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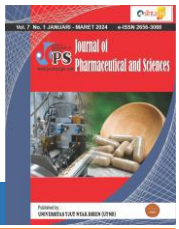

Sedative Activity of Ethanol Extract of Tali Kuning (*Anamirta cocculus* (L.) Wight & Arn.) Stem Against Male Mice (*Mus musculus*)

Aktivitas Sedatif Ekstrak Etanol Batang Tali Kuning (*Anamirta cocculus* (L.) Wight & Arn.) Terhadap Mencit (*Mus musculus*) Jantan

Wiwit Ayu Fitrianti [a], A.M. Muslihin [b] and Ratih Arum Astuti [c]

[a] *Department of pharmacy, Faculty of pharmacy, Universitas Pendidikan Muhammadiyah Sorong, Sorong, Papua Barat Daya, Indonesia.*

Corresponding Authors: am_muslihin@unimudasorong.ac.id

Abstract

Mental health is a state of well-being that includes where individuals recognize their personal potential, enabling them to manage life's pressures and be able to work productively. Treatment of mental health disorders usually involves the use of sedative drugs such as benzodiazepines, barbiturates, and several other drugs. This study aims to evaluate the sedative activity of the ethanol extract of tali kuning (*A. cocculus* (L.) Wight & Arn.) stem in male mice as an alternative therapy requiring sedative effects. The study was conducted using a quantitative experimental approach with a pretest–posttest control group design and the rotarod method. The experimental animals were divided into five groups: positive control, negative control, and three extract dose groups treated with the ethanol extract of tali kuning stem at 19.25 mg/kgBW, 38.5 mg/kgBW, and 77 mg/kgBW. The results indicated that the ethanol extract of tali kuning stem at all three doses produced sedative activity, as validated by a paired sample t-test that revealed significant differences condition between pre- and post-treatment measurements. The ethanol extract of tali kuning stem at doses of 19.25 mg/kgBW, 38.5 mg/kgBW, and 77 mg/kgBW demonstrated sedative activity, although statistical comparison with the positive control showed variable results depending on dose and observation time.

***Keywords*: Tali Kuning (Anamirta cocculus (L.) Wight & Arn.), Sedative Activity, Rotarod Test.**

Abstrak

Kesehatan mental merupakan sebuah keadaan sejahtera yang mencakup kesadaran individu atas kompetensi dirinya yang memungkinkan seseorang untuk mengatasi tekanan dalam kehidupan dan mampu bekerja secara produktif. Penanganan gangguan kesehatan mental biasanya melibatkan penggunaan obat-obatan golongan sedatif seperti benzodiazepine, barbiturate, dan beberapa obat lain. Penelitian ini ditujukkan untuk menguji aktivitas sedatif ekstrak etanol batang tali kuning (*A. cocculus* (L.) Wight & Arn.) pada mencit jantan sebagai alternatif terapi yang membutuhkan efek sedatif. Penelitian dilakukan dengan pendekatan eksperimental kuantitatif menggunakan *pretest-postest control group design* dan metode rotarod. Hewan uji dibagi menjadi lima kelompok, yaitu kontrol positif, negatif, serta tiga kelompok dosis ekstrak etanol batang tali kuning 19,25 mg/kgBB, 38,5 mg/kgBB, dan 77 mg/kgBB. Data penelitian mengungkapkan bahwa ekstrak etanol batang tali kuning pada ketiga dosis tersebut memberikan aktivitas sedatif yang divalidasi melalui *uji paired sample t-tes*t yang menunjukkan adanya perbedaan kondisi sebelum dan setelah perlakuan. Ekstrak etanol batang tali kuning pada dosis 19,25 mg/kgBB, 38,5 mg/kgBB, dan 77 mg/kgBB menunjukkan aktivitas sedatif, meskipun perbandingan statistik dengan kontrol positif menunjukkan hasil yang bervariasi tergantung pada dosis dan waktu pengamatan.

