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EVALUATION OF ANTHELMINTIC ACTIVITY OF *ZINGIBER OFFICINALE* AND ITS CHEMOMETRIC ANALYSIS

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ABSTRACT

The present study aims to collect and identify *Zingiber officinale,* to characterize the bioactive fraction by GC-MS, to evaluate the anthelmintic activity of extract in *Pheretima posthuma* and to optimize the biological action.The aqueous extract of *Zingiber officinale* was prepared using a Soxhlet extraction method to ensure efficient recovery of bioactive compounds and further analysis using Gas Chromatography system. It revealed the presence of fifteen distinct bioactive compounds. Among these, key constituents identified included Phenol, 2-methoxy-3-(2-propenyl), 4-Allyl-1,2-diacetoxybenzene, 1,3,4- eugenolacetate, Hexanedioic acid, and 1,2-Cyclohexanedicarboxylic acid. GC-MS analysis of the plant extract identified a complex mixture of fifteen bioactive compounds, among which eugenol (Phenol, 2-methoxy- 3-(2-propenyl)), 4-Allyl-1,2-diacetoxybenzene, and 1,3,4-eugenolacetate stood out for their known antimicrobial, antifungal, and antioxidant properties.

KEY WORDS: *Zingiber officinale,* Anti helmintic activity, *Pheretima posthuma*, Bioactive compounds

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INTRODUCTION

The infections caused by parasitic worms remain a major public health concern worldwide, particularly in low- and middle-income countries where inadequate sanitation, poor hygiene, and limited access to healthcare prevail. Helmintic infections are diverse and globally significant parasitic diseases caused by nematodes, cestodes and trematodes. Research continues to expand in epidemology, control measures, diagnostic methods for effective disease control and understanding of helminth biology. As this type of infections continue to rise in prevalence and geographic scope, there is an urgent need for integrated control strategies that combine improved sanitation, health education, environmental management, and sustainable deworming programs to effectively curb their spread and long-term impact.. A deeper understanding of helminth biology, host-parasite interactions, and transmission dynamics is essential to designing sustainable interventions and achieving long-term control and elimination goals (Baravkar et al. 2020).

Zingiber officinale (ginger) exhibits notable antiparasitic activity, making it an important natural remedy against a variety of parasitic infections. The bioactive compounds found in ginger, such as gingerols, shogaols, and paradols, have demonstrated efficacy against several parasites, including protozoans, cestodes, nematodes and trematodes. The antiparasitic potential of *Zingiber officinale* adds to its versatile medicinal profile, reinforcing its value in combating parasitic diseases worldwide (Promdam and Panichayupakaranant 2022).

Overall, the anti-helminthic activity of *Zingiber officinale* adds to its broad spectrum of medicinal benefits and highlights its potential role in integrated parasite control strategies, especially in traditional medicine systems. The objectives of the study are to collect and identify *Zingiber officinale,* to characterize the bioactive fraction by the employment of GC-MS, to evaluate the anthelmintic activity of extract in *Pheretima posthuma* and to optimize the biological action.
(Sheike P.S et.al 2020).

REVIEW OF LITERATURE

This paper describes the anthelmintic activity of *Zingiber officinale* Roscoe (family Zingiberaceae) rhizome, commonly known as ginger, to justify its traditional use in veterinary medicine. Crude powder (CP) and crude aqueous extract (CAE) of dried ginger (1–3 g/kg) were administered to sheep naturally infected with mixed species of gastrointestinal nematodes. Both CP and CAE exhibited a dose- and a time- dependent anthelmintic effect with respective maximum reduction of 25.6% and 66.6% in eggs per gram (EPG) of faeces on day 10 of post-treatment. Levamisole (7.5 mg/kg), a standard anthelmintic agent, exhibited 99.2% reduction in EPG. This study shows that ginger possesses in vivo anthelmintic activity in sheep thus justifying the age-old traditional use of this plant in helminth infestation (Iqbal et al. 2006).

