

Preliminary Phytochemical Screening and In-Vitro Evaluation of the Anthelmintic and Antibacterial Activities of the Aqueous Extract of the Rachis (*koor*) of *Azadirachta indica*

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Received: 25 November 2025

Accepted: 12 January 2026

Published: 28 February 2026

Citation: T. Vithuja, A. Sanjayan, & T. Thayalini. (2026). Preliminary Phytochemical Screening and In-Vitro Evaluation of the Anthelmintic and Antibacterial Activities of the Aqueous Extract of the Rachis (*koor*) of *Azadirachta indica*. Sri Lankan Journal of Applied Sciences (Online), Vol.4(2), 45–51. <https://doi.org/10.5281/zenodo.18533380>

Abstract

Helminthiasis and bacterial infections remain major public health challenges in developing countries, demanding safer plant-based alternatives to conventional drugs. *Azadirachta indica* is widely used in Siddha medicine; however, the rachis (*koor*) is an underexplored plant part despite its traditional use in aqueous decoctions. In this study, authenticated *A. indica* rachis collected from Jaffna, Sri Lanka, was shade-dried and extracted with distilled water (1:10 w/v), yielding 3g from 25g of dried material (12% w/w). Preliminary phytochemical screening revealed strong presence of tannins, glycosides, and steroids, and minimal presence of saponins and flavonoids. The aqueous extract exhibited significant dose-dependent in-vitro anthelmintic activity against *Pheretima posthuma*. Paralysis and death times at 200 mg/mL were 31.08 ± 0.19 min and 56.10 ± 0.24 min, respectively, which were significantly faster ($p < 0.05$) than albendazole (10 mg/mL) showing paralysis at 36.27 ± 0.27 min and death at 60.17 ± 0.41 min. At 150, 100, and 50 mg/mL, paralysis / death times were 56.10 ± 0.24 / 70.33 ± 0.50 min, 72.00 ± 0.00 / 90.17 ± 0.24 min, and 82.33 ± 0.52 / 100.17 ± 0.22 min, respectively, while no activity was observed in the control. Antibacterial evaluation using the agar well diffusion method demonstrated inhibition against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923), with zones of inhibition of 11.00 ± 0.00 mm and 12.17 ± 0.29 mm at 200 mg/mL, decreasing with concentration, and no inhibition in the control. This study provides the first scientific validation of the traditional Siddha use of *Azadirachta indica* rachis aqueous extract, highlighting its potent anthelmintic and antibacterial activities mediated by synergistic phytochemicals.

Keywords: *Azadirachta indica*, Rachis extract, Anthelmintic, Antibacterial, Phytochemicals, Siddha medicine

1. Introduction

Helminthiasis and bacterial infections continue to be serious threats to public health in many developing nations that affects millions of people globally [1,2]. A prevalent soil-transmitted parasite infection, helminthiasis causes anemia, malnutrition and impaired development, especially in children [1,3]. Communities in tropical and subtropical regions that lack access to clean water, sanitary facilities, and proper hygiene are affected by these infections, which mainly caused by helminths [1,3].

In humans, a gram-negative bacteria *Escherichia coli* is generally harmless and beneficial, but some strains have developed virulence properties which enables them to cause urinary tract infections such as cystitis, gastrointestinal diseases such as diarrhea and systemic invasive infections such as meningitis and sepsis [4]. About 30% of people are affected by gram-positive bacteria *Staphylococcus aureus*,

which is both a commensal organism and a significant human pathogen [2]. It serves as a major cause of bacteremia, infective endocarditis, osteoarticular infections, skin and soft tissue infections, pleuropulmonary diseases and device-and-implant related infections [2].

Despite being commonly used, typical anthelmintic medications like albendazole and antibacterial medications like amoxicillin can cause side effects, antimicrobial drug resistance, and ineffective worm eradication when used regularly [1-5]. Therefore, finding safer, plant-based substitutes with efficient anthelmintic and antibacterial activities is becoming ever more popular.

