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Exploration of the Anxiolytic Potential of Red Shoot Leaf Extract through Elevated Plus Maze and Light-Dark Box Tests in White Mice

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Abstract

Anxiety disorders remain a major global mental health concern, with increasing prevalence and limitations associated with long-term pharmacotherapy. This study aimed to evaluate the anxiolytic potential of ethanol extract of *Syzygium myrtifolium* leaves using in vivo behavioral models. Dried leaves were extracted by maceration with 96% ethanol, yielding 16.69% extract. The results of phytochemical screening demonstrated the presence of flavonoids, alkaloids, tannins, saponins, as well as terpenoids. Fifteen male white mice were randomly divided into five groups: negative control (0.5% Na-CMC), positive control (diazepam 1 mg/kgBW), and extract-treated groups (100, 200, and 300 mg/kgBW). Anxiolytic activity was assessed using the Elevated Plus Maze (EPM) and Light–Dark Box (LDB) tests. In the EPM model, extract doses of 200 and 300 mg/kgBW significantly increased the time spent in open arms compared to the negative control ($p < 0.05$). Similarly, in the LDB test, higher extract doses significantly prolonged the duration in the illuminated compartment ($p < 0.05$). Statistical analysis confirmed normal data distribution and significant differences among groups (ANOVA, $p < 0.05$). The findings indicate that *Syzygium myrtifolium* leaf extract exhibits significant anxiolytic-like activity, likely associated with its flavonoid content and antioxidant properties. These results support its potential development as a plant-based alternative therapy for anxiety management.

Keywords: Anxiolytic; Elevated Plus Maze; Flavonoid; Light Dark Box; *Syzygium myrtifolium*.

INTRODUCTION

As many as 301 million people worldwide experienced anxiety disorders in 2019, and this number increased dramatically by 25% throughout the COVID-19 pandemic period (Roest et al., 2021). In Indonesia, mental health issues, including anxiety, are a health challenge that is receiving increasing attention. The 2023 Indonesian Health Survey (SKI) reported that approximately 1 in 50 people aged 15 and over experience mental health problems, including emotional disorders. This data indicates that anxiety is not a rare condition and has the potential to continue to increase if appropriate prevention and treatment efforts are not implemented (Trihono, 2023).

Anxiety is a mental disorder characterized by excessive worry, fear, and anticipation of future threats, often accompanied by muscle tension and hypervigilance, and strong enough to interfere with daily activities and functioning. This disorder not only impacts quality of life but also has the potential to reduce life expectancy. Ironically, most sufferers do not receive adequate professional help due to limited access, stigma, or economic constraints (Wu et al., 2025).

Conventional antidepressants and anxiolytics are effective in treating anxiety disorders. although effective, long-term consumption can result in dependency, cognitive decline, and adverse toxic outcomes. Therefore, alternative therapies based on natural ingredients are a potential option due to their minimal side effects and comparable effectiveness. Various medicinal plants contain bioactive compounds that can modulate neurotransmitters such as serotonin, dopamine and GABA. Exploring anxiolytic plants is important to support the development of safe, affordable, and indigenous wisdom-based therapies, while also aligning with the "back to nature" trend of using phytopharmaceuticals in modern healthcare systems. One herb with potential as an anxiolytic is red shoot leaves (*Syzygium myrtifolium*) due to its various bioactive compounds, such as flavonoids, alkaloids, tannins, saponins, steroids, triterpenoids, and phenolic compounds, including gallic acid (Syafriana & Wiranti, 2022). Flavonoids are known to have anxiolytic activity (Rahangga et al., 2018). Anthocyanins belong to the flavonoid group, as evidenced by the red color at the tips of the leaves, indicating a high anthocyanin content (Syafriana & Wiranti, 2022).

This research innovation falls under the theme of health, presenting a new approach to addressing anxiety disorders, which are increasingly prevalent in society due to various factors. This study proposes the use of *Syzygium myrtifolium* leaf extract, which will be scientifically evaluated through in vivo testing as an alternative anxiety-reducing agent, aligning with Sustainable Development Goal (SDG) number 3. Containing bioactive compounds, particularly flavonoids, known to have anxiolytic activity, this extract is expected to modulate the central nervous system and reduce anxiety symptoms in test animals. Assessments were conducted using the elevated plus maze and light-dark box tests on white mice to measure anxiety responses. It is hoped that this research will provide the public and the scientific community with new scientific information on the use of local plants as safer and more effective herbal therapies in treating anxiety disorders.

MATERIALS AND METHODS

Study area

The research was conducted for 4 months, starting from July to November 2025, in the Biomedical Laboratory II, Faculty of Medicine, Universitas Adiwangsa Jambi.

