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The Effect of Sacha Inchi Leaf Extract (*Plukenetia volubilis*) with Different Doses on Reducing Uric Acid Levels in Mice (*Mus musculus*)

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Abstract

Sacha inchi (*Plukenetia volubilis*) is an ancestral plant native to the Amazon rainforest that has been adopted as a food source due to its high nutritional value, which has gradually been recognized for its potential health benefits for humans. One of its benefits is as a natural agent for lowering uric acid levels, due to its antioxidant content and active compounds such as flavonoids, which function as anti-inflammatory and cell protective agents. Although *P. volubilis* seeds have been extensively studied, the potential of its leaves as an alternative treatment for hyperuricemia remains largely unexplored. This study aims to determine the effect of sacha inchi (*P. volubilis*) leaf extract at different doses 0.35 mL/mouse, 0.40 mL/mouse, and 0.45 mL/mouse on reducing uric acid levels in mice (*Mus musculus*) and to identify the most effective dose for reducing uric acid levels in mice. This study used 24 male mice aged 3–4 months with a body weight not exceeding 25 g. The study design used a Randomized Block Design (RBD) with four treatments and six replicates. The treatments consisted of control (without extract), doses of 0.35 mL, 0.40 mL, and 0.45 mL of *P. volubilis* leaf extract. The *P. volubilis* leaf extract was administered orally via a mouth tube. The extract was obtained from the leaves of the *P. volubilis* using the maceration method. The mice were first induced with hyperuricemia using 1 mL of chicken liver juice per mouse. Uric acid levels were measured before and after treatment using a GCU test strip. Data were analyzed using a one way ANOVA test followed by a Duncan test at a significance level of 5%. The results showed significant differences between treatments ($p = 0.001 < 0.05$). The 0.45 mL dose was the most effective in lowering uric acid levels, from an average of 4.7 mg/dL to 3.4 mg/dL, and was significantly different from other doses based on Duncan's test. This study concluded that sacha inchi leaf extract has the potential to be used as a natural alternative for lowering blood uric acid levels.

Keywords: flavonoids; leaf extract; *Mus musculus; Plukenetia volubilis*; uric acid

INTRODUCTION

Uric acid is the end product of the catabolism (breakdown) of a substance called purine. Purine is a natural substance that is one of the chemical structures that make up DNA and RNA. There are two main sources of purines, produced by the body itself and those obtained from dietary intake, such as plants or animals. Uric acid actually has functions in the body, such as acting as an antioxidant and aiding in cell regeneration. The body's metabolism naturally produces uric acid. Uric acid becomes a problem when its levels in the body exceed the normal range, the global prevalence of gout in 2017 was 34.2% (Novianti *et al.*, 2019; Zainaro *et al.,* 2021). Gout is common in developed countries such as the United States. In many countries, such as the United States, the prevalence of gout is 26.3% of the total population there. The prevalence of gout in the United States is 13.6 per 1,000 men and 6.4 per 1,000 women (Prasetyaningrum & Amalia, 2018; Widiyawati & Muthoharoh, 2023). However,

an increase is also observed in developing countries, such as Indonesia. Indonesia has the fourth highest incidence of gout in the world, with an incidence rate of 81%. Meanwhile, according to the results of the Basic Health Research, Indonesia has three regions with high gout prevalence. These are East Nusa Tenggara at 33.1%, West Java at 32.1%, and Bali at 30%. The diagnose incidence of gout is 11.9%, and the asymptomatic incidence is 24.7% (Ministry of Health of the Republic of Indonesia 2014). Gout is estimated to affect 840 out of 100,000 people. Gout is more common in 37.2% of people aged 35–44 years, 45% in those aged 45–54 years, 24.3% of men, and 11.7% of women (Novianti *et al*., 2019; Widiyawati & Muthoharoh, 2023). It is around 81% of Indonesian who experienced gout, yet only 24% visit a doctor, while 71% tend to directly consume over the counter pain relievers women (Prasetyaningrum & Amalia, 2018).

