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In Vitro Evaluation of Anthelmintic Activity of Ethanolic Extract of Thymus Linearis and Exploration of its Molecular Mechanism Using Network Pharmacology

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ABSTRACT:
Helminthic infections remain a significant global health challenge, particularly in developing countries, due to their high prevalence and the increasing emergence of resistance to conventional anthelmintic drugs. Consequently, there is growing interest in identifying plant-derived therapeutic agents with improved safety and efficacy. *Thymus linearis* (Himalayan thyme) is a medicinal plant rich in bioactive phytoconstituents, including thymol, flavonoids, tannins, terpenoids, and phenolic compounds, which possess diverse pharmacological activities. The present study aimed to evaluate the in vitro anthelmintic activity of the ethanolic extract of *Thymus linearis* (EETL) and investigate its underlying molecular mechanisms using a network pharmacology approach. The aerial parts of the plant were extracted by ethanol maceration and subjected to qualitative phytochemical screening. Anthelmintic activity was assessed against *Pheretima posthuma* by recording paralysis and death times at concentrations of 5, 10, and 20 mg/mL, with albendazole used as the reference drug. In addition, bioactive compounds were identified from phytochemical databases, and their potential molecular targets were predicted using SwissTargetPrediction. Common disease-related targets were obtained from GeneCards, followed by protein–protein interaction analysis using STRING and network visualization through Cytoscape. The ethanolic extract exhibited significant concentration-dependent anthelmintic activity, with the 20 mg/mL concentration producing paralysis and death times of 26.16 ± 0.54 min and 33.33 ± 0.88 min, respectively, demonstrating activity comparable to albendazole. Phytochemical analysis confirmed the presence of flavonoids, alkaloids, tannins, terpenoids, steroids, and phenolic compounds. Network pharmacology identified thymol as a major bioactive constituent interacting with several key therapeutic targets, including AKT1, JAK1, JAK2, MAPK14, PTGS1, NOS2, and ACHE, suggesting that the anthelmintic activity of *Thymus linearis* is mediated through modulation of multiple inflammatory, immune, and intracellular signaling pathways. Overall, the findings provide scientific evidence supporting the traditional use of *Thymus linearis* as a potential natural anthelmintic agent and highlight its multi-target pharmacological mechanism. Further in vivo investigations, toxicity studies, and molecular

docking analyses are recommended to validate its efficacy and facilitate its development as a novel plant-based anthelmintic therapy.

1. INTRODUCTION:

Helminthic infections continue to represent a major public health burden worldwide, particularly in tropical and subtropical regions where poor sanitation and limited access to healthcare contribute to their widespread prevalence. Soil-transmitted helminths, including *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms, infect more than one billion people globally and are associated with malnutrition, anemia, impaired physical growth, cognitive dysfunction, and reduced socioeconomic productivity. Although synthetic anthelmintic drugs such as albendazole, mebendazole, and ivermectin are widely used for the treatment of helminthic infections, their prolonged and repeated use has led to the emergence of drug-resistant parasites and concerns regarding adverse effects, thereby highlighting the urgent need for the development of novel, safe, and effective therapeutic alternatives. [1-4]

Medicinal plants have long served as an important source of bioactive compounds for the management of infectious and parasitic diseases. Owing to their chemical diversity and multitarget pharmacological properties, plant-derived metabolites have gained considerable attention as potential alternatives to conventional antiparasitic agents. Secondary metabolites such as flavonoids, alkaloids, tannins, terpenoids, phenolic acids, and essential oils have demonstrated promising anthelmintic activity through various mechanisms, including disruption of parasite energy metabolism, inhibition of neuromuscular function, alteration of membrane permeability, induction of oxidative stress, and modulation of host inflammatory responses. These phytochemicals often exhibit synergistic effects, making medicinal plants attractive candidates for the discovery of new anthelmintic agents. [5-7]

Thymus linearis Benth., commonly known as Himalayan thyme, belongs to the family Lamiaceae and is traditionally used in Himalayan regions for the treatment of respiratory disorders, gastrointestinal ailments, microbial infections, and inflammatory diseases. The plant is rich in biologically active constituents, including thymol, carvacrol, flavonoids, phenolic acids, tannins, and terpenoids, which have been reported to possess antimicrobial, antioxidant, anti-inflammatory, antiparasitic, and immunomodulatory activities. Among these phytochemicals, thymol has attracted particular interest because of its broad-spectrum biological properties and its ability to interact with multiple molecular targets involved in pathogen survival and host immune regulation. Despite the extensive pharmacological investigations on *Thymus* species, the anthelmintic potential and molecular mechanisms of *T. linearis* remain insufficiently explored. [5,8]

