

**ANTI-PARASITIC ACTIVITY OF THREE INVASIVE
VERBENA SPECIES (*Premna odorata* B., *Lantana camara* L.
and *Duranta erecta* L.) AGAINST *Lumbricus terrestris*
(EARTHWORMS) AND *Ascaris lumbricoides*
(ROUNDWORMS)**

(RESEARCH PAPER)

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LOANZON, M.D.; S.C. DE CASTRO; AND M.A.C. PELISMINO. Anti Parasitic Activity of Three Invasive Verbena Species (*Premna odorata* B., *Lantana camara* L. and *Duranta erecta* L.) against *Lumbricus terrestris* (Earthworms) and *Ascaris lumbricoides* (Roundworms). Cavite National High School, Cavite City, Region IV-A CALABARZON

ABSTRACT

This study aims to provide an alternative source of parasiticide from *Premna odorata* (alagao), *Lantana camara* (kantutay) and *Duranta erecta* (duranta). Three setups were observed containing the plant extracts and controlled setup for *Lumbricus terrestris* (earthworms) and *Ascaris lumbricoides* (roundworms). Death time (in minutes) of the parasites at room temperature was recorded to determine the anti-parasitic activity of the plant extracts. Mean of the data obtained were used to determine the most effective plant extract. Results revealed that all of the plant extracts were active against earthworms and roundworms. The death time of earthworms in alagao extract ranges from 0.30 to 0.45 minutes and 32.52 to 47.48 minutes for roundworms. For kantutay extract, the death time of earthworms spans from 0.63 to 0.72 minutes and for roundworms, it ranges from 56.16 to 77.67 minutes. Duranta plant extract also showed anti-parasitic activity and killed the earthworms from 0.77 to 0.95 minutes and ranges from 60.53 to 75.7 minutes for roundworms. Controlled setups have not showed any effect on the parasites because both earthworms and roundworms were still alive even after the course of the experiment. In summary, alagao plant extract is the most active against earthworms with an average death time of 0.39 minutes and for roundworms with an average of 40.54 minutes. Results from this study can serve as a basis to provide an alternative source of parasiticide from local plants and help to find a more effective way of treating helminthic infections.

INTRODUCTION

Background of the Study

Parasitic infections become a huge threat to public health and increasing the rate of malnutrition nowadays (Bundy, 1994). Studies show that 7 out of 10 school children, approximately 67% of all school-age children are infected with intestinal worms in the Philippines (Asian Hospital, 2002).

Roundworm (*Nematoda*) is known to be one of the most common types of intestinal worms. It can be found on the dirt and can be transmitted through hands or skin (University of Maryland Medical Center, 2014). Roundworm infection causes malnutrition, decreases physical activity, and causes poor physical and mental development (Easton, 1999).

Drugs have been invented to combat health and nutritional impact of parasitic infections. But then, even the most common anthelmintic drug, piperazine salt showed side effects such as nausea and intestinal disturbances (Liu and Weller, 1996). Intestinal worms also become resistant to currently available anthelmintic drugs, causing a larger problem in treating helminthic infections (Tagbota and Townson, 2001). Because of this, people rely on traditional medical remedies and are known to use several plant-derived preparations to cure helminthic infections. (Satyavati, 1990).

Alagao (*Premna odorata* B.) is considered as one of the most effective medicinal herb in the Philippines. In recent studies, it is used to evaluate parasites and showed anti-parasitic activity. (Healing Wonders of Philippine Medicinal Plants, 2009; Philippine Medicinal Plants, 2014). Ethanolic extract of leaves of kantutay (*Lantana camara* L.) showed significant anthelmintic activity on earthworms according to the study of Patel, Kumar, Deviprasad, Deepika, Qureshi and Shamim (2011). Another plant under the Verbenaceae family, duranta (*Duranta erecta* L.) is also locally available here in Cavite City but it is not yet proven to have anti-parasitic activity.

With these prior knowledge, local plants here in the Philippines under the family Verbenaceae can aid this type of helminthic infection as it showed anti-parasitic activity. This study aims to provide an alternative source of parasiticide derived from local plant materials to be able to help people in finding a more effective way of treating parasitic infections.

Statement of the Problem

This study primarily aims to provide an alternative source of parasiticide derived from alagao (*Premna odorata* B.), duranta (*Duranta erecta* L.), and kantutay (*Lantana camara* L.). Specifically, this study aims to:

1. get plant extracts from alagao (*Premna odorata* B.), kantutay (*Lantana camara* L.) and duranta (*Duranta erecta* L.);

2. test anti-parasitic effect of the plant extracts to earthworms and roundworms by determining the death time (in minutes) of the parasites in each plant extract at room temperature; and
3. determine the most effective plant extract in terms of its anti-parasitic activity.

