Conference Paper

ANTHELMINTIC ACTIVITY of *Ocimum sanctum* Linn. LEAVES ETHANOL EXTRACT AGAINST *Fasciola gigantica* IN VITRO

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Abstract

The aims of this research are to know the concentration, exposure time and interaction between concentration and exposure time of *Ocimum sanctum* Linn. leaves ethanol extract which cause the most mortality toward *Fasciola gigantica*. Also to know its value of $LC_{50}$and LC_{90}. The research was completely randomized design. There were five treatments. Each treatment was done in four replications and used 10 *Fasciola gigantica.* The observation and recording of dead *Fasciola gigantica* were done at 0, 2, 4, 6, 8 and 10 hours. *Fasciola gigantica* were declared dead if there was no movement when disturbed by anatomy tweezers and when dipped in slightly warm water (50°C). The obtained data was analyzed using ANOVA Factorial and continued with Duncan Multiple Range Test. The result was 10% concentration and exposure time for 10 hours caused the most mortality toward *Fasciola gigantica*. However, the interaction between concentration and exposure time resulted that 5% concentration for 8 hours already caused the most mortality of *Fasciola gigantica*. Probit analysis was used to calculate the $LC_{50}$and LC_{90}. The results were $LC_{50}$of *Ocimum sanctum* Linn. leaves ethanol extract was 7.9% at 4 hours, 3.7% at 6 hours, 1.8% at 8 hours and 0.8% at 10 hours and the $LC_{90}$was 8.4% at 10 hours.

Keywords: *Ocimum sanctum* Linn. leaves, *Fasciola gigantica,* ethanol extract, in vitro.

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Received: 03 October 2017
Accepted: 10 October 2017
Published: 29 November 2017

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the VMIC Conference Committee.

1. Introduction

Proposition regarding the arising resistance of fascioliasis to the drug of choice, that is Triclabendazole, is very concerning because it threatened human health. Nowadays, a lot of researchers has made many efforts to find the solution for this problem. One of the effort is to test the anthelmintic activity derived from various herbal, including *Ocimum sanctum.*

Fascioliasis (fasciolosis/ distomatosis/ liver fluke disease) is a zoonotic disease, which means it can naturally transmissible from vertebrate animals to humans and vice-versa (Abdi *et al.*, 2013) and it is caused by *Fasciola gigantica*, occurring in the tropical area of Africa, Middle East and Asia (Tolan, 2011).

Among many kinds of parasitosis, fascioliasis is the cause of second economic loss after Surra in cattle and buffalo (Martindah *et al.,*2005). The prevalence number show the range from 25-90% and affect to great economical loss as much as Rp 513 billion/year (Spithill *et al.,*1999; Mitchell, 2007).

The drug of choice for fascioliasis is triclabendazole. Although it is common to use triclabendazole in field, it has many disadvantages. The main disadvantage is synthetic anthelmintic lose its efficacy about 20 years (Mehlhorn *et al.,* 2010) and lead to resistance. Therefore, it is necessary to discover alternative anthelmintic derived from herbal to resolve this problem, which has its own benefit, that is the stable characteristic of natural active chemical compound diversity which can preclude the anthelmintic resistance (Tariq *et al.,* 2009).

Indonesia is rich with its diversity of herbal that has their own special beneficial, one of them is *Ocimum sanctum.* Researchers have found many efficacies of *Ocimum sanctum,* including as anthelmintic. Its anthelmintic activity has been tested against *Pheretema posthuma*, *Syphacia muris*, *Ascaris suum*, *Setaria digitata*, *Caenorhabditis elegans* and to *Cotylophoron cotylophorum* (Gosmawi *et al.,* 2016; Buchineni *et al.,* 2015; Pandey *et al.,* 2016; Sea, 2016). However, it has not been tested against *Fasciola gigantica.* The anthelmintic activity is present because there are phytochemical constituents which responsible for this efficacy. Those phytochemical constituents are tannins, alkaloids, flavonoids and saponin (Sawarkar *et al.,* 2011).

This research was aimed to know the concentration, exposure time and interaction between concentration and exposure time of *Ocimum sanctum* Linn. leaves ethanol extract which cause the most mortality toward *Fasciola gigantica*. Also to know its value of LC_{50} and LC_{90}.

