ANTIPARASITIC POTENTIAL OF *Allium fistulosum* (GREEN ONION) CRUDE EXTRACT ON HELMINTHS OVA

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ABSTRACT

Allium fistulosum, also known as green onion, is a perennial plant that is a well-distinguished member of the *Amaryllidaceae* family, and has been considered to be a potential antiparasitic agent against *Ascaris lumbricoides* and *Trichuris trichiura*. This experimental research aimed to determine the anti-parasitic potential of *A. fistulosum* crude extract against ova of helminths, specifically, *A. lumbricoides* and *T. trichiura*. Stool specimens were collected from children to evaluate the extent of structural damage to parasite ova. Two diagnostic techniques were employed to isolate the ova: direct fecal smear and the formalin-ether sedimentation method. The treated samples were incubated for varying periods to determine the most effective and rapid action of the crude extract on the ova. The study achieved a 14.29% yield, demonstrating that the extraction method was effective in producing a substantial amount of crude extract. In addition, the 75% and 100% crude extracts were identified as the most effective concentrations. A 7-day exposure to *A. fistulosum* extract resulted in significantly greater effectiveness compared to shorter durations of 24, 48, and 72 hours. The study also revealed significant total changes in both qualitative and quantitative parameters, highlighting the impact of the *A. fistulosum* crude and fresh extracts. Moreover, the study reinforced the value of integrating scientifically supported herbal treatments into modern healthcare systems as a viable and accessible approach to managing parasitic infections.

Keywords: ascariasis, direct fecal smear, period of incubation, sedimentation technique, trichuriasis

INTRODUCTION

Background of the Study

According to the World Health Organization (WHO), about 80% of the world's population depends on medicinal plants for primary healthcare needs due to their accessibility, effectiveness, and minimal side effects (Bhatia et al., 2021). These plant extracts contain active components, such as phenolics, flavonoids, stilbenes, and tannins, that exhibit broad biological activities including antimicrobial properties. Parham et al. (2020) emphasize that these compounds can serve as potential sources for the development of antimicrobial drugs and food preservatives.

Despite advances in modern medicine, gastrointestinal (GI) parasitic infections remain a significant public health problem worldwide, especially in low- and middle-income countries. Kuete et al. (2015) report that intestinal parasitic infections, particularly those caused by helminths and protozoa, disproportionately affect impoverished communities. Globally, approximately 4.5 billion people are at risk of parasitic infections, with over two billion cases associated with morbidity and mortality each year (Eyayu et al., 2021). The WHO estimates approximately 800–1000 million cases of Ascaris lumbricoides infections, 700–900 million cases of Hookworm, 500 million cases of *Trichuris trichiura*, and millions more of protozoan infections such as Giardia lamblia and Entamoeba histolytica/dispar. These infections contribute significantly to the global disease burden, especially among vulnerable populations, such as children aged 2 to 4 years (Unasho, 2013; Tigabu et al., 2010).

The incidence of parasitic infections is linked to poor sanitation, unsafe drinking water, contaminated food, malnutrition, and overcrowding (Dangela, 2023). Additionally, the increasing emergence of multidrug resistance among parasites and adverse effects associated with synthetic anthelmintic drugs highlight the urgent need for alternative therapeutic interventions. Băieș et al. (2023) emphasize the importance of shifting the focus to medicinal plants as promising sources of new antiparasitic agents.

Among the plants studied for antiparasitic properties, *Allium fistulosum*, a perennial in the *Alliaceae* family of onions, is often known as "murang sibuyas". It is known for its sulfur-containing compounds and rich phytochemical profile, and has shown antimicrobial and antiparasitic potential (Balkrishna et al., 2023).

