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Research Article

## Evaluation of In-Vitro Anthelmintic Potential of *Nyctanthes Arbor-Tristis* Linn Leaves and Its Phytochemical Correlation

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### ABSTRACT

The present study investigated the in-vitro anthelmintic potential of *Nyctanthes arbor-tristis* Linn leaves and explored its correlation with phytochemical constituents. Leaf powder was extracted using [solvent, e.g., methanol/ethanol/aqueous], and preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, and phenolic compounds. The anthelmintic activity was evaluated using *Pheretima posthuma* as the test model, with Piperazine citrate as the standard comparator. Results demonstrated dose-dependent paralysis and mortality of worms, with higher concentrations producing effects comparable to the standard drug. The observed anthelmintic activity is likely attributable to the bioactive phytoconstituents present in the leaf extract. These findings support the traditional use of *Nyctanthes arbor-tristis* in treating helminthic infections and provide a basis for further studies aimed at isolating and characterizing its active compounds for potential therapeutic applications.

**Keywords:** *Nyctanthes arbor-tristis*, Anthelmintic activity, *Pheretima posthuma*, In-vitro study Phytochemical screening.**ARTICLE INFO:** Received 11 Feb 2025; Review Complete 05 April 2025; Accepted 18 August 2025. ; Available online 15 Oct. 2025**Cite this article as:**Chandrakant S, Choudhury P.K, Sharma M, Evaluation of In-Vitro Anthelmintic Potential of *Nyctanthes Arbor-Tristis* Linn Leaves And Its Phytochemical Correlation, Asian Journal of Pharmaceutical Research and Development. 2025; 13(5):01-06, DOI: <http://dx.doi.org/10.22270/ajprd.v13i5.1616>

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### INTRODUCTION

Helminth infections, caused by parasitic worms, are a major public health problem worldwide, particularly in tropical and subtropical regions. These infections can lead to malnutrition, anemia, stunted growth, and impaired cognitive development in humans, as well as significant economic losses in livestock due to reduced productivity. Conventional anthelmintic drugs, such as albendazole, mebendazole, and piperazine citrate, are commonly used for treatment. However, the increasing incidence of drug resistance, side effects, and the high cost of synthetic drugs have highlighted the need for alternative, safe, and effective therapies<sup>1</sup>.

Medicinal plants have been widely recognized in traditional medicine systems for their therapeutic potential against various diseases, including parasitic infections. Phytochemicals such as alkaloids, flavonoids, tannins, saponins, and phenolic compounds are known to exhibit

anthelmintic activity through mechanisms such as paralysis of worms, inhibition of nutrient absorption, or disruption of their metabolic processes<sup>2</sup>.

*Nyctanthes arbor-tristis* Linn (family: Oleaceae), commonly known as "Night-flowering Jasmine," has been traditionally used in Ayurvedic and folk medicine to treat fever, inflammation, skin diseases, and parasitic infections. Several studies have reported that leaves of *N. arbor-tristis* contain bioactive compounds with antimicrobial, antioxidant, anti-inflammatory, and anthelmintic properties. Despite its traditional use, there is limited scientific evidence documenting the in-vitro anthelmintic activity of this plant and its correlation with specific phytochemical constituents<sup>3</sup>.

This study aims to evaluate the in-vitro anthelmintic potential of *Nyctanthes arbor-tristis* leaf extract using *Pheretima posthuma* as a model organism and to correlate the observed activity with the presence of bioactive phytochemicals. The findings of this research are expected to provide a scientific

basis for the ethnomedicinal use of *N. arbor-tristis* and may contribute to the development of novel, plant-based anthelmintic agents<sup>4</sup>.

### Plant Profile

*Nyctanthes arbor-tristis* Linn, commonly known as Harsinghar or Night Jasmine, derives its botanical name from *Nyctanthes* meaning “night-flowering” and *arbor-tristis* meaning “the sad tree,” referring to the fading of its bright flowers during the day<sup>5</sup>.



