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Anthelmintic Activity of Jatropha (Jatropha curcas linn) Leaf Extract Against Ascaridia galli Worms In Vitro

Hasri Ainun[1], Adryani Ris[1], Muhammad Nur Amir[2], and Abdul Wahid Jamaluddin[1]
[1] Veterinary Study Program, Faculty of Medicine, Hasanuddin University, Indonesia
[2]Faculty of Pharmacy, Hasanuddin University, Indonesia.

*Corresponding author: abdulwahidjamaluddin@unhas.ac.id

Abstract

Ascariasis is an intestinal worm disease caused by Ascaridia galli. This disease can attack the small intestine and cause a decrease in productivity of local breed chickens. Worm infections in chickens can be suppressed by taking precautions and treatment measures such as improving the management of the cage and providing anthelmintic. Medicinal plants that can be used as an alternative anthelmintic are jatropha leaves (Jatropha curcas linn) which contain secondary metabolites such as alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, steroids, and glycosides. The study aims to determine the anthelmintic activity of Jatropha leaf extract against Ascaridia galli worms. 75 samples of Ascaridia galli worms were taken from chicken intestines in traditional markets. This research was conducted in vitro with five treatments consisting of one positive control group using Levamisole, one negative control group with 0.5% NaCMC, and three groups of Jatropha leaf extract (JLE) with concentrations of 10%, 15% and 20 %. The results of the study showed that jatropha leaf extract at a concentration of 20% gave a better anthelmintic effect compared with administration of Levamisole as a positive control (+) and jatropha leaf extract had LC50 and LT50 at concentrations of 5.49% and 96.006 minutes.
Key words: *Anthelmintic Activity, Jatropha leaf, Ascaridia galli, in Vitro*

Introduction

The animal protein needed by the population is increasing in line with the increase in welfare and population. The increase in the need for animal protein must be balanced with an increase in the livestock population. Free-range chicken is a source of animal protein, but there are still many obstacles faced in the development of native chickens in Indonesia. One of the causes is suffering from worms or ascariasis caused by Ascaridia galli worms (Fitriana, 2008). Ascariasis has occurred in all parts of the world, especially in continents or countries that have tropical climates such as Africa (Siamba et al. 2007), Asia (Lalchhandama et al, 2009) , and Indonesia (Darmawia et al. 2013). Worm infection in chickens can be suppressed by taking preventive measures and treatment such as improving cage management and administering anthelmintics. The type of anthelmintic that is commonly used comes from synthetic drugs which can cause several adverse side effects, such as the emergence of anthelmintic resistant parasites and residues in livestock products (Hanifah. 2010., Prastowo and Ariyadi. 2015). The development of anthelmintic derived from medicinal plants (herbs) can be an anticipation of the losses caused by synthetic anthelmintics. Jatropha leaves are one of the natural ingredients which are thought to be used as anti-worms because they contain secondary metabolites such as alkaloids,

saponins, tannins, phenolics, flavonoids, triterpenoids, steroids, and glycosides. This compound, especially tannins, is expected to be used as an anti-worm Ascaridia galli in local chickens (Suharti et al., 2010, Beriajaya et al., 2006)

Materials and Methods

Materials

The study was conducted from May to June 2020 at the Chemical Laboratory of the Faculty of Mathematics and Natural Sciences, Hasanuddin University and the Integrated Laboratory of the Hasanuddin University Veterinary Study Program. The samples used wereworms Acaridia galli obtained from chicken intestines infected with worms Acaridia galli in Makassar traditional markets.

Method

In this study there were 5 treatments in which 2 treatments were in the control group and 2 treatments were in the group giving jatropha leaf extract (10%, 15% and 20%). Then the t value used is 5. When entered in the formula above, the number of samples per treatment can be determined, namely:

$(T-1) \times (N-1) \geq 15$
$(5-1)\ (N-1) \geq 15$
$4\ (N-1) \geq 15$
$4N-4 \geq 15$
$4N = 19$
$N = 19/4$
$N = 4{,}75 \approx 5$

Then the number of samples treatment of at least 5. To maintain the reliability of the replication is performed 3 times, bringing the total number of samples required are: 5 x 5 x 3 (number of replication) = 75 samples.

