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# Activity Test of bitter melon Leaf Infusion (*Momordica charantia*) as an anthelmintic toward *Ascaridia galli* worms in vitro

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## Abstract

*Ascaridia galli* is a gastrointestinal parasite in poultry which can cause weight loss, slow growth and affect egg production. Chickens that are infected can cause damage to the integrity of the intestinal villi to severe inflammation of the intestinal mucosa. Bitter melon leaf (*Momordica charantia*) is known to have an anthelmintic effect which can be used as an alternative in the treatment of worms disease. The purpose of the research is to determine activity of bitter melon leaf infusion toward *Ascaridia galli* conducted in vitro. In the research, it uses 72 *Ascaridia galli* that divided into 4 groups consisting of positive control group (Levamid), negative control group (Aquadest), and 2 treatment group with 10%, and 20% bitter melon leaf infusion concentration, respectively. Each group replicated three times. The worms were immersed in the test solution and observations were performed every 15 minutes and counted the number of worms that died. The results showed that pare leaf infusion with concentrations of 10% and 20% had an anthelmintic effect but was no better than Levamid as a positive control.

*Keywords: Antihelmintic, bitter melon leaf, worm*



## Introduction

*Ascaridia galli* is a gastrointestinal parasite in poultry that can cause weight loss, slow growth and affect egg production. Severely infected chickens can cause damage to the integrity of the intestinal villi, damage to the intestinal mucosa (Darmawia et al. 2013), The intestinal lumen becomes narrow because the worms are in the intestinal lumen, enteritis, and the intestinal wall appears to be thickened with the mucosa appearing gray or cloudy (Salam 2015.

Worms caused by nematodes (roundworms) such as *Ascaridia galli* can be treated using anthelmintics such as albendazole and levamisole. However, in most developing countries such as Indonesia, small farmers lack access to provide commercial anthelmintics and animal health services due to high costs (Jabar et al. 2006). Many farmers in various countries rely

on ethnoveterinary medicine. The use of plants as anthelmintics is considered as an alternative method of eradicating worms in the breeder community because natural plant materials are easily obtained around the farm area.

The bitter melon plant (*Momordica charantia*) is one of the herbs that people believe to treat intestinal worms. Not only worms but also used for the treatment of several diseases including lowering blood sugar levels, lowering heat, increasing appetite, overcoming digestive disorders and as worm medicine (Herbie 2015).

Based on this, it is necessary to conduct research to test the activity of infusion of bitter gourd leaves (*Momordica charantia*) as an anthelmintic against *Ascaridia galli* worms in vitro.

## Meterials and Methods

This research took place in June 2020. The research was conducted at the Biochemistry Laboratory of the Faculty of Mathematics and Natural Sciences, Hasanuddin University for phytochemical screening testing and the Integrated Laboratory of the Veterinary Medicine Study Program, Faculty of Medicine for the manufacture of bitter melon leaf infusion and observation. This research is a kind of laboratory experimental research. Experimental research is an experimental activity that aims to determine a symptom or effect that arises as a result of certain treatments. The sample used in the study was *Ascaridia galli* worms. The determination of the number of samples is based on calculations using the Federer formula, namely:

$$(n-1)(t-1) \geq 15$$

*Ascaridia galli* worm samples were taken directly using anatomical tweezers from the intestinal lumen of native chickens infected with *Ascaridia galli* worms. Samples were selected with appropriate criteria such as active mobile worms, not anatomical defects and measuring 5-8 cm. Pare leaf infusion is obtained from the simplicia of bitter melon leaves mixed with distilled water with a ratio of 1:10 or the equivalent of 10% (50 gr / 500 ml distilled water) and 1: 5 or the equivalent of 20% (100 gr / 500 ml distilled water) then heated at 90 °C for 15 minutes. To identify the bioactive content contained, phytochemical screening was carried out against the infusion of bitter gourd, including examination of compounds such as alkaloids, saponins, flavonoids and tannins (Agustina et al. 2016). To make a levamide solution with a concentration of 0.2%, 0.2 grams of levamid powder are required. Levamid is classified as a drug that is difficult to dissolve, so 100 ml of solvent is needed to be able to dissolve the levamid powder completely.

