Efectiveness of *Ocimum sanctum* Linn. Leaves Ethanol Extract on Mortality *Raillietina echinobothrida In Vitro*

Efektivitas Daun Kemangi (*Ocimum sanctum* Linn.) Terhadap Kematian *Raillietina echinobothrida* Secara *In Vitro*

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Abstract

The aims of the research are to prove the anthelmintic effects of *Ocimum sanctum* Linn. leaves ethanol extract on *Raillietina echinobothrida in vitro* and to know the value of LC_{50} and $LC_{90}$of *Ocimum sanctum* Linn. leaves ethanol extract. The research used completely randomized design. The research had five treatments and each treatment was done in four replications. The research used 5 *Raillietina echinobothrida* adult worms for each treatment and all replications. The observation and the recording were done at the first hour until the sixth hour of the research. The *Raillietina echinobothrida* adult worms were noted dead if there was no movement when they were disturbed by anatomic tweezer, the death of *Raillietina echinobothrida* were confirmed when they dipped mildly in warm water (50°C) and they showed no movements. The acquired data was analyzed using ANOVA and continued with LSD (5%). The results were *Ocimum santum* Linn. ethanol extract has the anthelmintic activity in 1.25%, 2.5%, 5% and 10% concentrations. The LC_{50} and LC_{90} was analyzed using probit analysis. The results of LC_{50} calculation were 7.8% at the third hour, 6% at the forth hour, 3.2% at the fifth hour, 2.5% at the sixth hour and the results of LC_{90} were 6.5% at the fifth hour, 5.2% at the sixth hour. The higher concentration of the extract, the higher anthelmintic activity worked.

Key words : *Ocimum sanctum* Linn. leaves, ethanol extract, *Raillietina echinobothrida, in vitro*

Introduction

Tapeworm infection disease remains as a harm problem both in developed or developing countries. Raillietinasis is an important parasitic disease in poultry caused by the genus of *Raillietina*, particularly *Raillietina echinobothrida*, the most influential species in terms of prevalence and pathogenicity in domestic fowls (Khohlring, 2010). Infection of the tapeworms in livestock specifically in domestic fowls beside sucking the nutrients and blood of the host, they also cause the damage to certain organs. In mild infection of *Raillietina echinobothrida*, there are indigestion and growth problem. In heavy infection, there are anemia, conspicuous intestinal nodules in chicken with hyperplastic enteritis associated with the formation of granuloma, sometimes the nodule perforates the intestine causes peritonitis, manifestation of nerve to death (Khohlring, 2010; Tabbu, 2002; Shahin, 2011). Moreover, the infestation of tapeworms in domestic fowls could induce the economic loss of the poultry farmers incurred due to the fowls weight loss.

The control of Raillietiniasis massively was handled with modern synthetic chemical anthelmintics, such as Albendazole and Bunorat. the repeated usage of these chemical anthel-

mintic medicines, nevertheless, resulted various problems, there is development of resistance, chemical residues and toxicity issues (Husain, 2008). The idea of substituting the synthetic anthelmintic medicine with herbal anthelmintic if it is proven to react towards the mortality of *Raillietina echinobothrida* has considerably risen.

Indonesia as the rich country has many various kinds of herbal plants which have been observed as an anthelmintic, one of them is *Ocimum sanctum* Linn. *Ocimum sanctum* Linn., leaves contain phytochemical constituents, such as eugenol oil, saponin, tannin and flavonoid which are potential to be the alternative herbal anthelmintic (Sentana, 2010), it possessed some anthelmintic activity against nematodes, particularly ascarids, it tested *in vitro* showed potent anthelmintic activity towards the *Caenorhabditis elegans* (Kanojiya, 2015; Asha, M. K. *et al.*, 2001). The effectiveness of *Ocimum sanctum* Linn. leaves towards cestode worms, chiefly *Raillietina echinobothrida* has never been observed, however.

The present research aimed to know the effectiveness of *Ocimum* sanctum Linn. leaves ethanol extract on mortality of *Raillietina echinobothrida*, to know the most optimum concentration of *Ocimum sanctum* Linn. as anthelmintic and also to know the *Ocimum sanctum* Linn. leaves ethanol extract values of LC_{50} and LC_{90}.

