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In-Vitro Effectiveness of Ethanol Extract of Permot Leaf (*Passiflora foetida* Linn.) Towards Mortality of *Rhipicephalus sanguineus* Larvae

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Abstract

This study had three fold objective which were finding the effectiveness of ethanol extract of permot leaf (*Passiflora foetida* Linn.) towards mortality of *Rhipicephalus sanguineus* (*R. sanguineus*) larvae in vitro and also identifying lethal concentration and lethal time. Completely randomized design was used with six treatments and four replications. Five *R. sanguineus* were used in the treatment for all replication. The observation of mortality was done every ten minutes in five hours. *Rhipicephalus sanguineus* larvae were dead if there was no movement. The collected data was analyzed using ANOVA factorial and followed by Duncan's test. The obtained data showed that ethanol extract of permot's leaf ethanol extract was effective toward mortality of *R. sanguineus* larvae in vitro. The suspension of 10% extract concentration of permot leaf ethanol extract has a similar effectiveness as Noticks[®]. Probit analysis stated that LC₅₀ can be reached by using the 4% concentration in two hours and 2.4% concentration in five hours. LC₉₅ can be reached by using the 6.5% concentration in two hours and 3.93% concentration in five hours. The quickest LT₅₀ can be reached in 0.91 hours and LT₉₅ 2.37 hours after treatment with 10% concentration.

Keywords: ethanol extract, Permot leaf, in vitro, *Rhipicephalus sanguineus*, *Passiflora foetida*

Introduction

Permot plants have potential as biological larvicides. This plant grows wild and is found in large quantities in nature. Permot plants can be easily found in rice fields, gardens or growing vines between the main plants that are deliberately planted or propagated on fences (Susilowati and Sari, 2018). Indonesia is a country that has many plants that have benefits if explored properly. Permot (*Passiflora foetida* Linn.) is a plant that belongs to the types of weeds and shrubs. Permot leaf extract contains alkaloids, terpenoids and phenols. The flavonoids found in permot leaves include apigenin, ermanin, 7,4-dimethoxapigenin, isovitexin and viteksin. The group of alkaloids found in permot leaves include harmaline, hamine, harmol (Patel, 2011). *Passiflora foetida* contains alkaloids and approximately ten flavonoids, one of which is ermanin which caused the death of Nymphalid Butterfly Dione Juno. The content of ermanin contained in this plant can be used to kill pests and insects (Echeverri *et al.*, 2001). The tick *R. sanguineus* or commonly referred to as the Brown Dog Tick is

a three-hosted tick that attacks dogs, cats, rabbits, rodents, wild dogs, and humans (Dantas, 2008). Tick infestations in dogs affect the physical condition and health of dogs because the *R. sanguineus* can cause itching in the host so that the dog will be disturbed by this condition. If this condition is not treated, over time the dog will be stressed so that his appetite decreases, his weight decreases and he is susceptible to disease. Infestation of ticks on pets, especially dogs, can also cause economic losses because of the relatively high cost of treatment and must always be repeated to prevent re-infestation of the dog. Treatment for tick infestations is also not only for the host but also for the surrounding environment because the *R. sanguineus* tick is a tick that has three hosts. *Rhipicephalus sanguineus* tick infestation in dogs can cause hair loss, tick dermatitis, irritation, and trauma that make owners reluctant to play with their beloved dog (Puri *et al.*, 2014) Tick infestations can cause severe anemia to the point of death in the host and can cause tick paralysis in the host. These ticks are vectors of disease agents such as *Ehrlichia canis*,

Rickettsia rickettsii, and *Rickettsia conorii* which can cause zoonotic diseases (Dantas-Torres, 2008). *Rhipicephalus sanguineus* is also the main vector of blood protozoa that attack erythrocytes, namely *Babesia canis* in dogs (Lubis, 2006, in Paramita and Widyastuti, 2019). Control of tick infestations using chemical insecticides such as Coumaphos, Ivermectin, Diazinon, and other parasitic drugs often have side effects that can cause death if the insecticide levels used are not correct through contamination of drinking water, feed and (14) (Riyadi *et al.*, 2018). Vegetable pesticides are pesticides whose basic ingredients are derived from plant secondary metabolites. The working system of plant-based pesticides kills pests in real time and will soon decompose in nature (Syakir, 2011). The use of bioacridicides as a substitute for synthetic acaricides is expected to reduce environmental losses and pollution caused by the use of synthetic acaricides. Bioacridicides have properties that are easily and quickly degraded by nature so that they do not cause residues that are harmful to the environment, humans and animals.

