Trop J Nat Prod Res, November 2023; 7(11):5216-5220
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Anxiolytic and Anti-Depressant Activities of Ethanol Extract of *Mikania micrantha* Kunth Leaves in Mice

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ARTICLE INFO

Article history:
Received 10 September 2023
Revised 09 October 2023
Accepted 02 November 2023
Published online 01 December 2023

ABSTRACT

Sembung rambat (*Mikania micrantha* Kunth) is a weed that grows easily and may hinder cultivation of plants. However, it also has medicinal benefits. *M. micrantha* contains various secondary compounds such as linalool, quercetin, α-terpinene, and terpinene-4-ol, which have anti-depressant effects. To our knowledge, no studies have been conducted on the anxiolytic and anti-depressant activities of the leaves of this plant. This study determined the anxiolytic and anti-depressant activities of the ethanol extract of *M.micrantha* Kunth leaves. 48 male Swiss-Webster mice were assigned into four groups. All extracts, control, and Amitriptylilne groups were administered the same treatment. The forced swimming test (FST) and tail suspension test (TST) were carried out to examine anti-depressant activity, whereas the elevated plus maze test (EPM) was used to assess anxiolytic activity. The FST and TST data showed that immobility times were significantly reduced when *M. micrantha* Kunth was administered at 250 mg/kg and 500 mg/kg doses ($p<0.001$, $p<0.05$, $p<0.012$, and $p<0.033$, respectively), while 250 mg/kg of *M. micrantha* Kunth increased the time spent in the open arms against the control group, although it was slightly lower than the amitriptyline group ($p < 0.05$). There were no significant differences in the open-arm entries within the groups. *M. micrantha* Kunth leaves ethanol extract reduces the immobility time in FST and TST with increased entries on open arms and time spent in EPM.

Keywords: micania micrantha kunth, anti-depressant, anxiolytic

Introduction

Depression is a condition characterized by impaired mental health, leading to excessive anxiety, hopelessness, anhedonia, low self-esteem, loss of appetite and a persistent sad mood.[1,2] Meanwhile, anxiety is mental health disorders defined by symptoms such as panic, fatigue, fear, chest pain, sweating, insomnia.[3] Depression and other mental health problems are priority health concerns in the Indonesia national health program due to the annual increase in reported cases every year.[4] Anti-depressants are among the most commonly prescribed drugs globally and used to treat various conditions, including depression and other disorders such as panic disorder, neuropathic pain, and anxiety disorder, which often necessitate medical intervention.[5]

Recent studies have focused on natural compounds, such as foods or medications, that have demonstrated significant anti-depressant efficacy with minimal side effects.[6,7] These herbal preparations contain various natural metabolites with anti-depressant properties, such as saponins, flavonoids, terpenes, phenylpropanoids, carbohydrates, phenols, and alkaloids. These secondary metabolites exhibit anti-depressant effects by influencing neurotransmitter and receptor expression, affecting the hypothalamic-pituitary-adrenal (HPA), modifying brain's plasticity through various signaling pathways. [8]

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Citation: Su'aida N, Hasniah H, Mardiana L, Soemarie YB. Anxiolytic and Anti-Depressant Activities of Ethanol Extract of *Mikania micrantha* Kunth Leaves in Mice. Trop J Nat Prod Res. 2023; 7(11):5216-5220. http://www.doi.org/10.26538/tjnpr/v7i11.27

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Weeds are any plants whose presence is typically considered a nuisance, capable of harming the growth of neighboring plants [7]. However, certain weeds also find use in traditional medicine, such as Sembung rambat (*M. micrantha* Kunth). It holds potential as an antidepressant and anxiolytic agent due to its abundance of numerous compounds such as flavonoids, alkaloids, phenolics, saponins, proteins, tannins, amino acids, and terpenoids.[8] This study aims to determine the anti-depressant and anxiolytic effect of *M. micrantha* Kunth.

Materials and Methods

Materials

The materials were *M. micrantha* Krunth leaves, ethanol (merck, Germany), amitriptyline (PT. Indofarma, Indonesia), normal saline 0,9% (PT. Widatra Bhakti, Indonesia).

Extract Preparation

M. micrantha Kunth leaves was obtained from the backyard of the Faculty of Pharmacy at the Universitas Islam Kalimantan in March 2023. Plant identification and authentication was conducted at the Biology Laboratory, Lambung Mangkurat University, under reference number IV-23-005. The leaves were dried using oven at 65ºC approximately 10 hours and subsquently powdered. Maceration was used to extract the samples using 70% ethanol as the solvent. The samples were macerated with a ratio of 1:10 in solvent at room temperature for 3 days. The mixture was then filtered and evaporated using a rotary evaporator.

