous system (Cordell *et al*., 2001), tannins were also positive indicating strong antioxidant activity in counteracting oxidative stress that plays a role in the pathophysiology of depression (Maes *et al*., 2011), flavonoids were identified positively which are known to be able to increase monoamine

levels and BDNF expression thereby contributing to antidepressant effects (Kumar & Khanum, 2012; Pan et al., 2010), saponins showed positive results with the formation of foam related to neuroprotective effects and modulation of the serotonin system (Lacaille-Dubois & Wagner, 2000), and terpenoids were also positive which were reported to have neuromodulatory and antioxidant activity through interactions with the central nervous system (Mahato & Sen, 1997), so that the presence of these compounds as a whole supports the potential of the extract's antidepressant synergistically.

The Chronic Mild Stress (CMS) model is used to induce chronic stress in mice. This model is thought to replicate several aspects of the pathophysiology of depression in humans, particularly through activation of the hypothalamic-pituitary-adrenal (HPA) axis and increased stress hormone levels (Willner, 2016). Chronic stress is known to decrease serotonin and norepinephrine levels and increase oxidative stress in the brain, which plays a role in the development of depressive symptoms (Malhi & Mann, 2018)

In this study, antidepressant activity was tested on the methanol extract of *Etlingera calophrys* rhizome using three dosage variations: 100, 200, and 300 mg/kgBW. Tail Suspension Test (TST) and Forced Swimming Test (FST) results showed that all three doses reduced immobility duration compared to the negative control group, but the most significant effect was achieved at the 300 mg/kgBW dose. The reduction in immobility duration reflects increased motor activity and motivation in the mice, indicating an antidepressant effect (Porsolt *et al*., 1977). The results of this study indicate that the methanol extract of *Etlingera calophrys* rhizome has significant antidepressant activity in mice. The decrease in immobility time in the treatment group indicates increased motor activity and motivation to cope with stress, which are key indicators of an antidepressant effect (Porsolt *et al.,* 1977; Cryan *et al*., 2002). It can be seen in Table 2 that the administration of methanol extract of *Etlingera calophrys* rhizome at various doses showed a decrease in the average duration of immobility of mice in Tail Suspension Test (TST) and Forced Swimming Test (FST) compared to the negative control group, and this effect approached the activity of the positive control group given 25 mg/kgBW of amitriptyline.

The 300 mg/kgBW dose was considered the best because it produced the shortest immobility time in the TST and FST tests, demonstrating the strongest antidepressant effect in a dose-dependent manner. The decrease in immobility reflects an increase in antidepressant activity, consistent with behavioral testing principles reported in the literature (Cryan *et al*., 2005; Castagn *et al*., 2011)

The findings of this study demonstrate that the methanolic extract of *Etlingera calophrys* rhizome exhibits significant antidepressant activity, as evidenced by the reduction in immobility time in both the Tail Suspension Test (TST) and Forced Swim Test (FST). This effect is likely associated with the presence of secondary metabolites such as flavonoids, alkaloids, tannins, saponins, and terpenoids identified in the phytochemical screening. These classes of compounds have been widely reported to contribute to antidepressant effects through multiple biological mechanisms, including modulation of monoaminergic neurotransmission and reduction of oxidative stress in the central nervous system. Recent studies have shown that flavonoids and polyphenolic compounds can enhance serotonergic and dopaminergic signaling, inhibit monoamine oxidase (MAO) activity, and increase brain-derived neurotrophic factor (BDNF) expression, all of which are key pathways involved in the pathophysiology and treatment of depression. Furthermore, natural compounds with strong antioxidant properties are known to mitigate oxidative stress-induced neuronal damage, which plays a crucial role in depressive disorders (Salom & Cardoso, 2023) In addition, alkaloids and saponins have been reported to exert neuroprotective and neuromodulatory effects by interacting with neurotransmitter systems such as serotonin and dopamine, thereby enhancing mood regulation and stress response.

Therefore, the antidepressant activity observed in this study may result from a synergistic interaction of these bioactive compounds, supporting the potential of *Etlingera calophrys* rhizome as a natural antidepressant candidate.

CONCLUSION

The methanolic extract of Etlingera calophrys rhizome was confirmed to contain various bioactive secondary metabolites, including flavonoids, alkaloids, tannins, saponins, and terpenoids, which are known to possess important pharmacological activities. In vivo evaluation using the Tail Suspension Test (TST) and Forced Swim Test (FST) demonstrated that the extract significantly reduced immobility time in mice subjected to chronic mild stress, indicating notable antidepressant activity. Among the tested doses, 300 mg/kgBW showed the most pronounced effect, approaching that of the standard drug amitriptyline. The observed antidepressant activity is likely associated with the synergistic action of these phytochemical constituents through mechanisms involving modulation of monoaminergic neurotransmitters and reduction of oxidative stress. Therefore, Etlingera calophrys rhizome has strong potential to be developed as a natural antidepressant agent, although further studies, particularly at the molecular and clinical levels, are required to confirm its efficacy and safety.

ACKNOWLEDGEMENTS

The author would like to thanks for appreciation and gratitude to supervisor and all teaching staff who provided direction, guidance, and scientific support throughout the research process and the preparation of this scientific paper. Furthermore, they would like to express his gratitude to all parties who have assisted, both directly and indirectly, in ensuring the successful completion of this research.

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