Risk factors contributing to gout include age, excessive intake of purine compounds, excessive alcohol consumption, obesity, lack of physical activity, hypertension and heart disease, use of certain medications (especially diuretics), and kidney dysfunction (Dewi & Rini, 2020; Fitriyani *et al*., 2022; RJ *et al*., 2023). Hyperuricemia in humans characterized by uric acid levels exceeding 7.5 mg/dL is caused by excessive consumption of high purine foods or impaired renal excretion and in addition to triggering gout or gouty arthritis due to the accumulation of uric acid crystals in the joints, it is also a strong predictor of death from cardiovascular damage, particularly due to the low awareness of the public in maintaining a healthy diet and lifestyle (Yong *et al*., 2016). Indonesian society tends to prefer self medication using herbal ingredients as an alternative. Some traditional medicinal plants commonly used to help lower uric acid levels include African leaf (*Vernonia amygdalina* Del), soursop leaf, cat's whiskers, meniran, and sambiloto. According to research by Humaira *et al*. (2022), African leaf extract effectively lowers blood glucose levels in mice, with an optimal dose of 0.40 mL. This demonstrates the potential of herbal plants in the treatment of hyperglycemia and hyperuricemia. Herbal plants are considered safer for long term use compared to synthetic drugs because they have fewer side effects (Pane *et al*., 2021).

One plant that has the potential to lower uric acid levels is sacha inchi (*P. volubilis*), which originates from the Amazon rainforest and is now cultivated in Indonesia. The leaves of this plant are known to contain active compounds such as flavonoids, saponins, tannins, and phenolic compounds that act as antioxidants and anti-inflammatory agents (Wang *et al*., 2018; Sari *et al.*, 2024). Research by Nurhamidah *et al*. (2022) shows that ethanol extract of kaik-kaik root (*Uncaria cordata*) can lower blood uric acid levels in mice induced with potassium oxonate and fed a high-purine diet. This indicates the potential of this plant as a natural alternative therapy for anti-hyperuricemia. Indonesia is the country with the greatest biodiversity in the world, with more than 30,000 species of plants that have medicinal properties, as proven by scientific research. Only about 180 of these species have been used in traditional medicine by the Indonesian traditional medicine industry (Masriana *et al*., 2023; Nisa *et al*., 2024).

P. volubilis can be grown in various regions of Indonesia, particularly in areas with elevations between 100 and 1,700 meters above sea level (Wang *et al*., 2018; Tianara *et al*., 2024). Morphologically, the fruit has a star shaped form, with each fruit containing 4–5 seeds. Young fruits are green in color, while mature fruits are dark brown (Noormansyah *et al*., 2023). *P. volubilis* is relatively easy to cultivate in tropical regions, including Indonesia, and has begun to be cultivated in several countries such as Thailand, Vietnam, Malaysia, and China (Van *et al*., 2022; Rahman *et al*., 2023). Almost all parts of the plant can be utilized, including the seeds, shells, and leaves (Cárdenas *et al.*, 2021; Rodzi & Lee, 2022). In this study, the part of the plant used is the leaves. *P. volubilis* leaves are known to contain active compounds such as flavonoids, tannins, saponins, and phenolic compounds that have antioxidant and anti-inflammatory activities (Chirinos *et al.,* 2016; Sari *et al*., 2024).

Previous studies have investigated the potential of *P. volubilis* seed as a source of oil rich in omega 3 fatty acids, which are beneficial for lowering cholesterol levels, improving lipid profiles, and acting as anti-inflammatory agents (Gutiérrez *et al.*, 2019; Supriyanto *et al*., 2022). Oil extracted from the seed also has various additional benefits, both in the fields of health and cosmetics. In the cosmetic

field, this oil functions as a moisturizer and skin brightener. Meanwhile, in the health field, *P. volubilis* oil is known to have the potential to lower cholesterol and uric acid levels, improve cognitive function, reduce the risk of heart failure and stroke, inhibit tumor activity, alleviate knee joint inflammation, enhance vision, and reduce tingling sensations (Noormansyah *et al*., 2023; Maya *et al*., 2024; Rodzi *et al*., 2025). However, research focusing specifically on the use of *P. volubilis* leaves to lower blood uric acid levels remains limited. Meanwhile, research focused on the use of other plant parts as agents to lower uric acid levels remains limited. A recent study revealed that ethanol extract from the roots of *Uncaria cordata*, significantly reduced uric acid levels in potassium oxonate induced hyperuricemic mice and also anti hyperuricemia activity of nettle plant extract (*Urtica dioica* L.) in mice (Fadilah & Susanti, 2020; Nurhamidah *et al.* 2022). These findings confirm that the utilization of local biological resources can be developed as a safer herbal therapy alternative in the treatment of gout (uric acid arthritis).

