

ANTIDEPRESSANT ACTIVITY TEST OF RED BETEL LEAF ETHANOL EXTRACT (*Piper crocatum*) ON MALE WHITE MICE (*Mus musculus*) USING THE FORCED SWIM TEST METHOD

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Abstract Red betel leaves (*Piper crocatum*) contain flavonoids, alkaloids, polyphenols, tannins, and which is known for its antidepressant properties. The purpose of this study was to determine the effect of antidepressant activity of ethanol extract of red betel leaves on male white mice (*Mus musculus*) orally. The method used in testing antidepressants is the Forced Swim Test. Fifteen mice were divided into five groups: a positive control group receiving 3.5 mg/kg BW amitriptyline, a negative control group, and three groups receiving 200, 400, and 800 mg/kg BW of the extract. The antidepressant effect was measured by observing the immobility of mice during swimming. The results of the study using paired sample t-Test analysis showed that there was a difference in immobility before and after, in the 400 mg/kg BW extract and 3.5 mg/kg BW amitriptyline dose groups had significant differences.

Keywords *Piper crocatum* • Antidepressants • Mice • Immobility Time • Forced Swim test.



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Introduction

Depression is a common and serious mental health disorder, is characterized by persistent sadness, loss of interest or pleasure in activities, and a range of associated emotional, cognitive, physical, and behavioral symptoms (Paykel, 2008). Depression affects over 280 million people worldwide, making it a leading cause of disability (Santomauro et al., 2021). The disorder is complex, involving genetic, biological, environmental, and psychological factors (American Psychiatric Association, 2022).

The determination of eugenol content in essential oils using Gas Chromatography-Mass Spectrometry (GC-MS) from red betel leaves showed an average eugenol content of 0.24 % (v/b) with main constituents were monoterpene (such as sabinene, mircene, alpha tuhjene, alpha terpinene, gamma terpinene) and sesquiterpene (such as trans caryophyllene and germacrene D) (Utami, 2017). Recent research has focused on the potential of natural compounds as alternative treatments for depression. Compounds such as eugenol, found in red betel leaves, have shown promise due to their antidepressant properties (Nisar et al., 2021; Lely et al., 2023). These natural substances offer a potential advantage over conventional antidepressants by reducing side effects and improving patient compliance. The exploration of plant-based treatments is gaining traction as researchers seek more holistic approaches to mental health (Pertiwi et al., 2024).



The Forced Swim Test (FST) is a widely used method for evaluating the antidepressant activity of compounds in animal models. This test involves placing rodents in a container of water and measuring their immobility time, which is indicative of depressive-like behavior (Unal & Canbeyli, 2019). The FST is favored for its cost-effectiveness, simplicity, and reliability, making it a standard in preclinical antidepressant research. Antidepressant treatments typically reduce immobility time, suggesting improved mood and motivation (Molendijk & de Kloet, 2019).

The findings from studies using the FST and natural compounds like eugenol highlight the potential for developing new, effective treatments for depression. As the global burden of depression continues to rise, there is an urgent need for innovative approaches that address the limitations of current therapies (Patel, 2012). Future research should focus on understanding the mechanisms of action of these natural compounds and conducting clinical trials to validate their efficacy and safety in humans (Shamabadi et al., 2023). This could pave the way for more accessible and holistic treatment options for those suffering from depression.

Materials and Methods

Materials

The equipment used in this study included a mesh sieve (mesh size 60, Endecotts), a maceration apparatus (IKA), beaker glass (Pyrex), measuring cylinders (Pyrex), Erlenmeyer flasks (Pyrex), a blender (Philips), mouse cages (Tecniplast), trays, oral gavage needles (Instech), glass jars, porcelain dishes, stirring rods, an analytical balance (Sartorius), aluminum foil, and a stopwatch (Casio).

The materials used were fresh red betel leaves (*Piper crocatum Ruiz & Pav*) collected from Samarinda, East Kalimantan, weighing 2.8 kg. A total of 200 g of dried powdered leaves (simplicia) were prepared. The mature red betel

leaves were dark green, taken from the 5th leaf upwards. Other materials included 70% ethanol (Merck), distilled water, sodium carboxymethyl cellulose (Na CMC, Sigma-Aldrich), and amitriptyline tablets (generic).

