In-vitro anti-helminthic activity of crude hexane extract of *Raphanus raphanistrum subsp. sativus* (L.) Domin (Radish) root in *Ascaris suum*

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Abstract

Soil-transmitted helminthiasis remain a major public health problem affecting hundreds of millions of people, particularly in tropical developing country. According to the World Health Organization, about 10 percent of the developing world is infected with intestinal worms. Majority of the world's population depends on traditional medical remedies because of the limited availability and affordability of pharmaceutical medicines. Parasitic worms or helminthes, such as *Ascaris lumbricoides* causing ascariasis is one of the most common parasitic infection. This parasite is characteristically related to *Ascaris suum* causing weakness and diseases to the host through disrupting their nutrient absorption. This research determined the antihelminthic activity of the crude hexane extract of *Raphanus raphanistrum subsp. Sativus* (L.). Domin (radish) in an *Ascaris suum*. 1200 µg/ml, 1400 µg/ml, 1600 µg/ml, 1800 µg/ml and 2000 µg/ml concentrations of the extract were made and each concentrations was tested to fifteen *A. suum*. Mortality of the worms were observed after 48 hours. After the statistical analysis, it was found out that *R. raphanistrum* root extract exhibited the most toxic effect in the concentration of 2000 µg/ml with a percent mortality of 53.33 percent. It was also observed that as the concentration of the extract increases, the number of dead worms also increases. The result of the study indicates that the crude hexane root extract of *R. rapahanistrum* has an antihelminthic effect against *Ascaris suum.*

Keywords: *Raphanus raphanistrum,* anti-helminthic, pyrantel embonate, *Ascaris suum*

INTRODUCTION

Soil-transmitted helminthiasis (STH) is one of the most prevalent parasitic infections in impoverished communities, remarkably in children (Mationg et al., 2017). The infections with STH are presumed to affect two billion people globally, equating to approximately one-third of the world's population (Magalhães et al., 2015). This disease is commonly caused by *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm) and *Ancylostoma duodenale* and *Necator americanus* (hookworms) (Gabrielli, Montressor, & Savioli, 2014). Among the different age group, preschool-aged and school-aged children have the highest prevalence of STH infections (Sakari, Mbugua, & Mkoji, 2017). A baseline nationwide parasitological survey conducted in 2004 revealed that 66.0% of preschool-aged children aged 12-71 months old were infected with soil-transmitted helminthiasis (STH) (Belizario, Totanes, De Leon, Ciro, & Lumapao, 2013). Among the STH infections, the intestinal nematode, *Ascaris lumbricoides* is considered to be one of the most common cause of helminthiasis (Riess et al., 2013). Approximately twelve to twenty-two million people worldwide are infected with ascariasis (Kanneganti, Makker, & Remy, 2013). Infection with *Ascaris lumbricoides* may lead to diminish physical strength, growth hindrance, and respiratory and gastrointestinal problems (Schule et al., 2014). Also, *Ascaris lumbricoides* excretes neurotoxin that generate spasticity which may lead to intestinal obstruction, and intestinal inflammation (Andrade et al., 2015).

Ascaris suum, a parasitic nematode that infects pigs which is closely identical to *A. lumbricoides* (Masure et al., 2013). Due to its similar disease progression, pig roundworms can be chosen for comparison with the human roundworms (Siggers et al., 2008). There were studies about *Ascaris lumbricoides* and *Ascaris suum* being a single species (Leles, Gardner, Reinhard, Iñiguez, & Araujo, 2012). Through the analysis of the microRNA of *Ascaris suum* and *Ascaris lumbdicoides,* it seems to be impossible to differentiate them even by using additional molecular targets of genotyping (Liu et al., 2012). This is simply because these differences do not exist (Alves, Conceicão, & Leles, 2016). Demonstration of experimental cross-infections determine that *Ascaris suum* can infect humans and that *Ascaris lumbricoides* can infect pigs, with host preference in the efficiency of infection establishment (Betson, Nejsum, Bendall, & Stothard, 2014).