Materials

Tools used in extract preparation and anxiolytic testing include aluminum foil, stirring rods, beakers, blenders, porcelain cups, funnels, measuring cylinders, hotplates, parchment paper, droppers, analytical balances, filter paper, test tube racks, probes, spatulas, injection syringes, test tubes, animal scales, and a vacuum rotary evaporator. The materials used are distilled water, diazepam (drug purchases must be accompanied by an official request letter from the university), 96% ethanol, 2% FeCl3, H2SO4, concentrated HCl, 2N HCl, NaOH, 0.5% Na-CMC, sodium citrate, Dargendorf reaction, Mayer reaction, *Syzygium myrtifolium* leaf extract test samples, and Mg powder.

Figure 1. Syzygium mytrifolium plant found by author on Tebing Tinggi, Jambi.

Procedures

Plant Determination

The plant species was identified by the Herbarium Jatinangor, Universitas Padjadjaran (UNPAD), and the result is *Syzygium myrtifolium* [Roxb.] Walp. (Myrtaceae), as stated in certificate No. 125/HB/7/2025.

Plant Materials and Preparation of Extract

Syzygium myrtifolium leaves were collected from Tebing Tinggi District, West Tanjung Jabung Regency, Jambi Province, Indonesia. A total of 500 g of dried leaves were pulverized to obtain a fine simplicia powder. The powdered material (500 g) was macerated in 96% ethanol at a material-to-solvent ratio of 1:10 for 3 × 24 hours, with daily agitation for 30 minutes. The solution was filtered to remove any remaining solids, and the resulting filtrate was then further concentrated at 50°C using a rotary evaporator, and the thickened extract was further evaporated on a water bath maintained at 60°C until a viscous extract was obtained (Sugihartini & Maryati, 2022).

Phytochemical Analysis of Syzygium myrtifolium Leaf Extract

Alkaloids Detection

Alkaloids detection was conducted by taking 2 mL of filtrate, dropping it on a watch glass, adding 4 drops of Mayer's reagent, then the presence of a cream-colored precipitates that clumps and dissolves in methanol (positive for alkaloids). The alkaloid test using the Dragendroff reagent formed a reddish-brown precipitate indicating a positive for alkaloids.

Tannin Detection

Tannin detection was conducted by putting 2 mL of sample filtrate into a test tube, heating it for approximately 5 minutes, adding 4 drops of 1% FeCl3 solution. The presence of a dark blue or blackish green color (positive for tannins).

Flavonoid Detection

Flavonoid detection two milliliters of the filtrate were placed in a reaction tube and gently heated for approximately five minutes. Magnesium powder (0.1 g) was then introduced, followed by the addition of four drops of concentrated hydrochloric acid. The emergence of an orange to red hue was interpreted as evidence of flavonoid compounds.

Steroid/Triterpenoid Detection

Steroid/triterpenoid detection uses 2 mL of extract filtrate dissolved in ethanol added with the Liberman-Bouchard reagent. The formation of a brownish or violet ring at the interface of the solution indicated a positive result for terpenoids, whereas the appearance of a greenish-blue ring suggested the presence of steroids.

Saponin Detection
For saponin analysis, 0.5 g of *Syzygium myrtifolium* leaf powder was dissolved in 10 mL of hot distilled water using a test tube as the container and shaken vigorously for ten seconds. The presence of foam that was still observed after the addition of one drop of 2 N hydrochloric acid along the wall of the tube was considered as confirmation of the presence of saponins.

Ethical Approval
This study was approved by the Research Ethics Committee of Universitas YARSI under ethical clearance No. 258/KEP-UY/EA.10/VII/2025.

Experimental Animals
The experimental animals used in this study were 15 white mice weighing 20 to 30 grams. All experimental animals were acclimatized prior to the experiment to allow adaptation to the new housing conditions and to minimize stress. The experimental animals were randomly distributed into five groups of three mice each. Group I received the negative control (0.5% Sodium CMC), Group II received the positive control (diazepam at a dose of 0.0026 mg), Group III received Treatment I (100 mg of *Syzygium myrtifolium* leaf extract), Group IV received Treatment II (200 mg of *Syzygium myrtifolium* leaf extract), and Group V received Treatment III (300 mg of *Syzygium myrtifolium* leaf extract).

Preparation of Test Solution
Preparation of Negative Control Solution
Sodium CMC (0.5 g) was gradually dispersed in 50 mL of hot distilled water under continuous stirring until a homogeneous and viscous dispersion was achieved. The dispersion was then transferred to a 100 mL volumetric flask, and distilled water was added to the mark to yield a final concentration of 0.5% (w/v).

