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Development of Herbals as an Alternative for Worms (Theme: Determine the Optimization of Herbal Anthelmintic Power in Experimental Animals)

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Abstract

Ascariasis is an intestinal infection caused by the parasite Ascaris lumbricoides. Wuluh starfruit leaves are a natural plant that can be used as a natural worm medicine because this plant contains several compounds that have the potential to act as anthelmintics, namely, flavonoids, saponins, and tannins. The purpose of this study was to determine the optimal anthelmintic power of starfruit leaf extract in experimental animals in vivo.The method in this study was experimental with a post test only group design. The subject of the research is Ascarisdia gallinarum. The research was conducted at the Parasitology Laboratory, Department of Medical Laboratory, Poltekkes Kemenkes Surabaya.

This study used 6 treatment groups namely 0.9% NaCl as negative control and pyrantel pamoate 0.25% as positive control and starfruit leaf extract with 100% concentration which were consumed for 3 days, 4 days, 5 days, 6 days. The data were analyzed by using the Kolmogrov-Smirnov test, if the data were normally distributed then continued with the Parametric test with Anova and if it was not normally distributed with the non-parametric test with the Kruskal-Wallis test then continued using the Post Hoc test to determine the optimization of the anthelmintic power of the ethanol extract of starfruit leaves (Averrhoa bilimbi) against the death of Ascarisdiagalli worms in vivo. The average number of Ascari diagalli worms found in experimental animals given PZ solution (as negative control) was 11.

The mean of Ascari diagalli worms found in experimental animals which were given Pyrantel Pamoate solution (as positive control) was 0.25 = 0 (not found). The average of Ascari diagalli worms found in experimental animals given a solution of ethanol extract of starfruit leaves for 3 days was 7 tails, for 4 days was 3, for 5 days was 2 and for 6 days was 0.5 = 1 tail. It can be concluded that the optimal giving of starfruit leaf ethanol extract solution is for 6 days, because the closest to the positive control is pyrantel Pamoate.

The ethanol extract of starfruit leaves has a high chance to be developed as an alternative worm medicine because it has anthelmintic power, especially in ascariasis. side effects are riskier than natural ingredients. However, in particular it is hoped that the community will increasingly utilize starfruit leaves and cultivate starfruit plants and be able to optimally utilize every part of the plant, especially in the leaves as a family medicinal plant.

Keywords: *Anthelminti; Ascaris diagall; Wuluh starfruit extract*

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1.0 INTRODUCTION

Ascariasis is a disease caused by the intestinal nematode parasite, Ascaris lumbricoides, which occurs frequently in tropical and developing countries. The prevalence of disease caused by roundworms (Ascaris lumbricoides) reaches 25% or 0.8 - 1.22 billion people of the world's total population (Riswanda, 2017). This parasite is found cosmopolitan. In Indonesia, the prevalence of Ascaris lumbricoides is still high, around 60-90% (Iman F, et.al, 2015). According to Carneiro 2002, this parasite is capable of infecting all age levels, however ascariasis often infects elementary school-aged children and toddlers due to lack of awareness of personal hygiene and low immunity (Mia ZR, 2015).

This disease is one of the Soil Transmitted Diseases because it requires soil as a medium for egg development to become an infective form (Pendit, 2016). Ascaris lumbricoides worms can harm the human body. In large numbers, these worms can cause intestinal obstruction, reduced appetite, diarrhea, constipation, impaired absorption of nutrients, and impaired child development, whereas in small numbers these worms rarely show symptoms and are only known after the worms leave the patient's body or find eggs in feces (Kazura, 2007). In the larval stage, these worms can also cause organ damage, Ascaris lumbricoides larvae are able to migrate to various tissues in the body which can cause mild inflammation in the liver and Loefller's Syndrome in the lungs, and can even cause intestinal obstruction in severe infections (Masih M, Banerjee T, Banerjee B, & Pal A, 2011).