Based on the issues outlined above, the low level of public awareness in dealing with gout and the limited research on the use of *P. volubilis* leaves as an alternative therapy encourage further exploration of the potential of this plant. Therefore, the author is interested in investigating the effect of *P. volubilis* leaf extract at different doses on reducing blood uric acid levels in white mice (*Mus musculus*) using the uric acid strip test (GCU) method. The objective of this study is to determine the effect of sacha inchi leaf extract (*Plukenetia volubilis*) at different doses 0.35 mL/mouse, 0.40 mL/mouse, and 0.45 mL/mouse on reducing uric acid levels in mice (*Mus musculus*) and to identify the most effective dose for reducing uric acid levels in mice.

METHOD

This study used a laboratory experimental method to determine the effect of *P. volubilis* leaf extract on reducing blood uric acid levels in *Mus musculus*. This method was chosen because it allows researchers to control variables and directly observe the effects of specific treatments. The study was conducted in June 2024 at the Biology Laboratory, Garut Regency. The part of the plant used in this study was the leaves, obtained from *P. volubilis* plants grown in the *P. volubilis* cultivation garden in Ciamis, West Java. The raw material, consisting of 2 kg of *P. volubilis* plant leaves, was first washed with running water and drained in a shaded area. The leaves were then cut into small pieces and subjected to pure extraction with the addition of 1 L of distilled water. The *P. volubilis* leaves were ground periodically using a blender. After grinding, the mixture was filtered using a sieve to remove any residue. The extract was divided into labeled containers for each treatment group, consisting of four groups with doses of control (without extract) 0.35 mL, 0.40 mL, and 0.45 mL. The experimental animals in this study were *Mus musculus* weighing approximately 20–25 g. The normal uric acid level in mice is 1.7–3.0 mg/dL, and levels above 3.0 mg/dL are considered hyperuricemia (Apriani *et al*., 2016).

This study used RBD consisting of four treatment groups: control (without extract) and three treatment groups with different doses of *P. volubilis* leaf extract. Each group consisted of six healthy male mice aged 2–3 months with relatively uniform body weight. The total number of samples was 24 mice randomly selected from local breeders in Karangpawitan, Garut. The mice were first induced with chicken liver juice for 7 days to increase uric acid levels (hyperuricemia). Subsequently, the extract was administered orally via a syringe, once daily for 7 consecutive days. Data collection was performed by measuring blood uric acid levels in mice using the EasyTouch GCU device with uric acid strips. The examination was conducted twice, before and after treatment. The results of the uric acid level measurements were statistically analyzed using the normality test (Shapiro-Wilk) and the homogeneity test (Levene's test) to ensure that the data distribution met the requirements for parametric analysis. Subsequently, a one-way ANOVA test was performed to determine whether there were differences between groups, and a Duncan's post-hoc test was conducted to identify which groups differed significantly ($\alpha = 0.05$). The statistic test uses the help of software IBM SPSS Statistic *25.*

RESULT AND DISCUSSION

The results of the one-way ANOVA test regarding the value of reducing uric acid levels in mice using *P. volubilis* leaf extract can be seen in Table 1.

Table 1. ANOVA test results of the decrease in uric acid levels in mice (*Mus musculus*)

ANOVA Uric Acid Levels	Sof Squares	Df	Mean Square	F	Sig.
Between Groups	4.943	2	2.472	7.987	0.004
Within Groups	4.642	15	0.309		
Total	9.585	17			

Based on the results of the normality test (Shapiro-Wilk) and homogeneity test (Levene's test), the data were declared to be normally distributed and have homogeneous variance, so they can be further analyzed using a one-way ANOVA test. The results of the ANOVA test at a significance level of 5% showed a sig. value of $0.001 < \alpha = 0.05$, indicating a significant difference between treatment groups. Therefore, it can be concluded that the administration of *P. volubilis* leaf extract has a significant effect on reducing blood uric acid levels in *Mus musculus*. The differences in uric acid levels can be seen in Table 2.

