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Original Article

Exploring the Anthelmintic and Krumighna Properties of *Vitex negundo* Linn. (VN) for Indian Earthworms and Annelid Worms

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ABSTRACT

Introduction: Helminth infections are a global health concern, especially in underdeveloped regions, contributing to anemia, malnutrition, and cognitive issues. Drug resistance to conventional anthelmintics like Albendazole and Piperazine Citrate necessitates alternative treatments. *Vitex negundo* Linn. (*Nirgundi*), known for its medicinal properties, shows promise as a natural anthelmintic. This study evaluates its ethanolic leaf extract against *Pheretima praepinguis* and *Tubifex*, comparing its efficacy with standard drugs to explore a safer alternative. **Aim:** To investigate the anthelmintic activity of *Vitex negundo* Linn. ethanolic leaf extracts in comparison with standard drugs. **Materials and Methods:** Ethanolic extracts were prepared using the Soxhlet apparatus from powdered aerial leaves. The anthelmintic assay involved exposing worms to VN extract concentrations of 10, 20, and 50 mg/mL, with mean period of paralysis and death times noted. Albendazole (20 mg/mL) and Piperazine Citrate (15 mg/mL) were used as standard drugs, with control as a normal saline. **Results:** Ethanolic extracts showed dose-dependent anthelmintic activity. At 50 mg/mL, *Pheretima posthuma* exhibited paralysis at 2.85±0.11 min and death at 13.92±0.30 min, while *T. tubifex* showed paralysis at 2.10±0.29 min and death at 8.75±0.54 min. Piperazine Citrate induced paralysis without mortality, and Albendazole demonstrated moderate efficacy with slower times to paralysis and death. **Conclusion:** Ethanolic extracts of *Vitex negundo* exhibited superior dose-dependent anthelmintic effects, particularly at higher concentrations, compared to standard drugs. The findings support its potential as a natural anthelmintic agent, warranting further research on its bioactive compounds.

Keywords: Anthelminthic Activity Charkokta Krumighna herb, Aquarium worms *Tubifex tubifex*, Indian earthworms *Pheretima posthuma*, *Vitex negundo* Linn. (VN).

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INTRODUCTION

A significant percentage of people worldwide suffer helminthic infestations, which impacted the top prevalent in human population. They are a serious public health concern in underdeveloped nations, where they also increase the incidence of pneumonia, eosinophilia, anemia, and malnutrition. Many helminths kept hidden in the gastrointestinal system, while others also live in tissues or their larvae move there. Anthelmintics are medications designed to eliminate or expel parasitic worms (helminths) from the body. They harm the host by toxin release, intestinal or lymphatic blockage, organ damage, blood loss, and food starvation. Although helminthiasis seldom results in death, it is a leading cause of illness.[1]

Vitex negundo Linn. (VN), also called Nirgundi or the Five-Leaved Chaste Tree, is a big, fragrant shrub that is a member belongs to Lamiaceae family. Every Parts of this plant has significant Medicinal properties and indicated in illnesses in traditional Indian medical systems such as Ayurveda, Siddha, Unani, and Chinese. *Vitex negundo* Linn. (VN) is known as '*sarvaroganivarani*', the cure-all for all ailments, in the Indian traditional medical system.[2] "A man cannot die of disease in an area where *Vitex negundo* Linn. (VN) are found (provided that he knows how to use them)" is a well-known local saying among the Bhangalis in the Western Himalayan region of India.[3] "Nirgundi" means "which protects the body from diseases" in Sanskrit.[4]

Many traditionally used herbs are important in today's medical regimens because they have been shown by science to have a variety of useful activities. The Plant *Vitex negundo* Linn., found pan India. This huge, fragrant shrub, which is a member of the Lamiaceae family, grows in damp areas or by waterways. Flavonoids, terpenes, lignans, stilbene derivatives,

