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# Anthelmintic Activity Test of Pare Leaves Extract (*Momordica charantia L.)* Against Worms *Raillietina* sp. by In Vitro

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**Abstract**

The purpose of this research is to prove that pare leaf extract (*Momordica charantia* L.) has anthelmintic activity against *Raillietina* sp. in vitro. This study consisted of four treatments and each treatment contained six replications, each of which consisted of six worms. The treatments in this research were K- with 1% CMC-Na, P1 with 2% extract concentration, P2 with 4% extract concentration, P3 with 8% extract concentration. Pare leaf extract has anthelmintic activity against *Raillietina* sp. in vitro with an effective dose of 8%. The higher the concentration of pare leaf extract, the higher the mortality rate of worms. The longer the immersion time, the higher the number of dead worms.

**Keywords:** *Momordica charantia*, *Raillietina* sp., in vitro, anthelmintic, activity test, Pare leaves

## Introduction

One of many diseases that threaten chicken farms is Raillietiniasis which is a parasitic disease infected by the cestodes *Raillietina* sp. such as *Raillietina cesticillus, Raillietina echinobothrida, and Raillietina tetragona.* In Indonesia, the prevalence of *Raillietina* sp. cases in chickens is still relatively high, found in the Jakarta market at 56% in the Pasar Minggu Market and Palmerah Market, while the market in Bogor City is 70% in the Anyar and Gunung Batu traditional markets (Kusumadewi *et al*., 2020). Raillietiniasis cases in free-range chicken are high due to environmental influences, one of which is the high humidity in Indonesia which makes the environment good for helminth development, as well as poor sanitation and hygiene conditions (Dachi, 2005). Spread can be through contaminated feed, water, and livestock equipment (Parede *et al.,* 2005). Infection from *Raillietina* sp. causes the growth and productivity of chickens to be hampered (Pranoto *et al.,* 2019). Chickens infected with Raillietina sp. experienced a decrease in egg production, decreased body weight, growth disorders, and weakness causing economic losses (Loliwu and Thalib, 2012).

Effective control of helminth infections can be done by combining good livestock management and the provision of anthelmintics (Tjay and Rahardja, 2007). Raillietiniasis treatment can use drug such as Niclosamide, Hexachlorophene, besides that the following drugs can be given: Praziquantel, Bensimidazole, Albendazole and Oxfendazole (Oka and Dwinata, 2017). Synthetic anthelmintic treatments are generally used by breeders to treat Raillietiniasis, although they are quite effective, there are side effects which if used continuously for a long time can result in resistance and residues to livestock products that are harmful to livestock production (Ekawasti *et al.,* 2020), in addition to that, synthetic anthelmintics are relatively expensive and difficult to obtain by residents in rural areas (Ajaiyeoba *et al*., 2001; Zahir *et al*., 2009).

Alternative treatments against Raillietiniasis can be in the form of developing drugs that are sourced from natural ingredients. The use of anthelmintics sourced from natural ingredients has the potential as a worm exterminator, besides that, it is easier to obtain (Ajaiyeoba *et al.*, 2001; Zahir *et al.,* 2009). One of the plants that is easy to obtain but rarely used by the community is pare leaf (*Momordica charantia* L.).

According to Tjokropranoto and Nathania (2011), pare leaf can be used to kill Ascaris suum worms that attack the digestive system. Based on this background, this study aims to prove that pare leaf extract (*Momordica charantia* L.) has anthelmintic activity against *Raillietina* sp. in vitro so that it can be an alternative treatment for Raillietiniasis. In addition, to determine the optimal concentration and time required for pare leaf extract to cause 50-90% death in *Raillietina* sp.

## Methods

Research on the activity test of pare leaf extract (*Momordica charantia* L.) by maceration method against *Raillietina* sp. in vitro was carried out at the UPT Laboratory of Animal Health, Agriculture and Food Service, Gunungkidul Regency, Special Region of Yogyakarta. The preparation of pare leaf extract was carried out at

the Pharmacology Laboratory, Division of Veterinary Basic Medicine, Faculty of Veterinary Medicine, Airlangga University. This research was conducted in January - February 2021. The material used in this study was pare leaves extracted in several concentrations, *Raillietina* sp. Physiological NaCl 0.9%, CMC-Na 1%, Ethanol 96%. This study used a post-test control only group design with a completely randomized design. Each treatment used six repetitions and each replication used 6 *Raillietina* sp. worms. The treatment consisted of 1% (K-) CMC-Na solvent, pare leaf extract at a concentration of 2% (P1), 4% (P2), and 8% (P3). Observation of the time of death of worms was carried out every 1 hour for 7 hours. The time required for the worms to die is set as the deadline for observing the worms, which is the 7th hour. To distinguish that the worms are just paralyzed or have died, can be done by touching using a stir rod. The worms that do not move are then dropped with water at a temperature of 50$^0$C. If it is shaken it still remains still, it means that the worm can be declared dead, but if there is movement, it indicates that the worm is only experiencing paralysis (Meritha, 2019).

