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Premna odorata Blanco against Ascaris spp. Ova Found in Dewormed Swines of Tanauan, Philippines

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Abstract. This study investigated the effectiveness of *Premna odorata* Blanco (Alagao) aqueous leaf extract against swine Ascaris spp. ova. Ascaris is a common intestinal parasite in the Philippines, affecting animals and humans. Recent studies have highlighted the increasing antiparasitic resistance among parasites, which has led to the exploration of alternative conventional options and therapeutics to combat parasitic infections effectively. This growing resistance underscores the need for novel treatments, such as plant-based extracts, to address the limitations of current anthelmintic drugs. Researchers aimed to assess the ovicidal activity of the aqueous leaf extract. The study involved collecting *Premna odorata* Blanco (Alagao) leaves from Cavite City, verifying the plant identity, and preparing various extract concentrations. Swine fecal samples were collected in Tanauan City, Batangas, and examined for Ascaris spp. prevalence and intensity using Modified Kato-Katz. Results showed a moderate infection rate; 15 out of 90 pigs tested positive. Farms 1 and 6 had light infections, suggesting partial effectiveness of previous deworming. Farm 5 showed a high infection even after recent deworming. The *Premna odorata* Blanco (Alagao) aqueous leaf extract exhibited low efficiency against Ascaris spp. ova, with 15 to 45 minutes exposure times. However, extended exposures of 60 to 120 minutes showed promising results. The 25% extract concentration was ineffective, while 50% and 75% concentrations partially disrupted the eggs. Notably, the 100% concentration successfully penetrated and disrupted the ova. These findings suggested that higher concentrations of *Premna odorata* Blanco (Alagao) aqueous leaf extract have a better ovicidal effect against Ascaris spp.; however, it requires prolonged exposure. Further research is needed to optimize the extract's use as a potential alternative anthelmintic drug.

Keywords: Ascaris spp.; Ovicidal; *Premna odorata* Blanco; Soil-transmitted helminths; Swine.

1.0 Introduction

Universal Health Coverage (UHC) is a crucial target established by the world's nations when they adopted the 2030 Sustainable Development Goals (SDGs) in 2015. UHC ensures everyone can access the full range of quality health services without financial hardship (Department of Health, 2019). In 2019, the Philippines took a significant step toward achieving this goal with the passage of Republic Act No. 11223, the Universal Health Care Act (UHC Act). This law guarantees all Filipinos free access to a comprehensive array of quality healthcare services at the point of delivery (Department of Health, 2019). The National Government, through the Department of Health (DOH), funds essential preventive and public health programs offered free at the point of service (Department of Health, 2020). However, achieving UHC requires more than just human healthcare; it demands a holistic approach that recognizes the interconnectedness of human, animal, and environmental health. This is where the One Health framework becomes essential.

The One Health framework acknowledges the critical link between animal health and global health outcomes, addressing challenges that transcend traditional healthcare boundaries (World Health Organization, 2019). In the Philippines, One Health has been instrumental in tackling neglected tropical diseases (NTDs) (Tenorio, 2022), such as soil-transmitted helminths (STH), which are prevalent in resource-limited rural areas. For instance, Ascaris spp., a common STH, not only infects humans but also companion animals, which can act as reservoirs for human re-infection (Mationg et al., 2021; Labana et al., 2021). While mass drug administration (MDA) for humans is a critical component of STH control, neglecting animal deworming and inadequate community education can perpetuate the cycle of infection (Ackley et al., 2021). Despite the nationwide MDA program, STH prevalence in the Philippines remains high, ranging from 24.9% to 97.4%, due to limited awareness, program mistrust, and misconceptions (Mationg et al., 2021). Integrating UHC with the One Health framework can address these gaps by ensuring a coordinated approach to health that includes animal and environmental interventions, ultimately strengthening the Philippines' efforts to achieve sustainable health outcomes for all.

Across the globe, the World Organization for Animal Health (WOAH) leads swine health and welfare (World Organization for Animal Health, 2024). In the Philippines, the Philippine government prioritizes aggressive disease control through policies like Administrative Order No. 22, which enforces strict adherence to the "National Zoning and Movement Plan for ASF." This plan, outlined in DA Circular No. 12 (S.2019), aims to contain the highly contagious virus through targeted movement restrictions and control measures (Official Gazette, 2020). The Bureau of Animal Industry implements programs to enhance productivity, safeguard health, and promote sustainability (PCAARRD's Industry Strategic Science and Technology Programs, n.d). Thus, local programs like the Swine R3 Credit Program in Batangas collaborate with national authorities for coordinated control measures and industry revitalization (Office Portal of the Department of Agriculture, 2021).

Furthermore, the Philippines, a tropical archipelago famed for its vibrant culinary heritage, holds a deep affinity for pork. This succulent meat, ranking Filipinos among the world's top consumers and producers, finds its way onto countless plates nationwide (Yan, 2020). However, amidst this love affair with pork lies a concealed public health concern: the potential presence of diverse parasites within Philippine swine. Studies have revealed both well-known and newly discovered species residing within these animals, such as the common gastrointestinal (GIT) parasites, which include Trichuris spp., Strongyloides spp., Taenia spp., Isospora spp., *Balantidium coli*, and Ascaris spp., some of which pose a potential zoonotic threat, meaning they can be transmitted from animals to humans(Ybañez et al., 2017; Ybañez et al., 2019).

