

# In Vitro Anthelmintic Activity of Formulated Syrup from *Ocimum basilicum* (Sweet Basil) Leaves Ethanolic Extract against *Ascaris suum*

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## ABSTRACT

Ascariasis, caused by either *Ascaris suum* and *Ascaris lumbricoides*, is one of the most prevalent and most neglected tropical diseases (NTDs) in the world. In this study, the researchers assessed the anthelmintic capability of *Ocimum basilicum* (Sweet Basil) in attempt to combat ascariasis. This utilized true experimental research design, specifically pretest-posttest control group method. Using logarithmic scale method, the approximate effective concentration (AEC) of the *O. basilicum* (Sweet Basil) leaves ethanolic extract was determined. Three concentrations (3.98%, 15.84% and 63.06%) used have shown effectivity in paralyzing *A. suum*. The AEC used in formulating medicated syrup was 3.98% (1.99mL). The formulated syrup was assayed and its density, viscosity, and pH were identified as 1.26g/mL, 0.29cp, and 5, respectively. Moreover, in the in vitro anthelmintic assay, three treatment groups were utilized mainly pyrantel pamoate (positive control), formulated syrup from *O. basilicum* (Sweet Basil) leaves ethanolic extract (experimental group), and distilled water (negative control). A total of 45 *A. suum* roundworms were used, divided into three groups, each group had three trials, comprising of five test subjects in each trial. The percentage paralysis index of each treatment group was calculated in accordance to 1-hour and 3-hour post treatment. The formulated syrup from *Ocimum basilicum* showed 100% paralysis index, which means that it has the ability to induce paralysis to the helminth. Its action was comparable to the action of pyrantel pamoate against the *Ascaris suum*. Hence, it can be a potential anthelmintic agent.

KEYWORDS: *O. basilicum*; Ascariasis; *A. suum*; Pyrantel Pamoate; Formulated Syrup

## INTRODUCTION

Helminthic infections are ubiquitous. Among helminthic infections, ascariasis, caused by soil-transmitted helminths (STH), is the most prevalent and one of the most neglected tropical diseases (NTDs) in the world (Mahmood, 2021). Ascariasis, either caused by *Ascaris lumbricoides* (human roundworm) or *Ascaris suum* (pig roundworm) (Mahmood, 2021), show less symptoms among infected people. These two types of parasitic worms are hard to tell apart but both can widely infect humans. However, *Ascaris suum* is commonly postulated as a research subject on human infection by *Ascaris lumbricoides* in in vitro studies (Dmitryjuk, Szczotko, Kubiak, Trojanowicz, Parashchyn, Khomitska, & Lubenets, 2020).

Accordingly, over 807 million – 1.2 billion people are infested with human ascariasis worldwide (CDC, 2023). Such parasitic disease mostly affects younger generations, particularly in areas with poor sanitation, hygiene, and access to water (Dmitryjuk et al. 2020). In the Philippines, several studies across the country reported that STH are highly prevalent and considered as a major health problem particularly in school- aged children with prevalence rate of 33.8-75.9% (Mationg, Williams, Tallo, Olveda, Aung, Alday, Donald, Daga, Landicho, Demonteverde, Santos, Bravo, Bieri, Li, Steinmann, Halton, Stewart, McManus, & Gray, 2021). Kajero, Janoušková, Bakare, Vicente, Divina, Jethro, Manalo, Paller, Betson, & Prada (2022) reported that STHs marked a significant health problem in the country with prevalence of about 74% STH infections among school-children and Department of Health (DOH) considered ascariasis as one of the major helminth infections (Milanez, Lacorte, Gabon, Ahmad, Hagosojos, Alvarez, Quinto, & Ormita, 2020). In Mindanao, 20, 200 prevalence cases of STH are reported among school-aged children (Owada, Lau, Leonardo, Clements, Yakob, Nielsen, Carabin, & Magalhães, 2018). In General Santos City, in a survey conducted by Almida, Cahilsot, Fuentes, Salvatierra, and Tato (2023), prevalence rate of STH is reported particularly with 50% cases of *Ascaris spp.* infection, 56.7% with *Trichuris trichiura* infection, and 50% with hookworm infection.