The main challenges in determining treatment are the variability in preferred drug of choice and dose for specific worm infestations, as well as formulation and acquisition concerns (Lloyd, Honey, John, & Condren, 2014). Elimination of adult worms from the gastrointestinal tract is the main goal of

treatment for STH infections. Mebendazole and albendazole are the most frequently used drugs to treat these infections; pyrantel embonate and levamisole can be used as an alternative (Bethony et al., 2006). Pyrantel is broadly used in Southeast Asia and Latin America for various helminth control programs (Albonico et al., 2008). Pyrantel embonate releases acethylcholine and inhibits cholinesterase leading to paralysis of helminths (Lloyd et al., 2014). Though current anti-helminthic drugs on the market demonstrated high effectiveness, side effects are still determined in clinical setting. Different side effects such as gastrointestinal upset, headache, dizziness, hypersensitivity, alopecia, pancytopenia, leukopenia and elevation of liver enzymes had been reported in some clinical literatures (Gulani, Nagpal, Osmond, & Sachdev, 2007). Thus, studies are being rendered to think of new substances from plants that are safe and tended for humans, acting as anti-helminthic which have the best source of bioactive substances capable of killing helminths (Nunomura et al., 2006).

Figure 1. *R.raphanistrum* (A) whole plant (B) Root Crop

Raphanus raphanistrum subsp. sativus (L.) Domin (radish) is an ubiquitous vegetable, mainly because it can tolerate a wide range of climates. Due to its availability, it is used as a food product all over the world (Lim, 2015). It belongs to the Brassicaceae family which includes other edible vegetables such as cauliflower, cabbage and broccoli. Vegetables from genus Brassica are proven to be rich in sulphur-containing glucosides called glucosinolates which account for the bitter flavor and sulphurous aroma of Brassica and other Crusiferae (Tedeschi et al., 2011). The main bioactive compounds found in *R. raphanistrum* are glucosinolates and isothiocyanates (Baenas et al., 2016). Some studies have proven that glucosinolates have anti-tumor and anti-parasitic activities (Chaskar, Bandgar, Modhave, Patil, & Yewale, 2009). Glucosinolates are known to possess anti-helmintic properties (Dhiman, Prashar, Kalia, & Gill, 2015).

There were studies about various medicinal properties of *R. raphanistrum subsp. sativus* (L.) Domin (radish). Fresh *R. raphanistrum* has been reported to magnify protein digestibility. Roots, flowers, and pods of the plant exhibits anti-microbial activity against gram-positive bacteria like *Staphylococcus aureus* and *Bacillus subtilis*. Its hypoglycemic effect was also observed on induced diabetic rat models (Shetty, Kamath, Bhat, Hegde, & Shabaraya, 2011). Its roots have been found out to have anti-diabetic effect found in unprocessed extract which contains antioxidant enzyme activities and L-tryptophan (Banihani, 2017). Some studies also proved the effectiveness of anti-helmintic medicinal plants on *Ascaris suum* such as shea (Ramsay, Williams, Thamsborg, & Mueller-Harvey, 2016), and mahogany (Rabina et al., 2017).

After a thorough research of studies related to *R. raphanistrum,* the researchers found out that currently, there are no published studies regarding the anti-helminthic activity of *R. raphanistrum root extract* against *A. suum.* The aim of this study is to determine the anti-helminthic activity of *R. raphanistrum* root extract on an ascaris model and determine the concentration which exhibits the most toxic effect. If proven to be significantly effective, this study can contribute to lessen cases of soil transmitted helminthiasis and to provide an affordable treatment to people with STH.

MATERIALS AND METHOD

Plant Material

R. raphanistrum roots were freshly collected in San Andres, Malvar Batangas. Taxonomic identification and authentication were performed at University of the Philippines, Los Baños.