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iridoid glycosides, essential oils, and flavonoid glycosides are all detected by phytochemical analysis. Anxiolytic, larvicidal, nephroprotective Anti-inflammatory, antioxidant, analgesic, antipyretic, antibacterial, antitumor, anti-arthritic, anti-amnesic, anti-HIV, anti-eosinophilic, and anti-snake venom activities are just a few of the many pharmacological characteristics that *Vitex negundo* Linn. (VN) possesses. Numerous powerful bioactive chemicals found in natural goods target the intricate network of proteins implicated in a variety of illnesses. Commonly referred to as the "chaste tree," *Vitex negundo* Linn. (VN) is a significant ethnobotanical species with numerous therapeutic uses. The chemical makeup of several Vitex species varies, resulting in the production of distinct phytochemicals. Numerous bioactive substances, including volatile oils, flavonoids, lignans, iridoids, terpenes, and steroids, have been isolated from leaves, seeds, and roots. These bioactive substances have antibacterial, anticancer, antidiabetic, antioxidant, and anti-inflammatory properties.[5] VN is generally recognized for its function in controlling cellular processes such as apoptosis, the cell cycle, sperm motility, polycystic ovarian syndrome, and the menstrual cycle. Numerous pathways of cancer signaling, such as p-p38, p-ERK1/2, p-JNK, c-Jun N-terminal kinase (JNK), COX-1, MAPK, NF-κB, Tumor Necrosis Factor α (TNF-α), Akt, mTOR, Vascular Endothelial Growth Factor (VEGF), and Hypoxia-Inducible Factor (HIF-1α), have been shown to be disrupted by *Vitex negundo* Linn. (VN). Numerous bioactive substances derived from VN are currently being studied, while others have been marketed. This review is the first to provide current details regarding the VN, its bioactive components, and how they work.[6]

Pheretima praepinguis (Annelida), an Indian earthworm with an average size of 6-8 cm, was collected from soil that had been flooded. To get rid of the filth that stuck to them, they were cleaned with tap water. *Tubifex tubifex* (Annelida) aquarium worms were gathered from the neighborhood marketplace. Worms ranged in size from 1 to 1.5 cm on average. The anthelmintic assay was conducted using Ajayieoba *et al.*'s methodology with minor modifications.[7] Because adult Indian earthworms (*Pheretima praepinguis*) resemble human intestinal roundworm parasites in both anatomy and physiology, they were chosen for the testing. The readily available *Pheretima posthuma* is a good model for anthelmintic medication screening. Furthermore, the fact that they belong to the same phylum makes them more pertinent to these kinds of investigations.[8]

MATERIALS AND METHODS

Plant material

The herb *Vitex negundo* Linn. (VN) leaves were procured from the Salaikala village, Wardha district, State Maharashtra, in April 2024. The specimen was authenticated by a botanist at the J.B. College of Science, Wardha and Ayurveda Herb expert, Dravyaguna department of Mahatma Gandhi Ayurved College and research Centre, Salod (H); Datta Meghe Institute of Higher Education and Research (Deemed to be University), Sawangi, Wardha, A voucher specimen of *Vitex negundo* Linn. (VN) has been deposited at Herbarium Dravyaguna department, Mahatma Gandhi Ayurved College and research Centre, Salod (H) with the accession number [24121-I], ensuring its future reference and authentication.

VN, often called the five-leaved chaste tree, is a tiny shrub of Lamiaceae family which grows quickly indicated for therapeutic qualities. Its leaves are compound and palmate, typically consisting of five lanceolate leaflets with serrated margins and a slightly rough texture due to fine hairs shown in Figure 1.

The lower surface is pale green with noticeable veins, while the upper surface is glabrous and dark green. A transverse section of the leaf shows dorsiventral anatomy with a single-layered epidermis covered by a thick cuticle and stomata on the lower side. The Mesophyll (E) is differentiated into *Palisade Parenchyma* (Pa), rich in chloroplasts, and intercellular gaps for gas exchange in spongy *parenchyma*. A bundle sheath encloses the vascular bundle in the midrib. with Xylem (Xy) oriented upward and Phloem (Ph) downward. Non-glandular Trichomes (T) are present on both surfaces, serving as a defensive feature. This unique anatomy supports the plant's adaptability and contributes to its therapeutic uses, including anti-inflammatory, analgesic, and antimicrobial properties. The observations are summarized in Figure 2.