Animals

The test animals were male mice (*Mus musculus*) Swiss Webster, aged 2–3 months, weighing 20–40 g, obtained from the animal facility at the Faculty of Mathematics and Natural Sciences, Mulawarman University.

Methods

Plant Identification

Plant identification was performed before the study to confirm the species and authenticity of the plant material. Complete plant parts including flowers, leaves, stems, and roots were collected. Identification was carried out at the Plant Physiology Laboratory, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda. The plant used was confirmed as *Piper crocatum Ruiz & Pav*.

Preparation of Red Betel Leaf Powder

Mature red betel leaves (dark green, from the 5th leaf upwards) were collected from a single household garden in Samarinda to ensure sample uniformity. The fresh leaves were sorted, washed, air-dried for one week, then ground using a Philips blender. The powder was sieved through a mesh size 60 (Endecotts) to obtain a fine powder. The powder was stored in airtight containers to protect it from dust and contaminants.

Preparation of 70% Ethanol Extract of Red Betel Leaves

Two hundred grams of powdered red betel leaves were extracted by maceration using 1.5 liters of 70% ethanol (Merck) in a 1:10 ratio. The mixture was macerated with an IKA macerator for 3 hours, then soaked for 21 hours. The extract was

filtered using a Buchner funnel. The residue was re-macerated with 500 ml of 70% ethanol for another 3 hours and soaked for 21 hours, then filtered again. The combined filtrates were concentrated using a rotary evaporator (Buchi) at 30–40°C to prevent degradation of active compounds, resulting in a thick extract.

Preparation of Extract Suspensions

The thick red betel leaf extract was weighed according to the doses of 200 mg/kg, 400 mg/kg, and 800 mg/kg body weight. Each dose was suspended in 0.5% Na CMC (Sigma-Aldrich) and diluted to a final volume of 12.5 ml.

Animal Preparation

Mice were acclimated for one week before testing to adapt to the new environment, monitor health, and standardize feeding. The average body weight of mice used was 26.10 g.

Antidepressant Activity Test

The experiment involved five groups, each with three mice: negative control, positive control, and three treatment groups receiving 200, 400, and 800 mg/kg BW of extract. The Forced Swim Test (FST) was used to assess antidepressant activity. On day one, all mice were placed individually in a cylindrical glass container (diameter 10 cm, height 25 cm) filled with water to a depth of 15 cm for 6 minutes. Immobility time was recorded during the last 4 minutes (from minute 2 to 6). On day two, each group received their respective treatment. One hour after administration, the FST was repeated, and immobility time was recorded again for 4 minutes (Petit-Demouliere et al., 2005).

Data Analysis

The immobility time data were used to calculate the percentage of antidepressant activity using the formula: % Antidepressant Activity = [(Mean immobility time of control group – immobility

time of test group) / Mean immobility time of control group] × 100% (Costa et al., 2013).

Results and Discussion

Extraction of Red Betel Leaf Simplex

Fresh red betel leaves weighing 2.8 kg were processed during the orientation phase. From this amount, 650 g of dried red betel leaves were obtained, resulting in a drying loss of 23.22%. The thick extract obtained from maceration of 200 g of simplisia weighed 29.12 g, yielding an extractive value of 14.56%. The thick extract was dark green, oily, and had a characteristic spicy aromatic odor.

Antidepressant Activity Test of Red Betel Leaf Ethanol Extract

The immobility time data for each group before and after treatment are presented in table 1. The selection of 200, 400, and 800 mg/kg doses was based on preliminary studies and literature reports on plant extract dosing in FST models, which commonly use a range of doses to identify effective concentrations while avoiding toxicity (Chen et al., 2019). This dose range allows for observing dose-dependent effects and determining the most effective therapeutic dose. Statistical analysis that also conducted between immobility times before and after treatment in each group with showed a significant correlation, with a paired sample correlation p-value < 0.05. Paired sample t-tests indicated a significant decrease in immobility time after treatment in the positive control group (amitriptyline 3.5 mg/kg BW) and the 400 mg/kg BW extract group, demonstrating meaningful antidepressant activity. In contrast, the negative control group and the extract groups at 200 mg/kg BW and 800 mg/kg BW did not show significant differences in immobility time before and after treatment, indicating no significant antidepressant effect at these doses.

