In Vitro Ovicidal Activity of*Tabernaemontanapandacaqui* (Pandakaki) Leaf Ethanolic Extract AgainstAscaris lumbricoidesin Varying Concentrations

Abstract

Soil-transmitted helminth (STH) infections, particularly caused by Ascaris lumbricoides, continue to pose significant public health challenges in developing countries. In response to the limited alternative anthelmintic agents, this study investigates the in vitro ovicidal activity of Pandakaki (Tabernaemontana pandacaqui) leaf ethanolic extract against Ascaris lumbricoides ova at varying concentrations (25%, 50%, 75%, and 100%) and exposure durations (15, 30, and 45 minutes). Using the Formalin-Ether Concentration Technique (FECT) to isolate parasite ova and standardized morphological grading, the study found that higher concentrations and longer exposure durations significantly increased ovicidal activity. Notably, the 100% concentration at 45 minutes induced extreme shell thinning and deformation, with effects statistically comparable to the standard drug mebendazole (500 mg). Statistical analyses using One-Way ANOVA and Independent T-tests confirmed significant morphological changes at 75% and 100% concentrations ($p < 0.05$), while lower concentrations showed minimal efficacy. These results highlight the potential of Tabernaemontana pandacaqui as a natural ovicidal agent and support its future exploration as a plant-based alternative for helminth control in resource-limited settings.

Key words: Soil-transmitted helminths; Ascaris lumbricoides ova; Tabernaemontanapandacaqui leaf extract; ovicidal activity; natural anthelmintic

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Introduction:-

Soil-transmitted helminth (STH) infections are among the most prevalent infections globally, affecting an estimated 1.5 billion people or approximately 24% of the world's population (World Health Organization, 2023) and these infections are most common in tropical and subtropical regions, with the highest prevalence observed in sub-Saharan Africa, China, South America, and Asia (World Health Organization, 2023). The Pan American Health Organization highlights that the main risk factors for helminth infections include lack of access to water, basic sanitation, and poor hygiene.

According to Gilmour et al. (2021), Soil-Transmitted Helminth (STH) infections in Southeast Asia, covering countries such as Malaysia, India, Thailand, Laos, the Philippines, Vietnam, China, and Nepal, reported an overall prevalence of 61.4%, with species-specific rates of 32.3% for Ascaris lumbricoides, 43.6% for Trichuris trichiura, 19.9% for hookworm, and 6.3% for Strongyloidesstercoralis. Additionally, Ascaris lumbricoides prevalence was higher in minority populations (41%) than non-minority groups (25%) and in the Western Pacific (40%) compared to Southeast Asia (17%), with China (68%) reporting the highest and Thailand (14%) the lowest rates.

Most of the regions in the Philippines are still in danger because of their prevalence and infection rates of 24.9% to 97.4% (Mationg et al., 2021). In fact, even after interventions started in 2006, especially the Mass Drug Administration (MDA), infections are still more than the threshold that aims to bring STH cases in areas such as Laguna to less than 20% (Mationg et al., 2021). STH infections are caused by parasitic worms, with Ascaris lumbricoides being a primary example (de Lima Corvino & Horrall, 2023). The worms live in regions where there is poor sanitation and are usually spread through dirty soil, food, and contaminated water (World Health Organization, 2023).

Ascaris lumbricoides is the largest roundworm infecting humans that causes Ascariasis and the most common helminthic infection worldwide (Ahmed, 2023). Adult females can grow to lengths of 20 – 30 cm, while males typically range from 15 – 20 cm. Female worms are thicker with a straight tail, whereas males are slimmer and have a curved tail with two retractable copulatory sspicules (e Lima Corvino & Horrall, 2023).

According to Giri (2019), Ascaris lumbricoides eggs can be found in two forms: unfertilized and fertilized, unfertilized eggs are larger, approximately 90 µm x 45 - 90 µm in size, while fertilized eggs are round to oval in shape, measuring 50 - 70 µm x 40 - 50 µm. Some eggs found in feces lack the outer mamillated albuminous coat and are referred to as decorticated eggs. Decorticated infertile eggs may be easily mistaken for the eggs of other parasites (Mathison & Pritt, 2022).

Plant extracts have garnered interest due to their potential use against bacteria and helminths, possibly aiding in the use in medicine. Pandakaki (Tabernaemontanapandacaqui) belongs to the Apocynaceae family, which is found in tropical areas, especially in Southeast Asia. Plants in this family are known for their healing properties and have been used to treat various health problems (Saldo et al., 2023). According to the article on Leaves and Beaks (2021), Pandakaki (Tabernaemontanapandacaqui), contains phytochemicals with potential antiviral, antifungal, anti-inflammatory, antibacterial, antioxidant, and anticancer properties. Additionally, its bark juice can treat mouth sores, and the leaves are used to treat skin disorders like psoriasis and dermatitis. This study aims to assess the in vitro ovicidal activity of Pandakaki (Tabernaemontanapandacaqui) leaf ethanolic extract against Ascaris lumbricoides ova at varying concentrations. Specifically, it seeks to determine whether the morphological appearance of Ascaris lumbricoides ova is altered when exposed to different concentrations of the extract, whether the duration of exposure affects these morphological changes, and how the concentration of the extract and the length of exposure interact in influencing the morphological appearance of the ova.

Research Design

This study will use experimental quantitative research design. The independent variable will be the concentration of ethanolic extract measured in triplicate at different concentrations while the dependent variable will be the morphological alterations seen in the Ascaris lumbricoides ova. To determine the extract's ovicidal activity, we will expose the ova to the different concentrations of the extract and evaluate their morphology. Our aim in gathering this data is to provide reliable and accurate answers to our research questions.