**Table 1.** Division of in vitro treatment groups

| | |
|---|---|
| Group 1 | Aquadest |
| Group 2 | *Levamid* 0,2% |
| Group 3 | 10% Bitter Melon Infusion |
| Group 4 | 20% Bitter Melon Infusion |

*Ascaridia galli* worms, totaling 24 worms, were inserted 6 each into a petri dish containing 20 ml of each solution of the treatment group which was grouped into 4 groups.

Analysis of the results of data using the Kruskal Wallis test to analyze the differences in each treatment group given as a whole. If the value obtained is significant $P \leq 0.05$, then proceed with the Mann Whitney test to determine whether there is a significant effect between one treatment and another given with a P value $\leq 0.05$.

## Results and Discussion

Observation data on the time of death of *Ascaridia galli* worms is presented in table 2.

| Period (Minutes) | Repetition | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | C- | | BMI 10% | | BMI 20% | | C+ | |
| | | TD | M | TD | M | TD | M | TD | M |
| I (0–105 Minutes) | P1 | - | - | 0 | 0% | 0 | 0% | 5 | 83 % |
| | P2 | - | - | 0 | 0% | 0 | 0% | 5 | 83 % |
| | P3 | - | - | 0 | 0% | 0 | 0% | 6 | 100 % |
| II (105–210 Minutes) | P1 | - | - | 0 | 0% | 3 | 50% | 6 | 100 % |
| | P2 | - | - | 0 | 0% | 2 | 33.2 % | 6 | 100 % |
| | P3 | - | - | 0 | 0% | 2 | 33.2 % | 6 | 100 % |
| III (210 – 315 Minutes) | P1 | - | - | 2 | 33.2 % | 6 | 100% | 6 | 100 % |
| | P2 | - | - | 3 | 50% | 6 | 100% | 6 | 100 % |
| | P3 | - | - | 2 | 33.2 % | 6 | 100% | 6 | 100 % |
| IV (315 – 420 Minutes) | P1 | - | - | 6 | 100 % | 6 | 100% | 6 | 100 % |
| | P2 | - | - | 6 | 100 % | 6 | 100% | 6 | 100 % |
| | P3 | - | - | 6 | 100 % | 6 | 100% | 6 | 100 % |

* BMI is Bitter Melon Infusion, C is Control, TD is Total Deaths, M is Mortality

**Tabel 2**. The time of death for Ascaridia galli worms is based on a period of time

Based on table 2 it can be seen that in the 10% pare leaf infusion treatment group in the first period there were no dead worms. In the second period there were no dead worms. In the third period of the first repetition there were two worms that died (33.2%), in the second repetition there were three worms that died (50%), in the third repetition there were two worms that died (33.2%). In the fourth period of the first, second and third

repetitions, each there were six worms that died (100%). The fourth period shows the time of death of 100% *Ascaridia galli* worms.

In the treatment group of 20% bitter melon leaf infusion, it can be seen that in the first period there were no worm deaths. In the second period of the first repetition there were three worms that died (50%), in the second repetition there were two worms that died (33.2), in the third repetition there were two worms that died (33.2%). In the third period of the first, second and third repetitions, each there were six worm deaths (100%). The third period shows the time of death of 100% *Ascaridia galli* worms.

In the negative control treatment group, it can be seen that in the first, second, third and fourth periods each of the repetitions did not show any worm mortality, while for the positive control group in the first period of the first and second repetitions there were five worms that died (83%), on the third repetition there were six worms that died (100%). In the second period of the first, second and third return there were six worms each that died (100%). The second period shows the time of death of 100% *Ascaridia galli* worms.