Extensive research has highlighted the antiparasitic properties of *A. fistulosum* against various parasites, yet its efficacy against the resilient ova of soil-transmitted helminths, such as *A. lumbricoides* and *T. trichiura*, remains underexplored. These helminth eggs are a major source of environmental contamination and reinfection, particularly in tropical and subtropical regions. Most existing studies focus on adult or larval stages, leaving a critical gap in understanding the potential of *A. fistulosum* to inactivate parasite eggs. This study aims to evaluate the antiparasitic potential of fresh and crude *A. fistulosum* extracts, specifically against helminth ova, addressing a significant global health need. In the context of medical technology, where accurate diagnosis and treatment of parasitic infections are vital, discovering effective natural agents like *A. fistulosum* could improve disease management, particularly in developing countries where children are most at risk. Furthermore, this research supports efforts to combat drug resistance and contributes to improved diagnostics, more effective therapeutic strategies, and stronger public health programs targeting parasitic diseases.

Therefore, this study has significant potential in addressing the global health challenges posed by parasitic diseases. It can contribute valuable insights in the field of pharmaceuticals and medicine toward the development of effective anti-parasitic medications and create opportunities for new therapies that manage high-prevalence parasitic infections. Furthermore, it may help overcome critical issues such as drug resistance, which remains a major threat to public health. Beyond healthcare, the study's applications extend to agriculture, where it can serve as a natural pesticide or water treatment to control parasitic contamination in crops and livestock, enhancing food safety and reducing economic and life losses from parasite-related diseases.

Statement of the Objectives

The study aimed to determine the antiparasitic potential of *A. fistulosum* leaves extract against helminths. Specifically, the objectives were achieved in a duration of one month, from March to April 2025:

1. To determine the percent yield of *A. fistulosum* crude extract
2. To assess the concentration of *A. fistulosum* crude extract that exhibits antiparasitic potential on helminths in terms of:
   a. Period of Incubation
   b. Total Changes
      b.1 Quantity
      b.2 Characterization of the changes in the appearance of ova

METHODOLOGY

Research design

This study utilized an experimental research method. The crude extract from *A. fistulosum* was prepared at different concentrations, and a fresh extract was prepared as well. The different concentrations and the fresh extract were added directly to the test tube containing the stool suspension, and the reaction was examined under a microscope and described based on different parameters.

Study Site and Sample Collection

This study was performed at the research laboratory of the Center for Natural Sciences of Saint Mary's University, Bayombong. Plant samples were purchased at NVAT, Bambang. The biological samples were collected from children's stool samples in Don Mariano Marcos and Barangay Salvacion, both in Bayombong.

Specimen Identification

The plant sample was submitted to the College of Forestry, Environment and Resources Management at Nueva Vizcaya State University, Bayombong, Nueva Vizcaya, for taxonomic identification, and it was identified as green onion *(A. fistulosum).*

Data Gathering Procedure

Collection and Maceration of A. fistulosum

A 20kg two (2) sacks of *A. fistulosum* leaves were washed and cleaned with distilled water, then sliced into pieces. The plant samples were air-dried for 7 days until thoroughly dry, then ground to a fine powder in a blender. The extraction was performed by soaking 350 g of the pulverized *A. fistulosum* leaves and 95% ethanol to extract bioactive compounds (Oyawoye *et al*., 2022). To avoid evaporation, the mixture was kept at room temperature for 48 hours in a sterile flask covered with aluminum foil. A filter paper and a funnel were used to separate the residue and the filtrate. The filtrate was obtained as an extract and kept in the refrigerator. The crude extract was then dried by solvent removal using a Rotary Evaporator (Rota-vapor R300) at below 40°C to obtain the crude extract (Fonkeng *et al*., 2015; Assefa *et al*., 2016). The fresh extract was prepared by squeezing the fresh plant sample directly into the beaker and storing it in the refrigerator.