Research Flow chart

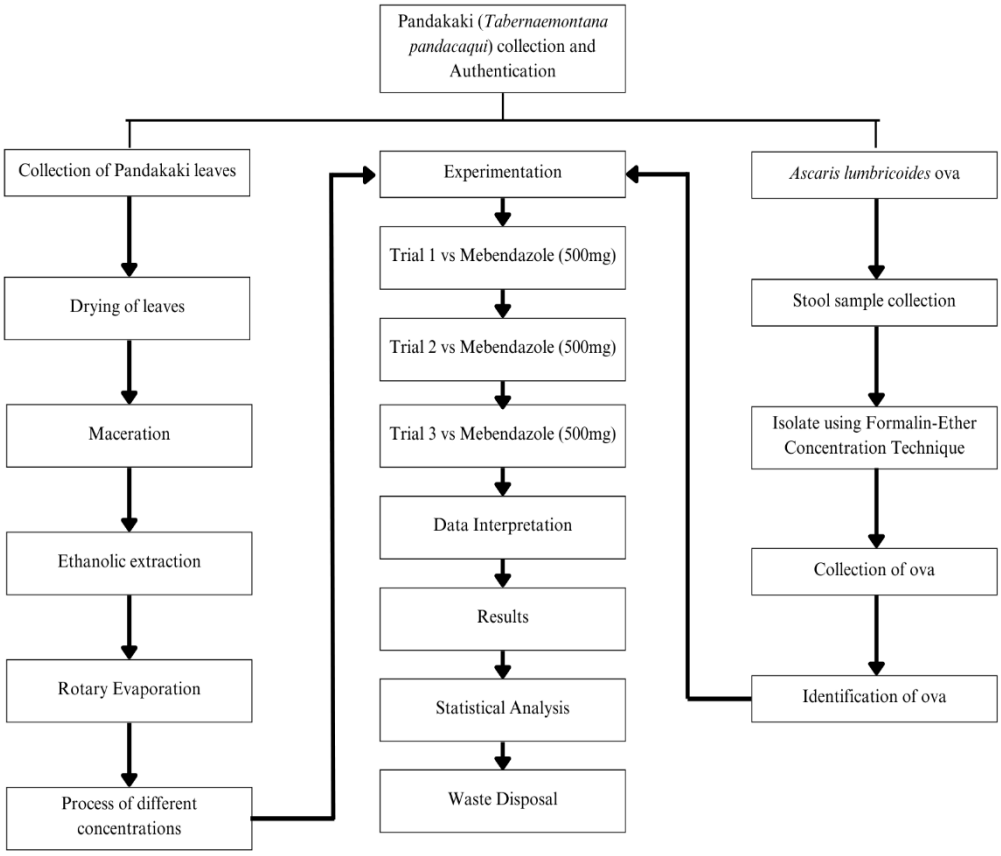


Figure 3.1 Process of the Experiment for In Vitro Ovicidal Activity of Pandakaki (Tabernaemontanapandacaqui) Leaf Ethanolic Extract Against Ascaris lumbricoides ova in Varying Concentrations

Plant Acquisition

The plant Pandakaki (Tabernaemontanapandacaqui) were sourced from a local farm, Maria's Garden in Talisay, Batangas. The farm was carefully selected to ensure the quality of the plant samples. To preserve the plant's bioactive compounds, the Pandakaki (Tabernaemontanapandacaqui) plant specimens were freshly harvested from the garden and were therefore available for use in purposes of intended research.

Verification of Plant

The acquired Pandakaki (Tabernaemontanapandacaqui) will be submitted to the University of Santo Tomas Herbarium located at Thomas Aquinas Research Complex, University of Santo Tomas, España Boulevard, Sampaloc, Manila, for verification. This is to ensure proper identification and authenticity of the plant to guarantee the integrity of the research.

Plant Extraction

The verified plant source for Pandakaki (Tabernaemontanapandacaqui) Ethanolic extract will be sourced from Maria's Garden in Talisay, Batangas and will be limited only to the use of its leaves. The leaves are hand-picked,

then washed thoroughly using tap water to remove dirt and unwanted residues. The cleaned leaves are dried in a conventional oven to avoid degradation of chemical integrity. The optimal drying temperature of 50℃ - 55℃ will be maintained for the leaves to preserve their bioactive compounds (El Gamal et al., 2023). The dried leaves will then be powderized using a blender. Approximately 100 g of the powdered leaves will be measured in a 1000 mL beaker to which 500 mL of 95% ethanol will also be added. The extraction ratio will be based on a 1:5 ratio. The mixture of the solvent will then be stirred, and the beaker's mouth will be covered with aluminum foil to prevent contamination and entry of particles. The mixture shall be kept at room temperature for 24 hours, the mixing will be done every 8 hours. The 24-hour extraction time was based on the study of Aranda-Ledesma et al. (2024) which indicates that the optimal extraction of bioactive compounds is within this period. After 24 hours, the mixture will be filtered through a funnel and Whatman filter paper grade 1. The extract would be then distilled under low-pressure by means of rotary evaporation to remove the solvent and obtain the residue of the extract. The water bath of the rotary evaporator will be set at a temperature of 30℃ - 40℃, with a 15℃ - 20℃ ethanol vapor temperature to prevent thermal decomposition of bioactive compounds. The confirmation that 95% ethanol is no longer present will be the flame test, whereby a flame occurs in the presence of 95% ethanol. If so, repeat the process of low-pressure distillation using a rotary evaporator. This method was adapted from Hidyatik et al (2023), with minor modifications made by the researchers to meet the specific requirements of this study. The entire ethanolic extraction process will be repeated in triplicates to ensure the reproducibility and validity of the procedure.

Varying Concentrations

This study will evaluate the in vitro ovicidal activities of different concentrations (25%, 50%, 75%, and 100%) in triplicate of extracts of Pandakaki's (Tabernaemontanapandacaqui) against Ascaris lumbricoides, with mebendazole as a positive control and normal saline solution (NSS) as a negative control. Six eggs will be included for each trial, whereas one egg will be used for one concentration or control. The percent solution will be used for the calculation of varying concentrations by the researchers. In particular, the preparation of each concentration will be: 25% (1 mL of extract + 3 mL of distilled water), 50% (2 mL of extract + 2 mL of distilled water), 75% (3 mL of extract + 1 mL of distilled water), and 100% (4 mL of extract with no dilution).