Samples were obtained from the small intestines of chickens infected with Raillietinesis at the Srimartani Piyungan Chicken Slaughterhouse, Yogyakarta by taking the small intestines of chickens before they were put into boiling water. After obtaining the small intestine of the chicken, the small intestine was dissected using surgical scissors and the process of taking the worms was carried out carefully using anatomical tweezers so that the scolex of the worms was not cut off then put in a jar containing 0.9% physiological NaCl to keep it alive and immediately taken to the UPT Animal Health Laboratory, Agriculture and Food Service, Gunung Kidul Regency, Special Region of Yogyakarta for observation and anthelmintic testing.

This study analyzed with Statiscal Program Service and Solution (SPSS) 25 for windows ANOVA test method, if there was a significant difference between treatments, it was continued with Duncan's Multiple Distance test to compare between treatments. The values of LC50, LC90, LT50, and LT90 can be analyzed using probit analysis.

## Result and Disscussion

Identification of *Raillietina* sp. in this study carried out macroscopically and microscopically. The results of the identification showed that macroscopically the worm *Raillietina* sp. flat, segmented, and yellowish white. The length of the worm is between 11-18 cm. Microscopically it appears to have a rostellum and sucker on the anterior. Research conducted at the Srimartani Chicken Slaughterhouse found species of *Raillietina tetragona* and *Raillietina echinobothrida*. The identification results can be seen in Figure 1.

Based on the results of research that has been done, *Raillietina* sp. treated with pare leaf extract showed a difference compared to the negative control, namely a dark green color change in the worm's body due to immersion in dark green pare leaf extract, besides that other changes were also obtained, namely the worm's body became softer, this This is because the higher the concentration of the extract given, the more obvious the changes that occur and changes in the cuticle can occur due to protein degradation (Imam *et al.,* 2015). Differences in the characteristics of *Raillietina* sp. given negative control with pare leaf extract treatment can be seen in Figure 2.

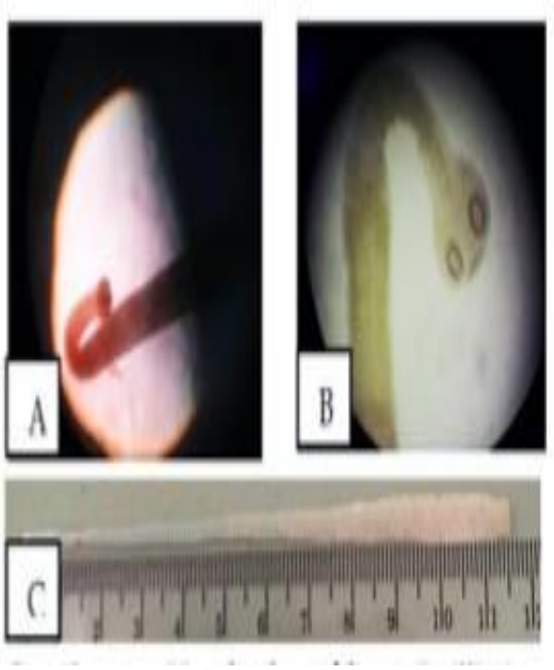


**Figure 1**. Identification results of *Raillietina tetragona* (A), *Raillietina echinobothrida* (B), worm length 11-18 cm and yellowish white (C).


**Figure 2.** Differences in the characteristics of negative control worms (A) and treatment of pare leaf extract (B).

**Table 1.** Average and Standard Deviation of Mortality of *Raillietina* sp. Due to Administration of Pare Leaf Extract

| | Observation Time (hour) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| K- | 0,00[a] ± 0,00 | 0,00 [a] ± 0,00 | 0,00[a] ± 0,00 | 0,00[a] ± 0,00 | 0,00 [a] ± 0,00 | 0,00[a] ± 0,00 | 0,00[a] ± 0,00 |
| P1 | 0,00[a] ± 0,00 | 0,00[a] ± 0,00 | 13,90[b] ± 12,54 | 50,00 [b] ± 19,48 | 50,00[b] ± 10,56 | 72,21[b] ± 13,58 | 100,00[b] ± 0,00 |
| P2 | 0,00[a] ± 0,00 | 8,35 [b] ± 9,15 | 27,76 [c] ± 8,57 | 50,00[c] ± 10,56 | 69,46 [c] ± 6,77 | 88,86[c] ± 8,62 | 100,00[b] ± 0,00 |
| P3 | 0,00[a] ± 0,00 | 27,78[c] ± 13,59 | 52,78[d] ± 12,57 | 72,21[d] ± 13,58 | 91,65[d] ± 9,15 | 100,00[d] ± 0,00 | 100,00 [b] ± 0,00 |

Based on the results of statistical data analysis using ANOVA, it is shown that there is a significant difference, namely at each hour of observation ($p < 0.05$). The mean and standard deviation of the mortality of *Raillietina* sp. The effect of giving pare leaf extract at every hour of observation can be seen in Table 1.