Azadirachta indica A. Juss. is a medicinal plant belonging to the family Meliaceae, commonly known as *Vembu* in Tamil and *Neem* in English [6]. The classical Siddha literature has widely described its therapeutic potential and applications due to its antimicrobial, antifungal, anthelmintic, insecticidal, and anti-inflammatory properties [6,7]. All parts of the plant, including the leaves, bark, seed, flower, and rachis (*koor*), have traditionally been

used for the treatment of gastrointestinal disorders, intestinal worms, gum inflammation, fever, measles, smallpox, and other diseases [6,7]. While several components of *Azadirachta indica*, including leaves, bark, seeds, and flowers, have been examined with diverse organic solvents such as ethanol, methanol, and chloroform, comparative analyses that involve the rachis and aqueous extraction are still remain limited. Aqueous decoctions are widely used in traditional Siddha medicine because they are safe, easily accessible, and suitable for human consumption. Therefore, the present study specifically focuses on the aqueous extract of the rachis to scientifically validate its traditionally claimed anthelmintic and antibacterial properties. Comparative evaluation with other solvents and plant parts is suggested as important and is recommended for future investigations to further demonstration of their relative effectiveness.

The rachis is one among these understudied plant parts, even though it is mentioned in traditional formulas of Siddha Medicine as a component of decoctions for the manufacture of medicines for anthelmintic and antibacterial properties [7]. Although earlier studies focused on the anthelmintic and antibacterial effects of ethanolic extracts and other parts of the plant, there aren't numerous scientific studies focusing on rachis's aqueous extract, especially in Sri Lanka [8,9]. There are no previously published studies evaluating the aqueous extract of the rachis were found after a systematic literature search using keywords like '*Azadirachta indica* rachis', 'aqueous rachis extract', 'anthelmintic activity', and 'antibacterial activity' in databases like Google Scholar and PubMed. To the best of our knowledge, this study is the first to evaluate the anthelmintic and antibacterial effectiveness of the aqueous rachis extract, thereby addressing an unexplored research gap.

Phytochemicals, the bioactive substances which include tannins, saponins, flavonoids, steroids, and glycosides have been identified in several plants and their different extracts; where these compounds are known for their pharmacological activities such as antibacterial and anthelmintic efficacy [9]. These findings provide the aqueous extract of *A. indica* rachis, a scientific basis for being considered as a potential anthelmintic and antibacterial agent.

Therefore, the aim of this study is to assess the *A. indica* rachis aqueous extract's dose-dependent in-vitro anthelmintic activity against *Pheretima posthuma* (Earthworms), in-vitro antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, validate the results with traditional Siddha theories, and offer a phytochemical understanding for its effectiveness. This study approaches a fresh element of *A. indica* pharmacology by emphasizing on the rachis and aqueous extraction, which is useful in developing secure and efficient plant-based anthelmintic and antibacterial medications.

2. Materials and Methods

2.1. Plant Material Collection and Authentication

Azadirachta indica rachis (*koor*) was collected from Kaithady, Jaffna, Sri Lanka, from its natural habitat. The plant was verified and authenticated by the Department of Gunapadam, Faculty of Siddha Medicine, University of Jaffna and a voucher specimen was deposited in the regional herbarium under the voucher number FSM/DG/PGV/005.

The collected rachis was properly cleaned under running water and allowed to dry in the shade for 10 days at room temperature (25 - 30°C). After being dried, the material was then ground into fine powder with a mechanical grinder and was stored in airtight containers.

2.2. Preparation of Aqueous Extract

250 mL of distilled water was used to dissolve 25 g of powdered dried *Azadirachta indica* rachis (solid-to-solvent ratio 1:10, w/v) for 24 hours at room temperature while stirring occasionally. Then, after 30 minutes of gentle boiling, the mixture was allowed to cool to room temperature before being reduced to 125 mL in a water bath set at 40-50°C. The extract was filtered initially with a muslin cloth and then followed by using Whatman No. 1 filter paper. Finally, the resultant stock solution of 200 mg/mL aqueous extract was prepared for further use.

The extract was further dried to constant weight, yielding 3 g of crude aqueous extract from 25 g of dried rachis powder, corresponding to a percentage yield of 12% (w/w).

The aqueous extract was prepared from 25 g of dried plant material to obtain a total volume of 125 mL and was directly used for anthelmintic and antimicrobial assays at concentrations of 200, 150, 100 and 50 mg/ml, of which 100 mL was utilized for biological evaluations. After completion of the assays, the remaining 25 mL of the aqueous extract was dried using a water bath until constant weight (0.6 g) to determine yield and the percentage yield was calculated based on the initial dry weight of the plant material. The dried extract was not used for biological assays; drying was performed solely for yield determination.