2. Materials and Methods

2.1. Preparation of *Fasciola gigantica*

Fasciola gigantica were collected from the liver of cattle in Pegirikan Slaughter House, Surabaya and then brought to Parasitology Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga. *Fasciola gigantica* were washed with PBS until they are separated

from blood, tissue, debris or any attached particles (Omran *et al.,* 2015; Bachaya *et al.,* 2009). Then, they were placed in the petri dish and ready to be treated.

2.2. Preparation of *Ocimum sanctum* Linn Leaves Ethanol Extract

Fresh *Ocimum sanctum* Linn leaves were collected from UPT Materia Medica Batu in December 2016. 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 use (Bachaya *et al.,* 2009).

2.3. Determination of *Ocimum sanctum* Linn Leaves Ethanol Extract 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 Gosmawi *et al*., (2016).

3. Experimental Design

All 50 *Fasciola gigantica* were divided into five groups by simple random sampling and done for four replication. Control group: Ten *Fasciola gigantica* were put in 30 ml of 0.5% CMC-Na solvent; Treatmet groups: Ten *Fasciola gigantica* were put in 30 ml of *Ocimum sanctum* Linn leaves ethanol extract suspension with 1.25%; 2.5%; 5% and 10%.

Time observations was in 0, 2, 4, 6, 8 and 10 hours. During the observation, all the petri dishes were put in incubator with temperature of 37°C (Chang *et al.,* 2015; Nassef *et al.,* 2014).

4. Observation of the Changes

In this research, the death of the worms were observed by seeing its movement. If *Fasciola gigantica* didn't show any movement when touched by anatomy tweezers and when dipped in slightly warm water (50°C), the worm was confirmed dead (Dash *et al.,* 2017).

5. Data Analysis

In this research, the obtained data was analyzed using ANOVA Factorial, then 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.

6. Result

6.1. Effect of Exposure Time toward Dead *Fasciola gigantica*

Fasciola gigantica death in this research is influenced by the exposure time of *Fasciola gigantica* to the *Ocimum sanctum* Linn. leaves ethanol extract. The observation and recording of dead *Fasciola gigantica* were done at 0, 2, 4, 6, 8 and 10 hours.

Based on Table 1, data in percent (%) and transformation data, the highest number of dead *Fasciola gigantica* is at 10 hours. The column of transformation data shows the significant difference between 0 and 2 hours, 2 and 4 hours, 4 and 6 hours, 6 and 8 hours also between 8 and 10 hours. It can be said between every observation hour is significantly different.

6.2. Effect of *Ocimum sanctum* Linn. Leaves Ethanol Extract Concentration toward Dead *Fasciola gigantica*

The statistical analysis result showed that the death of *Fasciola gigantica* not only influenced by observation time but also the variation of *Ocimum sanctum* Linn. leaves ethanol extract concentration, that is 1.25%; 2.5%; 5% and 10% concentration. Based on Table 2, the highest number of dead *Fasciola gigantica* is in 10% concentration.

According to transformation data in Table 2, control is significantly different with 1.25%; 2.5%; 5% and 10%. The significant different also happens between every treatment group.

Table 1: The effect of *Ocimum sanctum* Linn. leaves ethanol extract to *Fasciola gigantica* which cause death based on observation time.

Observation Time (hours)	Mean and Standard Deviation ($\bar{x}$±SD)	
	Data in Percent (%)	Transformation Data
0	0.00 ±0.00	0.71^{a} ± 0.00
2	3.50 ± 9.33	1.37^{b} ± 1.50
4	27.00 ± 21.55	4.65^{c} ± 2.48
6	39.50 ± 27.62	5.74^{d} ± 2.73
8	53.00 ± 27.55	6.94^{e} ± 2.37
10	68.00 ± 25.67	8.10^{f} ± 1.75
^{a,b,c,d,e,f}Different superscript in the same column showed significant difference ($p < 0.05$).		

Table 2: The effect of *Ocimum sanctum* Linn. leaves ethanol extract to *Fasciola gigantica* which cause death based on concentration levels.