Significance of the Study

Determining the effect of anti-parasitic property of plants will be a great help to the society. Moreover, the following will be the beneficiaries of this research project:

Pharmaceutical Scientists. Extracts from different plants used in the study can be developed by the pharmaceutical scientists into drugs. They are capable of determining if these extracts are safe to humans. Once it is proved, the scientists can further develop them into a new anti-parasitic drug that can be available to consumers.

Pharmaceutical Companies. With sufficient knowledge and sources of plant extracts, these companies can help the society because they are the ones actually investing finances to develop these anti-parasitic drugs.

Households. Once the plants extracts are proven by scientists to be safe for humans, it is possible for mothers to use these extracts as a deworming drug for their child.

School Clinics. The drugs developed by the scientists can also be used as first aid and alternative deworming drug for the students and teachers, thereby; they can afford it in a lower cost.

Researchers. This investigatory project can give idea and can serve as a prior knowledge for future researchers. They can widen the scope of this research and help the society by discovering other plants with anti-parasitic properties in their locality.

Scopes and Limitations

This study was conducted to provide an alternative source of parasiticide derived from alagao (*Premna odorata* B.), kantutay (*Lantana camara* L.) and duranta (*Duranta erecta* L.) plant extracts. Earthworms were gathered from a garden and cultured in a large container while roundworms were taken from intestines of a pig from a slaughterhouse. Plant materials used in this study were collected from San Antonio, Cavite City. This research will only focus on the effect of the plant extracts on parasites. Testing for the effectiveness of the plant extracts in treating parasites in humans was beyond the scope of this study. The results of this study will be based from the recorded average death time (in minutes) of both earthworms and roundworms subjected in each plant extract at room temperature.

The creation and testing of the plant-derived extracts were done from June to August, 2015 at Cavite National High School.

METHODOLOGY

Materials

- Leaves of alagao (*Premna odorata* B.), kantutay (*Lantana camara* L.) and duranta (*Duranta erecta* L.)
- 12 earthworms (*Lumbricus terrestris*)
- 12 roundworms (*Ascaris lumbricoides*)
- 8 petri dishes
- mortar and pestle
- 720 mL of 70% ethanol
- 3 evaporating dishes
- rubber gloves
- timer/stopwatch
- thermometer
- beaker (10 mL)
- tap water

Preparation and Creation of Plant Extracts

In determining the effect of plant extracts from alagao (*Premna odorata* B.), kantutay (*Lantana camara* L.), and duranta (*Duranta erecta* L.) in earthworms and roundworms, four setups were prepared. The worms in the first three setups were subjected to each plant extract while the fourth setup was untreated.

Following Szewczuk, Mongelli and Pomilio's (2003) method, the leaves of *Premna odorata*, *Lantana camara*, and *Duranta erecta* were shade dried and crushed using mortar and pestle. Crushed leaves (40 g each plant extract) were extracted using

70% ethanol (240 ml each) and left uncovered for 24 hours for the ethanol to evaporate. The remaining solutions were filtered using a cloth and then squeezed to get the plant extracts. The plant extracts were stored under room temperature.



(a) leaves of alagao



(b) leaves of lantana



(c) leaves of duranta



(d) mortar and pestle



(e) 3 containers



(f) weighing scale



(g) strainer and cloth



(h) beakers



(i) 720 mL of ethanol

Figure 1. Materials used in preparing the plant extracts

The plant extracts prepared were used to test its effect in earthworms and roundworms. A total of 12 earthworms and 12 roundworms were used in the assessment of the anti-parasitic activity of each plant extract. Earthworms were collected from a garden in Sta. Cruz, Cavite City and then cultured based on Footprints Environmental Centre's (2008) method of culturing earthworms. Roundworms were collected from intestines of a pig from a slaughterhouse in Cavite City and stored in a glass bottle with water.

Treatment of the Earthworms and Roundworms with each Plant Extract

A total of 15 mL from each plant extract were obtained from the extraction process. Table 1 summarizes the contents of each setup.

Table 1. Overview of the treatments done

Setup	Helminth	Amount of extract applied (mL)	Treatment
A - 1	Earthworm	3	<i>Premna odorata</i> plant extract
A - 2	Roundworm	3	<i>Premna odorata</i> plant extract
B - 1	Earthworm	3	<i>Lantana camara</i> plant extract
B - 2	Roundworm	3	<i>Lantana camara</i> plant extract
C - 1	Earthworm	3	<i>Duranta erecta</i> plant extract
C - 2	Roundworm	3	<i>Duranta erecta</i> plant extract
D - 1	Earthworm	3	Tap water
D - 2	Roundworm	3	Tap water

Random assignment of earthworms was done in each setup. Petri dishes were labelled in A, B, C and D for each setup. The same assignment was also done in setups with roundworms.