2.3. Preliminary Phytochemical Screening

The presence of bioactive compounds, which include tannins, saponins, glycosides, steroids, and flavonoids, on the freshly prepared aqueous extract of *A. indica* rachis was identified chemically using standard procedures and the qualitative identification was performed by observing distinctive color changes [9,10,11].

2.3.1 Test for Tannins

Few drops of 1% Ferric Chloride (FeCl₃) were added into 1 mL of the aqueous extract, and the presence of tannins was identified by the Ferric Chloride test for tannins, which shows a blue-black/greenish color shift.

2.3.2 Test for Saponins

1 mL of aqueous extract was added with 9 mL of distilled water in a sterile test tube and the mixture was vigorously shaken for 15 seconds. The extract was left to stand for about 10 minutes. According to the foam test, the existence of saponins is indicated by the formation of stable foam.

2.3.3 Test for Glycosides

To 1 mL of the aqueous extract, a few drops of glacial acetic acid were first added. A few drops of 1% ferric chloride (FeCl_3) were then added, followed by 3-4 drops of concentrated sulfuric acid (H_2SO_4). The Keller-Killiani test for glycosides shows the presence of glycosides when a reddish-brown ring at the junction of the layers.

2.3.4 Test for Steroids

2 mL of chloroform were mixed with 2 mL of the aqueous extract, and then 2 mL of concentrated sulfuric acid (H_2SO_4) were added along the test tube's walls. The Salkowski test for steroids shows the presence of steroids when a blue-green color development appears at the junction.

2.3.5 Test for Flavonoids

A few fragments of Magnesium ribbons were added into the 2 mL of the aqueous extract, and then a few drops of concentrated hydrochloric acid (HCl) were added. The Shinoda test, which detects the presence of flavonoids, shows the creation of a pink or red color.

2.4. Anthelmintic Activity Assay

The in vitro anthelmintic assay followed the standard method with slight modifications [12]. Before beginning the experiment, the petri dishes and equipment were sterilized, and all test and standard drug solutions were freshly newly prepared.

2.4.1 Standard Drug Solution Preparation

Albendazole 400 mg tablets were crushed into powder. 200 mg of that was dissolved in 15 mL of distilled water, which was then gradually heated to about 40°C. The final volume was then adjusted to 20 mL, producing a 10 mg/mL solution which serves as the standard reference.

2.4.2 Preparation of Serial Dilutions

Serial dilutions were prepared using the initial extract (200 mg/mL) by adding distilled water to it as shown in table 1. Group V, which consists 20 mL of distilled water (0 mg/mL) will be the control solution.

Table 1:

Serial dilutions of the prepared aqueous extract with distilled water.

Group	Concentration (mg/mL)	Aqueous Extract (mL)	Distilled Water (mL)
I	200	20	0
II	150	15	5
III	100	10	10
IV	50	5	15
V	0	0	20

2.4.3 Cultivation and Preparation of Earthworms

Adult earthworms (*Pheretima posthuma*) in identical size (7-10 cm length, 0.2-0.3 cm width) have been grown in soil containing 3:1 decomposed cow dung at a temperature of 25-28°C and a humidity of 70-80%. Twice a week, the worms were fed with decomposed organic materials, and after eight weeks, they were taken out. Worms were cleaned with normal saline before to being used in the anthelmintic assay.

2.4.4 Assessment of the Anthelmintic Activity Assay

Six different groups of experimenters were created in separate petri dishes, with six earthworms in each group; as shown in the Table 2.

Table 2:

Experiment groups and their contents in the anthelmintic activity assay.

Group No.	Extract / Content	Concentration (mg/mL)	Volume (mL)
1	Control [Distilled Water]	0	20
2	Standard reference solution [Albendazole]	10	20
3		200	20
4	Aqueous extract of	150	20
5	<i>Azadiracta indica</i> rachis	100	20
6		50	20

The worms' paralysis time was recorded by observing how long it took them to stop moving even after mild stimulation, and the death time was recorded by observing how long it took for them to stop movement. For each concentration, the mean paralysis and death times were calculated using six worms.