Preparation of Positive Control Solution
Diazepam tablets were administered according to the human dose converted to mice. The human dose of diazepam is 1 mg/kg (Rahman et al., 2019). The solution was prepared by weighing the crushed tablets and then adding 0.5% Sodium CMC little by little until homogeneous.

Preparation of *Syzygium myrtifolium* Leaf Extract Suspension
The red shoot leaf extract test suspension was prepared at various doses: 100 mg/kgBW, 200 mg/kgBW, and 300 mg/kgBW (Hasti et al., 2022). The extract was quantitatively transferred into a 10 mL volumetric flask and made up to the mark with 0.5% sodium CMC solution.

Anxiolytic Testing Using the Elevated Plus Maze Test and Light-Dark Box Methods
Elevated Plus Maze Test
Mice that received the test solution were positioned on an elevated plus-shaped apparatus placed 50 cm above the floor. The apparatus consisted of two open arms and two closed arms. Each mice was monitored for 5 minutes, during which the duration spent in the open and closed arms was recorded.(Rahangga et al., 2018).

Light-Dark Box
The light–dark test is a behavioral assessment method used to evaluate the exploratory behavior of experimental animals in response to environmental and light-related stressors. The testing apparatus consists of a box measuring 46 × 27 × 30 cm, which is divided into two compartments: a dark compartment and an illuminated compartment. Animals exhibiting anxiety-like behavior tend to avoid the illuminated area (Widyastiwi et al., 2023).

Data Analysis
Data were analyzed using a descriptive normality test (Shapiro-Wilk) to determine data distribution. If the data were normally distributed ($p>0.05$), an Analysis of Variance (ANOVA) test was performed, assuming normal and homogeneous data, with a 95% confidence level and a p value of <0.05. If the results were not normally distributed, the Friedman test was used.

RESULTS AND DISCUSSION

RESULTS

***Syzigium myrtifolium* Leaf Extract**
The leaves of *Syzygium myrtifolium* were extracted by maceration employing 96% ethanol as the solvent. A total of 500 grams of dried leaf powder was extracted, resulting in 83.457 grams of thick extract. Based on these results, the extraction yield obtained was 16.69%. This yield value indicates that the maceration method with ethanol was effective in extracting secondary metabolites from *Syzygium myrtifolium* leaves. The results of the extraction yield are presented in Table 1.

Table 1. Results of *Syzygium myrtifolium* Leaf Extract Yield.

Weight of Simplicia Powder	Extract Weight	Extract Yield
500 grams	83.457 grams	16.69%

Phytochemical Screening of *Syzygium myrtifolium* Leaf Extract
Phytochemical screening of *Syzygium myrtifolium* leaf extract was performed to confirm the presence of secondary metabolite constituents within the extract. The results demonstrated that the extract contained several

major bioactive constituents, namely alkaloids, flavonoids, tannins, saponins, and steroids/triterpenoids, as summarized in Table 2.

Table 2. Phytochemical Screening Results of Ethanol Extract of *Syzygium myrtifolium* Leaves.

Type of Compound	Test Method	Result	Description
Alkaloids	Mayer Reagent	+	Cream precipitate
	Dragendorff Reagent	+	Reddish–brown precipitate
Flavonoids	Magnesium powder + concentrated HCl	+	Reddish coloration
Tannins	$FeCl_3$ 1% reaction	+	blackish-green coloration
Saponins	10 mL hot distilled water	+	Formation of stable foam
Steroids / Terpenoids	Liebermann–Burchard reaction	+	Brown ring formation (+terpenoids)

(+) Indicates a positive result, meaning the presence of the tested secondary metabolite compounds.

Elevated Plus Maze Test

The anxiolytic activity of *Syzygium myrtifolium* leaf extract was evaluated using the Elevated Plus Maze (EPM) model. The main parameter observed was the duration spent by mice in the open and closed arms during a 5-minute session. The mean time in the open and closed arms for each treatment group is presented in Table 3.

Table 3. Time Spent in Open and Closed Arms in Elevated Plus Maze Test.