The use of plant extracts to control poultry helminths is increasing in different rearing systems. The anthelmintic activity of ginger and curcumin was studied on the nematode *Ascaridia galli*.Ginger in all concentrations used exhibited a higher death rate observed than curcumin. Their wormicidal effect is concentration–time dependent.(Bazh and El-Bahy 2013)

The present study was undertaken to evaluate the anthelmintic activity of ethanol extracts of *Zingiber officinalis* (Zingiberacae), *Zingiber zerumbet* (Zingiberacae) and *Curcuma longa* on Indian earthworm, *Pheretima posthuma.* Various concentrations (10ug/ml, 25ug/ml, 50ug/ml) of all extracts were tested and results were expressed in terms of time of paralysis and time of death of the earthworm. Piperazine citrate (10ug/ml) was used as reference standard and sterile water as control group (Raul et al. 2012).

The present research work deals with the establishment of a standardization parameter for the pharmacognositical evaluation of the plant *Embelia ribes* and *Camellia sinensis. Camellia sinensis* (ethanolic extract) extract and affords protection against helminthes (*Pheritima Posthuma)* and results show that *Camellia sinensis* is as effective as *Embellia ribes* (Rojas-Vargas et al. 2022). Our results indicate that AILE exhibits powerful anthelmintic and anticoccidial activities and it could be exploited further for the development of a novel therapeutic agent (Abu Hawsah et al. 2023).

This article aims at reviewing the most salient recent reports on ethnobotany, pharmacology, phytochemistry and biological activities of *Z. officinale* (Dhanik et al. 2017). These findings highlight the therapeutic prospects of *Sida cordifolia* and ginger as natural alternatives for managing inflammation and parasitic infections, supporting their traditional use and encouraging further pharmacological exploration. The extraction of essential oil methods and post-harvest process are the most traditional methods for Mango ginger production in developing countries. Further research on oil extraction methods, maximizing yield per hectare and optimum preservation are needed, especially in post-harvest of mango ginger rhizomes, leaves and roots (Al-Qudah et al. 2017).

The present invention discloses novel synergistic anthelmintic compositions used for prevention and treatment of gastrointestinal nematodes comprising combination of therape tically effective amount of ginger extract with proteolytic enzyme and fibre degrading enzymes. The novel anthelmintic compositions provide synergistic effect at comparatively lower doses which are affordable and impart minimal/no side effect on general health as well as milk yield of dairy animals .

MATERIALS AND METHODS

The leaves of *Zingiber officinale* were dried powdered and seived until the plant material reached a consistent level of dryness suitable for further processing (Nakra, Tripathy, and Srivastav 2025). The aqueous extract of *Zingiber officinale* was prepared using a Soxhlet extraction method to ensure efficient recovery of bioactive compounds. In this study, the bioactive constituents of the aqueous extract of *Zingiber officinale* were analyzed using a Gas Chromatography system (Agilent 6890 series) equipped with an HP-5MS capillary column, coupled to a mass spectrometer. The mass spectra obtained were compared against the National Institute of Standards and Technology (NIST) library database, with compounds exhibiting a similarity index of ≥70% being considered as positively identified. This analysis provided valuable insights into the bioactive molecules present in the aqueous extract, which may contribute to its pharmacological and biological activities (Kasi et al. 2017).

Adult specimens of *Pheretima posthuma* were collected for experimental assays or kept under laboratory conditions in moist soil until further use, ensuring humane handling throughout the process (Baravkar et al. 2020). The anthelmintic activity of *Zingiber officinale* was evaluated using adult Indian earthworms (*Pheretima posthuma*). Test solutions were prepared by dissolving the extract in normal saline to achieve various concentrations ranging from 200 mg/mL to 1000 mg/mL. In multiple animal studies ginger extract showed no lethality when administrered orally in high doses between 100-5000 mg/kg. For the assay, individual worms were placed into separate sterile Petri dishes, each containing 10 mL of the respective test solution. The mean values for paralysis and death times were calculated and compared to those of control and standard drug-treated groups to evaluate the dose-dependent anthelmintic efficacy of the *Zingiber officinale* aqueous extract. . *Pheretima Posthuma* was placed in Petri dish containing 10 ml of test solution of varying concentrations of *Zingiber officinale* aqueous extract ranging from 200mto 1000 mg/ml. Each Petri dish was placed with1 worm and observed for paralysis or death. M