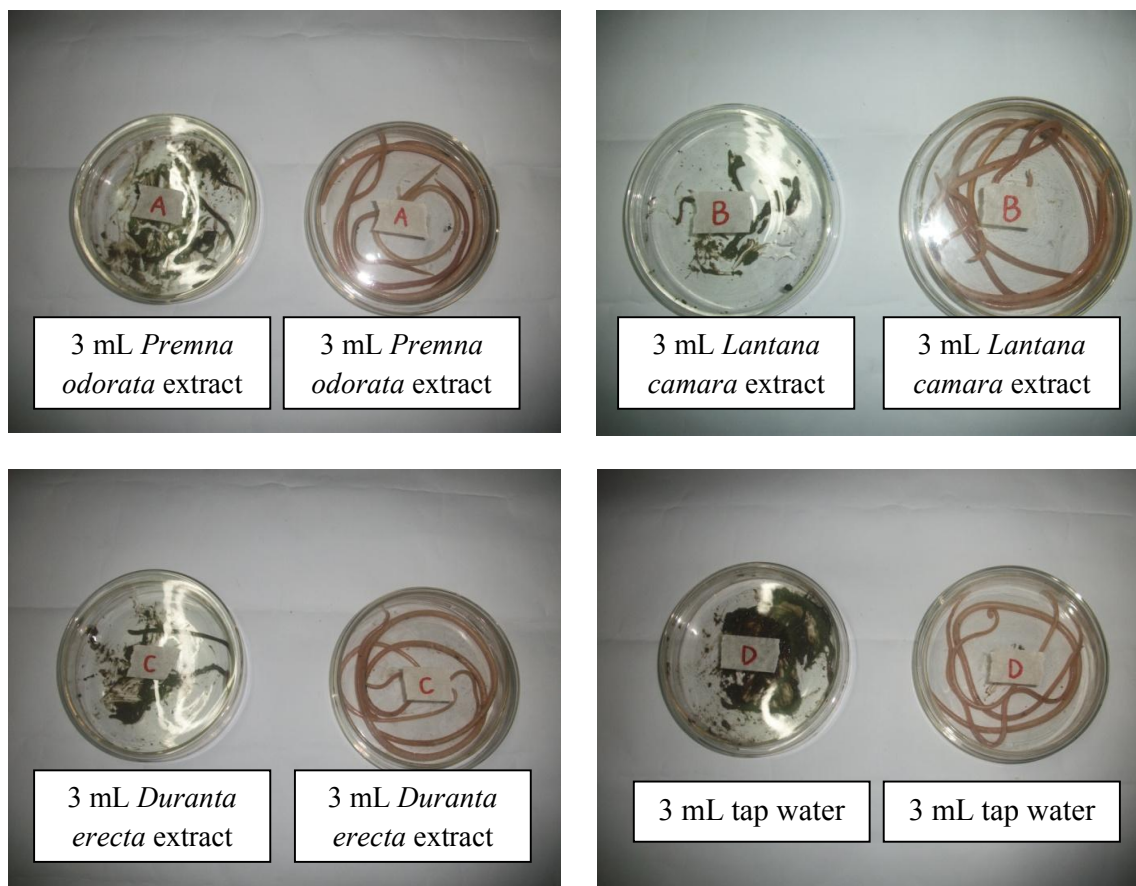


Figure 2. Experimental setups used in the testing the anti-parasitic activity of plant extracts

Three setups were observed in the course of the experimentation. While one setup remained controlled and untreated. Each resulting solutions were poured into petri dishes. Three earthworms and roundworms were placed in separate petri dishes for each plant extract and control group. The death time (in minutes) of earthworms and roundworms were recorded at room temperature. Worms were transferred into a beaker containing hot water at 50°C to ascertain the death of the worms (Szewczuk, Mongelli & Pomilio, 2003).

Data Analysis

Mean was used for all the obtained data from the three trials conducted. Mean scores were gathered by determining the death time (in minutes) of the helminthes using a stop watch. Figure 3 summarizes the methodology used in this study.

