

DOI: <http://dx.doi.org/10.33846/hn40502>
<http://heanoti.com/index.php/hn>



RESEARCH ARTICLE

URL of this article: <http://heanoti.com/index.php/hn/article/view/hn40502>

Identification of the Anthelmintic Power of *Anting-anting* Leaf and Starfruit Leaf

Sri Sulami Endah Astuti^{1(CA)}

^{1(CA)}Medical Laboratory Department, Poltekkes Kemenkes Surabaya; srisulamiea@gmail.com (Corresponding Author)

ABSTRACT

Ascariasis is an intestinal infection caused by the parasitic worm *Ascaris lumbricoides*. Starfruit leaf (*Averrhoa bilimbi*) and leaf anting-anting (*Acalypha indica* L) is a plant that is often used as a worm remedy in the community. Starfruit leaves (*Averrhoa bilimbi*) and Leaf anting-anting (*Acalypha indica* L) contain several compounds that have the potential as anthelmintics, namely saponins, tannins and flavonoids. The purpose of this study was to determine the effect of ethanol extention of starfruit leaf leaves (*Averrhoa bilimbi*) and leaf anting-anting (*Acalypha indica* L) as anthelmintic on the time of death of *Ascaris suum* Goeze worms in vitro. The method in this study is experimental with post test only group design. Test animals from the study are *Ascaris suum*. The study was conducted at the Parasitology Laboratory, Medical Laboratory Department, Poltekkes Kemenkes Surabaya in 2019. This study used 6 treatment groups, NaCl 0.9% as a negative control and pirantel pamoate 0.25% as a positive control and ethanol extract of *wuluh* starfruit leaf and ethanol extract of Anting-anting with concentrations of leaves. 40%, 60%, 80% and 100%. Data were analyzed using the Kolmogrov-Smirnov test, the Anova two way Block Design test, then continued using the Post Hoc test to determine differences between blocks and between treatments, against the time of *Ascaris suum* worm's death. The average time of death of *Ascaris suum* caused by ethanol extract of starfruit leaves concentration of 40% for 492.3 minutes and ethanol extract of leaf anting-anting 396.5 minutes, concentration of 60% ethanol extract of *wuluh* starfruit leaves for 278.5 minutes and leaf extract ethanol leaf anting-anting for 283.5 minutes, 80% concentration of ethanol extract of *wuluh* starfruit leaf for 141.8 minutes and ethanol extract of anting-anting leaf for 174 minutes, 100% concentration of ethanol extract of *wuluh* starfruit leaves for 66.75 minutes and extract ethanol leaf anting-anting for 92 minutes. So it can be concluded that the ethanol extract of starfruit leaves has an anthelmintic effect on the *Ascaris suum* worm more optimum than the ethanol extract of the Anting-anting leaves, because the time required is close to positive control that is 60 minutes.

Keywords: anthelmintic; *Ascaris suum*; starfruit leaf; anting-anting leaf

INTRODUCTION

Ascariasis is a worm disease that often occurs in tropical and developing countries.⁽¹⁾ Worming is a disease that is based on the environment. This can be influenced by tropical climate and high humidity. Indonesia is a good environment for worm development, as well as sanitation and hygiene conditions that do not meet health requirements and inadequate socio-economic conditions and education.⁽²⁾ This state of helminthiasis can result in decreased health, nutrition, intelligence, and productivity of sufferers.⁽³⁾

In 2015, the prevalence of ascariasis in the world was 807 million, while in Southeast Asia it was 589 million. Indonesia has an average prevalence of ascariasis in 33 provinces in 2012 of 31.8% with the highest percentage occurring at school age.⁽⁴⁾ This is influenced by several factors, such as individual hygiene, environmental sanitation, and maternal knowledge.⁽⁵⁾

Ascariasis is an infection caused by *Ascaris lumbricoides* or roundworms.⁽⁶⁾ Adult worm predilection is found in the lumen of the human small intestine, but is sometimes found in other parts of the intestine.⁽⁷⁾ Early ascariasis infection is characterized by worms coming out with feces or worms coming out of the mouth, nose

and anus.⁽⁸⁾ Worm infections can cause a decrease in nutrients and blood loss.⁽⁹⁾ These worms can also cause intestinal obstruction, reduced appetite, diarrhea, constipation, and impaired child development.⁽¹⁾