Percent Yield

The yield of the crude extract was calculated in grams and converted into a percentage. Yield is the amount of crude extract obtained from plant powder. In practice, it is determined by dividing the weight of the solids content after evaporation by the weight of the dry powder of the plant material used for the extraction, multiplied by 100 (Dagne *et al*., 2021). The percentage yield was calculated by:

$$Percent\ yield\ = \frac{Weight\ of\ dried\ crude\ extract}{Weight\ of\ dried\ plant\ sample} x100$$

Preparation of the Different Concentrations of Ethanolic Crude Extract

The ethanolic extract was dissolved in distilled water. Adapted from the study of Moya *et al*. (2019), an analytical balance was used to measure the following concentrations in four (4) different beakers:

Collection and Preparation of Helminths for the Antiparasitic Activity

Stool samples were collected from children after obtaining parental consent and providing proper collection instructions. Samples were placed in sterile containers while wearing gloves to prevent contamination. Clean, uncontaminated morning samples were preferred; if immediate processing was not possible, samples were preserved with formalin or polyvinyl alcohol or kept refrigerated.

The presence of *Ascaris lumbricoides* and *Trichuris trichiura* ova was examined using the direct fecal smear (wet mount) method. About 1–2 mg of stool was mixed with normal saline (0.9%) and Lugol's iodine on a microscope slide. The slide was observed under 10× and 40× magnification to identify and characterize the parasite ova.

Sedimentation Technique

Sedimentation techniques use fluids with lower specific gravity than the parasite organisms, thus concentrating them in the sediment, which allows for easier isolation of helminth ova (Centers for Disease Control and Prevention, 2019). Moreover, it is used in general diagnostic laboratories because it is simpler to implement and less prone to technical faults. The researchers utilized the formalin-ethyl acetate sedimentation technique, a diphasic method that eliminates issues associated with ether flammability and can be used with specimens preserved in 10% formalin, merthiolate-iodine-formalin, or sodium acetate-acetic acid-formalin. Furthermore, the formalin-ether concentration technique, also known as the formalin-ethyl acetate sedimentation method, is a frequently used sedimentation technique for the identification of intestinal protozoa in preserved stool samples (Becker *et al.,* 2011).

The procedure for the formalin-ethyl acetate sedimentation technique was adopted from the Centers for Disease Control and Prevention. Two to three grams of stool were mixed with 7 mL of 10% formalin, strained into a conical tube, treated with 3 mL of ethyl acetate, and centrifuged at 1500 rpm for 5 minutes. After removing the supernatant, 3 mL of formalin was added to resuspend the sediment.

Seven Wasserman test tubes were prepared and labeled for different concentrations (25%, 50%, 75%, 100%), negative and positive controls, and fresh extract. Equal volumes (seven drops) of stool suspension and respective test solutions were added to each tube. Normal saline served as the negative control, and liquid mebendazole as the positive control.

The seven (7) tubes were incubated at 37°C for 24 hours, 48 hours, 72 hours, and 7 days to simulate conditions within the human body. The study by Legesse et al. (2002) evaluated the efficacy of 100 mg mebendazole, administered twice daily for 3 consecutive days, for the treatment of *A. lumbricoides* and *T. trichiura* infections. Therefore, the researchers incubate the anthelmintic activity of the extracts and set a waiting period of up to 7 days to allow enough time for the extracts to take effect and enable us to assess the impact of potential treatments on worm eggs in a controlled setting, thereby facilitating the evaluation of their efficacy and the identification of their mechanisms of action (Hernando & Bouzat, 2024).

Evaluation of the Period of Incubation

The incubation period was used to evaluate the antiparasitic activity of *Allium fistulosum* crude and fresh extracts on helminth ova. Samples were incubated for 24, 48, and 72 hours, and up to 7 days, with daily observations of changes in the ova. After each incubation period, a wet mount was prepared by placing one drop of the incubated stool suspension and one drop of Lugol's iodine on a slide, then examined microscopically.

Evaluation of the Quantity of Ova

Simultaneously, the quality of helminth ova was closely monitored. The number of ova was counted by scanning the 10 fields of the prepared wet mount. The counting and characterization of disintegrated ova were validated by a registered medical technologist. This was done to determine the antiparasitic potential of A. fistulosum fresh and crude extracts.