Phytochemical Screening

Phytochemical tests were carried out using ethanol extract from *M. micrantha* Kunth leaves following standard procedure as described by Trease & Evans [9] for alkaloids, saponins and tannins; Edeoga [10] for steroids and terpenoids; Sofowora [11] for flavonoid.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: Experimental Design

Group	Treatment	Total
Negative Control	Normal saline 0.9%	12
Positive Control	Amitriptyline (10 mg/kg)	12
MMLE	*Mikania micrantha* Kunth Extract (250 mg/kg)	12
MMHE	*Mikania micrantha* Kunth Extract (500 mg/Kg)	12

Experimental animals
Male Swiss-Webster mice aged 12-15 weeks were used in this study. Mice were divided into four group (Table 1). All mice underwent a one-week adaptaion periode in a laboratory atmosphere under same treatment and freely accessed food and water. This study adhered to the Animal Reasearch guidelines of Universitas Islam Kalimantan and was approved by the Research Ethics Comission of Universitas Muhammadiyah Banjarmasin (No. 328/UMB/KE/V/2023).

Behavioral Assessments
Forced Swimming Test (FST)
The FST was carried out in accordance with the method described by Alves *et al.* (2020). [12] Mice were required to swim for 6 minutes in a cylindrical glass tube containing 15 cm^3 of water at 23ºC. Immobility time (time spent in the water without attempting to escape) during the last 4 minutes of the test was recorded. Following the behavioral assessment, mice were dried and returned to their cages with all evaluations were recorded using a digital camera.

Tail Suspension Test (TST)
The TST was performed according to the IACUC (2021) guidelines [13]. Mice were hanged by their tails using adhesive tape and recorded with a camera for 6 minutes. Mice were placed in a position where they could not escape or cling to nearby objects. The tape ought to be sufficient to keep the mice from falling without causing damage to the tail skin. The length of the tape varied based on the specific system setup and tail tip, leaving 3 mm of the tail outside the tape.

Elevated Plus Maze Test (EPM)
The EPM was conducted by modifying the method by Aduema *et al*. (2018), [14] following the description by Lister's (1987) description. Mice were positioned in the center of the EPM, adjacent to the open arm and the trial lasted for 5 minutes. Mice were taken from the maze after 5 minutes. Behavioral assessment included open arm and closed arm entries, with all four paws inside either the closed or open arms, and the amount of time spent in closed or open arms.

Stastical Analysis
The data obtained was analyzed using the IBM SPSS Statistics software version 27.0. The data was expressed as mean ± SEM. Immobility time and time spent were analyzed using one-way ANOVA, while total entries were analyzed using the Kruskal-Wallis test with a significance level of $p < 0.05$. Significant differences within groups were determined by using Tukey's HSD and Dunn's post-hoc tests.

Results and Discussion

Preliminary Phytochemical Analysis
The preliminary analysis indicated that the ethanol extract of *M. micrantha* Kunth leaves contained terpenoids, flavonoids, saponins, alkaloid and tannins while steroids was absent in this tested (Table 2).

Effect Of Ethanol Extract M. micrantha Kunth Leaves via Oral Administration on Forced Swimming Test
The administration of *M. micrantha* Kunth leaves ethanol extract showed significantly decreased the immobility time of mice in the FST, as shown in (Figure 1). When administered orally, *M. micrantha* Kunth extract at dose of 250 mg/kg and 500 mg/kg exhibited significant differences compared to control group (normal saline 0,9%) and Amitriptyline group (10 mg/Kg).

Effect Of Ethanol Extract M. micrantha Kunth Leaves via Oral Administration on Tail Suspension Test
The administration 500 mg/kg of *M. micrantha* Kunth showed significantly decreased on immobility time of mice in the TST compared to the control group (Figure 2). Meanwhile, it showed that no significant differences were observed between *M. micrantha* Kunth extract dose of 250 mg/kg and 500 mg/kg against the Amitriptyline group (10 mg/kg).

Effect of Ethanol Extract M. micrantha Kunth Leaves via Oral Administration on Elevated Maze Plus Test
The administration of *M. micrantha* Kunth leaves ethanol extract in mice showed no significant differences of time spent and entry on open arms compared to the control group and the Amitriptyline group. However, it can be observed in (Figure 3A) that there was a marked rise in time spent and entry on open arms in contrast to control group. Meanwhile, the Amitriptyline group showed a significant difference from the control group in time spent on open arms.
Behavioral assessment of mice administered *M. micrantha* Kunth leaves extracts were conducted using the FST, TST, and EPM models to assess depression and anxiety-like behaviors.[15] Both the FST and TST are assessments of behavioral despair or helplessness.[16] The administration of *M. micrantha* Kunth extract reduced the immobility time as measured by the FST and TST tests.