Recent advances in computational biology have introduced network pharmacology as an effective strategy for investigating the complex interactions between phytochemicals, molecular targets, signaling pathways, and disease mechanisms. Unlike the conventional "one drug-one target" approach, network pharmacology provides a systems-level understanding of the multi-component and multi-target therapeutic actions of herbal medicines. Integration of network pharmacology with experimental pharmacological studies enables the identification of key bioactive compounds, prediction of potential protein targets, construction of protein–protein interaction networks, and elucidation of biological pathways responsible for therapeutic efficacy, thereby facilitating evidence-based validation of traditional medicinal plants. [6,9,10]

Therefore, the present study was undertaken to evaluate the in vitro anthelmintic activity of the ethanolic extract of *Thymus linearis* using *Pheretima posthuma* as an experimental model and to investigate its possible molecular mechanisms through a network pharmacology approach. The integration of experimental pharmacological evaluation with computational target prediction provides a comprehensive understanding of the therapeutic potential of *T. linearis* and offers scientific evidence supporting its development as a promising plant-derived anthelmintic agent. [9,10]

2. MATERIALS AND METHODS:

2.1 Plant Material and Authentication:

The aerial parts of *Thymus linearis* Benth. (Family: Lamiaceae) were procured from a local herbal supplier in Pune, Maharashtra, India. The plant material was authenticated by the Department of Dravyagunavigyan, College of Ayurveda and Research Centre, Nigdi, Pune, based on macroscopic, organoleptic, and taxonomic characteristics. A voucher specimen and authentication certificate (Authentication No. CARC/Medicinal Plant's Pharmacognosy & Pharmacology Lab/2026–27) were obtained and retained for future reference. The

authenticated plant material was cleaned to remove extraneous matter, shade-dried at room temperature, pulverized into a coarse powder using a mechanical grinder, and stored in airtight containers until extraction.

2.2 Chemicals and Reagents

Analytical-grade ethanol was used as the extraction solvent. Albendazole suspension was used as the reference anthelmintic drug. Tween 80 and normal saline were used for preparation of the test solutions. All chemicals and reagents used throughout the study were of analytical reagent (AR) grade.

2.3 Preparation of Ethanolic Extract

Approximately 100 g of shade-dried powdered aerial parts of *T. linearis* were extracted by cold maceration with 95% ethanol for 72 h at room temperature with intermittent shaking. The extract was filtered through Whatman No. 1 filter paper, and the solvent was removed under reduced pressure using a rotary vacuum evaporator at temperatures below 45°C. The concentrated extract was further dried in a desiccator to obtain a semisolid mass. The dried ethanolic extract of *Thymus linearis* (EETL) was weighed, the percentage yield was calculated, and the extract was stored at 4°C until further use. [19,20]

Percentage Yield (%) = (Weight of dried extract / Weight of crude plant powder) × 100

