Anthelmintic Activity Ethanol Extract of *Ocimum sanctum* Linn. Leaves Against *Ascaridia galli* In Vitro

Aktivitas Antelmintik Ekstrak Etanol Daun Kemangi *Ocimum sanctum* Linn. Terhadap *Ascaridia galli* Secara In Vitro

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Abstract

The aims of this research are to determine concentration, exposure time, interaction between concentration and exposure time of ethanol extract of *Ocimum sanctum* Linn. Leaves to cause death toward *Ascaridia galli* in vitro, and the value of LC$_{50}$ and LC$_{90}$ ethanol extract of *Ocimum sanctum* Linn. Leaves. Research design that has been used in the research was completely randomized design. This research used 200 samples of *Ascaridia galli* with length 7-11 cm without differentiating their sex. The concentration ethanol extract of *Ocimum sanctum* Linn. leaves were 1.25%, 2.5%, 5%, 10%. The control was using CMC-Na 0.5%. Each treatment then being replicated four times. The observation and recording of dead worm were done at 0, 3, 6, 12 and 24 hours. *Ascaridia galli* were declared dead if there was no movement while disturbed by anatomy tweezers and dipped in slightly warm water (50ºC). The obtained data was analyzed using Anova Factorial and continued with Duncan Multiple Range Test by SPSS for Windows 22. The result were 10% concentration and exposure time for 24 hours caused the most mortality toward *Ascaridia galli*. Interaction between concentration and exposure time resulted 10% concentration ethanol extract of *Ocimum sanctum* Linn. leaves in 24 hours caused the most mortality towards *Ascaridia galli*. Probit analysis was used to calculate the LC$_{50}$ and LC$_{90}$ of *Ocimum sanctum* Linn. leaves. The results were LC$_{50}$ ethanol extract of *Ocimum sanctum* Linn. leaves at 6 hours was 14.8%, at 12 hours was 4.8% and at 24 hours was 3.0% and the LC$_{90}$ at 24 hours was 9.1%.

Key words: *Ocimum sanctum* Linn. leaves, *Ascaridia galli*, ethanol extract, in vitro.

Introduction

Nematodosis prevalence especially ascariasis caused by infection of *A. galli*, this worm attacks small intestine of domestic chicken especially in the traditional farm method and cause decreasing meat productions, which make farmers have financial loss (Parmin *et.al.*, 2002 and Subekti, 2011).

The disease is highly prevalent particular in world countries due to poor management practices. FAO (2001) showed that prevalence number of round-worms in Indonesia is ranged about 92.3% and 48% of them is *Ascaridia galli*. One of helminthiasis caused by Nematodes.

Most common drugs for anthelmintic treatment to treat ascariasis are Piperazine. Although these medicine are the most common drugs which used in field, various problems have been found in the parasite control using synthetic anthelmintic drugs, such as chemical residues, toxicity issues, long withdrawal period, possibility lead to resistance, not economical and unavailability of these drugs in remote areas (Hussain, 2008 and Veerakumari, 2015). Small holder farmers in many developing countries often do not have access to expensive anthelmintic drugs and low income farms are not able to prophy-
lactically treat animals with synthetic drugs. Therefore, there is an urgent need to investigate alternative or complementary options for the control of this parasite (Andrew et al., 2014).

Many researchers have been working to find the solutions. One of the works is by researching the anthelmintic activity derived from various herbal, included using Ocimum sanctum as one kind of herbal. Researchers have found many efficacies of Ocimum sanctum, including as anthelmintic. Recent work of its consumption has shown that holy basil had no genotoxic or organ toxic effects (Chandrasekaran et al., 2013). Its anthelmintic activity has been tested against Pheretima posthuma, Cotylophoron cotylophorum, Syphacia muris, Setaria digitata, Caenorhabditis elegans (Goswami et al., 2016; Buchineni et al., 2015; Pandey et al., 2016; Karumari et al., 2014; Joshi et al., 2013; Verma et al., 2013). However, it has not been done against Ascaridia galli. The anthelmintic activity presents because there are some phytochemical constituents which responsible for this efficacy. Those are tannins, alkaloids, flavonoids and saponin (Athanasiadou et al, 2001).