2.5. Antibacterial Activity Assay

The standard agar well diffusion method was used to evaluate the antibacterial effects of *Azadiracta indica* rachis aqueous extract at 200 mg/mL, 150 mg/mL and 100 mg/mL concentrations. Strict sterilization procedures were maintained throughout all of the tests [11,13,14].

2.5.1 Test microorganisms and Preparation of bacterial inoculums

Escherichia coli (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) colonies that had just been isolated were transferred into separate sterile universal bottles with roughly 2 mL of sterile normal saline. After vortexing the suspensions for 5 seconds, the turbidity was adjusted to meet the 0.5 McFarland standard solution.

2.5.2 Agar cut well method assessment

Muller Hinton Agar (MHA) plates were inoculated with 1 mL of each standardized liquid bacterial suspension (approximately 1×10^6 CFU/mL). After evenly spreading the inoculum over the agar surface, it was left to dry for about 15 minutes at 37 °C. Wells were created in the agar using

sterile cork borer (8 mm in diameter and 4 mm in depth), and the rachis aqueous extract was fully filled into the well.

The plates were left to stand at room temperature for 30 minutes to promote diffusion of the extract and then incubated at 37°C for 24 hours. The diameter of the Zone of Inhibition (ZOI) around each well was measured in order to evaluate the antibacterial activity. All tests were conducted in triplicate, and the mean ZOI values were recorded.

2.6. Data Collection and Statistical Analysis

Data collection and preliminary computations were performed using Microsoft Excel 2019. Statistical analyses were conducted using one-way analysis of variance (ANOVA) to evaluate differences among multiple groups, followed by Tukey's honestly significant difference (HSD) post-hoc test for pairwise comparisons. In addition, Welch's two-sample t-test was used to compare means between two groups while accounting for unequal variances. A p-value of < 0.05 was considered statistically significant.

3. Results and Discussions

Previous studies on *A. indica* have mainly focused on the leaves, bark, and seeds which were extracted using organic solvents like methanol and ethanol, which are known to extract a wider range of non-polar phytochemicals. Instead, the current study used aqueous extract of the rachis to selectively extract water-soluble bioactive components and to reflect traditional Siddha decoction techniques. Although direct experimental comparison with other plant parts was not conducted, reported aqueous extraction yields of neem leaves and seeds range between 7–13%, which is comparable to the 12% yield obtained for the rachis in the present study. Comparative extraction of different plant parts using multiple solvents is recommended for future studies.

Since the extract was homogeneous, the total extract from the full 125 mL was estimated as $(0.6 \text{ g} / 25 \text{ mL}) \times 125 \text{ mL} = 3 \text{ g}$, giving an extrapolated percentage yield of 12% (w/w).

All extraction, stock preparation, serial dilutions, and assay volumes are mass-balanced and internally consistent. No numerical or conceptual inconsistencies are present.

The preliminary phytochemical screening results summarized in Table 3, indicates that the aqueous extract of *A. indica* rachis contained a variety of types of bioactive chemicals, including a high concentration of tannins, glycosides, and steroids, along with saponins and flavonoids.

Table 3: Results of the Preliminary Phytochemical Screening of the aqueous extract of *Azadirachta indica* rachis.

No.	Test Method	Phytochemical tested for	Results
1	Ferric Chloride Test	Tannins	+++
2	Foam Test	Saponins	+
3	Keller-Killiani Test	Glycosides	+++

4	Salkowski test	Steroids	+++
5	Shinoda test	Flavonoids	+

(Phytochemical presence was classified as; '+++': Strong presence; '++': Moderate presence; '+': Minimal presence; and '-': Absence)

The presence of all these substances supports previous studies that they are abundantly present in *A. indica* while being the reason for a variety of pharmacological actions, such as anthelmintic, antioxidant, and antibacterial effects [15,16].

The anthelmintic effect of the aqueous extracts was assessed against the standard medication, albendazole (10 mg/mL). From the results of the anthelmintic activity assay (table 4, 5, 6 and figure 1), it was evident that aqueous extract of *A. indica* rachis exhibited dose dependent anthelmintic activity against *Pheretima posthuma*, causing progressive decrease in both mean paralysis and mean death times as the concentration increased from 50 mg/mL to 200 mg/mL.

Table 4: Paralysis times of six replicated groups of *Pheretima posthuma* treated against different content experimental groups.