Table 1. Immobility Time Data of Red Betel Leaf Extract Before-After Treatment and Percentage of antidepressant activity

No	Group	Replication	Before (s)	After (s)	AA**** (%)
1	NC*	1	102	80	
		2	127	60	
		3	135	42	
		Average	121.33	60.67	
2	PC**	1	71	31	
		2	64	33	
		3	62	32	
		Average	65.67	32	47.26
3	EI ***	1	36	13	
		2	54	38	
		3	45	38	
		Average	45	29.67	51.10
4	EII ***	1	95	47	
		2	65	28	
		3	65	36	
		Average	75	37	39.01
5	EIII ***	1	92	23	
		2	110	30	
		3	65	42	
		Average	89	31.67	47.80

*Negative Control (NC),

**Positive Control (Amitriptyline), (PC), 3.5 mg/kg BW

***Ethanol extract (E) dose of red betel leaf 200, 400, and 800 mg/kgBW

****Percentage of antidepressant activity (AA)

Although there is limited literature directly reporting antidepressant effects of red betel leaf (*Piper crocatum*), several active compounds present in the plant, such as alkaloids, flavonoids, glycosides, saponins, and terpenoids, have been associated with antidepressant activity (Hartini & Nugroho, 2017; Li et al., 2019). Previous phytochemical analyses identified flavonoids, alkaloids, polyphenols, quinones, steroids, and monoterpenes in red betel leaf extracts (Garabadu et al., 2011; Bahramsoltani et al., 2015). The presence of eugenol, alkaloids, and terpenoids likely contributes to the observed antidepressant effects.

The Forced Swim Test (FST) was chosen as the method to evaluate antidepressant activity

because it is a widely accepted, reliable, and cost-effective behavioral assay for screening antidepressant compounds in animal (Petit-Demouliere et al., 2005). The FST measures immobility time, which reflects behavioral despair or depressive-like states. Antidepressant treatments typically reduce immobility time by increasing active escape behaviors, indicating improved mood and motivation (Detke et al., 1995). The FST has predictive validity for antidepressant efficacy and is commonly used in preclinical research.

Comparing our results with studies on other *Piperaceae* family plants, such as *Piper nigrum*, shows similar antidepressant-like effects using the FST. For example, *Piper nigrum* essential oil significantly reduced immobility time in mice, likely through serotonergic mechanisms (Ghosh et al., 2021). Hydroethanolic extracts of *Piper betle* leaves at doses of 200 and 400 mg/kg also demonstrated significant antidepressant activity by reducing immobility time in the FST. These findings support the potential of *Piper crocatum* extract as an antidepressant agent.

Among the tested doses, the 400 mg/kg BW extract showed the most consistent and significant reduction in immobility time, with a 39.01% antidepressant activity, compared to 29.67% and 31.67% for 200 mg/kg and 800 mg/kg, respectively. This suggests that 400 mg/kg is the optimal dose for eliciting antidepressant effects in mice. The lower efficacy at 200 mg/kg may be due to insufficient active compound concentration, while the slightly reduced effect at 800 mg/kg could be related to possible saturation effects or mild toxicity reducing activity, a phenomenon observed in other phytochemical studies (Hossain & Sultana, 2019).

Conclusion

In conclusion, the ethanol extract of red betel leaf exhibits significant antidepressant activity at 400 mg/kg BW in the Forced Swim Test, likely due to its bioactive compounds such as eugenol, alkaloids, and terpenoids. The FST method provided a reliable and valid assessment of antidepressant-like effects, consistent with previous studies on related Piperaceae species. Further studies are recommended to elucidate the exact mechanisms of action and to evaluate clinical efficacy.

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Conflict of interest

The authors declare that they have no conflict of interest.

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