Several studies investigated the prevalence and impact of parasitic diseases in swine across the Philippines. One research by Ybañez et al. (2017) focused on gastrointestinal parasites in industrial swine farms in Cebu. They found a high prevalence of 78.2% and identified parasites with zoonotic potential. The study also investigated the link between farm equipment and parasite numbers, highlighting the need for further research on transmission pathways and their impact on swine health and productivity (Ybañez et al., 2017). Another study by Ybañez et al. (2019) examined the prevalence of *Toxoplasma gondii* in humans, cats, and swine in Cebu. They found a 13.4% seroprevalence in swine, indicating a potential risk of transmission to other animals (Ybañez et al., 2019). Moreover, studies in the Philippines have documented widespread resistance among livestock parasites to deworming drugs, posing a significant challenge to the livestock industry. One particularly concerning example is *Haemonchus contortus*, a prevalent and pathogenic ruminant parasite that Cabardo and Portugaliza (2017) identified as having developed resistance to common anthelmintic treatments such as Ivermectin (Cabardo & Portugaliza, 2017). In swine, there are limited reports of resistance against pyrantel, levamisole, and benzimidazoles in *Oesophagostomum dentatum*. Also, benzimidazole, including fenbendazole and flubendazole, demonstrates resistance activity against both adult and immature stages of the Ascaris suum (Vercruysse & Claerebout, 2023).

In connection with the world of animal health, the effectiveness of anthelmintic drugs can diminish over time due to a phenomenon known as anthelmintic resistance (Center for Veterinary Medicine, 2023). This process, unfortunately, poses a significant challenge to animal health management, necessitating the development of new strategies to combat resistant parasites. After exposure to an anthelmintic drug, susceptible parasites succumb, yielding resistant parasites to reproduce and pass on their resistant genes to their offspring, creating a lineage increasingly immune to the medication, rendering the once-effective drug ineffective. Notably, parasites are often called worms and anthelmintic drugs as dewormers (Center for Veterinary Medicine, 2023; Campbell & Faulkner, 2023).

According to the Morphology, Anatomy, and Secondary Metabolites Investigations of *Premna odorata* Blanco (P. odorata B) study by Youssef et al. (2021), *Premna odorata* Blanco, also known as alagao, is a small evergreen tree or shrub in the Lamiaceae family, is around 10 m tall and has a diameter breast height (DBH) of 15–30 cm (Youssef et al., 2021). It is from the Greek word premon, which means tree stump and refers to the short and twisted trunks (Youssef et al., 2021). Preliminary phytochemical screening of *Premna odorata* Blanco (Alagao) crude leaves extract showed the presence of iridoids, triterpenes, flavonoids, phenylethanoid, and acylated rhamnopyranosides (Elmaidomy et al., 2019). Flavonoids offer antioxidant and anti-inflammatory qualities; phenyl ethanoids have anti-inflammatory action, antibacterial, and anticancer properties; acylated rhamnopyranoside functions in defense mechanisms, and iridoids contribute to anti-inflammatory, antioxidant, and neuroprotective activities. (Youssef et al., 2021; Elmaidomy et al., 2019). In recent years, flavonoids have generated interest as potential therapeutic agents, particularly in an anthelmintic activity (Mead & McNair, 2006; Toklo et al., 2021), that can be used to develop alternatives to commercial anthelmintic drugs further.

Driven by the need to find an alternative anthelmintic source that could be a potential solution for managing anthelmintic drug resistance in swine raised in selected animal farms of Tanauan City, Batangas, this study aims to determine the ovicidal activity of *Premna odorata* Blanco (Alagao) aqueous leaf extract in varying concentrations against Ascaris spp., ova collected from recently dewormed swine. Despite its objective, the study acknowledges its limitations. It does not apply to in vivo conditions; further research involving live animals is crucial to confirm the effectiveness in a practical setting. The study focuses on ovicidal activity and other parasitic life stages requiring different extraction methods or concentrations. Further studies are needed to determine the ideal dosage for maximum effectiveness while ensuring animal safety. Hence, this research aims to determine the prevalence rate of infected swine intensity of infection, investigate the efficacy of alagao against the parasitic activity of swine that focuses on the time exposure measurement needed for the complete ovicidal activity of the Ascaris spp., and determine the minimum concentration required for the elimination of Ascaris spp., ova.

2.0 Methodology

2.1 Study Area

The fecal samples were collected from six different swine farms in Tanauan, Batangas, under the supervision of a registered veterinarian and the swine farm owner to guarantee adherence to proper collection procedures. Also, *Premna odorata* Blanco (Alagao) leaves were obtained from a designated farm in Cavite City.

2.2 Plant material

Green, healthy, and mature leaves of *Premna odorata* Blanco (Alagao) were collected from a designated farm in Cavite City, verified and certificated by Mr. Alex S. Pedroso of the Bureau of Plant Industry - Crop Research and Production Support Division (BPI-CRPSD), and screened its phytochemical content at the University of Santo Tomas Research Center for the Natural Sciences and Applied Sciences- Analytical Services Laboratory (UST RCNAS-ASL).

2.3 Plant extraction

Premna odorata Blanco (Alagao) leaves were extracted through the decoction process. The leaves are cleaned with tap water to remove contaminants; after washing, they are crushed using a mortar and pestle to achieve powdered alagao leaves. The alagao powder was mixed with distilled water with a ratio of leaves: water = 1:1 (w:v). Then, the mixture was heated to the boiling point under cooling reflux to avoid water loss with evaporation. After that, the mixture was removed from heat, stood for 20 minutes, and filtered through filter paper (Khajehei et al., 2017).

2.4 Different concentrations of Plant Extract

The varying concentrations of *Premna odorata* Blanco (Alagao) aqueous leaf extracts were achieved using the crude extract and the addition of distilled water. 20 ml of the plant extract was diluted with 80 mL of distilled water to achieve a 25% concentration. For 50% concentration, 40 ml of the extract was diluted with 80 ml of distilled water. Alongside 70%, 60 ml of alagao was diluted to 80 ml of distilled water. Lastly, 80 ml of alagao crude extract was diluted with 80 ml of distilled water to achieve 100% concentration (Garcia et al., 2022).

2.5 Data analysis

The fecal samples were collected from six Tanauan City, Batangas swine farms. Each swine farm collected 15 swine stool samples. They transported the specimen using the cooler with a temperature of 40 degrees Celsius and utilized 10% formalin for its preservative while on transport. After collection, they proceeded to the experimentation process, where Modified Kato-Katz Techniques were tested to determine the prevalence and intensity of infection in the collected sample. After Modified Kato-Katz, the positive samples were subjected to the Formalin Ether Concentration Technique (FECT) to extract sediments containing Ascaris spp., ova. They subjected the samples from FECT for ova destruction using the varying concentrations prepared. The experiment proceeded until the samples were disposed of. The disposal of the samples complied with the standard protocols of the World Health Organization (1947) and Clinical and Laboratory Standards Institute (2017), which consider all stool samples highly infectious to avoid spreading disease (World Health Organization, 2019).