Kata Kunci: Tali Kuning (Anamirta cocculus (L.) Wight & Arn.), Aktivitas Sedatif, Uji Rotarod.

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Introduction

In recent years, the incidence of mental health disorders has increased significantly. The World Health Organization (WHO) defines health as a state of well-being in which individuals are aware of their own capacities and are able to cope with the normal stresses of daily life while participating productively in their activities. Mental health problems may arise from pressures experienced in various life domains, including family, academic, and social environments [1]. Globally, it is estimated that nearly half of the manifestations of mental health disorders including depression, anxiety disorders, and aggressive behaviors begin to emerge by the age of 14, with a prevalence ranging from 10% to 20% [2]. The 2018 Basic Health Research Report indicates that approximately 706,688 individuals or 9.8% of the Indonesian population experience emotional psychological disorders, with an average age of over 15 years. This public health phenomenon is characterized by a high prevalence of depressive and anxiety symptoms detected among the adolescent population, increasing from 6% in 2013 for the same age group [3].

Until now, management of mental health disorders usually involves the use of sedative drug classes such as benzodiazepines, barbiturates, and several other agents, including paraldehyde, chloral hydrate, ethchlorvynol, and meprobamate. These drugs act as Central Nervous System (CNS) depressants, thereby suppressing mental activity and reducing an individual's response to emotional stimuli. Their mechanism of action induces a feeling of calmness and facilitates the relaxation process in individuals who consume them [4]. However, if these drug classes are used in the long term, they may cause toxic effects.

Meanwhile, in Indonesia approximately 28,000 plant species have been recorded, of which around 7,500 are considered to have potential as medicinal materials [5]. The plant tali kuning (*Anamirta cocculus* (L.) Wight & Arn.) is suspected to be one of the plants possessing sedative activity. This plant has a relatively large stem, with grayish bark and a whitish to yellowish inner wood. It is commonly used in traditional medicine, including in the inherited treatment of malaria [6,7]. Phytochemical analysis of tali kuning has confirmed the presence of various secondary metabolites, including compounds belonging to the flavonoid, alkaloid, tannin, and terpenoid groups [8,9]. Several subsequent studies have also indicated that alkaloid, saponin, flavonoid, terpenoid, tannin, and polyphenol compounds have the potential to exert sedative activity [4,10,11].

Alkaloid compounds are known to interact with GABA receptors, leading to the opening of chloride ion channels, which results in hyperpolarization of the neuronal membrane and reduces the ability of neurons to be excited thereby producing a sedative effect [12]. Gamma-aminobutyric acid (GABA) functions as the primary inhibitory neurotransmitter in the Central Nervous System (CNS). GABA reduces the activity of target neurons by binding to GABA receptors located on the cell surface [13]. Flavonoid compounds are known to interact with benzodiazepine receptors, thereby suppressing locomotor activity and potentiating the sedative effect. Terpenoid compounds exert a mild influence on the central nervous system, inducing muscular relaxation and reducing motor activity thus producing a sedative effect. Tannins can bind to the GABA α site within the benzodiazepine receptor complex leading to suppression of locomotor activity and enhancement of the sedative effect [10].

Based on the secondary metabolite content of tali kuning plant, it is presumed to contain sedative compounds that may exert a sedative effect. However, there is currently no experimental evidence demonstrating sedative activity of tali kuning plant. Through this study, it is expected that the ethanol extract of tali kuning stem could serve as a natural-product-based alternative therapy for diseases requiring a sedative effect.

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Experimental Section

This study employed a quantitative experimental method using a pretest–posttest control group design to identify differences in motor activity of experimental animals before and after treatment administration. Sedative activity was evaluated using the rotarod method, which was selected to assess motor coordination and balance in mice. A decrease in the ability of mice to remain on the rotarod apparatus after treatment was used as an indicator of reduced motor activity associated with the depressant effect on the central nervous system [12,14].