Extract / Content	Concentration (mg/mL)	Paralysis Time (min)					
		I	II	III	IV	V	VI
Distilled Water	0	0.0	0.0	0.0	0.0	0.0	0.0
Albendazole	10	36.0	36.6	36.0	36.5	36.0	36.5
Aqueous extract of <i>A. indica</i> rachis	200	31.0	31.0	31.5	31.0	31.0	31.0
	150	56.0	56.6	56.0	56.0	56.0	56.0
	100	72.0	72.0	72.0	72.0	72.0	72.0
	50	82.0	82.0	82.0	83.0	82.5	82.5

Table 5: Death times of six replicated groups of *Pheretima posthuma* treated against different content experimental groups.

Extract / Content	Concentration (mg/mL)	Death Time (min)					
		I	II	III	IV	V	VI
Distilled Water	0	0.0	0.0	0.0	0.0	0.0	0.0
Albendazole	10	60	60	61	60	60	60
Aqueous extract of <i>A. indica</i> rachis	200	56	56.6	56	56	56	56
	150	70	70	70	71	70.5	70.5
	100	90.5	90.5	90	90	90	90
	50	100	100.5	100	100.5	100	100

Table 6: Mean paralysis and mean death times of *Pheretima posthuma* treated against different concentrations of aqueous extract of *Azadirachta indica* rachis, Standard drug solution and control.

No.	Extract / Content	Concentration (mg/mL)	Mean ± SD Paralysis Time (min)	Mean ± SD Death Time (min)
1	Control [Distilled Water]	0	No activity	No activity

2	Standard reference solution [Albendazole]	10	36.27 ± 0.27	60.17 ± 0.41
3	Aqueous extract of	200	31.08 ± 0.19	56.10 ± 0.24
4	<i>Azadiracta</i>	150	56.10 ± 0.24	70.33 ± 0.50
5	<i>indica</i> rachis	100	72.00 ± 0.00	90.17 ± 0.24
6		50	82.33 ± 0.52	100.17 ± 0.22

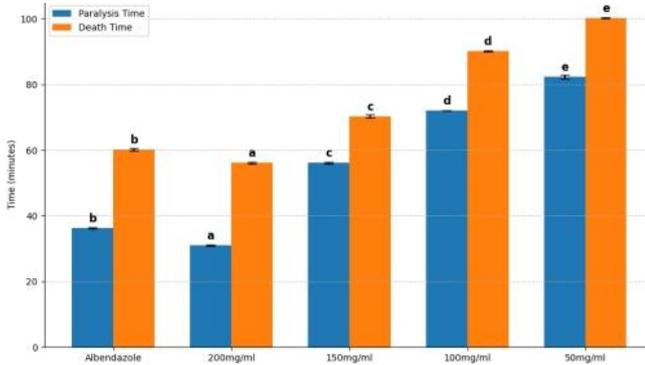


Fig. 1. Anthelmintic activity assay results of the aqueous extracts of *A. indica* rachis, control and standard reference solution against *P. posthuma* [Statistical differences ($p < 0.05$) between all groups confirmed by Tukey HSD post-hoc test (a = fastest, e = slowest)]

The aqueous extract performed higher than to the effects of standard albendazole (10 mg/mL) at the highest tested concentration (200 mg/mL), causing paralysis in 31.08 ± 0.19 minutes and death in 56.10 ± 0.24 minutes; where the time taken for paralysis and death at 10 mg/mL of albendazole was 36.27 ± 0.27 minutes and 60.17 ± 0.41 minutes respectively. Even the lowest concentration 50 mg/mL, resulted significant activity, with paralysis in 82.33 ± 0.52 minutes and death in 100.17 ± 0.22 minutes indicating effectiveness across all tested doses. The absence of any activity in the control group confirmed that the extract's bioactive ingredients were the cause of the effects observed.

Statistical analysis was performed using one-way ANOVA followed by Tukey's HSD post-hoc test; values were expressed as mean \pm SD ($n = 6$), and $p < 0.05$ was considered statistically significant. All treatment groups showed statistically significant differences compared to the control ($p < 0.05$). A clear concentration-dependent increase in paralysis and death times was observed with decreasing extract concentration, with all treatment groups differing significantly from each other ($p < 0.05$).