Group	Mice	Time in open arms (min:sec)	Time in closed arms (min:sec)
Negative control (Sodium CMC)	1	00:18	04:42
	2	00:11	04:49
	3	00:12	04:48
		Mean : 00:13	Mean : 04:47
Extract dose 100 mg/kgBW	1	00:43	04:17
	2	00:54	04:06
	3	01:08	03:52
		Mean : 00:55	Mean: 04:05
Extract dose 200 mg/kgBW	1	01:16	03:44
	2	01:43	03:17
	3	02:13	02:47
		Mean : 01:44	Mean : 03:16
Extract dose 300 mg/kgBW	1	02:34	02:26
	2	02:46	02:14
	3	03:02	01:58
		Mean : 02:47	Mean : 02:13
Positive control (Diazepam 1mg/kgBW)	1	03:42	01:18
	3	03:44	01:16
	3	03:55	01:05
		Mean: 03:47	Mean: 01:13

The Elevated Plus Maze findings demonstrated that mice in the negative control group exhibited the lowest mean duration in the open arms, averaging 0:13 minutes. In contrast, treatment with *Syzygium myrtifolium* leaf extract produced a dose-dependent increase in open-arm exploration. Administration of 100, 200, and 300 mg/kgBW resulted in mean open-arm durations of 00:55, 01:44, and 02:47 minutes, respectively. The positive control group treated with diazepam at 1 mg/kgBW displayed the longest average time in the open arms (03:47 minutes), reflecting a strong anxiolytic effect.

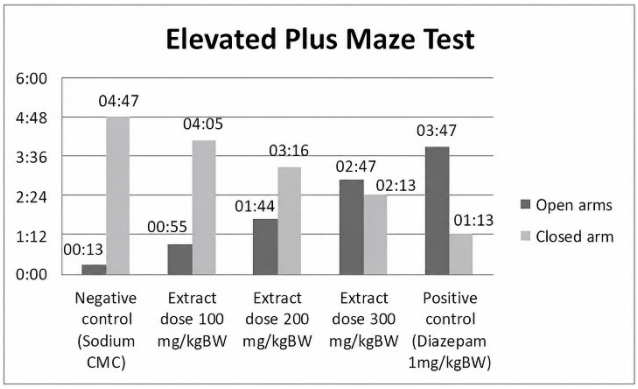

Figure 2. Diagram of Elevated Plus Maze Test Results.

Figure 2 presents a graphical comparison of the duration spent in the open and closed arms by mice across all treatment groups during the Elevated Plus Maze evaluation. The negative control group exhibited the shortest time in the open arms, reflecting heightened anxiety-like behavior. Conversely, administration of *Syzygium myrtifolium* leaf extract enhanced open-arm exploration in a dose-dependent fashion. Doses of 100, 200, and 300 mg/kgBW produced a gradual increase in open-arm duration compared to the negative control group. The positive control group receiving diazepam at 1 mg/kgBW demonstrated the longest time in the open arms and the briefest duration in the closed arms. This behavioral profile was consistent with the statistical findings, indicating that diazepam and higher doses of the extract significantly attenuated anxiety-like responses in mice.

Assessment of data normality using the Shapiro–Wilk test showed a normal distribution ($p = 0.150$; $p > 0.05$). Accordingly, parametric analysis with One-Way ANOVA was performed, revealing statistically significant differences among groups in both open- and closed-arm durations ($p < 0.001$).

Light-Dark Box Test

The anxiolytic effect of *Syzygium myrtifolium* leaf extract was further evaluated using the Light-Dark Box (LDB) test. The main parameter recorded was the duration of time the mice spent in the light compartment during a 5-minute observation period. The average time allocated to the light and dark compartments for each treatment group is presented in Table 4.

Table 4. Time Spent in Light and Dark Compartments in Light–Dark Box Test

Group	Mice	Time in light compartment (min:sec)	Time in dark compartment (min:sec)
Negative control (Sodium CMC)	1	00:16	04:44
	2	00:51	04:09
	3	00:06	04:54
		Mean: 00:24	Mean: 04:36
Extract dose 100 mg/kgBW	1	01:20	04:40
	2	01:10	04:50
	3	01:28	03:32
		Mean : 01:19	Mean: 03:41
Extract dose 200 mg/kgBW	1	02:15	02:45
	2	02:33	02:27
	3	03:20	01:40
		Mean : 02:42	Mean: 02:18
Extract dose 300 mg/kgBW	1	03:57	01:03
	2	04:26	00:34
	3	04:40	00:20
		Mean : 04:21	Mean : 00:39
Positive control (Diazepam 1mg/kgBW)	1	04:35	00:25
	3	04:38	00:22
	3	05:00	00:00
		Mean : 04:44	Mean : 00:16

The results of the Light-Dark Box test showed that rats in the negative control group spent the least amount of time in the light compartment, with an average of only 00:24 minutes, indicating high anxiety-like behavior. Administration of *Syzygium myrtifolium* leaf extract resulted in a gradual increase in the duration of stay in the light compartment corresponding to increasing doses. Mice given the extract at doses of 100, 200, and 300 mg/kgBW spent an average of 01:19, 02:42, and 04:21 minutes in the light compartment, respectively. The 300 mg/kgBW dose resulted in a substantial increase in exploration of the light compartment, approaching the effect observed in the positive control group. Rats given diazepam at a dose of 1 mg/kgBW showed the longest stay in the light compartment (04:44 minutes), consistent with its proven anxiolytic efficacy.

