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In vitro efficacy of patchouli oil on *Rhipicephalus sanguineus*

Risnawati[1], Muh Nur Amir[2], Isra Wahid[3], Adryani Ris[1], Baso Yusuf[1]*

[1]Veterinary Medicine Study Program, Faculty of Medicine, Hasanuddin University
[2]Faculty of Pharmacy, Hasanuddin University
[3]Departement of Parasitology, Faculty of Medicine, Hasanuddin University

*Corresponding author: Baso Yusuf (basoyusuf_frefor@yahoo.co.id)

Abstract

Patchouli oil is an essential oil derived from the patchouli plant, and it has many benefits, including the insecticide effect. Rhipicephalus sanguineus tick is a parasite often found in dogs in Indonesia. This study aims to determine the effect of patchouli oil on the mortality of R. sanguineus ticks in dogs. Samples of this study were 25 R. sanguineus ticks, and they were divided into five treatments: positive control, negative control, 5% patchouli oil, 10% patchouli oil, 20% patchouli oil. The result of this study showed that all ticks in the negative control using hexane were found alive. All ticks in the positive control using Ivermectin were found dead. Ticks in 5% patchouli oil showed that 4 of 5 ticks were found dead. Ticks in 10% and 20% patchouli oil showed that 5 of 5 ticks were found dead. This study conclude that patchouli oil can be used as an insecticide and 20% patchouli oil concentration has statistically no significant difference from the positive control. Moreover, this study suggests using patchouli oil as an alternative insecticide due to its low price compared to Ivermectin.
***Keywords** : Patchouli oil, mortality, rhipicephalus sanguineus*

Introduction

Pets are very popular in Indonesia, including dogs and cats. Dogs are in demand as pets because they can be playmates, guard homes and farms, and are useful in the field of policing. Dogs have very unique abilities and a friendly human habitus. At first, dogs were only used as hunting animals, but now there are developments in dogs' function and use. Increased use of dogs in the community also increases the chances of a dog experiencing health problems.

Ectoparasite infestation in pets, especially dogs, is a serious problem because it has a fairly high prevalence rate. The disorders that can be caused by ectoparasite infestation are quite diverse, such as weight loss, hair loss, trauma, irritation, anemia to death if not treated. According to Puri et al. (2014), the tick that most often found in dogs is *Rhipicephalus sanguineus.* Ticks are a class of ectoparasites that infect by sucking blood on the surface of the body. The *R. sanguineus* tick is spread in America, Africa, Australia, and Asia, including Indonesia. An adult female tick can suck up to 0.3 ml of blood a day. During blood sucking *R. sanguineus* can act as a vector for transmitting several dangerous blood protozoa diseases such as babesiosis, anaplasmosis and theileriosis which cause death (Leliana, 2014). Given the considerable losses due to the

infestation of the *R. sanguineus* tick itself and the diseases it causes, it is necessary to find a way out to overcome this problem (Abdjul et al. 2018).

The patchouli plant developed by the farming community of Tiara Village, West Malangke Subdistrict, North Luwu Regency, has morphological characteristics: thick leaves, not flowering, leaves on the upper surface are green, and the lower surface is purplish green. Research on patchouli oil shows that patchouli oil has pharmacological activities such as antifungal, antibacterial, antiemetic, Ca2 + antagonistic activity, and trypanocidal activity (Zhao et al. 2005). Research by Aisyah et al. (2008) stated that the antibacterial activity in patchouli was caused by the presence of patchouli alcohol, which is a cyclic tertiary alcohol sesquiterpen compound.

The role of patchouli oil as an insecticide raw material is based on secondary metabolites in the vacuole, which stimulate insect chemoreceptors so it is not preferred. The presence of active ingredients in patchouli plants causes this plant to be resistant to P. brachyurus with a resistance mechanism occurring before the plant is infected (Mustika et al. 2002). Patchouli oil quality is determined by patchouli alcohol. The alpha-pinene and beta-pinene compounds in patchouli oil can be used to control insect populations because of their properties as repellents and inhibitors of insect growth. Patchouli oil shows antimicrobial activity against Escherichia coli, Staphylococcus aureus, Candida albicans, Aspergillus niger, and Microsporum gypseum (Farizal, 2014). The patchouli oil that has been refined so far is still for export, and there is no further preparation or processing of the patchouli oil. Patchouli oil acts as an antimicrobial and antiparasitic has not been developed in Indonesia. The results of Prasetyo (2011) conducted research using patchouli with three concentrations, namely 5%, 10%, and 20%, gave a real difference to the time of mortality of ticks.