In comparison to a commercial synthetic medicine, this suggests that the aqueous extract has a strong anthelmintic effectiveness.

Since *Pheretima posthuma* resembles human intestinal roundworm parasites anatomically and physiologically, healthy adult earthworms were utilized to investigate the anthelmintic activity [17]. Based on the dose dependent response pattern, it may indicate the phytochemicals in the extract work together to interfere with the worms' processes in the body.

It is widely understood that these phytochemicals facilitate anthelmintic action through their modes of actions [16,18]. Condensed tannins, also known as tannic acid, bind

to free proteins in the host animal's digestive tract or the parasite's cuticle, causing surface destruction and loss of motion, which paralyzes and kills the worm [18,19,20]. Saponins' surfactant effects allow the membrane's integrity to be disrupted, which results in cell lysis and paralysis and it also disrupts mitochondrial function, that kills helminths [18,21]. Flavonoids have an effect on ATPase and the calcium pump, causing the helminths to die, while steroids and glycosides have been reported to disrupt the metabolism and energy generation of worms [18,20,22]. The synergistic effect of these phytochemicals possibly strengthens the overall anthelmintic activity of the aqueous extract of the *A. indica* rachis.

Even though ethanolic and other extracts of this *A. indica* plant have been studied before, this study is focused on the rachis's aqueous extract, which at 200 mg/mL displays strong anthelmintic activity in compared to the standard medicine, albendazole. This reflects and supports the traditional Siddha reference in decoction practices.

Table 7 summarizes the results of the Zone of Inhibitions (mm) of the antibacterial assay of different tested concentrations of the aqueous extract of *Azadirachta indica* rachis using the cut well method against *Escherichia coli* and *Staphylococcus aureus* bacteria.

Table 7:

Results of the Zone of Inhibitions (mm) of the antibacterial assay of different concentrations of the aqueous extract of *A. indica* rachis.

Organisms	Diameter of the Zone of Inhibition (mm)			
	[Mean \pm SD]			
	Aqueous extract of <i>A. indica</i> rachis (mg/mL)			Control (Distilled Water)
	200	150	100	
<i>Escherichia coli</i> (ATCC 25922)	11.0 \pm 0.0	10.17 \pm 0.15	9.33 \pm 0.29	Nil
<i>Staphylococcus aureus</i> (ATCC 25923)	12.17 \pm 0.29	11.5 \pm 0.0	10.0 \pm 0.0	Nil

(SD: Standard Deviation)

The results of the antibacterial activity (Table 7) indicates that different concentrations of tested *A. indica* rachis aqueous extract have significant antibacterial activity against both gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus* bacteria, as each concentration was performed in triplicate. The mean zone of inhibitions of *E. coli* and *S. aureus* were measured at highest tested concentration 200 mg/mL as 11.0 ± 0.00 mm and 12.17 ± 0.29 mm respectively, with a relatively greater inhibitory capability against *S. aureus*. Even the lowest tested concentration 100 mg/mL, resulted significant activity, with zone of inhibition for *E. coli* as 9.33 ± 0.29 mm and for *S. aureus* as 10.0 ± 0.00 mm, indicating effectiveness across all tested doses.

Statistical analysis was performed using Welch's two-sample *t*-test to account for unequal variances between treatment and control groups. Control samples showed no zone of inhibition (0 mm), resulting in zero variance; therefore, Welch's *t*-test was applied. The aqueous extract exhibited a statistically significant inhibitory effect against

E. coli at both 200 mg/mL ($p < 0.001$) and 100 mg/mL ($p < 0.001$) compared to the control. Similarly, significant inhibition was observed against *S. aureus* at 200 mg/mL ($p < 0.001$) and 100 mg/mL ($p < 0.001$). These results demonstrate a strong concentration-dependent antibacterial activity of the aqueous extract.

The partial extraction of water-soluble phytochemicals such as tannins, glycosides, saponins, and other bioactive substances possessing antimicrobial properties which are generally linked with mode of actions such as inhibition of bacterial efflux pumps, modification of target sites and antimicrobial binding sites, disruption of membrane integrity, and inhibition of ATP synthase, that contributes to the significant antimicrobial activity of the aqueous extract of *A. indica* rachis [23,24]. The absence of activity in the control indicated that the aqueous extract and its bioactive components caused the noted antibacterial effects.