Table 2: Secondary Metabolites of *M. micrantha* Kunth Leaves Ethanol Extract

Compounds	Results
Alkaloids	+
Flavonoids	+
Saponins	+
Steroids	-
Tannins	+
Terpenoids	+

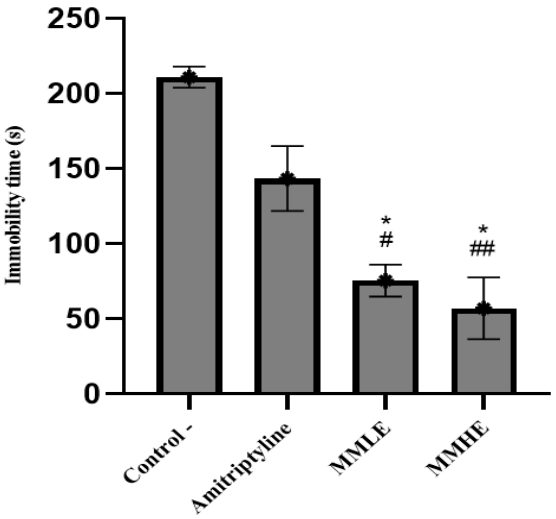

Figure 1: Effect of *M. micrantha* Kunth Leaves Ethanol Extract by p.o on forced swimming test
Effect of *M. micrantha* Kunth extract decrease immobility time on FST. Control vs MMLE; MMHE; *p<0.001; Amitriptyline vs MMLE; MMHE; #p<0.05; ##p<0.012. Significance value between groups p<0.001. Each column describes as Mean ± SEM of four mice.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

The anti-depressant activity of *M. micrantha* Kunth extract was comparable to the positive control (Amitriptyline). All the extract doses remarkably decreased immobility time in the FST effectively than Amitriptyline. In contrast, the 500 mg/kg of the extract showed better results than 250 mg/kg and Amitriptyline in the TST test. This suggests that the 500 mg/kg of *M. micrantha* Kunth extract has greater anti-depressant potential.

Terpenoids, saponins, flavonoids, alkaloids, and tannins were found in ethanol extracts of *M. micrantha* Kunth leaves, consistent with preliminary qualitative phytochemical screenings. These findings were comparable to those of a prior study conducted by Dev *et al.* [17] using a mEthanol extract of *M. micrantha* Kunth leaves. It has been reported that *M. micrantha* Kunth leaves contain an abundance of metabolites compounds, including derivatives of terpenoids, such as limonene, ß-terpinene, ß-copaene, α-longipinene, linalool, α-humulene, 2-butylamine, ß-caryophyllene, α-terpinene, carveol, menthol, curcumin, α-zingiberene, p-cymene-2-ol, α-bergamotene, ß-himachalene, verbenone, terpinene-4-ol, ß-cubebene, cadinene ß-germacrene, geraniol, and flavonoid derivatives, such as quercetin [18]. Some secondary metabolites, such as linalool, quercetin, and terpinene-4-ol, have anti-depressant effects [19-21]. According to previous research by Nicollier & Thompson,[22] and Perez-Amador *et al.*[23] linalool is one of the most abundant metabolites in *M. micrantha* Kunth constituting 15.86%. The anti-depressant activity of *M. micrantha* Kunth extract may be mediated by more than one mechanism. Each metabolite has an underlying mechanism of action against depression. Quercetin enhances hippocampal neuron regeneration and improves the HPA axis. Quercetin also acts as regulator of neurotransmitter such as monoaminergic, norepinephrine, choline and GABA [24,25]. Terpenoid derivates such as linalool and terpinene-4-oil are known to increase the concentrations of neurotransmitters including glutamate, GABA, dopamine, noradrenaline, and acetylcholine. They also modulate the expression of genes linked to stress in the hypothalamus and elevates oxytocin levels. Linalool has an anti-depressant effect comparable to fluoxetine.[26]

M. micrantha Kunth extract additionally raised the number of entrances and time spent in the open arms in the EPM in contrast to the control group. Increasing time spent in the open arms implies less nervousness in the mice, whereas spending more time in closed arms implies anxiety.[27-29] Anxiolytic-like assessment is linked to particular test drugs when the mice preferred the open arms without altering the amounts of closed arms entrances against the control.[30] In this study, 250 mg/kg of *M. micrantha* Kunth extract showed better anxiolytic effect than 500 mg/kg but was still lower than Amitriptyline. These findings demonstrated that the extract's anxiolytic effect is dose-dependent. The test given without any pre-trial/treatment to animals such as early animal handling, enrichment of the surroundings, or modest exposure to the EPM apparatus. It was described that a brief treatment before the test reduced anxiety-like behavior and thereby altered the reaction to anxiolytic drugs. [31,32]

The mechanism of action of *M. micrantha* Kunth extract as an anxiolytic agent may be derived from Linalool and Quercetin. A previous study by Cheng *et al*. [33] reported similar results, with a lower dose of linalool (250 mg/kg) spending longer time in the open arms than the higher dose (500 mg/kg). Linalool exerts a potential anxiolytic effect by altering 5-HT levels. Furthermore, quercetin is a well-known anxiolytic agent. Quercetin acts as a prodrug, transforming into its active hydroxyphenyl acetic acid metabolite by intestinal microflora.[34] At the same time, *M. micrantha* Kunth extract is reported to have antibacterial activity, and it can inhibit quercetin's anxiolytic effect.