The prohibition on the use of several types of synthetic chemical pesticides, such as organophosphates and carbamates, will increase the opportunity for pesticide products made from essential oils to be commercially developed and produced and are expected to be able to compete with synthetic chemical pesticides (Hartati, 2012). The use of anti-flea and tick shampoo, namely Ivermectin, is a good solution in overcoming tick infestations, however, these products are difficult to obtain in remote areas and are also expensive, so that patchouli oil can be used as an alternative. Therefore this research was conducted in addition to increasing the use of patchouli oil as a product that can be useful in the medical field, namely as an anti-tick drug.

Materials and Methods

This research was conducted in the Veterinary Medicine laboratory of Hasanuddin University. Tick samples were obtained from dogs in residential areas in Panakukang District, Makassar. The 25 *R. sanguineus* tick samples were collected from 11 dogs suffering from tick infestation. Patchouli oil was obtained from a patchouli distillery in Tuara Village, West Malangke, North Luwu Regency.

The selection of sample in this study was carried out selectively by measuring the physiological status and the same size of tick samples. Twenty-five female ticks (*R. sanguineus*) have a body length of about 0.7-085 cm. According to Federer (in Rahayu, 2018) to obtain valid data, repetition is carried out according to Federer's formula (n-1) (t-1)> 15. Then the number of treatment samples is at least 5. In this study, 5 samples of ticks were determined for each treatment so that the total The sample consisted of 25 ticks consisting of 10 ticks in the control group and 15 ticks in the treatment group.

The female tick will be taken from the dog's body and then put into a petri dish, and then taken to the laboratory for examination. Variations in the solution's concentration were made by diluting with hexane solution to obtain a solution volume of 25 ml with variations in the concentration of patchouli oil content of 5%, 10%, 20%.

To obtain a solution with thirteen concentrations, namely 5%, 10% and 20%, it is carried out based on the applicable provisions. PO solution with a concentration of 5% is obtained by taking 1.25 ml of patchouli oil which will be dissolved with hexane and the volume will be sufficient to 25 ml. PO solution with a concentration of 10% is obtained by taking 3.5 ml of patchouli oil which will be dissolved with hexane and the volume is sufficient to 25 ml. PO solution with a concentration of 20% is obtained by taking 5 ml of patchouli oil which will be dissolved with hexane, and the volume is sufficient to 25 ml. A total of 25 samples of ticks (R. sanguineus) were grouped into 5 treatment groups.

Each group will be given treatment by dropping per-tick with treatment A, namely negative control by giving 1 drop / 0.3ml of hexane solution, treatment B, namely positive control by administering Ivermectin, treatment C which is given 5% patchouli oil, treatment D namely given patchouli oil 10%, treatment E that is given patchouli oil 20%. Observations were made for 9 hours. Observation and recording were made of the number of dead ticks. The data obtained were analyzed by one-way analysis of variance (ANOVA oneway). If there is a significant difference, it will be followed by the LSD (Least Significant Differences) test.

Result & Discussion

The study results showed that in vitro test of the effectiveness of patchouli oil against ticks can cause death to the *R. sanguineus* tick. The results showed that there were differences in the number of tick deaths at each concentration. Data regarding the number of tick deaths based on observation time can be seen in Table 1.

Table 1. Number of tick deaths and time of death of ticks treated with patchouli oil

Time of Death	N-	K+		PO 5%		PO 10%		PO 20%	
	D	D	L	D	L	D	L	D	L
3 hours	0	5	100%	0		1	20%	5	100%
6 hours	0	5	100%	1	20%	5	100%	5	100%
9 hours	0	5	100%	4	80%	5	100%	5	100%
Death Presentation	0%	100%		80%		100%		100%	

Note :
D : Dead (dead sample) L : Life
K- : Negative control (Heksan) K+ : Positive control (Ivermectin)
3 hours: 9.00 – 12.00 WITA (1-3 hours after treatment)
6 hours : 12.00 – 15.00 WITA (3-6 hours after treatment)
9 hours : 5.00 – 18.00 WITA (6-9 hours after treatment)
PO 5%, 10%, 20% : Patchouli oil concentration 5% , 10%, 20%

Based on Table 21, it can be seen that there are differences in the number of ticks that died in each treatment group. It is clear that, in the negative control group, none of the ticks died in the three time period or 9 hours of treatment. In the negative control group, the treatment given was by dripping the hexane, and this proved that hexane was safe as a solvent from patchouli oil because it did not cause death in the test sample. Whereas in the positive control group and all test groups, the death of ticks started in the first period or 2 hours after treatment. In the positive control group, the treatment given was by dripping with Ivermectin. It can be

seen clearly that the highest number of dead ticks was in period 3 in each patchouli oil treatment group in terms of the tick death period.

Based on Table 2 and Figure 6 above, it can be seen that the death of ticks starts from PO 5%. However, when viewed from the number of dead ticks, 20% PO has been able to kill more than 50% of the number of tick samples. This means that the higher the concentration, the average value of tick mortality increases. Different things happened to 5% PO; the rate of death of ticks did not increase sharply. If we pay attention to the positive control group and the 20% PO concentration group, it has the same average value in almost all periods except in the first period. This is because the active ingredient of ivomec, namely Ivermectin, has the same mechanism of action as the active ingredient of patchouli oil, which works by affecting the central nervous system (Morsy, 2000).