The study's finding that *S. aureus* was more susceptible to the plant extracts than *E. coli* is attributed to the fact that gram-positive bacteria are generally more susceptible because they lack the lipopolysaccharide-rich outer membrane found in gram-negative bacteria [25].

This study is among the first that demonstrates that the aqueous rachis extract of *A. indica* has dose-dependent anthelmintic activity, and antibacterial activity proving that the rachis, which is frequently undervalued as compared to leaves and bark, is a potential natural anthelmintic and antibacterial agent.

Further studies are required to identify and isolate the active components causing this activity in order to develop the extract into adequate dosage forms and analyse their safety and in-vivo mechanisms of action.

4. Conclusion

The aqueous extract of *Azadirachta indica* rachis has notable anthelmintic action against *Pheretima posthuma*, which is dose-dependent and significant antibacterial effect against *Escherichia coli* and *Staphylococcus aureus* bacteria. The presence of tannins, saponins, glycosides, steroids, and flavonoids have been identified by preliminary phytochemical screening; these compounds probably function synergistically to deliver the anthelmintic and antibacterial effects that have been seen. These results establish the rachis of *Azadirachta indica* as a promising, unexplored natural dual-action anthelmintic and antibacterial agent and provides scientific evidence for traditional Siddha claims about its therapeutic usage.

Conflicts of Interest

There are no conflicts to declare.

References

[1] Soil-transmitted helminth infections (2023). World Health Organization. Available at: <https://www.who.int/news-room/factsheets/detail/soil-transmitted-helminth-infections> (Accessed: 02 October 2025).

[2] Tong S. Y. C., Davis J. S., Eichenberger E., Holland T. L., Fowler V. G. (2015). *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management, *Clinical Microbiology Reviews*, 28(3), 603-661. DOI:10.1128/CMR.00134-14.

[3] Ojo F. T., Idowu O. A., Ademolu K. O., Olukunle, J. O. (2023). In vivo anthelmintic potentials of *Gongronema latifolium* and *Picralima nitida* against gastrointestinal parasite (*Heligmosomoides bakeri*), *Helminthologia*, 60(4), 336-347. <https://doi.org/10.2478/helm-2023-0033>

[4] Alhadlaq M. A., Aljurayyad O. I., Almansour A., Al-Akeel S. I., Alzahrani K. O., Alsaman S. A., Yahya R., Al-Hindi R. R., Hakami M. A., Alshahrani S.D., Alhumeed N. A., Al Moneea A. M., Al-Seghayer M. S., AlHarbi A. L., Al-Reshooi F. M., Alajel S. (2024) Overview of pathogenic *Escherichia coli*, with a focus on Shiga toxin-producing serotypes, global outbreaks (1982-2024) and food safety criteria, *Gut Pathogens*, 16(1):57. doi: 10.1186/s13099-024-00641-9.

[5] Jalalpure S. S., Alagawadi K. R., Mahajanashetti C. S., Shah B. N., Salahuddin, Vijay S., & Patil, J. K. (2007). In vitro anthelmintic property of various seed oils against *Pheretima posthuma*. *Indian Journal of Pharmaceutical Sciences*. 69(1), 158-160. DOI:10.4103/0250-474X.32138.

[6] Khare C. P. (2007). *Indian Medicinal Plants*, Springer New York, NY, 1, USA, DOI: <https://doi.org/10.1007/978-0-387-70638-2>

[7] Murugesamuthaliyar K. S. (2002). *Gunapadam Mooligai Vaguppu*, Madurai: Siddha Academy, 3, 614-616.

[8] Adams E. G., Ekott E. J., Usip L. P. & Opara K. N. (2023). Efficacy of *Azadirachta indica* in the Treatment of Gastrointestinal Helminthiasis, *Biotechnology Journal International*, 27(5), 47-55. DOI: 10.9734/BJI/2023/v27i5695

[9] Parekh J. & Chanda S. (2007). In vitro Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants. *Turkish Journal of Biology*, 31(1), 53-58.

[10] Edeoga H. O., Okwu D. E. & Mbaebie B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, 4(7), 685-688.