With the predominant cases of ascariasis around the globe, the search for anthelmintic drugs from plant extracts in treating all stages of nematode development has become part of the current treatments of helminthic infections (Williams, Fryganas, Ramsay, Mueller-Harvey, & Thamsborg, 2014). Many of the plant species that provide medicinal herbs have been scientifically evaluated for their potential anthelmintic activity (Veerakumari, 2015).

Several research studies conducted on the possible anthelmintic action of *Ocimum basilicum* (Sweet Basil). According to Akoto, Acheampong, Boakye, Naazo and Adomah (2020), *Ocimum basilicum* (Sweet Basil) reportedly contains phytochemicals contributing to its anthelmintic activity specifically saponin, which help by inhibiting the acetylcholinesterase, the enzyme that hydrolyses and inactivates acetylcholine for the regulation of cholinergic transmission, hence the worm paralyzes and dies. Further, the phytochemicals of this plant kill helminths by binding to free proteins in the gastrointestinal tract of a host animal or binding to glycoprotein on the cuticle of the parasite (Sea, Hariadi, Koesdarto, Kusnoto, & Widjaja, 2019). Hence, with the reported pharmacological potential of *Ocimum basilicum* in aiding helminthic infections, this study was tailored to find and assess the anthelmintic capability of *Ocimum basilicum* in an attempt to combat ascariasis caused by *STH*. The aim of this study was, therefore, to evaluate the in vitro Anthelmintic activity of formulated syrup from *Ocimum basilicum* (Sweet Basil) leaves ethanolic extract against *Ascaris suum*.

### Statement of the Problem

The researchers sought to answer the following pertinent questions:

1. What is the approximate effective concentration (AEC) of *Ocimum basilicum* (Sweet Basil) leaves ethanolic extract as anthelmintic agent against *Ascaris suum*?
2. What is the quality control values of the formulated syrup in terms of:
  - 2.1 density;
  - 2.2 viscosity; and
  - 2.3 pH?
3. What is the percentage paralysis index after one hour and three hours post-treatment with the following:
  - 3.1 formulated syrup from *Ocimum basilicum* (Sweet Basil) leaves ethanolic extract (experimental group);
  - 3.2 Pyrantel Pamoate (Positive control); and
  - 3.3 Distilled water (Negative control)?
4. Is there a significant difference in the percentage paralysis index between formulated syrup from *Ocimum basilicum* (Sweet Basil) leaves ethanolic extract (experimental group), Pyrantel Pamoate (positive control), and distilled water (negative control)?

## METHODOLOGY

### Research Design

A true experimental, post-test-only control group design was used, with *A. suum* randomly assigned to three groups: experimental (formulated syrup), positive control (Pyrantel Pamoate), and negative control (distilled water). The percentage paralysis index was measured after one- and three-hours post-treatment.

### Sampling Technique

Purposive sampling was employed to collect 70 *A. suum* roundworms. Of these, 45 were randomly assigned to three treatment groups— formulated syrup (experimental), Pyrantel Pamoate (positive control), and distilled water (negative control)—with each group having three Petri dishes containing five worms each. Mature *O. basilicum* leaves (30+ days old) were harvested and stored in a dry, room-temperature environment to prevent mold and contamination.

### Data Gathering Procedure

**Extraction of Plant Material and Preparation of Ethanol Extract.** The leaves of *O. basilicum* were thoroughly washed three times with clean tap water to remove any form of adulterants. Subsequently, to exclude heavy metals in tap water, the leaves were rinsed in distilled water. Then, the leaves were sun-dried completely, pulverized with electric machine grinder separately and obtained a coarse powder (Hassen et al., 2022). A 100 g powdered sample of *O. basilicum* was soaked in 1 dm<sup>3</sup> 80% ethanol for three days at room temperature. After four days of maceration, the macerated sample was condensed and evaporated to dryness using the rotary evaporator at 78°C. The extract was further heated to achieve the syrupy consistency. Subsequently, the extract was stored at 4°C in a refrigerator (Akoto et al., 2020).

**Approximate Effective Concentration Determination.** This experiment had 25 samples of *Ascaris suum* from the total number of 70 test subjects, which underwent the approximate effective concentration determination. The *A. suum* roundworms were subjected to four concentrations in which each concentration used five helminths with 1:5 ratio and another 5 helminths received no treatment using distilled water. During the test, there was a

control group labeled as C and an experimental group labeled as PE for the treatment of *Ocimum basilicum* leaves ethanolic extract.

