RESEARCH PAPER

Evaluation of phytochemical and Anthelmintic Activity of ethanolic Extracts of seed of plants *Apium leptophyllum* and *Apium graveolens* of family Apiaceae

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ABSTRACT

Background:
Helminthic infections remain an important health concern, and the search for effective plant-based anthelmintic agents has increased interest in medicinal plants. The present study evaluated the in vitro anthelmintic activity of aqueous and ethanolic seed extracts of *A. leptophyllum* and *A. graveolens* against *Pheretima posthuma.*
Methods:
The aqueous and ethanolic extracts of *A. leptophyllum* and *A. graveolens* were evaluated at concentrations of 10 and 20 mg/ml using volumes of 10 and 15 ml. Albendazole was used as the standard drug. The time of paralysis (TOP) and time of death (TOD) of the worms were recorded as indicators of anthelmintic activity. The results were expressed as mean ± SD and analysed using one-way ANOVA followed by Dunnett's multiple-comparison test.
Results:
Both aqueous and ethanolic extracts produced dose dependent anthelmintic effects, as shown by decrease in the time required for paralysis and death of the worms. The ethanolic extracts generally exhibited stronger activity than the corresponding aqueous extracts. Significant differences were observed among the treatment groups for both TOP and TOD ($P < 0.0001$). Dunnett's multiple-comparison analysis showed significant differences from the albendazole-treated group for most treatment groups, while *A. leptophyllum* 20 mg/ml (15 ml) and A. graveolens 20 mg/ml (15 ml) showed no significant difference from albendazole for TOD.
Conclusion:
The findings demonstrate that both *A. leptophyllum* and *A. graveolens* seed extracts possess promising in vitro anthelmintic activity. The ethanolic extracts showed comparatively greater activity, particularly at higher concentrations and volumes. Further studies are required to identify the active phytoconstituents and elucidate their mechanisms of anthelmintic action.

Keywords: *Apium leptophyllum*; *Apium graveolens*; anthelmintic activity; *Pheretima posthuma*; albendazole; aqueous extract; ethanolic extract.

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1.INTRODUCTION

The term helminths originate from the Greek word helmins, meaning "worm," and describes a diverse group of parasitic worms capable of infecting humans and animals (1). Globally, gastrointestinal (GI) helminth infections remain a significant health challenge, especially in low- and middle-income countries. It is estimated that around 3.5 billion individuals are infected worldwide, leading to over 200,000 deaths annually and symptomatic disease in roughly 450 million people (2). Beyond human health, helminth infections cause substantial economic losses in the livestock industry; for instance, estimated annual losses in the European ruminant sector exceed €1.8 billion (3). Soil-transmitted helminths (STH) such as Ascaris lumbricoides, Trichuris trichiura, Ancylostoma duodenale, and Necator americanus are major contributors to helminthiasis (4). According to the World Health Organization, over two billion people remain affected, and by 2025, more than half the population in certain developing areas could be at risk (5). The effectiveness of widely used anthelmintics like albendazole, mebendazole, and levamisole is threatened by emerging drug resistance, prompting increased research into alternative agents (6,7). Although resistance in human helminthiasis is less advanced compared to veterinary medicine, the

need for new molecules and monitoring strategies remains critical (8,9). Historically, natural products have played a pivotal role in drug discovery, and research into traditional medicinal plants continues to yield promising bioactive compounds (10,11).

The distribution of helminth infections typically follows a negative binomial pattern, where a small subset of the population harbors high parasite burdens, contributing disproportionately to transmission (12). Anthelmintics administered orally to expel or kill worms remain the cornerstone of treatment; however, common side effects include nausea, abdominal pain, and diarrhoea (11). Additionally, helminth infections can increase susceptibility to other diseases, compounding public health challenges (13). In many developing regions, over 80% of the population relies on plant-based remedies for primary healthcare (5). This underscores the value of evaluating traditional medicinal plants, particularly given rising drug resistance and ecological concerns related to synthetic anthelmintics (14).

Research has identified several helminth targets, including neuromuscular coordination, energy metabolism, and nucleic acid synthesis, that may inform future drug design (6). The Apiaceae family, encompassing more than 3,700 aromatic plant species, is notable for its ethnomedicinal use against gastrointestinal ailments (15). *Apium leptophyllum* and *Apium graveolens* are traditionally recognized for their carminative, sedative, diuretic, antimicrobial, and anthelmintic properties. Seeds and essential oils from these species have also demonstrated antifungal and anticonvulsant activities, highlighting their continued potential in modern pharmacological research (15).

