

Phytochemical Analysis, Acute Toxicity Test, and Activity Test of Salam Leaf Dipping Preparation with Natural Sweetener Stevia Leaves as Supportive Therapy for Patients at High Risk of Hypertension

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ABSTRACT

South Kalimantan ranks first as the province with the highest prevalence of hypertension in Indonesia. Salam leaves are widely used empirically by the community for the treatment of hypertension. The preparation of Salam leaf tea dip combined with the natural sweetener stevia leaves is an innovation in the use of Salam leaves. The objectives of the study were to determine the content and levels of phytochemicals, the formulation of the tea dip preparation, toxicity, and activity of Salam leaf tea dip preparation with natural sweetener stevia leaves in patients at high risk of hypertension. The research methods include analysis of the content and levels of phytochemicals, the formulation of the tea dip preparation, toxicity testing on white wistar rats, and testing the activity of the Salam leaf tea dip preparation in patients at high risk of hypertension. This study will produce tea bags from Salam leaves with natural sweeteners in the form of experimental proof of concept. The results of testing Salam leaf tea bags contain compounds of the phenolic group, flavonoids, tannins, alkaloids, saponins, terpenoids, and steroids. The phytochemical content of fresh Salam leaves and dried Salam leaves shows that the total phenolic content is 8.62%, while the total flavonoid content is 5.43%. The optimum formula for Salam leaf tea bags with natural sweetener stevia leaves shows the physical properties of tea bag powder and organoleptically has a distinctive leaf odor, sweet taste, powder form, and green color. The results of testing physical properties based on flow rate parameters are known to be 9.33 seconds. The acute toxicity of Salam leaf tea bags tested on male Wistar rats, normal test animal behavior including lethargy, convulsions, tremors, and diarrhea did not occur. The test animals were still alive for 14 days of observation. The activity of a Salam leaf tea bag with natural sweetener tested on humans at high risk of hypertension in the control group showed that without Salam leaf tea bags, systolic blood pressure increased by 3.8 mmHg, while in the treatment group, blood pressure decreased in patients who consumed Salam leaf tea bags for 15 days. These patients were not taking blood pressure-lowering medication, while the patients receiving the treatment were at high risk of hypertension or pre-hypertension. Salam leaf tea bags were found to lower blood pressure by up to 11.4 mmHg.

KEYWORDS: Bay leaves, phytochemicals, hypertension, dipping preparations

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INTRODUCTION

Hypertension is a condition characterized by elevated blood pressure. The WHO explains that 40% of developing countries have hypertension, while 35% of developed countries have it. In Indonesia, it accounts for 32% of the total population (AL Berek et al., 2021). The 2018 Basic Health Research

(Riskesdas) report shows that the prevalence of hypertension by sex is 31.34% for men compared to 36.85% for women. South Kalimantan has the highest prevalence in Indonesia at 44.13% (Kemenkes, 2019). Chronic diseases such as high cholesterol, diabetes, and heart failure increase the risk of developing hypertension. In addition to primary hypertension,

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secondary hypertension can also result from other diseases such as kidney and thyroid disorders (Marlina & Tantan, 2007).

Prevention is essential to prevent further increases in blood pressure. Hypertension control can reduce the risk of stroke, coronary artery disease, congestive heart failure, end-stage renal disease, peripheral vascular disease, and mortality (Quang et al., 2010). Plants that are often used by the public are Salam leaves which contain many chemical compounds and are often used to treat gastritis, diarrhea, high blood pressure, hypercholesterolemia by lowering total cholesterol levels and many other diseases (Kemenkes, 2015).

The use of Salam leaves as a therapy for hypertension has been studied by Andoko (Andoko, 2016), Hidayat (Hidayat et al., 2018), and Dafriani (Dafriani, 2016). Fresh Salam leaves were boiled and the decoction drunk by patients. The results showed a difference in systolic blood pressure reduction after consuming the Salam leaf decoction. Several studies have shown that Salam leaves can be beneficial in efforts to control the incidence of hypertension. Patients must provide raw materials and must store them properly to prevent them from spoiling quickly. It also takes a long time to boil the Salam leaves until they are ready to drink. Therefore, a preparation that is easy and safe for patients to use is needed, namely a Salam leaf dip preparation. For sweetening, a natural sweetener from stevia leaves is used (Indiraphasa, 2022). To obtain a safe preparation, analysis of the content and levels of fresh and dried Salam leaves is necessary. To date, no examination of the content of Salam leaves has been conducted to provide a scientific basis for the use of Salam leaves. Furthermore, toxicity testing of Salam leaf dip preparations on test animals is also necessary. This testing is important to provide a basis for determining the safety of natural products.