The research procedure was started by collecting and identifying Ascaridia galli samples, making jatropha extract (Jatropha curcas L), phytochemical screening, treatment of Jatropha leaf extract on the test material. In the treatment of Jatropha leaf extract on the test material, 25 worms were put in a petri dish soaked with 8 ml of each treatment then they were grouped into 5 groups without differentiating gender, weight and length were assumed to be the same. Replicated 3 times with different groups of worms. The worm population from each petri dish was treated as follows:

Treatment A: Negative control (0.5% NaCMC)
Treatment B: Positive control with Levamid® (Levamisole)
Treatment C: Jatropha leaf extract 10%
Treatment D: Jatropha leaf extract 15%
Treatment E: Jatropha leaf extract 20%

Jatropha leaves extract solution was prepared using 0.5% NaCMC solvent. It is known that the Jatropha leaf extract contains active alkaloid compounds, flavonoids, saponins and tannins. Petri dishes that already contain 10 ml of 0.5% NaCMC plus JLE so that the final concentrations are 10%, 15%, and 20% w / v, and as a comparison, the same petri dishes plus Levamisole

solution were also prepared. A total of 5 worms regardless of gender, assuming the same weight and length, were inserted into each petri dish (Tarbiat, 2015., Pabala et al., 2017).

In this study, the data obtained from the observations will be analyzed using comparative descriptive, with 5 (five) treatments and 3 (three) repetitions. So, from the results of this processing, it can be seen whether or not there is an anthelmintic effect based on the difference in the time of death of worms between the control group and the treatment group. The research data were then processed using probit analysis to calculate the strength of the test extract as an anthelmintic based on the LC50 value (Lethal Concentration 50), namely the concentration where 50% of the test worms died and the LT50 value (Lethal Time 50), which is the time at which 50% of the test worms died.

Data analysis

Data analysis was carried out using theapplication IBM SPSS Statistics 23 with the Kruskal Wallis test to analyze the differences in each control and treatment group. If the value obtained is significant, namely $p \leq 0.05$, then proceed to thetest Mann-Whitney to determine whether there is a significant effect between one treatment and another with a value of $p \leq 0.05$.

Results and Discussion

Phytochemical testing of jatropha leaf extract is used to determine the content of the types of secondary metabolite compounds contained in the material being tested. From the results of phytochemical testing, Jatropha leaf extract was positive for tannins, saponins, and flavonoids, but was active in alkaloids. The results of the phytochemical analysis of the active compound content are presented in Table 1.

Table 1. Phytochemical analysis results of Jatropha leaf extract

No.	Test	Reactor	Result	Explanation
1.	Flavonoid	$MgCl_2$	Positive (+)	Orange
2.	Alkaloid	*Mayer*	Negative (-)	No white deposits are formed
3.	Saponin	Aquades	Positive (+)	Emulsion formation
4.	Tanin	$FeCl_2$	Positive (+)	Blackish green color

In testing the flavonoid extract of Jatropha leaves with the addition of MgClreagent$_2$ had a positive result which was marked by a change in color to orange. The addition of magnesium powder and hydrochloric acid to the flavonoid test will reduce the existing flavonoid compounds, causing a red, yellow or orange reaction which is a characteristic of flavonoids (Habibi et al, 2018). In alkaloid testing, HCl is added before adding reagents because alkaloids are alkaline so they are extracted with a solvent containing acid. In the alkaloid test, negative results were obtained because no precipitate was formed from ligand replacement. The nitrogen atom which has a lone pair on the alkaloid replaces the iodine ion inreagents *Dragendorff* and *Mayer*. Withreagent you *Mayer's* will get a white solution if it contains positive alkaloids. In the saponin test, jatropha leaf extract had positive results, indicated by the formation of a stable emulsion that did not disappear after the addition of distilled water. Saponins have glycosyls as

polar groups and steroid or triterpenoid groups as nonpolar groups so that they are surface active and form micelles when shaken with water. In the micellar structure the polar groups are facing outwards while the nonpolar groups are facing inwards and this is what looks like foam. In the tannin test, the Jatropha leaf extract had positive results, indicated by the occurrence of a blackish green color change. The color change is due to the covalent bond between the Fe2 + ion and the O- atom of the OH functional group of the tannin compound which releases the H atom and produces a black-green, blue, or black complex compound (Susanty. 2014).

Based on the compound content of jatropha leaves, tests were carried out by immersingworms *Ascaridia galli* in various extract concentrations to determine the effect onworms *Ascaridia galli*. The effect of jatropha leaf extract was observed for 180 minutes which was divided into 3 periods with each period once every 60 minutes. The observation data of *Ascaridia galli* are presented in table 2.