Percent solution formula

$$\%\ \frac{Volume\ of\ Solute}{Volume\ of\ Solution} = \frac{Number\ of\ milliliters\ of\ Solute}{Number\ of\ milliliters\ of\ Solution}\ x\ 100$$

I. FIGURE 3.2 *PERCENT SOLUTION FORMULA*

Table 3.1 Varying Concentrations of Pandakaki Extract

VARYING CONCENTRATIONS OF PANDAKAKI EXTRACT	
Concentration (%)	**Formula**
To obtain 25% concentration:	$\frac{1mL\ (100\%\ Pandakaki\ Ethanolic\ Extract)}{4mL}$ x 100
To obtain a 50% concentration:	$\frac{2mL\ (100\%\ Pandakaki\ Ethanolic\ Extract)}{4mL}$

	x 100
To obtain a 75% concentration:	$\frac{3mL\ (100\%\ Pandakaki\ Ethanolic\ Extract)}{4mL}$ x 100
To obtain 100% concentration:	$\frac{4mL\ (100\%\ Pandakaki\ Ethanolic\ Extract)}{4mL}$ x 100

Parasite Acquisition

Ascaris lumbricoides eggs were collected from stool samples that were collected from children residing in Baclaran. The feces, once collected, were handled with proper care as per biosafety requirements to prevent contamination and protect the researchers. The feces were subsequently added to leak-proof, sterile containers and sealed inside a resealable plastic container to prevent drying and stored at temperature control to preserve them. Stool samples were kept in an ice box while in transit to keep the temperature at a required level necessary. The samples were labeled and handled carefully to facilitate safe and efficient transport to the laboratory. Collection was done strictly by ethical standards and with full consent of parents or guardians of the children. In this way, the participants'rights were protected.

Once the samples were in the laboratory, the researchers started to extract the ova from the feces using the Formalin-Ether Concentration Technique (FECT). It is formulated on the principle of sedimentation in which formalin preserves the sample and ethyl acetate acts as a debris separator. It is utilized since it increases the sensitivity of microscopic analysis and makes it easier to detect and identify parasites.

For fixation of Formalin-Ether, 1 g of stool is mixed with 10 mL of 10% formalin in a test tube and kept at room temperature for at least 30 minutes. The suspension after fixation is strained through a double layer of gauze into a 15 mL conical tube and centrifuged for 10 minutes at 500 g relative centrifugal force. Afterwards, decant the supernatant, leaving 0.5 - 1.5 mL of sedimented material. Resuspend the sediment by adding 7 mL of saline and re-centrifuge for 10 minutes at 500 g. Add 3 mL of ethyl acetate to extract fats and debris. Seal with a rubber stopper and shake vigorously for 30 seconds. Allow you to stand for 15 - 30 seconds, then carefully remove the rubber stopper. Centrifuge again for 10 minutes, allowing the contents of the tube to separate into four layers: sediment, saline, fecal debris, and ethyl acetate from bottom to top. Detach the plug of debris from the tube using an applicator stick. Afterwards, decant the top three layers by inverting the tube with a brisk movement. Using a pipette, mix the sediment with the remaining liquid that drains from the sides of the tube. Prepare a wet mount examination by placing a drop of the sediment on a glass slide and covering it with coverslip. Examine the sediment under the microscope. This methodology is adapted from the World Health Organization, with minor modifications made by the researchers to suit the requirements of the study.

Statistical Analysis

The data of Pandakaki's ovicidal effect of different concentrations were analyzed for the means and standard deviation. The significance of the results was evaluated using the Independent T-test and One-Way ANOVA .

Ethical Consideration

The researchers must go through an ethics review and approval from a Research Ethics Committee (REC) that is certified by PHREB and follow the guidelines provided by the NU MOA Ethics Review Committee (ERC) before undertaking the study. There must be a risk-benefit assessment in the protocol of the study, where any risks involved will be minimized and justified (PHREB, 2022). The process ensures that the study complies with national ethical standards and safeguards the welfare and rights of minor participants (PHREB, 2022).

Additionally, ethical research on children must not use coercion and undue inducement. Compensation that is given must be reasonable and not applied as undue inducement (PHREB, 2022). It must be fully voluntary, and the children must not be forced or required to participate (PHREB, 2022). For studies with 5 minor participants, such ethical considerations should be stringently adhered to for adherence to both Philippine government policy and ethical research principles by the NU MOA Ethics Review Committee (ERC) (PHREB, 2022). Additionally, once the samples are collected, participants will be equitably compensated in order to appreciate their effort without acting as an undue inducement. This maintains ethical standards for the research process.

To provide appropriate informed consent, the researchers implemented a two-stage consent process. In the first stage, the research details such as the aim, process, possible risks and benefits, were explained in terms the guardian of the minor participants understood. Guardians were asked to sign a signed written informed consent form prior to their child's participation. Second, age-appropriate verbal consent was also obtained from the children to ensure they knew their place in the study and their right to withdraw at any time with no consequences. Due to the sensitive nature of taking stool samples, further efforts were made to preserve participant dignity, including offering explicit instructions for the collection of samples.

The Ethical Review Committee has certified this research, and the researchers have taken due cognizance of the recommendations emplaced by the committee and made suitable modifications in the affairs of the participants while still recognizing that other ethical considerations must be incorporated. Ethical review is the process by which research is ensured to fulfill the guidelines regarding the maintenance of rights and well-being of participants. Ethics review documentation is also there to ensure transparency and accountability to ethical principles.

Waste Disposal

Proper waste disposal research is required for the use of ethanolic extracts of Pandakaki (Tabernaemontanapandacaqui) and Ascaris lumbricoides ova with respect to the environment and biosafety regulations. Indeed, the first important element of destruction is to sort the waste into biological and chemical waste. The biological waste included Ascaris lumbricoides eggs, which must be disposed of in special disposal boxes termed biohazard containers so that there is no contamination (Smith et al., 2020). Stool samples suspected of harboring parasites must also be detoxified, as this may reduce transmission of infection; a common method is using sodium hypochlorite-based bleach solutions-preferred being Zonrox. A 1:10 dilution (1-partZonrox to 9 parts water) is recommended for disinfecting contaminated materials, with a contact time of at least 30 minutes to inactivate the parasite eggs and larvae (University of Waterloo Safety Office, 2022).