Conclusion

Ethanol extract of *M. micrantha* Kunth leaves has been proven to reduce immobility time in FST and TST while increasing entries in open arms and time spent on EPM. Additional study is needed to determine the underlying mechanism of action of *M. micrantha* Kunth as an anxiolytic and anti-depressant agent.

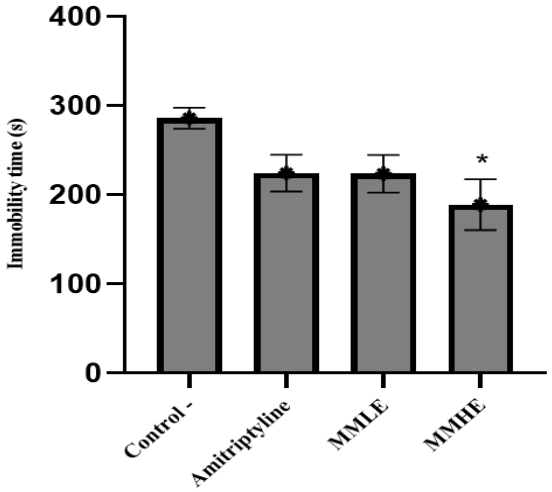

Figure 2: Effect of *M. micrantha* Kunth Leaves Ethanol Extract by p.o on tail suspension test

Effect of *M. micrantha* Kunth extract decrease immobility time on TST. Control vs MMHE; *p<0.033. Meanwhile, significance value between groups p<0.048. Each column describes as Mean ± SEM of four mice.

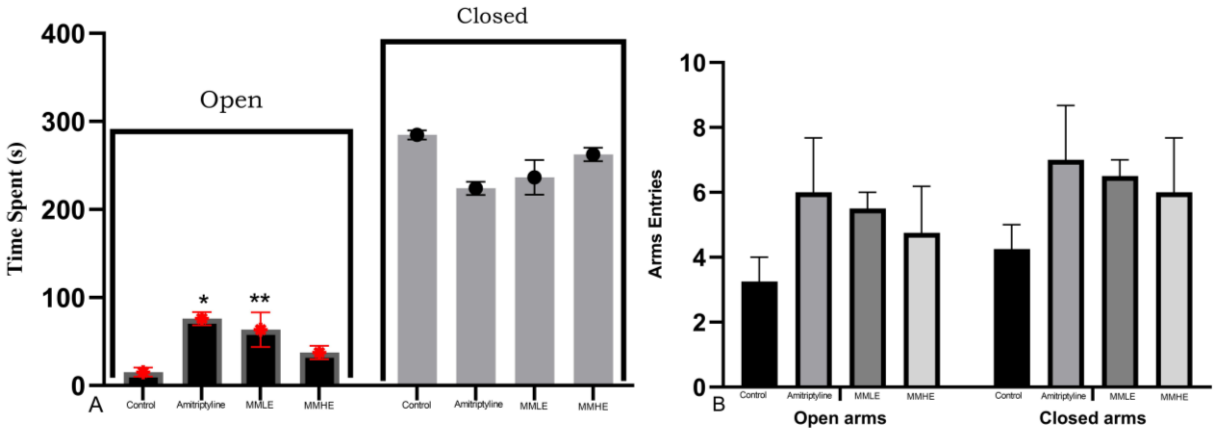

Figure 3: Effect of *M. micrantha* Kunth Leaves Ethanol Extract by p.o on elevated maze test

Effect of *M. micrantha* Kunth extract increase time spent on open arms (A) and open arms entries (B). (A) Control vs Amitriptyline; MMLE; *P<0.013; ** p<0.05. Significance value between groups (A) P<0.012 (B) P<0.284 Each column describes as Mean ± SEM of four mice.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

Gratitude is due to our colleagues from Faculty of pharmacy, Universitas Islam Kalimantan Muhammad Arsyad Al-Banjari for their technical support in this study. This research is supported by Universitas Islam Kalimantan Muhammad Arsyad Al-Banjari research Grant No.109/UNISKA-PUSLIT/III/2023.

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