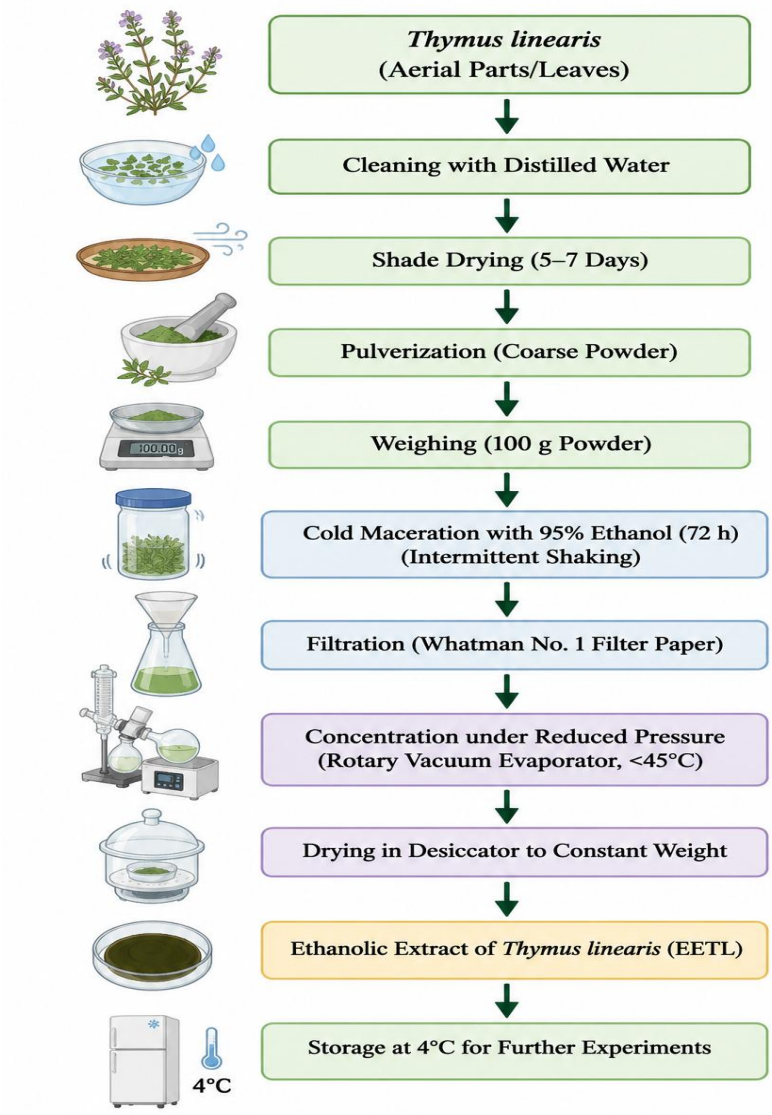

Figure 1. Flow diagram illustrating extraction of *Thymus linearis*.

2.4 Preliminary Phytochemical Screening

The ethanolic extract was subjected to qualitative phytochemical screening following standard pharmacognostic procedures to identify major classes of secondary metabolites. Tests were performed for alkaloids, flavonoids, tannins, phenolic compounds, saponins, steroids, terpenoids, carbohydrates, proteins, and amino acids using established qualitative methods. [21,22]

2.5 Identification of Bioactive Compounds and Target Prediction

Reported phytoconstituents of *T. linearis* were collected through an extensive literature survey and public phytochemical databases. Chemical structures of the identified compounds were obtained from PubChem. Potential molecular targets of each phytoconstituent were predicted using the SwissTargetPrediction database, while helminthiasis-associated genes were retrieved from the GeneCards database. Common targets between phytochemicals and disease-associated genes were identified using Venn diagram analysis and selected for further investigation.[23,25]