2.6 Ethical Considerations

This study adhered to strict guidelines to ensure the safety of researchers, animals, and the environment. No direct handling of swine was performed during the study. Instead, researchers patiently waited for the natural defecation of the swine to collect fecal samples, ensuring minimal disturbance to the animals. The collection of fecal samples was conducted under the supervision of a registered veterinarian and the swine farm owner to guarantee compliance with proper procedures and animal welfare standards.

Safety protocols were strictly followed throughout the study. Researchers wore appropriate personal protective equipment (PPE), including gloves and masks, during sample collection and handling to minimize exposure to fecal samples. Local government regulations treated and disposed of all waste to prevent environmental contamination.

3.0 Results and Discussion

3.1 Prevalence Rate and Intensity of Infection

Table 1 shows the Ascaris spp., ova count, prevalence rate, eggs per gram, and intensity of infection found in recently dewormed swine on selected farms in Tanauan City, Batangas, Philippines. Out of 90 swine tests examined, 15 (17%) were positive for Ascaris spp. infection. Farm 1 has 2 Ascaris spp., with a 13% prevalence rate. Farms 2,3,4 have a 0 Ascaris spp.; the count indicates no infection was detected. Farm 5 has 7 Ascaris spp., with a prevalence rate of 46.67%, and Farm 6 has 6 Ascaris spp., counted with a prevalence rate of 40%. In addition, only Farms 1,5,6 yield a positive intensity of infection. Farm 1 had an EPG (eggs per gram) of 48, while Farm 6 had an EPG of 1152, resulting in a light-intensity infection. Farms 2, 3, and 4 had an EPG of 0. Farm 5 had a significantly higher EPG of 70176, indicating a high-intensity infection.

Table 1. *Prevalence and intensity of Ascaris spp. infections in selected Swine farms in Tanauan City, Batangas*

Study Site	No. Examined	% Positive	EPG	Intensity of Infection
F1	15	13.0%	48	Light Intensity Infection
F2	15	0.00%	0	0
F3	15	0.00%	0	0
F4	15	0.00%	0	0
F5	15	47.0%	70176	High Intensity Infection
F6	15	40.0%	1152	Light Intensity Infection
Total	**90**	**17.0%**		

Legend: >*50,000 epg, Heavy Intensity Infection, 5,000-49,999 epg, Moderate Intensity Infection, 1-4,999 epg, Light Intensity Infection. F signifies Farm.*
**EPG = Eggs per gram*

Overall, 15 out of 90 swine (17%) tested positive for Ascaris spp., indicating a relatively common occurrence of this parasite among the sampled farms. The result suggests that deworming might not have been practical or that re-infection is happening relatively quickly. Additionally, the prevalence of infection varied significantly between farms. Farms 1, 5, and 6 had positive cases, whereas Farms 2, 3, and 4 showed no detected infection. This variation could be due to differences in sanitation practices, swine housing conditions, or the timing of deworming relative to the parasite's life cycle (Pettersson et.al, 2021). The intensity of infection is based on the numerical grading, in which light-intensity infection only has an EPG of <4999, moderate-intensity infection contains 5000-49999 EPG and high-intensity infection with an EPG of >50,000 (Eyayu et al., 2021). Among the infected farms, Farms 1 and 6 displayed a light intensity of infection with EPG values below 5000. It indicates that deworming might have been partially successful in these cases. However, Farm 5 has a significantly high EPG with 70176 eggs per gram, indicating a high-intensity infection despite recent deworming, highlighting the need to investigate the effectiveness of the deworming program used on this farm.

In contrast to the findings of Pettersson et al. (2021), a study by Petterson et al. (2021) reported a lower prevalence rate of Ascaris spp. in swine. Petterson et al. (2021) also noted that altered farming routines focusing on improved pig welfare have not solely resulted in a higher occurrence of parasites, most likely due to the adequate biosecurity and hygiene practices instituted. Thus, there seems to be no conflict between implementing measures to promote pig welfare and adequately control the more pathogenic and economically important parasites. Additionally, the intensity of infection in Petterson et al. (2021) study produced 50−8,250 EPG values, showing light to moderate intensity, unlike the high-intensity infection observed. This further underscores the variability in infection dynamics and the potential influence of farm-specific factors such as sanitation and deworming efficacy. Both studies agree on the importance of monitoring EPG levels to assess the success of deworming programs and the need for tailored interventions based on farm-specific conditions.

3.2 Time of Exposure Required for Ovicidal Activity of Ascaris spp.

Figure 1 illustrates Trial 1 of the experiment that shows results of a low-level efficiency to satisfactory efficacy of the *Premna odorata* Blanco (Alagao) aqueous leaf extract. An undestroyed egg envelope with no recorded egg decay and embryo dissolution (Low efficiency) was observed between 15 minutes, 30 minutes, and 45 minutes. Hence, satisfactory efficiency, meaning a partially destroyed egg envelope, partial egg decay, and destroyed larvae egg, was seen between 60 minutes, 75 minutes, 90 minutes, 105 minutes, and 120 minutes. The positive and negative controls used showed low efficiency throughout the experiment's time requirement, showing no significant effect on the Ascaris spp., ova.