This research was aimed to know the concentration, exposure time, interaction between concentration and exposure time of ethanol extract of Ocimum sanctum Linn. leaves which cause the most mortality toward Ascaridia galli, and to know its value of LC50 and LC90.

Materials and Methods

Research Location and Date

This research was held at Laboratory of Parasitological Department Faculty of Veterinary Medicine Universitas Airlangga, Badan Penelitian dan Konsultasi Industri Surabaya, UPT Materia Medica Batu and Wonokromo Slaughter House in Surabaya. It was started in January 2017 and has been done in March 2017.

Research Materials and Equipments

Materials used in this research were leaves of Ocimum sanctum Linn. which was obtained from UPT Materia Medica Batu, Ascaridia galli from Wonokromo Slaughter House in Surabaya, PBS solution, 96% ethanol and CMC Na.

Equipments used in this research were petri dish with 15 cm diameter, anatomy tweezers, thermometer, baker glass, glass rod, oven, stove, grinder, rotavapor, plastic bag, electronic scale, incubator with 120°C capacity and camera for documentation.

Preparation of Ocimum sanctum Linn Leaves Ethanol Extract

Fresh Ocimum sanctum Linn leaves were collected from UPT Materia Medica Batu in January 2017. The Ocimum sanctum Linn leaves were washed thoroughly with fresh water and dried with oven in 50-60°C. The dried Ocimum sanctum Linn leaves were mashed up with a grinder into powder and the powder was used for the extraction. The extraction was done in Badan Penelitian dan Konsultasi Industri, Surabaya.

Ocimum sanctum Linn leaves powder were macerated in 96 % ethanol for five days. Filtration was done to separate the dregs from the solution. Then the dregs were macerated again in 96 % ethanol (remacerated), maceration was performed three times and the pooled macerated then evaporated using a rotavapor at 50 °C for 4-5 hours to obtain a viscous extract. The ethanol extract was stored in 4°C until being used (Bachaya et al., 2009).

Preparation of Ascaridia galli

Ascaridia galli were collected from the small intestine of domestic chicken in domestic chicken slaughtered house in Wonokromo traditional market, Surabaya then brought to Parasitology Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga. Ascaridia galli were washed with PBS until they are separated from blood, tissue, debris or any attached particles (Amin et al., 2009 and Bachaya et al., 2009). Then, the alive and cleaned were used as the sample and were placed in the petri dish then ready to be treated.

Determination Ethanol Extract of Ocimum sanctum Linn Leaves Suspension Concentration

This research used four different concentration, those were 1.25%, 2.5%, 5% and 10%. These concentration was based on previous research which was done by Goswami et al., (2016).
Experimental Design

All 50 Ascaridia galli were divided into five groups by simple random sampling and done for four replications.

Control (C) : Ten Ascaridia galli were put in 40 ml of 0.5% CMC-Na solvent. Treatment 1 (T1) : Ten Ascaridia galli were put in 40 ml ethanol extract of Ocimum sanctum Linn leaves suspension with 1.25% concentration. Treatment 2 (T2) : Ten Ascaridia galli were put in 40 ml ethanol extract of Ocimum sanctum Linn leaves suspension with 2.5% concentration. Treatment 3 (T3) : Ten Ascaridia galli were put in 40 ml ethanol extract of Ocimum sanctum Linn leaves suspension with 5% concentration. Treatment 4 (T4) : Ten Ascaridia galli were put in 40 ml ethanol extract of Ocimum sanctum Linn leaves suspension with 10% concentration.

Time observations was in 0, 3, 6, 12 and 24 hours. During the observation, all the petri dishes were put in incubator with temperature of 37°C (Ali., et al, 2012; Chang et al., 2015; Nassef et al., 2014).

Observation of the Changes

In this research, death of the worms were observed by seeing its movement. If Ascaridia galli did not show any movement while disturbed by anatomy tweezers and while dipped in slightly warm water (50°C), the worm was confirmed dead (Ali., et al, 2012).