2.Materials and Methods

2.1 Plant Material and Extract Preparation

2.1.1 Collection and Authentication

Seeds of *Apium leptophyllum* and *Apium graveolens* were purchased from the local market in Chandigarh, India, and authenticated by Dr. Anita Mahiswar, Head, Department of Botany, Govt. Digvijay Autonomous PG College Rajnandgaon (C.G.).

2.1.2 Drying and Extraction

Seeds were air-dried and ground into coarse powder. Aqueous extracts were prepared by macerating 50 g of powdered seeds with 400 ml distilled water for 72 hours, followed by vacuum filtration and evaporation to dryness. Ethanolic extracts were obtained similarly using 400 ml ethanol and evaporated under reduced pressure in a rotary evaporator. Both extracts were dissolved in 2% Tween 80 saline solution to prepare concentrations of 10 and 20 mg/ml, with final volumes adjusted to 10 ml and 15 ml.

2.2 Animals

Adult Indian earthworms (*Pheretima posthuma*), due to their physiological similarity to intestinal helminths were collected from moist soil and cleaned with saline before use. Worms measuring 4–7 cm in length and 0.1–0.2 cm in diameter were selected. (16-17)

2.3. Reference Standard and Chemicals

Albendazole (Ankur Drugs and Pharma Ltd., Solan, HP) was used as the reference standard. Other chemicals included Tween® 80 (Molychem, Mumbai) and normal saline (Nirlife). Albendazole was dissolved in 2% Tween 80 saline to achieve 10 and 20 mg/ml concentrations, and normal saline was used as the negative control.

2.4. Experiment Method

2.4.1 Phytochemical screening

Phytochemical screening of *Apium leptophyllum* and *Apium graveolens* seed extracts was conducted using standard qualitative methods by Bibi et al., 2023; Nawaz et al., 2022; Singh et al., 2021 revealing the presence of alkaloids, flavonoids, saponins, tannins, glycosides, steroids, and triterpenoids. These bioactive compounds are known to contribute to various pharmacological effects, including antimicrobial and anthelmintic activities. The analysis supports traditional claims and highlights the therapeutic potential of these plants. Recent research also emphasizes that systematic phytochemical profiling is crucial for guiding the isolation of active constituents. (18-20)

2.4.2 Acute Toxicity Test

Present investigation included an acute toxicity test in this preliminary study, single oral doses of *Apium leptophyllum* and *Apium graveolens*. Ethanolic extract and aqueous extract 500, 1000, 1500 and 2000 mg/kg were administered by oral gavages to 5 female mice in each dose group respectively. After administration of test drugs, rats were observed for first 4 h to check any possible adverse effect. While 24 h later rat groups were examined again to check the death rate for 14 days. (21-23)

2.4.3 Anthelmintic Activity

Indian adult earthworms (*Pheretima posthuma*), widely accepted as a suitable model for screening anthelmintic activity due to their physiological resemblance to human intestinal roundworms (24-26), were used to assess the ethanolic and aqueous extracts of *Apium leptophyllum* and *Apium graveolens* seeds. Extracts and standard albendazole were prepared at concentrations of 10 and 20 mg/ml in 2% Tween 80 saline solution. Worms were placed in petri dishes (10 and 15 ml volumes), and paralysis and death times were observed, defined by complete loss of movement in warm water (50 °C) and no response to stimuli. Each group comprised five worms, and mean times for paralysis and death were recorded (27-28).

2.5 Statistical analysis

All experimental data were expressed as mean ± standard deviation (SD) (n = 5). Statistical analysis was performed using GraphPad Prism software.

Differences among the treatment groups were analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparisons test, with the albendazole-treated group considered as the standard control. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1 Result of Phytochemical screening

Preliminary phytochemical screening of *Apium leptophyllum* and *Apium graveolens* confirmed the presence of alkaloids, amino acids, steroids, glycosides, cardiac glycosides, saponins, flavonoids, tannins, and phenolic compounds (Table 1)

Table 1: Show results of phytochemical screening of *Apium Lyptophyllum* and *Apium graveolens.*

