

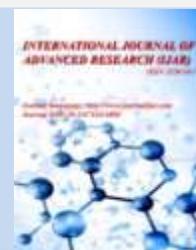


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RESEARCH ARTICLE

IN VITRO OVICIDAL ACTIVITY OF TABERNAEMONTANA PANDACAQUI (PANDAKAKI) LEAF ETHANOLIC EXTRACT AGAINST ASCARIS LUMBRICOIDES IN VARYING CONCENTRATIONS

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Soil-transmitted helminths; Ascaris lumbricoides ova; Tabernaemontana pandacaqui leaf extract; ovicidal activity; natural anthelmintic

Abstract

Soil-transmitted helminth (STH) infections, particularly those caused by *Ascaris lumbricoides*, remain a significant global health challenge in tropical regions like the Philippines. This study investigated the in vitro ovicidal activity of *Tabernaemontana pandacaqui* (Pandakaki) leaf ethanolic extract at varying concentrations (25%, 50%, 75%, and 100%) and exposure durations (15, 30, and 45 minutes). Using an experimental quantitative design, *A. lumbricoides* ova were extracted from stool samples via the Formalin-Ether Concentration Technique (FECT) and evaluated using a standardized morphological grading scale (0–3). Results indicated a significant dose-response and time-dependent relationship; the mean morphological damage score increased from 0.04 at a 25% concentration to 1.00 at a 100% concentration, while exposure duration saw a peak mean damage of 1.14 after 45 minutes. Statistical analysis using One-Way ANOVA and Dunn's Post-Hoc test revealed that higher concentrations (75% and 100%) at the 45-minute mark were statistically comparable to the positive control, Mebendazole, in inducing severe structural alterations like shell thinning and rupture. These findings conclude that *T. pandacaqui* leaf ethanolic extract is a highly effective natural ovicidal agent. Its efficacy at higher concentrations suggests it could serve as a sustainable, cost-effective, and locally available alternative to synthetic anthelmintics in managing STH infections in resource limited communities.

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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 *Strongyloides stercoralis*. 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 spicules (de 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 (*Tabernaemontana pandacaqui*) 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 (*Tabernaemontana pandacaqui*), 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 (*Tabernaemontana pandacaqui*) 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.