In this research, the obtained data was analyzed using Anova Factorial and continued with Duncan Multiple Range Test. While the LC_{50} and LC_{90} were calculated using probit analysis. SPSS 22 for windows software was used as statistical analysis program.

Result and Discussion

Effect of Ocimum sanctum Linn. Leaves Ethanol Extract Concentration toward Dead Ascaridia galli

The statistical analysis result showed that the death of Ascaridia galli influenced by the variation of ethanol extract of Ocimum sanctum linn. leaves concentrations, that are 1.25% (T1), 2.5% (T2), 5% (T3), and 10% concentration (T4).

Based on Table 4.1 and Figure 4.1, the highest number of dead Ascaridia galli is in 10% concentration (T4). According to transformation data in Table 4.1, control is significantly different with 1.25% (T1). T1 has significant difference with 2.5% (T2). T2 has significant difference with 5% (T3), and significant difference also occur between T3 and 10% (T4). It can be concluded that significant difference happens between every treatment groups.

Table 1. The Effect of Ethanol Extract of Ocimum sanctum Linn. Leaves to Ascaridia galli Which Cause Death Based on Concentration Treatment Groups.

| Treatment Group | Mean ($\bar{x}$) | Data in Percent | Transformation Data |
|-----------------|-----------------|-----------------|---------------------|
| Control         | 00.00           |                 | 0.71$^a$            |
| 1.25% (T1)      | 05.50           |                 | 1.70$^b$            |
| 2.5% (T2)       | 16.00           |                 | 3.04$^c$            |
| 5% (T3)         | 22.00           |                 | 3.70$^d$            |
| 10% (T4)        | 36.50           |                 | 4.77$^e$            |

$^{a,b,c,d,e}$ Different superscript in the same column showed significant difference (p < 0.05).
Effect of Exposure Time toward Dead Ascaridia galli

Ascaridia galli death in this research is influenced by the exposure time of Ascaridia galli to the extract of Ocimum sanctum linn. leaves. The observation and recording of dead Ascaridia galli were done at 0, 3, 6, 12 and 24 hours.

Based on Table 1.1 and Figure 1.1, data in percent (%) and transformation data, the highest number of dead A. galli is at 24 hours. The column of transformation data showed that there is no significant difference between observation at 0 to 3 hours. The significant difference occur between observation at 3 to 6 hours, 6 to 12 hours, also between 12 to 24 hours.

Table 2. The Effect of Ethanol Extract of Ocimum sanctum Linn. Leaves to Ascaridia galli Which Cause Death Based on Observation Time

| Observation Time (hours) | Data in Percent (%) | Transformation Data |
|--------------------------|---------------------|---------------------|
| 0                        | 00.00               | 0.71<sup>a</sup>     |
| 3                        | 00.00               | 0.71<sup>a</sup>     |
| 6                        | 06.50               | 2.03<sup>b</sup>     |
| 12                       | 29.50               | 4.58<sup>c</sup>     |
| 24                       | 44.00               | 5.90<sup>d</sup>     |

<sup>a,b,c,d</sup> Different superscript in the same column showed significant difference (p < 0).
**Table 3.** The Interaction Between Observation Time and Concentration Level of Ethanol Extract of *Ocimum sanctum* Linn. Leaves to *Ascaridia galli* Death