Ascariasis can be treated using worm medicine. Worm medicine which is the choice of ascariasis is pirantel pamoate which is a single dose drug and is the first line in the treatment of worm infections. However, these drugs have side effects in the form of digestive disorders such as abdominal pain and diarrhea. Some deficiencies in the anthelmintic drugs above are relatively expensive. So we need to find other alternatives that can suppress the prevention of ascariasis with natural ingredients that are easily available.⁽⁶⁾

Traditional medicines contain many chemicals that have anthelmintic effects, among these chemicals are flavonoids, tannins, and saponins. One of the plants in Indonesia that contains flavonoid compounds, tannins, and saponins is starfruit (*Averrhoa bilimbi* Linn) and leaf anting-anting (*Acalypha indica* L). As has been found from previous studies.⁽¹⁰⁾ that the results of photochemical screening show that simplicia from starfruit leaves contain flavonoids, saponins, tannins and steroids / triterpenoids.

Ascaris suum Goeze test animals are used as research subjects, because *Ascaris lumbricoides* Linn as an obligate parasite in humans cannot be found living outside the human body. *Ascaris suum* Goeze is a roundworm found in the pig's small intestine.⁽⁴⁾ Although genetically different, judging by its morphological characteristics, *Ascaris lumbricoides* (L) has many similarities with *Ascaris suum* Goeze, as well as several traits such as the way of life and breeding, worms of this genus are the same.⁽¹¹⁾

METHODS

Types of Study

This type of study was an experimental research in order to determine the effect of ethanol extract of starfruit leaf (*Averrhoa bilimbi*) and *anting-anting* leaf (*Acalypha indica* L) as anthelmintic on the time of death of *Ascaris suum*, Goeze roundworm in vitro by using a post test only with control group design.

Material

The materials used in this study were starfruit leaves (*Averrhoa bilimbi*) and *anting-anting* leaves (*Acalypha indica* L) obtained from the UPT Materia Medika, in Pesanggrahan Village, Batu sub-district, Batu, East Java, Indonesia.

Animals

The test animal used was *Ascaris suum*, adult Goeze has a length of 30-35 cm that is still actively obtained from the intestine of pigs from the slaughterhouse on Jl. Pegirian Surabaya, East Java, Indonesia.

Location and Time

The study was conducted in the Parasitology Laboratory, Medical Laboratory Department, Poltekkes Kemenkes Surabaya and at the Faculty of Veterinary Medicine, Universitas Airlangga in 2019.

Sampling

This study used a purposive sampling technique by equalizing the length of the worm and the type of worm and did not differentiate the sex of the worm. Worm samples used in this study were five animals per treatment. Determination of the number of research replications was carried out using the Frederer formula.

Data Collection Method

This study used observational data collection techniques (direct observation) by observing the time of death of *Ascaris suum* worm after giving ethanol extract of starfruit leaf (*Averrhoa bilimbi*) and ethanol extract of *anting-anting* leaves (*Acalypha indica* L) with a concentration of 40%, 60%, 80% and 100%. Negative control was carried out to determine the quality of the *Ascaris suum* worm sample used in this study.

Making Simplisia Wuluh Starfruit Leaves (*Averrhoa bilimbi*, L) and Anting-anting Leaves (*Acalypha indica*, L)

Washing the leaves of starfruit and leaves of anting-anting using running water, drain, then dried by aerating (without sunlight) to dry completely. Smooth the dried *wuluh* starfruit leaves and *anting-anting* leaves using mortar and pestle and then the sifting of the starfruit leaves and the leaves of the *anting-anting* have dried to get the starfruit leaf powder and anting-anting powder.

Making Ethanol Extract of Wuluh Starfruit Leaves (*Averrhoa bilimbi*, L) and Anting-anting leaves (*Acalypha indica* L)

Weigh the dried starfruit leaf powder as much as 1000 grams then put it into a maceration container and perform the extraction process using maceration method with 96% ethanol solvent.