Albendazole was used as the standard reference drug and prepared at a concentration of 10 mg/mL in normal saline. (Horton J , 2001). Adult earthworms of equal size (7–10 cm) and weight (2–3 g) were collected from the vermiculture unit and acclimatized in laboratory conditions. The worms were washed with normal saline and divided into seven groups (n = 6 worms per group). The groups were assigned as follows: Group I (control, normal saline), Group II (Albendazole 10 mg/mL), and Groups III–VII (*Zingiber officinale* extract at 200, 400, 600, 800, and 1000 mg/mL respectively). Each worm was placed in a separate Petri dish containing 10 mL of the respective solution. And observed for or deathwas recorded for each worm using a stopwatch. The mean paralysis time and mean death time were calculated for each group.

The results were statistically analyzed using one-way ANOVA followed by Tukey's post hoc test to compare the activity of ginger extract with that of Albendazole. Tukeys test revealed that the high dose extract group differed significsntly from the control and was comparable to albendazole.A p-value less than 0.05 was considered statistically significant (Vadakkan et al. 2021)

Adult specimens of *Pheretima posthuma* were collected anesthetized by placing them in a 30% ethanol solution for 5–10 minutes to immobilize them for observation. External morphological features such as body length, diameter, segmentation, pigmentation, and presence of clitellum were observed using a stereomicroscope and recorded. The prostomium, number and arrangement of setae, position of the clitellum, and the location of genital openings were examined using a hand lens and dissection microscope (Sims et al. 2024).

After the incubation period the treated samples were collected and sections were sectiones were prepared for histological evaluation. To evaluate the anthelmintic activity of *Zingiber officinale* under varying physicochemical conditions, experiments were conducted to assess the influence of extract concentration, pH, and temperature. The data collected were analyzed to determine the optimal conditions for maximizing the anthelminthic activity of *Zingiber officinale* extract.

RESULTS

1.1 Characterization of Bioactive fraction using GC-MS Analysis

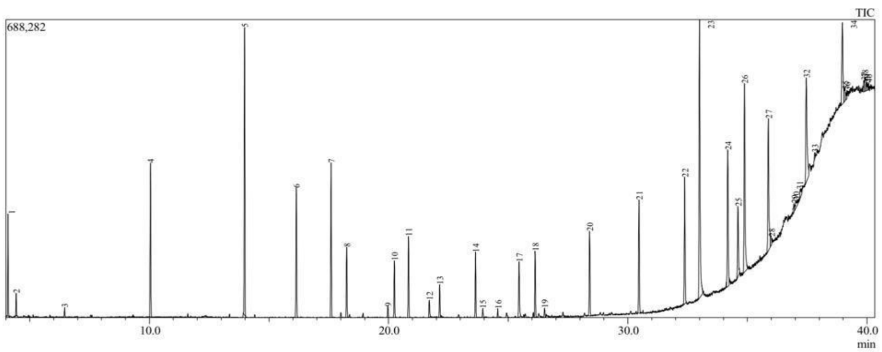


Figure 1 : GC-MS chromatogram of *Zingiber officinale* extract

Gas Chromatography–Mass Spectrometry (GC-MS) analysis of the plant extract exhibiting anti- helmintic activity revealed the presence of fifteen distinct bioactive compounds. Among these, key constituents identified included Phenol, 2-methoxy-3-(2-propenyl), 4-Allyl-1,2-diacetoxybenzene, 1,3,4- eugenolacetate, Hexanedioic acid, and 1,2-Cyclohexanedicarboxylic acid.

The anthelmintic activity of aqueous extract of *Zingiber officinale* was evaluated using *Pheretima posthuma* as a model organism, with extract concentrations ranging from 200 to 1000 mg/mL. Observations indicated a concentration-dependent effect on the anatomical, morphological, and behavioral responses of the earthworms. At lower concentrations (200–400 mg/mL), the organisms displayed a pronounced escape behavior characterized by rapid locomotion, wriggling, and curling movements, indicative of a stress response. In contrast, higher concentrations (800–1000 mg/mL) elicited immediate and severe physiological responses, including reduced mobility and rapid onset of paralysis. At the highest concentration tested (1000 mg/mL), paralysis occurred within 1 minute post-exposure, followed by mortality at approximately 6 minutes. death, supporting its efficacy as a bioactive agent with anthelmintic properties.