Preparation of extracts

Formulations of 20 mL were prepared, each containing a concentration of crude alcoholic of aerial leaf parts at 10 mg/mL in with 90% ethanol.

Preparation of Ethanolic Extract

The yield percentage should be detailed using a specific formula, such as:

[(Weight of extract obtained ÷ weight of initial plant material)×100]

The choice of ethanol as the solvent can be justified due to its polarity, which effectively extracts a broad range of phytochemicals. Information on whether other solvents, such as water or methanol, were tested for comparative extraction efficiency should be included. Comparative analysis would provide insights into the optimal solvent for bioactive compound isolation. Powdered aerial leaf material of *Vitex negundo* Linn. (VN) was placed in a thimble and with Soxhlet extracted out with 90% ethanol period of 8 to 12 hr. An oven was used to evaporate the solvent at a temperature lower than 50°C. For research purposes, the resultant residue (extract) was gathered and kept at 4°C. A calculation and record of the extraction concentration

(% w/w) were made.[9] In Table 1 the phytochemical screening of *Vitex negundo* Linn. (VN) Ethanolic Extract is enlisted.

Drugs and chemicals used

The Reference standards suspension Piperazine Citrate (Antepar 750 mg by Glaxo SmithKline Pharmaceuticals Ltd.,) and suspension Albendazole (Pfizer Pharmaceuticals, Mumbai) were used Chemicals: Ethanol (95% w/v) (Sigma-Aldrich Merk co.), Petroleum Ether 60-80 extrapure grade AR and Acacia (Gum Acacia) (enzyme free) extrapure AR (Sisco Research Laboratories Pvt. Ltd.,) (Gum Arabic).

Controls

To ensure the validity of the experimental results, several controls were implemented. Environmental factors such as temperature and pH were carefully monitored and standardized. To minimize variability, the experiments were performed at room temperature (25°C±2°C), and the solutions' pH values were kept at neutral levels (about pH 7) to eliminate any potential impact of pH variations on the extracts' activity. Negative controls were included in the form of Normal saline and pure ethanol solvent to validate the observed biological effects. These controls confirmed that the anthelmintic activity was attributable to the ethanolic extracts of *Vitex negundo* Linn. and not to the solvents or water used in the preparation of test solutions. In Table 2: Anthelmintic Activity of Ethanolic Extract of *Vitex negundo* Linn. (VN) extract presented.

Worms (Helminths)

Indian adult Indian earthworm *Pheretima praepinguis, Annelida* with an average length of 6-8 cm was collected from water logged areas of Bordharan area and Aquarium worms *Tubifex tubifex* (Annelid) of average length recorded as 1-1.5 cm worms were obtained from stagnant water of Bor dam of Wardha district. Identification of worms done in department of Zoology, J.B. College of Science, Wardha (Figures 1 and 2).

Sampling Procedure

Earthworms and aquarium worms were selected based on their maturity and full length. Indian earthworms (*Pheretima praepinguis*, Annelida) in Figure 3 and aquarium worms (*Tubifex tubifex*, Annelida in Figure 4) with an average length of 1-1.5 cm were used. For the anthelmintic assay, Same type of two worms were inoculated in 9 cm sized Petri dishes containing crude extracts solutions of concentrations of 10, 20, and 50 mg/mL and alcoholic extract solutions.

For every type of worm, this was done twice. With the exception of severe shaking, the mean paralysis times (P, in minutes) were noted when no movement was seen. Final verification done worms did not move after being shook briskly and submerged in warm water at 50°C, the periods of death (D, in minutes) were recorded. The controls piperazine citrate (15 mg/mL) and Albendazole (20 mg/mL) and were employed as reference substances, while control distilled water was utilized.[10]

Assessment of Anthelmintic Potential

The anthelmintic activity was evaluated on adult Indian earthworms Annelid (*Pheretima posthuma*) and *Tubifex tubifex* (aquarium annelid worms), both regarded as appropriate models for human parasite helminths (Figures 3 and 4).