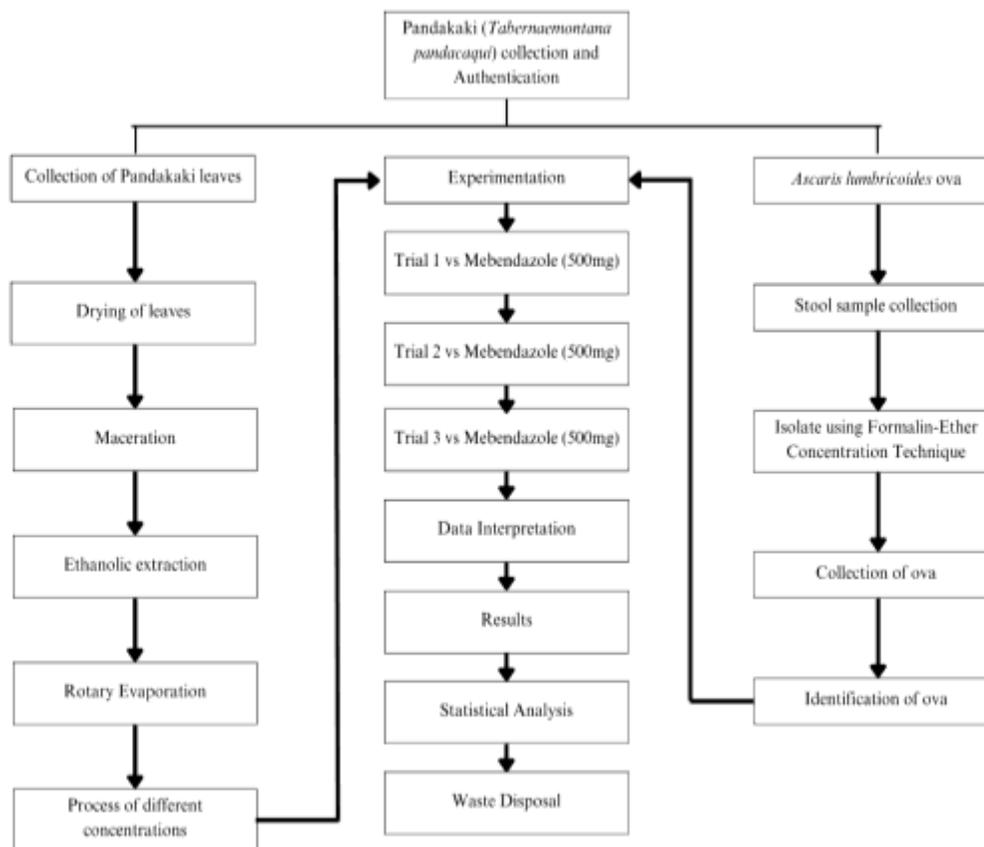
Research Flowchart:-

Figure 1. Process of the Experiment for In Vitro Ovicidal Activity of Pandakaki (Tabernaemontana pandacaqui) Leaf Ethanolic Extract Against Ascaris lumbricoides Ova in Varying Concentrations

Plant Acquisition:-

The plant Pandakaki (*Tabernaemontana pandacaqui*) 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 (*Tabernaemontana pandacaqui*) 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 (*Tabernaemontana pandacaqui*) 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 (*Tabernaemontana pandacaqui*) 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°C - 55°C 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°C - 40°C, with a 15°C - 20°C 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:-

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

Figure 2 Percent solution formula

Table 1 Varying Concentrations of Pandakaki Extract

VARYING CONCENTRATIONS OF PANDAKAKI EXTRACT	
Concentration (%)	Formula
To obtain 25% concentration:	$\frac{1\text{mL (100\% Pandakaki Ethanolic Extract)}}{4\text{mL}} \times 100$
To obtain a 50% concentration:	$\frac{2\text{mL (100\% Pandakaki Ethanolic Extract)}}{4\text{mL}} \times 100$
To obtain a 75% concentration:	$\frac{3\text{mL (100\% Pandakaki Ethanolic Extract)}}{4\text{mL}} \times 100$
To obtain 100% concentration:	$\frac{4\text{mL (100\% Pandakaki Ethanolic Extract)}}{4\text{mL}} \times 100$

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:-

Before the commencement of the study, the research protocol underwent a rigorous ethics review and was granted formal approval by the National University Mall of Asia (NU MOA) Ethics Review Committee (ERC), a body certified by the Philippine Health Research Ethics Board (PHREB). Ethical clearance was officially issued under Approval Number NUMOA-PR084 on March 28, 2025. The researchers strictly adhered to the PHREB 2022 guidelines, ensuring the study complied with national ethical standards to safeguard the welfare and rights of all participants. A core component of the protocol was a comprehensive risk-benefit assessment. Given that the study involved five minor participants, the researchers ensured that any potential risks were minimized and ethically justified. Participation was entirely voluntary, and the researchers strictly avoided coercion or undue inducement. While participants received equitable compensation to acknowledge their effort and cooperation, the amount was carefully calibrated to remain reasonable and not serve as an improper incentive for participation.

The informed consent process was implemented in two distinct stages. In the first stage, the guardians of the minor participants were provided with a detailed explanation of the study's objectives, procedures, potential risks, and benefits in a language they fully understood. Written informed consent was obtained from the guardians prior to any data or sample collection. In the second stage, age-appropriate verbal assent was obtained from the children to ensure they understood their role and their right to withdraw from the study at any time without consequences. To preserve participant dignity during the collection of stool samples, explicit and sensitive instructions were provided. Furthermore, the study maintained strict protocols for the management of biological materials. All laboratory waste, including stool samples and used extract solutions, were disposed of according to the institutional biosafety and waste management guidelines of the university to prevent environmental contamination or health hazards. By incorporating the recommendations of the Ethics Review Committee and maintaining detailed documentation, the researchers ensured transparency, accountability, and a steadfast commitment to the protection of human participants.

Waste Disposal:-

Proper waste disposal research is required for the use of ethanolic extracts of Pandakaki (*Tabernaemontana pandaciqui*) 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-part Zonrox 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:-

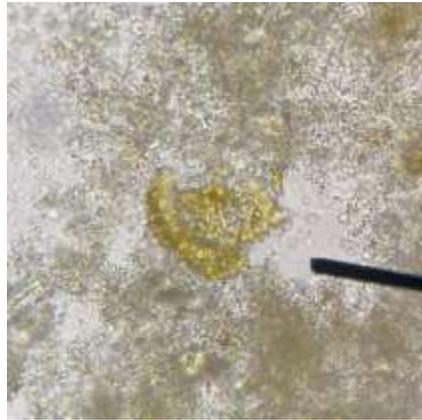
Due to the limited availability of articles and established guidelines regarding the morphological grading or assessment of *Ascaris lumbricoides* ova, the researchers will verify the criteria they have created through three different licensed Medical Technologists to ensure accurate and reliable evaluation.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