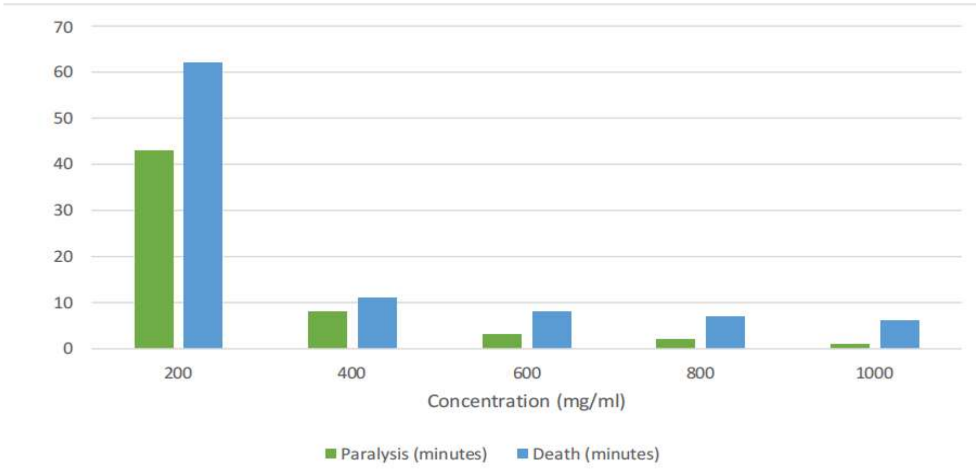


Fig.1 Effect of Zingiber officinale on paralysis and death of *Pheretima posthuma*

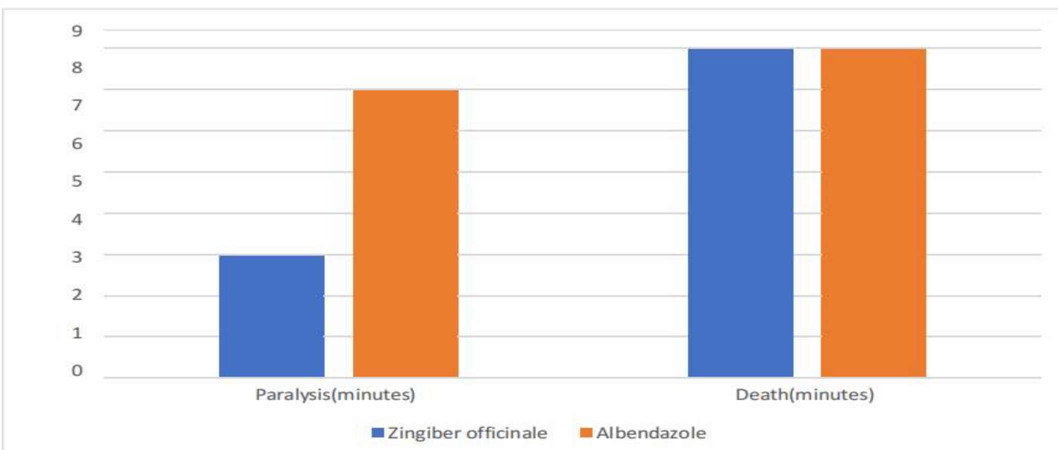
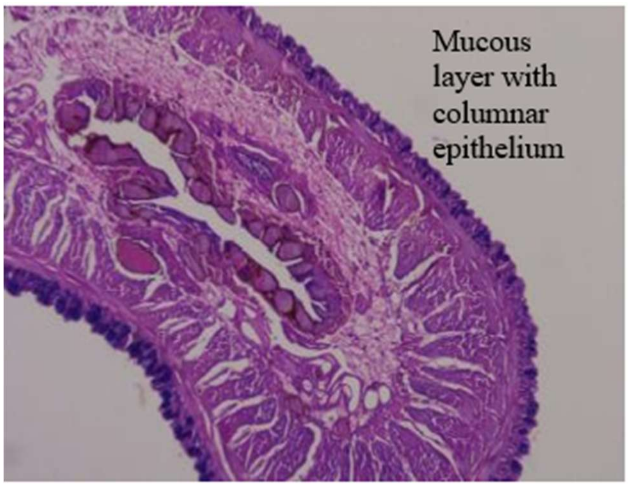