| Observation Time (hours) | Treatment | Data in Percent | Mean (x̄) | Transformation Data |
|-------------------------|-----------|-----------------|----------|---------------------|
|                         | Control   | 00.00           | 0.71a    |                     |
|                         | 1.25% (T1)| 00.00           | 0.71c    |                     |
|                         | 2.5% (T2) | 00.00           | 0.71c    |                     |
|                         | 5% (T3)   | 00.00           | 0.71c    |                     |
|                         | 10% (T4)  | 00.00           | 0.71a    |                     |
| 3                       | Control   | 00.00           | 0.71a    |                     |
|                         | 1.25% (T1)| 00.00           | 0.71a    |                     |
|                         | 2.5% (T2) | 02.50           | 1.34a    |                     |
|                         | 5% (T3)   | 10.00           | 2.93b    |                     |
|                         | 10% (T4)  | 20.00           | 4.45c    |                     |
| 6                       | Control   | 00.00           | 0.71a    |                     |
|                         | 1.25% (T1)| 05.00           | 1.66a    |                     |
|                         | 2.5% (T2) | 20.50           | 5.73d    |                     |
|                         | 5% (T3)   | 42.50           | 6.54cde  |                     |
|                         | 10% (T4)  | 67.50           | 8.23g    |                     |
| 12                      | Control   | 00.00           | 0.71a    |                     |
|                         | 1.25% (T1)| 05.00           | 1.66a    |                     |
|                         | 2.5% (T2) | 32.50           | 5.73d    |                     |
|                         | 5% (T3)   | 42.50           | 6.54cde  |                     |
|                         | 10% (T4)  | 67.50           | 8.23g    |                     |
| 24                      | Control   | 00.00           | 0.71a    |                     |
|                         | 1.25% (T1)| 22.50           | 4.70c    |                     |
|                         | 2.5% (T2) | 45.00           | 6.74ef   |                     |
|                         | 5% (T3)   | 57.50           | 7.61fg   |                     |
|                         | 10% (T4)  | 95.00           | 9.77h    |                     |

Different superscript in the same column showed significant difference (p < 0.05).

**LC₉₀ and LC₉₀**
Ethanol extract of *Ocimum sanctum* linn. leaves LC₉₀ and LC₉₀ calculation is using probit analysis. From the calculation, the LC₉₀ and LC₉₀ can be known in every observation time that can be seen in Table 4.4. Based on Table 4.4, can be concluded that LC₉₀ of ethanol extract of *Ocimum sanctum* Linn. leaves at 6 hours is 14.8%, at 12 hours is 4.8%, and at 24 hours is 3.0% concentration. The 10% concentration is enough to kill 50% of *Ascaridia galli* population at 6, 12 and 24 hours. The LC₉₀ of ethanol extract of *Ocimum sanctum* Linn. leaves at 6 hours is 45.5%, at 12 hours is 14.7%, and at 24 hours is 9.1% concentration. The 10% concentration is enough to kill 90% of *Ascaridia galli* total population in the longest observation time, which is 24 hours.

![Figure 3](image-url)
**Table 4.** Various Concentration of Ethanol Extract of *Ocimum sanctum* Linn. Leaves Suspension That Cause Death of 50% and 90% *Ascaridia galli* Population in Every Observation Time

| Observation Time (hours) | LC$_{50}$ | LC$_{90}$ |
|--------------------------|-----------|-----------|
| 0                        | -         | -         |
| 2                        | -         | -         |
| 6                        | 14.8%     | 45.5%     |
| 8                        | 4.8%      | 14.7%     |
| 10                       | 3.0%      | 9.1%      |

**Discussion**

Based on statistical analysis of the research result, there is no significant difference of dead *Ascaridia galli* between every treatment groups at 0 and 3 hours. At 6 hour, the result showed that the ethanol extract of *O. sanctum* Linn. leaves at 2.5% concentration (T2), 5% concentration (T3) and 10% concentration (T4) already has potential anthelmintic activity to kill *Ascaridia galli*, which means the onset of action of the ethanol extract of *O. sanctum* Linn. leaves has started at 6 hours. Those concluded based on the statistical analysis data which showed that control has no significant difference to T1 and T2 while T2 has significant difference to T3, also the T3 which significantly difference to T4.

Statistical analysis result at 12 hours showed there is no significant difference between control to 1.25% concentration (T1). T1 showed significant difference to 2.5% concentration (T2). But T2 is not significantly difference to 5% concentration (T3) which can be concluded that control to T1 has similar activity with T2 to T3. T3 showed there is significant difference to 10% concentration (T4).

The result of observation at 24 hours revealed that the number of dead *Ascaridia galli* are keep increasing based on statistical analysis result. The interaction between observation of exposure time and concentration in control showed significant difference to all of treatments, so are in between 1.25% concentration (T1) to 2.5% concentration (T2). But not between 2.5% concentration (T2) to T3. This can be concluded that T2 has similar anthelmintic activity with T3. While 10% concentration (T4) is significantly different to other treatments, control, T1, T2 and T3. From the data above, can be concluded that the highest average of dead *Ascaridia galli* were in 10% concentration (T4) at 24 hours of time observation..