Materials and Methods

Research Location and Time

This research was finished in four places, the sequence was UPT Materia Medica Batu, Badan Penelitian dan Konsultasi Industri Surabaya, Wonokromo poultry market Surabaya and Parasitology Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga.

Research Materials and Equipments

The materials used in this research were the *Ocimum sanctum* Linn., leaves ethanol extract, the *Raillietina echinobothrida* tapeworms from Wonokromo poultry market, CMC-Na, 0.9% PBS solution, 96% ethanol, warm water (40°-45°C).

The equipments that used in this research were anatomic tweezers, petri dish with 15cm diameter, electronic scale, beaker glass, glass rod, oven, stove, thermometer, incubator, canon microscope, stationery for recording and camera for documentation.

Preparation of *Ocimum sanctum* Linn. Leaves Ethanol Extract

Fresh *Ocimum sanctum* Linn. leaves was purchased from UPT Materia Medica, Batu, Malang, East Java on February 2017. The basil leaves was washed thoroughly with fresh running water. The *Ocimum sanctum* Linn. leaves which had been cleaned were dried with oven in 50---60°C heat. The dried *Ocimum sanctum* Linn. leaves were pulverized with grinder into powder, the powder used for the extraction.

The *Ocimum sanctum* Linn. leaves powder was macerated in 96% ethanol for five days at room temperature and on the first six hours it was stirred with a glass rod manually and repeated once twice a day in five days. After five days, the macerated solutions were filtered with cotton pad and filter paper. The residues were macerated again until the color turned to clear green, the obtained filtrate was combined and condensed with rotary evaporator 50°C until the extract became viscous (Noviana, 2015) and the color turned to clear brown. The extract was stored in refrigerator with temperature about 4°C.

Collection of *Raillietina echinobothrida*

The adult tapeworms of *Raillietina echinobothrida* were collected from the small intestine of the domestic chicken which slaughtered in Wonokromo poultry market, Surabaya. The whole small intestines of chicken were brought to Parasitology Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga. The adult *Raillietina echinobothrida* worms were identified by their scolex which attached to the mucosa of the chicken duodenum then confirmed under the light microscope and also by the average body length from the measurement.

Research Design and Procedure

The research was an experimental research which completely randomized to determine effect of basil leaves (*Ocimum sanctum* Linn.) ethanol extract on mortality of *Raillietina echinobothrida in vitro*. The extraction used maceration method to prepare the *Ocimum*

sanctum Linn. leaves extract and there were five treatments groups in this research which contained 0.5% CMC-Na solvent, the *Ocimum sanctum* Linn. leaves ethanol extract suspension with 1.25%, 2.5%, 5% and 10% concencentrations. Five treatment groups in this research were: Control (C): 5 tapeworms was put into petri dish with 40 ml of 0.5% CMC-Na solvent. Treatment 1 (T1) : 5 tapeworms were put into petri dish with 40 ml of *Ocimum sanctum* Linn., leaves ethanol extract suspension with 1.25% concentrations. Treatment 2 (T2) : 5 tapeworms were put into petri dish with 40 ml of *Ocimum sanctum* Linn., leaves ethanol extract suspension with 2.5% concentrations. Treatment 3 (T3) : 5 tapeworms were put into petri dish with 40 ml of *Ocimum sanctum* Linn., leaves ethanol extract suspension with 5% concentrations. Treatment 4 (T4) : 5 tapeworms were put into petri dish with 40 ml of *Ocimum sanctum* Linn., leaves ethanol extract suspension with 10% concentrations.

Time observations had been done every one hour for six hours. All the petri dishes was put in incubator with temperature 37°C (Jagadeesan *et al*., 2014).

Observation of the Changes

The observation was done by recorded the death of the tapeworm or the live tapeworm by its movements. The worm was disturbed with anatomic tweezer or was shaken vigorously. The death of the worms was confirmed when the worms did not show any movements after vigorous shake and after introduced them into warm water (50°C) by mildly dipping the worms into the petridish with 50°C water inside (Jagadeesan *et al.*, 2014).

In this research, the researcher analyzed the data used Analysis of Variance (ANOVA), then continued with LSD and Duncan Multiple Range Test to analize the data variance between the treatment groups and control group. The calculation of LC_{50} and LC_{90} was analyzed using probit analysis. The analysis was done using SPSS version 20 for windows as the statistical analysis program.

