

# Exploring The Antibacterial Efficacy of *Nyctanthes Arbor-tristis*: Phytochemical Insights and Activity Against *Xanthomonas Campestris*

Prof. Manoj Kumar<sup>1</sup> & Dr. Vinay Bhushan Kumar<sup>2\*</sup>

<sup>1</sup>Department of Botany, College of Commerce, Arts and Science, Patliputra University, Patna

<sup>2</sup>Department of Botany, T.P.S. College, Patliputra University, Patna, Bihar

## ABSTRACT

*Nyctanthes arbor-tristis*, commonly known as night-flowering jasmine or parijat, is a medicinal plant widely valued in traditional healing systems such as Ayurveda and folk medicine. With the growing challenge of antimicrobial resistance and increasing prevalence of infectious diseases, there is renewed interest in plant-based therapeutics. This study evaluates the antibacterial and antifungal potential of *Nyctanthes arbor-tristis* leaves, highlighting their scientific relevance alongside traditional uses. Leaf extracts prepared using ethanol and methanol were tested against the pathogenic microorganism *Xanthomonas campestris* using the disc diffusion method, using the disc diffusion method. Among the extracts, the ethanol extract demonstrated the strongest antibacterial activity against all tested organisms, indicating its superior efficacy compared to methanol and chloroform extracts. Additionally, antifungal activity was observed against *Penicillium expansum*, further supporting the plant's broad-spectrum antimicrobial properties. Phytochemical investigations reveal that *Nyctanthes arbor-tristis* contains bioactive compounds such as flavonoids, alkaloids, terpenes, phenols, steroids, and iridoid glycosides. These constituents contribute to a wide range of pharmacological activities, including anti-inflammatory, antioxidant, antidiabetic, hepatoprotective, immunomodulatory, and wound healing effects. Traditionally, different parts of the plant—leaves, bark, roots, seeds, and flowers—have been used to treat fever, arthritis, liver disorders, parasitic infections, and skin conditions. Despite its extensive traditional use and promising pharmacological profile, scientific data on its mechanisms of action remain limited. Therefore, further research is necessary to validate its therapeutic efficacy, isolate active compounds, and understand their biological pathways. Overall, *Nyctanthes arbor-tristis* represents a valuable source of natural medicine with potential for developing effective, low-side-effect treatments in modern healthcare.

**Keywords:** *Traditionally, Nyctanthes arbor-tristis, Therapeutic agents, Metabolites, Phytoconstituents*

## Introduction:

The use of plants for medicinal purposes dates back to the earliest stages of human civilization. Ancient healthcare systems in regions such as Egypt, Mesopotamia, Greece, India, and China relied extensively on plant-based remedies, and this traditional knowledge has been preserved through generations (Lemonnier et al., 2017; Thomas et al., 2020). Medicinal plants are derived from different parts of flora, including leaves, stems, roots, flowers, seeds, and bark, and have been widely utilized in traditional medical systems for the prevention and treatment of diseases (Kala, 2017). These plants are

rich in biologically active compounds such as alkaloids, flavonoids, glycosides, tannins, saponins, and terpenoids, which contribute significantly to their pharmacological properties (Riaz et al., 2023). Due to their natural origin, affordability, and relatively fewer side effects compared to synthetic drugs, medicinal plants play a crucial role in primary healthcare, especially in rural and economically disadvantaged regions (Singhal, 2005). It is estimated that approximately 80% of the population in developing countries depends on traditional medicine for their healthcare needs, a fact acknowledged by the World Health Organization.

Medicinal plants are also recognized as valuable sources of antimicrobial agents. Their bioactive compounds exhibit activity against a wide spectrum of microorganisms, including bacteria, fungi, and viruses. Different plant parts may be used either individually or in combination to achieve therapeutic effects. To assess their antimicrobial potential, plant materials are often subjected to extraction using solvents of varying polarity, which helps isolate different groups of phytochemicals. The choice of solvent plays a critical role in determining the efficiency of extraction and the nature of compounds obtained.