After disinfection, reusable items should be rinsed with water to avoid damage, especially to metal surfaces. Disposable materials like gloves and wipes should be securely sealed in biohazard bags for safe disposal. Importantly, bleach should never be mixed with other chemicals such as ammonia or acids because toxic gases can form (Stanford Environmental Health & Safety, 2021; University of Waterloo Safety Office, 2022).

Treated biological waste should be disposed of in approved landfills, while chemical waste should be incinerated or processed at specialized facilities to prevent environmental contamination (Johnson & Lee, 2021). These measures ensure the safe and ethical handling of laboratory waste, safeguarding public health and the environment.

Ethanol and formalin must be stored in labeled, air-tight hazardous waste containers to prevent evaporation and contamination. Ethanol waste should be collected in fire-resistant containers, stored in a dedicated flammable storage cabinet, and disposed of through accredited hazardous waste management services. Formalin, due to its toxicity and volatility, should be kept in a well-ventilated area and handled with fume hoods when necessary. Any spills must be contained immediately using absorbent materials, which should then be sealed in hazardous waste bags and disposed of according to safety protocols.

Researchers must wear PPE, including gloves, lab coats, masks, and face shields. Work should be performed in a biosafety cabinet, and contaminated surfaces disinfected with a 10% bleach solution (Smith et al., 2020). These measures ensure researcher safety and environmental protection.

Assessment of Ova

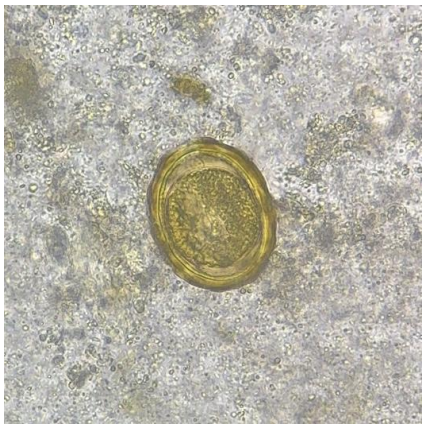
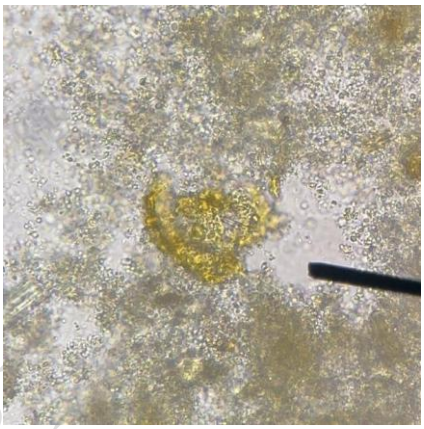
Due to the limited availability of articles and established guidelines regarding the morphological grading or assessment of Ascaris lumbricoides ova, the researchers will verify the criteria they have created through three different licensed Medical Technologists to ensure accurate and reliable evaluation. This collaboration will provide expert insights and enhance the validity of the morphological observations in this study.

Table 3.2 *Criteria for observing the alteration in the morphological aspect of Ascaris lumbricoides*

3	2	1	0
Shell ruptured or collapsed; extreme thinning, leakage, or complete deformation.	Major distortion, irregular shape, severe thinning.	Includes moderate damage, mild distortion, and localized thinning.	Normal development and morphology; no damage, regular in shape, normal thickness and texture.

The table above (Table 3.2 Criteria for observing the alteration in the morphological aspect of Ascaris lumbricoides ova) will serve as a guideline for the researchers to assess the ovicidal activity of Pandakaki (Tabernaemontanapandacaqui). Each criterion is scored from 0 to 3, with higher scores indicating greater morphological abnormalities. A score of 3 indicates severe thinning or shell rupture, leakage of contents, or severe deformation. 2 represents major distortion, severe thinning, and irregularity in shape. 1 shows moderate damage characterized by mild distortion and localized thinning. Lastly, 0 denotes normal development and morphology, characterized by the absence of damage, regular shape, and normal thickness and texture. The information used to accomplish the criteria was based on the study of Hass et al. (2024). To ensure accuracy and reliability, conducting inter-rater reliability testing among trained observers, and utilizing reference images and objective criteria to minimize subjectivity; this approach will be verified in collaboration with three registered medical technologists to avoid unbiased assessments.

RESULTS AND DISCUSSION

***Ascaris lumbricoides* ova: Negative control**	***Ascaris lumbricoides* ova: Positive control**
	
The ova of *Ascaris lumbricoides* were observed under a microscope at 400x magnification (High power objective).	

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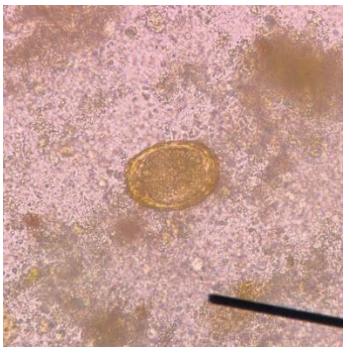
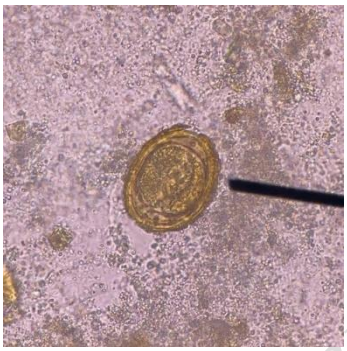
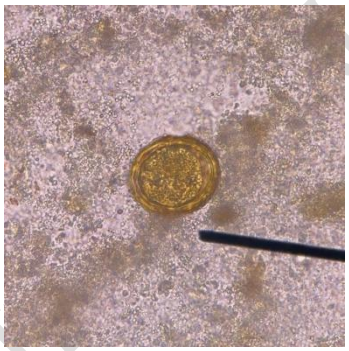
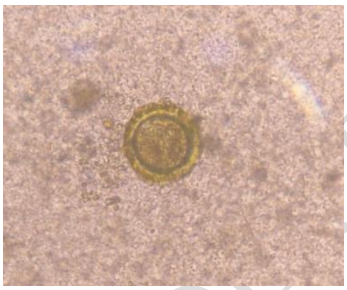
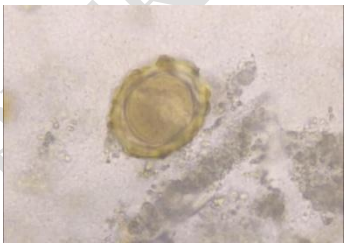
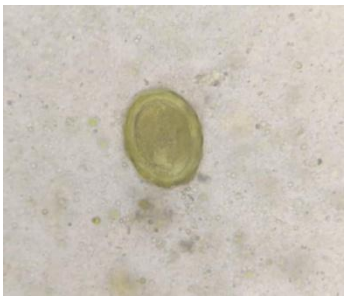
***Ascaris lumbricoides* ova in 25% Pandakaki Ethanolic Extract**		
TRIAL 1		
		
