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Therapeutic Potential of Aceh's *Syzygium polyanthum* in Reducing Uric Acid in *Rattus Norvegicus*

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Abstract

This research aims to evaluate the anti-hyperuricemic activity of *Syzygium polyanthum* ethanolic extract in hyperuricemic male rats (*Rattus norvegicus*) induced by liver juice. A total of 25 animals were divided into five groups: a negative control group, a positive control group, and three treatment groups receiving *S. polyanthum* extract at doses of 150, 200, and 250 mg/kg body weight, respectively. The result showed that the dose of 250 mg/kg body weight resulted in the highest decrease of uric acid plasma, measuring 3.44 ± 2.03 mg/dL. This reduction is comparable to the effect of allopurinol, which showed a decrease of 3.70 ± 1.54 mg/dL. A minimum dose-dependent activity was observed. To conclude, the administration of ethanolic extract of *S. polyanthum* for 14 days significantly reduced uric acid levels. Further exploration of higher doses or a long-term treatment period to enhance its effectiveness is needed.

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1. Introduction

Hyperuricemia in gout is characterized by increased uric acid levels in the blood [1]. The documented occurrence of gout globally varies widely, ranging from a prevalence of <1% to 6.8% and an incidence of 0.58–2.89 per 1,000 person-years. The management of gout remains inadequate, with fewer than half of the patients receiving effective 'curative' urate-lowering treatment. Obesity and associated health conditions are significant risk factors for gout and play a major role in the increasing prevalence and incidence of the disease [2, 3].

Uric acid is primarily the final product of an exogenous and endogenous of purines [4]. Uric acid is produced from nucleic acids, adenine, and guanine breakdown [5]. This process finally produces xanthine, oxidized to uric acid by the enzyme xanthine oxidase. The process occurs in the liver, intestines, and various tissues, such as muscles, kidneys, and vascular endothelium [6]. A standard drug, allopurinol, reduces uric acid by inhibiting the enzyme xanthine oxidase, an essential enzyme for the metabolic pathway of purines, converting hypoxanthine to xanthine and then xanthine to uric acid [7]. Despite its evidence-based effectiveness, allopurinol poses unwanted side effects, such as hypersensitivity [8] and agranulocytosis [9]. Consequently, there is growing interest in exploring alternative therapies, particularly traditional medicines, including herbs, for managing uric acid-related conditions. Traditional medicines are often perceived as safer due to their relatively lower incidence of side effects compared to conventional pharmaceuticals [10–12]. This has encouraged further

Figure 1. *Syzygium polyanthum* plant.

research into identifying and validating herbal remedies that could offer effective and safer options for managing elevated uric acid levels and related diseases.

A recent study showed the effectiveness of several plants in reducing uric acid levels, including *Sida rhombifolia, Syzygium polyanthum, Cyperus rotundus, Ruellia tuberosa,* and *Phaleria macrocarpa* [13]. Among these plants, *S. polyanthum* is a tree that typically produces more plant materials usable for medicinal purposes due to its large size, while the other is shrubs. Trees survive better in nature than smaller shrubs, making them more resilient and capable of thriving in various challenging environments. Their larger size and extensive root systems provide greater access to nutrients and water, producing secondary metabolites essential for their survival and medicinal properties.

S. polyanthum (Figure 1) contained alkaloids, saponin, terpenoids, and steroids [14]. This plant has been investigated for its antioxidant activity. The methanolic extract of *S. polyanthum* collected from East Java, Indonesia, showed potential antioxidant activity with an IC_{50} value of 44.35 µg/mL and 17.69 µg/mL measured with DPPH (2,2-diphenyl-2-picrylhydrazyl) and ABTS *(2,2'-azinobis (3-ethylbenzothiazole-6-sulfonic acid)* methods respectively [15]. The methanol extract of *S. polyanthum* leaves collected from Aceh showed an IC_{50} value of 25.68 µg/ml in the antioxidant activity test measured by the DPPH method [16]. This lower IC_{50} value indicates that the Aceh-sourced extract has high antioxidant activity, which is beneficial for managing oxidative stress-related diseases, including hyperuricemia. High antioxidant activity may help eliminate oxidative damage and inflammation associated with elevated uric acid levels.

This study aims to investigate the effectiveness of ethanol extract from *S. polyanthum* at doses of 150 mg/kg BW, 200 mg/kg BW, and 250 mg/kg BW in reducing uric acid levels in male Wistar rats (*Rattus Norvegicus*) with induced hyperuricemia. The induction was achieved using a high-purine chicken liver diet to simulate conditions of elevated uric acid. By evaluating different doses of the ethanol extract, this research seeks to identify the optimal dosage for managing hyperuricemia. The findings will contribute to the existing body of knowledge by providing insights into the efficacy of *S. polyanthum* in lowering uric acid levels. They may offer a potential therapeutic approach for managing hyperuricemia. This study's outcomes could influence future research directions and potential clinical applications of herbs in managing conditions related to elevated uric acid.