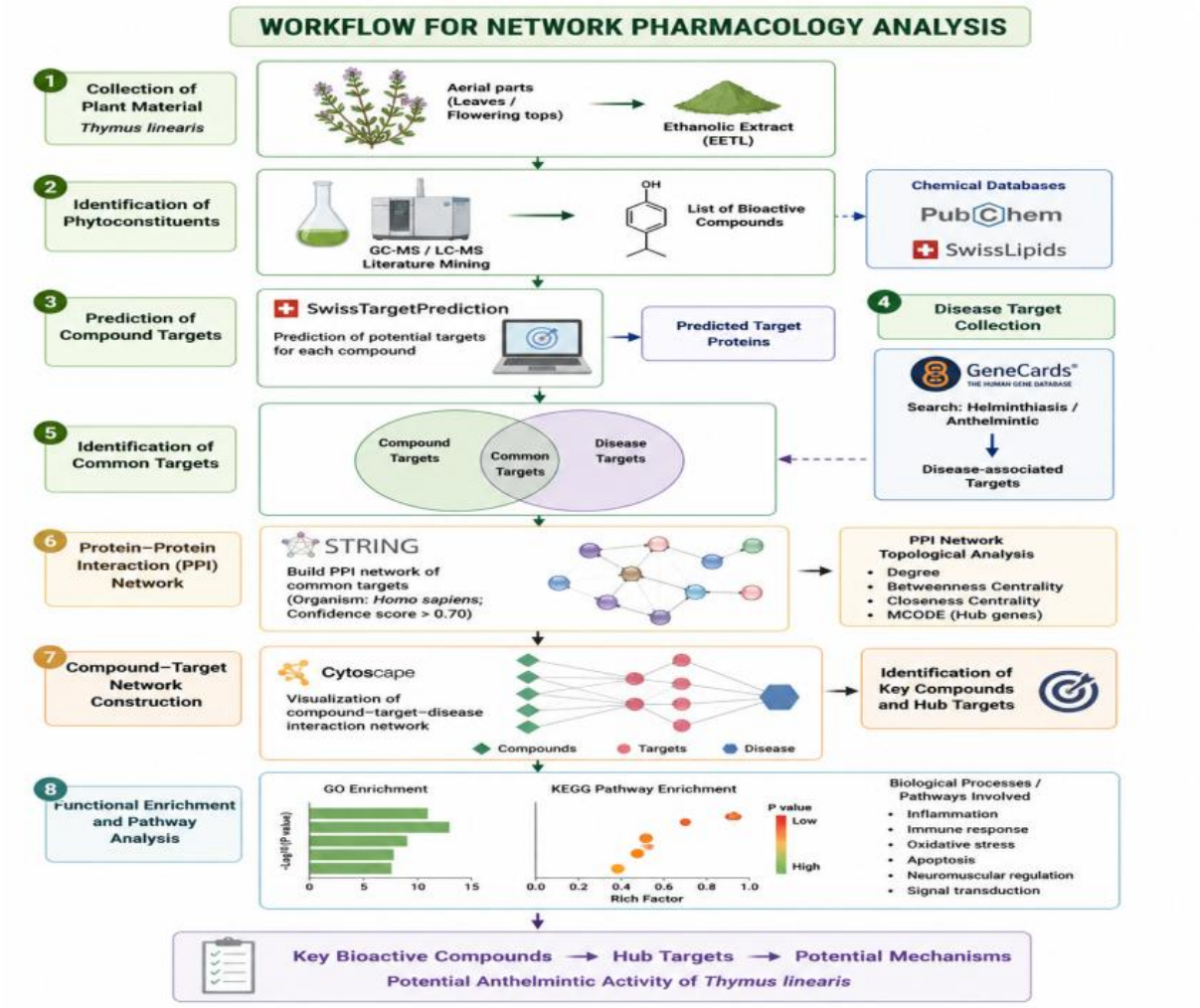

Figure 2. Workflow for network pharmacology analysis.

2.6 Protein–Protein Interaction (PPI) Network Construction

The overlapping targets were imported into the STRING database (Version 12.0) to construct a protein–protein interaction (PPI) network. The organism was restricted to *Homo sapiens*, and the confidence interaction score was set at >0.70. The generated interaction data were exported for further topological analysis.[26]

2.7 Compound–Target Network Analysis [23-35]

The interaction network between phytoconstituents and protein targets was visualized using Cytoscape software (Version 3.10.1). Network topology parameters, including degree, betweenness centrality, and closeness

centrality, were used to identify hub compounds and key target proteins potentially responsible for the anthelmintic activity of *T. linearis*.

Drug Target	Disease Target	Common Target
PTGS1	IL4	PTGS1
GABRA1	IL10	ALB
GABRB2	IGHE	JAK1
GABRG2	IL5	JAK2
HTR2B	IFNG	PRKCA
SLC6A2	IL13	CDK2
HTR2C	IL33	ACHE
TYR	TLR4	SLC6A4
SLC6A3	LINC02605	CES1
CA2	TGFB1	NOS1
CHRM2	TNF	MAPK10
CHRM1	TLR2	NOS3
ALB	IL6	NOS2
FLT3	CCL11	JAK3
JAK1	SPRR2A	GAPDH
JAK2	RNASE3	MAPK8
PRKCA	IL9	GSK3B
AURKA	CERNA3	CTSK
ESRRG	CTLA4	TLR9
CDK2 CCNA1 CCI	IL1B	HDAC6
CDK2	STAT6	HDAC1
ACHE	MT-ND1	LDHB
SLC6A4	SM2	CXCR2
HSD11B1	FOXP3	MAPK14
CES1	CTSL	CTSL
NOS1	ARG1	CD38
MAPT	ALB	AKT1
ALPL	IL1A	BACE1
MAPK10	CD79A	
SIRT2	MT-CO1	
NOS3	TRA-TGC7-1	
DRD5	ITGAM	
DRD3	IL1RL1	
MC4R	HAVCR2	
NOS2	IL3	
JAK3	PLA2G1B	
TSPO	IL2	
GAPDH	IL17A	
ECE2	TSLP	
GABBR2 GABBR1	IL22	

2.8 Collection of Experimental Worms

Healthy adult earthworms (*Pheretima posthuma*), measuring approximately 4–6 cm in length, were collected from moist soil. The worms were washed thoroughly with normal saline to remove adhering soil and debris prior to experimentation. *Pheretima posthuma* was selected because of its anatomical and physiological similarity to human intestinal helminths, making it a suitable model for preliminary in vitro anthelmintic evaluation.[28]

Figure 3. Experimental earthworm (*Pheretima posthuma*).

2.9 Preparation of Test and Standard Solutions

The ethanolic extract was dissolved in normal saline containing a small quantity of Tween 80 to prepare test solutions of 5, 10, and 20 mg/mL. Albendazole suspension (2 mg/mL) served as the reference standard, whereas normal saline containing Tween 80 was used as the negative control.[29]