Materials and Apparatus

The equipment required in this study included a glass stirring rod, glassware, a water bath, a funnel, labels, filter paper, dishes, an oven, a rotarod TEQ-IND® apparatus, a balance, a box container, a syringe, glass containers, a mask, and gloves. The materials required were tali kuning stem simplicia (*A. cocculus* (L.) Wight & Arn.), 1% Na CMC, 70% ethanol, and diazepam tablets 5 mg.

Research Procedure

Sample Collection and Preparation

The tali kuning stem samples were collected from Kampung Folley, Raja Ampat Regency, Papua Barat Daya. A total of 1.4 kg of tali kuning stem samples were washed under running water to remove foreign materials and dirt, then weighed to determine the initial weight. Subsequently, the samples were dried in an oven at 50°C for 8 hours. After drying, the tali kuning stems were ground into a fine powder [15].

Extract Preparation

Extraction of tali kuning stem was carried out using the maceration technique. A total of 500 g of tali kuning stem powder was weighed and then placed into a glass container, followed by immersion in 70% ethanol using a ratio of 1:4 until all of the sample was completely submerged. The maceration process was carried out for 72 hours under light-protected conditions with periodic stirring. Afterwards, the mixture was filtered to obtain the macerate. The maceration process was repeated up to three times using the same solvent. All macerates were then combined and evaporated using a water bath until a thick extract was obtained [16].

1% Na CMC Suspension Preparation

A total of 1 g of 1% sodium carboxymethylcellulose (Na CMC) was weighed and added into 50 mL of warm distilled water under gradual heating while being stirred until a homogeneous solution was formed. Once the solution was fully mixed, distilled water was added gradually until the final volume reached 100 mL, followed by stirring until the solution became completely homogeneous [17].

Determination of Diazepam Dose for Mice

Diazepam was selected as the reference drug for the positive control. The dose used was 5 mg. The diazepam dose for mice (20 g) was calculated based on the human dose (5 mg/70 kg) using a conversion factor of 0.0026, resulting in 0.013 mg/20 gBW. When prepared as a 10 mL solution, a total of 0.13 mg of diazepam was weighed to prepare a 10 mL suspension formulation.

Test Solution Preparation

The extract solution of tali kuning stem was formulated into three different dose levels: 19.25 mg/kgBW, 38.5 mg/kgBW, and 77 mg/kgBW. For the 19.25 mg/kgBW dose, 9.24 mg of the thick extract was weighed and suspended in 1% Na CMC solution up to a total volume of 10 mL. The 38.5 mg/kgBW dose was prepared by weighing 19.25 mg of the thick extract and suspending it in 1% Na CMC solution up to a final volume of 10 mL. The 77 mg/kgBW dose was obtained by weighing 36.96 mg of the thick extract and then adding 1% Na CMC solution until the final volume reached 10 mL.

Experimental Animal Treatment

The criteria for the experimental animals included mice aged 2 to 3 months with a body weight of 20–35 g, declared healthy and free from physical defects. Prior to testing, the mice were acclimatized for one week in their cages and were trained on the rotarod apparatus for 15 minutes each day to habituate them to the rotarod. The rotarod method was selected to evaluate motor coordination ability in mice [12].

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During testing, each mouse was placed on the rotarod apparatus at a speed of 30 rpm, and the time at which the mouse fell was recorded using a stopwatch as the measurement parameter. The experimental animals consisted of five groups, with three mice in each group. The positive control group was administered a diazepam suspension at a dose of 0.013 mg/20 gBW, whereas the negative control group received 1% Na CMC solution. The treatment groups were given an ethanol stem extract suspension of tali kuning at three dose levels, namely 19.25 mg/kgBW, 38.5 mg/kgBW, and 77 mg/kgBW. Assessment of the sedative effect in all treatment groups was performed repeatedly four times, with observation intervals at 30, 60, 90, and 120 minutes after administration of the preparation.

