

Influence of Curry leaves (*Murraya koenigii* L.) against of bleeding time of *Rattus norvegicus*

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ABSTRACT Bleeding is when blood is removed from damaged blood vessels and can occur during dental procedures. Curry leaves (*Murraya koenigii* L.) contain tannins and flavonoids, which have the potential as a hemostatic agent to stop bleeding. The purpose of this study was to determine the effect of curry leaf extract (*Murraya koenigii* L.) on the bleeding time of Wistar rats (*Rattus norvegicus*). The test animals used were 5 Wistar rats (*Rattus norvegicus*), which were divided into a control group that was applied with distilled water and the treatment group with the extract of curry leaves. The first treatment group was given a concentration of 25%, the second treatment group had a concentration of 50%, the third treatment group had a concentration of 75%, and the fourth treatment group had a concentration of 100%. Bleeding time was calculated using the Duke method on the tail of the rats. The results showed that curry leaf extract concentrations of 25%, 50%, 75%, and 100% were able to shorten the bleeding time with an average time of 140 seconds, 81.67 seconds, 138.33 seconds, and 73.33, respectively. Second. One-way ANOVA data analysis showed that the bleeding time in all treatment groups was significantly different from the control group ($p < 0.05$). This study concludes that the extract of curry leaves (*Murraya koenigii* L.) affects the bleeding time in Wistar rats (*Rattus norvegicus*).

Keywords: Bleeding, curry leaf extract, Wistar rat

INTRODUCTION

Bleeding is the process by which blood is removed from damaged blood vessels. In dentistry, bleeding can occur during dental procedures such as tooth extraction or other oral surgery procedures.^{1,2} The prevention of blood loss is known as hemostasis. Hemostasis occurs through several mechanisms consisting of vascular constriction, blood platelet formation, blood platelet formation resulting from blood coagulation, and fibrous tissue growth in platelets to cover leaks in blood vessels.³ One of the ways to control bleeding is to use local hemostatic agents.^{2,4}

Currently, traditional medicines derived from plants have been developed as alternative treatments. One of the plants used as traditional medicine is curry leaves (*Murraya koenigii* L.). Curry leaves are often used to remedy bruises, insect bites, fevers, burns, and spice in cooking. Some of the curry leaves properties are anti-inflammatory, anti-analgesic, antimicrobial, anti-cytotoxic, anticancer, and anti-oxidant. Curry leaves have compounds that have potential as a hemostatic agent, namely

flavonoids, alkaloids, tannins, terpenoids, vitamins A and C.^{5,6}

Kalaiselvan reported curry leaves to have useful activity in shortening wound healing time.⁷ The study of Kainde et al., who applied spoon leaf extract (*Plantago major* L.) to Wistar rats' tails, showed that the treatment group's bleeding time was shorter than the control group because it contained tannins and flavonoids, which accelerated the hemostasis process.⁸ Research report showed that betel leaf extract (*Piper betle* L.) at concentrations of 10%, 20%, and 40% could shorten the bleeding time in male Swiss Webster mice because they contain flavonoids and tannins.⁹ Besides, based on other research, the bleeding time in male Swiss Webster mice became shorter after applying *Jatropha curcas* L. stem sap at concentrations of 40%, 80%, and 100%, which also contain tannins and flavonoids. These materials are functional as hemostatic agents.¹⁰ This study evaluated the effect of curry leaf extract on the bleeding time of Wistar rats (*Rattus norvegicus*).

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MATERIALS AND METHODS

Research Design

This research is a laboratory experimental study to determine the effect of curry leaf extract (*Murraya koenigii* L.) on bleeding time in Wistar rats (*Rattus norvegicus*) conducted at the Pharmacology Laboratory and Experimental Animal Laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University in August 2019.

The sample used is curry leaves (*Murraya koenigii* L.) taken from Ulee Kareng, Banda Aceh Indonesia, and five Wistar rats (*Rattus norvegicus*) obtained from the animal pen of the Faculty of Veterinary Medicine, Syiah Kuala University.