The effectiveness of ethanolic extracts made from powdered aerial leaves of *Vitex negundo* Linn. (VN) was evaluated in six groups of worms. Worms in Group A, the control, were submerged in regular saline. Ethanolic extracts of *Vitex negundo* Linn. (VN) produced in a 1% gum acacia suspension in normal saline were administered to groups 2 through 4 at doses of 10, 20, and 50

Figure 1: External morphological characteristics of Arial leaf.[19]

mg/mL, respectively. Albendazole (20 mg/mL) in normal saline, another typical anthelmintic drug, was administered to Group 6 as a standard reference, whereas piperazine citrate (15 mg/mL) in normal saline was administered to Group 5. Six worms of each species were included in each group for the study. It was noted how long it took the worms to paralyze (P) and die (D). The definition of paralysis was the inability to move at all, even after being shaken violently. Death was determined when the worms stayed motionless after being violently shook or immersed in warm water at 50ºC. Albendazole and piperazine citrate were used as research reference substances.

Experimental Design

The significance of group differences was evaluated using One-Way ANOVA, followed by pairwise comparisons using Tukey's *post hoc* test. Numerical value $p<0.05$ was the cutoff point for statistical significance. The data's mean±Standard Deviation (SD) was shown. For statistical analysis, SPSS software was utilized.

Table 1: Phytochemical screening of *Vitex negundo* Linn. (VN) Ethanolic Extract.

Type of Compounds	Compounds present	Status
Alkaloids	Tannins	+
	Saponins	+
	Glycosides	+
	Fixed Oils	+
	Flavonoids	+
	Triterpenoids	-
Macronutrients	Carbohydrate	+
	Fats	+
	Proteins	-

Note : '+' Present; '-' Absent.

RESULTS

The preliminary phytochemical analysis findings were summarized in Table 1. The assessment identified the presence of alkaloids, tannins, flavonoids, saponins, fixed oils, fats, and glycosides in the extract and its fractions. In the anthelmintic assay, the extracts of *Vitex negundo* Linn. (VN) exhibited significant activity by inducing both paralysis and mortality in the tested worm species. As detailed in Table 2: Anthelmintic Activity of Ethanolic Extract of *Vitex negundo* Linn. (VN) extract, the ethanolic extract demonstrated dose-dependent anthelmintic effects, with the shortest times for Paralysis (P) and Death (D) recorded at a concentration of 50 mg/mL. The Same effects were observed with the reference drugs, piperazine citrate and albendazole. However, while piperazine citrate induced paralysis, it did not cause worm mortality. The ethanolic extract of *Vitex negundo* Linn. (VN) displayed anthelmintic efficacy comparable to albendazole, as it caused both paralysis and death in the worms.

The observed actions of herb extract and drugs on *Pheretima posthuma* and *Tubifex tubifex* were assessed as a Paralysis (P) and Death (D) times, with the data presented as Mean±SEM. In the control group, no significant activity was observed in either species (denoted as "A" for absence of activity) within 24 hr. For Ethanol Extract (10 mg/mL), *Pheretima posthuma* showed paralysis at 48.75±0.38 min and death at 92.85±0.89 min, while *Tubifex tubifex* exhibited paralysis at 32.12±0.60 min and death at 58.30±0.72 min. This indicated a significant effect of the ethanol extract, with *Pheretima posthuma* taking longer to die compared to *Tubifex tubifex*. At 20 mg/mL, *Pheretima posthuma* experienced paralysis at 14.95±0.68 min and death at 42.55±0.45 min, while *Tubifex tubifex* showed paralysis at 10.03±0.81 min and death at 25.90±0.59 min. Both species died and became paralyzed more quickly at a concentration of 20 mg/mL, demonstrating a dose-dependent impact. *Pheretima posthuma* demonstrated paralysis at 50 mg/mL in 2.85±0.11 min. and death at 13.92±0.30 min, while *Tubifex tubifex* experienced paralysis at 2.10±0.29 min and death at 8.75±0.54 min. The highest concentration caused rapid effects in both species, with *Tubifex tubifex* being more

Table 2: Anthelmintic Activity of Ethanolic Extract of *Vitex negundo* Linn. (VN) extract.