[11] Tharshika S., Vidhya M. P., Shivatharsiny R., Thavaranjit A. C. & Thayalini T. (2017). Preliminary Phytochemical Screening and Anti-Bacterial Activity of Leaves of *Moringa oleifera* Lamk, *Asian Journal of Medicine and Health*, 6(1), 1-5. DOI: 10.9734/AJMAH/2017/34816

[12] Ajaiyeoba E. O., Onocha P. A. & Olarenwaju O. T. (2001). In vitro Anthelmintic Properties of *Buchholzia coriacea* and *Gynandropsis gynandra* Extracts. *Pharmaceutical Biology*, 39(3), 217-220. DOI: 10.1076/phbi.39.3.217.5936.

[13] Balouiri M., Sadiki M. & Ibensouda S. A. (2016). Methods for in vitro evaluating antimicrobial activity: A review, *Journal of Pharmaceutical Analysis*, 6(2), 71-79. DOI: 10.1016/j.jpha.2015.11.005

[14] Thayalini T., Thevanesam V., and Kathirgamanathar S. (2017). Antimicrobial Activity of Seeds and Leaves of *Myristica*

fragrans against Multi-resistant Microorganisms, Journal of Agricultural Science and Technology, 302-308. DOI: 10.17265/2161-6256/2017.05.002

[15] Kumar S. & Upadhyay K. (2025). Validation of anthelmintic properties of neem *Azadirachta indica* in the livestock, World Journal of Pharmaceutical Research, 14 (17), 438-457, DOI: 10.20959/wjpr202517-38104

[16] Swargiary A., Daimari A., Daimari M., Basumatary N. & Narzary E. (2016). Phytochemicals, antioxidant, and anthelmintic activity of selected traditional wild edible plants of lower Assam. Indian Journal of Pharmacology, 48, 418-423. DOI: 10.4103/0253-7613.186212

[17] Esther J., Sangeetha N., Balabhaskar R. & Gunalan G. (2018). In Vitro Anthelmintic Activity of *Achyranthes aspera* Linn. (Whole Plant) Against *Pheretima posthuma*. Journal of Research in Siddha Medicine, 1(1), 68-71.

[18] Ahmad S., Humak F., Ahmad M., Altaf H., Qamar W., Hussain A., Ashraf U., Abbas R.Z., Siddique A., Ashraf T. & Mughal M. A. S. (2023). Phytochemicals as alternative anthelmintics against poultry parasites: A review. Agrobiological Records, 12, 34-45, DOI: <https://doi.org/10.47278/journal.abr/2023.015>

[19] Rubini D., Sudhahar D. & Anandarajagopal K. (2012). Phytochemical investigation and anthelmintic activity of *Celosia Cristata* leaf extracts, International Research Journal of Pharmacy, 3(5), 335-337.

[20] Narayanan R. R., Gopal T. K. & Chamundeeswari, D. (2020). In vitro Evaluation of Anthelmintic Activity of *Gymnema sylvestre* Plant, Pharmacognosy Journal, 12(4), 809-814. DOI: 10.5530/pj.2020.12.116

[21] Maestrini M., Tava A., Mancini S., Tedesco D., & Perrucci S. (2020). In vitro anthelmintic activity of saponins from *Medicago spp.* against sheep gastrointestinal nematodes, Molecules, 25(2), 242. DOI: 10.3390/molecules25020242

[22] Goel V., Sharma S., Chakroborty N. K., Singla L. D. & Choudhury D. (2023). Targeting the nervous system of the parasitic worm, *Haemonchus contortus* with quercetin, Heliyon, 9(2), 1-11. DOI: 10.1016/j.heliyon.2023.e13699

[23] Cowan M. M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12(4), 564-582. DOI: 10.1128/CMR.12.4.564

[24] Ashraf M.V., Pant S., Khan M. A. H., Shah A. A., Siddiqui S., Jeridi, M., Alhamdi H. W. S., Ahmad S. (2023). Phytochemicals as Antimicrobials: Prospecting Himalayan Medicinal Plants as Source of Alternate Medicine to Combat Antimicrobial Resistance. Pharmaceuticals, 16, 881. DOI: 10.3390/ph16060881

[25] Nikaido H. (2003). Molecular basis of bacterial outer membrane permeability revisited. Microbiology and Molecular Biology Reviews, 67(4), 593 - 656. Doi: 10.1128/MMBR.67.4.593-656.2003