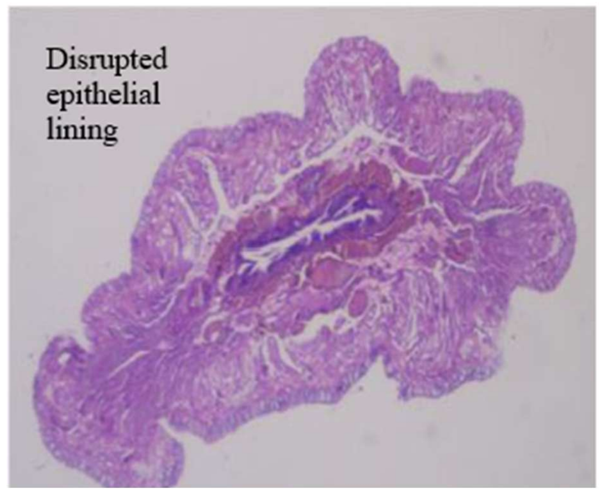
Figure 2: Comparison study on the plant extract with albendazole

Following exposure to varying concentrations of *Zingiber officinale* aqueous extract, *Pheretima posthuma* exhibited distinct morphological alterations indicative of stress and physiological disruption. The most prominent features observed across all concentrations included segmental bulging and cutaneous peeling, which suggest damage to the cuticle and underlying tissues. These effects were more severe at higher concentrations (800–1000 mg/mL), where rapid onset of integumentary breakdown was evident. Additionally, increased secretion of coelomic fluid was noted, especially at the initial stages of exposure, possibly reflecting a defensive or inflammatory response to the phytochemical constituents of the extract. The body of the worm became progressively opaque and swollen, and in several instances, longitudinal ruptures were observed post-mortem. Discoloration and flaccidity were also apparent as the worms progressed from paralysis to death.

These morphological manifestations reflect the potential of *Z. officinale* extract to disrupt the structural integrity of helminths, likely due to the presence of bioactive compounds such as gingerols and shogaols, which may interfere with membrane stability and neuromuscular coordination. The consistency of these changes across multiple specimens supports the hypothesis that the plant extract exerts a direct toxic effect on the helminth's somatic tissues, affirming its anthelmintic potential.



Untreated



Treated

Figure 3 : Histological analysis of extract upon *Pheretima posthuma*.

The histological analysis of earthworm intestinal tissue revealed distinct differences between the normal and treated groups, indicating the impact of the anti-helmintic compound. In the normal tissue (left image), the intestinal architecture appeared intact and well-organized. The mucosal layer was lined with healthy columnar epithelial cells and numerous goblet cells, while the submucosal and muscular layers were clearly defined and structurally preserved. Regular mucosal folds suggested normal absorptive capacity and tissue function. In contrast, the treated tissue (right image) displayed marked degeneration. The epithelial lining was severely disrupted, with evident sloughing of cells and a notable reduction in goblet cell population. The mucosal structure appeared collapsed, and there were signs of vacuolation and cellular disintegration, indicative of cytotoxic stress. Additionally, the muscular layers showed reduced integrity, with thinning and disorganization. These observations suggest that the anti-helmintic compound exerts significant damage to the gastrointestinal tract of the earthworm,