Test	Concentration of Drug (mg/mL)	Time period for paralysis (P) and of worms (min)			
		Pheretima posthuma (P)		*Tubifex tubifex*	
		P	D	P	D
Control	--------	A	A	A	A
VN Ethanol Extract	(10 mg/mL)	48.75±0.38	92.85±0.89	32.12±0.60	58.30±0.72
	(20 mg/mL)	14.95±0.68	42.55±0.45	10.03±0.81	25.90±0.59
	(50 mg/mL)	2.85±0.11	13.92±0.30	2.10±0.29	8.75±0.54
Piperazine Citrate	(15 mg/mL)	17.50±0.32	A	10.75±0.87	A
Albendazole	(20 mg/mL)	33.10±0.69	62.50±0.75	22.05±0.80	40.25±0.27

Notes: A indicates no observed effect or data not applicable. Values represent mean±standard deviation (SD) of the observed effects. Tabular representation of Mean±SEM based on six observations. 'A' denotes no observed activity within 24 hr of administration.

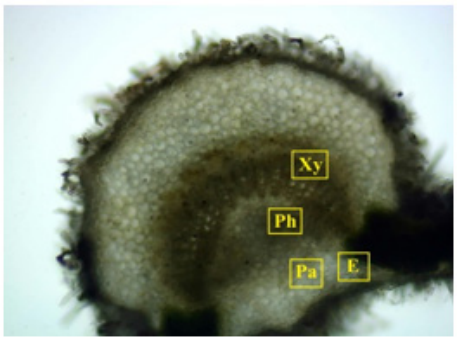

Plate 1: Transverse Section-1 (Zoom Mode): Arial Leaf.

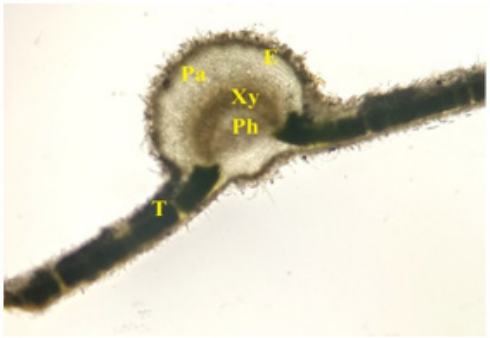

Plate 2: Transverse Section-2: Arial Leaf.

Figure 2: Microscopic characteristics of Arial leaf.[20]

Figure 3: Indian Earth worms. *Pheretima praepinguis* (Annelid).[21]

sensitive. For Piperazine Citrate (15 mg/mL), *Pheretima posthuma* had paralysis at 17.50±0.32 min, with no death observed ("A"), and *Tubifex tubifex* showed paralysis at 10.75±0.87 min, with no death observed ("A") (Table 2), (Figure 5). Piperazine Citrate was effective in causing paralysis but did not induce mortality. Lastly, Albendazole (20 mg/mL) caused paralysis in *Pheretima posthuma* at 33.10±0.69 min and death at 62.50±0.75 min, while *Tubifex tubifex* experienced paralysis at 22.05±0.80 min and death at 40.25±0.27 min. Albendazole demonstrated moderate efficacy in causing both paralysis and death, with longer times for paralysis and death compared to ethanol extracts. In summary, ethanol extract exhibited the strongest dose-dependent paralytic and lethal effects, especially at higher concentrations, while Piperazine Citrate caused paralysis but did not lead to death. Albendazole showed moderate effects, with slower times to paralysis and death than ethanol extract.