In recent decades, the increasing and often indiscriminate use of conventional antimicrobial drugs has led to the emergence of resistant microbial strains. Microbial resistance is frequently associated with genetic factors, including the presence of resistance genes within bacterial DNA. As a result, many pathogenic microorganisms have developed the ability to withstand commonly used antibiotics, posing a significant challenge to global healthcare systems. This growing issue has intensified the search for alternative therapeutic agents, particularly from natural sources. Medicinal plants, with their diverse chemical constituents and multiple mechanisms of action, offer promising alternatives for combating microbial resistance and controlling infectious diseases.

*Nyctanthes arbor-tristis* is a small ornamental tree that typically grows between 10 and 30 feet in height. It has simple, opposite leaves measuring approximately 6–12 cm in length and 2–6.5 cm in width, with an entire margin. The plant is often referred to as the “Tree of Sorrow” due to the characteristic shedding of its flowers during the night. In addition to its ornamental value, the plant is deeply rooted in traditional medicine and is mentioned in ancient texts such as the Vedas, highlighting its historical significance.

Various parts of *Nyctanthes arbor-tristis*, including leaves, flowers, bark, seeds, and roots, have been extensively used in traditional medical systems such as Ayurveda, Unani, and Siddha. These

plant parts are utilized for the treatment of a wide range of ailments, including fever, arthritis, sciatica, malaria, liver disorders, and parasitic infections (Parekh & Soni, 2020). The leaves, in particular, are widely used in home remedies and herbal formulations for managing pain, inflammation, and infectious diseases.

Phytochemical investigations have revealed that *Nyctanthes arbor-tristis* contains a variety of bioactive compounds that contribute to its medicinal properties. These include D-mannitol,  $\beta$ -sitosterol, flavonoid glycosides such as nicotiflorin, oleanolic acid, tannic acid, ascorbic acid, methyl salicylate, carotene, glucose, and benzoic acid. Additionally, iridoid glycosides such as arbortristoside A and B have been identified as important constituents responsible for several pharmacological effects. These compounds are known to exhibit significant biological activities, including antioxidant, anti-inflammatory, and hepatoprotective effects.

Modern pharmacological studies have validated many of the traditional uses of *Nyctanthes arbor-tristis*. Both in vitro and in vivo investigations have demonstrated that the plant possesses a wide range of biological activities, including antibacterial, antifungal, antiviral, anti-inflammatory, antipyretic, analgesic, antioxidant, antimalarial, antihelminthic, and hepatoprotective effects (Parekh & Soni, 2020; Thapa & Kumar, 2024). These diverse pharmacological properties are primarily attributed to the presence of various phytoconstituents that act synergistically to produce therapeutic effects.

In addition to its therapeutic importance, *Nyctanthes arbor-tristis* represents a valuable source of chemically diverse compounds that can be developed into novel drugs (Vijayalakshmi & Ravindran, 2012). The plant has been widely studied for its antimicrobial potential, and several reports have highlighted its effectiveness against pathogenic microorganisms. Plant-derived compounds are known to exert antimicrobial effects through different mechanisms, such as disrupting microbial cell membranes, inhibiting enzyme activity, and interfering with genetic material. These mechanisms

make plant-based antimicrobials particularly valuable in overcoming drug resistance.

Fungal contamination is another significant concern in both agriculture and food industries. Fungi can cause spoilage, reduce crop yield, and produce harmful mycotoxins that pose serious health risks. Common fungal genera such as *Aspergillus*, *Fusarium*, and *Penicillium* are known to affect food quality and safety (Bhatnagar & McCormick, 1988; Terzi et al., 2014). Although chemical fungicides are widely used to control fungal infections, their excessive use can lead to environmental pollution and health hazards. Therefore, there is a growing interest in identifying natural antifungal agents from plant sources that are safer and environmentally friendly.

In this context, the present study focuses on evaluating the antimicrobial potential of *Nyctanthes arbor-tristis* leaves collected from different geographical locations. The study specifically investigates the antibacterial activity of the plant against *Xanthomonas campestris*, a significant plant pathogenic bacterium known to cause various agricultural diseases. The objective is to assess the effectiveness of plant extracts and to explore their potential application as natural antimicrobial agents. Overall, the integration of traditional knowledge with modern scientific approaches offers a promising pathway for the development of novel, effective, and safer therapeutic agents. *Nyctanthes arbor-tristis*, with its rich phytochemical composition and diverse pharmacological properties, holds significant potential in this regard. However, further research is required to fully understand its mechanisms of action, standardize its extracts, and validate its clinical applications.