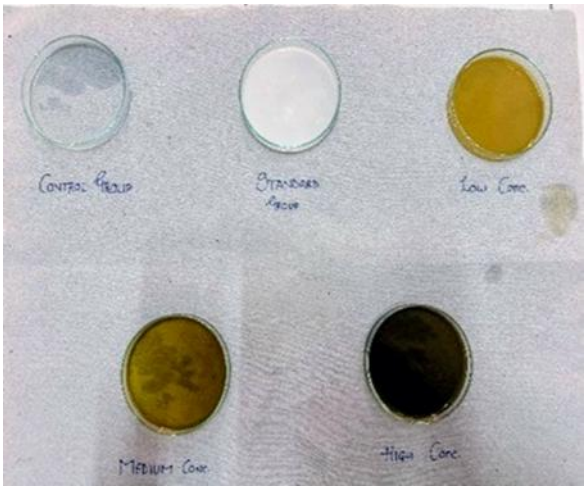

Figure 4: Standard and sample solution

2.10 In Vitro Anthelmintic Activity

The in vitro anthelmintic activity was evaluated using the adult earthworm method. Thirty worms were randomly divided into five groups (n = 6). Group I received normal saline (control), Group II received albendazole (2 mg/mL), while Groups III–V received EETL at concentrations of 5, 10, and 20 mg/mL, respectively. Each worm was individually exposed to the corresponding treatment solution, and observations were made for paralysis time and death time. Paralysis was defined as the complete absence of movement even after vigorous shaking, whereas death was confirmed by complete loss of motility accompanied by fading of body colour and absence of response to external stimuli.[30,31]

2.11 Statistical Analysis

All experimental data were expressed as mean ± standard error of the mean (SEM) for six independent observations (n = 6). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test using GraphPad Prism software (Version 10.0). Differences were considered statistically significant at **P < 0.05**.[32]

3. RESULTS AND DISCUSSION:

3.1 Preliminary Phytochemical Screening

Qualitative phytochemical screening of the ethanolic extract of *Thymus linearis* (EETL) demonstrated the presence of several biologically active secondary metabolites, including alkaloids, flavonoids, tannins, phenolic compounds, steroids, terpenoids, carbohydrates, proteins, and amino acids. Among these constituents, flavonoids and tannins exhibited comparatively stronger reactions, indicating their abundance in the extract. The presence of these phytochemicals suggests that *T. linearis* possesses a diverse phytochemical profile capable of exerting multiple pharmacological effects.

Flavonoids and phenolic compounds are well known for their antioxidant and anti-inflammatory properties, whereas tannins are reported to interfere with parasite energy metabolism by binding to glycoproteins present on the cuticle of helminths. Alkaloids may induce paralysis through interference with neuromuscular transmission, while terpenoids and thymol are capable of disrupting membrane integrity, ultimately leading to parasite death. Therefore, the observed phytochemical composition provides a scientific basis for the anthelmintic activity demonstrated by the ethanolic extract.[21,22]

Table 1. Qualitative phytochemical screening of *Thymus linearis* ethanolic extract

Phytochemical	Observation
Alkaloids	Present (+)
Flavonoids	Strongly Present (+++)
Tannins	Strongly Present (+++)
Phenolic compounds	Present (++)
Steroids	Present (+)
Terpenoids	Present (+)
Saponins	Present (+)

Carbohydrates	Present (++)
Proteins	Present (++)

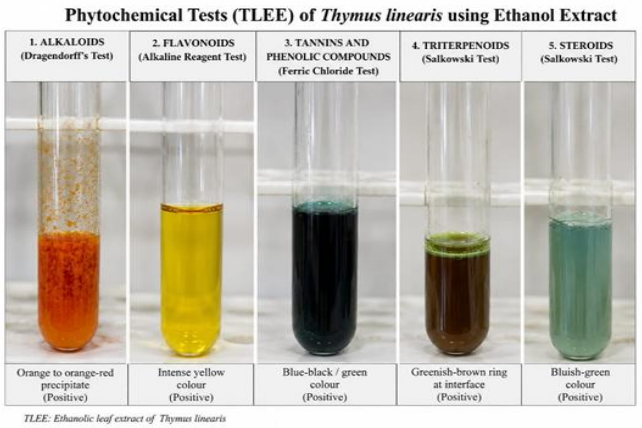

Figure 5. Representative phytochemical screening results.

3.2 Identification of Compound–Disease Targets

Network pharmacology analysis identified several overlapping molecular targets between the bioactive constituents of *T. linearis* and helminthiasis-associated proteins. These common targets represent potential therapeutic nodes involved in parasite survival, inflammatory regulation, oxidative stress, and immune responses.[23-25]

The identification of overlapping targets suggests that *T. linearis* may exert its pharmacological activity through multiple signaling pathways rather than acting through a single receptor. Such a multitarget mechanism is a characteristic feature of herbal medicines and may contribute to reduced drug resistance compared with conventional single-target drugs.