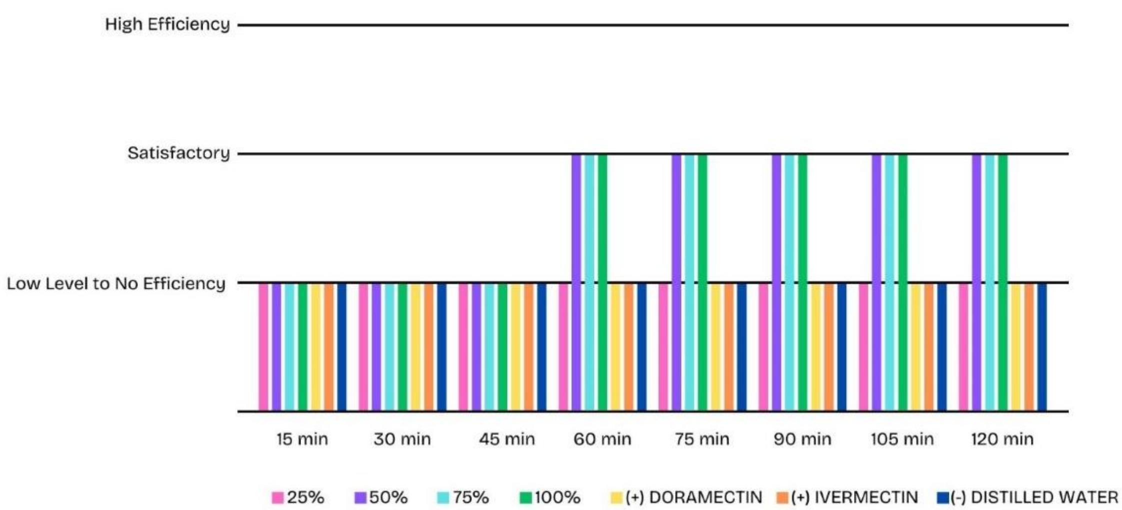

Figure 1. *Trial 1 of Experiment to Determine the Time of Exposure Required for Ovicidal Activity of Ascaris spp.*
*Note: *Low Level to No Efficiency (LL) = undestroyed egg envelope, no recorded egg decays, and embryo dissolution; Satisfactory (S) = partially destroyed egg envelope, partial egg decays, and destroyed larvae egg; High-Level Efficacy (HLE) = entire destruction of the egg envelope of Ascaris spp. ova, decays of eggs into separate fragments, larvae that may present are found dead and embryo is dissolved.*

Figure 2 presented the results of Trial 2, which demonstrate a gradual increase in the effectiveness of the *Premna odorata* Blanco (Alagao) aqueous leaf extract against Ascaris spp., ova. An undestroyed egg envelope with no recorded egg decay and embryo dissolution (Low efficiency) was seen earlier during the 15, 30, and 45 minutes. However, the efficacy improved to satisfactory levels, meaning a partially destroyed egg envelope, partial egg decay, and destroyed larvae egg are observed at 60, 75, 90, 105, and 120 minutes later. The positive and negative controls exhibited consistently low efficacy throughout the experiment, indicating minimal impact on the Ascaris spp., ova.

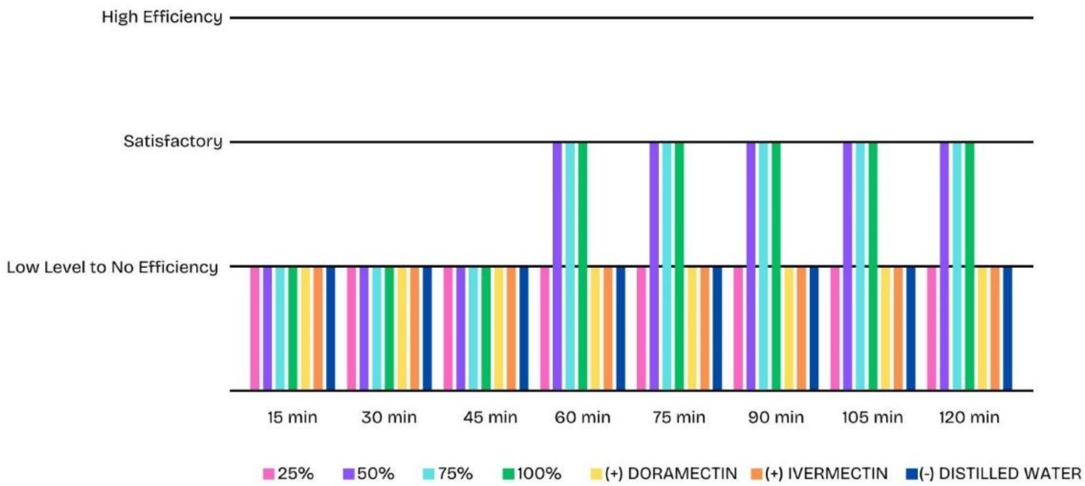

Figure 2. *Trial 2 of Experiment to Determine the Time of Exposure Required for Ovicidal Activity of Ascaris spp.*
*Note: *Low Level to No Efficiency (LL) = undestroyed egg envelope, no recorded egg decays, and embryo dissolution; Satisfactory (S) = partially destroyed egg envelope, partial egg decays, and destroyed larvae egg; High-Level Efficacy (HLE) = entire destruction of the egg envelope of Ascaris spp. ova, decays of eggs into separate fragments, larvae that may present are found dead and embryo is dissolved.*

Figure 3 illustrated the effectiveness of the *Premna odorata* Blanco (Alagao) aqueous leaf extract against the ovicidal activity of Ascaris spp. in Trial 3. The results reveal a varied effect on ova disruption times. Lower concentrations, corresponding to 15, 30, and 45 minutes, exhibited low effectiveness, demonstrating an undestroyed egg envelope with no recorded egg decay and embryo dissolution. However, there was a significant improvement in ova disruption between 60 and 120 minutes, partially destroyed egg envelope, partial egg decay, and destroyed larvae egg (satisfactory efficacy) observed at these concentrations. Both the positive and negative controls displayed minimal impact throughout the experiment.