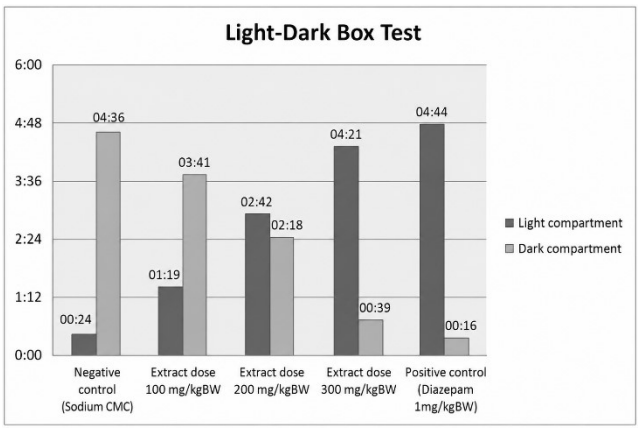

Figure 3. Diagram of Light–Dark Box Test Results

Figure 3 illustrates the comparative duration spent in the light and dark compartments during the Light–Dark

Box assessment. Mice in the negative control group predominantly occupied the dark chamber throughout the observation period, reflecting heightened anxiety-like behavior. Conversely, treatment with *Syzygium myrtifolium* leaf extract resulted in a dose-dependent increase in the time spent in the illuminated compartment. A gradual rise in light-compartment duration was noted from the 100 mg/kgBW group to the 300 mg/kgBW group. The positive control group treated with diazepam at 1 mg/kgBW exhibited the longest duration in the light area, followed by the group receiving 300 mg/kgBW of the extract. This trend further substantiates the anxiolytic activity of the extract, particularly at higher doses.

Normality testing using the Shapiro–Wilk method demonstrated that the data were normally distributed ($p = 0.573$; $p > 0.05$). Therefore, statistical evaluation was conducted using a parametric One-Way ANOVA. The ANOVA results indicated statistically significant differences among treatment groups in terms of time spent in both the light and dark compartments ($p < 0.001$).

DISCUSSION

This study was conducted to evaluate the anxiolytic potential of ethanol extract of *Syzygium myrtifolium* leaves using experimental animal models. Anxiety is a common neurobehavioral disorder, and the exploration of plant-based alternatives has become an important approach in the development of safer therapeutic agents. Therefore, this research investigated the effectiveness of *Syzygium myrtifolium* leaf extract through a series of stages, including extraction, phytochemical screening, and behavioral testing using Elevated Plus Maze (EPM) and Light–Dark Box (LDB) methods.

The yield of *Syzygium myrtifolium* leaf ethanol extract obtained in this study was 16.69%. This result is consistent with previous studies, where (Puspaningrum & Muflihah, 2025) reported a yield of 12.88%, while (Sugihartini & Maryati, 2022) obtained a yield of 20.13%, both using a maceration method with 96% ethanol as a solvent. Variations in yield values between studies are normal because they are influenced by differences in raw material conditions, extraction methods, and process parameters used.

Phytochemical analysis was performed to determine the presence of secondary metabolites within the extract. Phytochemical screening revealed that the ethanol extract of *Syzygium myrtifolium* leaves contained several groups of secondary metabolites, including flavonoids, alkaloids, saponins, tannins, and terpenoids. Alkaloid compounds in the extract were detected using both Mayer and Dragendorff reagents. The addition of Mayer reagent produced a cream-colored precipitate, while the addition of Dragendorff reagent resulted in an orange-red to reddish-brown precipitate, both indicating positive reactions for alkaloid presence in the sample. These qualitative tests are standard procedures in phytochemical screening for alkaloids and have been previously reported in phytochemical studies (Ahmed et al., 2020).

Flavonoid identification was performed using the Shinoda reaction by introducing magnesium (Mg) powder and concentrated hydrochloric acid (HCl) into the extract solution. After the addition of the reagent, the solution changed color to reddish, indicating the presence of flavonoid compounds in the extract. This color change occurs due to the reduction reaction of the benzopyrone nucleus in the flavonoid structure by magnesium in an acidic environment, thus forming a colored compound that can be observed visually. research that reported that the flavonoid test with Mg and HCl reagents resulted in a color change from pink to orange-red as a positive indicator of flavonoids (Humbare et al., 2022).