To maximize the anthelmintic efficacy of *Zingiber officinale* aqueous extract, optimization studies were conducted by varying key physicochemical parameters, including concentration, pH, and temperature. The experimental outcomes demonstrated that these variables significantly influence the onset of paralysis and time to death in *Pheretima posthuma*, the chosen model organism. Among the tested concentrations, the extract at 1000 mg/mL exhibited the most potent anthelmintic activity, with complete mortality observed within 6 minutes of exposure. To evaluate the effect of pH on extract activity, the test mixtures were adjusted across a pH range of 4 to 10 using appropriate buffer systems—acetate buffer (pH 4–5.6), phosphate buffer (pH 5.8–7.4), and carbonate buffer (pH 9.2–10.6). The results indicated a positive correlation between pH and anthelmintic efficacy, with higher activity observed at alkaline pH levels. This suggests that the bioactive components of *Z. officinale* may be more stable or more readily absorbed by the organism under basic conditions. Similarly, the effect of temperature was assessed by incubating the reaction mixtures at increasing temperatures. The extract showed optimal activity at 40 °C, beyond which a decline in efficacy was noted, especially at elevated temperatures such as 60 °C. It should be noted that a temperature of 120 °C is likely above the thermal stability threshold for most plant-derived bioactive compounds and may lead to degradation. Therefore, the observed reduction in activity at higher temperatures could be attributed to thermal denaturation of active constituents. These findings underscore the critical role of environmental factors in enhancing the therapeutic potential of *Z. officinale* as a natural anthelmintic agent.