2 Materials and Methods

The samples used in this study were 120 larvae of *R. sanguineus* which were taken randomly from the rearing of female *R. sanguineus* in the engorged phase of dogs in East Java. Permot leaves that have been weighed with a weight of 7.5 kilograms are cut to get smaller sizes first. The small size can affect the speed in drying the leaves. Leaves aerated for approximately one week. After it is dry enough, (9) the permot leaves are ground to get simplicia. Extraction was carried out by maceration method using 96% ethanol. The dried simplicia was weighed as much as 1000 grams and then put into a maceration vessel. Ethanol was added in a maceration vessel containing permot leaf simplia then stirred and allowed to stand for 3 x 24 hours. The extraction process stops if the solvent obtained is clear after the filtering (3) process. The filtrate was concentrated using a rotary evaporator at a temperature of 50°C to obtain an extract that still contained a small amount of solvent. Evaporation was carried out using an oven at 40°C to obtain a thick extract (Patmasari *et al.*, 2013). The thick extract was diluted according to the concentration to be used, namely 2.5%; 5%; 7.5%; and 10% (Prasetya, 2015). This study used a higher concentration of 10%. *R. sanguineus* female taken from the dog was put into a medicine pot. *Rhipicephalus*

sanguineus was then reared by placing it in a pot covered with gauze as a cover. During the rearing process the humidity needs to be maintained by wetting the gauze cover. Every day *R. sanguineus* was observed until the ticks lay eggs. Tick eggs are waited until they hatch into larvae. Larvae that hatch awaited until the age of 7 days. The 7-day-old *R. sanguineus* tick larvae were randomly assigned to six treatment groups and 20 *R. sanguineus* larvae were placed in each treatment group which were divided into four petri dishes in each group. Each petri dish contained five *R. sanguineus* larvae. The negative control group was sprayed using aquadest + Tween 80 1%. The positive control group was sprayed using Noticks® containing 80% Coumaphos. The first treatment group was sprayed with a suspension of 2.5% concentration of Permot leaf ethanol extract. The second treatment group was sprayed with a suspension of Permot leaf ethanol extract with a concentration of 5%. The third treatment group was sprayed with a suspension of 7.5% permot leaf ethanol extract. The fourth treatment group was sprayed using a suspension of 10% permot leaf extract. Spraying is carried out five times with the same pressure and a distance of 5 cm from the top of the larva. Observations of mortality were carried out every 10 minutes for 5 hours. The data (11) obtained were analyzed using two-way and one-way analysis of variance (ANOVA), followed by Duncan's multiple-distance test to compare the mean larval mortality. Lethal concentration (12) and Lethal Time were analyzed using probit analysis. Statistical analysis was carried out using the SPSS 20 program.

2 Results and Discussion

The results of observations made on the mortality rate of *R. sanguineus* larvae based on various treatments and associated (5) with the fastest required time of death can be seen in Table 1.

The death of *R. sanguineus* larvae was indicated by the larvae's legs rolling inward (Wardhana, 2005) and there was no movement. Signs of larval death with rolled legs can be seen in Figure 21.

It can be seen in Table 1 that the use of ethanol extract of permot leaves also showed effective results on the mortality of *R. sanguineus* larvae although they had different levels of effectiveness.

Table 1. Average Mortality and Standard Deviation Observation of Mortality of *R. sanguineus* Larvae

No.	Treatment	Mean ± Standard Deviation (head)				
		1 hour	2 hours	3 hours	4 hours	5 hours
1	Aquadest + Tween 80	0.50 ^a ± 0.58	1.00 ^a ± 0.00	1.75 ^a ± 0.5	2.00 ^a ± 0.82	2.25 ^a ± 0.96
2	Noticks [®]	3.75 ^c ± 0.96	5.00 ^c ± 0.00	5.00 ^b ± 0.00	5.00 ^b ± 0.00	5.00 ^c ± 0.00
3	Permot Ethanol Extract 2.5%	0.25 ^a ± 0.5	1.50 ^a ± 1.73	2.50 ^a ± 2.08	3.50 ^{ab} ± 1.92	3.75 ^b ± 1.50
4	Permot Ethanol Extract 5%	0.00 ^a ± 0.00	1.25 ^a ± 0.50	2.50 ^a ± 1.73	4.25 ^b ± 1.5	4.75 ^{bc} ± 0.5
5	Permot Ethanol Extract 7.5%	1.75 ^b ± 0.50	3.00 ^b ± 1.41	5.00 ^b ± 0.00	5.00 ^b ± 0.00	5.00 ^c ± 0.00
6	Permot Ethanol Extract 10%	3.00 ^c ± 1.41	4.25 ^{bc} ± 0.50	5.00 ^b ± 0.00	5.00 ^b ± 0.00	5.00 ^c ± 0.00

Note: Different superscripts in the same column, showing significant differences (P<0.05).