The qualitative tannin test in this study showed a positive result, indicated by a blackish-green color change in the extract after the addition of 1% $FeCl_3$ reagent. Phytochemical testing using 1% $FeCl_3$ was used to determine phenol content. The occurrence of phenolic groups is demonstrated by the appearance of a blackish-green or dark blue coloration following the addition of 1% $FeCl_3$. Accordingly, a positive reaction with 1% $FeCl_3$ confirms the presence of phenolic constituents in the sample, possibly tannins, as tannins are polyphenolic compounds. These results align with previous research, which reported that the addition of 1% $FeCl_3$ to an extract containing tannins also produces a blackish-green color, a positive indicator of the presence of tannins (Triana Kusumaningsih et al., 2015).

Positive results were obtained from the saponin test due to the presence of stable foam was produced after vigorous shaking with hot distilled water, and remained stable after the addition of 2 N HCl. This result is in line with previous research which reported that the method of adding hot water followed by vigorous shaking produces stable foam as a positive indicator of saponins (Dhamapada et al., 2024).

A positive result for terpenoids was obtained due to the formation of a brown ring at the solution boundary after the addition of Liebermann–Burchard reagent. The appearance of this color suggests the presence of terpenoid compounds in the extract, which is caused by the dehydration and condensation reactions of the terpenoid compounds with the reagent, forming a distinctive colored complex. These results are consistent with previous research that reported that in the Liebermann–Burchard test, extracts containing terpenoids will show a color change to brown or violet as a positive indicator of the presence of terpenoids (Muzani et al., 2021).

The Elevated Plus Maze (EPM) is used as an anxiety testing method because it exploits the instinctive response of test animals, such as mice, which naturally avoid open and exposed areas. In this apparatus, the open

arms without walls are considered an anxiogenic stimulus, so the animals spend more time in the closed arms. This behavior allows researchers to assess anxiety levels through parameters such as the time spent in each arm during the test (Cerqueira et al., 2023). Results of the Elevated Plus Maze test indicated that the *Syzygium myrtifolium* leaf extract enhanced open-arm exploration, reflecting reduced anxiety levels. Doses of 200 and 300 mg/kgBW yielded the strongest responses, approaching the effect of diazepam. Assessment of data normality demonstrated that the values were normally distributed ($p > 0.05$). Further evaluation with a One-Way ANOVA showed significant differences between the treatment groups ($p < 0.05$), suggesting that administration of the extract had a meaningful effect on anxiety-like behavior in mice.

In the light-dark box test, the anxiolytic effect is based on the natural characteristics of test animals, particularly mice, which tend to avoid bright areas and prefer dark areas. This response reflects a state of anxiety when the animals are in an open, bright environment. When experiencing anxiety, mice will spend less time in the lighted room and more time in the dark. Thus, the difference in time spent in these two areas can be used to assess anxiety levels (Tucker & McCabe, 2021). In this test, mice given *Syzygium myrtifolium* leaf extract demonstrated a longer duration in the light compartment and a reduced duration in the dark compartment. The most substantial effects were observed at doses of 200 mg/kgBW and 300 mg/kgBW, yielding responses comparable to diazepam. Normality testing indicated that the data were normally distributed ($p > 0.05$), and an ANOVA test ($p<0.05$) confirmed that the extract had a significant anxiolytic effect.

The anxiolytic effects observed in this study are strongly suspected to be related to flavonoids were detected as one of the secondary metabolites present in the ethanolic extract of *Syzygium myrtifolium* leaves. Flavonoids are bioactive compounds reported to play a crucial role in regulating emotional behavior through their ability to modulate central neurotransmitter systems. These compounds are capable of influencing several neurochemical pathways involved in regulating anxiety, including the serotonergic, dopaminergic, GABAergic, and glutamatergic systems. Furthermore, flavonoids exhibit potent antioxidant and anti-inflammatory activities, which may reduce oxidative stress and neuroinflammation in the brain, two key factors known to contribute to the development of anxiety disorders. Epidemiological evidence also suggests that higher dietary flavonoid intake is significantly associated with a lower prevalence of anxiety symptoms in adults, suggesting that flavonoids may function as natural anxiolytic agents (Jin et al., 2025).

Anthocyanins, which belong to the flavonoid subclass, are likely to contribute substantially to the anxiolytic potential of the extract. Anthocyanins are natural pigments widely distributed in plants and are well recognized for their strong antioxidant capacity. These compounds can neutralize free radicals and reduce the formation of reactive oxygen species, thereby safeguarding neuronal cells against oxidative injury. Furthermore, anthocyanins possess anti-inflammatory and neuroprotective effects that contribute to the maintenance of normal central nervous system function. Through these mechanisms, anthocyanins help to preserve neuronal integrity, improve neurochemical balance, and prevent oxidative stress–induced neuronal dysfunction, all of which are closely related to emotional regulation and anxiety reduction (Singla et al., 2025).