S. No.	Phytochemical Test	*A. leptophyllum* Aqueous	*A. leptophyllum* Ethanolic	*A. graveolens* Aqueous	*A. graveolens* Ethanolic
1	**Alkaloids**				
	Dragendorff's reagent	–	+	–	+
	Mayer's reagent	–	+	–	+
	Wagner's reagent	+	+	–	–
	Hager's reagent	+	+	–	–
	Tannic acid test	+	+	+	+
2	**Amino acids**	+	+	+	+
3	**Proteins**	–	–	–	–
4	**Starch**	–	–	–	–
5	**Steroids & Triterpenoids**	+	+	+	+
	Salkowski test (yellow)	+	+	+	+
	Sulfur powder test	+	+	+	+
6	**Carbohydrates (aqueous only)**	+	–	+	–
7	**Glycosides**	+	+	+	+
8	**Cardiac glycosides (Keller-Killiani, Legal)**	+	+	+	+
9	**Cyanogenetic glycosides**	–	–	–	–
10	**Saponins (Froth test)**	+	+	+	+
11	**Flavonoids**	+	+	+	+
12	**Diterpenes**	–	–	–	–
13	**Volatile oil**	–	+	–	+
14	**Tannins & Phenolic compounds**	+	+	+	+
	Ferric chloride test (green)	+	+	+	+
	Test for chlorogenic acid	+	+	–	–
15	**Naphthoquinones (Juglone, Dam-Karrer tests)**	–	–	–	–

Note: '+' indicates presence; '–' indicates absence.

3.2 Acute toxicity Study

Present investigation included an acute toxicity test in this preliminary study, single oral doses of *Apium leptophyllum* and *Apium graveolens* ethanolic and aqueous extract 500, 1000, 1500 and 2000 mg/kg were administered by oral gavages to 6 mice of either sex, in each dose group respectively. After administration of test drugs, rats were observed for first 4 h to check any possible adverse effect. While 24 h later rat groups were examined again to check the death rate for 14 days.

3.3 Result of Anthelmintic Activity

The ethanolic extracts of both plants exhibited stronger anthelmintic activity compared to their aqueous extracts and showed efficacy comparable to the standard drug albendazole. In vitro anthelmintic activity was evaluated using Indian adult earthworms (*Pheretima posthuma*) at two concentrations (10 mg/ml and 20 mg/ml) and two volumes (10 ml and 15 ml). The time to paralysis (P) and death (D) was recorded. The ethanolic extracts at 20 mg/ml demonstrated highly significant activity in both volumes when compared with albendazole (10 mg/ml) in a volume of 15 ml.

3.3.1 Anthelmintic Activity aqueous extracts

The effects of the standard drug and test aqueous extracts of *Apium lyptophyllum* and *Apium graveolens* on the time of paralysis and time of death are presented in Table 2. A statistically significant difference was observed among the treatment groups for both time of paralysis and time of death, as determined by one-way ANOVA ($F(8,36) = 1510$, $P < 0.0001$) and ($F(8,36) = 1548$, $P < 0.0001$), respectively. Dunnett's multiple-comparison test

revealed that all test groups differed significantly from the standard albendazole group (adjusted P < 0.0001 for all comparisons).

Table 2: Result of Anthelmintic activity of Aqueous extract.

S.No	Plant Name	Aqueous Extract				Aqueous Extract			
		10mg/ml volume taken 10 ml		10mg/ml volume taken 15 ml		20mg/ml volume taken 10 ml		20mg/ml volume taken 15 ml	
		Time of paralysis	Time of Death	Time of paralysis	Time of Death	Time of paralysis	Time of Death	Time of paralysis	Time of Death
1.	***Apium lyptophyllum***	61.83 ± 1.373	71.50 ± 1.225	53.38 ± 0.6073	67.14 ±0.5721	50.60 ± 0.4266	59.24 ± 0.3870	49.03 ± 0.2400	55.77 ± 0.4963
2.	***Apium graveolens***	44.41 ± 1.318	58.46± 0.9712	41.37± 0.8518	48.41 ± 0.2735	34.97 ± 0.6542	46.87± 0.5685	31.78 ± 0.8324	44.82 ± 0.6486
3.	**Standard Albendazole**			12.16 ± 0.4902	28.12± 0.9803				

Values are expressed as mean ± SD (n = 5). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparisons test. Comparisons were made with the standard (albendazole) group. P < 0.05 was considered statistically significant.