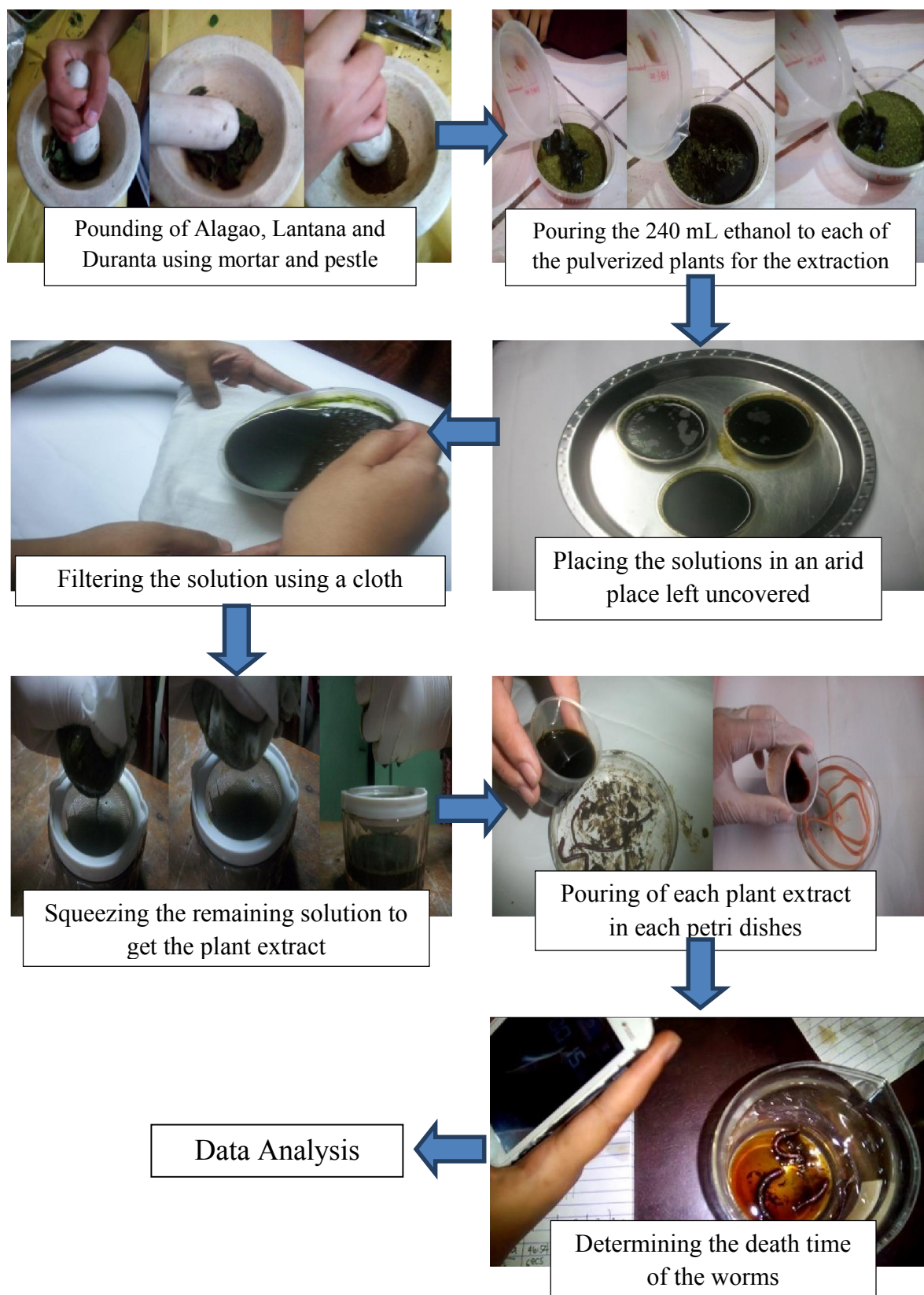


Figure 3. Summary of the methodology used in this study

RESULTS AND DISCUSSION

Alternative Parasiticide From the Three Plant Materials

Before getting the plant extracts, first, the leaves were collected from different local areas in Cavite City. They were shade-dried and pounded until pulverized. Ethanolic extraction was done for 24 hours. After 24 hours, the plant extracts were collected by squeezing and straining the mixture of the pulverized plant and ethanol. Figure 4, 5 and 6 shows the extracts obtained from the plant materials.



Figure 4. *Premna odorata* plant extract



Figure 5. *Lantana camara* plant extract



Figure 6. *Duranta erecta* plant extract

The figures above show the physical appearance of the three plant extracts. Figure 4 shows alagao (*Premna odorata* B.) plant extract which is dark green in color, has a strong and pungent smell. Kantutay (*Lantana camara* L.), plant extract in Figure 5, is green in color but lighter than *Premna* extract and has a sweet smell as well as duranta (*Duranta erecta* L.) plant extract, in Figure 6, which has a brownish-green color.

Effect of the Plant Extracts to Earthworms and Roundworms

Three earthworms and roundworms were placed in separate Petri dishes for each setup; three mL of each plant extract were later poured. A timer was set to determine the death time of the earthworms and roundworms when subjected to plant extracts at room temperature. Worms were then placed on a container with warm water to ascertain its death. Table 2 shows the death time of earthworms while the results in Table 3 shows the death time of roundworms in each setup.