15 minutes	**30 minutes**	**45 minutes**
TRIAL 2		
		
15 minutes	**30 minutes**	**45 minutes**

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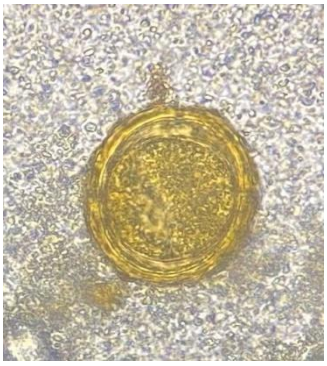
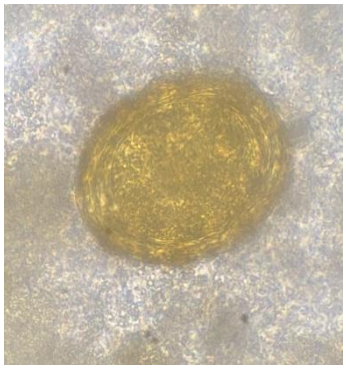
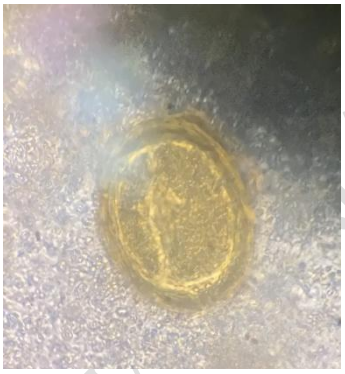
TRIAL 3		
		
15 minutes	**30 minutes**	**45 minutes**
The ova of *Ascaris lumbricoides* were observed under a microscope at 400x magnification (High power objective).		

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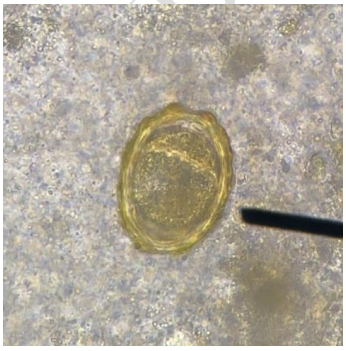
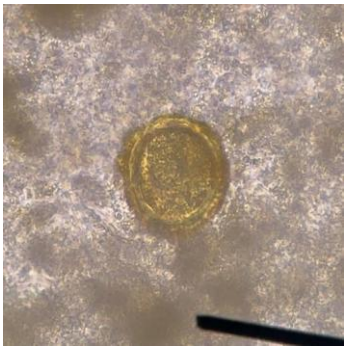
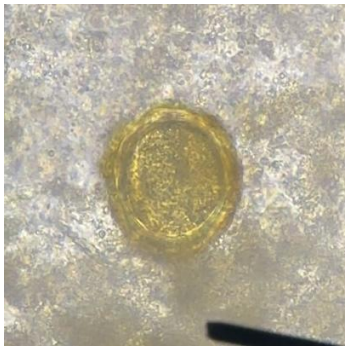
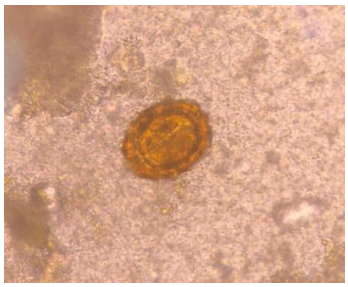
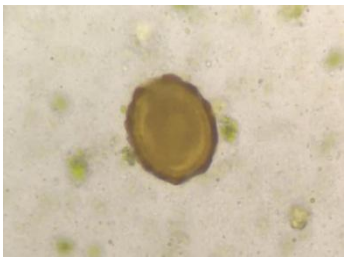
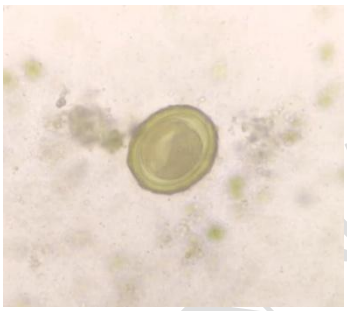
***Ascaris lumbricoides* ova in 50% Pandakaki Ethanolic Extract**		
TRIAL 1		
		

15 minutes	30 minutes	45 minutes
TRIAL 2		
		
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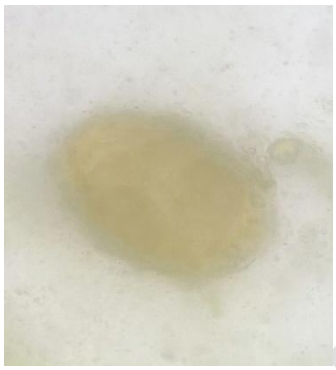	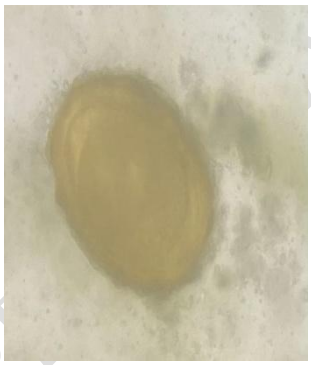	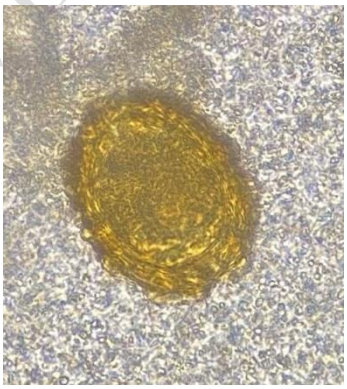
15 minutes	**30 minutes**	**45 minutes**

The ova of *Ascaris lumbricoides* were observed under a microscope at 400x magnification (High power objective).