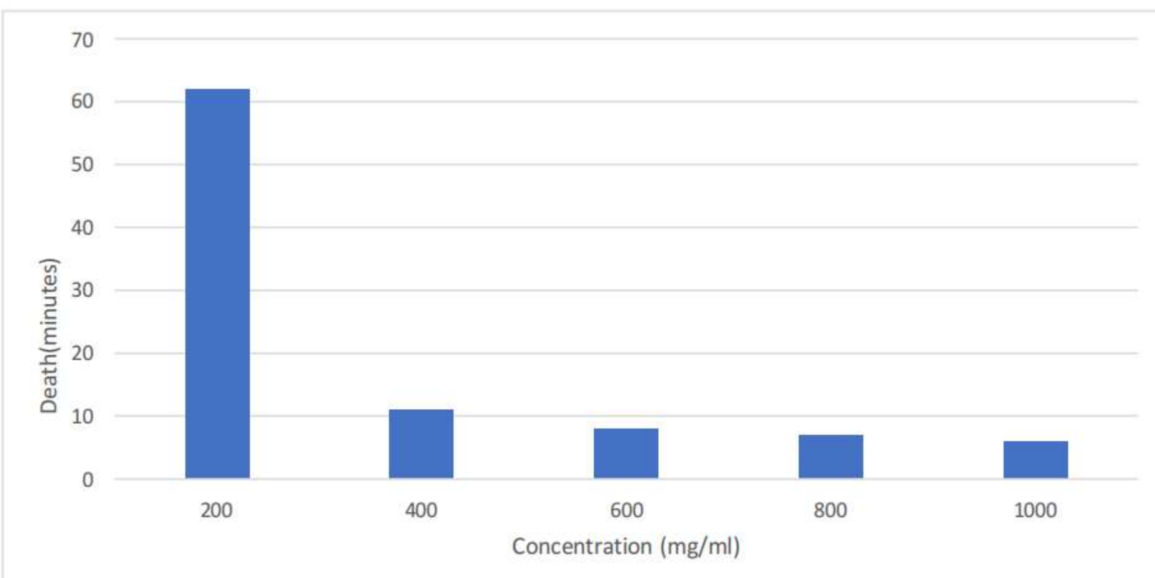


Fig.4 Effect of concentration on death of *Pheretima posthuma*

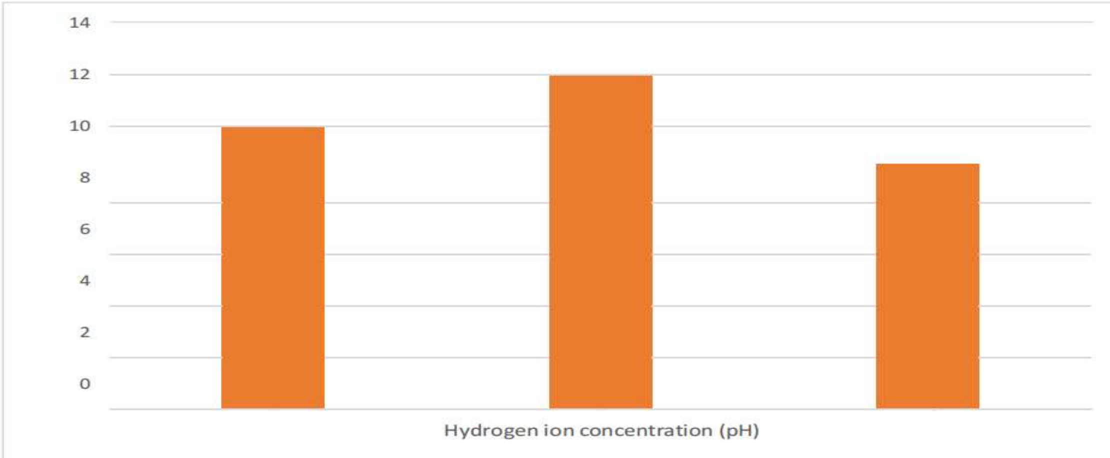


Fig.5 Effect of pH on death of *Pheretima posthuma*

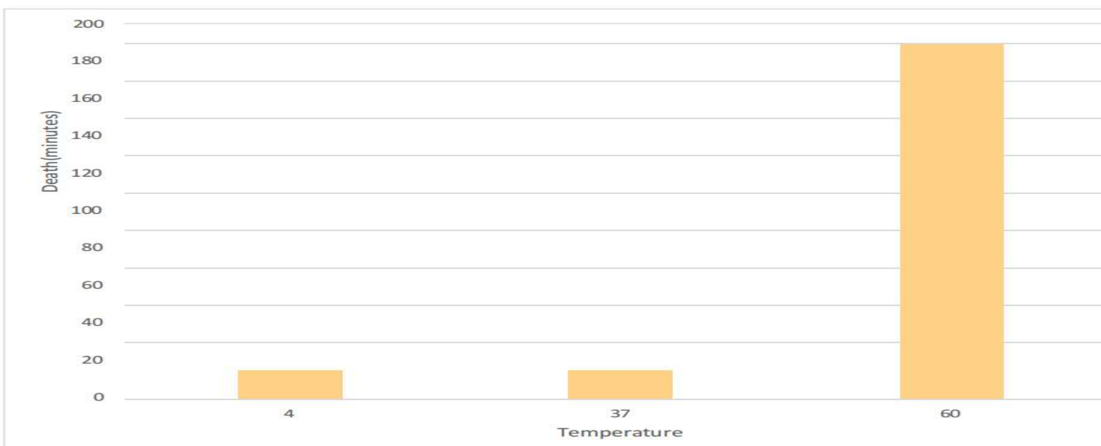


Figure 6: Effect of temperature on death of *Pheretima posthuma*

DISCUSSION

In the study, the chemical profile of *Zingiber officinale* essential oil (EO), the *in vitro* anthelmintic effect against the murine helminth parasite model *Heligmosomoides polygyrus.* Chromatographic profile of *Zingiber officinale* (EO) extracted from the leaves of *Zingiber officinale* have shown the presence of monterpens 1.8-cineol (Eucalyptol) (29.47%), D-Limonène (18.51%) and Linalool (10.84%) in high fractions. The *in vitro* anthelmintic potential was expressed by an ovicidal effect against *H. contortus* egg hatching with inhibition value of 1.72 mg/mL and 87.5% of immobility of adult worms after 8 h of exposure to 4 mg/mL of *Zingiber officinale* EO. Regarding, the *in vivo* anthelmintic potential, *Zingiber officinale* (EO) at 2400 mg/kg bw completely eliminated the egg output of *H. polygyrus* after 7 days of oral treatment, together with a 79.2% of reduction in total worm counts. Based on the obtained results, *Zingiber officinale* EO showed promising *in vitro* and *in vivo* anthelmintic capacities against gastrointestinal parasites (Kiambom et al. 2020).

The present study investigated the anthelmintic potential of *Zingiber officinale* (ginger) aqueous extract using *Pheretima posthuma* as a model organism, revealing promising bioactivity supported by behavioral, morphological, and histological observations. The GC-MS analysis of the plant extract identified a complex mixture of fifteen bioactive compounds, among which eugenol (Phenol, 2-methoxy- 3-(2-propenyl)), 4-Allyl-1,2-diacetoxybenzene, and 1,3,4-eugenolacetate stood out for their known antimicrobial, antifungal, and antioxidant properties. These compounds likely act synergistically to disrupt the parasite's physiological integrity, thereby exerting a potent anthelmintic effect.