Table 2. Death time (in minutes) of earthworms in each setup at room temperature

Treatments	Death time of earthworms (in minutes)		
	1	2	3
A <i>Premna odorata</i>	0.41	0.45	0.30
B <i>Lantana camara</i>	0.72	0.63	0.66
C <i>Duranta erecta</i>	0.77	0.88	0.95
D Control (water only)	>1	>1	>1

Results revealed that the earthworms were killed after subjecting it into the plant extracts. *Premna odorata* extract, the death time of worms ranges from 0.30 to 0.45 minutes for the three trials which is the fastest among the three. In setup B, with *Lantana camara* plant extract also showed anti-parasitic activity. The death time of earthworms ranges from 0.63 to 0.72 minutes in three trials which is slower compared to the effect of *Premna odorata* extract. While for the setups containing *Duranta erecta* plant extracts, the death time of earthworms ranges from 0.77 to 0.95 minutes and is much slower than

the first two setups. On the other hand, the controlled setups containing water in all trials have not showed any effect on the worms. Death time of roundworms in each setup is shown on the table below (Table 3).

Table 3. Death time (in minutes) of roundworms in each setup at room temperature

Treatments	Death time of roundworms (in minutes)		
	1	2	3
A <i>Premna odorata</i>	32.52	41.61	47.48
B <i>Lantana camara</i>	56.15	72.98	77.67
C <i>Duranta erecta</i>	60.53	65.8	75.7
D Control (water only)	>120	>120	>120

Table 3 shows the effect of the plant extracts in roundworms at room temperature for the three trials. *P. odorata* extract killed the roundworms that spans from 32.52 to 47.48 minutes, which suggests that it is active against roundworms. For setups B, containing *L. camara* plant extract, the death time of roundworms ranges from 56.16 to 77.67 minutes. The effect of the plant extract is much slower than of the *P. odorata* extract. While for the setups C, death time of roundworms ranges from 60.53 to 75.7 minutes which also signifies that *D. erecta* is effective in killing the parasite. Results from this setup showed that it kills roundworms faster than *Lantana camara* plant extract but slower than *Premna odorata* extract. Setups D for all trials have not showed any significant effect because the worms were still alive even after 120 minutes. The death time of earthworms (Figure 7) and roundworms (Figure 8) in different setups are shown below.

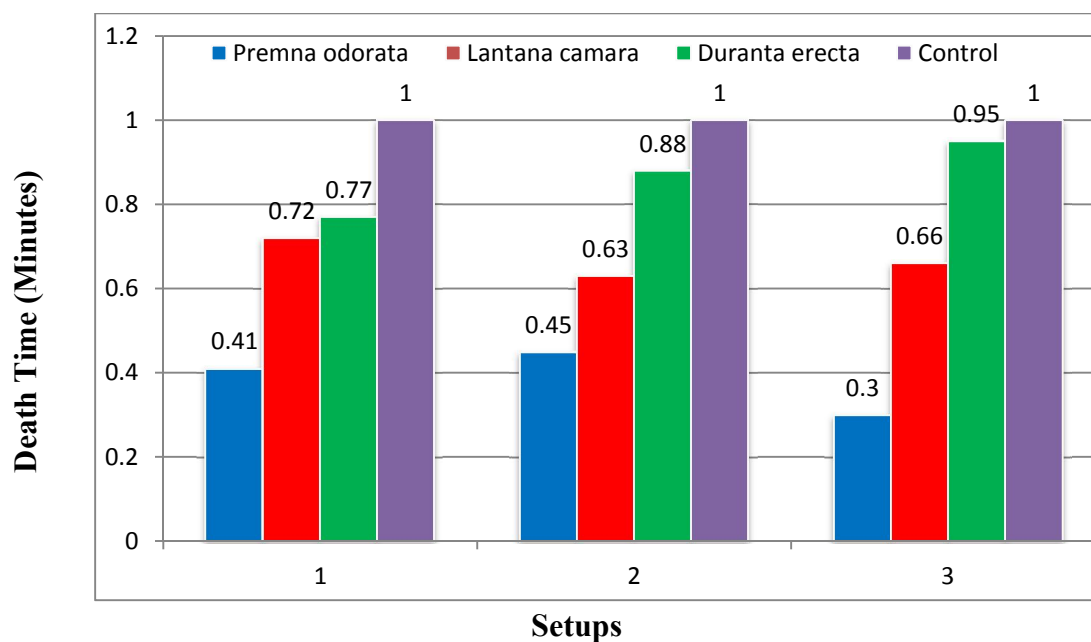


Figure 7. Death time of earthworms treated with *P. odorata*, *L. camara* and *D. erecta*

