Research Article

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Stomach-affecting intestinal parasites as a precursor model of *Pheretima posthuma* treated with anthelmintic drug from *Dodonaea viscosa* Linn.

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Abstract: The preponderance of helminth contagions is constrained to tropical regions and can cause massive vulnerability to malnutrition, anemia, pneumonia, and eosinophilia. Many human parasitic diseases cause severe illness in endemic populations. The helminths transmitting through the gastro-intestinal tract may develop resistance to anthelmintic drugs. The phytotherapy, anthelmintic, and antimicrobial efficacy of *Dodonaea viscosa* leaf solvent extracts were examined. Phytochemical screening was carried out by ultraviolet (UV) and Fourier transform infrared spectroscopy (FTIR). The anthelmintic activity was performed against a South Indian earth worm as a model by measuring the paralytic time. The antibacterial activity was performed against *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*, which stimulate the stomach helminths. Phytochemical screening revealed the presence of chemical compounds from the UV spectrum, and alkenes and aromatic compounds were confirmed by FTIR. Higher concentrations of the *D. viscosa* extract showed a rapid paralytic effect with a rapid death rate and histopathology. The zone-of-inhibition study indicated the potent antibacterial activity of the ethanolic and methanolic extracts of *D. viscosa* against different bacterial species. The current research revealed that *D. viscosa* has significant anthelmintic and antibacterial activities and it can be used for further elucidation and characterization.

Keywords: *Pheretima posthuma*, anthelmintic, *Dodonaea viscosa*, paralysis, histopathology

Abbreviations

* D. viscosa* *Dodonaea viscosa*
* P. posthuma* *Pheretima posthuma*
* GIT* gastro-intestinal tract
* UV* ultraviolet
* FTIR* Fourier transform infrared spectroscopy
* ZOI* zone of inhibition

1 Introduction

Helminthic infections are the major problem because host–parasite interactions are not defined, and the research is still in the commencement of the twenty first century. The nematodes are widespread in humans and animals, leading to health distress. Gastro-intestinal nematodes in ruminants are also important cases of animal diseases in tropical regions [1,2]. In contrast, the preponderant infections of helminths are constrained to tropical regions. These complications increase nematode resistance in the
prolonged chronic treatment of commercially existing drugs [3]. They cause vulnerability to malnutrition, anemia, pneumonia, and eosinophilia. Human parasitic diseases cause severe morbidity in the endemic population. The helminth infection caused through the gastro-intestinal tract (GIT) becomes more resistant to the available anthelmintic drugs markedly. In the past few decades, there has been a renewed curiosity into the natural therapy of conventional plant therapeutics and the role of innate products in drug invention [4]. An explanation for this comprises the immense requirement for novel molecular models, which leads to prospective new drugs for authenticating habitual therapeutic applications [5]. Infectious diseases are the most prevalent nowadays, and approximately 57 million people are affected by various infectious diseases universally every year [6]. So, there was an early promotion in the consistency of certain plant-based drugs impending beneficiary accomplishments. In spite of the contemporary techniques, recognition of plant drugs is most expected in further consistency [7].

Contagious helminthic infection, frequently unconstrained Helminthiasis, is among the invasive illnesses and a prime degenerative sickness upsetting a vast section of inhabitants. It has become a hefty risk to community health and makes a payment to the hectic condition of the helminth population [8], especially in developing and underdeveloped countries. GIT is the appropriate locality for most of the helminths. However, larvae show easy transmission of larvae in the direction of GIT and also accommodate several places in different tissues [9]. Chemical organization of attached helminths with enhanced supervision has become an imperative worm management approach worldwide. Nevertheless, the development of conservative anthelmintic resistance in helminths is a key challenge in eradicating helminth infection. It has become vital to look for substitute approaches against nematodes present in GIT, which has been linked with the liable selection of medicinal plants for the identification of potential therapeutic moieties for better anthelmintic activity [10].