Results

The Observation of the Dead *Raillietina echinobothrida*

The observation of the dead *Raillietina echinobothrida* was done by noted the number of dead *Raillietina echinobothrida* in five treatment groups, which are control group, 1.25% concentration (T1), 2.5% concentration (T2), 5% concentration (T3) and 10% concentration (T4) every one hour in six observation hours.

The Table 1 and the Figure 1 showed the observation data of dead *Raillietina echinobothrida,* at the first observation hour, the 10% concentration was the only concentration that had showed anthelmintic activity, it was significantly different with other concentrations. At the second observation hour, the 10% concentration still the only concentration that had showed the anthelmintic activity with significant difference.

The third observation hour showed the treatment group of 5% concentration had showed the death of *Raillietina echinobothrida*, there was significant difference compared to 2.5% concentration. The 10% concentration at this hour also showed significant difference with 5% concentration.

Table 1. The Dead *Raillietina echinobothrida* in Every Treatments at All Observation Hours

Treatment	1	2	3	4	5	6
Control (To)	0.00^{a}±0.0	0.00^{a}±0.0	0.00^{a}±0.0	0.00^{a}±0.0	0.00^{a}±0.0	0.25^{a}±0.5
1.25% (T1)	0.00^{a}±0.0	0.00^{a}±0.0	0.00^{a}±0.0	0.50^{ab}±0.6	1.50^{b}±0.6	3.00^{b}±0.8
2.50% (T2)	0.00^{a}±0.0	0.00^{a}±0.0	0.00^{a}±0.0	1.00^{b}±0.0	2.25^{c}±0.5	3.25^{b}±0.5
5.00% (T3)	0.00^{a}±0.0	0.00^{a}±0.0	1.00^{b}±0.0	2.25^{c}±0.5	3.00^{d}±0.0	4.00^{c}±0.0
10% (T4)	0.25^{b}±0.5	1.75^{b}±0.5	3.50^{c}±0.6	4.00^{d}±0.0	4.75^{e}±0.5	5.00^{d}±0.0

Values are expressed as mean ± SD, the different superscript of $^{a, b, c, d, e}$ in same column show significant difference ($p<0.05$)

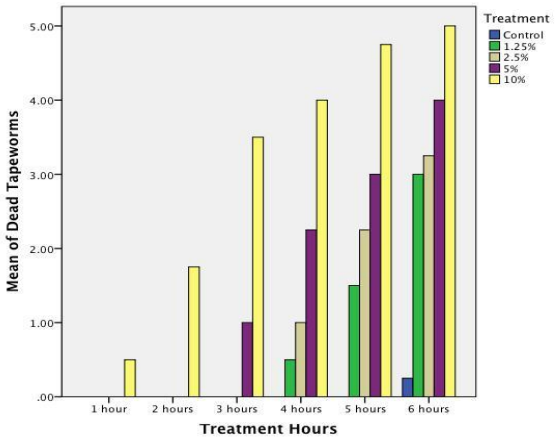

Figure 1. Showing The Effect of *Ocimum sanctum* Linn. Leaves Ethanol Extract to The Average Numbers of Dead *Raillietina echinobothrida* in Each Concentrations at Each Observation Hours.

The forth observation hour in treatment group of control and 1.25% had not been showed difference significantly. The 1.25% concentration and the 2.5% concentration had showed the death of *Raillietina echinobothrida*, but no significantly different. The 2.5% concentration and the 5% are significantly different at this hour. The 5% concentration and the 10% concentration showed significant difference as well.

The fifth observation hour in the treatment group of control and the 1.25% concentration had showed significant difference. The 1.25% the 2.5% concentration had also showed significant difference, the 2.5% and the 5% concentration had also showed significant difference and the 5% and 10% concentration has also showed the significant difference, but the 10% concentration showed the highest average number of dead *Raillietina echinobothrida* at the fifth hour. The dead *Raillietina echinobothrida* was found in all treatments groups at sixth observation hour, the significant difference was happened between every treatment groups at this last observation hour.