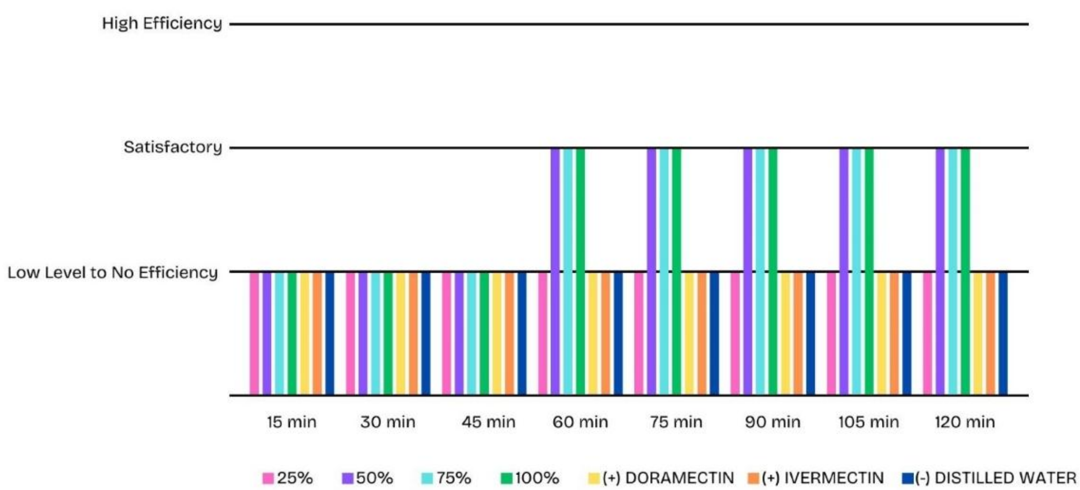

Figure 3. *Trial 3 of Experiment to Determine the Time of Exposure Required for Ovicidal Activity of Ascaris spp.*
*Note: *Low Level to No Efficiency (LL) = undestroyed egg envelope, no recorded egg decays, and embryo dissolution; Satisfactory (S) = partially destroyed egg envelope, partial egg decays, and destroyed larvae egg; High-Level Efficacy (HLE) = entire destruction of the egg envelope of Ascaris spp. ova, decays of eggs into separate fragments, larvae that may present are found dead and embryo is dissolved.*

Figure 4 presents the results of Trial 1, which investigated the minimum concentration of Premna odorata Blanco (Alagao) aqueous leaf extract required for ovicidal activity against Ascaris spp., ova. The three trials showed that the 25% concentration consistently exhibited low efficacy across all exposure times.

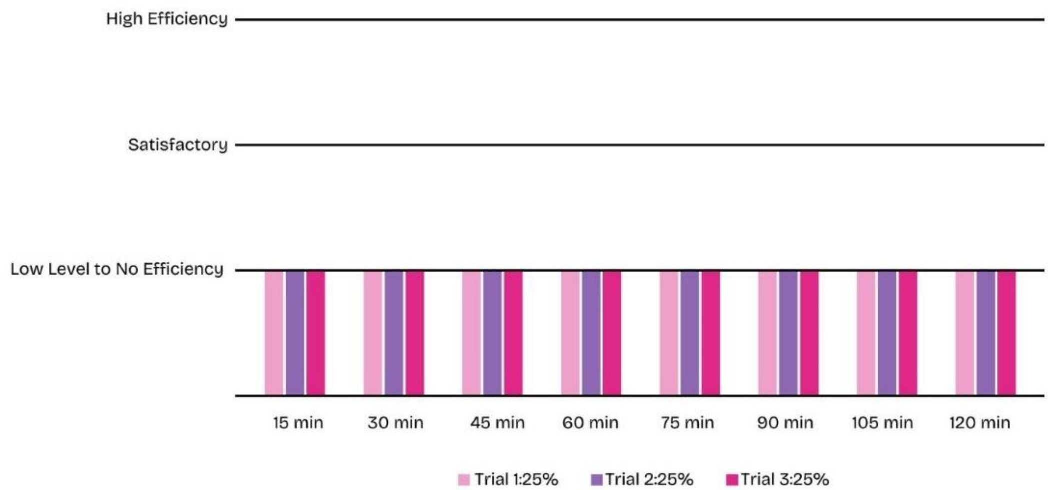

Figure 4. *25% Concentrated Premna odorata Blanco (Alagao) for Ovicidal Activity of Ascaris spp.*
*Note: *Low Level to No Efficiency (LL) = undestroyed egg envelope, no recorded egg decays, and embryo dissolution; Satisfactory (S) = partially destroyed egg envelope, partial egg decays, and destroyed larvae egg; High-Level Efficacy (HLE) = entire destruction of the egg envelope of Ascaris spp. ova, decays of eggs into separate fragments, larvae that may present are found dead and embryo is dissolved.*

Figure 5 revealed that the 50% concentration of *Premna odorata* Blanco (Alagao) demonstrates satisfactory efficacy in disrupting Ascaris spp., ova. It also showed that effective ovicidal activity with the 50% concentration begins at the 60-minute exposure time point.

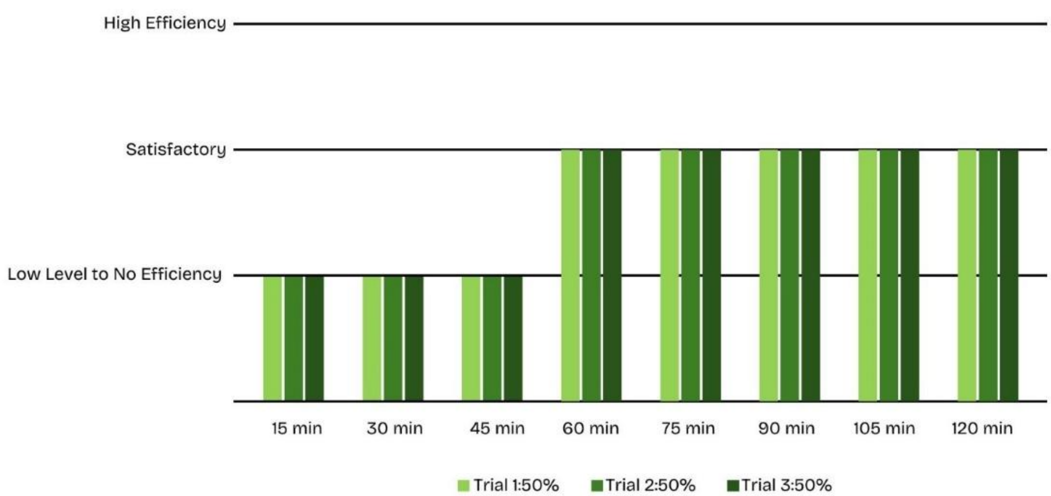

Figure 5. *50% Concentrated Premna odorata Blanco (Alagao) for Ovicidal Activity of Ascaris spp.*
*Note: *Low Level to No Efficiency (LL) = undestroyed egg envelope, no recorded egg decays, and embryo dissolution; Satisfactory (S) = partially destroyed egg envelope, partial egg decays, and destroyed larvae egg; High-Level Efficacy (HLE) = entire destruction of the egg envelope of Ascaris spp. ova, decays of eggs into separate fragments, larvae that may present are found dead and embryo is dissolved.*