DISCUSSION

The pharmacological effects observed in this study suggest that both ethanol extracts and pharmaceutical agents like Piperazine Citrate and Albendazole have significant anthelmintic activity, albeit with varying degrees of effectiveness on *Pheretima posthuma*

Figure 4: Aquarium worms. *Tubifex tubifex* (Annelid).[22] FPO

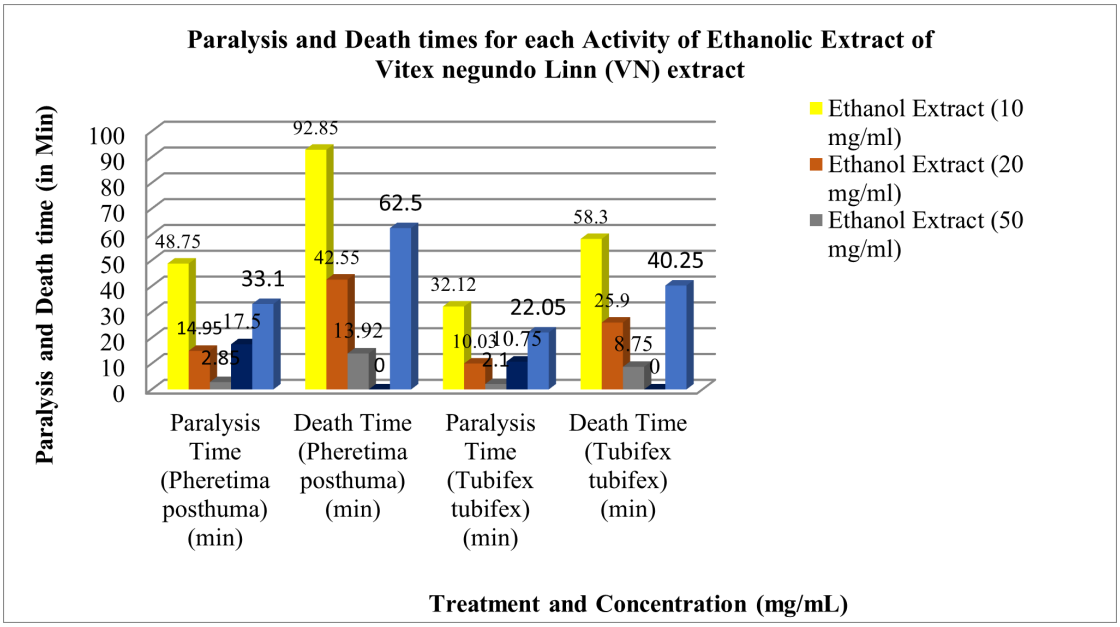

Figure 5: Paralysis and Death times for each Activity of Ethanolic Extract of *Vitex negundo* Linn. (VN) extract.

and *Tubifex tubifex*. The ethanol extracts, particularly at higher concentrations, showed strong dose-dependent effects in both paralysis and death, indicating that the active compounds present in these extracts may possess potent anthelmintic properties.

The ethanol extract at 10 mg/mL induced moderate paralysis and death in both worm species, with *Pheretima praepinguis*, Annelida taking longer to experience death compared to *Tubifex tubifex* Annelida. This suggests that the anthelmintic action of ethanol extracts may involve multiple pathways or mechanisms, affecting species differently. The results became more pronounced at higher concentrations. At 20 mg/mL and 50 mg/mL, both species exhibited quicker paralysis and death, with *T. tubifex* being more sensitive than *P. posthuma*. The rapid onset of effects at 50 mg/mL confirms that higher concentrations of ethanol extracts have an enhanced ability to induce paralysis and death, possibly due to a higher concentration of active alkaloid compounds or other bioactive molecules.

Alkaloids are known for their pharmacological effects, particularly their anthelmintic properties. They often interact with the neuromuscular system of worms, causing paralysis and eventually death. The rapid paralysis and death seen with ethanol extracts, especially at 50 mg/mL, due to the presence of alkaloid piperine specifically affect nervous system of helminths, interfering with muscle function and coordination. These alkaloids induce of paralytic action due to inhibition of acetylcholinesterase activity by large surge of accumulation of acetylcholine in the synapses.