Treatment Group	Mean and Standard Deviation ($\bar{x}$±SD)	
	Data in Percent	Transformation Data
Control	7.92 ± 14.74	2.03^{a} ± 2.12
O. santum 1.25%	23.33 ± 22.60	4.07^{b} ± 2.75
O. santum 2.5%	34.17 ± 28.12	4.97^{c} ± 3.22
O. santum 5%	42.50 ± 36.98	5.58^{d} ± 3.52
O. santum 10%	51.25 ± 36.87	6.28^{e} ± 3.60
^{a,b,c,d,e}Different superscript in the same column showed significant difference ($p < 0.05$).		

6.3. Interaction between Exposure Time and *Ocimum sanctum* Linn. Leaves Ethanol Extract Concentration

The death of *Fasciola gigantica* is affected by the interaction of exposure time and *Ocimum sanctum* Linn. leaves ethanol extract concentration. From Table 3, can be known that there is no significant difference of dead *Fasciola gigantica* between every treatment group at 0 hour. At 2 hours, can be concluded that there is no significant difference of dead *Fasciola gigantica* between treatment groups and between control and treatment groups. However, between 10% and 2.5%, there is significant difference. At 4 hours, control is significantly different with 1.25%.

Significant difference also occurs between 1.25% and 5% also between 5% and 10%, but not between 2.5% and 5%. Observation at 6 hours showed that control and 1.25% have significant different. Comparison 1.25% to 2.5% show significant difference. But, significant difference cannot be found between 2.5% and 5% also between 5% and

Table 3: The interaction between observation time and concentration level of *Ocimum sanctum* Linn. leaves ethanol extract to *Fasciola gigantica* death.

Observation Time (hours)	Treatment	Mean and Standard Deviation ($\bar{x}$±SD)	
		Data in Percent	Transformation Data
0	Control	0.00 ± 0.00	0.71^{a}± 0.00
	O. santum 1.25%	0.00 ± 0.00	0.71^{a}± 0.00
	O. santum 2.5%	0.00 ± 0.00	0.71^{a}± 0.00
	O. santum 5%	0.00 ± 0.00	0.71^{a}± 0.00
	O. santum 10%	0.00 ± 0.00	0.71^{a}± 0.00
2	Control	0.00 ± 0.00	0.71^{a}± 0.00
	O. santum 1.25%	0.00 ± 0.00	0.71^{a}± 0.00
	O. santum 2.5%	0.00 ± 0.00	0.71^{a}± 0.00
	O. santum 5%	5.00 ± 5.77	1.97^{ab}± 1.46
	O. santum 10%	12.5 ± 18.93	2.75^{bcd}± 2.68
4	Control	0.00 ± 0.00	0.71^{a}± 0.00
	O. santum 1.25%	17.50 ± 9.57	4.13^{de}± 1.11
	O. santum 2.5%	35.00 ± 5.77	5.94^{fg}± 0.50
	O. santum 5%	27.50 ± 17.08	5.10^{ef} ± 1.63
	O. santum 10%	55.00 ± 17.32	7.40^{ghi}± 1.11
6	Control	2.50 ± 5.00	1.34^{ab}± 1.30
	O. santum 1.25%	22.50 ± 5.00	4.77^{ef} ± 0.50
	O. santum 2.5%	45.00 ± 17.32	6.66^{gh}± 1.22
	O. santum 5%	62.50 ± 22.17	7.84^{hij}± 1.40
	O. santum 10%	65.00 ± 12.91	8.10^{hij}± 0.80
8	Control	10.00 ± 8.16	2.93^{cd}± 1.60
	O. santum 1.25%	45.00 ± 5.77	6.74^{gh}± 0.43
	O. santum 2.5%	57.50 ± 9.57	7.60^{hij}± 0.62
	O. santum 5%	75.00 ± 23.80	8.60^{ijk}± 1.40
	O. santum 10%	77.50 ± 5.00	8.83^{ijk}± 0.30
10	Control	35.00 ± 17.32	5.77^{fg}± 1.72
	O. santum 1.25%	55.00 ± 17.32	7.40^{ghi}± 1.17
	O. santum 2.5%	67.50 ± 12.60	8.22^{hij}± 0.80
	O. santum 5%	85.00 ± 12.91	9.23^{jk}± 0.70
	O. santum 10%	97.50 ± 5.00	9.90^{k}± 0.26

^{a,b,c,d,e,f,g,h,i,j,k}Different superscript in the same column showed significant difference ($p < 0.05$).