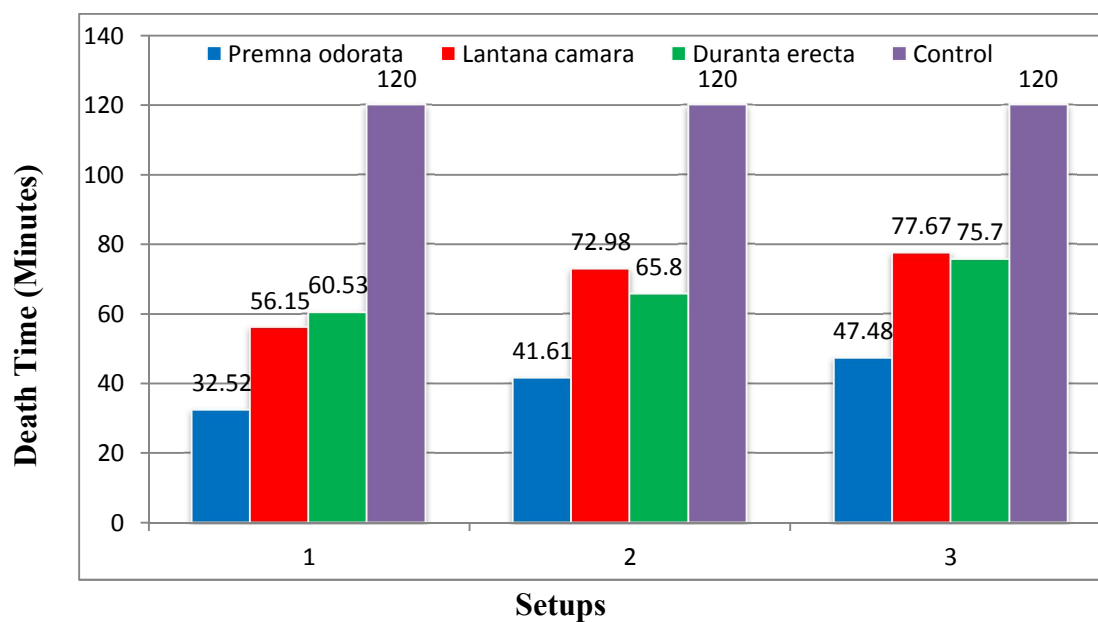


Figure 8. Death time of roundworms treated with *P. Odorata*, *L. camara* and *D. erecta*

The figures above show the death time of earthworms (Figure 7) and roundworms (Figure 8) in *Premna*, *Lantana* and *Duranta* plant extracts. Results above suggest that the plant extracts were active against earthworms and roundworms.

Plants from Verbenaceae family are well known for their uses in the traditional medicinal systems of various countries. Previous studies cited the feasibility of the plant extracts from this family to eliminate parasites but local species of these plants in the Philippines were not yet tested. They are reported to contain bio-active phytochemicals with important pharmacological effects (Rahmatullah, M., Jahan, R., Azam, F.S., Hossah, S., Molik, M. & Rahman, T., 2011). To strengthen the claim, 18 medicinal plants in New Caledonia have been studied and it is proven that *Premna odorata* can eliminate several parasites (Philippine Medicinal Plants, 2014). Ethanolic extracts of leaves of *Lantana camara*, on the other hand, was evaluated and showed significant anthelmintic activity according to the study of Patel, Kumar, Quereshi, Deviprasad S. P. and Deepika S. in 2011. *Duranta erecta* had not been studied yet in other researches to have anti- parasitic property. And since the plants are from same family, it had been tested and proven in this study that it can also eliminate parasites.

These findings support the claim of using *P. odorata*, *L. camara* and *D. erecta* plant extracts as an alternative source of parasiticide.

Most Effective Plant Extract

Determining the most effective plant extract is based on the fastest average death time of earthworms and roundworms in each set up using the three plant extracts.

In summary, *Premna odorata* plant extract showed the fastest time to kill earthworms at an average of 0.39 minutes and roundworms with an average of 40.54 minutes. Table 4 below shows the summarized data with the means of the death time of worms subjected in each plant extract.

Table 4. Death time of earthworms and roundworms in each plant extract

Treatments	Death Time of Worms (in minutes)							
	EARTHWORMS				ROUNDWORMS			
	1	2	3	Average	1	2	3	Average
A <i>Premna odorata</i>	0.41	0.45	0.30	0.39	32.52	41.61	47.48	40.54
B <i>Lantana camara</i>	0.72	0.63	0.66	0.67	56.15	72.98	77.67	68.93
C <i>Duranta erecta</i>	0.77	0.88	0.95	0.87	60.53	65.8	75.7	67.24
D Control (water only)	>1	>1	>1	>1	>120	>120	>120	>120

Table 4 shows the response of earthworms and roundworms in the three different plant extracts. From the testing done, ethanolic extracts of alagao (*Premna odorata*), kantutay (*Lantana camara*) and duranta (*Duranta erecta*) have proven to eliminate parasites such as earthworms and roundworms.