Table 2. Duncan test results of the decrease in uric acid levels in mice (*Mus musculus*)

Uric Acid Levels Duncan				
		Subset for alpha = 0.05		
Treatment	N	1	2	3
Control	6	3.283	2.472	7.987
Dose of sacha inchi leaf exctract 0.45mL	6	3.433	0.309	
Dose of sacha inchi leaf exctract 0.40mL	6		4.100	
Dose of sacha inchi leaf exctract 0.35mL	6			4.716
Sig		0.601	1.000	1.000
Means for Groups in homogeneous subsets are displayed				
Uses harmonic mean sam;e size=6.000				

Based on the results of Duncan's test, there were differences in blood uric acid levels between treatment groups. However, to determine whether these differences were statistically significant, the significance value (p-value) needed to be examined. From Table 1, the one-way ANOVA test shows a significance value of 0.004 ($p < 0.05$), indicating a significant difference between the treatment groups of *P. volubilis* leaf extract. This serves as evidence that the administration of *P. volubilis* leaf extract does indeed have a significant effect in reducing uric acid levels in mice. The pattern of uric acid level reduction shows that the higher the dose of extract administered, the greater the reduction in uric acid levels. The 0.45 mL dose showed the most significant reduction compared to the 0.40 mL and 0.35 mL doses. This phenomenon is consistent with the theory that increased concentrations of active compounds, such as flavonoids, tannins, and phenols, can provide stronger pharmacological effects on the inhibition of xanthine oxidase, which plays a role in uric acid (Chirinos *et al*., 2016; Sari *et al*., 2024). Comparison with the literature shows supporting results. Research by Nurhamidah *et al.* (2022) states that ethanol extract of kaik-kaik root effectively lowers blood uric acid levels in mice after induction with potassium oxonate, with a significant percentage decrease at a dose of 21.2 mg/30g. Additionally, the African plant *Vernonia amygdalina*, which belongs to the same group of tropical herbal plants, has also been shown to lower blood glucose levels in mice through its flavonoid content, which operates via a similar mechanism to the inhibition of uric acid formation (Humaira *et*

al., 2022; Adewuyi *et al*., 2025).

The possible cause of this decrease in uric acid levels is the secondary metabolite content in sacha inchi leaves, particularly flavonoids and phenols, which are known to inhibit the xanthine oxidase enzyme. This mechanism was also reported by Chirinos *et al*. (2016) who studied the antioxidant activity of Amazonian plants, including *P. volubilis*, which contains high levels of phenolic compounds. These compounds contribute to anti-inflammatory effects and the reduction of hyperuricemia. Thus, the higher the dose of extract administered, the greater the concentration of active compounds working in the mice's bodies, explaining the increasingly significant reduction in uric acid levels at the 0.45 mL dose. The effect of giving *P. volubilis* leaf extract on decreasing uric acid levels in *Mus musculus*. Based on the results of the study, the graph can be seen in the Figure 1.