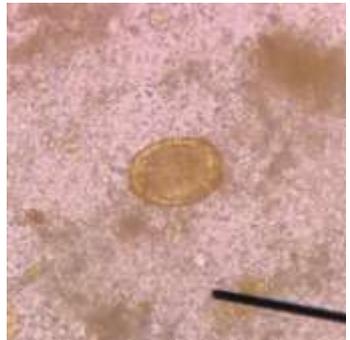
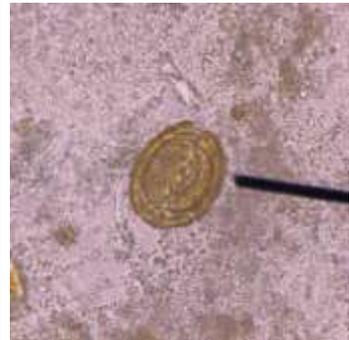
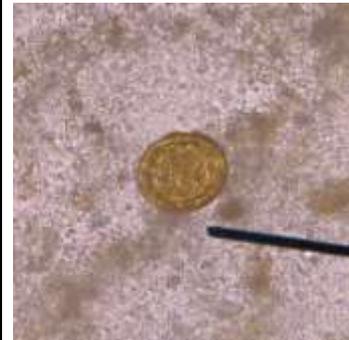
Table 2. Criteria for observing the alteration in the morphological aspect of *Ascaris lumbricoides*

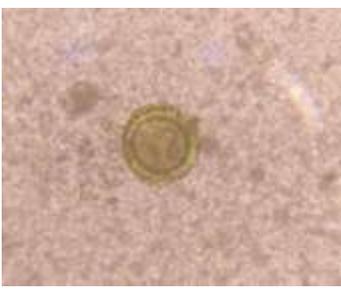
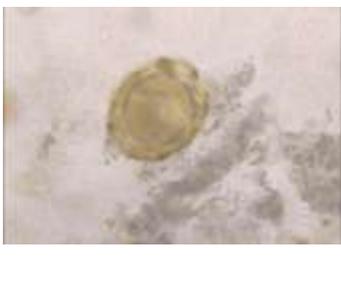
3	2	1	0
Shell ruptured or collapsed; extreme thinning, leakage, or complete deformation.	Major distortion, irregular shape, severe thinning.	Includes moderate damage, mild distortion, and localized thinning.	Normal development and morphology; no damage, regular in shape, normal thickness and texture.

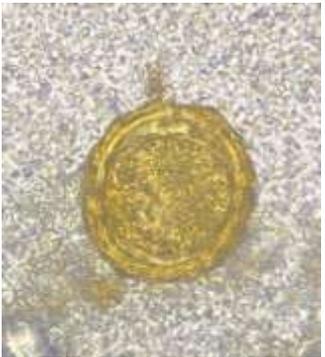
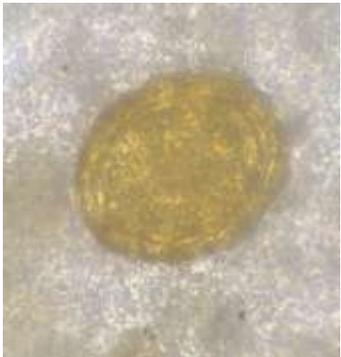
The table above (Table 2. Criteria for observing the alteration in the morphological aspect of *Ascaris lumbricoides* ova) will serve as a guideline for the researchers to assess the ovicidal activity of Pandakaki (*Tabernaemontana pandacaqui*). Each criterion is scored from 0 to 3, with higher scores 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.

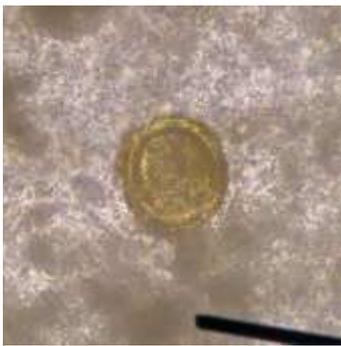
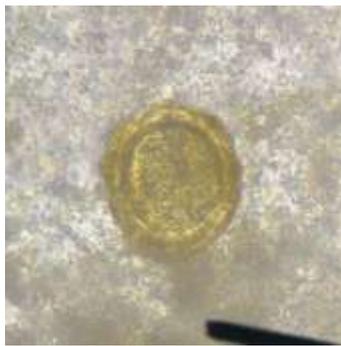
Results and Discussion:-