Salam leaf dip preparations are an alternative for controlling hypertension because the ingredients are well-known to the community, readily available, and relatively inexpensive, making them suitable for use as a traditional or complementary treatment. The objectives of this study are: 1. To determine the phytochemical content of fresh and dried Salam leaves. 2. To determine the phytochemical levels in fresh and dried Salam leaves. 3. To determine the optimum formula for a Salam leaf dip with the natural sweetener stevia. 4. To determine the acute toxicity of the Salam leaf dip tested on male Wistar rats. 5. To determine the activity of the Salam leaf dip with the natural sweetener tested on humans at high risk of hypertension.

METHODOLOGY

This research design is an experimental study that includes phytochemical analysis, preparation formulation, acute toxicity testing on experimental animals, and activity testing on human subjects. The study will be conducted at the Pucuk Sirih Banjarmasin Herbal Medicine Factory Laboratory, which has a Good Traditional Medicine Manufacturing Practice (CPOTB) certificate. The test subjects are male Wistar rats and humans with a high risk of hypertension. Fresh Salam leaves will be

used for phytochemical analysis, while the remaining leaves will be dried. The Salam leaves are washed thoroughly and cut into approximately 1x1 cm pieces. They are then dried using a drying cabinet for 3 x 24 hours. The dried Salam leaves are then blended until smooth (Rizki et al., 2022). Qualitative phytochemical analysis includes compounds from the tannin, phenolic, flavonoid, alkaloid, saponin, and terpenoid groups. Quantitative analysis includes total phenolic and total flavonoid compounds using gallic acid and quercetin standards. Determination of levels uses a UV-Vis spectrophotometer. The formula for the Salam leaf dip with stevia leaf sweetener can be seen in Table 1. Dried Salam leaf powder was added to the stevia leaves and mixed thoroughly. The powder was sterilized with UV light, placed in a brewing bag, and labeled. The toxicity test used 30 male Wistar rats, divided into six treatments and five replications. Observations were conducted for 14 days. Clinical symptoms, mortality, and body weight were recorded. At the end of the study (day 14), the rats were sacrificed for organ evaluation (Ayun et al., 2021). The Salam leaf dip was tested on patients at high risk of hypertension. A total blood count was measured on 20 patients. The patients then used the Salam leaf dip twice daily, in the morning and evening, for 15 days. Furthermore, on day 16, blood was drawn for a total blood count. The results of the patients' blood parameters before and after treatment were compared.

Table I. The formula for the Salam leaf dip with stevia leaf sweetener

Material	Function of material	Ingredient composition (gram)		
		Formula I	Formula II	Formula III
Salam leaf powder	Active ingredients	3,5	3,5	3,5
Stevia leaf powder	natural sweetener	0,5	1	1,5

RESULTS

1. Qualitative Phytochemical Analysis

Active compound content was tested through phytochemical screening. Phytochemical screening uses specific reagents characterized by color changes or the formation of precipitates (Sudira et al., 2024). The results of the active compound content testing are presented in Table II.

Table II. Results of Active Compound Content Testing

No	Parameter	Salam Leaf
1	Phenolic	positive
2	Flavonoids	positive
3	Tannins	positive
4	Alkaloids	positive
5	Saponins	positive

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6	Terpenoids	positive
7	Steroids	positive

2. Quantitative Phytochemical Analysis

Salam leaves were analyzed quantitatively to determine the phenolic and flavonoid content. The average total phenolic content is 8.62%b/b while the total flavonoid content is 5.43%b/b. The results of the determination of the compound content of Salam leaves are presented in Table III.

Table III. Results of Determination of Salam Leaf Compound Content

No	Parameter	Replication	Level (%b/b)	Mean (%b/b)
1	Phenolic Total	I	8,41	8,62
		II	8,62	
		III	8,83	
2	Flavonoid Total	I	5,62	5,43
		II	5,14	
		III	5,53	

3. Formulation of Dip Preparations

Salam leaf powder was formulated into tea bags with the addition of stevia leaf powder as a sweetener. The physical properties of the powder were evaluated using organoleptic parameters, flow rate, homogeneity, and angle of repose. The results of the physical properties test for the tea bag powder are presented in Table IV.