Evaluation of the Changes in the Appearance of Ova

The ovum was characterized by its appearance after the specified incubation period. The ova of *A. lumbricoides* and *T. trichiura* were evaluated based on the appearance of the embryo or larva inside the shell. If the plant crude extract is effective, the embryo or larva within is completely inactivated, with many tiny globules that were formed within the embryo or larva. According to Flota-Burgos *et al.* (2020), the morula, embryo, or larva of hookworm ova exposed to chemical substances and antiparasitic agents, such as extracts, shows signs of degeneration and a dehydrated appearance. Moreover, in a study conducted by Schmitz *et al*. (2016), the organism A. lumbricoides ova died and appeared non-viable, with a disfigured, dark-oval structure and bubbled yolk or refractile granules inside the shell. Aside from the appearance inside the shell, another sign of the effectiveness of chemical substances is the disintegration or removal of the uterine layer of a mamillated *A. lumbricoides* ova (Brownell & Nelson, 2006).

Treatment of Data

The antiparasitic potential of the fresh and crude extracts of A. fistulosum was assessed by characterizing morphological changes in ova of the two tested organisms. The descriptive statistics present the calculated mean and standard deviation of the disintegration grade for each examined organism. The degree of degradation was recorded using a grading scale (0-3) for the various concentrations of the crude extract (25%, 50%, 75%, 100%), fresh extract, and the positive and negative controls.

Ethical Consideration

The study was approved by Saint Mary's University Research Ethics Board (SMUREB) with address and contact information at 2nd Floor, Rev. John Van Bauwel Hall, SMU Main Campus, Ponce Street, Don Mariano Marcos, Bayombong, 3700 Nueva Vizcaya, Philippines (email: reb@smu.edu.ph; cellphone: 09177053041), with the code 2025 0861.

RESULTS AND DISCUSSIONS

Section 1. Percent Yield of *A. fistulosum* Crude Extract

To determine the efficiency of the plant extraction process, the crude extract yield was calculated in grams and subsequently converted to a percentage. To obtain the percent yield of the Allium fistulosum crude extract, 350 g of powdered plant material was extracted with ethanol. In this experiment, 50g of solid residue (crude extract) after evaporation was obtained from 350g of dry powder of A. fistulosum, yielding a significant 14.29% yield. As a result, the high yield obtained indicates that the extraction method is effective in producing a substantial amount of crude extract.