Soak the dried 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 with 96% ethanol was filtered using filter paper. Maserat results were collected and concentrated using a rotatory vacuum evaporator at a temperature of 50°C until a concentrated extract was obtained. Leave the concentrated extracts produced at room temperature until all the ethanol solvents evaporate. Concentrated ethanol extract of starfruit leaves (*Averrhoa bilimbi*) 100%. Likewise for anting-anting - branches done the same thing as in the leaves of *wuluh* starfruit.

Positive Control Making (Pirantel Pamoat 0.25%)

Dissolve 250 mg of Pirantel Pamoat with 100 ml of distilled water.

Negative Control Making (NaCl 0.9%)

Dissolve 0.9 grams of NaCl in 100 ml of distilled water.

Making Ethanol Extract of Wuluh Starfruit Leaves and Anting-anting Leaves (*Acalypha indica* L) Concentration of 40%

Dissolve 8 grams of concentrated extract of ethanol starfruit leaves with 0.1 mL of Tween 80 surfactant solution and then add 20 mL of 0.9% NaCl slowly. The same thing was done for Anting-anting leaves.

Making Ethanol Extract of Wuluh Starfruit Leaves and Anting-anting Leaves (*Acalypha indica* L) Concentration of 60%

Dissolve 12 grams of concentrated extract of ethanol starfruit leaves with 0.1 mL of Tween 80 surfactant solution and then add 20 mL of 0.9% NaCl slowly. The same thing was done for *anting-anting* leaves.

Making Ethanol Extract of Wuluh Starfruit Leaves and Anting-anting Leaves (*Acalypha indica* L) Concentration of 80%

Dissolve 16 grams of concentrated extract of ethanol starfruit leaves with 0.1 mL of Tween 80 Surfactant solution and then add 20 mL of 0.9% NaCl slowly. The same thing was done for Anting-anting leaves.

Making Ethanol Extract of Wuluh Starfruit Leaves and Anting-anting Leaves (*Acalypha indica* L) Concentration of 100%

Made using thick extract without dilution. The same thing was done for leaf anting-anting.

Observation of the Anthelmintic Effect of *Wuluh* Starfruit Leaf Extract and *Anting-anting* Leaf (*Acalypha indica* L)

Prepare a test container to be used for observing the anthelmintic power. Fill each test container with a solution of ethanol extract of starfruit leaf (*Averrhoa bilimbi*) with 40%, 60%, 80%, and 100% constructions, negative control, and positive control. The negative control contained 0.9% NaCl solution. The positive control contained a 0.25% pirantel pamoate solution.

Add five *Ascaris suum* worms to each petri dish which contains a solution of ethanol extract of starfruit leaf (*Averrhoa bilimbi*), negative control solution and positive control solution. Observe the movements of *Ascaris suum* worms every minute by touching the body of *Ascaris suum* worms using anatomical tweezers. Record the number of worms that died and the time of death *Ascaris suum* worms. The same was done for the same thing with anting-anting leaf ethanol extract solution.

Data Analysis Techniques

Quantitative data analysis techniques were taken from primary data that was data obtained from observations of the number of *Ascaris suum* worm deaths and the time of *Ascaris suum* worm deaths after being treated with ethanol extract of *wuluh* starfruit leaf (*Averrhoa bilimbi*) and ethanol extract of *anting-anting* leaves (*Acalypha indica* l) will then be presented using tables and graphs. The data obtained will be analyzed using the Kolmogrov-Smirnov statistical test to determine the normality of the data obtained and proceed with the homogeneity test using the SPSS application. Anova Two Way test was then performed to determine differences between blocks and between treatments.