Table IV. Results of Testing the Properties of Tea Bag Powder

No	Parameter	Replication 1	Replication 2	Replication 3	Mean
1	Organoleptic	Smell: Typical of leaves Taste: Sweet Form: Powder Color: Green	Smell: Typical of leaves Taste: Sweet Form: Powder Color: Green	Smell: Typical of leaves Taste: Sweet Form: Powder Color: Green	Smell: Typical of leaves Taste: Sweet Form: Powder Color: Green
2	Flow rate	9.2 seconds	9,2 seconds	9,6 seconds	9,33 seconds
3	Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
4	Angle of repose	29°	29°	29°	29°

4. Acute Toxicity Testing

Salam leaf tea bags were tested for safety using the acute toxicity test method. Acute toxicity testing uses test animals to determine the response to toxicity symptoms, changes in body weight, and macroscopic images of major

organs in the animals. The results of observations of acute toxicity symptoms are presented in Table V, changes in body weight in Table VI, and macroscopic images of animal organs in Figure 1.

Table V. Observation of Acute Toxicity Symptoms in Testing Salam Leaf Tea Bags at Doses of 300 mg/kgBW and 2000 mg/KgBW for 14 Days (n=10).

Observation	Dose	30 minutes	4 hours	24 hours	7 days	14 days
Skin and Fur	300 mg/KgBW	Normal	Normal	Normal	Normal	Normal
	2000 mg/KgBB	Normal	Normal	Normal	Normal	Normal
Lethargy	300 mg/KgBW	Not Showing Up	Not Showing Up	Not Showing Up	Not Showing Up	Not Showing Up
	2000 mg/KgBB	Not Showing Up	Not Showing Up	Not Showing Up	Not Showing Up	Not Showing Up
Convulsions	300 mg/KgBW	Not Showing Up	Not Showing Up	Not Showing Up	Not Showing Up	Not Showing Up
	2000 mg/KgBW	Not Showing Up	Not Showing Up	Not Showing Up	Not Showing Up	Not Showing Up
Tremor	300 mg/KgBW	Not Showing Up	Not Showing Up	Not Showing Up	Not Showing Up	Not Showing Up

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	2000 mg/KgBW	Not Showing Up	Not Showing Up	Not Showing Up	Not Showing Up	Not Showing Up
Diarrhea	300 mg/KgBW	Not Showing Up	Not Showing Up	Not Showing Up	Not Showing Up	Not Showing Up
	2000 mg/KgBW	Not Showing Up	Not Showing Up	Not Showing Up	Not Showing Up	Not Showing Up
Mati	300 mg/KgBW	Not Showing Up	Not Showing Up	Not Showing Up	Not Showing Up	Not Showing Up
	2000 mg/KgBW	Not Showing Up	Not Showing Up	Not Showing Up	Not Showing Up	Not Showing Up

The results of the study in the table above show observations of acute toxicity symptoms from Salam leaf tea bags administered at doses of 300 mg/kgBW and 2000 mg/kgBW for 14 days based on BPOM (BPOM is the Food and Drug Monitoring Agency) standards. Based on these results, it is known that at both doses there was no loss of skin and fur in the test animals. The behavior of the test animals was also normal, including

lethargy, convulsions, tremors, and diarrhea did not occur. Observations were made at 30 minutes, 4 hours, 24 hours, 7 days, and 14 days. The test animals were still alive during the 14 days of observation. These results indicate that administering Salam leaf tea bags at very high doses does not cause problems, including no deaths.

Table VI. Average Body Weight of Mice in Acute Toxicity Test of Tea Bags at Doses of 300 mg/kgBW and 2000 mg/KgBW for 14 Days (n=10).

Mice	Average Body Weight of Mice (grams) on Day			
	0	1	7	14
Mice Dose 300 mg/KgBW	27,8	28,4	30,2	31,4
Mice Dose 2000 mg/KgBW	27,7	29,2	30,8	32,1

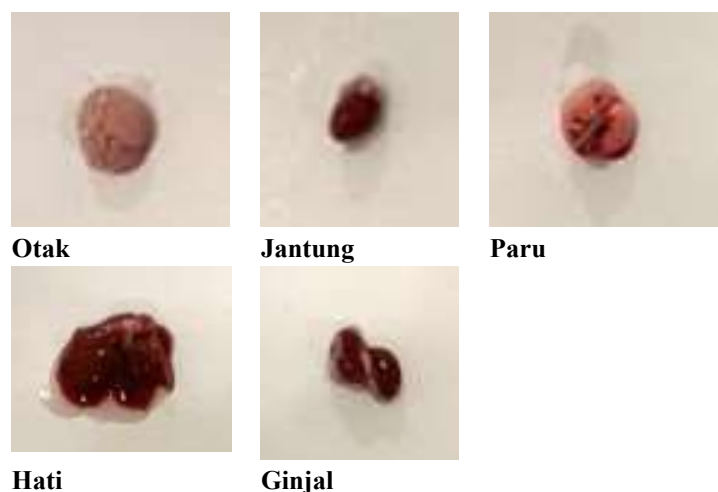


Figure 1. Macroscopic Test Results on Brain, Heart, Lungs, Liver, and Kidney Organs