Preparation of *Murraya koenigii* L ethanol extract

Curry leaves (*Murraya koenigii* L.) collected were washed, dried, then mashed and weighed, and extracted using the maceration method. The leaf powder is soaked using 96% ethanol solvent for three days. After that, the ethanol filtrate was filtered and evaporated using a 40°C rotary evaporator to obtain a thick extract.^{11,12}

Screening of Phytochemical

Phytochemical tests then carry out the extract of curry leaves (*Murraya koenigii* L.), which has been obtained to determine the presence of active compounds that are expected to affect the bleeding time of Wistar rats (*Rattus norvegicus*). The tests carried out are as follows. Alkaloid examination is done by mixing the extract with hydrochloric acid drops and filtering it. In Mayer's test, the filtrate is put into Mayer's reagent, and if a yellow precipitate is formed, it indicates the presence of alkaloids. In Dragendroff's trial, the filtrate is inserted into the dragendroff reagent if a red precipitate is formed, indicating the presence of alkaloids. Tannin examination was carried out by mixing 1% gelatin solution and added sodium chloride to the extract. If a white precipitate is formed, it indicates the presence of tannins. Examination of flavonoids is done by mixing a few drops of sodium hydroxide solution into the extract of curry leaves. The presence of flavonoids is indicated by a yellow precipitate that fades when the acid solution is added. Saponin examination was carried out by dissolving the curry leaf extract in 20ml of water in a test tube and shaking it for 15 minutes. If a foam

layer is formed measuring 1cm, it indicates the presence of saponins.^{13,14}

Extract application on the wounds of animal model

Wistar rats (*Rattus norvegicus*) used in this study were 2-3 months old, had an average weight of 250-300 grams, and had good nutritional status. Besides, spare mice were prepared to avoid exclusion criteria.

Wistar rats (*Rattus norvegicus*), which are used as experimental animals, are placed in individual cages with holes in the tail so that the tails of the mice can come out to facilitate wound making.

After that, the rats were disinfected using 70% alcohol. Furthermore, the rats were stabbed using a sterile 27gauge disposable lancet in one-third of the tail end area as deep as 2.5mm.¹⁵

Wistar rats (*Rattus norvegicus*) in the treatment group were applied curry leaf extract (*Murraya koenigii* L.) by dipping the tail which had been injured for 2 minutes then calculating the bleeding time. In the control group, the tails of the rats' given injuries were immersed in distilled water for 2 minutes and evaluated the bleeding time.

Bleeding Time

The rats' bleeding time was calculated when the blood first came out until the blood stopped coming out, that is when there were no bloodstains blotting paper. Calculation of the bleeding time is done using a chronometer.

The blood that comes out is rubbed every 10 seconds using a blotting paper without pressing the wound, so it does not affect blood clots' formation. After no more blood is absorbed on the blotting paper, stop the chronometer and calculate the amount of bleeding time.

RESULTS

Phytochemical tests then carry out an extract of curry leaves (*Murraya koenigii* L.) obtained through the extraction process to see compounds such as tannins flavonoid, saponins, and alkaloids. **Table 1** shows the results of the phytochemical test of curry leaf extract (*Murraya koenigii* L.), while **Table 2** shows the results of the calculation of bleeding time.

Table 1. Phytochemical Test Results of Curry Leaf Extract (*Murraya koenigii* L.)

No.	Phytochemical Screening	Prouct Assay
1.	Alkaloid	
	a. Dragendroff	+
	b. Mayer	-
	c. Wagner	+
2.	Flavonoid	+
3.	Tannin	+
4.	Saponin	+
5.	Steroid	+
6.	Terpenoid	+
7.	Phenolic	+

Table 2. Results of Calculation of Bleeding Time (seconds)

Treatment	Control	T1 (25%)	T2 (50%)	T3 (75%)	T4 (100%)
1	172	130	70	155	50
2	230	120	75	110	80
3	180	170	100	150	90
Average	194	140	81,67	138,33	73,33

Data analysis was performed using the SPSS application. The normality test used was the Shapiro-Wilk test because this study had a small sample size. The results of data analysis showed usually distributed and homogeneous with $p > 0.05$. One way ANOVA test results showed a p-value < 0.05 , which indicates that there is an effect

of curry leaf extract (*Murraya koenigii* L.) on bleeding time of Wistar rats (*Rattus norvegicus*). The Post Hoc analysis results using the Least Significant Different (**Table. 3**) test showed that the bleeding time in all treatment groups was significantly different from the control group.