Patchouli oil is used as an insecticide due to its secondary metabolite compounds that stimulate chemoreceptors not to be liked by insects. Research results from El-Shazly and Hussein (2004) that patchouli oil contains sesquiterpenes, especially alcohol sesquiterpenes, which can function as antimicrobial and larvacidal. According to Farizal (2014), the amount of sesquiterpene compounds in patchouli oil is 40-45% by weight of patchouli oil. There are many sesquiterpenes in patchouli oil, including α-bulnesene (δ-guaiene) (14.7%), α-guaiene (13.4%), αpatchoulene (8.0%), seychellene (7.5%) (Aisyah , 2008). The test results on the effectiveness of patchouli oil against agricultural insects showed that 20% of patchouli refined waste extract gave mortality to Heliopeltis and Ostremia purnacali (Usmiati et al. 2004).

When viewed from the increase in concentration, it is clear that the treatment group with a concentration of 20% showed the highest mortality. Mortality at a concentration of 20% is considered the best treatment because the highest active substance content is found at 20% PO. According to Purnamaningsih (2002) the mechanism of action of Ivermectin is to interfere with the activity of chloride ion flow in the arthropod nervous system. These preparations can bind to receptors that increase the parasite's membrane's permeability to chloride ions, thereby causing chloride channels to open and prevent gamma aminobutyric acid release (GABA) neurotransmitters. As a result, the neuromuscular transmission will be blocked, and neurons' polarity will be disturbed, which will cause paralysis and the death of parasites (ticks).

In giving patchouli oil in all concentrations it reacts to ticks, including paralysis to death because it is influenced by monoterpenes in patchouli oil which act as acetylcholinesterase inhibitors (AChE). According to (Zhu et al. 2003), monoterpenes can inhibit the acetylcholinesterase enzyme action, which plays a role in the transmission of nerve impulses. Nerve impulses are transmitted from one neuron to another via synapse by the neurotransmitter acetylcholine (ACh). If the acetylcholinesterase enzyme is inhibited, regular nerve activity will be disrupted. Disruption to the acetylcholinesterase enzyme causes nerve impulses to be transmitted continuously resulting in incoordination, seizures, weakness, and death (Riyanto, 2009).

According to Beek (2017) Monoterpene compounds identified in patchouli oil include eugenol, limoncello, linalool. The eugenol content identified was 1.15%. Some of the components of essential oils are antagonists of octopamine receptors. Toxicity can be significantly affected by the poison's ability to penetrate the membrane and reach the target site. Certain essential oils have been reported to exhibit a neurotoxic mode of action, regardless of administration route (oral, topical, by fumigation or from residual contact to surfaces). Common symptoms of the neurotoxic mode of action include hyperactivity, convulsions, tremors, and paralysis. Our observations of tick behavior indicate that patchouli oil in various administration concentrations has a neurotoxic mode of action against the ticks. Patchoulol content in patchouli oil is at least 32%. Patchoulol toxicity may have several actions against ticks, including neurotoxicity,

penetrating and damaging cuticles and membranes, and causing internal tissue damage (Zhu et al. 2003)

Based on the results of the ANOVA test, the significance probability value is 0.000. With 95% confidence because the value <0.05, it is known that there is a significant difference in tick mortality using the three patchouli oil concentrations, namely PO 5%, PO 10%, and PO 20%, which means that the concentration has a significant effect on the time of tick mortality. Based on ANOVA data, a significant value of 0.042 (0.042 <0.05) was obtained for measuring the effect of time/period on tick mortality, meaning that with 95% confidence, there was a significant effect between exposure time on tick mortality.

The price of Ivermectin which is used as a positive control, is IDR 400,000 with a 50ml package, while the price of patchouli oil in the market, which is sold commercially, has a standard price of 400,000 per/kg (Nismawati, 2019) or more than 1 liter (1000ml). If viewed from an economic point of view, there is a comparison of the price of Ivermectin with patchouli oil, namely 1: 20. In the business of making patchouli oil 1kg, it is IDR 250,000.

Conclusion

The study showed that patchouli oil could be used as an alternative insecticide due to its effectiveness in killing ticks starting from a concentration of 5%, 10%, and 20%. The highest tick mortality found at a concentration of 20% was not statistically significantly different from the positive control. Comparison of patchouli oil price with positive control is 1:20 with almost the same effectiveness. This study also suggests that further research related to direct application in dog should be conducted. Moreover, for massive application, it is necessary to show socialization to the community regarding patchouli oil as an alternative insecticide to solve the ectoparasite problem.

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