5. Activity Testing in Patients

The formulated Salam leaf tea bags were then tested on patients. The testing procedures were approved by the research ethics committee. The patients involved also completed informed consent forms. The results of the tea bag testing on patients are presented in Table VII.

Based on Table VII, the systolic blood pressure data for the control and treatment groups can be seen. The control group experienced an average increase in systolic blood pressure, while the treatment group experienced a tendency to decrease

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Table VII. Results of Testing Salam Leaf Tea Bags on Patients

Control Respondents	Systolic Blood Pressure		Treatment Respondents	Systolic Blood Pressure	
	Control			Treatment	
	Before	After		Before	After
1	135 mmHg	138 mmHg	11	136 mmHg	125 mmHg
2	138 mmHg	135 mmHg	12	133 mmHg	128 mmHg
3	130 mmHg	136 mmHg	13	134 mmHg	124 mmHg
4	128 mmHg	130 mmHg	14	129 mmHg	120 mmHg
5	132 mmHg	140 mmHg	15	132 mmHg	115 mmHg
6	140 mmHg	138 mmHg	16	139 mmHg	126 mmHg
7	138 mmHg	142 mmHg	17	140 mmHg	132 mmHg
8	130 mmHg	138 mmHg	18	136 mmHg	118 mmHg
9	129 mmHg	137 mmHg	19	132 mmHg	121 mmHg
10	131 mmHg	135 mmHg	20	136 mmHg	124 mmHg
Mean	133,1 mmHg	136,9 mmHg	Mean	134,7 mmHg	123,3 mmHg

DISCUSSION

1. Qualitative Phytochemical Analysis

Test results showed that Salam leaf tea bags contain phenolic compounds, flavonoids, tannins, alkaloids, saponins, terpenoids, and steroids. The presence of phenolics is indicated by the formation of a bluish-black color when the sample solution is mixed with ferric chloride. Phenolics are largely responsible for antioxidant activity, including analgesic or pain-relieving properties (Ekayanti, 2022). The sample was found to contain flavonoids, indicated by the formation of a yellow color when mixed with an alkaline reagent. Flavonoids have the ability to inhibit increased blood pressure, which is responsible for the development of hypertension (Wahyudi et al., 2024).

Tannins are identified by the formation of a white precipitate when added to a gelatin solution. Tannins have the ability to relieve pain by inhibiting inflammatory mediators (Nifinluri et al., 2019). Alkaloid compounds are identified by the formation of a precipitate when added with Mayer's reagent. Alkaloids are widely known as analgesic agents that work through opioid and neurotransmitter pathways. Morphine and codeine are among the medicinal products included in the alkaloid group, so the alkaloid group has great potential as a dysmenorrhea agent (Kamelan et al., 2023). The presence of saponins is known because of the formation of foam that persists when the sample solution is shaken vigorously. Terpenoids and steroids were identified using the Salkowski reagent and the Libermann reagent, which formed a golden yellow color and a brown ring. The test results in this study are consistent with other studies that state that Salam leaves contain phenolics, flavonoids, tannins, alkaloids, saponins, terpenoids, and steroids (Lestari, Devi Eka, Indriyani, Susanti, 2025).

2. Quantitative Phytochemical Analysis

Total phenolic and total flavonoid levels were determined using a spectroscopic method. Gallic acid was used as the standard for determining total phenolic content, while quercetin was used as the standard for determining total

flavonoid content. The results showed that the total phenolic content was 8.62%, while the total flavonoid content was 5.43%. Compound content determination is carried out as a quality parameter for natural product ingredients. Compound content varies from plant to plant, so determining the content for each plant is necessary to ensure consistent levels of active compounds (Utami, 2020). Consistent levels of active compounds contribute to consistent pharmacological activity of a natural product.