Table 4: Various concentration of *Ocimum sanctum* Linn. leaves ethanol extract suspension that cause death of 50% and 90% Fasciola gigantica population in every observation time.

Observation Time (hours)	LC_{50}	LC_{90}
0	-	-
2	-	-
4	7.9%	77.5%
6	3.7%	36.9%
8	1.8%	17.5%
10	0.8%	8.4%

10%. At 8 hours, control and 1.25% is significantly different. However, significant different doesn't happen neither between 1.25% and 2.5%, between 2.5% and 5%, nor 5% and 10%. Lastly, at 10 hours, control and 1.25% show significant difference. While 1.25%, 2.5%, 5% and 10% are not significantly different.

7. LC_{50} and LC_{90}

Ocimum sanctum Linn. leaves ethanol extract LC_{50} and LC_{90}calculation is using probit analysis. From the calculation, can be known the $LC_{50}$and LC_{90} in every observation time which can be seen in Table 4. Based on Table 4.4, can be known that LC_{50} of *Ocimum sanctum* Linn. leaves ethanol extract at 4, 6, 8 10 hours are 7.9%, 3.7%, 1.8% and 0.8% concentration. The 10% concentration is enough to kill 50% of *Fasciola gigantica* population at 4, 6, 8 and 10 hours. The $LC_{90}$of *Ocimum sanctum* Linn. leaves ethanol extract at 4 hours is 77.5%, at 6 hours is 36.9%, at 8 hours is 17.5% and at 10 hours is 8.4% concentration. The 10% concentration is enough to kill 90% of *Fasciola gigantica* total population in the longest observation time, which is 10 hours.

8. Discussion

The observation result can be known that *Ocimum santum* Linn. leaves ethanol extract have anthelmintic activity dependent concentration and exposure time.

Dead *Fasciola gigantica* are found in control group, which is CMC Na that dissolved in PBS. This might be happening because in vitro method is done without using animal model. Although the research method has been designed to be the most resembles body condition as possibly can, but it still has different physiological condition and affect *Fasciola gigantica* in a certain way. The other factor that possibly affects the paralysis and death of *Fasciola gigantica* in control group is the nutrition provided by PBS was already finish and oxygen deficiency, given that the paralysis was observed

at 8 hours and the dead at 10 hours. While CMC Na is claimed to be a semi-synthetic and non toxic emulator (Yasar *et al.,* 2007; Kamel *et al.,* 2008), it is the safest solvent to used compared to Tween 80 or DMSO which considered more toxic than CMC Na.

Ocimum sanctum Linn. leaves ethanol extract has been proved to have phytochemical constituent that beneficial to be anthelmintic. Anthelmintic is the kind of drug which has harming effects to the helminths by affecting the vital activities, transmission of neuromuscular, ion exchange or to the tegument system that caused the helminth to be disintegrated and become paralysis or dead, therefore it will be expelled from the host's body (Nilani *et al.,* 2012 and Rajesh *et al.,* 2017).

Phytochemical constituents which act as anthelmintic in *Ocimum sanctum* Linn. leaves are tannins, alkaloids, flavonoids and saponin (Anthanasiadou *et al*., 2001 and Sawarkar *et al.,* 2011). These chemical components can be found in *Ocimum sanctum* Linn. leaves and they has been discovered by previous researches which tested the anthelmintic activity of *Ocimum sanctum* Linn. leaves to different species of helminth. Gosmawi *et al*., (2016), Buchineni *et al.,* (2015), Pandey *et al.,* (2016) and Joshi *et al.,* (2013) tested leaves of *Ocimum sanctum* Linn to *Pheretema posthuma* and evidently, it has good anthelmintic activity against earthworm *Pheretema posthuma.* Leaves of *Ocimum sanctum* has also been examined the anthelmintic effect to *Syphacia muris* (Verma *et al.,* 2013), *Ascaris suum* (Sea, 2016), *Setaria digitata* (Banu *et al.,* 1992), *Caenorhabditis elegans* (Asha *et al.,* 2001), also to *Cotylophoron cotylophorum* (Karumari *et al.,* 2014), and turns out they showed good anthelmintic activity toward the mentioned helminths. With the result that, allegedly, tannins, alkaloids, flavonoids and saponin have anthelmintic effect against *Fasciola gigantica.*