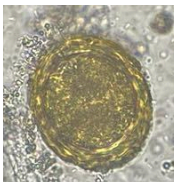
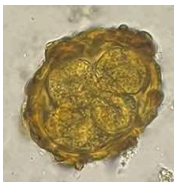
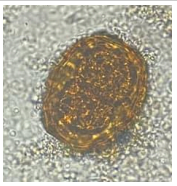
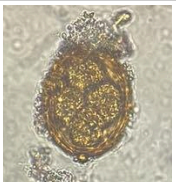
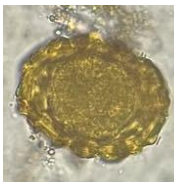
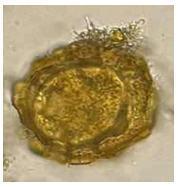
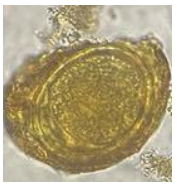
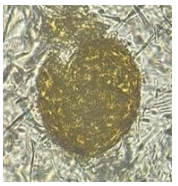
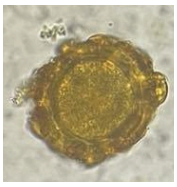
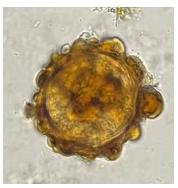
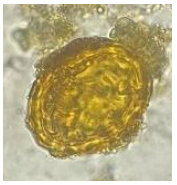
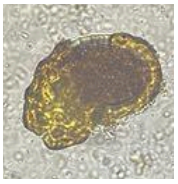
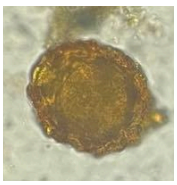
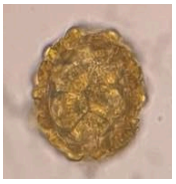
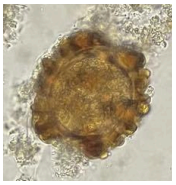
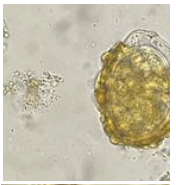
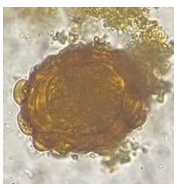
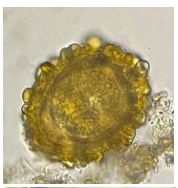
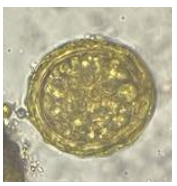
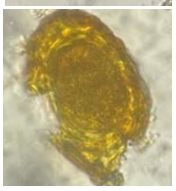
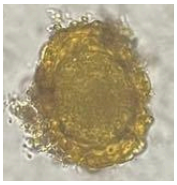
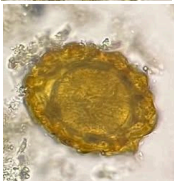
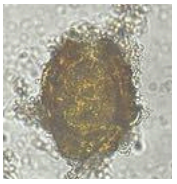
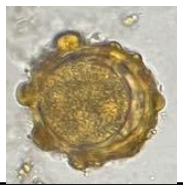
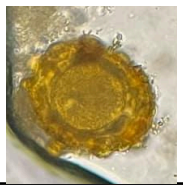
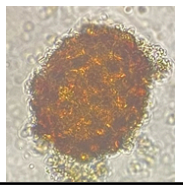
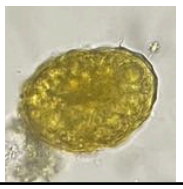
Therefore, the consistency of the yield obtained implied the efficiency of ethanol in extracting compounds from *A. fistulosum*. Specifically, the 14.29% yield indicates an effective extraction process, strongly suggesting that a substantial yield of active compounds was successfully isolated.

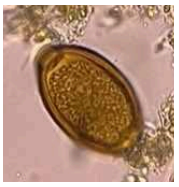
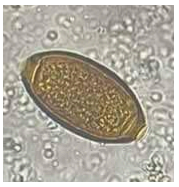
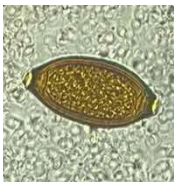
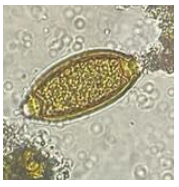
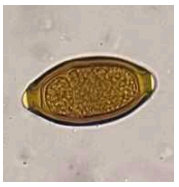
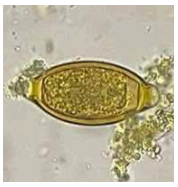
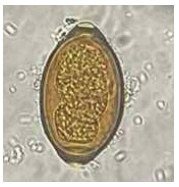
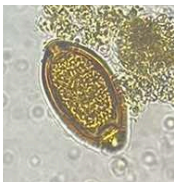
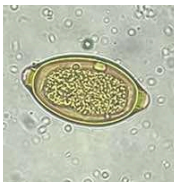
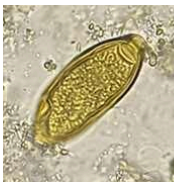
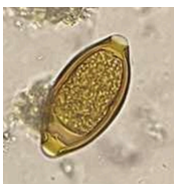
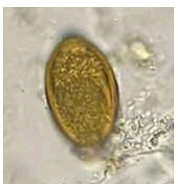
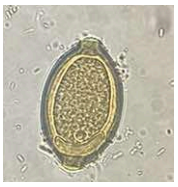
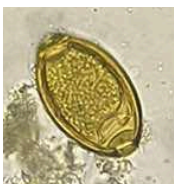
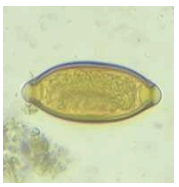
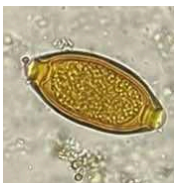
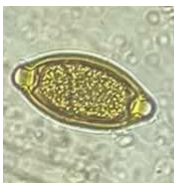
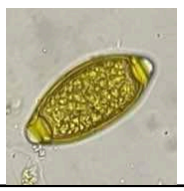
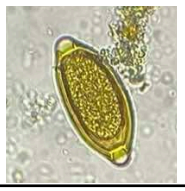
Section 2. Antiparasitic Activity of *Allium fistulosum* on Helminth Ova in Terms of Period of Incubation and Total Changes