The total phenolic content of 8.62% is considered high for natural products because active compounds are usually present in small amounts. This demonstrates the significant potential of salam leaves, supported by their high total phenolic content. The total flavonoid content of 5.43% strengthens the potential of Salam leaves as an antihypertensive. The presence of phenolics and flavonoids synergizes to prevent and reduce hypertension. Phenolics have been found to affect the renin-angiotensin system, which plays a role in regulating blood pressure. Phenolics can influence the production of hormones that affect blood vessels (Subawa et al., 2023). Flavonoids may mediate antihypertensive effects by increasing nitric oxide (NO) bioavailability, reducing endothelial cell oxidative stress, and modulating vascular ion channel activity (Maaliki et al., 2019).

3. Formulation of Dip Preparations

The results of the physical properties test of the tea bag powder revealed that organoleptically, it has a distinctive leafy odor, a sweet taste, a powdery consistency, and a green color. The distinctive leafy odor is common in tea bags derived from leaves. The sweetness is due to the presence of stevia leaf powder. The use of stevia leaves as a sweetener can make it easier for diabetics to consume this product. The tea bag contains Salam leaves and stevia leaves that have been powdered using a blender to form a powder. The powder form facilitates the active compounds' dissolution when immersed in hot water. The larger surface area of the powder facilitates interaction with the hot water, thus facilitating the penetration

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of the active compounds into the water (Asworo & Widwastuti, 2023). The organoleptic results showed the powder to be green in color, derived from the color of the leaves used.

The results of the physical properties test, based on the flow rate parameter, were found to be 9.33 seconds. Powders with a flow rate of less than 10 seconds have good flow properties (Haryono & Noval, 2022). The flow properties of the powder are beneficial in the product packaging process because the flow into the packaging is not obstructed. The resulting powder was homogeneous based on uniformity of color and size. Inhomogeneous powder can cause uneven distribution of active compounds, thus affecting the consistency of pharmacological activity. The angle of repose of the powder was 29°. These results indicate that the powder has an angle of repose that meets the requirements, namely in the range of 25-35° (Cheiya et al., 2023). The angle of repose also affects the flow properties of the powder. The results of physical property tests showed that the tea bag powder met the physical requirements.

4. Acute Toxicity Testing

The results of the physical properties test of the tea bag powder revealed that organoleptically, it has a distinctive leafy odor, a sweet taste, a powdery consistency, and a green color. The distinctive leafy odor is common in tea bags derived from leaves. The sweetness is due to the presence of stevia leaf powder. The use of stevia leaves as a sweetener can make it easier for diabetics to consume this product. The tea bag contains Salam leaves and stevia leaves that have been powdered using a blender to form a powder. The powder form facilitates the active compounds' dissolution when immersed in hot water. The larger surface area of the powder facilitates interaction with the hot water, thus facilitating the penetration of the active compounds into the water (Asworo & Widwastuti, 2023). The organoleptic results showed the powder to be green in color, derived from the color of the leaves used.

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5. Activity Testing in Patients

The control group was not given Salam leaf tea bags; patients' blood pressure was only measured before and after the study. The treatment group consumed Salam leaf tea bags for 15 days. Blood pressure was measured before and after consuming the Salam leaf tea bags on the 16th day. Results in the control group showed that without Salam leaf tea bags, there was a slight increase in systolic blood pressure of 3.8 mmHg. Patients in the control group were not given any intervention, including education. Patients at high risk of developing hypertension, including those in a pre-hypertensive state, are more likely to experience an increase in blood pressure, leading to hypertension (Yunus et al., 2021).

Results in the treatment group showed a decrease in blood pressure in patients who consumed Salam leaf tea bags for 15 days. These patients were not taking blood pressure-lowering medication. Patients receiving treatment were at high risk for hypertension or pre-hypertensive patients. Administering Salam leaf tea bags can lower blood pressure by up to 11.4 mmHg. Comparing the results in the control group with the treatment group, it was found that administering Salam leaf tea bags had a pharmacological effect on lowering patients' blood pressure. Similar research also demonstrated the ability of Salam leaf to lower blood pressure (Azim, 2022). Phenolic and flavonoid compounds play a significant role in lowering blood pressure in hypertensive patients. This reduction occurs through vasodilation of blood vessels (Wahyudi et al., 2024).

CONFLICTS OF INTEREST

There are no conflicts of interest declared by the authors of this study.

AUTHORS' CONTRIBUTION

Marwansyah literature review and writing, presenting research findings in the form of quality reports, scientific articles and presentations. Suroto contribute to interpreting analysis results and identifying key findings. Akhmad Rizani design, collection, analysis and writing. Muhammad Ikhwan Rizki supervision of all stages of the study and Assist in providing research raw materials. All authors acknowledge having reviewed and approved the final version.

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