The manifestation of plant-based drug therapy is evolved for treating pathogenic human bacterial species and animal bacterial species. Also, redundant adverse reactions of certain antibiotics have generated extensive attention in the discovery of new antimicrobial substitutes of plant origin. Noteworthy advantage asserted for counteractive utilization of therapeutic medicinal plants in various diseases in their fortification as wellbeing competent, simple convenience, and economical [11]. Significant bioactive compounds in potential medicinal plants are alkaloids, tannins, phenolic compounds, flavonoids, saponins, and terpenoids to treat various diseases. Researchers are interested in such compounds because of the beneficial impact of minimal toxic effects and no adverse reactions. The plant-based chemical derivatives could be used to develop medicines to suppress or kill parasites and fungal and bacterial pathogens [12]. *Dodonaea viscosa* is a widespread native species, which is simple to yield and exchange for a diversity of therapeutic agents. The selected plant *D. viscosa* has many medicinal uses such as antidiabetic, antimicrobial, insecticidal, antioxidant, cytotoxic, anti-diarrheal, antifertility, anti-spasmodic, wound, analgesic, anti-ulcer, anti-inflammatory, and detoxification effects. No such findings of adverse reactions or toxicity are reported in *D. viscosa*, although there are animal toxicological studies nor any clinical trial on humans to officially declare claims of effectiveness and safety [13]. This research intends to revise the potential anthelmintic and antimicrobial activities of *D. viscosa* in earthworm *Pheretima posthuma* and pathogenic bacteria species, respectively. The present study aims to explore the latent anthelmintic and antimicrobial activities of the extracts of *D. viscosa*, which will be useful in future research in the pharmaceutical and food industry.

## 2 Materials and methods

### 2.1 Sample collection

The leaves of *D. viscosa* were collected from Tiruchirappalli, Tamil Nadu, India. The collected plant was authenticated by Tamil Nadu Agricultural University at Coimbatore and recognized from the herbarium of the Botanical Survey of India, with specimen BSI/SRC/5/23/2016/Tech/12. Adult earthworms (*P. posthuma*) of equal size of around 6–7 cm were grabbed from humid soil from Munnar, Kerala. Worms used to study anthelmintic potential were pretreated with fresh saline to detach all fecal matters prior to the start of the study. For all the experimental procedures, the earthworms selected were 6–7 cm in length and 0.2–0.5 cm in width. Four widespread human pathogenic microbes were chosen, and some were very much related to oral wounds and diseases. The Gram-positive bacterial strains were *Bacillus subtilis* and *Staphylococcus aureus*, and Gram-negative bacterial strains were *Escherichia coli* and *Klebsiella pneumoniae* used for this analysis.

### 2.2 Preparation of plant extracts

The *D. viscosa* leaves were dried in the shade and ground to a coarse powder using a laboratory pulverizer. The dried powder was extracted with different solvents such
as water, ethanol, methanol, chloroform, and petroleum benzene for 48 h by using a soxhlet extractor according to the experimental protocol. The *D. viscosa* filtrate was taken into a Petri plate and dried in a hot air oven for 2 days at 50°C. The dried samples were collected into an Eppendorf tube and stored in desiccators for further procedure to estimate the anthelmintic potential and antimicrobial activities.

### 2.3 Anthelmintic activity

The anthelmintic potential of *D. viscosa* was studied by collecting Indian adult earthworms (*P. posthuma*) from a muggy area and rinsing with freshly prepared normal saline to eliminate all fecal matter and dust materials. The earthworm, approximately 5–7 cm in length and 0.3–0.5 cm in width were utilized for all the experiments. The concentrations of 12, 24, 36, and 48 mg · mL⁻¹ of all extracts were prepared for the bioassay, wherein the paralytic time and death rate are measured. Albendazole was used as a standard reference drug for positive control, and saline water was used as a negative control. The time taken to paralyze or individual death of worms was observed. The mean paralytic time was observed when there was no movement of any kind except when the worms were intensely shaken. The worms are observed that the shaken or given external stimuli, the paralyzing time was recorded [14]. Histopathology was performed on earthworms and the results were obtained by hematoxylin and eosin staining and the anatomy was observed through microscopical examination.

### 2.4 Antimicrobial activity

The Gram-positive bacteria *B. subtilis* and *S. aureus*, and Gram-negative bacteria *E. coli* and *K. pneumoniae* were screened for antibacterial activity with the extracts of *D. viscosa* (100 μg · mL⁻¹). Microorganisms were cultured in nutrient broth at 37°C; the concentration of approximately 10⁵–10⁶ CFU · mL⁻¹ was used for the antibacterial activity. Lawns of different species were prepared using sterile swabs, then labeled appropriately, and retained for a few minutes. Then, 100 μg of the extracted samples and the corresponding antibiotics were added to the respective tube and the plates were incubated at 37°C for 24 h. The individual zone diameters were measured to determine the zone of inhibition (ZOI). The antibacterial activity of *D. viscosa* extracts was performed against virulent oral bacterial species, and the ZOI was calculated using the agar diffusion method [15]. The activity was expressed against different concentrations of the extract across the well in terms of ZOI.