Piperazine Citrate, at a concentration of 15 mg/mL, also induced paralysis in both *P. posthuma* and *T. tubifex* However, unlike the ethanol extracts, it did not result in death within the 24-hr observation period. Piperazine works by paralyzing the worms' muscles through the stimulation of GABA receptors, but it generally does not have a lethal effect unless prolonged exposure or higher doses are applied. This suggests that while Piperazine Citrate is effective for controlling worm movement and providing a therapeutic response, it may require additional treatment or higher doses to cause mortality, especially for more resilient species like *P. posthuma*.

Table 3: Probable Correlations between Phytochemicals and Anthelmintic Activity.

Phytochemical	Presence (+/-)	Anthelmintic Activity Observed
Alkaloids	+	Neuromuscular paralysis and death via AChE inhibition
Tannins	+	Interference with digestive enzymes, causing m
Saponins	+	Disruption of membrane integrity
Flavonoids	+	Oxidative stress on helminths leading to mortality
Fixed Oils	+	Enhanced bioavailability of active components
Glycosides	+	Potential contribution to metabolic interference

Albendazole, at 20 mg/mL, showed moderate anthelmintic effects, with both species experiencing paralysis and death, but at slower times compared to the ethanol extracts. Albendazole works by inhibiting the polymerization of tubulin, which is essential for the survival and mobility of helminths. This mechanism, while effective, seems to be less rapid than the action of ethanol extracts, which could be attributed to the fact that Albendazole needs to interfere with the metabolic processes of the worms at a cellular level, rather than producing an immediate neuromuscular disruption like the alkaloid components in ethanol extracts.

Mode of action of Activity of Ethanolic Extract concentration of *Vitex negundo* Linn. (VN) extract

bioactive compounds and alkaloids possess Anthelmintic activity of *Vitex negundo* Linn. (VN) is due to action of inhibiting Acetylcholinesterase (AChE), causing neuromuscular paralysis and eventual death (Table 3).[11] Flavonoids in the extract may induce oxidative stress, disrupting worm cellular defenses and leading to mortality, while saponins and tannins affect membrane integrity and digestive enzyme activity,[12] respectively. Compared to other herbal extracts like neem (*Azadirachta indica*) and holy basil (*Ocimum sanctum*), *Vitex negundo* Linn. (VN) showed faster, dose-dependent effects due to its potent combination of compounds. Synergistic interactions between alkaloids, flavonoids, and fixed oils likely amplify its efficacy, targeting multiple physiological systems in worms.[13] However, the study's reliance on *in vitro* assays limits direct applicability to humans or animals without further *in vivo* validation. Additionally, the absence of toxicity profiling raises concerns about the extract's safety in higher organisms. The use of a single solvent (ethanol) also precludes understanding the efficacy of other solvent-based extracts. These limitations, combined with a narrow focus on two worm species, suggest further research is essential to generalize the findings and confirm the mechanisms of action. Despite these constraints, the rapid effects observed make *Vitex negundo* Linn. (VN) a promising candidate for developing novel, plant-based anthelmintic therapies.

Effectiveness of Extracts

Based on the observed results, the ethanol extract at 50 mg/mL was the most effective in causing both paralysis and death in *Pheretima posthuma* Annelida and *Tubifex tubifex* Annelida, with rapid onset compared to the other treatments. The dose-dependent effects of the ethanol extract suggest that it may contain potent bioactive alkaloids or other compounds that directly target the neuromuscular system of the worms (Table 3). While Piperazine Citrate and Albendazole were also effective, they showed slower onset and did not result in mortality within the observation period (for Piperazine Citrate) or exhibited less rapid effects (for Albendazole). Therefore, ethanol extracts, particularly at higher concentrations, emerge as the most promising for their anthelmintic potential.[14]