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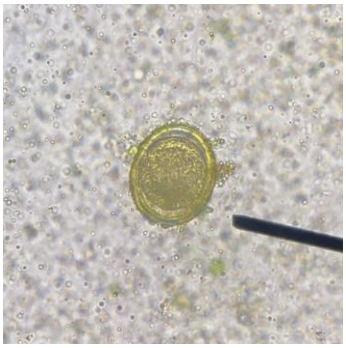
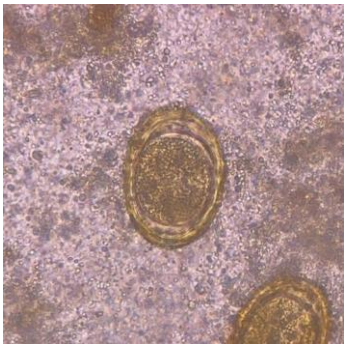
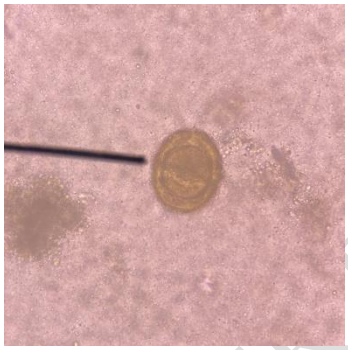
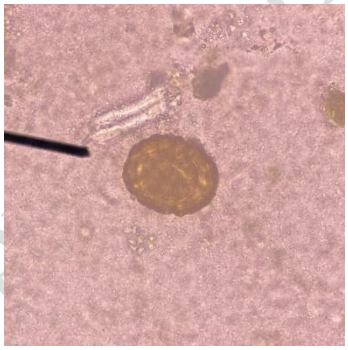
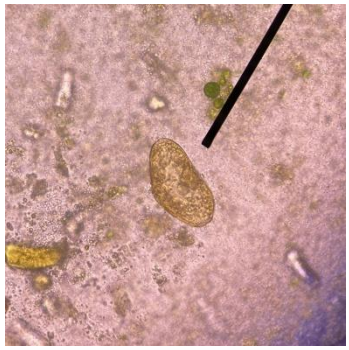
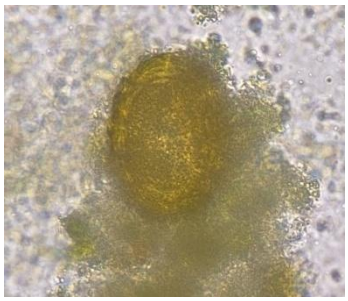
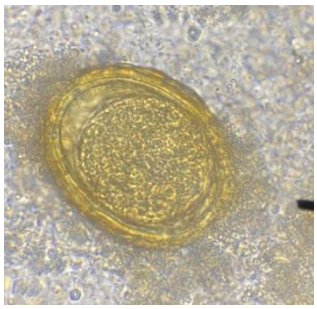
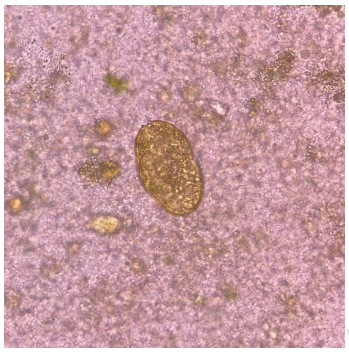
***Ascaris lumbricoides* ova in 75% Pandakaki Ethanolic Extract**		
TRIAL 1		
		
15 minutes	**30 minutes**	**45 minutes**
TRIAL 2		
		
15 minutes	**30 minutes**	**45 minutes**
TRIAL 3		

		
15 minutes	**30 minutes**	**45 minutes**
The ova of *Ascaris lumbricoides* were observed under a microscope at 400x magnification (High power objective).		

