BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 14, Number 1, April 2025 | Pages: 239-242 | DOI: 10.14421/biomedich.2025.141.239-242 ISSN 2540-9328 (online)

In Vivo Evaluation of Antidepressant Potential of Temulawak (*Curcuma xanthorriza* Robb.) Ethanol Extract in *Mus musculus*

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Manuscript received: 18 February, 2025. Revision accepted: 20 May, 2025. Published: 23 June, 2025.

Abstract

Temulawak (*Curcuma xanthorriza* Roxb.) is a medicinal plant from the Zingiberceae family widely used in Indonesia for its various bioactives, including antiviral, antibacterial, and hepatoprotective properties. This research aims to evaluate the antidepressant potential of *Curcuma xanthorriza* ethanol extract in *Mus musculus* through in vivo testing. The extraction used the maceration method with 96% ethanol as a solvent, yielding 5.2504% extract. The primary bioactive compounds identified in the extract were terpenoids and curcuminoids, which are known for their neuroprotective and antidepressant effects. The antidepressant activity was assessed using the tail-suspension test (TST) and forced-swimming test (FST), with immobility time as the main parameter. Amitriptyline, a standard antidepressant, was used as a positive control, while sodium carboxymethylcellulose served as a negative control. The results showed that increasing doses of *Curcuma xanthorriza* extract significantly reduced immobility time, indicating an antidepressant-like effect. Statistical analysis revealed that the immobility time in mice treated with a 650 mg/Kg BW dose of *Curcuma xanthorriza* extract was not significantly different from the amitriptyline group, suggesting comparable efficacy. These findings highlight the potential of *Curcuma xanthorriza* ethanol extract as a natural antidepressant, warranting further investigation into its mechanisms and clinical applications.

Keywords: Antidepressant; *Curcuma xanthorriza* Roxb.; Forced-swimming test Phytochemistry; Tails-suspension test.

INTRODUCTION

Temulawak (*Curcuma xanthorriza* Robb.) is one of the plants from the Zingiberaceae family. Temulawak has a very similar shape to turmeric. Java Island is the place where temulawak thrives. Indonesians tend to use temulawak as a food flavoring additive and herbal medicine. Based on research results, temulawak ethanol extract has many bioactivities such as antiviral, antibacterial, hepatoprotective, and other bioactivities (Syamsudin et al., 2019). The bioactivity possessed by temulawak ethanol extract is due to the presence of the chemical compounds it contains. Scientific studies showed that the most abundant essential phytochemicals obtained from the temulawak ethanol extract were terpenoids and curcuminoids (Zhang et al., 2014). Other contents possessed by temulawak ethanol extract are vanillin, 3-hydroxy-6-methylacetophenone, 12,13-epoxy-xanthorrhizol, 3-hydroxy-6-methylacetophenone, and dehydro-6-gingeridone (Zhang et al., 2015) as well as several amino acids such as methionine, phenylalanine, tryptophan, valine, alanine, lysine, and leucine (Rahmat et al., 2021).

Mental health has become a hotly debated issue, especially among young people. One of the mental health issues that can happen to anyone is depression. Depression is a mood disorder that causes a persistent feeling of sadness and loss of interest (Suma & Hasan, 2023). Depression is one of the leading causes of disability, and, if left unmanaged, it can increase the risk of suicide. An imbalance of neurotransmitters in the brain such as serotonin and dopamine is the cause of depression. Based on data from the Global Burden of Disease Study, by 2020 there will be at least 322 million people in the world suffering from depression (Charlie et al., 2024). The treatment of depression cases varies depending on the condition of the patient ranging from psychological assistance to the use of antidepressant drugs.

Antidepressants refer to psychotropic drugs that are used to treat mental illness with prominent emotional depression symptoms (Yunxi et al., 2023). The use of antidepressant drugs is closely monitored and regulated by the FDA. Antidepressants are the last resort for people with depression. Antidepressants work to relieve the symptoms experienced by people with depression such as

anxiety, increased fatigue, impaired concentration, and emotional outbursts. The mechanism of action of antidepressant drugs varies from inhibiting monoamine reuptake, inhibiting monoamine breakdown, and increasing serotonin reuptake and release of serotonin and norepinephrine (Chittaranjan & Sanjay, 2010).