In conclusion, this study highlights the pharmacological relevance of ethanol extracts, likely due to alkaloid compounds, in providing a potent and fast-acting solution for controlling parasitic worm infestations. Further isolation and characterization of the active compounds in the ethanol extract could help in developing more targeted and effective anthelmintic therapies. From an Ayurvedic perspective, *Vitex negundo* Linn. (VN) (*Nirgundi*) is recognized for its *Kirimighna* (antiparasitic)[15] and *Vishghna* (detoxifying or antitoxic) properties,[16] which align with its potential in combating parasitic infections. According to Sushrut, *Nirgundi* is classified under the *Sursaadi Gana* group, which includes herbs that help balance *Kapha*, exhibit wormicidal effects, and are beneficial in treating conditions such as *Pratishyaaya* (nasal congestion), *Aruchi* (loss of appetite), *Kasa* (cough), and are also noted for their effectiveness in wound healing (*Vranashodhan*).[17] These traditional uses support the findings of this study, suggesting that *Vitex negundo* could be a valuable herbal remedy for managing parasitic worm infestations.[18]

CONCLUSION

Vitex negundo shows great promise as a safer and more cost-effective alternative to synthetic anthelmintic agents like Albendazole and Piperazine Citrate. Its rapid, dose-dependent efficacy, combined with the abundance and affordability of the plant, highlights its potential for addressing drug resistance and accessibility challenges in low-resource settings. Future studies should focus on isolating and characterizing the active compounds, such as alkaloids and flavonoids, to understand their precise mechanisms. Conducting *in vivo* trials will help confirm the extract's effectiveness and safety in complex biological systems, alongside comprehensive toxicity evaluations. These steps are crucial to developing *Vitex negundo* Linn. (VN) into a reliable, natural anthelmintic therapy.

The ethanol extract of *Vitex negundo* Linn. (VN) showed superior dose-dependent efficacy compared to Albendazole and Piperazine Citrate. At 50 mg/mL, it caused rapid paralysis and death in *Pheretima posthuma* and *Tubifex tubifex*, outperforming Albendazole's slower action and Piperazine Citrate's ineffectiveness. The extract's rapid action suggests a potent mechanism, likely due to synergistic effects of alkaloids, flavonoids, and other phytochemicals. (Tables 1 and 3) These compounds may target multiple worm systems, enhancing effectiveness. The extract demonstrated comprehensive anthelmintic effects in less time, highlighting its potential as a strong anthelmintic agent.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

VN: *Vitex negundo* Linn.; **LD:** Lethal Dose; **Pa:** Parenchyma; **Xy:** Xylem; **Ph:** Phloem; **E:** Mesophyll; **Xy:** Xylem; **Ph:** Phloem; **T:** Non-glandular trichomes; **AChE:** Acetylcholinesterase; **P:** Paralysis; **D:** Death; **(A):** No Death Observed; **ANOVA:** Analysis of Variance, **p-p38:** Phosphorylated **p38**; **p-ERK1/2:** Phosphorylated Extracellular Signal-Regulated Kinase 1/2; **p-JNK:** Phosphorylated c-Jun N-terminal Kinase; **JNK:** c-Jun N-terminal Kinase; **COX-1:** Cyclooxygenase-1; **MAPK:** Mitogen-Activated Protein Kinase; **NF-κB:** Nuclear Factor Kappa B; **TNF-α:** Tumor Necrosis Factor Alpha; **Akt:** Protein Kinase B; **mTOR:** Mammalian Target of Rapamycin; **VEGF:** Vascular Endothelial Growth Factor**; HIF-1α:** Hypoxia-Inducible Factor-1 Alpha.

SUMMARY

The focus of above research study was to assess the anthelmintic potential of *Vitex negundo* Linn. (VN) ethanol extract. The study evaluated the activity against common parasitic worms, namely *Pheretima posthuma* and *Tubifex tubifex*, against synthetic anthelmintics like Albendazole and Piperazine Citrate. Results indicate that the extract has a better LD-dependent anthelmintic activity, where paralysis and death were observed swiftly within 24 hr at 50 mg/mL, outperforming Albendazole and Piperazine Citrate in speed and efficacy. The extraction activity may be rapid due to the synergistic effect exerted by alkaloids, flavonoids, and other phytochemicals that jointly target different systems of the worms. These findings lend credence to the potential for VN to act as a viable natural alternative to synthetic anthelmintics. Further work should focus on isolating and characterizing the bioactive compounds and validating the safety and efficacy of the extract through *in vivo* studies.

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