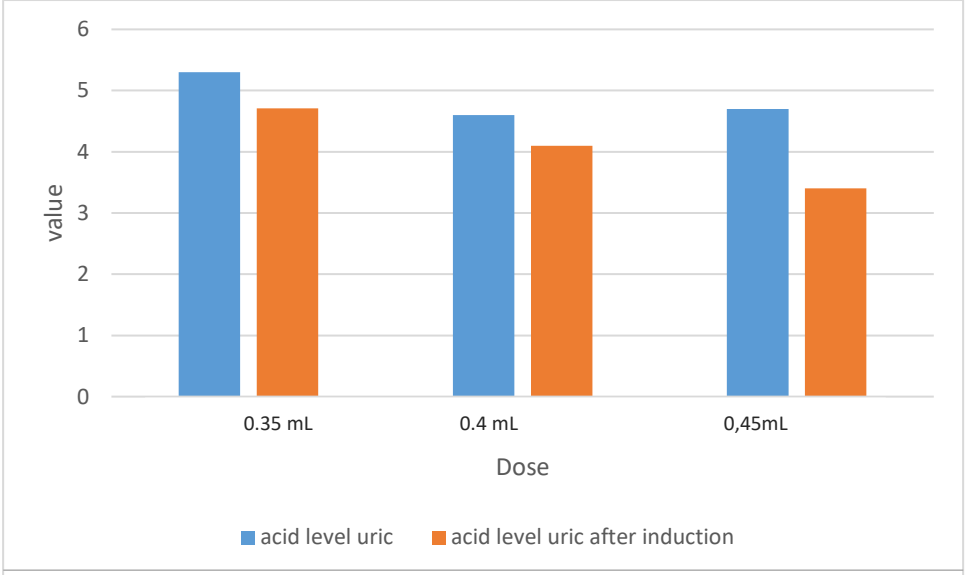

Figure 1. Graph of the decrease in uric acid levels given various doses of sacha inchi leaf extract (*Plukenetia volubillis*)

Based on the graph above, it is evident that the average decrease in uric acid levels at each dose shows varying results. At a dose of 0.35 mL, uric acid levels decreased from 5.3 mg/dL to 4.71 mg/dL, with an average decrease of 0.59 mg/dL. The 0.40 mL dose reduces uric acid levels from 4.6 mg/dL to 4.1 mg/dL (a decrease of 0.5 mg/dL), and the 0.45 mL dose shows the most significant decrease, from 4.7 mg/dL to 3.4 mg/dL (a decrease of 1.3 mg/dL). Next, hypothesis testing was performed using one-way ANOVA, the analysis yielded a significance value of 0.001, which is less than $\alpha = 0.05$. This indicates that the administration of *P. volubilis* leaf extract has a significant effect on reducing uric acid levels in *Mus musculus*.

These results are not only statistically proven, but can also be scientifically explained through the bioactive content in *P. volubilis* leaves. These leaves are known to contain secondary metabolites such as flavonoids, tannins, saponins, and phenolic compounds (Sari *et al*., 2024). Flavonoids and phenolic compounds exhibit high antioxidant and anti-inflammatory activity and are capable of inhibiting the activity of xanthine oxidase, an enzyme involved in the formation of uric acid from purines (Chirinos *et al*., 2016; Ullah *et al*., 2024). This mechanism of xanthine oxidase inhibition is one of the primary pharmacological strategies in the treatment of hyperuricemia and gout (Saigal & Agrawal, 2015; Aryan *et al*., 2024). Mice do not have xanthine oxidase enzymes, so they are unable to metabolize uric acid, but in mice, uric acid metabolism is influenced by uricase enzymes. The function of uricase enzymes is to convert uric acid into allantoin, which is easily excreted by the mouse body (Rahmawati & Kusumastuti, 2015; Feng *et al*., 2024). These results are consistent with

the findings of Nurhamidah *et al*. (2022), which showed that administration of ethanol extract of kaik-kaik root at various doses (5.3; 10.6; and 21.2 mg/30g) resulted in a significant decrease in uric acid levels in mice. This effect is believed to be due to the presence of secondary metabolites such as flavonoids, alkaloids, tannins, and saponins, which are known to inhibit the activity of xanthine oxidase, thereby preventing uric acid formation (Kumar *et al*., 2020; Singanusong & Jiamyangyuen, 2020). Additionally, these compounds also act as antioxidants capable of counteracting oxidative stress, one of the triggers of hyperuricemia. Kumar *et al.* (2014) and Lewis *et al.* (2021) reported that *P. volubilis* leaf extract contains strong antioxidant compounds that have the potential to counteract oxidative stress and free radicals, one of the triggering factors for hyperuricemia.

A similar effect was also reported that *Vernonia amygdalina* leaf extract on blood glucose reduction in mice (Singanusong & Jiamyangyuen, 2020; Humaira *et al*. 2022). Although not on uric acid parameters, the mechanism of flavonoids in modulating metabolism and inhibiting oxidative stress shows similar potential for hyperuricemia. The active compounds in soursop leaves can lower uric acid levels through antioxidant pathways, reinforcing the idea that flavonoids and phenolic compounds play a crucial role in herbal therapy for uric acid. The content of bioactive compounds in *P. volubilis* leaves has been confirmed in various studies. The seeds and leaves of this plant contain high levels of phenolic compounds and tocopherols, which exhibit strong antioxidant activity even after physical processing (Gutiérrez *et al*. 2019; Sharfina *et al*., 2025). This biological activity provides a strong basis for explaining that the higher the dose administered, the greater the pharmacological effect produced. Therefore, the administration of a 0.45 mL dose has been scientifically and statistically proven to be the most effective dose in reducing uric acid levels in mice. The graph of the research results can be seen in Figure 2.