CONCLUSIONS

The ethanolic extract of *Syzygium myrtifolium* leaves exhibited anxiolytic potential in white mice. Administration at doses of 200 mg/kgBW and 300 mg/kgBW significantly increased exploratory behavior in both the Elevated Plus Maze and Light–Dark Box tests compared to the negative control group ($p < 0.05$), indicating a reduction in anxiety-like responses. Phytochemical screening revealed the presence of flavonoids and other secondary metabolites that may underlie the observed pharmacological activity. Collectively, these findings suggest that *Syzygium myrtifolium* leaf extract may serve as a promising plant-derived candidate for the management of anxiety.

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Authors' Contributions: Sari Nurdian designed the research, conducted laboratory experiments, performed data analysis, and prepared the manuscript. Triyana Agustin contributed to laboratory analysis as well as data processing and statistical analysis. Novita Yelica Umina and Aziz Suhada prepared and managed all research instruments and materials required during the study. Apt. Helman Kurniadi, S.Farm., M.Farm supervised the research process and provided guidance in manuscript preparation and publication requirements. All authors reviewed the manuscript and provided necessary input before approving the final version for publication.

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REFERENCES

Ahmed, Z., Sohail, A., Hanif, M., Mohiuddin, S. G., Sayed Hurmat Ali Khan1, R. A., & Siti Maisharah Sheikh Ghadzi, A. N. B. (2020). Phytochemical Screening and Enzymatic and Antioxidant Activities of Erythrina suberosa (Roxb) Bark. *Journal of Pharmacy and Bioallied Sciences*, *12*(2), 192–200. https://doi.org/10.4103/jpbs.JPBS

Cerqueira, M. M. de F., Castro, M. M. L., Vieira, A. A., Kurosawa, J. A. A., Junior, F. L. do A., Mendes, F. de C. C. de S., & Kronka, M. C. (2023). Heliyon Comparative analysis between Open Field and Elevated Plus Maze tests as a method for evaluating anxiety-like behavior in mice. *Heliyon*, *9*(4), e14522. https://doi.org/10.1016/j.heliyon.2023.e14522

Dhamapada, N., Made, N., Sanjiwani, S., Nyoman, N., & Udayani, W. (2024). Pengujian Fitokimia dan Penentuan Kadar Senyawa Saponin Pada Ekstrak Etanol Bunga Telang (Clitoria ternatea L .) Phytochemical Identification and Determination of Saponin Compound Content in the Ethanol Extract of Butterfly Pea Flower (Clitoria ternatea. *USADHA: Jurnal Integrasi Obat Tradisional*, *3*(1), 8–13.

Hasti, S., Renita, L., Santi, F., Anggraini, S., Sinata, N., & Rusnedy, R. (2022). Sub-Chronic Toxicity of Syzygium Myrtifolium Walp on Liver and Kidney Function. *Jurnal Ilmu Kefarmasian Indonesia*, *20*(1), 30–37.

Humbare, R. B., Sarkar, J., Kulkarni, A. A., Juwale, M. G., Deshmukh, S. H., Amalnerkar, D., Chaskar, M., Albertini, M. C., Rocchi, M. B. L., Kamble, S. C., & Ramakrishna, S. (2022). Anti-Proliferative Properties of Rubia cordifolia L . Extracts Prepared with Improved Extraction Conditions. *Antioxidants*, *11*(1006), 1–20.

Jin, L., Wu, L., Zhu, G., Yang, L., Zhao, D., He, J., & Zhang, Y. (2025). Association between dietary flavonoid intake and anxiety : data from NHANES 2017 – 2018. *BMC Public Health*, *25*(1477), 1–17.

Muzani, C. U., Handayani, R., Farmasi, J., Kemenkes, P., & Besar, A. (2021). Efek Perasan Daun Pandan Wangi (Pandanus amaryllifolius Roxb .) Untuk Membunuh Larva Nyamuk Aedes aegypti PENDAHULUAN Indonesia merupakan salah satu negara terbesar yang beriklim tropis . Nyamuk masih sering menjadi salah satu penyebab penyakit diiklim. *Jurnal JIFS : Jurnal Ilmiah Farmasi Simplisia*, *2021*(1), 104–111.