LC50 and LC90

The value of LC50 and LC90 of Ocimum sanctum Linn. Ethanol extract was calculated using probit analysis with log base 10. The LC_{50} and the LC_{90} in all observation hours can be seen in Table 2. Based on the Table 2, it can be known the value of LC_{50} at 1, 2, 3, 4, 5, and 6 observation hours are 13.8%, 12.1%, 7.8%, 6%, 3.2% and 2.5%. The 10% concentration had been able to kill 50% population of *Raillietina echinobothrida* at the third hour, the 5% and 2.5% concentration had been enough to kill 50% population of *Raillietina echinobothrida,* but in longer exposure time. The LC_{90} of *Ocimum sanctum* Linn. ethanol extract are 6.5% at the fifth hour and 5.2% at the sixth hour.

The 10% concentration is the only concentration that was able to kill 90% population of *Raillietina echinobothrida.*

Table 2. The Value of LC50 and LC90 of *Ocimum sanctum* Linn. Ethanol Extract in Every Observation Hours.

Observation Hour	LC_{50}	LC_{90}
1	13.8%	28.1%
2	12.1%	24.5%
3	7.8%	15.8%
4	6%	12%
5	3.2%	6.5%
6	2.5%	5.2%

Discussion

The research results from the data analysis with ANOVA continued with LSD can be found out that the result was significant difference ($p<0.05$). The observation at the first hour showed there was still no anthelmintic activity of *Ocimum sanctum* Linn. ethanol extract in the 1.25% concentration group (T1), the 2.5% concentration group (T2) and the 5% concentration group (T3), but the 10% concentration group (T4) had showed the anthelmintic activity and there was already significant difference between 10% concentration and other concentrations. The observation result at the second hour, the anthelmintic activity of *Ocimum sanctum* Linn. was not clearly showed in the concentrations of 1.25% (T1), 2.5% (T2), 5% (T3), that can be inferred from the not significant difference average number of the dead *Raillietina echinobothrida* among these groups, but still only the 10% concentration group (T4) had showed the anthelmintic activity to *Raillietina echinobothrida*, it can be seen from the significant difference of 10% concentration (T4) group with others group.

The observation at the third hour showed the anthelmintic activity of *Ocimum sanctum* Linn. leaves ethanol extract began to appear at 5% concentrations (T3). The 5% concentration group has also showed significant difference with the 1.25% and 2.5% concentrations group, it also was significantly different with the 10% concentration group. The 10% concentration group showed the anthelmintic activity of *Ocimum sanctum* Linn. leaves ethanol extract increased during the immersion time got longer, it showed higher average number of dead *Raillietina echinobothrida* and also significant difference with 5% concentration group which means the *Ocimum sanctum* Linn. leaves ethanol extract with 10% concentration is more effective.

The *Ocimum sanctum* Linn. leaves ethanol extract in each concenctrations at the forth observation hour had showed the anthelmintic activity. The *Ocimum sanctum* Linn. leaves ethanol extract with 1.25% concentration had even showed the anthelmintic activity, but from the statistic analysis, the result showed that the 1.25% concentration did not make the significant difference with the control group. The 2.5% concentration also showed anthelmintic at this hour, but statistically had no significant differrence with 1.25% concentration which means the 1.25% was more effective at this hour. The 5% concentration showed the increased number in the average number of the dead *Raillietina echinobothrida*. Beside that, the 10% concentration at this observation hour continually increased the amount of dead *Raillietina echinobothrida,* from the analysis data the average number of dead *Raillietina echinobothrida* in *Ocimum sanctum* Linn.

The *Ocimum sanctum* Linn. leaves ethanol extract at the fifth observation hour showed the anthelmintic activity against the *Raillietina echinobothrida* more effective. The average number of the dead *Raillietina echinobothrida* in each concentration groups was increased. At this hour, the difference between each concentration appeared significantly. The *Ocimum sanctum* Linn. leaves ethanol extract with 10% concentration in this hour successfully almost killed all the *Raillietina echinobothrida,* it can be inferred from the death percentage that has reached more than 90% of tapeworms

population in this hour. The *Ocimum sanctum* Linn. at the sixth observation hour which is the last observation hour showed the anthelmintic activity clearly with the highest effectivity, it can be seen from the number of the average number of the dead *Raillietina echinobothrida* was highly increased in each concentration group. The *Ocimum sanctum* Linn. leaves ethanol extract with 1.25% and 2.5% concentration showed no significant difference in their anthelmintic activity, because there was only small amount difference in average number of dead *Raillietina echinobothrida*, that means 1.25% concentration was enough and more effective than 2.5% concentration at this hour. The average number of the dead *Raillietina echinobothrida* in the 2.5% concentration and 5% concentration showed significant difference, the statistical analysis showed 5% concentration worked more effective than the 2.5% concentration. The last observation hour showed that the *Ocimum sanctum* Linn. leaves ethanol extract with 10% concentration has the highest average number of the dead *Raillietina echinobothrida.*