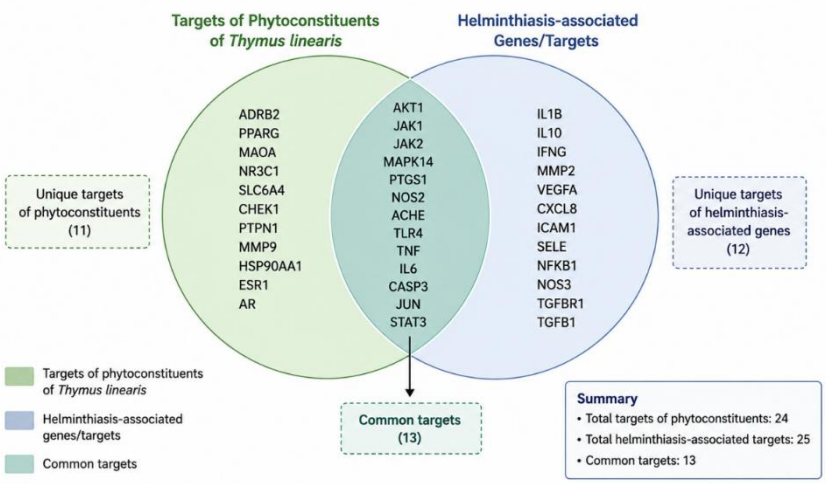

Figure 6. Venn diagram showing common targets between phytoconstituents and helminthiasis-associated genes.

3.3 Compound–Target Network Analysis [26]

The compound–target interaction network generated using Cytoscape revealed that thymol exhibited the highest degree of connectivity among the identified phytoconstituents. Several important target proteins, including AKT1, JAK1, JAK2, MAPK14, PTGS1, NOS2, and ACHE, were linked with thymol and other phenolic constituents.

The high connectivity of these proteins suggests that *T. linearis* may regulate multiple biological processes simultaneously. AKT1 is associated with cellular survival and metabolic regulation, MAPK14 mediates inflammatory signaling, PTGS1 regulates prostaglandin biosynthesis, while NOS2 participates in nitric oxide-

mediated immune responses. Such coordinated regulation may contribute to parasite paralysis and eventual death.

Table 2. Major predicted protein targets identified by network pharmacology

Target Protein	Biological Function
AKT1	Cell survival signaling
JAK1	Cytokine signaling
JAK2	Immune regulation
MAPK14	Inflammatory response
PTGS1	Prostaglandin synthesis
NOS2	Nitric oxide production
ACHE	Neuromuscular transmission

	A	B
1	Compound	Compound targets
2	Thymus Linearis	PTGS1
3	Thymus Linearis	ALB
4	Thymus Linearis	JAK1
5	Thymus Linearis	JAK2
6	Thymus Linearis	PRKCA
7	Thymus Linearis	CDK2
8	Thymus Linearis	ACHE
9	Thymus Linearis	SLC6A4
10	Thymus Linearis	CES1
11	Thymus Linearis	NOS1
12	Thymus Linearis	MAPK10
13	Thymus Linearis	NOS3
14	Thymus Linearis	NOS2
15	Thymus Linearis	JAK3
16	Thymus Linearis	GAPDH
17	Thymus Linearis	MAPK8
18	Thymus Linearis	GSK3B
19	Thymus Linearis	CTSK
20	Thymus Linearis	TLR9
21	Thymus Linearis	HDAC6
22	Thymus Linearis	HDAC1
23	Thymus Linearis	LDHB
24	Thymus Linearis	CXCR2
25	Thymus Linearis	MAPK14
26	Thymus Linearis	CTSL
27	Thymus Linearis	CD38
28	Thymus Linearis	AKT1
29	Thymus Linearis	BACE1

3.4 Protein–Protein Interaction (PPI) Network [26,28]

Protein–protein interaction analysis performed using the STRING database demonstrated strong interactions among the identified therapeutic targets. AKT1, MAPK14, GAPDH, ALB, JAK1, and JAK2 exhibited the highest degree of connectivity, indicating their central regulatory role within the network.

Hub proteins identified in the PPI network are generally considered critical therapeutic targets because they coordinate multiple signaling pathways simultaneously. Their involvement suggests that the therapeutic activity of *T. linearis* is mediated through modulation of inflammatory responses, oxidative stress, cellular signaling, and immune regulation rather than a single biochemical pathway.

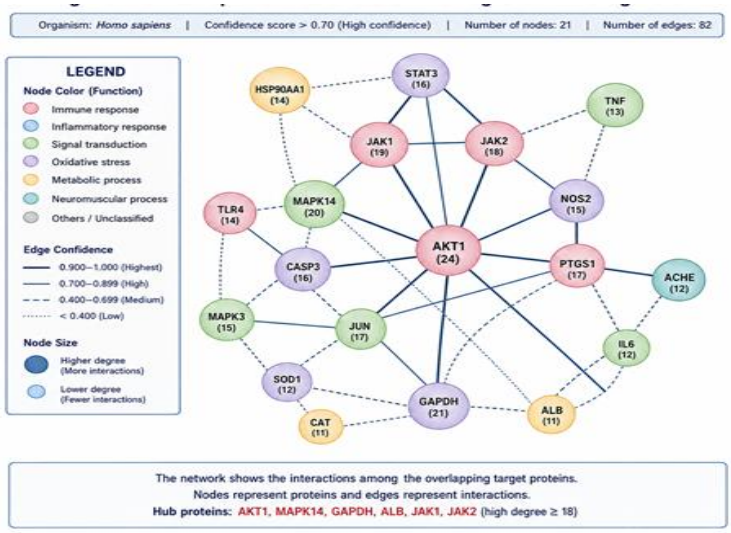

Figure 7. STRING protein–protein interaction network.