### 2.5 Preliminary chemical analysis

The phytochemical study of the different extracts of the leaves was performed on samples prepared by subsequent extraction. These solvent extracts were subjected to chemical analyses to identify various phytochemical substances such as alkaloids, saponins, phenolics, flavonoids, proteins, amino acids, carbohydrates, mucilage, and resins. Chemical testing of aqueous and ethanolic extracts was performed using the standard procedures for the identification of the phytochemicals [16–18]. Preliminary qualitative analysis was carried out on alkaloids, flavonoids, carbohydrates, phenols, tannins, gums, mucilage, fixed oils, fats, and saponins.

### 2.6 Ultraviolet (UV) and Fourier transform infrared spectroscopy (FTIR) analysis

The prepared plant extracts were subjected to the UV absorption range of 200–800 nm. The aqueous and ethanolic extracts of *D. viscosa* were used for FTIR analysis. To prepare a translucent sample tablet, 10 mg of dehydrated extract powder was mixed with 100 mg of KBr pellet. The prepared samples were analyzed by FTIR (Shimadzu, IR Affinity 1, Japan), at a scanning range of 400–4,000 cm⁻¹ with a 4 cm⁻¹ resolution to examine the stretching of chemical bonds.

### 2.7 Statistical analysis

Statistical comparisons were made using IBM SPSS Statistics version 19, by regression analysis. Differences at the *p* < 0.05 level were significant, and all data were represented as mean ± standard deviation (*n* = 3).

### 3 Results and discussion

In the evaluation of the anthelmintic property of *D. viscosa*, significant paralytic effect was produced by the higher extract concentration and the time to death was shorter for all *P. posthuma* organisms, as shown in Figures 1 and 2. For all types of extracts, aqueous extracts showed dose-
dependent anthelmintic activity and possessed the earliest paralytic time \((P)\) and a rapid worm death rate \((D)\) at 48 mg mL\(^{-1}\). The anthelmintic potential was measured for different plant extracts and compared with that of drug albendazole as the reference standard (Figure 3). The anthelmintic activities of aqueous, ethanolic, methanolic, chloroform, and petro benzene extracts were compared with that of the standard drug, as shown in Figure 3a–f. The anthelmintic activity of the extracts showed significance in controlling the parasitic disease caused by worms. Undeveloped forms of parasites invade humans through the GIT or skin and proceed to well-defined mature worms with tissue circulation. Anthelmintic drugs may work in the vicinity to drive worms out of the GIT or to eliminate systemically developed helminths or growth forms that attack tissues and organs [19].

Phytochemical examination of *Baliospermum montanum* crude extracts shows that tannins have significant anthelmintic activity [20]. Generally, tannins are chemically polyphenolic in nature. Some synthetic phenolic anthelmintic drugs, e.g., oxyclozanide, bithionol, and niclosamide, are exposed to block energy production of helminth parasites by disconnecting oxidative phosphorylation. It is probable that tannins have noteworthy effects of *D. viscosa* extracts. The potential anthelmintic effect of tannins may be connected to their binding with free proteins of the GIT of the test animal or glycoprotein of cuticle of the parasite [21]. It was observed that aqueous (Figure 3a), ethanolic (Figure 3b), methanolic (Figure 3c), chloroform (Figure 3d), and petro benzene (Figure 3e) extracts showed a notable anthelmintic property against earth worms. In addition to anthelmintic activity, synergistic active phytoconstituents like flavonoids, saponins, alkaloids, and tannins were present in the *D. viscosa* extracts [22]. In additional, the active principles of *D. viscosa* leaves chemical entities for their anthelmintic action to unlock the

![Figure 1](image1.png)

**Figure 1:** Paralytic activity of *P. posthuma* treated upon *D. viscosa*.

![Figure 2](image2.png)

**Figure 2:** Mortality rate of *P. posthuma* treated upon *D. viscosa*.
novel admission for innate anthelmintic. This evidence supports using *D. viscosa* as a wealthy resource of compounds with high beneficial properties for medications. The histopathological physiology of *P. posthuma* is shown in Figure 4. The histopathological analysis shows the significant anthelmintic activity against earthworms.

Earthworm paralysis in the anthelmintic activity was most responsive to the chloroform extract of *D. viscosa*, as shown in Figure 1. Figures 1 and 2 show the dose-dependent paralytic ranges from immobility to the absence of response to external stimuli, which finally led to death. The different solvent extracts have been quite effective in triggering the worms to die and inducing paralysis. Utmost earthworm expellers like albendazole leads to paralysis of the worms; thus, they are eliminated through the feces. The leaf extract has confirmed anthelmintic properties and destroyed the worms as well. The *D. viscosa* extract shows anthelmintic activity regardless of the involvement of polyphenolic compounds [23]. The extract of *D. viscosa* has shown wormicidal activity and indicates that it may be efficient against parasitic human infections.