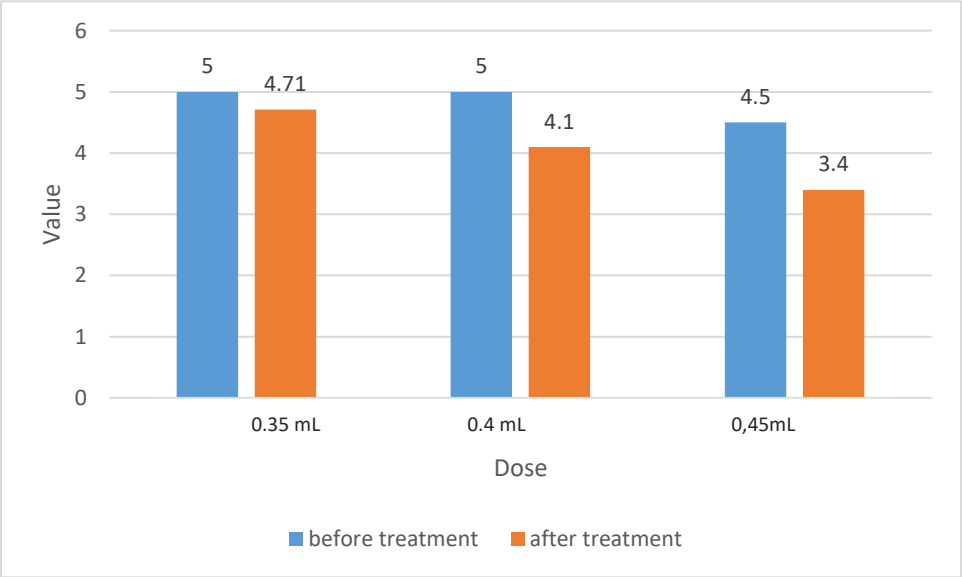

Figure 2. Graph of uric acid levels of mice before and after giving sacha inchi leaf extract (*Plukenetia volubilis*)

Based on Figure 2, the 0.45 mL dose of *P. volubilis* leaf extract showed the highest reduction in blood uric acid levels in mice compared to the 0.40 mL and 0.35 mL doses. The reduction in uric acid levels at the 0.45 mL dose was nearly twice as high as that at the 0.35 mL dose. This indicates that the 0.45 mL dose is the most effective in lowering blood uric acid levels in mice with hyperuricemia. Further one-way ANOVA testing using Duncan's test also supports this conclusion, where the group with the 0.45 mL dose statistically showed a significant difference compared to the other dose groups. The significance value obtained was below the 0.05 threshold, indicating a real difference between treatments in lowering

uric acid levels. When compared to the literature, the results of this study showed that ethanol extract of kaik-kaik root (*Uncaria cordata*) effectively reduced blood uric acid levels in mice induced by potassium oxonate and a high purine diet (Nurhamidah *et al*. 2022; Yunarto *et al*., 2024). Although the plants used differ, the biological mechanisms underlying the antihyperuricemic effects appear to involve secondary metabolites such as flavonoids, phenolics, saponins, and tannins. These compounds, such as flavonoids, phenolics, saponins, and tannins, are known to possess antioxidant, anti-inflammatory activities, as well as the ability to inhibit xanthine oxidase, which is the primary pharmacological target in the treatment of hyperuricemia (Nascimento *et al*., 2013; Chirinos *et al*., 2016; Gutiérrez *et al*., 2019). Additionally, a study by Humaira *et al.* (2022) on African leaves (*Vernonia amygdalina*), a plant also containing flavonoids, showed that an optimal dose of 0.40 mL of leaf extract effectively reduced blood glucose levels in mice. This indicates that the efficacy of secondary metabolites is significantly influenced by the concentration of active compounds entering the body, as well as their absorption rate and mechanism of action within the biological system of mice. Thus, the results of this study not only indicate the optimal dose for lowering uric acid levels but also reinforce the hypothesis that the pharmacological effects of sacha inchi leaves are dose dependent where an increase in dose yields more significant effects within certain limits. The effectiveness at a dose of 0.45 mL is believed to result from the accumulation of bioactive compounds that work synergistically to inhibit uric acid formation and enhance its excretion from the body.