Puspaningrum, D., & Muflihah, C. H. (2025). AKTIVITAS SITOTOKSIK EKSTRAK ETANOL DAN METANOL DAUN PUCUK MERAH (Syzygium myrtifolium Walp.) TERHADAP SEL HELA CYTOTOXIC ACTIVITY OF ETHANOL AND METHANOL EXTRACTS OF RED SHOOTS LEAFS (Syzygium myrtifolium Walp.) AGAINST HELA CELLS. *Usadha: Journal of Pharmacy*, *4*(2), 160–173. https://jsr.lib.ums.ac.id/index.php/ujp

Rahangga, D. G. O., Hair, L., Sasmita, W. O. I., & Sahidin, S. (2018). Efek Ansiolitik Ekstrak Etanol Kangkung Air (Ipomea aquatica) dalam Mengurangi Perasaan Cemas. *Pharmauho: Jurnal Farmasi, Sains, Dan Kesehatan*, *4*(1), 34–38. https://doi.org/10.33772/pharmauho.v4i1.4632

Rahman, S. M. M., Rana, S., Islam, M. N., Kumer, A., Hassan, M. M., Biswas, T. K., & Atikullah, M. (2019). Evaluation of Anxiolytic and Sedative-Like Activities of Methanolic Extract of Euphorbia hirta Leaves in Mice. *Pharmacology & Pharmacy*, *10*(06), 283–297. https://doi.org/10.4236/pp.2019.106023

Roest, A. M., De Vries, Y. A., Al-Hamzawi, A., Alonso, J., Ayinde, O. O., Bruffaerts, R., Bunting, B., Caldas De Almeida, J. M., De Girolamo, G., Degenhardt, L., Florescu, S., Gureje, O., Haro, J. M., Hu, C., Karam, E. G., Kiejna, A., Kovess-Masfety, V., Lee, S., McGrath, J. J., … De Jonge, P. (2021). Previous disorders and depression outcomes in individuals with 12-month major depressive disorder in the World Mental Health surveys. *Epidemiology and Psychiatric Sciences*, *30*(Cidi), 3–9. https://doi.org/10.1017/S2045796021000573

Singla, R., Kamboj, S., Kumar, B., Bhargava, A., & Chaudhary, J. (2025). Medicine in Drug Discovery Flavonoids and anxiety : decoding their role in brain function and pathophysiology. *Medicine in Drug Discovery*, *27*(May), 100214. https://doi.org/10.1016/j.medidd.2025.100214

Sugihartini, A., & Maryati, M. (2022). UJI AKTIVITAS ANTIOKSIDAN EKSTRAK DAUN PUCUK MERAH (Syzygium myrtifolium) DAN PENETAPAN KADAR FENOL TOTAL. *Usadha Journal of Pharmacy*, *1*(3), 267–277. https://doi.org/10.23917/ujp.v1i3.77

Syafriana, V., & Wiranti, Y. (2022). POTENSI DAUN TANAMAN PUCUK MERAH (Syzygium myrtifolium Walp.) SEBAGAI AGEN ANTIBAKTERI TERHADAP Streptococcus mutans. *Farmasains*, *9*(2), 65–75.

Triana Kusumaningsih, Asrilya, N. J., Wulandari, S., Tri, D. R., Wardani, & Fatikhin, K. (2015). PENGURANGAN KADAR TANIN PADA EKSTRAK STEVIA REBAUDIANA DENGAN MENGGUNAKAN KARBON AKTIF. *ALCHEMY Jurnal Penelitian Kimia*, *11*(1), 81–89.

Trihono. (2023). Survei Kesehatan Indonesia 2023 (SKI). *Kemenkes*, 235.

Tucker, L. B., & McCabe, J. T. (2021). Measuring Anxiety-Like Behaviors in Rodent Models of Traumatic Brain Injury. *Frontiers in Behavioral Neuroscience*, *15*(October), 1–16. https://doi.org/10.3389/fnbeh.2021.682935

Widyastiwi, Muzaki, Y. A. R., & Roseno, M. (2023). Aktivitas Antiansietas Ekstrak Etanol Kulit Buah Pisang Cavendish (Musa acuminata Cavendish): Studi In Vivo dengan Metode Elevated Plus Maze (EPM), Light Dark Test (LDT), and Open Field Test (OFT). *MPI (Media Pharmaceutica Indonesiana)*, *5*(1), 60–70. https://doi.org/10.24123/mpi.v5i1.5576

Wu, Y., Li, X., Ji, X., Ren, W., Zhu, Y., Chen, Z., & Du, X. (2025). Trends in the epidemiology of anxiety disorders from 1990 to 2021: A global, regional, and national analysis with a focus on the sociodemographic index. *Journal of Affective Disorders*, *373*(October 2024), 166–174. https://doi.org/10.1016/j.jad.2024.12.086