**Figure 1:** Leaf of *Nyctanthes arbor-tristis* Linn



**Figure 2:** Plant of *Nyctanthes arbor-tristis*

### Vernacular Names

| Language  | Name                    |
|-----------|-------------------------|
| Sanskrit  | Parijata                |
| English   | Night Jasmine           |
| Hindi     | Harsinghar              |
| Bengali   | Sephalika               |
| Malayalam | Parijatakam             |
| Kannada   | Parijata                |
| Gujarati  | Jayaparvati, Pagdamalle |
| Oriya     | Gangasiuli              |

### Geographical Source

*Nyctanthes arbor-tristis* is native to India, predominantly found in the Himalayan region from Chhattisgarh to Nepal, Assam, Bengal, Central India (Chhattisgarh, Rajasthan, Madhya Pradesh), and extending southwards to the Godavari region<sup>6</sup>.

### Taxonomical Classification

| Rank     | Classification       |
|----------|----------------------|
| Kingdom  | Plantae              |
| Division | Magnoliophyta        |
| Class    | Magnoliopsida        |
| Order    | Lamiales             |
| Family   | Oleaceae             |
| Genus    | <i>Nyctanthes</i>    |
| Species  | <i>arbor-tristis</i> |

### Botanical Description

*Nyctanthes arbor-tristis* is a shrub or small tree, reaching up to 10 m in height, with gray or greenish rough bark and stiff whitish hairs. Young branches are sharply quadrangular<sup>7</sup>.

- Leaves:** Opposite, 2.5–6.3 cm, ovate, acute or acuminate, entire or with few large distant teeth; short bulbous hairs; main nerves few, conspicuous beneath; petiole ~6 cm, hairy<sup>8</sup>.

2. **Flowers:** Small, fragrant, sessile in pedunculate bracteate fascicles of 3–5; peduncles 4-angled, slender, hairy, axillary, and solitary. Bracts broadly ovate or suborbicular, 6–10 mm, apiculate, hairy on both sides. Calyx 6–8 mm, narrowly campanulate, hairy outside, glabrous inside. Corolla glabrous, >13 mm; tube 6–8 mm; limb equal; lobes white, unequal; color orange<sup>9</sup>.
3. **Fruit:** Capsular, 1–2 cm diameter, obcordate or nearly orbicular, compressed, 2-celled, separating into 2 flat, 1-seeded carpels, reticularly veined, glabrous<sup>10</sup>.
4. **Seeds:** Exalbuminous; testa thick, outer layer with large transparent cells and heavily vascularized<sup>11</sup>.

### Collection of Plant Material

Fresh leaves of *N. arbor-tristis* were collected from Gandhwari, Madhubani, Bihar, in February. Approximately 1 kg of fresh leaves was collected, cleaned to remove unwanted materials, shade-dried, and homogenized to obtain a coarse powder. After slicing and powdering, 82 g of leaf powder was obtained and stored in an airtight container for subsequent solvent extraction<sup>12</sup>.

### Authentication of Plant

The plant material was authenticated by a botanist at the Regional Plant Resource Centre (R.P.R.C.), Bhubaneswar. A herbarium specimen and authentication certificate were deposited at the Department of Pharmacognosy, I.G.I.P.S., Bhubaneswar<sup>13</sup>.

### Pharmacognostical Investigation

Pharmacognostical studies are essential for confirming the identity, quality, and purity of a plant drug, as well as for detecting any adulteration. In the present study, the leaves of *Nyctanthes arbor-tristis* Linn were subjected to detailed morphological, microscopical, and physicochemical analyses<sup>14</sup>.

### Morphological Study

- a) **Colour:** Greenish brown
- b) **Surface:** Glabrous
- c) **Odour:** Characteristic
- d) **Taste:** Bitter
- e) **Nature:** Oily

### Microscopical Study

#### (i) Transverse Section (T.S.)

Fresh leaves were washed and treated with a solution of chloral hydrate in water, then boiled in a water bath for 45 minutes. The softened leaves were examined under a microscope to study the internal cellular structure<sup>15</sup>.