Table 2. The number of deaths ofworms *Ascaridia galli* treated with Jatropha leaf extract

Repetition	Period	Treatment									
		C-		JLE 10%		JLE 15%		JLE 20%		C+	
		ND	M	ND	M	ND	M	ND	M	ND	M
I	P1	-	-	-	0%	2	40%	3	60%	-	0%
	P2	-	-	-	0%	5	100%	5	100%	3	60%
	P3	-	-	5	100%	5	100%	5	100%	5	100%
II	P1	-	-	-	0%	2	40%	2	40%	-	0%
	P2	-	-	-	0%	5	100%	5	100%	3	60%
	P3	-	-	4	80%	5	100%	5	100%	5	100%
III	P1	-	-	-	0%	1	20%	3	60%	-	0%
	P2	-	-	-	0%	5	100%	5	100%	2	40%
	P3	-	-	4	80%	5	100%	5	100%	5	100%

Explanation:

- JLE : Jatropha Leaf Extract
- JLE 10% : Jatropha Leaf Extract 10%
- JLE 15% : Jatropha Leaf Extract 15%
- JLE 20% : Jatropha Leaf Extract 20%
- Control (-) : Negative Control (NaCMC 0,5%)
- Control (+) : Positive Control (Levamid 0,2%)
- P1 : Period 1 (0 – 60 minute)
- P2 : Period 2 (60 – 120 minute)
- P3 : Period 3 (120 – 180 minute)
- ND : Number of Deaths
- M : Mortality