**In vitro Anthelmintic Assay.** The protocol used in the study was the combined methods based on authors Akoto et al. (2020), Hassen et al. (2022), Maregesi, Kidukuli, Nkungu, and Mkupete, (2016), and Zenebe, Feyera, and Assefa (2017). To determine the in vitro anthelmintic activity of the formulated syrup from the leaves ethanolic extract derived from *Ocimum basilicum* (Sweet Basil), the formulated syrup was examined and compared with pyrantel pamoate (reference drug) and distilled water (negative control). Before the start of the experiment, pyrantel pamoate (reference drug) was freshly prepared using distilled water (Akoto et al., 2020). Using Adult Motility Assay (AMA), the test was performed in a 5cm diameter glass Petri dish. 45 roundworms were used in the study (Zenebe, Feyera, & Assefa, 2017). Three groups that contained 15 *A. suum* roundworms per group and released into three separate Petri dishes (with 5 helminths in each Petri dish) for each treatment group, mainly experimental, positive, and negative, respectively (Akoto et al., 2020). Indication of time of paralysis of the roundworms was recorded based on the inhibitions of motility. Motility of worms were observed and counted at 1- and 3-hour post treatment. Assessment for the individual worms were done as followed; it was determined as paralysis when sign of movement was observed when soaked in lukewarm 5% Dextrose in Lactated Ringer's Injection (D5LR) for 10 minutes while death was determined when the worms showed no motility even after being subjected to an external stimulus (Maregesi et al., 2016). Further, roundworms that showed motility even after the post treatment was concluded as alive. Consequently, roundworms that showed no motility were taken out and kept for 10 minutes in lukewarm 5% Dextrose in Lactated Ringer's Injection (D5LR). When the roundworms showed any motility after being subjected to the D5LR (external stimulus), they were counted as paralyzed; otherwise, they were counted as dead (Hassen et al., 2022). The number of paralyzed *A. suum* roundworms at 1- and 3-hours post treatment was calculated for each test solution. The formula used in calculating the percentage paralysis index was (Hassen et al., 2022):

$$\text{Percentage Paralysis index (\%)} = \frac{\text{total no. of paralysis worms total}}{\text{no. of worms per Petri dish}} \times 100$$

### Statistical Analysis

Data were analyzed using JASP software. Mean and standard deviation were determined for the trials performed in the treatment groups.

### Ethical Considerations

The researchers obtained ethical approval from the school's Research Ethics Committee and strictly followed laboratory safety protocols under supervision during experimentation. Data were reported truthfully with integrity, and any errors were addressed responsibly. After the experiment, test subjects were properly disposed of in pit holes, and all used laboratory equipment was sterilized through autoclaving.

## RESULTS

### Approximate Effective Concentration

**Table 1: Approximate Effective Concentration of *Ocimum basilicum* (Sweet Basil) Leaves Ethanolic Extract**

Sample	Treatment	Concentration (%)	Volume (mL)	No. of Paralyzed <i>A. suum</i> after post-treatment		Description
				After 1 hr	After 3 hrs	
A	<i>O. basilicum</i>	1%	0.5mL	3	3	Negative
B	<i>O. basilicum</i>	3.98%	1.99mL	3	5	Positive
C	<i>O. basilicum</i>	15.84%	7.92mL	5	5	Positive
D	<i>O. basilicum</i>	63.06%	31.53mL	5	5	Positive
E	Distilled water	100%	50mL	0	0	Negative

Positive:  $\geq 4$  *Ascaris suum* paralyzed after post-treatment

Negative:  $\leq 3$  *Ascaris suum* paralyzed after post-treatment

## Assay of Formulated Syrup

**Table 2: Formulated Syrup (*O. basilicum*) Density, Viscosity, and pH Determination**

	Values	Interpretation
Density (g/mL)	1.26	denser than $H_2O$
Viscosity (cp)	0.29	more viscous than $H_2O$
pH	5	acidic

## In vitro Anthelmintic Assay

**Table 3: In vitro Anthelmintic Activity of Three Treatment Groups against *A. suum***

Treatment	$\bar{x}$	$\sigma^2$	% Paralysis Index	Description
Pyrantel Pamoate (Positive control)	5	0	100%	Positive
Formulated Syrup (Experimental group)	5	0	100%	Positive
Distilled water (Negative control)	0	0	0%	Negative