During the study, mice that were initially active experienced behavioral changes, becoming lethargic and inactive after being given chicken liver juice. This condition is thought to be due to the high purine content in chicken liver, which can significantly increase uric acid levels in the mice's bodies. Elevated uric acid levels in the blood can trigger inflammation and joint pain, causing the mice to exhibit passive and unresponsive behavior, as explained by Saigal & Agrawal (2015) that high uric acid levels can lead to gouty arthritis and other systemic symptoms. After being treated with *P. volubilis* leaf extract, the mice began to show increased activity again. This can be attributed to the presence of active compounds such as flavonoids and antioxidants in *P. volubilis* leaves, which have anti-inflammatory effects and reduce uric acid levels (Chirinos *et al*., 2016; Sari *et al*., 2024). Anti-inflammatory effect has the same character with land snail mucus (Pertiwi *et al*., 2025; Pertiwi *et al*., 2025). These compounds play a role in inhibiting the xanthine oxidase enzyme involved in uric acid formation, thereby gradually reducing hyperuricemia symptoms and helping the mice recover to normal conditions. After being given *P. volubilis* leaf extract, a few moments later the mice began to be active again. Visible differences from the first dose of 0.35 mL of *P. volubilis* leaf extract, where the mice still looked lethargic and less active, to the second dose of 0.40 mL of *P. volubilis* leaf extract, where the mice were sometimes lethargic and sometimes active, and to the third dose of 0.45 mL of *P. volubilis* leaf extract, where the mice were active as before. The effect of reduced uric acid levels in mice in this study is strongly suspected to originate from the flavonoid compounds in *P. volubilis* leaves. Flavonoids are known to have antioxidant and anti-inflammatory activities and can inhibit the activity of xanthine oxidase, an enzyme involved in uric acid production. By inhibiting this enzyme's activity, uric acid production can be suppressed, thereby reducing uric acid levels in the blood. Indicates that *P. volubilis* leaves contain high levels of phenolic compounds and flavonoids, which have potential as agents against hyperuricemia (Chirinos *et al*., 2016; Sari *et al*., 2024).

Thus, the administration of *P. volubilis* leaf extract has been statistically and biologically proven to reduce blood uric acid levels in mice, with the best effect observed at a dose of 0.45 mL. This finding contributes significantly to the development of herbal therapy alternatives for hyperuricemia based on local plants. This potential is also in line with initiatives to develop medicinal plants to strengthen national drug self-reliance (Sholikhah, 2016; Noormansyah *et al*., 2023). *P. volubilis* leaf extract, rich in flavonoids and phenols, has been shown to inhibit uric acid production through the mechanism of xanthine oxidase enzyme inhibition, thereby offering potential for development as a safe, natural, and effective phytotherapy (Nascimento *et al*., 2013; Kodahl & Sørensen, 2021).

CONCLUSION

Based on the results of the study, it can be concluded that administering *P. volubilis* leaf extract at different doses has a significant effect on reducing blood uric acid levels in mice. The 0.45 mL dose was found to be the most effective in lowering uric acid levels compared to other doses. These research findings that *P. volubilis*, commonly used for medicinal purposes, has been scientifically proven to have potential as a safe and natural source of phytopharmaceuticals for the treatment of hyperuricemia. This finding needs to be continued in the next test to be more valid. This discovery brings significant benefits at the global level, as it contributes to the advancement of local medicinal plants. By promoting the use of these plants, it supports the development of alternative treatment options that are not only more affordable and accessible, but also environmentally sustainable. Moreover, this approach is highly relevant to addressing the specific health needs of the Indonesian population, aligning with both local traditions and modern medical demands.

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