MATERIAL AND METHODS

Tools and Materials

The tools used are laboratory glassware, stative, syringe, analytical balance, pestle and mortar, yarn, feeding tube, beaker glass, spatula, and rotary evaporator.

The ingredients used in this research were *Curcuma xanthorriza* Roxb. rhizomes, ethanol 96%, amitriptyline, water, and sodium carboxymethyl cellulose.

Procedures

Preparation and acclimatization of the test animals

Animal acclimatization was carried out for 7 days by giving the mice standard feed and drinking water ad libitum. Feed is given as much as 4 grams/head/day. The mice used for treatment were 48 mice (n=8 head/group). Mice were weighed and the drum was separated in each treatment. All research procedures were approved by the Ethics Committee of the Faculty of Mathematics and Natural Sciences, IPB University.

Preparation of Curcuma xanthorriza ethanol extract

The temulawak rhizome was cleaned, peeled, and sliced before being dried in an oven at 45ºC for 6-8 hours. After drying, the sample was ground into powder and the simplisia was filtered using an 80-mesh sieve to obtain softer temulawak powder, which was extracted using the maceration method. For the extraction process, 100 grams of temulawak powder was extracted using ethanol 96% for 24 hours. The macerate was filtered using Whatman filter paper. Subsequently, the extracted solution was separated from the solvent using a rotary evaporator 45ºC and 80 cmHg pressure to obtain a concentrated extract. The extract yield was calculated and stored in a vial bottle at -20ºC to 4ºC for measuring antidepressant bioactivity.

Antidepressant activity test

Antidepressant activity testing with the tail suspension test (TST) and forced swimming test (FST) method was performed based on the procedure of Castagne et al., 2010. Mice that have been acclimated for 14 days were randomly divided into 4 groups, as follows the following:

Table 1. Test animal grouping data.

Group name	Treatment
Normal	Untreated mice
Negative control	Sodium carboxymethylcellulose 0,5%
Positive control	Amitriptyline
Group I	Dose 325 mg/kg BW
Group II	Dose 650 mg/kg BW

Mice were placed in the experimental room at least 60 minutes before starting the experiment. The decoction and positive control were administered once a day. Antidepressant activity testing was performed 1 hour after oral administration of the treatment (Castagne et al., 2010). Immobility time was observed, namely the duration of time not moving (body, legs, and hands in a state of rest) for ≥ 1 second per mouse for 6 minutes (Ueno et al., 2022)

Data analysis

The immobility time data were used to calculate the percent of antidepressant activity of the ethanol extract of *Curcuma xanthorriza* against the positive control amitriptyline. statistical analysis using SPSS 29.0 for Windows. Values expressed as mean ± SD were then compared with a one-way ANOVA test followed by an LSD post hoc test to find out whether the differences between treatment groups if the data are significantly different (H_0 is rejected if the probability <0,05).

RESULTS AND DISCUSSION

Curcuma xanthorriza Roxb extract

Curcuma xanthorriza Roxb. extraction was done by the maceration method by soaking *Curcuma xanthorriza* Roxb. powder in 96% ethanol solvent for 24 hours. The ethanol solvent was chosen because it is a polar solvent and can dissolve polar compounds such as terpenoids and curcuminoids (Aisyah & Erminawati, 2022). Terpenoids and curcuminoids have been shown to have antidepressant bioactivity. The extraction process produced a paste of *Curcuma xanthorriza* extract. A total of 5.2504 grams of *Curcuma xanthorriza* extract paste was produced from 100 grams of *Curcuma xanthorriza* powder. The yield of *Curcuma xanthorriza* maceration extraction was 5.2504% as shown in Table 1. The yield of a extraction process is influenced by several factor such as temperature, solute and solvent ratio, target compounds to be extracted, and solvent purity (Abin et al., 2024). Terpenoid and curcuminoid isolates have been shown to have antidepressant bioactivity. The yield value of 5.2504% obtained in this research is not much different from the yield value of 5.42% obtained in the research of Lidvina et al., 2017.