2. Materials and Methods

2.1. Plants and Animals

S. polyanthum leaves were harvested from Blang Bintang, Aceh Besar. The Wistar strain's male rats (*Rattus norvegicus*) were supplied from The Veterinary Faculty, Universitas Syiah Kuala. Animals were housed in cages with access to the water ad *libitum*. The test animals were acclimatized for seven days in a 12-hour light/12-hour dark cycle at a room temperature of 25 °C. They were fed pellets equivalent to 10% of their body weight (approximately 15-25 grams per animal per day) twice daily at 11:00 AM and 4:00 PM.

2.2. Preparation of Ethanolic Extract of Bay Leaves

S. polyanthum leaves (1500 grams) were washed with tap water and dried in a shaded area. The leaves were finely ground into a powder (500 grams) after drying. The powdered material was soaked in 1500 mL of 70% ethanol solvent for maceration. The mixture underwent a three-day extraction process with periodic stirring, replacing the solvent every 24 hours. All the filtrate was collected and concentrated using a vacuum rotary evaporator (Heidolph) at 60 °C to produce 15 grams of concentrated extract.

2.3. Induction of Hyperuricemia and Experimental Design

Fresh 300-gram chicken liver was washed, chopped into small pieces, and blended with 75 mL of distilled water. The relatively smooth liver juice was transferred into a container. Chicken liver juice was freshly prepared daily. The dosage of chicken liver juice induced in the test animals was 5 mL/200 g BW, given twice daily, and adjusted to the maximum fluid intake capacity of rats, which was 10 mL/200 g BW. Induction was administered orally after a 7-day acclimatization period. The induction of chicken liver juice at a dosage of 25 mL/kg BW given twice daily for seven days in male mice resulted in an average increase in uric acid levels of ± 3-4 mg/dL. Twenty-five test animals induced with chicken liver juice

Table 1. The uric acid levels of different groups.

Groups	Pre-test (mg/dL)	Post-test (mg/dL)	Decrease of uric acid (mg/dL)
NT	10.32±3.88	9.80±4.12	0.52±0.34
Alo	11.98±3.10	8.28±1.61	3.70±1.54
SP1	11.28±2.25	8.32±2.37	2.96±2.22
SP2	10.86±4.56	7.92±4.03	2.94±1.34
SP3	12.36±2.94	8.92±1.02	3.44±2.03

were randomly divided into five groups, each comprising 5 test animals.

- Group NT (no treatment): Test animals were only fed pellets and distilled water.
- Group Alo (Allopurinol): The test animals were fed pellets, distilled water, and allopurinol 5.4 mg/200 g BW.
- Group SP1 (Treatment 1): The test animals were fed pellets, distilled water, and 150 mg/kg BW of bay leaf extract.
- Group SP2 (Treatment 2): The test animals were fed pellets, distilled water, and 200 mg/kg BW of bay leaf extract.
- Group SP3 (Treatment 3): The test animals were fed pellets, distilled water, and 250 mg/kg BW of bay leaf extract.

Ethanol extracts of bay leaves and allopurinol were administered orally once daily to each group at 6 PM for 14 days, starting from day 15 of the study.

2.4. Sample Collection and Analysis

The test animals' uric acid levels were examined on day 14 (pretest) and day 28 (post-test) using the Glucose, Cholesterol, and Uric acid (GCU) Nesco Multi Check portable device. Blood was collected via the tail using a lancet. The first drop was wiped away with cotton, and the second was applied to a disposable strip. Uric acid measurement results appeared after 10 seconds, starting when the blood entered the strip. Uric acid levels were recorded in mg/dL.

2.5. Statistical Analysis

The data obtained were tested for normality (Shapiro-Wilk test) and homogeneity (Levene's test). A one-way ANOVA test was conducted to examine the significance of treatment for all groups, followed by the LSD (Least Significant Difference) method to determine differences between groups.

2.6. Ethical Considerations

Research ethics are based on medical ethics in treating test animals and the values that must be adhered to in the laboratory. Wound care for blood collection sites on the tails of test animals involves applying an Iodine solution to dry the wounds. After the post-test blood collection, test animals are euthanized using chloroform in a closed container until death, and then they are buried in the ground. Animal welfare ethics were conducted under the Faculty of Medicine Ethical Committee, Syiah Kuala University No 336/KE/FK/2015.

3. Results and Discussion

This study investigates whether administering ethanol extract of *S. polyanthum* for 14 days alleviates hyperuricemia induced by chicken liver juice. Liver juice contains high levels of purine, which stimulates increased uric acid. Table 1 shows that the uric acid level was about 10-12 mg/dL after administering a high purine diet for seven days. After 14 days of treatment (day 28), a decrease in the average uric acid levels was observed in all post-test groups with different grades of decline. The non-treated group showed the smallest decrease compared to other groups. The positive control group had the highest average decrease, followed by the three treatment groups.