Preparation of Plant Extracts

Frozen *R. raphanistrum* root was pulverized. It was placed in a sealed, glass container and allowed to stand at room temperature for three hours to effect enzymatic reaction. Three hundred milliliters of hexane were added and extraction was carried out for one hour with gentle stirring. The solvent was removed after by centrifugation. Then, another three hundred milliliters of hexane were added to the residue, and the extraction and centrifugation were carried out in the same manner. The residue was concentrated under reduced pressure by means of an evaporator to remove the remaining hexane the remaining hexane and the percentage yield was calculated. (Murata, Okunishi, Ugai, & Kobayashi, 2003).

Phytochemical Screening (Gupta & Dhawan, 2016)
Test for saponins: Ten mg of the *R. raphanistrum* root extract was diluted with twenty mL of distilled water. The test tubes were then shaken for 15 minutes. Formation of foam on top of the test tube shows the presence of saponins.

Test for tannins: Ten mg of the *R. raphanistrum* root extract was dissolved in 45% of ethanol. The test tubes were then boiled for 5 min and 1 mL of 15% ferric chloride solution was added. The appearance of greenish to black color confirms the presence of tannns.

Test for flavonoids: Few drops of diluted NaOH was added to ten mg of *R. raphanistrum* root extract. The appearance of yellow colour which disappears or become colorless after adding few drops of diluted H_2SO_4 confirms the presence of flavonoids in the plant extract.

Test for steroids: Ten mg of the *R. raphanistrum* root extract was taken and 1 mL of concentrated H_2SO_4 was added to the each by the side walls of the test tube. Appearance of dark reddish green color confirms the presence of steroids.

Test for alkaloids: Ten mg of the *R. raphanistrum* root extract dissolved in 2 mL of Wagner's reagent. The appearance of reddish brown colored precipitate confirms the presence of alkaloids.

Test Organisms

A. suum (pig roundworm) is used for the evaluation of the antihelminthic activity of the extract using a commercial drug, pyrantel embonate (Combantrin) for comparison. The parasites were collected from Lipa City Slaughterhouse and were confirmed by a parasitologist from Research Institute for Tropical Medicine (RITM) in Alabang, Makati City.

In Vitro Preparation

Baldwin and Moyle Solution was used as the medium to sustain the pig roundworm in vitro. The solution appears to mimic the conditions in the porcine intestine. The composition of Baldwin and Moyle Solution is as follows: 151 grams of NaCl (sodium chloride), 28.4 grams of KCl (potassium chloride), 13.4 grams of $CaCl_2$ (calcium chloride), and 20.4 grams $MgCl_2$ (magnesium chloride) dissolved in two liters of distilled water. In another container, 27.2 grams of KH_2PO_4 (monopotassium phosphate) were added in one liter of distilled water. One volume of KH_2PO_4 solution and four volumes of NaCl-KCl-$CaCl_2$-$MgCl_2$

solution were mixed and diluted with 35 volumes of distilled water. The pH was adjusted to 7.2 with 1M sodium bicarbonate (Baldwin & Moyle, 1947).

Collection and Selection of the Test Organisms (Austria & Villapando, 2006).

The worms were transported to the laboratory in a wide-mouthed container containing Baldwin and Moyle Solution and were subjected to testing on the same day. Screening and sorting of the worms were done according to length and motility. Fifteen to forty-centimeter-long worms were used in the study which corresponded to mature *A. suum*. For motility, adult worms exhibiting vigorous undulating reaction to the heat sensitivity test was used.

Pig roundworms that met the criteria for length and motility were transferred to fresh Baldwin and Moyle solution and were incubated at 37°C for 30 minutes to establish the norms and body temperature in an artificial environment. The worms were again subjected to the heat sensitivity test after thirty minutes.

Heat Sensitivity Test

The worms were placed in a dissecting pan. The posterior end of the worm was touched with a heated wire loop. A reaction or movement suggested that the worms were still alive. To avoid possible variations in data gathering, only one person performed this procedure (Austria & Villapando, 2006).

Determination of Approximate Lethal Concentration (Austria & Villapando, 2006)

Ten rectangular microwaveable containers with *R. raphanistrum* root extract and another ten for pyrantel embonate were prepared.