RESULTS

Data Presentation

Table 1. The results of research on the effect of giving *wuluh* starfruit leaf ethanol extract as anthelmintic against the time of death of *Ascaris suum*, Goeze by In Vitro (minutes)

Replication	Time of death <i>Ascaris suum</i> Goeze (minute)				Control	
	40%	60%	80%	100%	+	-
1	488	266	118.25	62	60	5760
2	482.5	297.25	147	71.5	60	5760
3	497	285	154.5	68	60	5760
4	501.25	286.5	148.75	64.5	60	5760
Mean	492.875	282.5625	142.25	66.75	60	5765

+ = using pirantel pamoat 0.25%
- = using NaCl 0.9%

Figure 1 illustrates the acceleration of the death of the *Ascaris suum* worm. This is shown by the decrease in the graph caused by the ethanol extract of starfruit leaves with concentration of 40% to ethanol extract of starfruit leaves with concentration of 100% and ethanol extract of anting-anting leaves with concentration of 40% to 100% concentration.

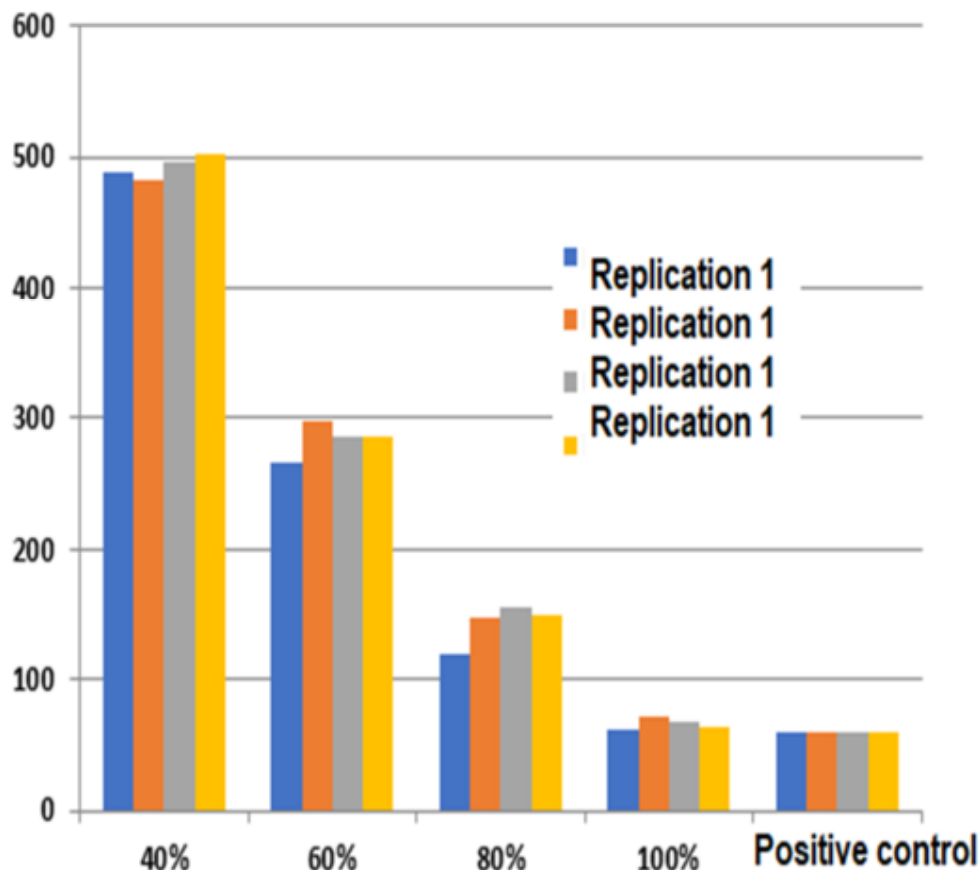


Figure 1. Time of the death of *Ascaris suum* worm caused by ethanol extract of starfruit leaf and ethanol extract of *anting-anting* leaf (in minutes)

Data Analysis

To find out the significant influence of worm death time from ethanol extracts of starfruit leaf leaves and *anting-anting* leaf ethanol extracts in the four concentration groups, a data normality test was first performed to find out whether the data was normally distributed or not and the homogeneity test of the data to find out the data homogeneous or not. If the data is normally distributed and homogeneous then the Two Way Anova parametric test is continued while if the data is not normally distributed and is not homogeneous then the Kruskal-Wallis non parametric test is continued. From the data and research results regarding the effect of ethanol extract of starfruit leaf (*Averrhoa bilimbi*) and ethanol extract of *anting-anting* leaf (*Acalypha indica* L) as anthelmintic on the time of death of *Ascaris suum* worm, Goeze in vitro will be statistically tested using Parametric test because normal distributed data, namely the two way Anova test.