According to Manoj (2008), rural communities or low-income people who are the main target of ascariasis feel reluctant to use synthetic drugs that are sold in the market due to the difficulty of finding these drugs and economic factors, most of them prefer to use traditional medicines that are prescribed regularly. hereditary although the benefits have not been proven scientifically. The use of natural medicine in society has begun to develop in the last decade because of the side effects that are almost non-existent if used correctly, starfruit (Averrhoa belimbi) is a traditional medicinal plant that is widely used by Indonesian people, this plant contains flavonoids, saponins, tannins and steroids. One of the uses of this part of the plant is that its leaves can be used as worm medicine because it has an anthelmintic effect. This is consistent with research conducted by Sri Sulami (2019) that extracting star fruit leaves with a concentration of 100% can kill Ascaris suum worms as a substitute for Ascaris lumbricoides in 66 minutes. This anthelmintic power is in accordance with the patent antelmetic drug, pyrantel Pamoate. Ascaris lumbricoides is a parasite that is often found in humans. While Ascari diagalli is a parasite that is often found in chickens and has similarities with Ascaris lumbricoides. Even though Ascari diagalli rarely attacks humans, it is possible that this worm infection can occur when humans consume chicken meat as one of their needs for animal protein which is the host of this worm (Sandy S, Irmanto M, 2014).

Treatment using medicinal plants is one of the alternatives chosen to minimize side effects due to the administration of synthetic drugs. It has been widely known that medicinal plants have anti-worm or anthelmintic properties. From several studies that have been done, it is found that plants that have anthelmintic properties include papaya, bitter melon and temu siring. From this, the researchers were interested in researching other plants that could be potential anthelmintics, in this case the extract of starfruit leaves (averrhoa belimbi) in vivo. Starfruit leaves, which are abundant in nature, can also be used as a natural ingredient that can be an alternative to synthetic worm medicine (Wiraputra H, 2016).

2.0 METHODOLOGY

This type of research is experimental research to determine the optimalization effect of 100% starfruit leaf extract given for 3 days, 4 days, 5 days and 6 days, as an anthelmintic power in experimental animals in vivo. The study design was Post-test only group design. This study used six treatment groups, namely a negative control group, a positive control group, and four experimental groups, namely giving 100% starfruit leaf extract for 3 days, 4 days, 5 days and 6 days. The test material used in this study was a 100% concentration of starfruit leaf extract (*Averrhoa bilimbi*). Test animal, Adult gally Ascarisda worms obtained from chicken slaughterhouses, and chicks as experimental animals. The samples used in this study were 5 experimental animals in each treatment, and replicated 4 times. The independent variable in this study was the administration of ethanol extract of e starfruit leaves with a concentration of 100%

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in experimental animals. The dependent variable in this study was the presence or absence of Ascaridia galli worms. This study used observational data collection techniques (direct observation) by observing the presence or absence of Ascari diagalli worms after giving 100% concentration of starfruit leaf extract, for 3 days, 4 days, 5 days and 6 days.

The data analysis technique used in this study is quantitatively taken from primary data, namely data obtained from observations of the number of Ascari diagalli worms found in experimental animals after being treated with ethanol extract of starfruit leaves (*Averrhoa bilimbi*) which will then be processed using tables and graphics. The data obtained will be analyzed using the Kolmogrov-Smirnov statistical test to determine the normality of the data obtained and followed by a homogeneity test using the SPSS application. If the data obtained produces homogeneous and normally distributed data, then it can be continued using the Annova One Way statistical test if the data is not normally distributed then using the Kruskal-Wallis statistical test with a confidence level of 95% or α = 0.05, then continue using the Post-Hoc test for Knowing the optimal anthelmintic power of ethanol extract of starfruit leaves (*Averrhoa bilimbi*) against worm mortality Asscaridiagalli in vivo.

3.0 RESULTS

3.1 Research Stage

3.1.1 Making Leaf Starfruit Simplicia

Starfruit leaf plants were obtained from the UPT Materia Medika garden yard, Batu City. The leaves are dried in the form of simplicia by drying them in the hot sun indirectly by covering them with a black cloth until they are completely dry. Make a simplicia powder by grinding it with a mortar and pestle.