During the preparation of the concentrations for the *R. raphanistrum* root extract, residues were separately dissolved with 10 milliliters of Baldwin and Moyle solution and were further diluted to make 300 milliliters each of 1200 µg/mL, 1400 µg/mL, 1600 µg/mL, 1800 µg/mL, 2000 µg/mL, 4000 µg/mL, 6000 µg/mL, 8000 µg/mL, 10000 µg/mL and 12000 µg/mL solutions, respectively using a volumetric flask.

The same procedure was done for the pyrantel embonate (positive control) but with different final concentrations of 70 µg/mL, 130 µg/mL, 200 µg/mL, 270 µg/mL, 330 µg/mL, 400 µg/mL, 470 µg/mL, 530 µg/mL, 600 µg/mL and 670 µg/mL, respectively. The ten varying concentrations were placed separately in each container. Five pig roundworms were placed in each prepared concentration and were examined after 48

hours for the final observation. The approximate lethal concentration was determined from the container that caused one death of the total number of worms.

Determination of Median Lethal Concentration (LC_{50}) (Austria & Villapando, 2006)

The determination of the approximate lethal concentration of *R. raphanistrum* root extract served as the basis for obtaining the median lethal concentration. Two higher concentrations and two lower concentrations than that of the approximate lethal concentration were prepared.

A total of 33 rectangular microwaveable containers were used, 15 for *R. raphanistrum* root extract, 15 for positive control, and 3 for negative control. In the negative control, 300 milliliters of Baldwin and Moyle solution were placed. These were observed for 48 hours.

Median lethal concentration was determined from the contained which caused 50 percent of the death of the total number of worms.

RESULTS AND DISCUSSION

Crude Hexane Extract

One hundred grams of *R. raphanistrum* root were used for the extraction which yielded to pale yellow, dry clumps of residue as seen in Figure 2 which is also similar with the study of Jakmatakul, Suttisri and Tengamnuay (2009) using hexane extract which gained 4.21% w/w. The extract obtained in the experiment weighed 7 grams and has a total percent yield of 7%.

Figure 2. *R. raphanistrum root extract*

Phytochemical Analysis

Table 1 shows a qualitative analysis of phytochemical constituent of the *R. raphanistum* root extract. The analysis revealed the presence of saponin, flavonoids and alkaloids. On the other hand, tannins and steroid were absent in the extract.

Table 1
Results of the Phytochemical Analysis of *R. raphanistrum* root extract

Phytochemical constituents	Results
Saponin	+
Tannins	-
Flavonoids	+
Steroid	-
Alkaloids	+

**(+) presence ; (-) absence*

Figure 3A shows the foam test for saponin, formation of persistent foam was observed indicating its presence. Figure 3B shows the Wagner's Test for the detection of alkaloid, a reddish brown precipitate was observed. In Alkaline Reagent Test, shown in Figure 3C and Figure 3D, disappearance of the intense yellow color was observed after addition of H_2SO_4 which indicates the presence of flavonoids. The test for tannins in Figure 3E and steroids in Figure 3F did not show any greenish to black and dark reddish green color appearance respectively which indicates that tannins and steroids were not present.

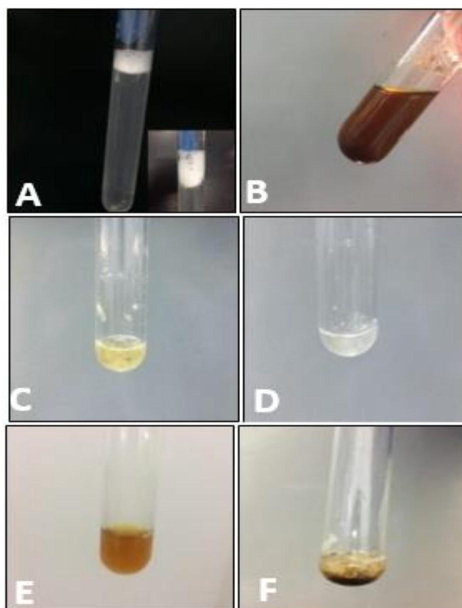

Figure 3. Results of the Phytochemical Analysis of *R. raphanistrum*

The presence of saponin, flavonoids, and alkaloids in *R. raphanistrum* agrees with the previous study of Bhat and Al-