Based on the results of statistical tests with the two way Anova for inter-block analysis produced p-values of 0.697, meaning that there was no difference between the blocks. The conclusions of *anting-anting* leaf extract and *wuluh* fruitstar leaf extract had the same function as pirantel pamoat as antelmintic. The results of the inter-treatment analysis obtained p-value of 0.002, meaning there were differences between treatments.

To find out the pairs of different treatment groups, a multiple comparison test was conducted using the Post-Hoc Test. The results of the multiple comparison test with the Post Hoc Test of ethanol extract of starfruit leaf leaves and ethanol extract of *anting-anting* leaves concentration of 40% had different values with ethanol extract of starfruit leaf leaves and ethanol extract of *anting-anting* leaves concentration of 60%, concentration of 80%, concentration of 100% , negative control and positive control.

The results of the multiple comparison test with the Post Hoc Test of ethanol extract of starfruit leaf leaves and ethanol extract of *anting-anting* leaves concentration of 40% had different values with ethanol extract of starfruit leaf leaves and ethanol extract of *anting-anting* leaves concentration of 60%, concentration of 80%, concentration of 100% negative control and positive control.

At concentrations of 80% and 100% ethanol extract of *wuluh* starfruit leaf and ethanol extract of *anting-anting* leaves did not differ from positive control namely pirantel pamoat. The 80% concentration and 100% concentration had the opportunity to be developed as an anthelmintic drug, especially in ascariasis, because it did not have a significant difference with positive control.

DISCUSSION

Based on the results of the study, it can be seen that the average death time of *Ascaris suum* worm on starfruit leaf extract and *anting-anting* leaves concentration of 40% is 492.3 and 396.5 minutes, at a concentration of 60%, the average time of death is 278, 5 and 283.5 minutes, at a concentration of 80%, the mean time of death was 141.8 and 174 minutes, and at a concentration of 100%, the mean time of death was 66.75 and 92 minutes. This shows that there was an acceleration of the death of *Ascaris suum* worm by increasing the concentration of ethanol extract of starfruit leaf and ethanol extract of the leaf of *anting-anting*, meaning that ethanol extract of starfruit leaf and ethanol extract of *anting-anting* leaf had anthelmintic effect by showing the faster time of death of worms on the higher concentration of ethanol extract of starfruit leaves was 100%.

Meanwhile, for the positive control used in this study is Pirantel Pamoat which is one of the standard drugs for ascariasis. Researchers used pirenthal pamoate with a concentration of 0.25% which is equivalent to the dose of one-time tablet that is as much as 250 mg per tablet.

This positive control can cause the death of *Ascaris suum* worms with an average of 60 minutes. This is because the pyrantel pamoate can inhibit the process of neuromuscular depolarization in the worm's body, so that it can cause spastic neuromuscular paralysis and worm death. In addition, it also inhibits the enzyme cholinesterase thereby increasing muscle contraction in the worm's body⁽⁷⁾. Pirantel pamoat used is tablet-shaped, so to get a concentration of 0.25%, a 100 mL aquadest solution is used.

The Pos-Hoc Test results show that there are significant differences between the treatment groups (concentrations) of ethanol extracts of starfruit leaf leaves and ethanol of extract of *anting-anting* leaf. Based on the results of these studies indicate that the ethanol extract of starfruit leaf and ethanol extract of *anting-anting* leaves have anthelmintic power, because there is an acceleration of the time of death of the *Ascaris suum* worm, Goeze. At the highest concentration of ethanol extract of starfruit leaves, 100%.