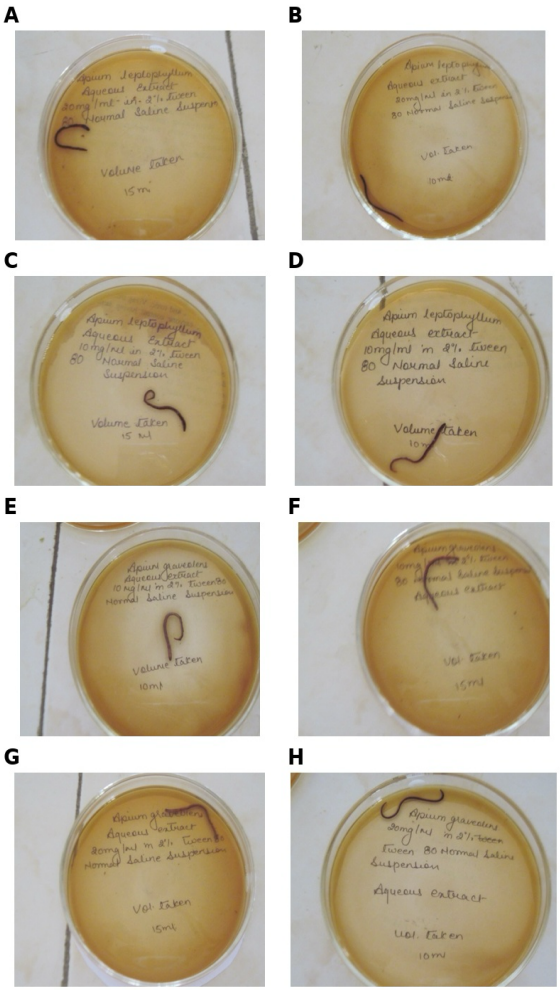

Fig. 1 Anthelmintic activity of Aqueous extract of *Apium lyptophyllum* and *Apium graveolens*. [A-D Aqueous extract of *Apium lyptophyllum*, E-H Aqueous extract of *Apium graveolens*.]

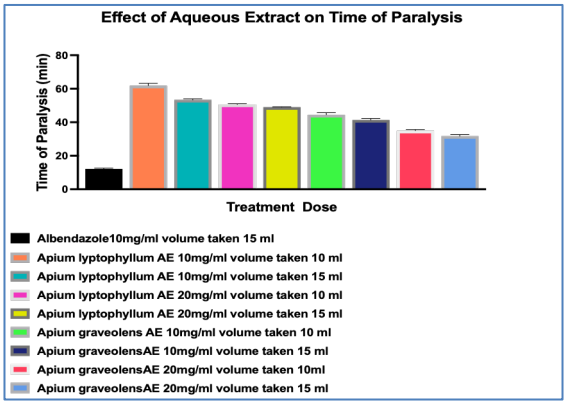

Fig. 2 Effect of aqueous extracts of *Apium lyptophyllum* and *Apium graveolens* compared with std. Albendazole on the time of paralysis of experimental worms. Values are expressed as mean ± SD (n = 5). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparisons test. Comparisons were made with the standard (albendazole) group.

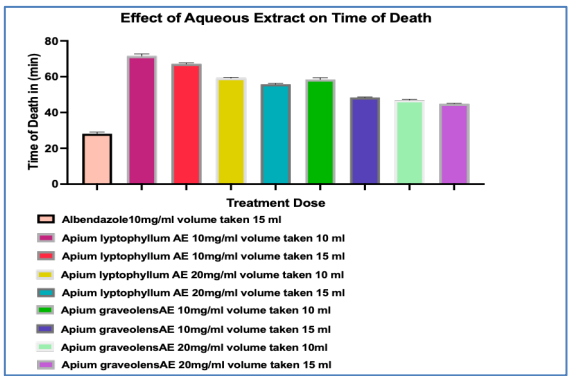

Fig. 3 Effect of aqueous extracts of *Apium lyptophyllum* and *Apium graveolens* compared

with std. Albendazole on the time of death of experimental worms. Values are expressed as mean ± SD (n = 5). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparisons test. Comparisons were made with the standard (albendazole) group.

3.3.2 Anthelmintic Activity ethanolic extracts

The effects of the standard drug and test ethanolic extracts of *Apium lyptophyllum* and *Apium graveolens* on the time of paralysis (TOP) and time of death (TOD) of the experimental worms are presented in Table 3. A statistically significant difference was observed among the treatment groups for both time of paralysis and time of death, as determined by one-way ANOVA ($F(8,36) = 403.6$, $P < 0.0001$) and ($F(8,36) = 117.9$, $P < 0.0001$), respectively. Dunnett's multiple-comparison test revealed that all treatment groups differed significantly from the albendazole-treated control for time of paralysis (adjusted $P < 0.0001$).