Daihan (2014) regarding the phytochemical constituents of the plant. According to the study of Sparg, Light and Staden (2004), saponin was proven to have anti-parasitic effect. Flavonoids also exhibit the same effect to helminths, in line with the study of Saddique, Maimoona and Khalid (2013). The absence of tannins and steroids on the extract is in contrary with the phytochemical analysis of the plant done by Khamees (2017) which showed that tannins and steroids were present in *R. raphanistrum.*

Heat Sensitivity Test

All of the collected worms were subjected to heat sensitivity testing prior to the determination of the approximate lethal concentrations and median lethal concentrations. It is found out that upon introducing the heated wire loop, the worms exhibited lateral movement. According to a research conducted by Hunter (2017), the lateral movement indicates that the worms are alive.

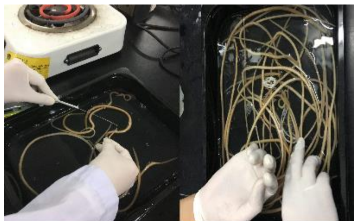

Figure 4. *A. suum* Heat Sensitivity Testing

Approximate Lethal Concentration

In the determination of the approximate lethal concentration of *R. raphanistrum* root extract, ten increasing concentrations were used with five *A. suum* in each concentration. As observed in Table 2, the two lowest concentrations (1200 µg/mL and 1400 µg/mL) revealed that they did not cause the death of any of the five worms. *R. raphanistrum* root extract produced toxic effect on one out of five *A. suum* in the concentrations of 1600, 1800 and 2000 µg/mL. At 4000 and 6000 µg/mL, a total of two out of five *A. suum* were killed. The highest concentrations which are 8000, 10000 and 12000 µg/mL gave the highest number of death of *A. suum*.

It is evident that as the concentration of the *R. raphanistrum* extract increases, the number of dead worms also increases. This is more likely due to the increased concentrations of the phytochemical substances on the *R. raphanistrum* root extract. According to the study of Sparg, Light and Staden (2004), saponin was proven to have anti-parasitic effect. Flavonoids also exhibit the same effect to helminths, in line with the study of Saddique, Maimoona and Khalid (2013).

Table 2
Approximate Lethal Concentration Of *R. raphanistrum* Root Extract against *A.suum*

Concentration µg/mL	Total no. of dead worms (N=5)
1200	0
1400	0
1600	1
1800	1
2000	1
4000	2
6000	2
8000	3
10000	3
12000	3

In the determination of the approximate lethal concentration of pyrantel embonate, ten increasing concentrations were used with five *A. suum* in each concentration. As what is observed in Table 3, the concentrations 70 µg/mL, 130 µg/mL, 200 µg/mL, 270 µg/mL, and 330 µg/mL of pyrantel embonate did not kill any *A. suum*. Pyrantel embonate exhibited toxic effect on one out of five *A. suum* at 400 µg/mL. In the concentration 470 µg/mL, two *A. suum* died. And in the concentrations of 530 and 600 µg/mL, three out of five *A. suum* died. The highest concentration, 670 µg/mL, exhibited the most toxic effect since four out of five *A. suum* died.

Table 3
Approximate Lethal Concentration of Pyrantel Embonate against *A. suum*

Concentration µg/mL	Total no. of dead *A. suum* (N=5)
70	0
130	0
200	0
270	0
330	0
400	1
470	2
530	3
600	3
670	4

It is also evident in Table 3 that as the concentration of pyrantel embonate increases, the number of dead *A. suum* also increases. The increased rate of mortality of the worms is most probably due to the highly effective anti-helminthic activity of pyrantel embonate. Pyrantel embonate induces the release of acetylcholine which inhibits the release of cholinesterase. This paralyzes the worms which later causes the death of the worms (Lloyd et al., 2014).