Data Analysis Techniques

Data analysis was performed statistically using SPSS software. The initial stage included normality testing using the Shapiro–Wilk method and homogeneity of variance testing using Levene's test [18]. Subsequently, data were analyzed using the Paired Sample T-Test to evaluate differences before and after treatment, as well as the Independent Sample T-Test with a 95% confidence level ($\alpha < 0.05$) to determine significant differences among treatment groups [4].

Results and Discussion

Tablel 1. Percentage of Tali Kuning Stem Extract Yield

Sample	Sample Weight (g)	Extract Weight (g)	Yield (%)
Tali kuning stem	500	33	6,6

The yield of ethanol extract obtained from tali kuning stem was 6.6%. This yield value is less than 10%. High or low extract yields reflect the efficiency of the extraction process, which is influenced by technical variables such as the degree of fineness of the simplicia powder, the choice of solvent, and the duration of extraction applied [12]. The stirring process during maceration may also influence the yield obtained. The longer the duration of stirring, the higher the extract yield [19].

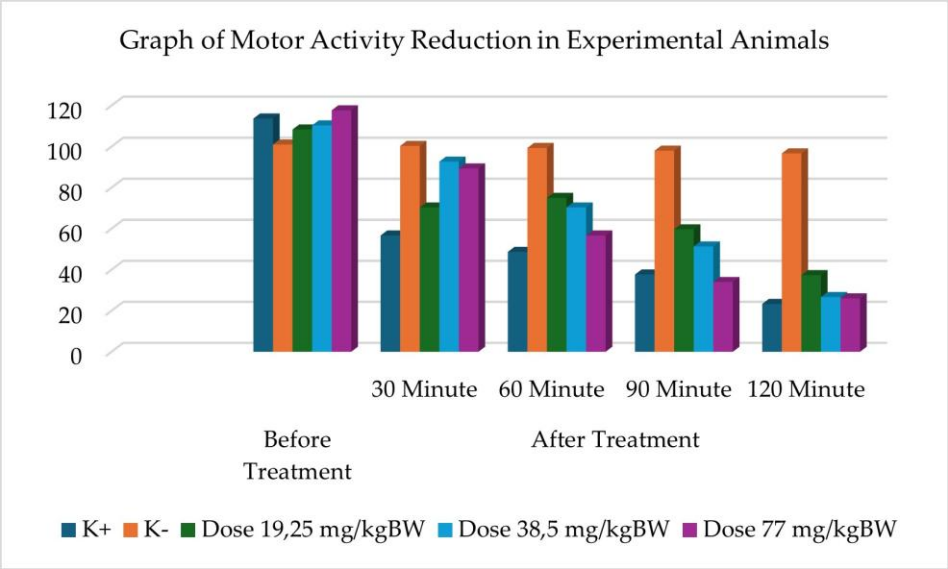

Figure 1. Graph of Motor Activity Reduction in Mice

Figure 1 presents a graph comparing the conditions before and after treatment administration in each group. The results show a decrease in activity following treatment, with the positive control group and the groups receiving extract doses exhibiting a significant reduction indicating the presence of a sedative effect. In contrast, the negative control group did not show a meaningful change in activity, suggesting that this group lacks sedative potential.