3.1.2 Making Ethanol Extract of Starfruit Leaves

Weighing the dried starfruit leaf powder as much as 500 grams then put it in a maceration container and carry out the extraction process using the maceration method with 96% ethanol solvent. Soak the dry starfruit leaf powder using 96% ethanol, covered with aluminum foil and left for 3 × 24 hours at room temperature. After 3 × 24 hours, the sample soaked in 96% ethanol is filtered using filter paper. The results of maceration were collected and concentrated using a rotatory vacuum evaporator at a temperature of 50 °C until a concentrated extract was obtained. Allow the resulting concentrated extract to stand at room temperature until all of the ethanol solvent evaporates. The ethanol extract of the earrings and starfruit leaves was 100% concentrated, positive control deworming *pirantel pamoate and* negative control (aquabides)

3.1.3 Giving Ethanol Extract of Starfruit Leaves with a concentration of 100% for 3,4,5 and 6 days according to the dosage

*Experimental Animal Treatment***:** Chickens were adapted to the study cage for two weeks. Before infection with A. galli worm eggs, all chickens were treated using *Pirantel Pamoate* according to the recommended dose of 0.2 gram / kg body weight to eliminate the possibility of worms in the body of the chicken, A. galli worms were put into beaker glasses containing NaCI physiological 0.85%. All worms were washed several times with this solution until they were clean of feces, then selected female worms which were marked by their large body size and straight posterior parts. The female worms obtained were then put in a beaker containing physiological NaCI. All female worms that have been collected are cut at the posterior porus genitalis, which is the boundary between dark and light, then the eggs are removed with the uterus by massaging the worm's body until two grams of worm eggs are obtained, then the worm's body marks are removed. The worm eggs are put into a beaker containing 50 ml 0.5 N NaOH. The eggs in the solution were stirred using a magnetic stirrer for 30 minutes. Then let stand for 10 minutes so that the worm eggs settle, the supernatant is removed. This process is repeated 3 times so that the worm eggs are clean from the uterus.

The solution containing worm eggs is centrifuged 3 times and the worm eggs are ready to be embryoned. Centrifuged worm eggs are put into an Erlenmeyer tube containing 150 ml of distilled water. Worm eggs are incubated at room temperature for two weeks. After two weeks the worm eggs contain stage II larvae, and are ready to be infected in chickens. Infection is carried out using a syringe equipped with a plastic cannula inserted into the esophagus of the chicken, waiting for 28 days. (Chicken has worm infection). Furthermore, the treatment was carried out

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by administering the ethanol extract of starfruit leaves for 3 days, 4 days, 5 days and 6 days. placed on the tray. The chicken intestine is surgically removed longitudinally, then the intestine is washed with water. The entire contents of the intestine are placed in a beaker and allowed to settle, the supernatant is removed. The sediment is then examined under a microscope to check for worms and worm larvae. Record the number of worms present in the experimental animal in each treatment group.

3.2 Research Data The number of Ascari worms was diagnosed in the negative control group, the positive control group and treatment with starfruit leaf extract for 3 days, 4 days, 5 days and 6 days

Table 1: The number of *Ascari diagalli* worms found in the negative control group, the positive control group and treatment with starfruit leaf extract for 3 days, 4 days, 5 days and 6 days.

No	Replication	The number of *Ascari diagalli* worms found					
		Control Negative	Positive Control	Providing 100% concentration of starfruit leaf extract			
				3 Days	4 Days	5 Days	6 Days
1	1	11	0	6	3	2	0
2	2	9	0	4	2	1	0
3	3	12	0	7	3	1	1
4	4	13	1	9	4	2	1
Average		11	0.25 = 0	7	3	2	0.5 = 1