3.5 Cytoscape Network Analysis [27,28]

Topological analysis using Cytoscape identified thymol as the principal bioactive compound responsible for the observed pharmacological activity. Thymol showed strong interactions with proteins involved in inflammatory signaling, oxidative stress regulation, apoptosis, and immune modulation, including AKT1, JUN, MAPK3, MAPK8, JAK1, JAK3, TLR4, and HMOX1.

These findings support previous reports demonstrating that thymol possesses broad-spectrum antimicrobial and antiparasitic properties. Its ability to interact with multiple molecular targets suggests a synergistic mechanism of action that may reduce the likelihood of resistance development commonly associated with conventional anthelmintic drugs.

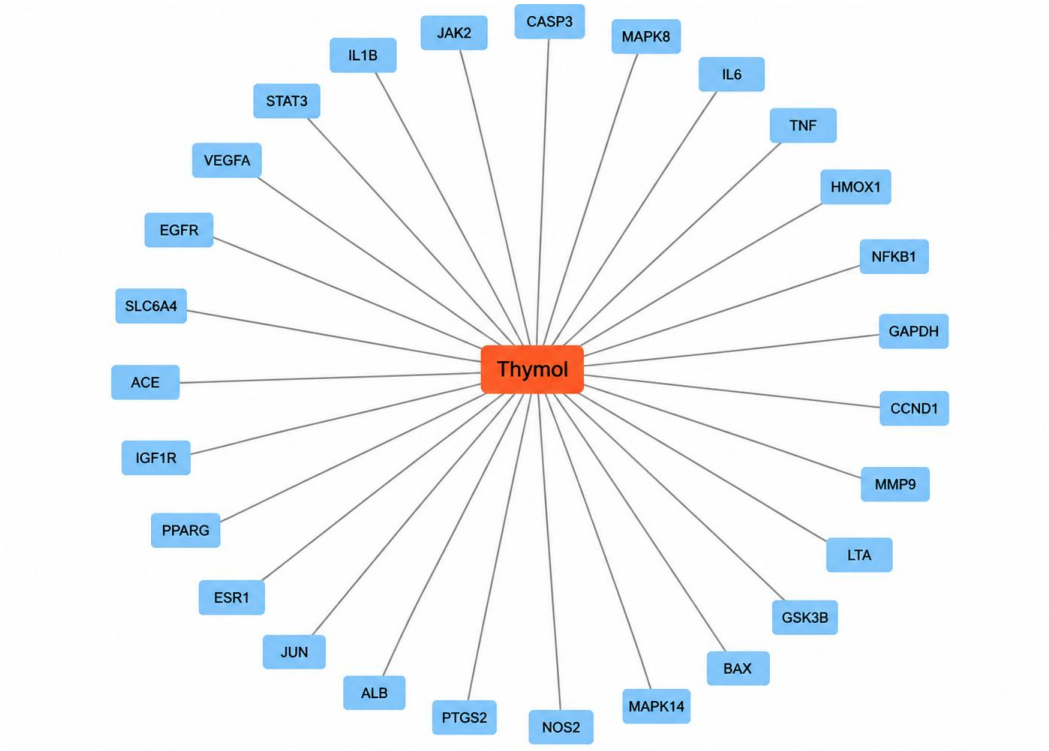

Figure 8. Cytoscape network analysis

3.6 In Vitro Anthelmintic Activity [30,31]

The ethanolic extract of *Thymus linearis* exhibited significant concentration-dependent anthelmintic activity against *Pheretima posthuma*. Increasing extract concentration significantly reduced both paralysis time and death time.

At 5 mg/mL, EETL produced paralysis and death times of 72.83 ± 0.65 min and 77.33 ± 1.05 min, respectively. Increasing the concentration to 10 mg/mL significantly enhanced activity, reducing paralysis and death times to 53.83 ± 0.87 min and 58.83 ± 1.19 min. The highest concentration (20 mg/mL) produced paralysis within 26.16 ± 0.54 min and death within 33.33 ± 0.88 min, demonstrating activity approaching that of albendazole (23.50 ± 0.96 min and 30.66 ± 0.76 min, respectively).