Median Lethal Concentration

For the determination of median lethal concentration, three trials were performed for each concentration of *R. raphanistrum* root extract containing 5 test organisms (total of 15 worms). Two lower concentrations (1200 µg/mL and 1400 µg/mL) and two higher concentrations (1800 µg/mL and 2000 µg/mL) of *R. raphanistrum* root extract were gathered from its approximate lethal concentration.

As observed in Table 4, the two lowest concentrations, 1200 and 1400 µg/mL, did not cause toxicity in any of the *A. suum.* At 1600, 1800 and 2000 µg/mL, four, five and eight *A. suum* died, respectively.

It is also revealed that the median lethal concentration of the *R. raphanistrum* root extract is most probably less than 2000 µg/mL since 53.33 percent of the worms died in this concentration. The increase in the mortality of the worms is more likely due to the presence of the main anti-helminthic component, glucosinolate (Dhiman, Prashar, Kalia, & Gill, 2015), and other phytochemicals such as saponin (Sparg, Light, & Staden, 2004), flavonoids (Saddique, Maimoona, & Khalid, 2013), and alkaloids, on the *R. raphanistrum* root extract.

Table 4
LC50 of the *R. raphanistrum* Root Extract against the *A. suum*

Concentration µg/ml	Total no. of dead *A. suum* (N=15)	Percentage mortality (%)
1200	0	0
1400	0	0
1600	4	26.67
1800	5	33.33
2000	8	53.33

Table 5 presents the multiple comparisons on the anti-helmintic effect of the *R. raphanistrum* root extract on varying

concentrations. When 1200 μg/mL was compared with 1400 μg/mL the resulting p-value is equal to 1.0, which shows there is no significant difference between the two concentrations. In Table 4, it is evident that both the 1200 μg/mL and 1400 μg/mL concentrations did not kill *A. suum*, which is why there is no significant difference between the two concentrations. On the other hand, when 1200 μg/mL was compared with 1600 μg/mL, 1800 μg/mL, and 2000 μg/mL, all the p-values obtained were greater than the 0.05 level of significance. In Table 4, it is evident that no *A. suum* was killed at 1200 μg/mL and that the concentrations 1600 μg/mL, 1800 μg/mL, and 2000 μg/mL were able to kill *A. suum*. When 1400 μg/mL was compared with 1200 μg/mL the resulting p-value is equal to 1.0, which indicates that there is no significant difference between the two concentrations. In Table 4, it is evident that both the 1400 μg/mL and 1200 μg/mL concentrations did not kill *A. suum*, which is why there is no significant difference between the two concentrations. On the other hand, when 1400 μg/mL was compared with 1600 μg/mL, 1800 μg/mL, and 2000 μg/mL, all the p-values obtained were greater than the 0.05 level of significance. In Table 4, it is revealed that no *A. suum* was killed on 1400 μg/mL. However, at 1600 μg/mL, 1800 μg/mL, and 2000 μg/mL, *A. suum* were killed. This shows that there is a significant difference between 1400 μg/mL and 1600 μg/mL, 1800 μg/mL, and 2000 μg/mL. When 1600 μg/mL was compared with 1800 μg/mL the resulting p-value is equal to 1.0, which shows there is no significant difference between the two concentrations. Table 4 shows that four *A.suum* were killed at 1600 μg/mL and five *A. suum* were killed at 1800 μg/mL. It is evident that the two concentrations (1600 μg/mL and 1800 μg/mL) produced the same effect, which is likely why there is no significant difference between the two concentrations. However, when 1600 μg/mL was compared with 1200 μg/mL, 1400 μg/mL, and 2000 μg/mL, all the p-values obtained were greater than the 0.05 level of significance. Table 4 shows that both 1200 μg/mL and 1400 μg/mL did not kill *A. suum* but at 2000 μg/mL, eight *A. suum* were killed. When 1800 μg/mL was compared to 1600 μg/mL and 2000 μg/mL the resulting p-value is equal to 1.0, which shows that there is no significant difference between the concentrations. Table 4 shows that at 1800 μg/mL, five *A. suum* were killed, at 1600 μg/mL, four *A. suum* were killed, and at 2000 μg/mL, eight *A. suum* were killed. This shows that the anti-helminthic effect produced on these concentrations were almost similar, which is more likely why there is no significant difference between the two concentrations. On the other hand, when 1800 μg/mL was compared with 1200 μg/mL and 1400 μg/mL, the p-values obtained were greater than the 0.05 level of significance. There is significant difference