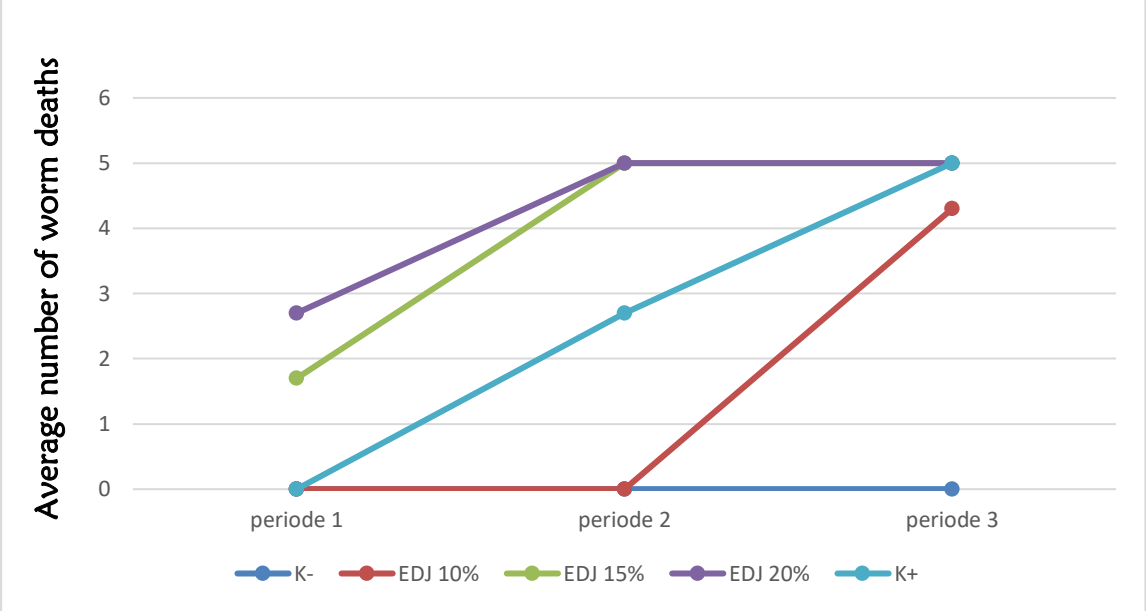

Figure 1. Graph Average number of worm deaths per concentration

Based on Table 2. It can be seen that in the first iteration with 10% fence leaf extract treatment in the first and second periods there were no dead worms, but in the third period there were 5 dead worms (100%). In the second iteration in the first and second periods there were no dead worms, but in the third period there were 4 dead worms (80%). As for the third iteration in the first and second periods there were no dead worms, but in the third period the number of dead worms was 4 (80%). Treatment of foliage extraction leaves 15% concentration for the first iteration of the first period there are 2 (40%) dead worms, for the second and third period there are 5 (100%) dead worms. In the second iteration of the first period there are 2 (40%) worms that die, for the second and third period there are 5 (100%) worms that die. For the third iteration of the first period there are 1 (20%) dead worms, for the second and third period there are 5 (100%) dead worms. Treatment of foliage leaf extract concentration 20% for the first iteration of the first period there are 3 (60%) dead worms, for the second and third period there are 5 (100%) dead worms. In the second iteration of the first period there are 2 (40%) worms that die, for the second and third period there are 5 (100%) worms that die. For the third iteration of the first period there are 3 (60%) worms that die, for the second and third period there are 5 (100%) worms that die. In the positive control treatment for the first iteration of the first period there were no dead worms, for the second period there were 3 (60%) dead worms and in the third period there were 5 dead (100%) worms. In the second iteration of the first period there are no dead worms, for the second period there are 3 (60%) dead worms and in the third period there are 5 dead (100%) worms. For the third iteration of the first period there are 2 (60%) worms that die, for the second and third period there are 5 (100%) worms that die. From the three groups of concentrated foliage leaf extract concentrations it can be seen that in the second period the concentration of 15% is more effective in killing worms when compared to positive control which is capable of killing 3 worms (60%) in the first iteration, 3 birds (60%) worms in the second iteration and 2 (40%) worms on the third iteration. In the negative control treatment showed the death of the whole worm that is 5 worms (100%) in minutes to 590 minutes. The use of negative control treatment was performed to test the carrier carrier of NaCMC 0.5% which did not affect the death of worms. According to Yudiatmoko (2010), the time of death ofworms *ascaridia galli* soaked in physiological solution (NaCl 0.9%) is about ± 28 hours. Based on these negative control results, no dead worms were found until 180 minutes after the first 0.5% NaCMC administration. So

it can be concluded that NaCMC 0.5% as a carrier of leaf extract leaves fence does not cause death to the worms tested. worm mortality was *Ascaridia galli* assessed by looking at the morphology of the worm. Worms are considered dead if in contact with a stirring rod the worm does not move or does not respond and the worm remains immobile if placed in aquadest at a temperature of ± 50 ° C. Worm deaths are affected by compounds contained in the foliage leaves themselves, such as saponin compounds as inhibitors of the work of the enzyme acetylcholine α insulin until the worm undergoes muscle paralysis and ends in death (Intannia et al. 2015). Tannins are part of large molecular phenol compounds that can form complex compounds with proteins. Tannin can also interfere with the work of enzymes in the body cells of worms. According to Naidu (2000), large molecular phenolic compounds are able to activate essential enzymes in cells even at very low concentrations and eventually worms will die due to decreased glycogen supply and reduced ATP formation. In addition to saponin and tannin compounds, fence leaf extract also has flavonoid compounds that are thought to be able to denaturate proteins in worm tissue to cause death in worms, and to degenerate neurons in the body of worms to cause death.

The time of death of worms is not only influenced by the content of tannins, saponins and other secondary metabolite compounds in Jatropha leaf extract, but also influenced by conditions *in vitro* which are not the same as environmental conditions when the worms are in the digestive tract of native chickens (Robiyanto et al., 2018).

Table 3. The average value of the Kruskal Wallis test

Ranks

	Treatment	N	Mean Rank
Repeat	Control(-)	3	14.0
	Control (+)	3	8.0
	JLE 10%	3	11.0
	JLE 15%	3	5.0
	JLE 20%	3	2.0
	Total	15	

Table 3.shows the average value of the grouping according to sequence against the time of death of worms. It is known that control (-) has the highest Mean Rank value (indicating the longest time of death of worms). While the 20% JLE treatment had the lowest Mean Rank value (indicating the fastest worm death time). The time sequence of worm mortality from fastest to longest was shown successively starting from JLE treatment 20%, JLE treatment 15%, control (+), JLE treatment 10% and control (-). This shows that the concentration of 20% castor leaf extract and 15% JLE has the ability to kill worms faster than the control (-) and positive controls.

Table 4. The significant value of the Kruskal Wallis Test P Value

Test Statistics[a,b]

	Death time
Chi-Square	13.856
df	4
Asymp. Sig.	0.008

a. Kruskal Wallis Test
b. Grouping Variable: Treatment

Time to death of worms was analyzed using the non-parametric *Kruskal Wallis test*. The *Kruskal Wallis test* can only determine the overall difference in the treatment given, so that to determine the differences between treatments, a further test was carried out using thetest *Mann Whitney*.

Table 4 shows that the P value is 0.008 which is smaller than 0.05 so that it can be seen that there is a significant difference in the treatment given. Or it can be concluded that $H_{1\ is}$ accepted and $H_{0\ is}$ rejected. Where:

H_0 : there is no significant effect of the treatment given to the worm samples
H_1 : there is a significant effect of the treatment given to the worm samples

Advanced test is one of the next test options. Based on a series of analyzes, a further test will be carried out if the previous test (shows *Kruskal Wallis test*) a significant difference between the treatments given. As for the follow-up test for the absence of a significant difference, it is only limited to further information and cannot be used as a conclusion to the analysis. The follow-up test used was the test *Mann Whitney*.