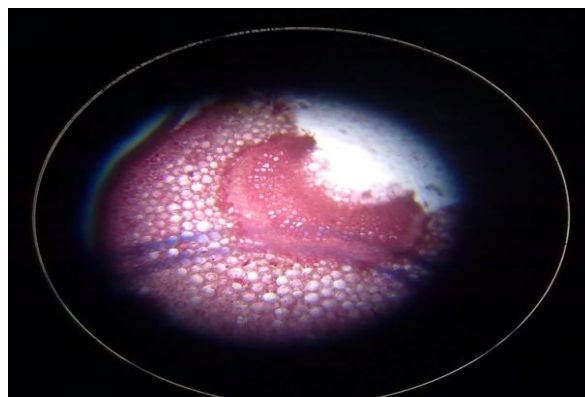


Figure 3: Transverse section of leaf

#### (ii) Powder Microscopy

A small quantity of powdered leaf was boiled with chloral hydrate for 15–20 minutes, filtered, partially dried, and treated with a staining solution of phloroglucinol and glycerine. The mixture was spread on a glass slide, and microscopical observations were performed to identify characteristic cell types and structures<sup>16</sup>.

#### Determination of Ash Value

Approximately 2 g of air-dried powdered leaf was incinerated in a tarred silica crucible by gradually increasing the temperature until carbon-free ash was obtained<sup>17</sup>. The crucible was cooled and weighed repeatedly until a constant weight was achieved. The total ash content was calculated with reference to the air-dried material and found to be 9.5% w/w.

#### Determination of Extractive Value

Five grams of air-dried coarse leaf powder was macerated with 100 mL of solvent for 24 hours, with intermittent shaking during the first 6 hours<sup>18</sup>. After filtration, 25 mL of filtrate was evaporated to dryness at 105°C. The percentage of extractive value in various solvents is shown below:

Table 2: Extractive Value of *N. arbor-tristis* Leaves

| S.No | Extract Name  | Wt. of Powder (g) | Wt. of Extract (g) | % Yield |
|------|---------------|-------------------|--------------------|---------|
| 1    | Ethyl alcohol | 5                 | 0.56               | 44.8    |
| 2    | Ether         | 5                 | 0.04               | 3.2     |
| 3    | Water         | 5                 | 0.42               | 32      |
| 4    | Chloroform    | 5                 | 0.06               | 4.2     |

### Preparation of Extracts

Shade-dried leaves (82 g) were successively extracted with petroleum ether (94 h 35 min), chloroform (15 h 6 min) by

reflux, and finally with hydro-alcoholic solvent (ethanol:water 60:40, 16 h 22 min)<sup>19</sup>. The extracts were filtered, concentrated, and dried. Fractionation and distillation were performed to minimize solvent loss<sup>20</sup>.

**Table 3:** Yield of Extracts from 82 g Leaf Powder

| S.No | Extract         | Yield (g) |
|------|-----------------|-----------|
| 1    | Petroleum ether | 2.36      |
| 2    | Chloroform      | 2.14      |
| 3    | Hydro-alcoholic | 20.42     |

## Phytochemical Screening

### Qualitative Chemical Analysis

Phytochemical screening was conducted to identify the bioactive constituents responsible for the pharmacological activity of the leaf extracts.

**Alkaloids:** Detected using Mayer's, Dragendorff's, Wagner's, and Hager's tests.

**Carbohydrates:** Identified by Molisch's test, indicated by the formation of a violet ring.

**Flavonoids:** Detected by the alkaline reagent test; yellow coloration turning colorless with acid indicates flavonoids.

**Cardiac Glycosides:** Detected by Keller-Killiani and Baljet tests; reddish-brown or orange coloration indicates presence.

**Steroids and Triterpenoids:** Libermann-Burchard test; formation of brown or deep red coloration indicates presence.

**Tannins:** Lead acetate test and catechin test; formation of precipitate or pink coloration indicates presence<sup>21</sup>.

**Table 4:** Qualitative Identification of Phytoconstituents

| S.No | Test                   | Petroleum Ether Ext. | Chloroform Ext. | Hydro-Alcoholic Ext. |
|------|------------------------|----------------------|-----------------|----------------------|
| 1    | Alkaloids              | +                    | –               | +                    |
| 2    | Carbohydrates          | –                    | +               | –                    |
| 3    | Flavonoids             | –                    | +               | –                    |
| 4    | Cardiac Glycoside      | –                    | –               | –                    |
| 5    | Tannins                | +                    | –               | +                    |
| 6    | Steroids/Triterpenoids | +                    | –               | –                    |