Table 3. Result of Post Hoc Least Significant Different (LSD)

Kelompok	P Value	Information
C - T1	$p < 0.05$; 0.022	Significant
C - T2	$p < 0.05$; 0.000	Significant
C - T3	$p < 0.05$; 0.019	Significant
C - T4	$p < 0.05$; 0.000	Significant
T1 - T2	$p < 0.05$; 0.015	Significant
T1 - T3	$p > 0.05$; 0.935	No Significant
T1 - T4	$p < 0.05$; 0.007	Significant
T2 - T3	$p < 0.05$; 0.018	Significant
T2 - T4	$p > 0.05$; 0.685	No Significant
T3 - P4	$p < 0.05$; 0.009	Significant

C (Control); T (Treatment)

DISCUSSION

Curry leaves are reported as an alternative medicine for insect bites, fevers, burns, and spice in cooking. Some of the curry leaves' properties are anti-inflammatory, anti-analgesic, anticancer,

antimicrobial, anti-cytotoxic, and anti-oxidizing.¹⁶ Curry leaves contain active compounds in flavonoids, alkaloids, tannins, terpenoids, and vitamins A and C, which have the potential as a

hemostatic agent.^{17,18} The extract of curry leaves (*Murraya koenigii* L.) was tested for phytochemicals to determine the presence of active compounds, which are expected to affect the bleeding time of Wistar rats (*Rattus norvegicus*). The curry leaf extract (*Murraya koenigii* L.) used in this study contained alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, and phenolics, which have potential as hemostatic agents.

This study's experimental animals were five male Wistar (*Rattus norvegicus*) white rats, 2-3 months old, with an average weight of 200-300 grams. White rats are one of the experimental animals that are often used and deliberately bred for research because they have relatively similar characteristics to humans.¹⁹ The mice used were male rats to provide more stable results because they were not influenced by the menstrual cycle and pregnancy as in female rats. The rats used in this study were 2-3 months, which are young adults and large enough so that the image of vascularity is easily visible.^{20,21} The body weight of mice in this study ranged from 200-300 grams, which is the average weight of healthy adult male rats.²⁰

Wistar rats were injured in the tail area, easily accessible compared to other body parts in mice. Before the injury, the rats were disinfected using 70% alcohol. The wound was made using a sterile 27 gauge disposable lancet, and the bleeding time was calculated using a chronometer.¹⁵ The assay material was applied to the wound area by immersing the rats' tails in the section for 2 minutes, while the control group was immersed in distilled water for 2 minutes. The blood that comes out is then rubbed with a

blotting paper every 10 seconds without pressing on the injured area so that it does not affect the process of stopping the bleeding.²¹

The bleeding time calculation results in Table 2 show that the rats in the control group had an average bleeding time of 194 seconds. It is following the average bleeding time in mice, which is 2-5 minutes.²² There was a decrease in bleeding time in mice applied with a 25% concentration of curry leaf extract with an average of 140 seconds in the treatment group. The used curry leaf extract with a concentration of 50% had an average bleeding time of 81.67 seconds. Meanwhile, the group with a concentration of 75% had an average of 138.33 seconds. The fastest reduction in bleeding time occurred in the group applied with 100% concentration of curry leaf extract with an average of 73.33 seconds. It can happen because curry leaf extract has compounds that have potential as a hemostatic agent, such as alkaloids, flavonoids, tannins, and terpenoids, to stop bleeding.⁶

Several factors can affect the results of the calculation of bleeding time in this study, one of which is the absence of laboratory tests on rats so that there is no known possibility of blood clotting function abnormalities. This study considers the mice to be in good health based on their general conditions and expected behavior. Besides, It's a possible difference in the number of compounds present at each extract concentration, or the result of a less homogeneous dilution can also affect the rats' bleeding time. In this study, there was no examination of which compounds had the most influence on bleeding time.

CONCLUSION

The results study concludes that the extract of curry leaves (*Murraya koenigii* L.) affects the bleeding time of Wistar rats (*Rattus norvegicus*). The administration of curry leaf extract (*Murraya*

koenigii L.) with a concentration of 100% had the most significant effect in shortening the bleeding time of Wistar rats (*Rattus norvegicus*).

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