The methanolic and ethanolic extracts of *D. viscosa* were screened for their effective antibacterial activity against two Gram-positive bacteria, such as *B. subtilis* and *S. aureus*, and two Gram-negative bacteria, such as *E. coli* and *K. pneumoniae*. Superior antibacterial activity was shown by the ethanolic and methanolic extracts of *D. viscosa* (Table 1). The methanolic and ethanolic extracts of *D. viscosa* showed noteworthy antibacterial activity against all the tested strains (Figures 5 and 6). Our results revealed that the extract has active constituents against other bacterial strains. The extract antimicrobial activity was evaluated by calculating the ZOI diameter shown by the extracts.

Plants’ antimicrobial efficiency is hypothetical due to flavonoids, essential oils, tannins, and phenolic compounds [24]. It is very exciting that even raw extracts
from these plants have achieved excellent quality action against multidrug-resistant strains when compared to modern antibiotic management, which was ineffective due to antibiotic resistance. In the earlier results, plant extracts are not effective in doses less than \(2 \times 10^5\) μg·mL\(^{-1}\) against Gram-negative bacteria [25]. The inhibition concentrations as low as 50 μg·mL\(^{-1}\) was recorded in this study. The different sensitivity of the microorganisms can be recognized according to their inherent properties linked to the permeability of their external cells to the extracts. The emergence of antibiotic-resistant pathogens in households and hospitals makes plants a remarkable therapeutical agent against the further rise in such microorganisms. The plant extracts exhibited various levels of antibacterial potential due to dissimilar chemical composition. Our findings revealed that \(K.\) pneumoniae, \(E.\) coli, \(P.\) aeruginosa, \(Proteus\) vulgaris, and \(S.\) aureus were mainly resistant to the extracts. The presence of an additional external membrane in the cell wall may explain this, which contributes to selective permeability of samples. The extracts can also suppress bacteria with active compounds that are able to act by blocking bacterial growth without actually reaching the bacterial cell itself [26]. Several researchers observed that the plant extracts showing antibacterial activities have phenols and flavonoids. We therefore presume that the antibacterial activities of these metabolites are evident of a significant therapeutical agent. Flavonoids are known as plant-synthesized hydroxylated phenolic compounds and are classified as antimicrobial substances associated with a wide range of microorganisms \textit{in vitro}. Their activity is conceivable because of their versatility with extracellular and soluble proteins and their complexities with the bacterial cells [27,28]. Since \(D.\) viscosa has a rich amount of phytoconstituents like flavonoids, phenols, alkaloids, and tannins, which were confirmed by

<table>
<thead>
<tr>
<th>Material</th>
<th>Extracts</th>
<th>Concentration (μg·mL(^{-1}))</th>
<th>BS</th>
<th>EC</th>
<th>KP</th>
<th>SA</th>
</tr>
</thead>
<tbody>
<tr>
<td>(D.) viscosa</td>
<td>Ethanol</td>
<td>50</td>
<td>10.1</td>
<td>9.4</td>
<td>11.3</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>15.3</td>
<td>8.4</td>
<td>7.6</td>
<td>11.3</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>50</td>
<td>12.3</td>
<td>8.6</td>
<td>10.1</td>
<td>11.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>12.9</td>
<td>10.4</td>
<td>11.6</td>
<td>10.7</td>
</tr>
</tbody>
</table>

EC: \(E.\) coli, KP: \(K.\) pneumonia, BS: \(B.\) subtilis, SA: \(S.\) aureus.
phytochemical screening and FTIR analysis, these results support that *D. viscosa* has antimicrobial activity when interpreted with previous studies. The high bacterial population may enhance the living rates of the helminths in the stomach. This could be a significant finding of this study; *D. viscosa* plant extracts control the bacterial population as it is able to control the stomach worms.

Qualitative phytochemical analyses for alkaloids, carbohydrates, tannins, flavonoids, phenols, saponins, proteins, and steroids are performed in the ethanolic extracts of *D. viscosa*. The presence of alkaloids, phenols, flavonoids, tannins, steroids, and saponins in alcoholic extracts is found by screening the extracts. This plant growing under normal conditions possesses a range of secondary metabolites such as flavonoids, tannins, phenols, coumarins, quinones, alkaloids, glycosides, essential oils, etc.; many researchers have emphasized the importance of these compounds as potential microbial agents against pathogens [16]. The preface phytochemical analysis revealed that these phytochemicals are mostly present in the alcoholic extracts. The plant material was found to have the necessary vital phytochemicals and additional nutritive compounds required by the pharmaceutical industries and food supplements [29,30]. Therapeutical plants comprise the foremost constituents of the majority of native medicines and a hefty number of allopathic medical preparations.