Table 4 presents a comparative overview of the incubation periods of tubes containing the *A. fistulosum* extracts with *A. lumbricoides* and *T. trichiura*, two common soil-transmitted helminths affecting human populations. The table highlights variations in incubation duration at an optimal temperature of 37°C for these two parasite ova, and it illustrates the affected ova after their incubation period.

Table 4

Period of incubation

Concentration	A. ***lumbricoides***			
	24 hrs	48 hrs	72hrs	7 days
Negative Control				
Positive Control				
Fresh Extract				
100% Crude extract				
75% Crude extract				
50% Crude extract				
25% Crude extract				
	T. ***trichiura***			
Concentration	24 hrs	48 hrs	72hrs	7 days

Negative Control				
Positive Control				
Fresh Extract				
100% Crude extract				
75% Crude extract				
50% Crude extract				
25% Crude extract				

The table above displays morphological differences in A. lumbricoides and *T. trichiura* ova across varying concentrations of *A. fitulosum* extract and incubation periods, suggesting a concentration- and time-dependent ovicidal effect. At lower concentrations and shorter incubation times, the ova retain much of their structural integrity, with only minimal deformation. However, as the extract concentration increases and the exposure duration extends, the ova exhibit progressive structural damage, such as shell disintegration and distortion of the internal material. The result shows that a 7-day incubation period for A. lumbricoides ova is more effective than shorter incubation periods of 24, 48, and 72 hours. The extended incubation period is due to the duration effect of the positive control, mebendazole. On the other hand, the results revealed no significant effect against *T. trichiura* among all tested incubation periods. Despite the varying durations, the extracts had no significant effects on the ova of *T. trichiura*.

The observed antiparasitic activity of the extract suggests that its mechanism of action may target the structural and inner components of *A. lumbricoides* ova in 7 days. This effect

highlights the susceptibility of soil-transmitted helminths after a sufficient amount of time. Clinically, the finding implies that the extract shows promising anthelmintic activity for *A. lumbricoides*, but it may not be suitable as a broad-spectrum treatment for other parasitic infections involving *T. trichiura.*

Table 5

Quantity and Characterization of the Changes in the Appearance of Ova

Concentration	*Period of incubation*	***A. lumbricoides***		***T. trichuria***	
		Quantity	Quality (Characterization)	Quantity	Quality (Characterization)
Negative Control	24 hrs	0	0	0	0
	48 hrs	0	0	0	0
	72 hrs	0	0	0	0
	7 days	0	0	0	0
Positive Control	24 hrs	0	1.22	0	0
	48 hrs	0.67	2	0	0
	72 hrs	1.33	2.56	0	0
	7 days	12.33	2.67	0	0
Fresh Extract	24 hrs	0	1	0	0
	48 hrs	0.67	3	0	0
	72 hrs	1	2.44	0	0
	7 days	3.67	2.56	0	0
100% Crude extract	24 hrs	0	1.44	0	0
	48 hrs	1	2.22	0	0
	72 hrs	0.67	2.89	0	0
	7 days	5.67	2.22	0	0
75% Crude extract	24 hrs	0	1.56	0	0
	48 hrs	1	2.33	0	0
	72 hrs	1	2.56	0	0
	7 days	6.33	2.33	0	0
50% Crude extract	24 hrs	0	1.33	0	0
	48 hrs	0	1.89	0	0
	72 hrs	1	2.33	0	0
	7 days	4.33	2.78	0	0
25% Crude extract	24 hrs	0	1	0	0
	48 hrs	0	1.89	0	0
	72 hrs	0	2.11	0	0
	7 days	1.67	2.11	0	0