Figure 6 demonstrated the results of all three trials, highlighting the efficacy of the 75% *Premna odorata* Blanco (Alagao) aqueous leaf extract concentration against Ascaris spp., ova. The extract displayed satisfactory efficacy in disrupting the Ascaris spp., across all trials. Hence, it also reveals that 60 minutes of exposure was required to achieve this 75% efficiency level.

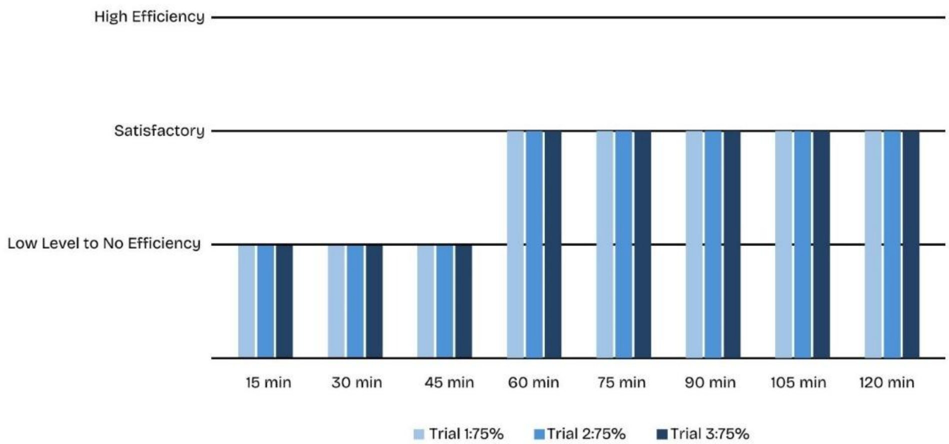

Figure 6. *75% Concentrated Premna odorata Blanco (Alagao) for Ovicidal Activity of Ascaris spp.*

*Note: *Low Level to No Efficiency (LL) = undestroyed egg envelope, no recorded egg decays, and embryo dissolution; Satisfactory (S) = partially destroyed egg envelope, partial egg decays, and destroyed larvae egg; High-Level Efficacy (HLE) = entire destruction of the egg envelope of Ascaris spp. ova, decays of eggs into separate fragments, larvae that may present are found dead and embryo is dissolved.*

Figure 7 confirmed the effectiveness of the 100% *Premna odorata* Blanco (Alagao) aqueous leaf extract concentration in disrupting Ascaris spp., ova across all three trials, potentially achieving even faster disruption than the 75% concentration.

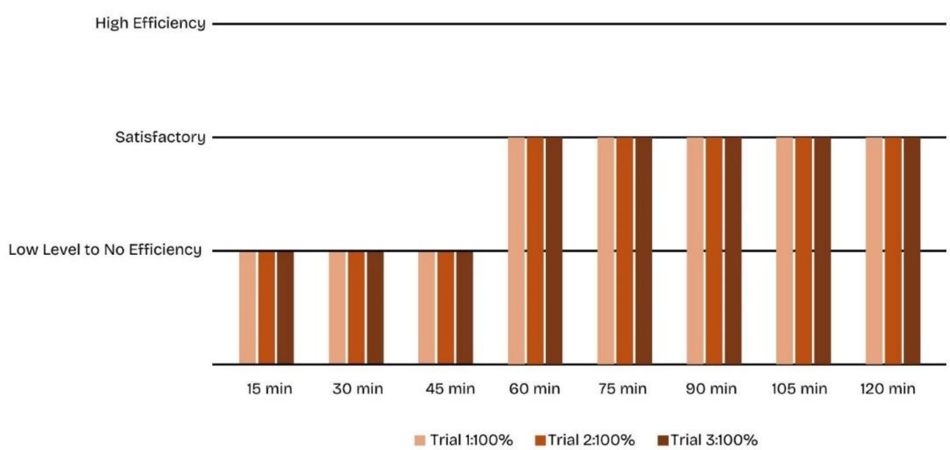

Figure 7. *100% Concentrated Premna odorata Blanco (Alagao) for Ovicidal Activity of Ascaris spp.*

*Note: *Low Level to No Efficiency (LL) = undestroyed egg envelope, no recorded egg decays, and embryo dissolution; Satisfactory (S) = partially destroyed egg envelope, partial egg decays, and destroyed larvae egg; High-Level Efficacy (HLE) = entire destruction of the egg envelope of Ascaris spp. ova, decays of eggs into separate fragments, larvae that may present are found dead and embryo is dissolved.*

Figure 8 revealed that neither positive nor negative control group had any significant ovicidal activity against Ascaris spp., ova over the tested period. Figures 2, 3, and 4 investigated the effectiveness of an aqueous leaf extract from *Premna odorata* Blanco (Alagao) against Ascaris spp., ova collected from recently dewormed swine. Three separate trials were conducted, and aqueous leaf extract was tested in varying concentrations: 25%, 50%, 75%, 100%. Doramectin and Ivermectin were used as positive controls, and distilled water served as the negative control; the required time for exposure ranged from 15 minutes to 120 minutes (2 hours). The results, consistent across three separate trials, reveal a variety of efficacy depending on the extract's concentration and the exposure duration. The efficacy was determined by examining the following characteristics (Melnychuk & Yuskiv, 2018). It consistently reveals a low-level efficiency to satisfactory efficacy of the *Premna odorata* Blanco (Alagao) aqueous leaf extract. Among the time required to disrupt the Ascaris spp., ova, there was a low efficiency observed between 15 minutes, 30 minutes, and 45 minutes. Hence, satisfactory efficiency was seen between 60 minutes, 75 minutes, 90 minutes, 105 minutes, and 120 minutes. The positive and negative controls used showed low efficiency throughout the experiment's time requirement, showing no significant effect on the Ascaris spp., ova.