The anthelmintic effect that comes from starfruit leaves and *anting-anting* leaves due to the presence of active substances saponins, tannins and flavonoids that act as anthelmintics as has been found from previous studies by Masduqi & Anggoro⁽¹²⁾ that the results of phytochemical screening, *wuluh* starfruit leaf extracts contains compounds including: alkaloids, flavonoids, tannins, saponins and triterpenoids.

According to a quote from Kristianto⁽¹³⁾ states that the content of saponins contained in starfruit leaves is 10.0%, and the content of tannins contained in starfruit leaves) as much as 6.0%. Whereas in previous studies Ramadan, et al.⁽¹⁴⁾ stated that the content of flavonoids in starfruit leaf extract with 96% ethanol solvent amounted to 2.265%.

Saponin compounds contained in extracts of starfruit leaf leaves and leaf *anting-anting* are compounds in the form of glycosides. The mechanism of saponin compounds as anthelmintic is that it has the potential to kill worms because it works by inhibiting the enzyme acetylcholinesterase and irritating the mucous membrane, so that the worm will experience muscle paralysis and lead to death⁽²⁾.

While the mechanism of action possessed by tannins is by interfering with 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 disrupts the metabolism and homeostasis of the worm's body.⁽⁶⁾

In addition to saponins and tannins there are flavonoid compounds that support the acceleration of worm death time. According to Ulya, et al.⁽⁷⁾ the ability of flavonoids in anthelmintics, namely flavonoids that come in direct contact with the worm's body, will be quickly absorbed into the worm's body and will cause denaturation of proteins in the tissues, causing death in the worms. Therefore, this research can produce an acceleration time of *Ascaris suum* worms followed by an increase in the concentration of ethanol extract of starfruit leaf leaves and *anting-anting* leaves.

Based on the results of the study in table 1 it can be concluded that the ethanol extract of starfruit leaves at a concentration of 100% has an influence on the time of death of *Ascaris suum* worms and can be used as an anthelmintic because it has a faster death time and is *anting-anting* the time of death caused by positive control, while the ethanol of extract of starfruit leaf and ethanol of extract of *anting-anting* leaves at concentrations of 40%, 60%, and 80% have a death time that is far from positive control, so that it can still be used as an anthelmintic but is less than optimal.

Ethanol extract of starfruit leaves has a high chance to be developed as an anthelmintic drug, especially in askariasis. Because, there is an acceleration of the death of the *Ascaris suum* worm caused by ethanol extract of starfruit leaves. In addition, the use of Pirantel pamoate has side effects in the form of digestive disorders,

fever and headaches, which may not be found in the use of ethanol extracts of starfruit leaves as a worm medicine⁽¹⁵⁾.

Conclusion

The ethanol extract of starfruit leaf and ethanol extract of *anting-anting* leaf has an influence on the time of death of *Ascaris suum*, Goeze worms in vitro. This can be seen from the average time of death of the *Ascaris suum*, Goeze worm as follows:

The time of *Ascaris suum* worm death caused by ethanol extract of starfruit leaves concentration of 40% was 482.3 minutes and ethanol extract of *anting-anting* leaves concentration of 40% was 396.5 minutes. The time of death of *Ascaris suum* worm caused by ethanol extract starfruit leaves concentration 60% for 278.5 minutes and ethanol extract of *anting-anting* leaf leaves concentration 60% for 283.5 minutes. The time of death of *Ascaris suum* worm caused by ethanol extract of starfruit leaves 80% concentration was 141.8 minutes and ethanol extract of *anting-anting* leaves 80% concentration was 174 minutes. The time of death of *Ascaris suum* worm caused by ethanol extract of starfruit leaves 100% concentration was 66.75 minutes and ethanol extract of *anting-anting* leaves was 100% concentration that was for 92 minutes. The most optimum concentration in killing *Ascaris suum* worms, Goeze based on the time of *A.suum* worm death approaching positive control was starfruit leaf extract with 100% concentration for 66.75 minutes.

Suggestion

1. For the next researcher, it is expected to test the active compounds in the ethanol extract of leaves and starfruit, especially the active compounds which act as anthelmintics.
2. For the community can utilize the parts of the starfruit leaf plant to be used as an alternative treatment for Askariasis.

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