between these concentrations due to the fact that at both 1200 µg/mL and 1400 µg/mL, no *A. suum* were killed but five *A. suum* were killed at 1800 µg/mL. When 2000 µg/mL was compared with 1800 µg/mL the resulting p-value is equal to 1.0, which shows there is no significant difference between the two concentrations. Table 4 shows that at 2000 µg/mL, eight *A. suum* were killed while at 1800 µg/mL, five *A. suum* were killed. On the other hand, when 2000 µg/mL was compared to 1200 µg/mL, 1400 µg/mL, and 1600 µg/mL, all the p-values obtained were greater than the 0.05 level of significance. Table 4 shows that at both 1200 µg/mL and 1400 µg/mL, no *A. suum* were killed while at 2000 µg/mL, eight *A. suum* were killed. The results produced show that there is significant difference between these concentrations. This only implies that among the concentrations, 2000 µg/mL exhibited the most anti-helminthic effect but 1600 µg/mL is the minimum concentration which produces the same anti-helminthic effect.

Table 5
Multiple Comparison on the Concentration of *R. raphanistrum* Root Extract

Concentration	Concentration	p-value	Interpretation
1200 µg/mL	1400 µg/mL	1	Not Significant
	1600 µg/mL	0.029	Significant
	1800 µg/mL	0.007	Significant
	2000 µg/mL	<0.1	Significant
1400 µg/mL	1200 µg/mL	1	Not Significant
	1600 µg/mL	0.029	Significant
	1800 µg/mL	0.007	Significant
	2000 µg/mL	<0.1	Significant
1600 µg/mL	1200 µg/mL	0.29	Significant
	1400 µg/mL	0.29	Significant
	1800 µg/mL	0.886	Not Significant
	2000 µg/mL	0.029	Significant
1800 µg/mL	1200 µg/mL	0.007	Significant
	1400 µg/mL	0.007	Significant
	1600 µg/mL	0.886	Not Significant
	2000 µg/mL	0.117	Not Significant
2000 µg/mL	1200 µg/mL	<0.1	Significant
	1400 µg/mL	<0.1	Significant
	1600 µg/mL	0.029	Significant
	1800 µg/mL	0.117	Not Significant

*. The mean difference is significant at the 0.05 level

For the determination of median lethal concentration of pyrantel embonate (positive control), three trials were performed for each concentration of pyrantel embonate containing 5 test

organisms (total of 15 worms). Two lower concentrations (270 µg/mL and 330 µg/mL) and two higher concentrations (470 µg/mL and 530 µg/mL) than the approximate lethal concentration of 400 µg/mL were used.

As observed in Table 6, the two lowest concentrations, 270 and 330 µg/mL, did not cause death in any of the *A. suum.* In the concentration of 400 and 470 µg/mL, four and eight out of fifteen *A. suum* died, respectively. The highest concentration, 530 µg/mL, exhibited the most toxic effect causing the death of twelve out of fifteen *A. suum.*

Table 6 shows that the median lethal concentration of pyrantel embonate was most probably less than 470 µg/mL since 53.33 percent of the worms died in this concentration. The increased rate of mortality of the worms was most probably due to the highly effective anti-helminthic activity of pyrantel embonate. Pyrantel embonate induces the release of acetylcholine which inhibits the release of cholinesterase. This paralyzes the worms which later causes the death of the worms (Lloyd et al., 2014).