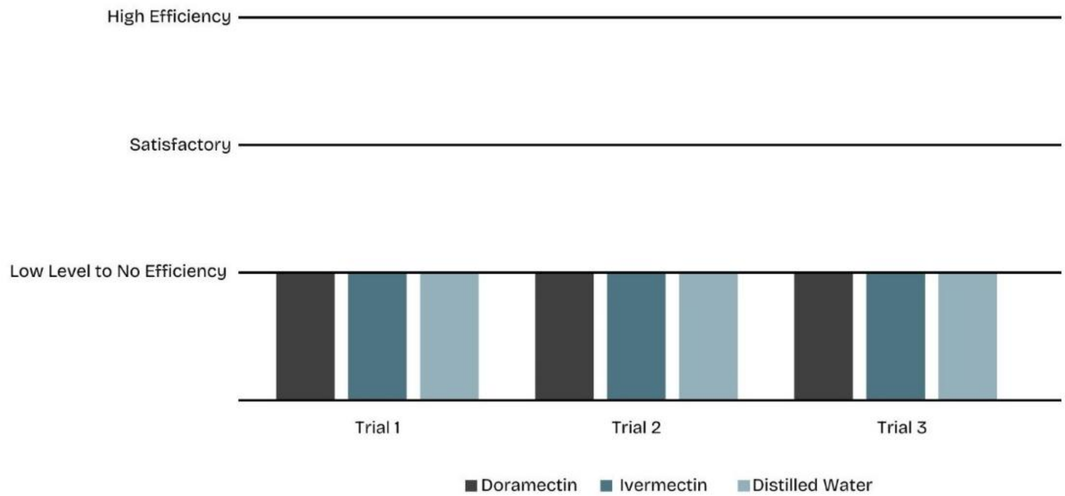

Figure 8. *Control Groups for Ovicidal Activity of Ascaris spp.*

*Note: *Low Level to No Efficiency (LL) = undestroyed egg envelope, no recorded egg decays, and embryo dissolution; Satisfactory (S) = partially destroyed egg envelope, partial egg decays, and destroyed larvae egg; High-Level Efficacy (HLE) = entire destruction of the egg envelope of Ascaris spp. ova, decays of eggs into separate fragments, larvae that may present are found dead and embryo is dissolved.*

Low-level efficiency at shorter exposure times could be attributed to several factors. Ascaris spp., ova possess a thick shell (Keyzers, 2019) that might require more than 15 to 45 minutes to penetrate and disrupt the internal structures. Also, the 25% concentration might be insufficient to disrupt the ova within a short timeframe, while higher concentrations such as 50%, 75%, and 100% might achieve better results. Since the specimens were collected from recently dewormed swine, the swine immune system might have already partially deactivated them (Sulik et.al, 2023), making them less vulnerable to the extract's action even at shorter exposure times.

The study demonstrated the ovicidal efficacy of *Premna odorata Blanco* (Alagao) aqueous leaf extract against *Ascaris spp.* ova at varying concentrations. Figure 5 revealed that a 50% concentration of the extract exhibited satisfactory efficacy, with effective ovicidal activity beginning at the 60-minute exposure mark. Similarly, Figure 6 highlighted the results of three trials using a 75% concentration, which also demonstrated satisfactory efficacy in disrupting *Ascaris spp.* ova, with a minimum of 60 minutes of exposure required to achieve this level of effectiveness. Figure 7 further confirmed the enhanced efficacy of the 100% concentration, which disrupted *Ascaris spp.* ova across all trials and potentially achieved faster disruption compared to the 75% concentration. In contrast, Figure 8 showed that neither the positive nor negative control groups exhibited any significant ovicidal activity over the tested period, underscoring the specific effectiveness of *Premna odorata Blanco* extract. These findings collectively suggest that higher concentrations of the extract yield faster and more efficient ovicidal activity, with the 100% concentration being the most effective. The lack of activity in the control groups further validates the extract's potential as a natural anthelmintic agent.

In comparison to the findings on *Premna odorata Blanco* (Alagao) aqueous leaf extract, a study by Panjaitan et al. (2021) demonstrated that *Cawat Anuman* (Bauhinia Sp.) leaf extract exhibited anthelmintic activity against *Ascaris spp.*, with a 50% concentration achieving significant disruption after 900 minutes of exposure, which is slower than the 60-minute efficacy observed with *Premna odorata Blanco* at the same concentration. Similarly, a study by Husori et al. (2016) found that *Allium fistulosum* (welsh onion) leaf extract required a 100% concentration and 232 minutes of exposure to achieve comparable anthelmintic activity, highlighting the superior efficacy of *Premna odorata Blanco* in disrupting *Ascaris spp.* ova at lower exposure times. However, both studies align with the observation that higher concentrations of plant extracts generally yield faster and more effective anthelmintic activity, as seen in the 100% concentration of *Premna odorata Blanco*. These comparisons emphasize the potential of *Premna odorata Blanco* as a promising natural anthelmintic agent, particularly due to its faster action at lower concentrations compared to other plant extracts.