Dead *Ascaridia galli* are not found in control group, which was CMC-Na dissolved in PBS. This might be happening because of CMC-Na is a suspensator that does not effect *Ascaridia galli* ability to live outside its host and also because of the duration of *A. galli* ability to live outside the host which known by the preliminary test result that was done before the main experiment started. The preliminary test resulted that *Ascaridia galli* could live outside its host up to ±40 hours while the main experiment was done in 24 hours because the anthelmintic effect of *O. sanctum* Linn. leaves towards *Ascaridia galli* only take 24 hours to kill almost all of the *Ascaridia galli* population in the highest concentration which was 10% concentration.

Ethanol extract of *O. sanctum* Linn. leaves has been proved to have phytochemical constituent that were beneficial to be anthelmintic. According to Karumari *et al.* (2014), *O. sanctum* Linn. leaves has anthelmintic activity. Its phytochemical constituents contain tannin, phenol, flavonoid and saponin. Tannins and phenol are known to interfere with the energy generation in helminth parasites by uncoupling oxidative phosphorylation. Thus blocking ATP synthesis in helminth parasite then causing paralysis and death, also works bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of parasite and leading to death (Athanasiadou *et al.*, 2001). Flavonoid have pharmacological effect to vascular and causing capillary vasoconstriction and decreasing vascular permeability, because of these pharmacological effects, it causing vascular disturbance so the worm’s nutrition and oxygen that required for the parasite sustainability will be disturbed and quickly quicken the mortality of the parasite. Saponin as an anthelmintic works by increasing the permeability and pore formation of the worm body wall, it causing vacuolization and disintegration cuticle (Sea, 2016).

Based on the statistical analysis of the result, the higher concentration of the extract and the longer the time of observation hours causing higher number of dead *Ascaridia galli*. 

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**Table 4** Various Concentration of Ethanol Extract of *Ocimum sanctum* Linn. Leaves Suspension That Cause Death of 50% and 90% *Ascaridia galli* Population in Every Observation Time

| Observation Time (hours) | LC$_{50}$ | LC$_{90}$ |
|--------------------------|-----------|-----------|
| 0                        | -         | -         |
| 2                        | -         | -         |
| 6                        | 14.8%     | 45.5%     |
| 8                        | 4.8%      | 14.7%     |
| 10                       | 3.0%      | 9.1%      |
According to statistical analysis of the research result to determine the effective concentration can be concluded that 10% concentration for 24 hours is the most effective concentration to kill *Ascaridia galli* population.

Based on Table 4.4, can be concluded that LC\textsubscript{50} of ethanol extract of *Ocimum sanctum* Linn. leaves at 6 hours is 14.8%, at 12 hours is 4.8%, and at 24 hours is 3.0% concentration. The 10% concentration is enough to kill 50% of *A. galli* population at 6, 12 and 24 hours. The LC\textsubscript{90} of ethanol extract of *Ocimum sanctum* Linn. leaves at 6 hours is 45.5%, at 12 hours is 14.7%, and at 24 hours is 9.1% concentration. The 10% concentration is enough to kill 90% of *A. galli* total population in the longest observation time, which is 24 hours.

**Conclusion**

Based on research result, it can be concluded that ethanol extract of *Ocimum sanctum* linn. leaves with 10% concentration and exposure time for 24 hours caused the most mortality toward *Ascaridia galli*. Means that the interaction between concentration and exposure time stated that 10% concentration for 24 hours is the effective concentration and time to cause the most mortality towards *Ascaridia galli*. Meanwhile, the LC\textsubscript{50} of ethanol extract of *Ocimum sanctum* linn. leaves is 14.8% at 6 hours, 4.8% at 12 hours and 3.0% at 24 hours. While the LC\textsubscript{90} of ethanol extract of *Ocimum sanctum* linn. leaves is 9.1% at 24 hours.

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