### Thin Layer Chromatography (TLC)

TLC was performed by applying the extract as a spot on a coated plate, which was developed in a suitable solvent system<sup>22</sup>. The Rf value was calculated as:

$$R_f = \text{Distance travelled by spot} / \text{distance travelled by solvent front}$$

**Table 5:** TLC Profile of Extracts

| S.No | Solvent System                          | Extracts                                     | Rf Value                                       |
|------|-----------------------------------------|----------------------------------------------|------------------------------------------------|
| 1    | Ethyl acetate:Methanol:Water (60:14:10) | Petroleum Ether, Hydro-alcoholic, Chloroform | 0.76, 0.92; 0.24, 0.68; 0.40, 0.59, 0.72, 0.93 |
| 2    | Toluene:Ethyl Acetate (40:60)           | Chloroform                                   | 0.98, 0.90, 0.72, 0.38                         |

**Detection Agents:** Vanillin-sulphuric acid spray, iodine chamber.

## PHARMACOLOGICAL STUDY

### Introduction to In-Vitro Anthelmintic Activity

Gastrointestinal parasitic infections are a major concern for livestock production, particularly in developing countries. Despite the development of resistance in economically important helminths, chemotherapy remains the most commonly used approach to control these infections. Helminthic infections pose significant social and economic challenges in many regions, affecting both human and animal health. Certain infections, such as filariasis, have limited therapeutic options<sup>23</sup>.

Continuous reliance on a narrow range of anthelmintic drugs has led to the emergence of drug-resistant helminth strains. Commonly used drugs, such as albendazole and mebendazole, may induce adverse effects including

gastrointestinal disturbances (epigastric pain, diarrhea, nausea, vomiting), nervous system symptoms (headache, dizziness), and allergic reactions (edema, rashes, urticaria). Additionally, some anthelmintic agents like praziquantel and albendazole are contraindicated in pregnant or lactating women and require caution in hepatitis patients and children under two years<sup>24</sup>.

*Nyctanthes arbor-tristis* Linn (Oleaceae) is a large shrub or small tree traditionally used as a bitter tonic, anti-inflammatory, digestive, laxative, febrifuge, expectorant, diuretic, and sedative. It is also reported for use in rheumatism, dyspepsia, skin diseases, bronchitis, asthma, cough, and as an anthelmintic. The present study aims to explore the potential of *N. arbor-tristis* leaf extracts as a natural anthelmintic agent with reduced side effects<sup>25</sup>.

## MATERIALS AND METHODS

### (i) Plant Extracts

Leaf extracts of *Nyctanthes arbor-tristis* were prepared using the following solvents:

- Petroleum ether
- Chloroform
- Hydro-alcoholic (Ethanol:Water 60:40)

### (ii) Test Organism

Indian adult earthworms (*Pheretima posthuma*) measuring 6–7 cm in length and 0.1–0.2 cm in width were collected from moist soil. The worms were washed with normal saline to remove adhering soil and debris. *Pheretima posthuma* is anatomically and physiologically similar to human intestinal roundworms, making it a suitable model for in-vitro anthelmintic studies<sup>26-28</sup>.

### (iii) In-Vitro Anthelmintic Assay

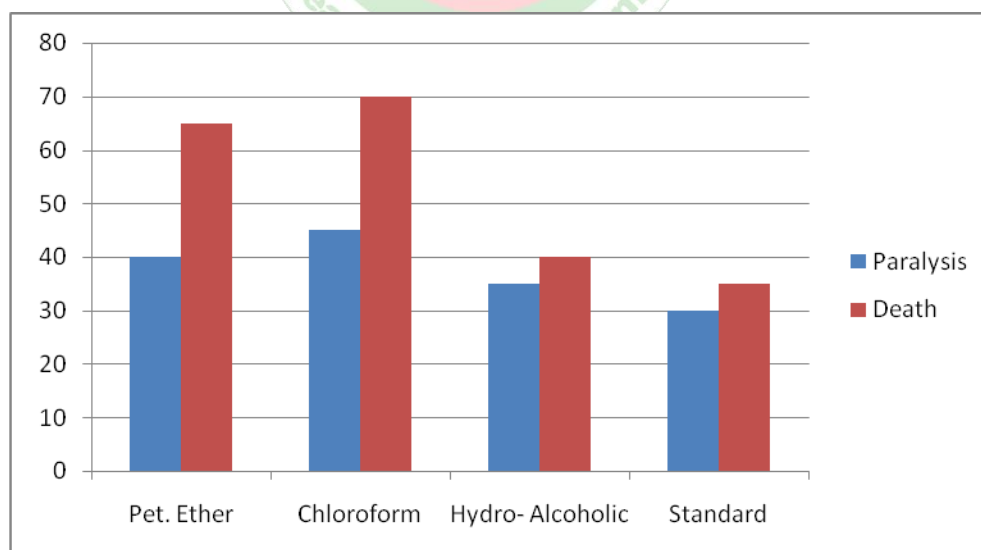
The earthworms were divided into ten groups of six worms each. Extracts and the standard drug albendazole were freshly prepared in normal saline. Different concentrations (25, 35, and 50 mg/mL) of each extract were tested. Distilled water served as the control<sup>29-30</sup>.