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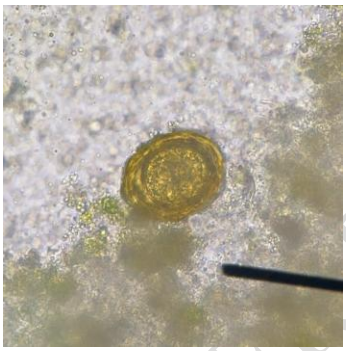
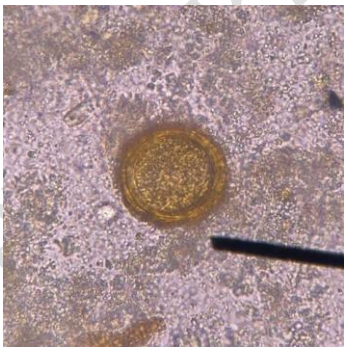
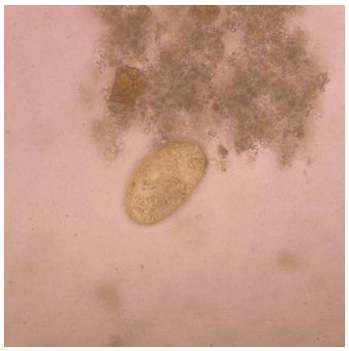
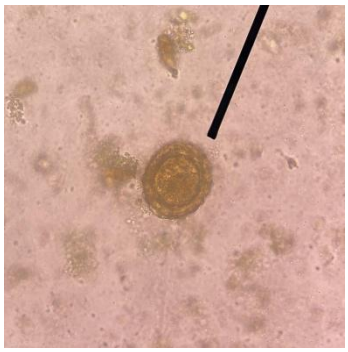
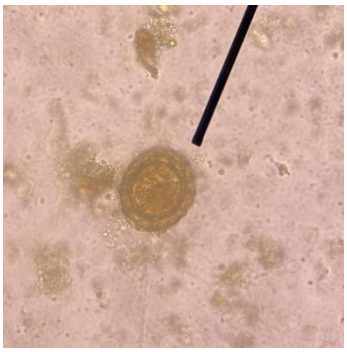
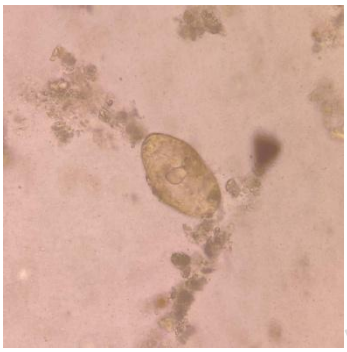
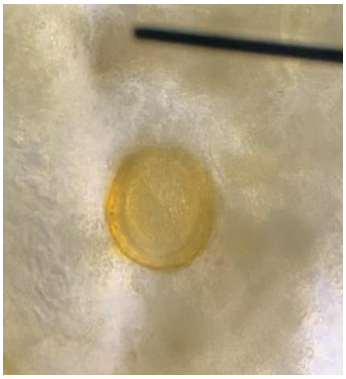
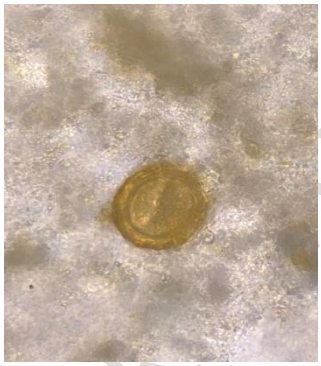
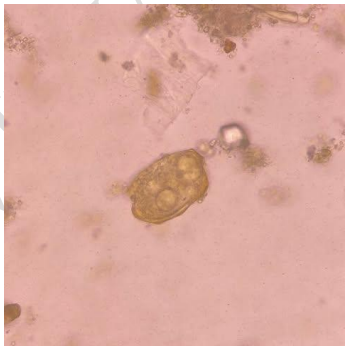
***Ascaris lumbricoides* ova in 100% Pandakaki Ethanolic Extract**		
TRIAL 1		
		
15 minutes	**30 minutes**	**45 minutes**
TRIAL 2		

		
15 minutes	**30 minutes**	**45 minutes**
TRIAL 3		
		
15 minutes	**30 minutes**	**45 minutes**

The ova of *Ascaris lumbricoides* were observed under a microscope at 400x magnification (High power objective).

I. **TABLE 4.1** *DESCRIPTIVE STATISTICS OF PANDAKAKI'S OVICIDAL ACTIVITY ACROSS VARYING CONCENTRATIONS*

DESCRIPTIVE STATISTICS					
	N	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
25%	27	0	1	.04	.192
50%	27	0	2	.56	.801

75%	27	0	2	.78	.801
100%	27	0	3	1.00	1.000
Valid N (listwise)	27				

Table 4.1 presents the data on Pandakaki's ovicidal activity across different concentrations. For each concentration, N represents the 27 data sets from each respondent, which were used to calculate the reported mean and Standard Deviation. The Mean indicates the average ovicidal activity observed at each corresponding concentration, and the Standard Deviation, quantifies the variability of the Pandakaki's ovicidal activity. The 100% concentration exhibited the highest means (1.00), indicating the strongest effect. The ova exposed to 100% concentration exhibited extreme thinning of the shell and complete deformation of their structure. The minimum effective concentration seems to be 50%, as there is a notable increase in the mean compared to 25%. Additionally, concentrations below 25% showed minimal activity, highlighting the importance of reaching at least 50% for significant results.

I. **TABLE 4.2** *DESCRIPTIVE STATISTIC OF PANDAKAKI'S OVICIDAL ACTIVITY ACROSS DIFFERENT EXPOSURE TIME*

DESCRIPTIVE STATISTICS					
	N	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
15 minutes	36	0	2	.28	.513
30 minutes	36	0	2	.36	.639
45 minutes	36	0	3	1.14	.990
Valid N (listwise)	36				

Table 4.2 presents the data of Pandakaki's ovicidal activity across different exposure times. For each concentration, N represents the 36 data sets from the corresponding time of exposure, which were used to calculate the reported Mean and Standard Deviation. The Mean indicates the average ovicidal activity observed at each corresponding length of exposure and the Standard Deviation quantifies the variability of the Pandakaki's ovicidal activity. With the highest mean value of 1.14 observed at 45 minutes, suggesting this duration produces the greatest impact; although some changes start to appear at 30 minutes, the most significant results occur after 45 minutes. The ova exposed to 100% concentration for 45 minutes exhibited extreme thinning of the shell and complete deformation of the structure.

Table 4.3 *ANOVA*

		Sum of Squares	df	Mean Square	F	Sig
25%	Between Groups	.130	3	.043	1.193	.335
	Within Groups	.833	23	.036		
	Total	.963	26			
50%	Between Groups	2.403	3	.801	1.291	.301
	Within Groups	14.264	23	.620		
	Total	16.667	26			
75%	Between Groups	10.958	3	3.653	14.718	.000
	Within Groups	5.708	23	.248		
	Total	16.667	26			
100%	Between Groups	17.528	3	5.843	15.861	.000
	Within Groups	8.472	23	.368		
	Total	26.000	26			

Table 4.3 shows that at 25% concentration, the p-values are 0.335 which is greater than 0.05, which means we fail to reject the null hypothesis. At 50% concentrations, the p-values are 0.301 which is still greater than 0.05, which means we still fail to reject the null hypothesis. The ova exposed to 25% and 50% concentrations displayed normal development: no shell damage, regular in shape, and normal thickness and texture. At 75% and 100% concentrations, the p-values are 0.000, which are less than 0.05. This means we reject the null hypothesis and accept the alternative hypothesis. The ova at 75% displayed major distortion and severe shell thinning, while those at 100% demonstrated extreme shell thinning and complete deformation.