## DISCUSSION

In the determination of approximate effective concentration (AEC) of *Ocimum basilicum* (Sweet Basil) leaves ethanolic extract, a logarithmic scale method was used for the study (Elevencionado et al., 2016). The study was based on four different concentration levels that were determined using the logarithmic scale method at 0.6 log interval. The percent concentrations were used to determine the equivalent volume of the plant extract by ratio and proportion using the formula  $C1V1 = C2V2$  (Elevencionado et al., 2016). Only concentrations of sample B, C, and D showed a positive effect on paralyzing helminths for each trial. The determination of approximate effective concentration (AEC) was based on the number of paralyzed *A. suum* after 1-hour and 3-hour post treatment. In this study, the approximate effective concentration (AEC) determined was the second concentration from sample B since it was the lowest possible concentration that elicited paralysis on *A. suum* roundworms after the three hours post-treatment.

Moreover, after determining the AEC, the extract was used as the active ingredient in formulating syrup. Formulating the extract into syrup extends the stability and shelf-life of the active ingredient because the simple syrup used contained a minimal amount of preservative which helps in retarding the growth of bacteria, fungi and mold (Devkar, Shaikh, Jadhoo, Sanap, & Patil, 2021). The formulated syrup from *Ocimum basilicum* (Sweet Basil) leaves ethanolic extract underwent an assay to determine its density, viscosity, and pH (Mali et al., 2019). The formulated syrup had a density which was greater than the density of water (standard reference), implying that the formulated syrup is denser than water. The same result has been observed in the study by Olayemi, John-Africa, Chikwendu, and Isimi (2020) wherein their formulated syrup containing the aqueous root extract of *Nauclea latifolia* exhibited a greater density than water. The viscosity of the formulated syrup was further calculated and determined with three trials using Ostwald viscometer. In comparison to distilled water (standard reference), the formulated syrup had a higher viscosity value, indicating that the syrup is more viscous than water. Identical result from the assay of the formulated syrup from the aqueous root extract of *Nauclea latifolia* was observed (Olayemi et al., 2020). This is mainly due to the presence of sugar incorporated with the distilled water, causing the increase in viscosity of the syrup (Goldfein and Slavin, 2015). Also, the pH of the formulated syrup was determined as acidic that is within the standard range pH of 3-6 for medicated syrups. Relative to this, Olayemi et al. (2020) provided a range of 4.45 to 5.78 for the pH levels in formulated syrups, in which the *Ocimum basilicum* formulated syrup conforms with. For density and viscosity, it was considerable that the range of acceptable values for such parameters can change due to the physicochemical properties of the active pharmaceutical ingredient in the formulated syrup (Patel et al., 2017). Further, with these determined parameters, the *O. basilicum* formulated syrup was significantly proven to be stable upon use during the anthelmintic activity bioassay (Nerkar & Ghadge, 2023). In the determination of the in vitro anthelmintic activity of formulated syrup from *Ocimum basilicum* (Sweet Basil) leaves ethanolic extract, three treatments were used with corresponding three trials to identify the percentage paralysis index of each treatment. 1 hour and 3-hour post treatments were used as parameters upon determining the number of paralyzed *Ascaris suum*. In the positive control (pyrantel pamoate), all trials resulted in the paralysis of all the test subjects after an hour and three hours. Identical results were also observed with the use of the formulated syrup for the experimental group which means that the *O. basilicum* significantly elicited a high efficacy as an anthelmintic agent. Similar result has been shown in the study by Shagana et al. (2021) which confirmed that *Ocimum basilicum* used by common people traditionally to treat intestinal worm infections, is proven to have efficient anthelmintic activity. In confirming the

paralysis, the treated roundworms were soaked in lukewarm D5LR solution for 10 minutes (Maregesi et al., 2016). Signs of motility within the given time frame were confirmed, thus differentiating paralysis from death of the *Ascaris suum* (Hassen et al., 2022). The negative control (distilled water) was recorded to have no paralyzed helminths during the 1-hour and 3-hour post treatment. Subsequently, all the numbers in the data set were equal, thus, the expected variance of the study was zero. However, since the variance was zero, the researchers were unable to proceed in using one-way ANOVA to compare the means of the three independent groups to determine whether there is a significant difference between the percent paralysis index of the formulated syrup, pyrantel pamoate, and distilled water.