Table 2: FTIR of methanolic extract of *D. viscosa*

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>Characteristic absorption (cm(^{-1}))</th>
<th>Functional groups</th>
<th>Types of vibration</th>
<th>Intensity</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>3434.57</td>
<td>Ketone</td>
<td>Stretch</td>
<td>Medium (12.00783)</td>
</tr>
<tr>
<td>2</td>
<td>3905.03</td>
<td>OH (alcohol)</td>
<td>stretch, free</td>
<td>Strong</td>
</tr>
<tr>
<td>3</td>
<td>2363.21</td>
<td>–C≡C– (alkyne)</td>
<td>Stretch</td>
<td>Variable, not present in symmetrical alkynes</td>
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<tr>
<td>4</td>
<td>2338.14</td>
<td>–C≡C– (alkyne)</td>
<td>Stretch</td>
<td>Variable, not present in symmetrical alkynes</td>
</tr>
<tr>
<td>5</td>
<td>2075.05</td>
<td>–C≡C– (alkyne)</td>
<td>Stretch</td>
<td>Variable, not present in symmetrical alkynes</td>
</tr>
<tr>
<td>6</td>
<td>1637.76</td>
<td>(C≡C) (alkene)</td>
<td>Stretch bending</td>
<td>Variable (0.05785)</td>
</tr>
<tr>
<td>7</td>
<td>672.94</td>
<td>===C–H (alkene)</td>
<td>Bending</td>
<td>Strong (8.252915)</td>
</tr>
</tbody>
</table>

Table 3: FTIR of ethanolic extract of *D. viscosa*

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>Characteristic absorption (cm(^{-1}))</th>
<th>Functional groups</th>
<th>Types of vibration</th>
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<tr>
<td>3</td>
<td>3906.63</td>
<td>OH (alcohol)</td>
<td>stretch, free</td>
<td>Strong</td>
</tr>
<tr>
<td>4</td>
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<td>Stretch</td>
<td>Variable, not present in symmetrical alkynes</td>
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<tr>
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<td>Stretch</td>
<td>Variable, not present in symmetrical alkynes</td>
</tr>
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<td>(C≡C) (alkene)</td>
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<td>1116.98</td>
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<td>Stretch</td>
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<tr>
<td>8</td>
<td>671.14</td>
<td>===C–H (alkene)</td>
<td>Bending</td>
<td>Strong (8.252915)</td>
</tr>
</tbody>
</table>
Figure 9: FTIR spectrum of ethanolic extract of *D. viscosa*.

Figure 10: FTIR spectrum of methanolic extract of *D. viscosa*. 
hold one or more components of plant origin. Beneficial plants possess organic compounds that provide a particular effect on cell biology and taking consideration of the presence of bioactive compounds. Knowledge of plant chemical substances is desirable as these data would be useful developing chemical composites [31].

The UV spectrum of D. viscosa in the selected solvent is shown in Figures 7 and 8. As a result of the FTIR spectrum, we recognize the functional group of active components based on the peak value area. The results of FTIR peak values with their functional groups are denoted in Tables 2 and 3. The results of FTIR analysis confirmed the presence of phenol, cycloalkane, alkene, and aromatic compounds (Figures 9 and 10). FTIR analysis of the leaf powder of D. viscosa revealed the presence of diverse functional groups. Comparative anthelmintic activity of different herbal and synthetic drugs is shown in Table 4. Consequently, the FTIR analysis of D. viscosa exhibited unique phytochemical markers as a helpful analytical contrivance not just to ensure the powder quality but also to discover the plant therapeutic importance [32]. The study has significant anthelmintic activity against the stomach worm model of P. posthuma treated with D. viscosa extracts.

4 Conclusion

In conclusion, D. viscosa has phytocompounds that demonstrated potential and significant anthelmintic and antimicrobial activities. The upshots of our revision divulged greater anthelmintic and antimicrobial activities with the ethanolic extract of D. viscosa. These results sustain the idea that a diet rich in plants and herbs probably will decrease helminths and microbial growth and be defensive against associated disorders. Additional studies are essential to segregate and disclose the active compounds in the extracts of leaves of D. viscosa liable for action and to institute the mechanism of action so that the common public can benefit from this vital therapeutical plant.

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Conflict of interest: Authors state no conflict of interest.
Data availability statement: Data and materials used in this manuscript are available duly request from the audience.

References