Means of the death time of the worms in the different setups were obtained to determine the most effective plant extract that can kill the parasites for the shortest period of time (Szewczuk, Mongelli & Pomillo, 2003). The following figures show the average death time of earthworms (Figure 9) and roundworms (Figure 10) at room temperature.

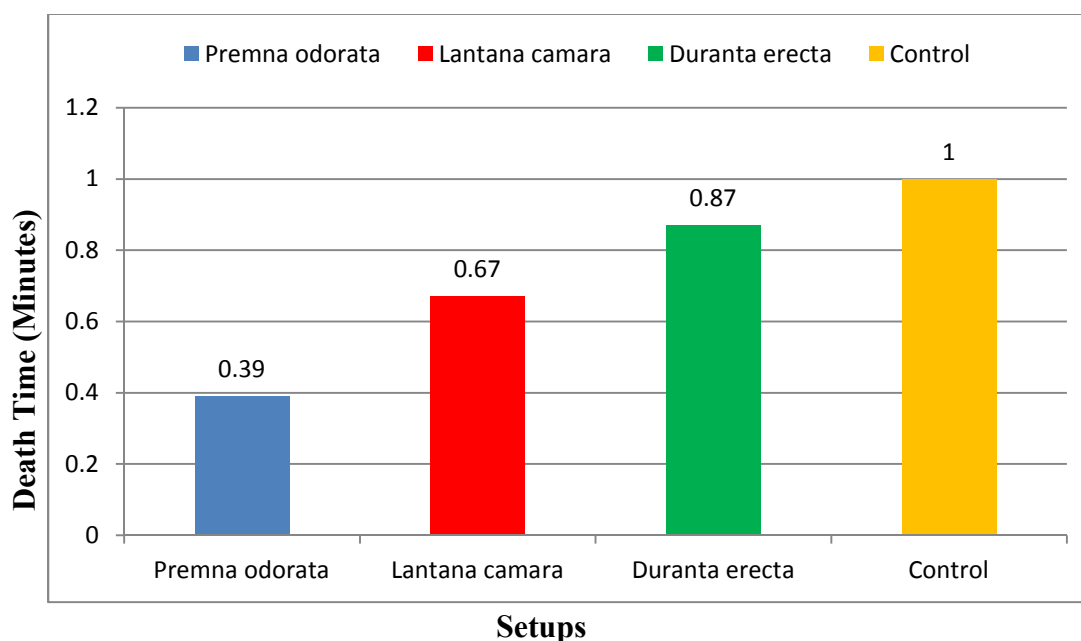


Figure 9. Average death time of earthworms, treated and untreated

The fastest recorded average death time for earthworms is 0.39 minutes for setup A which contains the *P. odorata* plant extract which can be considered as the most active against earthworms. It is followed by setup B, with *L. camara* plant extract which killed the worms with an average of 0.67 minutes. Results from setup C containing *D. erecta* plant extract also showed anti-parasitic activity but the average death time of the earthworms is much slower than of the first two setups. While setup D, which controlled with water have not shown activity against the worms.