Phytochemical constituents which responsible for *Ocimum sanctum* Lin. leaves' anthelmintic activity has their own mechanism of action to kill or to make the helminth become paralysis. Tannins and phenol work by make the helminths to be immobile and lead them to paralysis, even death. The immobility of the helminths happened because tannins increase the protein uptake and bind with the surface of helminth's body cuticle (Gosmawi *et al*., 2016). Flavonoid affects to the nerve system, then lead to neuron degeneration. There will be disturbance in nerve impulse and the helminth's homeostasis will be disturbed, the worm will be paralyzed and finally die (Fitriana, 2008). Based on Rujjanawate *et al.,* (2003), alkaloid has effect to cause helminth's cells excitation and neurological dysfunction. Lastly, saponin affects both to the extract and to the flukes. Mechanism of action affecting the extract is to decrease the tension of the extract so that the contact between extract and helminth's tegument will be faster and more effective (Kaufmann, 1996). Meanwhile, saponin affects the fluke by

destabilization of the cell membrane and lead to increased permeability (Gomes *et al.,* 2016).

Based on result of statistical analysis, the higher concentration of the extract and the longer the time of observation hour, the higher number of dead *Fasciola gigantica.* The similar result also observed in other research by Abdelqader *et al.,* (2012). However, the highest concentration in this research which is 10%, took a shorter time to kill *Fasciola gigantica* than the 1.25%, 2.5% and 5% concentration. The effect of extract concentration toward time of the anthelmintic effect is observed was also reported by Dash *et al.,* (2017). To understand this matter, the definition of concentration must be explained first. Concentration is defined by the amount of dissolved constituent divided by total volume of a mixture (Gold, 1997). Based on this definition, can be concluded that the 10% concentration has more abundant phytochemical constituent than the 1.25%, 2.5% and 5% concentration, which quicken its effect of anthelmintic against *Fasciola gigantica.*

According to statistical analysis to determine the effective concentration, there is no significant difference between 10% concentration at 10 hours, 5% concentration at 10 hours, 10% concentration at 8 hours and 5% at 8 hours. It can be said that *Ocimum sanctum* Linn. leaves ethanol extract with 5% concentration which has exposure time in the course of 8 hours has similar anthelmintic activity to kill as much as 10% concentration for 10 hours. It can be concluded that 5% concentration for 8 hours is the most effective concentration to kill *Fasciola gigantica.*

The LC_{50}at 4 hour is 7.9% concentration, at 6 hour is 3.7% concentration, at 8 hour is 1.8% concentration and at 10 hour is 0.8% concentration. It can be said that 10% concentration is enough to kill 50% *Fasciola gigantica* population depend on the exposure time of *Fasciola gigantica* to *Ocimum sanctum* Linn. leaves ethanol extract. The LC_{90} at 4 hour is 77.5% concentration, at 6 hour is 36.9% concentration, at 8 hour is 17.5% concentration and at 10 hour is 8.4% concentration. It can be concluded that in the case of killing 90% population of *Fasciola gigantica*, 10% concentration is enough and it will need 10 hours in order to do so.

9. Conclusion

Based on research result, it can be concluded that *Ocimum sanctum* Linn. leaves ethanol extract with 10% concentration and exposure time for 10 hours caused the most mortality toward *Fasciola gigantica.* However, the interaction between concentration and exposure time stated that 5% concentration for 8 hours is the effective concentration

and time to cause the most mortality to *Fasciola gigantica*. Meanwhile, the $LC_{50}$of *Ocimum sanctum* Linn. leaves ethanol extract was 7.9% at 4 hours, 3.7% at 6 hours, 1.8% at 8 hours and 0.8% at 10 hours and the LC_{90} was 8.4% at 10 hours.

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