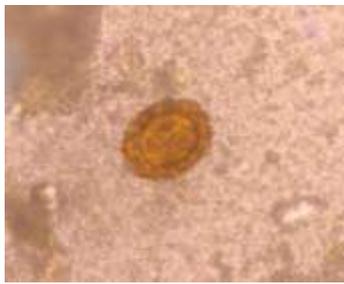
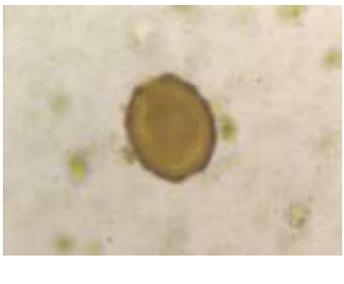
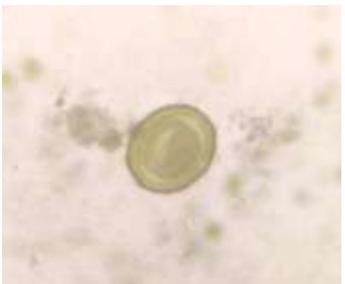
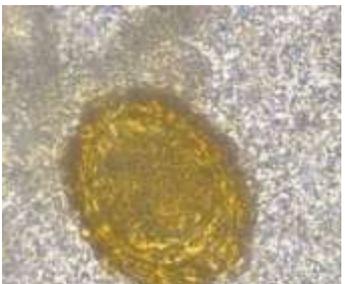
Ascaris lumbricoides ova: Negative control	Ascaris lumbricoides ova: Positive control
	
<p>The ova of <i>Ascaris lumbricoides</i> were observed under a microscope at 400x magnification (High power objective).</p>	

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

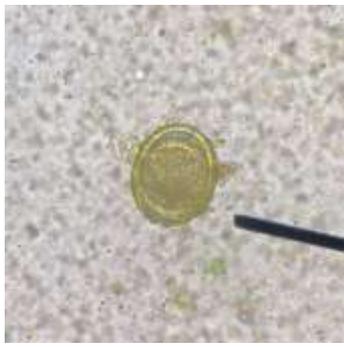
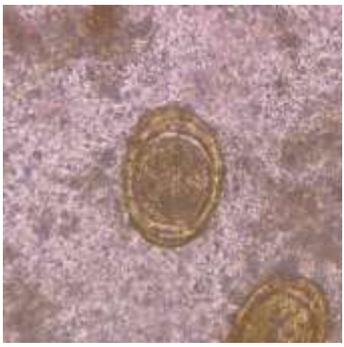
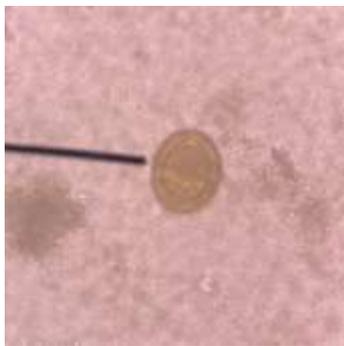
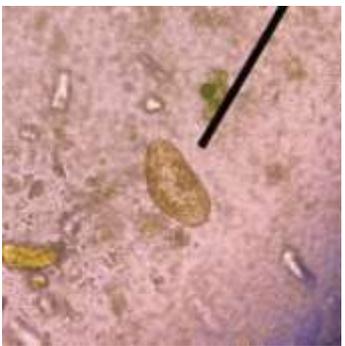
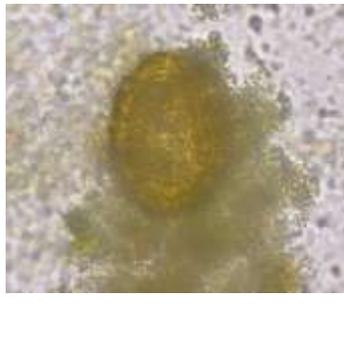
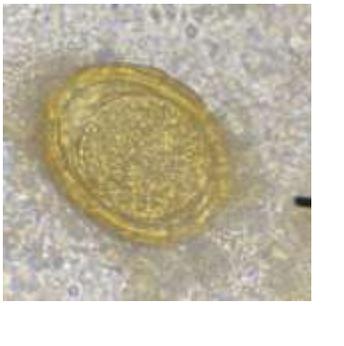
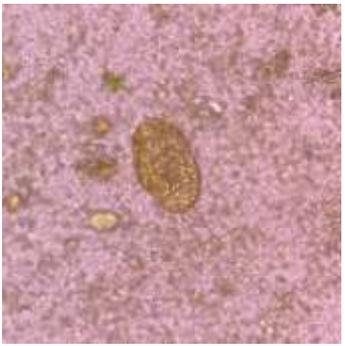
		