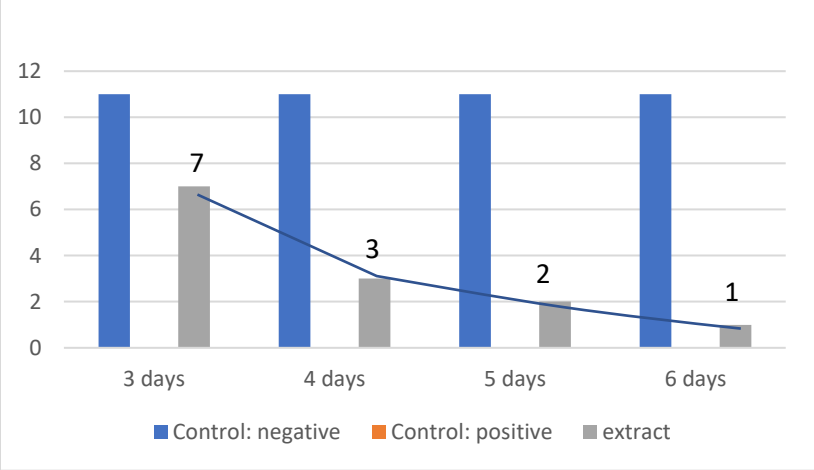

Figure 1. The number of worms found in experimental animals

Based on table 1 and figure 1, it can be seen that the longer the application of starfruit leaf extract to experimental animals, the number of worms found decreased (approaching positive control.). In the treatment group that was given starfruit extract solution for 3 days, it was found that an average of 7 *Ascari diagalli* worms was found, while in the treatment group that was given starfruit extract solution for 4 days, it was found that an average of 3 *Ascari diagalli* worms was found, in the treatment group that was given a solution of starfruit extract for 5 days. days found an average of 2 *Ascari diagalli* worms, while in the treatment group given a solution of starfruit extract for 6 days found an average of 1 *Ascari diagalli* worms. This shows that the results of this study on day 6 in experimental animals found 1 *Ascari diagalli* worms, while the positive control was not found (0), meaning that on the 6th day of giving Wuluh starfruit leaf extract, the closest to positive control was the provision of pyrantel *Pamoate* solution.

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3.3 The results of the analysis of the effect of starfruit leaf extract on the number of Ascari diagalli worms found in experimental animals after giving the ethanol extract of star fruit leaves to four treatment groups

To determine the effect of starfruit leaf extract on the number of *Ascari diagalli* worms found in experimental animals after giving the ethanol extract of starfruit leaves to the four treatment groups, namely giving starfruit extract for 3 days, 4 days, 5 days and 6 days, Anova One test was carried out. Way. Assuming the data is normally distributed and at least an interval or ratio scale. The data obtained are first performed data normality test to determine whether the data is normally distributed or not and the minimum data scale is interval or ratio scale. If the data is normally distributed, it is continued with the One way Anova parametric test, while if the data is not normally distributed then the non-parametric test is continued.

Based on the results of the SPSS Output for the One Way Anova Test, it produces a significant value of p = 0.000 at α = 0.05. means that p <α then Ho is rejected, meaning that there is a long time effect of giving ethanol extract of starfruit leaves on the number of *Ascari diagalli* worms found in the intestines of experimental animals against the positive control (those given Pirantek *Pamoate* solution). To determine the pairs of different treatment groups, a multiple comparison test was carried out using the Post-Hoc Test.

Based on the results of the multiple comparison test using the Post-Hoc Test, it was found that the pairs of different treatment groups (marked with *) were the treatment pairs with the ethanol extract of starfruit leaves for 3 days and 4 days, which was different from the Positive control, while in the treatment group the ethanol extract of the leaves was given. Wuluh starfruit for 5 days and 6 days Not different from positive control (no sign *).

The conclusion is that giving ethanol extract of starfruit leaves for 5 days, and giving ethanol extract of starfruit leaves for 6 days, the number of *Ascari diagalli* worms found in the intestines of experimental animals was not different from the number of *Ascari diagalli* worms found in positive control, (by giving *Pirantel Pamoate*) This means that the administration of starfruit leaf extract for 5 days and 6 days is an effective administration, but at 5 days of administration, *Ascari diagalli* worms were still found in the intestines of experimental animals, on the 6th day it was the most optimal giving of starfruit extract, because statistically or It is descriptively the same as positive control.