Evaluation of the Quantity of Ova

The table above shows the mean average of the three trials of the quantity of the disintegrated ova among the five (5) tested extracts and two (2) controls. This result was obtained by counting ten (10) ova (either normal or affected ova) in ten (10) fields; during counting, the number of affected ova showing degeneration was recorded from those ten (10) counted ova. The number of ova affected by the extract did not consistently increase with increasing concentration, which means that even though the extract has an antiparasitic effect on the ova, they often do not achieve complete eradication. The results revealed that the fresh extract had the highest number of disintegrated A. lumbricoides ova during the 7-day incubation period. Overall, the 75% and 100% crude extracts and the fresh extract have the highest number of disintegrated A. lumbricoides ova throughout the incubation period. The other crude extracts (25% and 50%), along with the negative control, had fewer or no inactive ova across the tested incubation periods, suggesting a weaker or no impact on egg integrity. However, the results

show that none of the extracts or controls worked against *T. trichiura* at any of the tested incubation periods, indicating that no inactive or disintegrated ova were observed.

This result highlights the potential potency of the 75% and 100% crude extracts and the fresh extract against A. lumbricoides ova, but not against *T. trichiura* ova. Therefore, even though *A. fistulosum* may offer a natural alternative for controlling nematode populations; their application may be more effective for permeable egg shells or when used in conjunction with other antiparasitic agents to enhance efficacy.

Evaluation of the Changes in the Appearance of Ova

Table 5 on page 20 presents the final grading of the ova, which shows the disintegration effects caused by the different extracts. The qualitative assessment was based on the standardized criteria scale from 0 (no effect) to 3 (complete degradation). The gradings of the affected ova were obtained by averaging the gradings of four medical technologists per trial. The results show that 75% and 100% crude extracts, as well as fresh extracts, exhibit the greatest changes in the appearance of A. lumbricoides ova. This suggests that higher concentrations of the extract were associated with significantly greater structural degeneration or alterations of the ova, supporting the hypothesis that *A. fistulosum* possesses antiparasitic properties. As expected, there are still no observable changes in the appearance of *T. trichiura* ova in all extracts and controls among the tested incubation periods.

This finding suggests a threshold effect, where concentrations at or above 25% already produce a maximal antiparasitic response. This implies that *A. fistulosum* extract may effectively compromise the morphology of helminths with relatively permeable egg shells. However, its limited effect on the resilient ova of *T. trichiura* highlights species-specific limitations and the need for combination therapies.

CONCLUSION AND RECOMMENDATIONS

Conclusion

The study evaluated the antiparasitic potential of *Allium fistulosum* leaf extract against helminth ova, yielding a crude extract yield of 14.29%. Results showed that longer incubation periods, particularly 7 days, enhanced the ovicidal activity against *Ascaris lumbricoides* ova, with 75% and 100% crude extracts and fresh extract demonstrating high efficacy comparable to mebendazole. The extracts likely contain potent compounds that damage the ova's structural integrity. However, no effect was observed on *Trichuris trichiura* ova, and quantitative assessment was limited by the lack of a precise basis for measuring disintegration. Overall, the findings suggest *A. fistulosum* extract may be a promising treatment for *A. lumbricoides* infections, especially in low-resource settings.