Table 3: Result of Anthelmintic activity of Ethanolic extract

S.No	Plant Name	Ethanolic Extract				Ethanolic Extract			
		10mg/ml volume taken 10 ml		10mg/ml volume taken 15 ml		20mg/ml volume taken 10 ml		20mg/ml volume taken 15 ml	
		Time of paralysis	Time of Death	Time of paralysis	Time of Death	Time of paralysis	Time of Death	Time of paralysis	Time of Death
1.	*Apium lyptophyllum*	26.63± 0.6733	36.17± 0.5430	23.40± 0.4552	33.05 ± 0.7220	20.93 ± 0.2406	30.17 ±0.5660	18.45± 0.6958	28.54± 0.3002
2.	*Apium graveolens*	28.29 ± 0.6199	38.02± 0.8334	26.52 ± 0.3080	35.67± 0.7052	24.02 ± 0.6797	31.49 ± 1.063	20.44± 0.6295	29.25± 0.7298
3.	Standard Albendazole			12.16 ± 0.4902	28.12± 0.9803				

Values are expressed as mean ± SD (n = 5). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparisons test. Comparisons were made with the standard (albendazole) group. $P < 0.05$ was considered statistically significant.

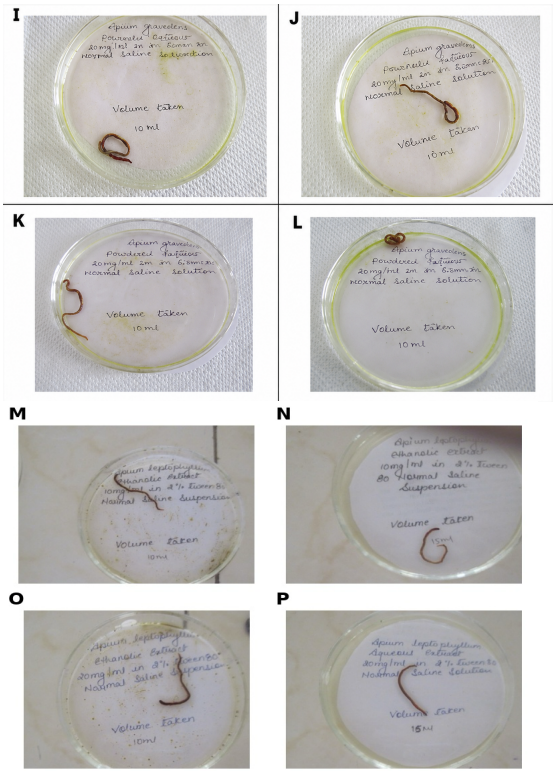

Fig. 4 Shows Anthelmintic activity of Ethanolic extract of *Apium lyptophyllum* and *Apium graveolens*. [I – L Ethanolic extract of *Apium graveolens*, M-P Ethanolicextract of *Apium lyptophyllum*]

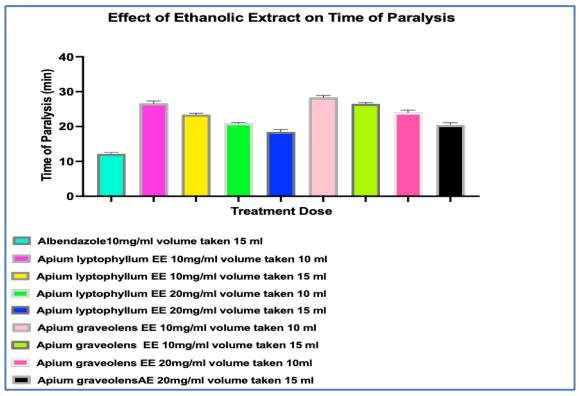

Fig. 5 Effect of Ethanolic extracts of *Apium lyptophyllum* and *Apium graveolens* compare with std. Albendazole on the time of paralysis of experimental worms. Values are expressed as mean ± SD (n = 5)

Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparisons test. Comparisons were made with the standard (albendazole) group.

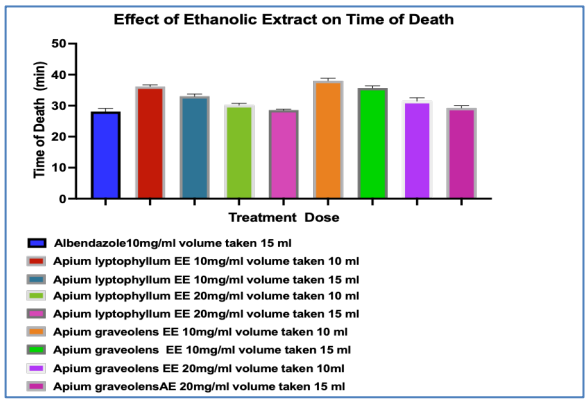

Fig. 6 Effect of Ethanolic extracts of *Apium lyptophyllum* and *Apium graveolens* compare with std. Albendazole on the time of Death of experimental worms. Values are expressed as mean ± SD (n = 5). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparisons test. Comparisons were made with the standard (albendazole) group.