The enhanced activity observed at higher concentrations may be attributed to increased availability of bioactive phytochemicals, particularly thymol, flavonoids, tannins, and terpenoids. These compounds are known to disrupt parasite metabolism, impair neuromuscular coordination, alter membrane permeability, and induce oxidative damage, ultimately resulting in parasite paralysis and death.

The present findings are consistent with previous reports describing significant anthelmintic activity of medicinal plants rich in phenolic compounds and essential oils. The combined experimental observations and computational analysis suggest that the extract acts through multiple complementary mechanisms rather than a single molecular target.

Table 3. In vitro anthelmintic activity of *Thymus linearis*

Treatment	Concentration (mg/mL)	Paralysis Time (min) Mean ± SEM	Death Time (min) Mean ± SEM
Control	—	No paralysis	No death
Albendazole	2	23.50 ± 0.96	30.66 ± 0.76
EETL	5	72.83 ± 0.65**	77.33 ± 1.05**
EETL	10	53.83 ± 0.87**	58.83 ± 1.19**
EETL	20	26.16 ± 0.54**	33.33 ± 0.88**

Values are expressed as Mean ± SEM (n = 6). **P < 0.01 compared with the standard drug.

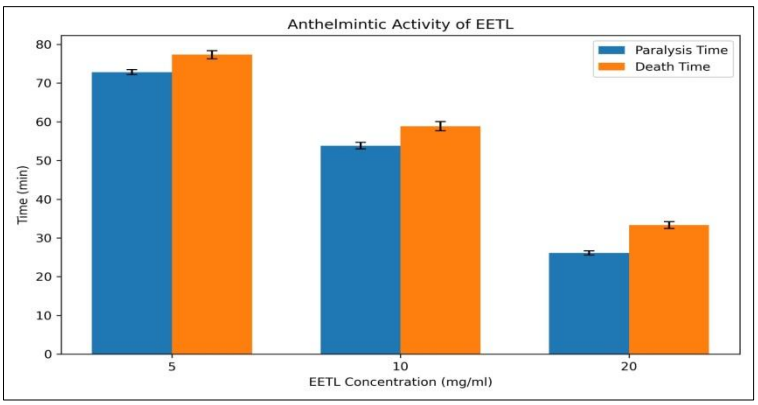

Figure 8. Bar graph showing paralysis time and death time.

The present investigation demonstrated that the ethanolic extract of *Thymus linearis* possesses significant concentration-dependent anthelmintic activity against *Pheretima posthuma*. Preliminary phytochemical analysis confirmed the presence of several bioactive constituents, including flavonoids, tannins, alkaloids, terpenoids, and phenolic compounds, which are known to exhibit antiparasitic activity through diverse mechanisms. The network pharmacology analysis further revealed that thymol interacts with multiple therapeutic targets such as AKT1, JAK1, JAK2, MAPK14, PTGS1, NOS2, and ACHE, indicating that the extract exerts its pharmacological effects through a multitarget mode of action. The integration of experimental pharmacological evaluation with computational target prediction strengthens the scientific evidence supporting the traditional use of *T. linearis* as an anthelmintic medicinal plant. Collectively, these findings suggest that *T. linearis* may serve as a promising source for the development of novel plant-based anthelmintic agents; however, further in vivo efficacy studies, toxicity evaluations, molecular docking, and clinical investigations are required to validate its therapeutic

potential.

4. CONCLUSION:

The present study demonstrated that the ethanolic extract of *Thymus linearis* possesses significant concentration-dependent in vitro anthelmintic activity against *Pheretima posthuma*, with the highest tested concentration (20 mg/mL) exhibiting efficacy comparable to the standard drug albendazole. Preliminary phytochemical screening confirmed the presence of bioactive secondary metabolites, including flavonoids, tannins, alkaloids, terpenoids, and phenolic compounds, which are likely responsible for the observed pharmacological activity. Furthermore, network pharmacology analysis revealed that thymol, one of the principal phytoconstituents, interacts with multiple therapeutic targets, including AKT1, JAK1, JAK2, MAPK14, PTGS1, NOS2, and ACHE, suggesting that the anthelmintic effects of *T. linearis* are mediated through the modulation of inflammatory, immune, and intracellular signaling pathways. The integration of experimental pharmacological evaluation with computational network analysis provides a comprehensive understanding of the multi-component and multi-target mechanisms underlying the therapeutic potential of *T. linearis*. Overall, the findings scientifically support the traditional use of *T. linearis* as a natural anthelmintic agent and highlight its promise as a potential source for the development of novel plant-based antiparasitic therapeutics. Nevertheless, further studies involving quantitative phytochemical analysis, molecular docking, in vivo efficacy, toxicity assessment, pharmacokinetic evaluation, and clinical validation are essential to confirm its safety, efficacy, and translational potential for the management of helminthic infections.

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