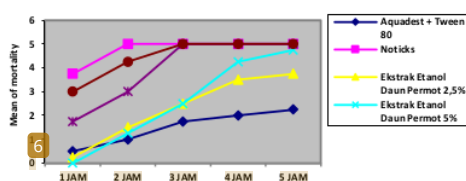


Figure 1. Graph of average mortality of *R. sanguineus* larvae

At a concentration of 2.5%, larval mortality increased but until the fifth hour it had not reached the optimum value of 75%. The use of ethanol extract of permot leaves with a concentration of 5% resulted in larval mortality that increased every hour but did not reach the optimum value until the fifth hour, only reaching 95%. The use of ethanol extract of permot leaves with a concentration of 7.5% and 10% has reached the optimum in the third hour. The use of 10% permot leaf extract is considered the most effective because in the second hour it has shown no significant difference with Noticks[®] administration. The results of observations every 10 minutes for 5 hours in all

treatments showed different results, but in the same treatment there was an increase in the average mortality of larvae. The use of Noticks[®] gave the average value of larval mortality at the 1st hour and has given the optimum value at the 3rd hour because it reaches a mortality percentage of 100%. The probit test results gave an LC50 in the second hour of 2,000 mg/50 ml equivalent to 4% with a lower limit of 1500 mg/50mL or equivalent to 3% and an upper limit of 2300 mg/50 mL or equivalent to 4.6%. Lethal Concentration 50 at the fifth hour of 1200 mg/50

ml is equivalent to 2.4% with an upper limit of 1500 mg/50 mL or equivalent to 2.95%. These results indicate that the dose interval that can be used to kill 50% of *R. sanguineus* larvae in vitro is 2.4% - 4.6%. The probit test results gave an LC95 in the second hour of 3.250 mg/50 ml equivalent to 6.51% with a lower limit of 2.780 mg/50mL or equivalent to 5.56% and an upper limit of 4.880 mg/50 mL or equivalent to 9.76%. Lethal Concentration 95 at the fifth hour was 1.965 mg/50 ml equivalent to 3.93% with a lower limit of 1.725 mg/50 ml or equivalent to 3.45% and an upper limit of 4.120 mg/50 mL or equivalent to 8.24%. These results indicate that the dose interval that can be used to kill 95% of *R. sanguineus* larvae in vitro is 5.56% as the lowest dose and 8.24% as the highest dose. The fastest time to reach the LT50 value is 0.91 hours by giving 10% permot leaf ethanol extract to kill 50% of the sample population, followed by 7.5% permot leaf ethanol extract with 1.36 hours, 5% concentration with time 2.77 hours, and a concentration of 2.5% with a time of 2.96 hours. The higher concentration of ethanol extract of permot leaves indicates the secondary metabolite levels are also higher. The time required by ethanol extract of permot leaves to kill 50% of *R. sanguineus* tick larvae in vitro was in the interval of 0.91 hours - 2.96 hours while the time needed to kill 95% of *R. sanguineus* larvae was at 2.37 intervals hours - 9.21 hours. The probit test for Lethal Concentration 50 in the second hour was carried out at the four concentrations because the four concentrations had already begun to show an effect on larval mortality, while the fifth hour was carried out because it was the last observation time.

Alkaloids work as a contact poison where the poison will be able to enter the digestive organs through direct contact with the tick's body. Alkaloids are toxic and can work to inhibit the work of the nervous system and damage cell membranes. The alkaloid group works by inhibiting the enzyme acetylcholinesterase, so that acetylcholine will accumulate at synapses. This causes the inhibition of the process of nerve transmission. In addition, alkaloids also cause inhibitory processes to chitin synthesis and inhibit hormone work (Soemirat, in Aseptianova *et al.*, 2017). Flavonoids contained in permot leaves can damage the permeability of cell walls and inhibit the work of enzymes that affect the metabolic process in insects. Flavonoids have a mechanism of attacking several nerves in several vital organs, causing weakness in the tick's nerves. Flavonoids also act as respiratory inhibitors and disrupt energy mechanisms in mitochondria by inhibiting the electron transport system (Muta'ali and Kristani, 2015). Permot plants also contain saponins which can cause irritation to the cuticle of insects. The waxy coating that protects the insect's body and will be lost due to saponins and causes death due to loss of a lot of body fluids. Saponins can also cause digestive enzyme activity and absorption to decrease and interfere with the body's metabolic processes (Novizan, 2002).

Conclusion

Based on the results of the study, the ethanol extract of permot leaves (*Passiflora foetida* L.) can kill the larvae of *R. sanguineus* in vitro. The effective time and concentration to kill *R. sanguineus* larvae in vitro was a concentration of 5% at the fourth hour to a concentration of 10% at the third hour.

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