4.0 DISCUSSION

Based on the results of the research in table 5.1, it can be seen that the average number of *Ascari diagalli* worms found in experimental animals after giving starfruit leaf extract with a concentration of 100% for 3 days was 11, and in giving starfruit leaf extract a concentration of 100% for 4 days. On average, there were 7 heads, while the administration of starfruit leaf extract with a concentration of 100% for 5 days on average was found 2 heads, whereas in giving starfruit leaf extract with a concentration of 100% for 6 days an average of 0.5 (1) heads was found. This shows that there was a decrease in the number of *Ascari diagalli* worms found in experimental animals with the increasing time of administration of starfruit leaf extract. In giving starfruit leaf extract with a concentration of 100% for 6 days the number of *Ascari diagalli* worms found was not different from the number of worms found in positive controls.

Meanwhile, the positive control used in this study was *Pirantel Pamoate*, which is one of the standard drugs for ascariasis. Researchers used pyental *Pamoate* with a concentration of 0.25% which is equivalent to a one-time dose of tablets of 250 mg per tablet. This positive control can cause the death of *Ascari diagalli* worms. This is because pyrantel *Pamoate* can inhibit spastic neuromuscular depolarization processes and worm mortality. In addition, it also inhibits the cholinesterase enzyme, thereby increasing muscle contraction in the worm's body (Ulya, et al, 2014). The pyrantel *Pamoate* used is in the form of tablets, to get the neuromuscular body in the worm, so that it can cause paralysis in the concentration of 0.25%, a 100 mL aquadest solution is used.

This research was conducted to determine the optimalization effect of giving starfruit leaf extract with a concentration of 100% given for 3 days, 4 days, 5 days and 6 days, as an anthelmintic power in experimental animals *in vivo*. To determine the effect of the duration of giving ethanol extract of starfruit leaves, a one-way ANOVA statistical test was performed. Based on the results of statistical test analysis with one way Anova, it was found that the sig p value = 0.000 at the 95% confidence level (α = 0.05) from these results it can be seen that the value of

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Sig p. <A, it can be concluded that there is a difference in the number of worms which was found in experimental animals for the duration of administration of 100% starfruit leaf ethanol extract. It can be concluded that there is a long effect of giving starfruit extract on the number of *Ascari diagalli* worms found in the intestines of experimental animals, namely the longer the starfruit leaf extract was given, the number of *Ascari diagalli* worms found was closer to the number of worms found in positive control. The value of p <α, meaning that at least there are a pair of different treatments, so to find out the different treatment pairs, a multiple comparison test is performed with the Post-Hoc test.

Based on the results of the multiple comparison test using the Post-Hoc Test, it was found that the pairs of different treatment groups (marked with *) were the treatment pairs with the ethanol extract of starfruit leaves for 3 days and 4 days, which was different from the Positive control, while in the treatment group the ethanol extract of the leaves was given. Wuluh starfruit for 5 days and 6 days Not different from positive control (no sign *). The conclusion is that giving ethanol extract of starfruit leaves for 5 days, and giving ethanol extract of starfruit leaves for 6 days, the number of *Ascari diagalli* worms found in the intestines of experimental animals was not different from the number of *Ascari diagalli* worms found in positive control, (by giving *Pirantel Pamoate*) This means that the administration of starfruit leaf extract for 5 days and 6 days is an effective administration, but at 5 days of administration, *Ascari diagalli* worms were still found in the intestines of experimental animals, on the 6th day it was the most optimal giving of starfruit extract, because statistically or It is descriptively the same as positive control.