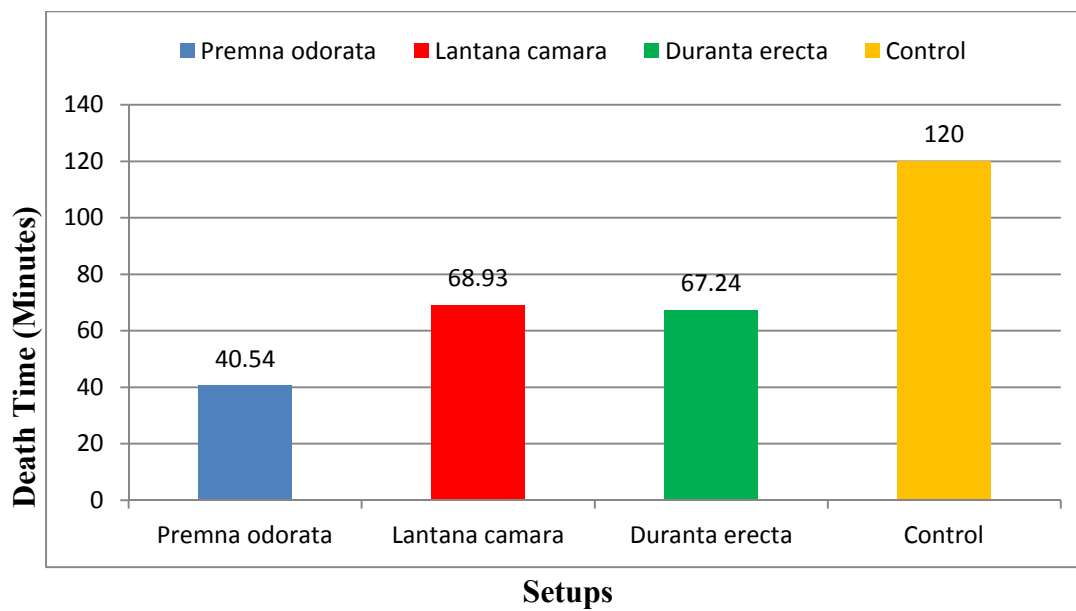


Figure 10. Average Death Time of Earthworms, Treated and Untreated

On the other hand, for roundworms, the fastest recorded average death time is 40.54 minutes for setup A with *P. odorata* plant extract. While *D. erecta* extract comes next after the first setup with an average death time for roundworms of 67.24 minutes. And *L. camara* plant extract was the least active against roundworms which has an average of 68.93 minutes but just a little bit slower than of the setup C. For setup D, which is controlled, have not shown any significant effect on roundworms since the parasites were still alive even after 120 minutes.

SUMMARY, CONCLUSION AND RECOMMENDATIONS

This study primarily aims to provide an alternative source of parasiticide derived from alagao (*Prema odorata* B.), durante (*Duranta erecta* L.), and kantutay (*Lantana camara* L.). Plant extracts were gathered by using ethanolic extraction of the leaves of the plants. Extracts were later poured in petri dishes for each setup and one setup is controlled with water only. Determining the death time of earthworms (*Lumbricus terrestris*) and roundworms (*Ascaris lumbricoides*) in each plant extract were based from Szewczuk, Mongelli and Pomilio's (2003) method of determining the activity of each extract against the parasites. Mean was used for all the obtained data from the three trials.

Premna odorata extract killed the earthworms with their death time ranging from 0.30 to 0.45 minutes. While death time of earthworms in setups containing *Lantana camara* plant extract ranges from 0.63 to 0.72 minutes which is slower than the effect of *Premna* extract. And lastly, for setups with *Duranta erecta* plant extract, death time of earthworms spans from 0.77 to 0.95 minutes. This suggests that *Duranta* extract exhibits anti parasitic activity against earthworms but the death time of worms were much slower than the first two plant extracts. Controlled setups have not shown effect on the earthworms because the worms were still alive even after one minute.

On the other hand, roundworms were also killed by all the plant extracts which strengthens the claim of these plants having anti parasitic activity. For *Premna odorata* plant extract, roundworms were killed with death time ranging from 32.52 to 47.48

minutes. Setups containing *Lantana camara* extract were also active against roundworms. The death time of the parasites spans from 56.16 to 77.67 minutes. This shows that *P. odorata* extract have killed the roundworms faster than *L. camara* extract. While *Duranta erecta* plant extract also exhibited activity against the roundworms with the death time of the parasites ranging from 60.53 to 75.7 minutes. The effect of this plant extract is much slower than of the *Premna* extract but more active against roundworms than *Lantana* extract. As for the controlled setup, roundworms were still alive even after 120 minutes which suggests that it does not take any effect on the parasites.

The plant extract with the fastest average death time recorded is the *Premna odorata* with an average of 0.39 minutes for earthworms and roundworms with an average of 40.54 minutes. *Lantana camara*, on the other hand, has the faster effect to kill the earthworms next to *Premna* extract with an average of 0.67 minutes but slower than *Duranta* extract to kill roundworms with an average of 68.93 minutes. While *Duranta* extract was also active for both parasites but it killed the earthworms slower than *Premna* and *Lantana* with an average death time of 0.87 minutes. However, it killed the roundworms faster than the *Lantana* extract with an average recorded death time of 67.24 minutes.

In summary, all of the plant extracts were active and showed anti parasitic activity against earthworms and roundworms. This proves that *Premna odorata*, *Duranta erecta*, and *Lantana camara* from the Verbenaceae family can provide an alternative source of parasiticide as they are proven to have anti parasitic property. As showed from the

experiment done, *Premna odorata* plant extract has the fastest time to kill parasites among the three plants. Therefore, it is considered to be the most effective plant extract in terms of its anti parasitic activity.

Since it is proven that *Premna odorata*, *Lantana camara* and *Duranta erecta* exhibit anti parasitic property, the researchers would like to suggest the following recommendations:

1. carry out phytochemical analysis to determine the composition and properties present in the extracts of *P. odorata*, *L. camara* and *D. erecta* that makes it active against earthworms and roundworms;
2. test if these plant extracts are safe to use by humans;
3. determine the anti parasitic activity of the plant extracts to other types of parasite;
4. find other methods of extraction in getting the extracts of the plants; and
5. know other species from the Verbenaceae family that can be also a source of parasiticide.

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