Based on the results of hourly observations, it was found that the difference in concentration affected the length of time the worms died. The higher the concentration, the faster the death of the worm, and vice versa. Based on the observations of the worm *Raillietina* sp. in the first hour showed that in the control group (K-) and the treatment group there was no significant difference as evidenced by the absence of worm deaths, this happened because the onset of action had not yet occurred. The results of observations on the worm *Raillietina* sp. showed that anthelmintic activity began to be seen at the 2nd hour with concentrations of 4% and 8%, this indicated that the onset of action was starting to occur which was marked by a decrease in body motility until the death of worms, while the observations of the death of *Raillietina* sp. at a concentration of 2% was first shown at the 3rd hour, thus the order of activity from highest to lowest was indicated by 8% pare leaf extract, 4% pare leaf extract and 2% pare leaf extract. The most effective anthelmintic effect was obtained at a concentration of 8%. The highest concentration variation showed the highest activity, this was due to the high content of active compounds and potential as anthelmintics so that the effects were faster. The best time for pare leaf extract to cause 100% death of *Raillietina* sp. occurred at 6th hour. The results of this study are similar to the results of research (Setiyawan, 2015) which showed that immersing the ethanol extract of pomegranate peel with concentrations of 2%, 4%, and 8% had anthelmintic effect against *Railietina* sp. in vitro. The most effective anthelmintic effect was obtained at a concentration of 8%. The results showed that the best time for the ethanolic steroids/triterpenoids, momordicin I, momordicin II, and momordicin III (Swadini, 2012). It is necessary to conduct a similar study using a extract of pomegranate rind in causing 100% mortality of *Raillietina* sp. occurred at 6th hour.

**Table 2**. LC50 and LC90 Pare Leaf Extract

| Observation Time (Hour) | LC50 | LC90 |
|---|---|---|
| 1 | 37,3% | 134,2% |
| 2 | 5,2% | 18,76% |
| 3 | 2,4% | 8,8% |
| 4 | 1,3% | 4,6% |
| 5 | 0,76% | 2,7% |
| 6 | 0,58% | 2,1% |
| 7 | 0,88% | 0,32% |

The potential of pare leaf extract (*Momordica charantia* L.) in vitro is the ability of pare leaf extract to kill 50% and 90% of *Raillietina* sp. by looking at the lethal concentration of pare leaf extract. The result of the probit analysis test in Table 2. shows that the LC50 of pare leaf extract against *Raillietina* sp. namely 0.88% and LC90 0.32%, this indicates that with a low concentration pare leaf extract was able to kill *Raillietina* sp. Research conducted by Robiyanto *et al.,* (2018) on *Raillietina tetragona* given ethanol extract of mango arumanis leaves had an LC90 value of 0.31%, it shows that pare leaf extract has an effectiveness that is not much different from the extract in Robiyanto *et al.,* (2015).

Based on Table 3. the results of the probit analysis showed that the LT50 and LT90 values of pare leaf extract (*Momordica charantia* L.) were the fastest at a concentration of 8%, namely at 2.61 and 4.62 hours, while the LT50 and LT90 values of pare leaf extract which takes a long time, namely the concentration of 2% at the 4,32 and 7,64 hours. This shows that the higher the concentration of pare leaf extract, the higher the active ingredient content of pare leaf extract and increases the effectiveness of the time caused to the death of *Raillietina* sp., this is in accordance with the research of Robiyanto *et al.*, (2018) regarding anthelmintic activity research using ethanol extract of mango arumanis leaves, the LT50 value of *Raillietina tetragona* was obtained for 4.5 hours. These results indicate that pare leaf extract takes the same time as mango arumanis leaf extract in killing 50% of worms (Robiyanto *et al*., 2018).

**Table 3.** LT50 and LT90 Pare Leaf Extract

| Extract Concentration | LT50 (Hour) | LT90 (Hour) |
|---|---|---|
| 2% (P1) | 4,32 | 7,64 |
| 4% (P2) | 3,36 | 5,95 |
| 8% (P3) | 2,61 | 4,62 |

Anthelmintic activity against *Raillietina* sp. This caused by the presence of secondary metabolites contained in pare leaf extract, including alkaloids, tannins, saponins, flavono concentration higher than 8% and further research in vivo so that the active compound in pare leaf extract (*Momordica charantia* L.) which

is thought to have an anthelmintic role can work more optimally than the concentration used in this reasearch and find out the possible effects that occur in chickens.

## Conclusion

Pare leaf extract (Momordica charantia L.) has anthelmintic activity against *Raillietina* sp. in vitro. The concentration of pare leaf extract which is effective in killing *Raillietina* sp. in vitro is the concentration of 8%.

The LC50 of pare leaf extract at 1, 2, 3, 4, 5, 6, 7 hours was 37.3%; 5.2%; 2.4%; 1.3%; 0.76%; 0.58%; 0.88% while the LC90 of pare leaf extract at 1, 2, 3, 4, 5, 6, 7 hours in a row was 134.2%; 18.76%; 8.8%; 4.6%; 2.7%; 2.1%; 0.32%.

LT50 of pare leaf extract at a concentration of 2% at 4.32 hours, 4% concentration at 3.36 hours, and a concentration of 8% at 2.61 hours while the LT90 concentration of pare leaf extract was 2% at 7.64 hours, a concentration of 4% at 5.95 hours, and a concentration of 8% at 4.62 hours.

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