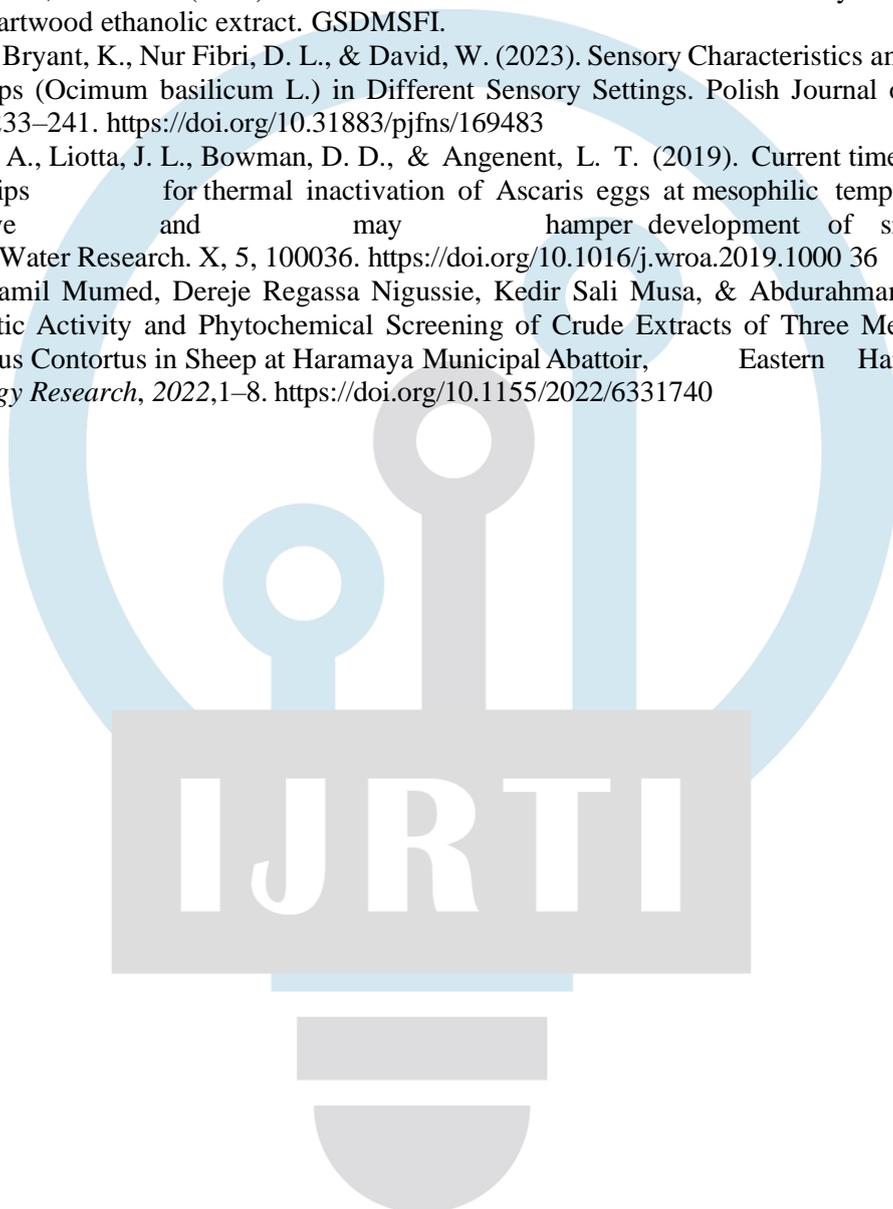
## Conclusion

The study found that *O. basilicum* can induce paralysis in *A. suum*, making it a potential anthelmintic agent. It suggests using *O. basilicum* in ascariasis treatment alongside current options and recommends public health centers incorporate it into anti-ascariasis campaigns. Further clinical studies by the FDA are advised, along with improving plant material collection and sourcing native-raised pigs for testing. Future research should explore other phytochemicals like flavonoids and saponins in *O. basilicum* to enhance its anthelmintic potential.

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