Each group of worms was exposed to 10 mL of the test solutions in separate Petri dishes. Observations were made for:

- Time to Paralysis:** Recorded when worms showed no movement, except upon vigorous shaking.
- Time to Death:** Concluded when worms lost all motility and did not respond when immersed in warm water (50°C), accompanied by fading of body color<sup>31</sup>.

**Table 6:** In-Vitro Anthelmintic Activity of *Nyctanthes arbor-tristis* Leaf Extracts

| S.No | Treatment                 | Concentration (mg/mL) | Paralysis Time (min) | Death Time (min) |
|------|---------------------------|-----------------------|----------------------|------------------|
| 1    | Petroleum ether extract   | 25                    | 55                   | 100              |
|      |                           | 35                    | 50                   | 80               |
|      |                           | 50                    | 40                   | 65               |
| 2    | Chloroform extract        | 25                    | 52                   | 92               |
|      |                           | 35                    | 48                   | 79               |
|      |                           | 50                    | 42                   | 70               |
| 3    | Hydro-alcoholic extract   | 25                    | 45                   | 50               |
|      |                           | 35                    | 37                   | 45               |
|      |                           | 50                    | 35                   | 40               |
| 4    | Standard (Albendazole)    | 25                    | 40                   | 45               |
|      |                           | 35                    | 33                   | 40               |
|      |                           | 50                    | 30                   | 35               |
|      | Control (Distilled water) | --                    | --                   | --               |



**Figure 4:** Comparative in-vitro anthelmintic activity of *N. arbor-tristis* extracts and standard drug (vertical values represent time in minutes for paralysis and death).

## RESULTS AND DISCUSSION

Preliminary phytochemical screening of the leaves of *Nyctanthes arbor-tristis* Linn revealed the presence of several bioactive constituents, including alkaloids, flavonoids, amino acids, steroids, carbohydrates,

triterpenoids, and tannins, in petroleum ether, chloroform, and hydro-alcoholic extracts. These phytoconstituents are likely responsible for the observed anthelmintic activity.

Among the tested extracts, the **hydro-alcoholic leaf extract** demonstrated the most potent anthelmintic effect.

Comparative observations indicated that all extracts of *N. arbor-tristis* exhibited significant activity against *Pheretima posthuma*, when compared with the standard reference drug, **albendazole**, at varying concentrations.

a. **Petroleum ether extract** (50 mg/mL) induced paralysis at 40 minutes and death at 65 minutes.

b. **Chloroform extract** (50 mg/mL) caused paralysis at 42 minutes and death at 70 minutes.

c. **Hydro-alcoholic extract** (50 mg/mL) exhibited paralysis at 35 minutes and death at 40 minutes.

In comparison, **albendazole** at the same concentration (50 mg/mL) caused paralysis at 30 minutes and death at 35 minutes.

The results indicate that the hydro-alcoholic extract of *N. arbor-tristis* exhibits **remarkable anthelmintic activity**, with paralysis and death times closely resembling those of albendazole. This suggests that the active constituents in the hydro-alcoholic extract may exert strong anthelmintic effects.

Future studies could focus on the **isolation and characterization of the bioactive phytoconstituents** responsible for this activity, as well as elucidating the underlying mechanisms of action. Such investigations may contribute to the development of novel, plant-derived anthelmintic agents with fewer side effects compared to conventional drugs.

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