3.3 Minimum Concentration Required for Ovicidal Activity of Ascaris spp.

The observed results suggests that higher concentrations of *Premna odorata* Blanco (Alagao) aqueous leaf extract might be more effective against Ascaris spp., ova, but requires a longer exposure time for optimal results. Among the varying concentrations prepared, 50% of the concentration shows significant results in partially disrupting the Ascaris spp., which serves as the minimum concentration required for the ovicidal activity of Ascaris spp. Figure 9 illustrated the Ascaris spp., ova at 50% concentrated *Premna odorata* Blanco (Alagao), the thick mammalian albuminous layer of Ascaris spp. was partially destroyed, showing half coverage only of its thick mammalian albuminous layer. Alongside 50% concentration efficacy, 75% and 100%, can also disrupt the Ascaris spp., ova.

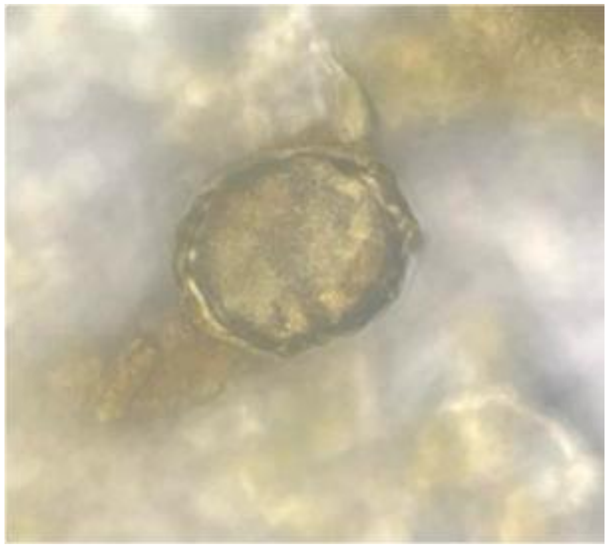

Figure 9. *Ascaris spp., ova at 50% concentration of Premna odorata Blanco (Alagao) aqueous extract under Oil Immersion Objective*

Figures 10 and 11 revealed that 100% of *Premna odorata* Blanco (alagao) aqueous leaf extract penetrated and disrupted the ova. It penetrated the thick mammalian albuminous layer of the Ascaris up until the germ cell, showing no content of its embryo. This result can significantly determine the effectiveness of *Premna odorata* Blanco (Alagao) aqueous leaf extracts as an alternative anthelmintic agent.

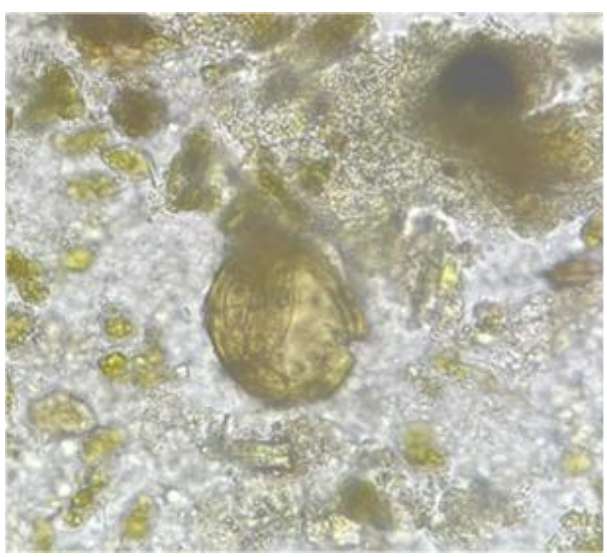

Figure 10. *A photo of Ascaris spp., ova partially destroyed of Premna odorata Blanco (Alagao) aqueous extract under Oil Immersion Objective*

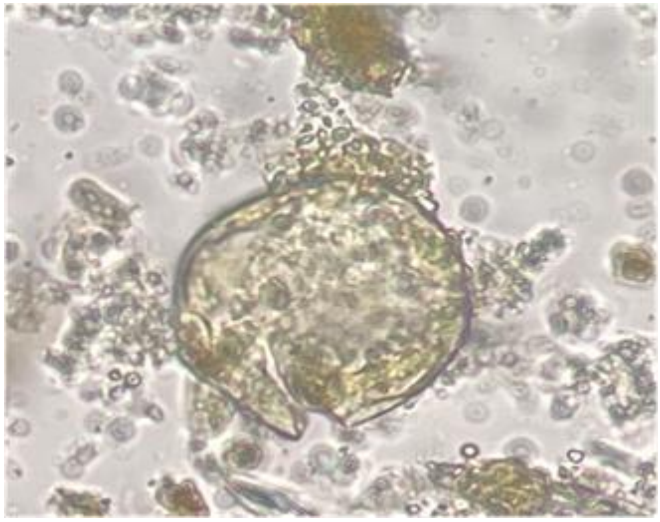

Figure 11. *A photo of Ascaris spp., ova partially penetrated by Premna odorata Blanco (Alagao) aqueous extract under Oil Immersion Objective*

4.0 Conclusion

The study's findings demonstrated the potential of *Premna odorata* Blanco (Alagao) aqueous leaf extract as an effective anthelmintic treatment against Ascaris spp., ova in swine. The extract, derived from the Lamiaceae family plant, provides a valuable alternative for managing emerging anthelmintic drug resistance in Tanauan City, Batangas, Philippines swine farms. In recent years, flavonoids have generated interest as potential therapeutic agents, particularly in an anthelmintic activity (Toklo et al., 2021) that can be used to develop alternatives to commercial anthelmintic drugs further. *Premna odorata* Blanco (Alagao) exhibits a multifaceted mode of action. The aqueous leaf extract effectively penetrates Ascaris spp., ova at various concentrations (50%, 75%, and 100%), disrupting their membranes and embryo development. Notably, compared to Doramectin and Ivermectin, the positive controls in this study showed lower effectiveness within the same timeframe. *Premna odorata* Blanco (Alagao) demonstrated significantly faster penetration within 60 minutes.

5.0 Contributions of Authors

R.O. Pumaras- Conceptualization; methodology; formal analysis; data curation; writing – original draft; writing – review & editing; supervision
S.M. Gozon- Conceptualization; methodology; formal analysis; data curation; writing – original draft; writing – review & editing; supervision
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7.0 Conflict of Interest

The authors declare that there is no conflict of interest.

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