Dead *Raillietina echinobothrida* was also found in the control group which contained CMC-Na that was dissolved in PBS solution. The death percentage was quite small, it was only about 5%, it might be happened because of the in vitro method. The research has been designed as simulant as the host body condition, but still the different physiological condition and the environment orientation might affected the condition of *Raillietina echinobothrida*. In fact, CMC---Na has already been used in many fields, especially in pharmaceutics, food and chemical industries (Musfiroh and Budiman, 2013).

The *Ocimum sanctum* Linn. leaves has been proven that it has important phytochemical constituents to be anthelmintic, there were phenolics, flavonoid, tannin, saponin and eugenol (Karumari *et al*., 2014). Tannin could act as anthelmintic indirectly by increasing the protein availability for enhancing the immunelogical responses of host to helminthes (Athanasiadou *et al*., 2001). Tannins and phenolics are known to act with the energy generation in helminthes by uncoupling the oxidative phosphorylation, binding to free proteins in the host gastrointestinal and leading to death, that ability made tannins could also inhibit enzymatic

activity in metabolic pathway of the worms (Athanasiadou *et al.*, 2001; Hoste *et al.*, 2006). Saponin is worked by increasing the permeability of the helminth body wall, it could cause the vacuolization (Parvathy *et al.*, 2012). The essential oil of *Ocimum sanctum* Linn. which named eugenol is showed possible utilization in the treatment of gastrointestinal helminthes, it has already been proven that eugenol has potent anthelmintic activity towards the *Caenorhabditis elegans* and *Haemonchus contortus* (Pessoa *et al.*, 2002). The effective anthelmintic activity of *Ocimum sanctum* Linn. leaves ethanol extract might be supported by the used of *Ocimum sanctum* Linn. leaves from UPT Materia Medica, Batu, Malang where the *Ocimum sanctum Linn.* was came from a good quality of seeds and grew with a good set maintenance.

The comparation in each treatment groups at every observation hours was analyzed statistically for the second time using probit analysis to determine the LC_{50} and the LC_{90} of *Ocimum sanctum* Linn. leaves ethanol extract at every observation hours. The LC_{50} at 1, 2, 3, 4, 5, and 6 observation hours are 13.8%, 12.1%, 7.8%, 6%, 3.2% and 2.5%. The LC_{90} of *Ocimum sanctum* Linn. ethanol extract at first hour is 28.1%, 24.5% at the second hour, 15.8% at the third hour, 12% at the forth hour, 6.5% at the fifth hour and at the sixth hour is 5.2%.

Based on the research results, that can be known that the longer immersion time of the *Raillietina echinobothrida* the lower concentration that needed to kill 50% or 90% population of *Raillietina echinobothrida* as well. The 10% concentration of *Ocimum sanctum* Linn. leaves ethanol extract in term of exposure time was the fastest concentration whom showed the anthelmintic activity, at the very first observation hour it had showed the anthelmintic activity, it even had been able to kill 50% population sample at the third hour and it had been able to kill 90% population sample at the fifth hour.

Conclusions

Based on the the research results, there can be concluded that the ethanol extract suspension of *Ocimum sanctum* Linn. leaves in 1.25%, 2.5%, 5% and 10% concentrations have the anthelmintic activity towards *Raillietina echinobothrida in vitro*. The LC_{50} of *Ocimum sanctum* Linn. leaves ethanol extract is 7.8% at the third hour, 6% at the forth hour, 3.2% at the fifth hour and the 2.5% at the sixth hour. The 5% had been effective to kill 50% population of *Raillietina echinobothrida* at fifth observation hour. The LC_{90} of *Ocimum sanctum* Linn. leaves ethanol extract is more than 10% at the first and until the forth hour, 6.5% at the fifth hour and 5.2% at the sixth hour. The 10% concentration in accordance of the LC_{50} and LC_{90} value of *Ocimum sanctum* Linn. leaves ethanol extract is the most optimum concentration as the anthelmintic towards *Raillietina echinobothrida*.

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