15 minutes	30 minutes	45 minutes

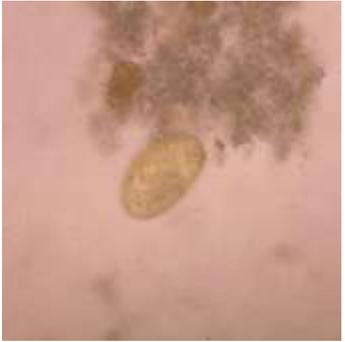
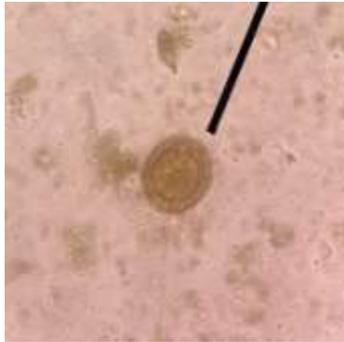
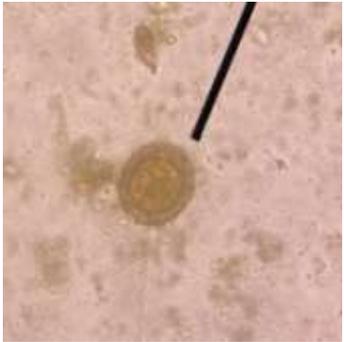
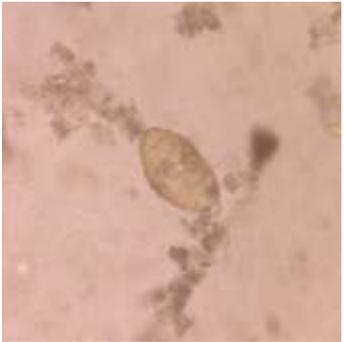
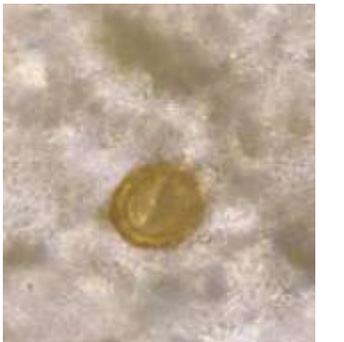
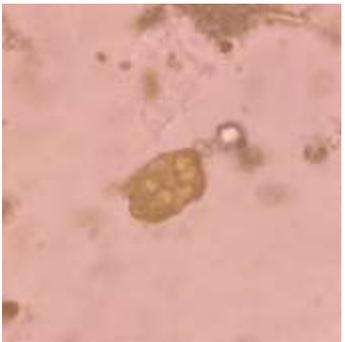
TRIAL 3		
		
15 minutes	30 minutes	45 minutes
The ova of <i>Ascaris lumbricoides</i> were observed under a microscope at 400x magnification (High power objective).		

Ascaris lumbricoides ova in 50% 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 <i>Ascaris lumbricoides</i> were observed under a microscope at 400x magnification (High power objective).		

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 <i>Ascaris lumbricoides</i> were observed under a microscope at 400x magnification (High power objective).		

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).

Table 3 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 (listwise)	N 27				

Table 3. 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.

Table 4. 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 (listwise)	N 36				

Table 4. 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 5. 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 5 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.

Table 6. 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 6, 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.

Checking for Normality Assumption (Shapiro-Wilk's Test):-**Table 7. Checking for Normality Assumption (Shapiro-Wilk's Test)**

	W	P- Value
Treatment at 15 minutes	0.85291	0.0191
Treatment at 30 minutes	0.87736	0.04333
Treatment at 45 minutes	0.87716	0.04304

The data was first checked for normality assumption using the Shapiro-Wilk's test. The data was first fitted in a one-way analysis of variance model. The residuals of the model were subjected to Shapiro-Wilk's test, wherein residuals with a p-value of more than 0.05 are considered to follow a normal distribution. The results provided that the residuals of all the treatment (25%, 50%, 75%, 100%, and mebendazole control) at 15, 30, and 45 minutes does not follow normal distribution. The violation of normality assumption prompted the researchers to utilize the non-parametric test Kruskal-Wallis test to determine significant differences between the five groups of treatment.