Table 2 shows that the positive control group (Alo) and the treatment groups (SP1, SP2, and SP3) differ significantly ($p < 0.05$) from the negative control group, which shows the importance of the treatment on the uric acid decrease. The p-value also shows that the use of *S. polyanthum* extract gives the same activity level to the positive control group ($p \geq 0.05$). The result of the study also indicates a minimal dose-dependent effect on uric acid reduction across SP1, SP2, and SP3 groups despite dose escalation. However, SP3 (250 mg/kg BB) demonstrated the most significant uric acid reduction among the three doses tested, suggesting potential effectiveness at higher concentrations. The limitations of this study include the short-term treatment. Longer-term studies may show whether the increased doses will affect uric acid levels in cases of hyperuricemia or explain a dose-dependent manner in this extract activity. This will provide insights into optimal dosing regimens and any potential need for adjustments.

The decrease in uric acid levels in *S. polyanthum* extract groups is thought to be related to secondary metabolites such as flavonoids, phenolic acids, and alkaloids (Figure 2). Flavonoids interact with xanthine oxidase through hydrophobic interactions and hydrogen bonding. They

Table 2. The statistical analysis of uric acid decreases in different groups.

Groups		*p-value*
NT	Alo	0.006
	SP1	0.029
	SP2	0.030
	SP3	0.011
Alo	NT	0.006
	SP1	0.483
	SP2	0.471
	SP3	0.804
SP1	NT	0.029
	Alo	0.483
	SP2	0.985
	SP3	0.648
SP2	NT	0.030
	Alo	0.471
	SP1	0.985
	SP3	0.634
SP3	NT	0.011
	Alo	0.804
	SP1	0.648
	SP2	0.634

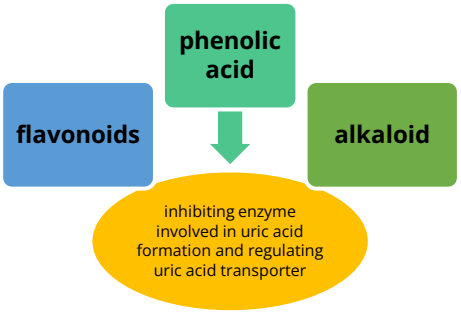

Figure 2. Role of secondary metabolites in uric elimination of uric acid.

may bind to the enzyme's hydrophobic cavity, potentially altering its structure and impacting both enzyme and substrate activity, which could decrease xanthine oxidase's catalytic efficiency. Additionally, flavonoids undergo various metabolic processes that contribute to reducing uric acid. They do this by modifying the internal environment of organs and affecting the expression of key genes and proteins (such as organic anion transporter 1, urate transporter 1, and glucose transporter 9) in the kidneys, liver, and intestines [17, 18]. Phenolic acids boost the levels of uric acid transporters like URAT1 (Urate Anion Transporter 1) and GLUT9 (Glucose Transporter 9), which help remove uric acid from the body [19]. Alkaloids enhance the expression of transporters such as ABCG2, transmembrane proteins that promote the secretion of urate, which aids in the elimination of uric acid from the body [20].

This research shows that the ethanolic extract of *S. polyanthum* was not much different from allopurinol, the positive control, in reducing uric acid levels in test animals. Allopurinol is an inhibitor of xanthine oxidase, which reduces the oxidation reactive group of xanthine oxidase. This allopurinol inhibition mechanism maintains stable uric acid synthesis [21]. Based on the results of this study, it can be seen that the ethanolic of *S. polyanthum* leaves can significantly reduce the uric acid levels of test animals compared to the negative control. Still, variations in the dose of 70% ethanol extract of bay leaves do not significantly affect the reduction in uric acid levels of test animals. However, the best ability to reduce uric acid levels in test animals was obtained from the positive control group, followed by a treatment dose of 250 mg/kg BW. Although this study did not conduct the identification of phytochemical compounds in *S. polyanthum*, the data on the role of phytochemicals described above can still be valuable. The information on how these compounds may influence biological activities or health outcomes can provide insights into the plant's potential benefits. This existing data can guide future research and support the exploration of specific phytochemicals in subsequent studies.

4. Conclusions

The result of this study supports the hypothesis that the administration of the 70% ethanol extract of *S. polyanthum* leaves for 14 days significantly lowers uric acid levels in *Rattus Norvegicus*. Altering the dosage of *S. polyanthum* did not significantly affect uric acid levels, suggesting that treatment duration may be more critical than dosage adjustments in achieving therapeutic outcomes. The limitations of this study include the relatively short observation period. Future studies should explore optimal dosing regimens and long-term effects to enhance the therapeutic efficacy of *S. polyanthum*. Toxicity evaluations are warranted to determine its safety in human subjects. Investigation of the mode of action is also important to comprehensively understand the underlying mechanisms, clarify the pathways involved, elucidate the biochemical interactions, and determine the precise molecular targets. The following work in the future can be to determine the most suitable dosage forms that can optimize the bioavailability, ensuring that the active components of the medication are effectively absorbed and utilized by the body. These findings suggest a shift in focus towards exploring treatment durations rather than solely adjusting dosages, potentially influencing future research directions and clinical strategies for hyperuricemia management. These findings indicate that *S. polyanthum* could be a promising therapeutic option for conditions associated with hyperuricemia, such as gout, and add knowledge on natural remedies for metabolic disorders. This finding

also provides evidence for the traditional use of *S. polyanthum* in preventing and treating hyperuricemia.

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