4. Discussion

In vitro assays offer a rapid, cost-effective method to screen large numbers of plant extracts for potential anthelmintic activity by directly assessing effects on parasites without host interference (29). In the present study, ethanolic extracts of *Apium leptophyllum* and *Apium graveolens* seeds demonstrated stronger anthelmintic activity compared to aqueous extracts. This may be attributed to higher concentrations of phenolic compounds and flavonoids extracted in ethanol, which is supported by previous findings that ethanol efficiently extracts lipophilic bioactive (30-32). Additionally, ethanol can penetrate plant cell walls more effectively and limit microbial growth during extraction, preserving active phytochemicals (33-34). The anthelmintic mechanism of these plant extracts is likely multifactorial. Tannins may act by binding to glycoproteins on the cuticle of worms,causing disruption of membrane integrity, uncoupling oxidative phosphorylation, and impairing energy production (35-36). Alkaloids may exert paralytic effects by interfering with neurotransmission and have been shown to block acetylcholine receptors or act directly on the central nervous system of helminths (37-38). Saponins can increase membrane permeability, promote vacuolation, and damage the tegument of parasites, like the action of established drugs like praziquantel (39). Phenolic compounds may also inhibit metabolic enzymes and bind to free proteins, affecting larval nutrition and survival (40). The observed activity differences between ethanolic and aqueous extracts may also be linked to transcuticular absorption, as lipophilic compounds in ethanol extracts can more readily cross the worm's cuticle (41). This aligns with previous evidence that lipophilic phytochemicals are more bioavailable and effective against parasites than hydrophilic compounds (42). The mucopolysaccharide layer of *Pheretima posthuma* helps worms move by reducing friction; damage to this layer by phytochemicals restricts mobility, resulting in paralysis and death (43). Tannins may additionally cross-link collagen in the worm cuticle, hardening it and further contributing to immobilization (44). Albendazole, the reference drug, disrupts glucose uptake by inhibiting microtubule polymerization, leading to energy depletion and death of the parasite (45). Similarly, the studied extracts likely disrupt multiple pathways, including nutrient uptake, neuromuscular coordination, and membrane integrity (46). Together, these effects could explain the dose-dependent and significant anthelmintic activity observed, particularly in ethanolic extracts. Phytochemical screening confirmed the presence of alkaloids, tannins, flavonoids, phenolic compounds, saponins, and steroidal compounds in both plant extracts, which may act synergistically to produce the observed anthelmintic effects (47-48). These findings highlight the value of medicinal plants as alternative sources for anthelmintic agents, supporting continued investigation into their bioactive constituents.

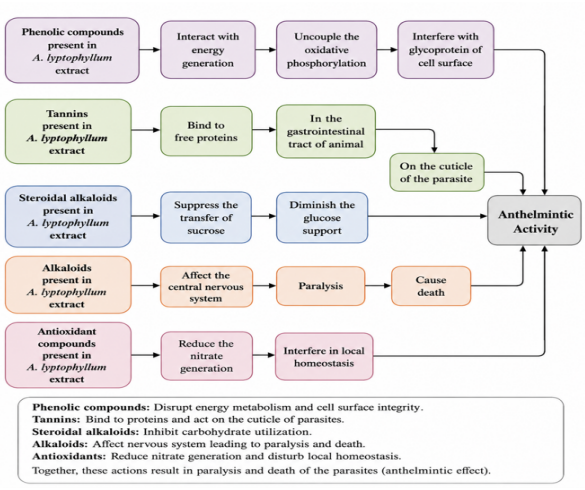

Fig. 7 Possible mechanism of Action

5. Conclusion

The present study indicates that the aqueous and ethanolic seed extracts of *Apium leptophyllum* and *Apium graveolens* possess anthelmintic activity against *Pheretima posthuma.* The observed activity suggests the presence of bioactive constituents that may contribute to the anthelmintic effects of these extracts. Further studies are needed to isolate and characterize the active constituents and to understand their possible mechanisms of action.

Conflict of Interest

The authors Declare no Conflict of Interest

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