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SPSS Data Analysis Results

Table 2. Results of Paired Sample T-Test dan Independent Sample T-Test

Variable	Group I Positive Control (Diazepam)	Group II Negative Control (Na CMC)	Group III Extract Dose 19,25 mg/kgBW	Group IV Extract Dose 38,5 mg/kgBW	Group V Extract Dose 77 mg/kgBW
Pre-treatment	113.67±3.215	101.00±14.17	108.33±16.07	110.33±18.17	117.67±17.21
Post-treatment T30	56.67±15,144*	100.33±13.05	70.33±21.59*	92.67±20.03*	89.33±5.85
Post-treatment T60	48.67±3.512*	99.33±13.05	75.00±25.94*	70.33±15.94*	56.67±20.55*
Post-treatment T90	37.67±7.024*	98.00±15.87	59.67±29.02*	51.33±7.76*	34.00±14.42*
Post-treatment T120	23.33±10.970*	96.67±13.31	37.33±15.01*	26.67±10.40*	26.00±17.08*
ΔT					
ΔT30	57.00±13.00b**	0.66±1.15a**	38.00±15.09b**	17.66±2.08ab**	28.33±15.30
ΔT60	65.00±1.00b**	1.66±1.15a**	33.33±10.06ab**	40.00±8.88ab**	61.00±3.60b**
ΔT90	76.00±10.14b**	3.00±1.73a**	48.66±13.50ab**	59.00±12.16b**	83.66±10.01b**
ΔT120	90.33±13.65b**	4.33±2.08a**	71.00±9.84b**	83.66±7.76b**	91.66±8.14b**

Data are presented as mean ± SD, n = 3, ΔT = Difference between pre-treatment and post treatment, *p < 0,05 paired sample t-test, **p < 0,05 independent sample t-test. avs. Positive control, bvs. Negative control.

Discussion

This study employed three dose levels of ethanol extract of tali kuning stem, namely 19.25 mg/kgBW, 38.5 mg/kgBW, and 77 mg/kgBW. The preparations were formulated as suspensions using Na CMC as an effective suspending agent. The use of Na CMC as a viscosity enhancing agent plays an important role in maintaining a homogeneous distribution of the extract's active compounds within the formulation. In addition, its inert nature prevents chemical interaction with the active components, thereby preserving the integrity of the drug's pharmacological effects [4]. The negative control group was treated with 1% Na CMC solution because all treatment groups also used Na CMC as a suspending agent, thereby allowing the effect of Na CMC on motor activity to be controlled. Sedative activity was evaluated using the rotarod test at 30 rpm, which was selected to assess motor function in mice. Although the rotarod is commonly used to test ataxia, this study adopted the method based on several previous studies that employed it as an indicator of central nervous system depressant effects, in which a reduced latency to fall from the rotarod reflects a sedative effect [4,9,17]. The sedative effect was measured based on the duration for which mice were able to remain on the rotarod apparatus. The ability of mice to remain on the rotarod for a prolonged period indicates that the sedative effect has not yet been exerted. In contrast, a shorter latency to fall from the rotarod reflects a reduction in motor performance, indicating the presence of a sedative effect of tali kuning stem extract [14].

The data obtained from the testing were then analyzed using SPSS with paired sample t-test and independent sample t-test. The analysis began with a normality test using the Shapiro–Wilk method, as the number of samples per group was less than 50 [21]. The results showed a p-value (sig) > 0.05, indicating that the data were normally distributed. Subsequently, homogeneity of variances was tested using Levene's test, which yielded a p-value (sig) > 0.05, indicating that the data were homogeneous. The next analysis was performed using paired sample t-test to compare the effects of diazepam suspension, Na CMC, and ethanol extract of tali kuning stem on sedative activity in mice before and after treatment.

Statistical analysis using paired sample t-test **(Table 2)** showed a significant decrease in motor activity, indicating a sedative effect, between pre- and post-treatment at 30 to 120 minutes for the positive control group, the 19.25 mg/kgBW group, and the 38.5 mg/kgBW group. In contrast, for the 77 mg/kgBW group, a significant difference between pre- and post-treatment was observed from 60 to 120 minutes, with a p-value < 0.05. The negative control group did not show any significant change between pre- and post-treatment, with a p-value > 0.05.

Statistical analysis using independent sample t-test **(Table 2),** comparing Group II (negative control), Group III (extract dose 19.25 mg/kgBW), Group IV (extract dose 38.5 mg/kgBW), and Group V (extract dose 77 mg/kgBW) with Group I (positive control), showed that at 30 minutes of testing, there was a significant difference ($p < 0.05$) between Group II and Group IV. At 60 minutes, a significant difference ($p < 0.05$) was observed between Group II, Group III, and Group IV. At 90 minutes, a significant difference with p-value < 0.05 was found between Group II and Group III, whereas at 120 minutes, a significant difference ($p < 0.05$) was observed only in Group II.