II. **TABLE 4.4** *SUMMARY OF THE INDEPENDENT T-TEST FOR THE VARYING CONCENTRATIONS*

Concentration	t-value	df	P-value (Sig. 2-tailed)	Interpretation
25%	-6.399	27.235	0.000	Significant difference from mebendazole

50%	-3.633	44.294	0.001	Significant difference from mebendazole
75%	-2.855	44.294	0.007	Significant difference from mebendazole
100%	-1.925	49.635	0.060	*Not* significantly difference from mebendazole

Based on table 4.4, the 25%, 50%, and 75% concentrations of Pandakaki extract demonstrated statistically significant differences from mebendazole ($p < 0.05$), indicating a lower ovicidal effect at these levels. In contrast, the 100% concentration yielded a p-value of 0.060, which, while not statistically significant at the 0.05 threshold, suggests a comparable ovicidal effect to that of mebendazole due to its close proximity to significance. The ova exposed to 100% concentration exhibited extreme thinning of the shell and complete deformation of their structure.

Summary of Results

This study aimed to evaluate the in vitro ovicidal activity of Tabernaemontanapandacaqui (Pandakaki) leaf ethanolic extract against Ascaris lumbricoides ova using different concentrations: 25%, 50%, 75%, and 100%. Results revealed that the extract's ovicidal effect increased with concentration. The 100% concentration had the highest mean morphological damage score, while the 25% concentration showed minimal effect. Based on statistical analysis using One-Way ANOVA, significant morphological changes were observed at 75% and 100% concentrations ($p < 0.05$), indicating that the extract at these levels has strong ovicidal properties. Conversely, 25% and 50% concentrations were not found to exhibit effective efficacy.

Compared to the clinical anti-helminthic drug mebendazole (500 mg), the 75% and 100% concentrations of Pandakaki extract did not show any significant difference in efficacy statistically. This suggests that Pandakaki's ovicidal activity at these higher concentrations is as effective as a clinically applied drug. On the other hand, the 25% and 50% concentrations were significantly less effective than mebendazole, emphasizing that lower doses are insufficient to match the drug's ovicidal performance. These findings highlight Pandakaki's potential as a natural alternative treatment provided it is used at sufficiently high concentrations.

The influence of exposure time was also tested at 15, 30, and 45 minutes. Progressive development of ovicidal action as exposure time to Tabernaemontanapandacaqui leaf ethanolic extract increases. Indeed, the average morphological damage score of Ascaris lumbricoides ova was 0.28 after 15 minutes, 0.36 after 30 minutes, and reached 1.14 after 45 minutes. This trend suggests that the extract's ovicidal becomes more effective with prolonged exposure. While minimal morphological alterations were observed at the 15-minute mark, and slight improvements became evident at 30 minutes, the most substantial damage to the ova was recorded after 45 minutes of exposure.

Therefore, 45 minutes appears to be the most effective exposure time for inducing observable morphological changes under the conditions of this study.

In support of this, for each exposure time, 36 data sets were analyzed to compute the reported means and standard deviations. The mean values reflect the average ovicidal activity observed at each length of exposure, while the standard deviation indicates the consistency of the results. The highest mean score of 1.14 at 45 minutes indicates the strongest ovicidal impact during this time period. Notably, the ova exposed to 100% concentration for 45 minutes exhibited extreme thinning of the shell and complete deformation of the structure, reinforcing the finding that both concentration and exposure duration are critical for maximizing ovicidal effect.

Conclusion

This study aimed to evaluate the in vitro ovicidal activity of Tabernaemontanapandacaqui (Pandakaki) leaf ethanolic extract against Ascaris lumbricoides ova at varying concentrations and exposure durations. The findings clearly demonstrate that the ovicidal activity of the extract is significantly concentration-dependent, with 75% and 100% concentrations producing the most pronounced morphological damage to the ova. At these higher concentrations, the extract's effect was comparable to mebendazole (500 mg), showing no statistically significant difference in ovicidal action.

Conversely, 25% and 50% concentrations showed minimal morphological changes, and statistical analyses revealed a significant difference from the positive control, indicating that lower concentrations are not sufficient to achieve effective ovicidal activity.

With respect to exposure time, the outcomes showed that longer exposure, specifically 45 minutes, caused more morphological damage than shorter exposure. While there was a progressive increase in ovicidal activity over time, differences among the exposure times were rather subtle. This reveals that the extract could start to manifest its ovicidal effect even within shorter exposure times, a factor that suggests its ability to act quickly when used at effective concentrations.

Overall, Tabernaemontanapandacaqui (Pandakaki) ethanolic leaf extract is highly ovicidal against Ascaris lumbricoides ova, especially at concentrations of 75% and higher. Its effectiveness similarity to mebendazole recommends it as a credible natural alternative for helminth control. Moreover, the extract's potency, even at brief exposure length, emphasizes its practical benefit. Enable the research to contribute towards developing plant ovicidal agents that could be cost-effective as well as locally available growth, which is very beneficial for the low-resource helminth-endemic societies.

Recommendation

The present study tends to motivate further work directed towards optimizing the extraction of ethanolic leaf extract of Tabernaemontanapandacaqui (Pandakaki) by using alternative solvents and advanced techniques to increase the dry yield of bioactive compounds. It also aims at assessing additive effects when combined with standard anti-

helminthics such as mebendazole in order to improve efficacy while reducing resistance. In addition, the study encourages the analysis of other plant parts, including roots and seeds, as well as another related species within the family Apocynaceae, in order to find further ovicidal agents. Last, it plans to check the ovicidal efficacy of extracts against other common soil-transmitted helminths to see if it possesses a broad-spectrum potential to control parasites in endemic areas.

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