Table 2. Calculation of yield from *Curcuma xanthorriza* extracts.

Weight of *C. xanthorriza* powder	Weight of extract	%Yield
100 grams	5.2504 grams	5,2504%

Antidepressant activity of *Curcuma xanthorriza* ethanol extract

Bioactivity testing was carried out by open tails-suspension test (TST) and forced-swimming test (FST) methods on each group of mice with the measurement

variable being immobility time. The antidepressant bioactivity of *Curcuma xanthorriza* ethanol extract can be described by the immobility time. The increase in immobility time is directly proportional to the antidepressant bioactivity. Stress in mice causes immobility in the mice's bodies, so a high immobility time indicates a high stress level. Amitriptyline was used as the positive control in this research. Amitriptyline was chosen as the positive control because amitriptyline has been commonly consumed as an antidepressant drug commonly used in the community. The mechanism of action of the drug amitriptyline is by inhibiting the reuptake of serotonin and norepinephrine at the pre-synapse cell membrane resulting in an increase in the concentration of serotonin and or norepinephrine in the central nervous system (Kim, 2017).

Based on Table 3, it can be seen through tails-suspension test (TST) and forced-swiming test (FST) untreated mice have the highest immobility time. Mice given amitriptyline have the lowest immobility time because the stress generated can be reduced by the presence of amitriptyline as an antidepressant. Sodium carboxymethylcellulose–was used as a negative control in this research. Sodium carboxymethylcellulose is commonly used as a suspending agent in drug preparations (Henny et al., 2019). Based on Table 3, it can be seen that increasing the dose of *Curcuma xanthorriza* decreases the immobility time so that it can be seen that there is linearity between increasing the dose of *Curcuma xanthorriza* extract and decreasing the immobility time. Based on the results of the least significant difference (LSD) test, it can be seen that there is no significant difference between amitriptyline and a dose of 650 mg/Kg BW of *Curcuma xanthorriza* extract.

Table 3. Antidepressant bioactivity testing result.

Group	Body weight (g)	TST Immobility Times (s)	FST Immobility Times (s)
Untreated mice	2.87 ± 0.35	155.43 ± 12.42	173.48 ± 12.42
Na-carboxy methyl cellulose	3.19 ± 0.58	135.00 ± 16.90	145.71 ± 11.58
Amitriptyline	2.31 ± 0.59	79.75 ± 21.02	75.57 ± 16.76
325 mg/Kg BW dose of *C. xanthorriza*	2.10 ± 0.29	102.75 ± 13.47	103.86 ± 14.16
650 mg/Kg BW dose of *C.xanthorriza*	2.04 ± 0.53	80.01 ± 17.47	77.81 ± 11.98

CONCLUSIONS

Ethanol can be used to extract *Curcuma xanthorriza* using the maceration method. Curcuminoids and terpenoids can be extracted using ethanol. The yield of *Curcuma xanthorriza* ethanol extract was 5.2504%. Antidepressant bioactivity testing can be done by tails-suspension test and also forced-swim test. The increase in *Curcuma xanthorriza* ethanol extract levels will be inversely proportional to the immobility time of the mice. Mice injected with amitriptyline had an immobility time in the tails-suspension test of 80.01±17.47s and in the forced-swimming test of 75.57±16.76s. The 650 mg/Kg dose has immobility time test result that are not significantly different from amitriptyline, namely in the tails-suspension test of 80.01±17.47 and 77.81±11.98 s in the forced-swimming test.

Acknowledgements: The author acknowledges the Department of Chemistry, Faculty of Mathematics and Natural Science, IPB University for providing material support for the research.

Authors' Contributions: Raja David Virgilius conceived the research design, conducted the antidepressant bioactivity testing, analyzed the data, and wrote the manuscript. Zest Lively Limbong dan Jericho L Ginting planted, cared for, and harvested *Curcuma xanthorriza* Robb. and conducted *Curcuma xanthorriza* Robb. extraction. All author read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no competing interests.

Funding: The author declares that this research is funded by the Ministry of education, culture, research, and higher education (Kemendikbud-ristekdikti RI) through the Program Kreativitas Mahasiswa (PKM) 2023 funding program.

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