The extract demonstrated a clear dose-dependent response, with increasing concentrations leading to a more rapid onset of paralysis and death in *P. posthuma*. At the highest concentration (1000 mg/mL), complete paralysis was achieved within 1 minute and death occurred by 6 minutes. These results indicate that the efficacy of *Z. officinale* is directly proportional to its concentration, likely due to higher availability of bioactive phytochemicals interacting with the helminth's neuromuscular system and cuticular structures. Notably, the observed morphological changes—including segmental bulging, cuticle peeling, and coelomic fluid excretion—suggest mechanical and chemical disruption of epidermal and muscular tissues, consistent with the action of membrane-active compounds such as gingerols and shogaols (Lin et al. 2010).Comparative studies with albendazole further validated the extract's efficacy. While albendazole induced paralysis at 7 minutes, *Z. officinale* achieved this at 3 minutes, with both treatments resulting in mortality by 8 minutes. Although the synthetic drug is more potent in terms of dosage, the rapid paralytic effect of the plant extract underscores its therapeutic potential and positions it as a viable natural alternative or complementary agent in helminth management strategies.

Histological analysis in treated specimens, intestinal tissue revealed significant structural degradation, including sloughing of epithelial cells, vacuolation, and reduction in goblet cells. These alterations reflect cytotoxic effects consistent with disruption of essential gastrointestinal functions such as nutrient absorption and motility. The damage to both mucosal and muscular layers suggests that the extract compromises tissue viability through multiple pathways, possibly involving oxidative stress and membrane destabilization(Wang et al. 2022).

Optimization studies further highlighted the influence of external variables on extract efficacy. Among the tested parameters, alkaline pH conditions (pH 10) enhanced the extract's activity, reducing time to death to 9 minutes. This suggests that the stability or bioavailability of active compounds is improved in basic environments, which may favor solubilization or facilitate uptake by the helminth. Temperature also played a significant role: optimal anthelmintic activity was observed at 40 °C, aligning with the thermal tolerance range of many bioactive plant compounds. However, a marked reduction in efficacy was observed at 60 °C and beyond, with death time extending to 180 minutes at 60 °C, likely due to degradation of thermolabile phytoconstituents. Collectively, these findings support the hypothesis that *Z. officinale* exerts potent anthelmintic effects through a combination of chemical toxicity and structural disruption.

Experimental studies using aqueous extracts of *Z. officinale* demonstrated significant efficacy against helminths, with the highest activity observed at a concentration of 1000 mg/mL. The extract's anthelmintic effect was found to be comparable to that of albendazole, a widely used standard drug (Tiki et al. 2024). The efficacy of *Z. officinale* was influenced by both pH and temperature. The extract exhibited maximum stability and activity at pH 7, with positive effects also observed at other pH levels. Optimal anthelmintic activity occurred at 37 °C, while exposure to extreme temperatures led to morphological changes in the helminths, such as tissue swelling, coiling, localized lesions, and paralysis. Observations during treatment revealed a progression from rapid, involuntary movements to reduced motility, followed by complete paralysis and eventual death of the parasites (Ghafar et al. 2021).

Chemical profiling using Gas Chromatography–Mass Spectrometry (GC-MS) identified 13 bioactive compounds in the *Z. officinale* extract. These included 3-methyl-2-butenyl, 3-ethyl-4- methyltetrahydrofuran-3-ol, pentadecane, eugenol, 1,2-benzenedicarboxylic acid, furo[2,3-c]pyridine, cyclotrisiloxane, 14,19-dioxoundecacyclo compounds, 1H-furo[3,4-c]pyrrole-4-carboxylic acid, as well as nickel, cesium, and various complex organic derivatives. These compounds are believed to contribute to the plant's observed anthelmintic effects(Sharifi-Rad et al. 2017).

CONCLUSION

Zingiber officinale exhibits strong potential as a plant-based anthelmintic agent, offering a viable alternative to conventional synthetic drugs. However, further in vivo studies and pharmacological evaluations are essential to fully understand its mechanism of action, safety profile, and therapeutic potential in managing helminth infections.

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