Table 6
LC50 of Pyrantel Embonate
against the *A. suum*

Concentration µg/mL	Total no. of dead *A. suum* (N=15)	Percentage mortality (%)
270	0	0
330	0	0
400	4	26.67
470	8	53.33
530	12	80

Table 7 represents the multiple comparison on the effects of pyrantel embonate on different concentration. At 270 µg/mL, there is the same effect as compared to 330 µg/mL with a p-value of 1 but there is a significant difference at 400 µg/mL, 470 µg/mL and 530 µg/mL with a p-value of 0.029, 0.001 and 0.000, respectively. At 330 µg/mL, it exhibits the same effect as compared to 270 µg/mL but there is a significant difference at 400 µg/mL, 470 µg/mL and 530 µg/mL with a p-value of 0.0029, 0.001 and 0.000, respectively. At 400 µg/mL, there is the same effect as compared to 470 µg/mL but there is a significant difference at 270 µg/mL, 330 µg/mL, and 530 µg/mL with a p-value of 0.001, 0.001 and 0.0029, respectively. At 470 µg/mL,

same effect is seen as compared to 400 µg/mL but there is a significant difference at 270 µg/mL, 330 µg/mL, and 530 µg/mL. At 530 µg/mL, there is a significant difference on all concentrations. This only implies that among the concentration, 530 µg/mL was the best concentration.

There is no significant difference between 270 µg/mL and 330 µg/mL, which is more likely due to the fact that both concentrations did not cause death to *A. suum*. The mortality of *A. suum* increases as the concentration of Pyrantel embonate increases. This is more likely due to the increasing concentrations of Pyrantel embonate. Pyrantel embonate induces the release of acetylcholine which inhibits the release of cholinesterase. This paralyzes the worms which later causes the death of the worms (Lloyd et al., 2014).

Table 7
Multiple Comparison on the Concentration of Pyrantel Embonate

Concentration	Concentration	p-value	Interpretation
	330 µg/mL	1	Not Significant
270 µg/mL	400 µg/mL	0.029	Significant
	470 µg/mL	0.001	Significant
	530 µg/mL	<0.1	Significant
	270 µg/mL	1	Not Significant
330 µg/mL	400 µg/mL	0.029	Significant
	470 µg/mL	0.001	Significant
	530 µg/mL	<0.1	Significant
	270 µg/mL	0.29	Significant
400 µg/mL	330 µg/mL	0.29	Significant
	470 µg/mL	0.117	Not Significant
	530 µg/mL	0.001	Significant
	270 µg/mL	0.001	Significant
470 µg/mL	330 µg/mL	0.001	Significant
	400 µg/mL	0.117	Not Significant
	530 µg/mL	0.029	Significant
	270 µg/mL	< 0.1	Significant
530 µg/mL	330 µg/mL	<0.1	Significant
	400 µg/mL	0.001	Significant
	470 µg/mL	0.029	Significant

*. The mean difference is significant at the 0.05 level

CONCLUSION

From the study conducted by the researchers, it is therefore concluded that crude hexane extract of *Raphanus raphanistrum subsp. Sativus* (L.). Domin (radish) possesses antihelminthic activity against *Ascaris suum.* The antiheminthic activity started at the concentration of 1600 ug/ml with a percentage mortality of 26.67% and the concentration that exhibited the most toxic effect to *A. suum* is 2000 ug/ml with a

percentage mortality of 53.33%. Since the study only focused on the crude hexane extract of the plant, the anti-helminthic activity might probably be due to the phytochemical constituents of the plant such as saponins, flavonoids and alkaloids. Based from the findings, the antihelminthic activity of the *R. raphanistrum* root extract increases with an increase in its concentration.

RECOMMENDATION

The researchers recommend that other plants under the Brassicaceae family be tested for their potential anti-helminthic activity. Isolation of the main antihelminthic component, glucosinolate, is also recommended through the use of different chromatographic methods. Furthermore, it is recommended to isolate different phytochemicals from other parts of the plant.

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