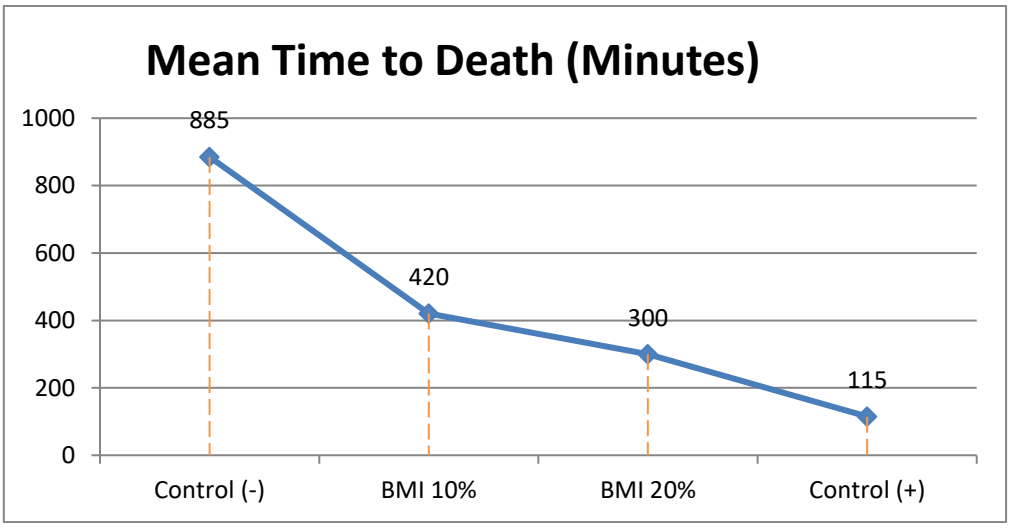


Figure 1. Graph of the average time of death of Ascaridia galli worms

Based on Figure 1, it can be seen that the average time of death of *Ascaridia galli* worms shows that in the first treatment, control (-) has an average time of death of *Ascaridia galli* worms as a whole for 885 minutes. In the second treatment, 10% IDP had an average time of death of *Ascaridia galli* worms as a whole for 420 minutes and in the third treatment, 20% IDP had an average time of death of *Ascaridia galli* worms as a whole for 300 minutes. In the fourth treatment, the control (+) had an average death time of *Ascaridia galli* worms as a whole for 115 minutes.

**Table 3**. The average value of the Kruskal Wallis test

| Treatment | N | Mean Rank |
|---|---|---|
| Control (-) | 3 | 11.00 |
| Control (+) | 3 | 2.00 |
| BMI 10% | 3 | 8.00 |
| BMI 20% | 3 | 5.00 |
| Total | 12 | |

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

| Test Statistics | Time of death |
|---|---|
| Kruskal-Wallis H | 10.722 |
| Df | 3 |
| Asymp. Sig. | .013 |

a. Kruskal wallis test
b. Grouping variable : Treatment

To determine the significant difference in mortality of *Ascaridia galli* worms as a whole the treatment given was used the Kruskal Wallis test. However, the Kruskal Wallis test could not find out the significant difference between treatments so that to find out the significant difference between treatments, the test would be continued with the Mann Whitney test.

The Mann Whitney test can be done if the P value obtained from the previous test, namely the Kruskal Wallis test, is smaller than the P value ≤ 0.05. Based on table 4, it is found that the value of P = 0.013, which means that the value is smaller than the value of P = 0.05, so it can be concluded that $H_1$ is accepted and $H_0$ is rejected.
$H_0$ : There is no significant effect of the treatment given
$H_1$ : There is a significant effect on the treatment given

Based on the Kruskal Wallis test series, a significant difference has been found in the overall treatment given and then the Mann Whitney test will be carried out to determine the significant difference between the treatments given.

**Table 5**. Significant value of the Mann Whitney test

| Treatment Group | Control (-) | Control (+) | BMI 10% | BMI 20% |
|---|---|---|---|---|
| Control (-) | | 0.046* | 0.037* | 0.037* |
| Control (+) | | | 0.034* | 0.034* |
| BMI 10% | | | | 0.025* |
| BMI 20% | | | | |

Based on table 5, the results of the Mann Whitney test between treatments show that the control (-) and control (+) treatment groups have a value of P = 0.046; the Control (-) treatment group with IDP 10% had a value of P = 0.037; the Control (-) treatment group with IDP 20% had a value of P = 0.037; the Control (+) treatment group with IDP 10% had a P value = 0.034; the Control (+) treatment group with IDP 20% had a P value = 0.034; The treatment group for IDP 10% with IDP 20% had a value of P = 0.025. The test results indicate that the value of P ≤ 0.05, which means it can be concluded that there are significant differences between the treatment groups given.

## Conclusion

The administration of bitter melon leaf infusion has an anthelmintic effect with 100% worm mortality, for bitter melon leaf infusion the concentration is 10% for ± 420 minutes and for bitter melon leaf infusion the concentration is 20% for ± 300 minutes.

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