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The test results comparing Group I (positive control), Group III (extract dose 19.25 mg/kgBW), Group IV (extract dose 38.5 mg/kgBW), and Group V (extract dose 77 mg/kgBW) with Group II (negative control) **(Table 2)** showed that at 30 minutes of testing, there was a significant difference ($p < 0.05$) in Groups I, III, and IV. At 60, 90, and 120 minutes, a significant sedative effect ($p < 0.05$) was observed in all treatment groups (Groups I, III, IV, and V). These findings indicate that sedative activity was exhibited in male mice after administration of tali kuning stem extract (*A. cocculus* (L.) Wight & Arn.) at doses of 19.25, 38.5, and 77 mg/kgBW, with significant differences ($p < 0.05$) compared to the negative control group. However, among these three doses, the 77 mg/kg BW group did not show a significant difference ($p > 0.05$) at 60, 90, and 120 minutes, indicating that, statistically, there is insufficient evidence to conclude that the sedative effect of the 77 mg/kgBW extract group differs from that of the positive control group.

Sedative effects in organisms are mediated by the GABAergic system through the interaction between γ-aminobutyric acid (GABA) and GABAA receptors. This binding triggers the opening of chloride ion channels, increasing chloride conductance into the cell and leading to membrane hyperpolarization. As a result, neuronal excitability is reduced and the cellular response to stimuli is diminished, which underlies the observed sedative effect [10]. Based on the literature, the ethanol extract of tali kuning stem is known to contain alkaloids, flavonoids, tannins, and terpenoids [8]. These compounds are reported to act via mechanisms that can influence the central nervous system, such as modulation of neurotransmitter receptors, enhancement of GABAergic activity, and depressant effects on the central nervous system, which may contribute to sedative properties [22,23]. As agonist compounds, alkaloids are able to elicit a maximal response at GABAA receptors, subsequently inducing membrane hyperpolarization. Tannins act by binding to the GABAA receptor site within the benzodiazepine complex; this interaction at the postsynaptic membrane triggers membrane hyperpolarization as a feedback response to the received stimulation. This process leads to the opening of ion channels that induce a hyperpolarized state, thereby producing a sedative effect in the subject [24].

Alkaloids, triterpenoids, and tannins bind to GABAA receptors, causing the ion channel to open and the membrane to become more permeable to K^+, which exits the cell. As a result, the intracellular compartment becomes more negative than the extracellular space due to the outward movement of K^+, which carries positive charge out of the cell, while Cl^- enters the cell, leading to membrane hyperpolarization [25]. This hyperpolarized state inhibits the propagation of action potentials, making the cell less excitable and resulting in decreased muscle tone and reduced motor activity, as evidenced by the fall of mice from the rotarod apparatus. Flavonoids belong to the class of polyphenolic compounds, which are secondary metabolites in plants and have potential as sedative agents. Flavonoids can interact with GABA receptors, thereby inducing the opening of chloride ion channels and promoting the influx of Cl^- into the cell. This leads to neuronal membrane hyperpolarization, which reduces the ability of neurons to become excited [10,12]. However, because specific phytochemical screening of the extract used in this study was not carried out, the causal relationship between particular compounds and the observed sedative effect remains hypothetical.

Research Limitations

This study used n = 3 per group, in consideration of the 3Rs (Replacement, Reduction, Refinement) guidelines for laboratory animals. However, these findings should be confirmed by further studies employing a larger sample size.

Conclusions

This study concludes that the ethanol extract of (*Anamirta cocculus* (L.) Wight & Arn.) stem, at doses of 19.25 mg/kgBW, 38.5 mg/kgBW, and 77 mg/kgBW exhibits sedative activity, as evidenced by a significant reduction in motor activity in mice when comparing pre-treatment and post-treatment conditions with a significance level of $p < 0.05$.

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