Kruskal-Wallis Test:-**Table 8. Kruskal-Wallis Test**

	Degree of Freedom	Kruskal-Wallis χ^2 statistic	P-Value
Treatment at 15 minutes	4	1.3628	0.8506
Treatment at 30 minutes	4	6.5274	0.1631
Treatment at 45 minutes	4	12.2170	0.0158

***Significant at alpha = 0.05. Proceed to Dunn's Post-Hoc Test.**

The data was subjected to Kruskal-Wallis test to determine significant differences across the five groups. A p-value of less than 0.05 suggests that at least one response from the five groups of treatments is statistically significantly different. The results show that durations 15 and 30 minutes did not show significant differences between the five groups of treatment. This suggest that 25%, 50%, 75%, 100%, and mebendazole during 15 and 30 minutes did not show any significant differences in its results. However, there is a significant difference between the five treatment groups observed at 45-minute duration, suggesting that during 45-minute mark duration, at least one of the treatment groups, either 25%, 50%, 75%, 100%, and mebendazole is statistically significantly different from others. To determine this, the treatment group will be subjected to further analysis using Dunn's Post-Hoc test

Dunn's post-Hoc Test (using Bonferroni Adjustment):-

Treatment at 45 minutes	Letter
25% extract	a
50% extract	ab
75% extract	ab
100% extract	ab
Mebendazole	b

***Treatment with different letters is statistically significantly different**

Dunn's post-Hoc test was used to determine the specific treatment group that is significantly different from the other treatment group. The Dunn's Post-Hoc test employs the usage of Bonferroni adjustment to adjust pairwise p-values to control Type I error due to the multiple pairwise comparison done by the Dunn's test. The results show that the effects of 25% concentration (at 45 minutes) and mebendazole (at 45 minutes) are statistically significantly different, while extract concentrations 50%, 75%, and 100% does not behave differently from the mebendazole control, as shown in the report, where treatment groups with similar letters are not statistically significant from each other. In short, the effect of 25% concentration at 45 minutes behaves differently from the positive control mebendazole.

Summary of Results:-

The statistical analysis of the in vitro ovicidal activity of *Tabernaemontana pandacaqui* (Pandakaki) leaf ethanolic extract against *Ascaris lumbricoides* revealed a clear dose-dependent and time-dependent relationship. As the

concentration of the extract increased from 25% to 100%, a progressive rise in morphological damage to the ova was observed. At the 25% concentration, the mean morphological damage score was minimal at 0.04, whereas the 100% concentration yielded a significantly higher mean score of 1.00. Exposure duration also played a vital role in the extract's efficacy; the average damage score across all groups rose from 0.28 at the 15-minute mark to 1.14 after 45 minutes of exposure. Comparative analysis using the Independent T-test and One-Way ANOVA showed that while lower concentrations (25%) were significantly less effective than the positive control (Mebendazole), the higher concentrations (50%, 75%, and 100%) demonstrated increasing parity with the synthetic treatment. Notably, Dunn's Post-Hoc test confirmed that at the 45-minute interval, there was no statistically significant difference between the 75% and 100% extract concentrations and the Mebendazole control. The most severe structural changes, characterized by scoring levels 2 and 3—indicating major distortion, shell thinning, and rupture—were most prevalent in the 100% concentration group at the maximum exposure time.

Conclusion:-

Based on the statistical findings, it is concluded that *Tabernaemontana pandacaqui* leaf ethanolic extract possesses significant ovicidal activity against *Ascaris lumbricoides* ova. The extract effectively disrupts the structural integrity of the ova, with its potency being directly proportional to both the concentration levels and the duration of exposure. Because the 75% and 100% concentrations achieved results statistically comparable to the conventional anthelmintic Mebendazole within 45 minutes, the extract serves as a highly effective natural alternative.

These results suggest that the bioactive phytochemicals present in *Pandakaki* leaves can penetrate or degrade the complex layers of the *Ascaris* eggshell. This study highlights the potential of *T. pandacaqui* as a locally accessible, cost-effective botanical agent for the management of soil-transmitted helminth infections. Furthermore, the findings support the integration of traditional ethnobotanical knowledge with laboratory-validated science to develop sustainable public health interventions against parasitic diseases in endemic regions.

Recommendation:-

The present study tends to motivate further work directed towards optimizing the extraction of ethanolic leaf extract of *Tabernaemontana pandacaqui* (*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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