Recommendations

1. Integrate bioactive compounds from green onions into functional foods or supplements to combat parasitic infections, especially in developing countries with high prevalence.
2. Study the effect of *A. fistulosum* crude extract against adult *T. trichiura*, as it may offer an effective natural alternative for treating this common parasite.
3. Formulate antiparasitic products against *A. lumbricoides* ova using *A. fistulosum* and evaluate their effects on living organisms to develop innovative treatments that can interrupt parasite transmission.
4. Explore the antiparasitic effect of *A. fistulosum* crude extract against other parasites, to test its efficacy on a broader range of parasites.

REFERENCES

Băieş, M. H., Gherman, C., Boros, Z., Olah, D., Vlase, A. M., Cozma-Petruț, A., Györke, A., Miere, D., Vlase, L., Crișan, G., Spînu, M., & Cozma, V. (2022). The Effects of Allium sativum L., Artemisia absinthium L., Cucurbita pepo L., Coriandrum sativum L., Satureja hortensis L. and Calendula officinalis L. on the Embryogenesis of Ascaris sum Eggs during an In Vitro Experimental Study. *Pathogens (Basel, Switzerland), 11*(9), 1065. https://doi.org/10.3390/pathogens11091065

Balkrishna, A., Chaudhary, M., Sharma, H., Srivastava, D., Kukreti, A., Kumar, A., & Arya, V., (2023). Phytochemistry, pharmacology, and medicinal aspects of Allium fistulosum L.: A narrative review. *Journal of Applied Pharmaceutical Science, 13*(10), 107-118. https://doi.org/10.7324/JAPS.2023.142822

Bhatia, P., Sharma, A., George, A., Anvitha, D., Kumar, P., Dwivedi, V., & Chandra, N. (2021). Antibacterial activity of medicinal plants against ESKAPE*: Heliyon, 7*(2), 2405-8440, https://doi.org/10.1016/j.heliyon.2021.e06310.

Eyayu, T., Kiros, T., Workineh, L., Sema, M., Damtie, S., Hailemichael, W., Dejen, E., & Tiruneh, T. (2021). Prevalence of intestinal parasitic infections and associated factors among patients attending at Sanja Primary Hospital, Northwest Ethiopia: An institutional-based cross-sectional study. *PloS one, 16*(2), 0247075. https://doi.org/10.1371/journal.pone.0247075

Kuete, T., Yemeli, F., Mvoa, E., Nkoa, T., Somo, R., & Ekobo, A. (2015). Prevalence and risk factors of intestinal helminth and protozoa infections in an urban setting of Cameroon: The case of Douala. *American Journal of Epidemiology and Infectious Disease, 3*(2), 36-44. https://doi.org/10.1186/s13104-018-3556-6

Parham, S., Kharazi, A. Z., Bakhsheshi-Rad, H. R., Nur, H., Ismail, A. F., Sharif, S., RamaKrishna, S., & Berto, F. (2020). Antioxidant, Antimicrobial and Antiviral Properties of Herbal Materials. *Antioxidants (Basel, Switzerland), 9*(12), 1309. https://doi.org/10.3390/antiox9121309

Tigabu, E., Petros, B., & Endeshaw, T. (2010). Prevalence of giardiasis and cryptosporidiosis among children in relation to water sources in selected village of Pawi Special District in Benishangul-Gumuz Region, Northwestern Ethiopia*. Ethiopian Journal of Health Development 24*(3). https://doi.org/10.4314/ejhd.v24i3.68387

Unasho, A. (2013). An investigation of intestinal parasitic infections among the asymptomatic children in Southern Ethiopia. *International Journal of Child Health and Nutrition, 2*(3), 212–222. https://doi.org/10.6000/1929-4247.2013.02.03.3

World Health Organization: WHO. (2023, January 30). *Integrating Traditional Medicine in Health Care*. https://www.who.int/southeastasia/news/feature-stories/detail/integrating-traditional-medicine