The anthelmintic effect that comes from starfruit leaves is due to the presence of active substances saponins, tannins and flavonoids which act as anthelmintics as has been found from previous research by Masduqi & Anggoro (2017) that the results of phytochemical screening tests, starfruit leaf extract (*Averrhoa bilimbi* Linn) contains compounds including: alkaloids, flavonoids, tannins, saponins and triterpenoids (Asih A, 2014). Saponin compounds contained in starfruit extract are compounds in the form of glycosides. The mechanism of saponin compounds as an anthelmintic has the potential to kill worms because it works by inhibiting the acetylcholinesterase enzyme and irritating the mucous membrane, so that the worms will experience muscle paralysis and lead to death (Intannia, et al, 2015). According to a quote from Kristianto (2013) states that the saponin content in starfruit leaves is 10.0%, and the tannin content contained in starfruit leaves is 6.0%. Whereas in previous research, Ramadhan et al. (2011) stated that the flavonoid content in the starfruit leaf extract with 96% ethanol solvent was 2.265%.

While the working mechanism possessed by tannins is by disrupting the negative ion charge of the worm's body into positive ions (protonization) which then these positive ions attract the worm's body protein in the digestive tract so that it interferes with the metabolism and homeostasis of the worm's body (Andaru PGR, 2012). Besides saponins and tannins, there are flavonoid compounds that support the acceleration of worm death time. According to Ulya, et al. (2014) the ability of flavonoids in anthelmintics, namely flavonoids that are in direct contact with the worm's body, will be quickly absorbed into the worm's body and will cause protein denaturation in the tissue, causing death in worms. In previous studies conducted by researchers using starfruit leaf extract where the optimum concentration was 100% with the average length of time of death for all worms was 66.25 minutes which was close to the length of time of death of all worms caused by positive control, namely pyrantel *Pamoate* (in vitro) resulted in an accelerated time of death of Ascaris suum worms followed by an increase in the concentration of the ethanol extract of starfruit leaves. In this study, researchers continued previous research, namely determining the optimization of herbal anthelmintic power in experimental animals (*in vivo*) 100% starfruit ethanol extract given to experimental animals (chickens) for 3 days, 4 days 5 days and 6 days.

Based on the research results in table 5.1 it can be concluded that the ethanol extract of starfruit leaves at a concentration of 100% has an effect on the number of *Ascari diagalli* worms found in experimental animals given starfruit leaf extract solution for 3 days, 4 days, 5 days and 6 days. It is proven that starfruit leaf extract solution can be used as an anthelmintic because the number of *Ascari diagalli* worms found in the intestines of experimental animals given for 5 days and 6 days is not different from the number of worms found in positive controls. While the optimum time of administration is on the sixth day, because on the sixth day the *Ascari diagalli* worms found were the same as those given positive control (Dharma YP, 2016). The anthelmintic

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effect caused by Pyrantel *Pamoate* itself is well known, because Pyrantel *Pamoate* is the drug of choice to treat cases of ascariasis. Pyrantel *Pamoate* can inhibit the neuromuscular depolarization process in the worm's body, so that it can cause spastic neuromuscular paralysis and worm death. In addition, it also inhibits the cholinesterase enzyme, thereby increasing muscle contraction in the worm's body (Anam K, 2017).

The ethanol extract of starfruit leaves has a high chance to be developed as an anthelmintic drug, especially for ascariasis. Because, it has the same anthelmintic power with pyrantel *Pamoate* (Ganestya S, 2011). In addition, the use of Pyrantel *Pamoate* has side effects in the form of indigestion, fever and headaches, which may not be found in the use of ethanol extract of starfruit leaves as worm medicine (Saputra, O., & Anggraini N, 2016).

5.0 CONCLUSION

Research on Herbal Development as an alternative worm medicine with the theme of determining the optimization of herbal anthelmintic power in experimental animals given a solution of ethanol extract of starfruit leaves for 3 days, 4 days, 5 days and 6 days, the average *Ascari diagalli* worms found in experimental animals given PZ solution (as a negative control) was 11 tails, which were given a solution of Pyrantel *Pamoate* (as a positive control) was 0.25 = 0 (not found), while those given a solution of ethanol extract of starfruit leaves for 3 days were 7 tails, for 4 days is 3 birds, for 5 days is 2 birds, and those given for 6 days is 0.5 = 1 head. So it can be concluded that the optimal giving of starfruit leaf ethanol extract solution is for 6 days, because the closest to the positive control is giving pyrantel *Pamoate* solution.

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