Table 5. The significant value of the Mann Whitney test for each treatment

Treatment Group	Control(-)	Control (+)	JLE 10%	JLE 15%	JLE 20%
Control (-)		0.034*	0.034*	0.025*	0.025*
Control (+)			0.043*	0.034*	0.025*
JLE 10%				0.043*	0.034*
JLE 15%					0.025*
JLE 20%					

Explanation:

- * : $p \leq 0.05$ (there is a significant difference)
- JLE : Jatropha leaf extract
- Control(-) : NaCMC 0,5%
- Control(+) : Levamid

Table 5. is the significant value of the Mann Whitney test for each treatment. The hypothesis formulation for the Mann Whitney test is:

H_0 : there is no significant difference between treatment group i and j given to worm samples
H_1 : there is a significant difference between treatment groups i and j given to worm samples

With (= Control (-), Control (+), JLE 10%, JLE 15%, JLE 20%;).

Based on the results of the Mann Whitney test, it shows that the treatment group Control (-) with Control (+) has a value of p = 0.034; the Control (-) treatment group with JLE 10% had a value of p = 0.034; the Control (-) treatment group with JLE 15% had a p value = 0.025; the Control (-) treatment group with JLE 20% had a p value = 0.025; the Control (+) treatment group with JLE 10% had a p value = 0.043; the Control (+) treatment group with JLE 15% had a p value = 0.034; the Control (+) treatment group with JLE 20% had a p value = 0.025; the JLE treatment group 10% with JLE 15% had a p value = 0.043; JLE treatment group 10% with JLE 20% had a value of p = 0.034; and the JLE 15% treatment group with 20% JLE had a p value = 0.025. The test results showed p value <0.5 for each treatment group between groups so that it can be concluded that there are significant differences between the treatments given.

Table 6. The results of the LC50 probit analysis of Jatropha curcas linn leaf extract against *Ascaridia galli* worms by In Vitro

Mortality Percentage (%)	LC (%)	Lower limit (%)	Upper limit (%)
10	1.73%	0.43%	3.15%
20	2.57%	0.83%	4.19%
30	3.42%	1.33%	5.14%
40	4.37%	2.00%	6.13%
50	5.49%	2.93%	7.24%
60	6.90%	4.27%	8.56%
70	8.80%	6.36%	10.30%
80	11.71%	9.88%	13.14%
90	17.40%	15.32%	21.86%

Table 6. shows that giving Jatropha leaf extract has an LC50 of 5.49% with a lower limit of 2.93% and an upper limit of 7.24%. This shows that to kill worms by 50%, it takes an average concentration of Jatropha leaf extract of 5.49%.

Table 7. The results of the LT50 probit analysis of Jatropha curcas (Jatropha curcas linn) leaf extract against Ascaridia galli worms by In Vitro

Mortality Percentage (%)	LT (minute)	Lower limit (minute)	Upper limit (minute)
10	33.81	24.35	41.78
20	55.162	47.652	61.648
30	70.557	64.243	76.174
40	83.711	78.193	88.813
50	96.006	90.964	100.894
60	108.301	103.419	113.291
70	121.455	116.390	126.909
80	136.850	131.188	143.229
90	158.200	151.262	166.309

Table 7 shows that giving jatropha leaf extract has a LT 50 of 96,006 minutes with a lower limit of 90,964 minutes and an upper limit of 100,894 minutes. This shows that to kill worms by 50%, it takes an average of 96,006 minutes.

The observations made to determine the time limit on the test of the anthelmintic effectiveness of jatropha leaf extract (*Jatropha curcas linn*) were determined by testing the life span of worms *Ascaridia galli* in 0.5% NaCMC solution. Determination of the length of life of worms was determined from the time the worms were immersed in 0.5% NaCMC and put in water with a temperature of ± 50ºC until all worms in each immersion died (observed every 10 minutes for 10 hours). Jatropha leaf extract with a concentration of 20% has a faster death time than other treatments because it contains a higher secondary metabolite concentration.

Conclusion

Jatropha leaves extract was given with an LC 50 value of 5.49% and a LT 50 value of 96.006 minutes. Jatropha leaf extract with a concentration of 10%, 15%, and 20% against Ascaridia galli worms provides an anthelmintic effect, especially at a concentration of 15% and 20%, but based on LC50 the anthelmintic power of Jatropha leaf extract is lower than that of Levamisole.

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