#### **Methodology :**

**Plant Material Collection:** Fresh leaves of *Nyctanthes arbor-tristis* were collected from different locations during the study period. The plant material was identified and cleaned thoroughly to remove dust and other contaminants. Only healthy, mature leaves were selected for further experimental procedures.

**Preparation of Plant Extracts :** The collected leaves were washed with distilled water and dried

under shade at room temperature to preserve their active constituents. After complete drying, the leaves were finely powdered using a mechanical grinder. Approximately 25 g of the powdered material was subjected to extraction using ethanol and methanol as solvents through a Soxhlet extraction process. The extraction was carried out at controlled temperatures below the boiling point of the respective solvents for efficient recovery of phytochemicals. The obtained extracts were filtered using Whatman No. 1 filter paper and then concentrated under reduced pressure using a rotary evaporator. The semi-solid residues were further dried to obtain crude extracts. The dried extracts were stored in sterile containers at low temperature until further use. The concentration of each extract was prepared in mg/ml for antimicrobial testing.

#### **Qualitative Phytochemical Analysis**

Preliminary phytochemical screening of *Nyctanthes arbor-tristis* leaf extracts was carried out to identify the presence of important bioactive compounds such as anthraquinones, terpenoids, saponins, flavonoids, and glycosides using standard qualitative procedures.

**Test for Anthraquinones:** Approximately 1 ml of the plant extract was mixed with 2 ml of concentrated sulfuric acid and gently heated. The mixture was filtered while hot, and the filtrate was subsequently shaken with 2 ml of chloroform. The chloroform layer was separated and treated with ammonia solution. The appearance of a blue, violet, or reddish coloration confirmed the presence of anthraquinones.

**Test for Terpenoids:** For detection of terpenoids, 1 ml of the extract was mixed with 4 ml of chloroform. Carefully, concentrated sulfuric acid was added along the side of the test tube to form a distinct layer. The development of a reddish-brown interface indicated the presence of terpenoid compounds.

**Test for Saponins :** A small quantity (0.5 ml) of the extract was diluted with 2 ml of distilled water and shaken vigorously. Persistent frothing or foam formation was observed. The addition of a few drops

of oil followed by further shaking led to stable emulsion formation, confirming the presence of saponins.

**Test for Glycosides :** In this test, a small volume of extract was treated with chloroform, followed by the careful addition of concentrated sulfuric acid. The appearance of a reddish-brown coloration at the interface suggested the presence of glycosides, while a greenish or yellowish color indicated steroidal components. These qualitative tests confirmed the presence of various phytochemicals in *Nyctanthes arbor-tristis*, which may contribute to its observed antibacterial activity.

**Test Microorganism and Culture Conditions:**

The antibacterial activity of the plant extracts was evaluated against *Xanthomonas campestris*, a plant pathogenic bacterium of significant agricultural importance. The bacterial culture was obtained from a recognized microbiology laboratory and maintained under appropriate conditions. The organism was cultured on nutrient agar medium and incubated at 37°C for 24 hours to obtain fresh bacterial growth. For experimental use, bacterial suspensions were prepared in nutrient broth.

**Antibacterial Assay:** The antibacterial activity of *Nyctanthes arbor-tristis* leaf extracts was determined using the agar disc diffusion method. Sterile nutrient agar plates were prepared and

inoculated with 0.1 ml of freshly prepared bacterial culture using a sterile spreader to ensure uniform distribution. Sterile filter paper discs were impregnated with different concentrations of ethanol and methanol extracts and placed gently on the inoculated agar surface. The plates were kept at room temperature for a short period to allow diffusion of the extracts and then incubated at 37°C for 24 hours. This methodology was designed to evaluate and compare the antibacterial potential of different solvent extracts of *Nyctanthes arbor-tristis* leaves against *Xanthomonas campestris*.

**Results and Didertation:**

**Antibacterial Activity:**

The antibacterial activity of ethanol and methanol extracts of *Nyctanthes arbor-tristis* leaves was evaluated against *Xanthomonas campestris* using the agar disc diffusion method. The results demonstrated that both extracts exhibited inhibitory effects; however, the ethanol extract showed comparatively higher antibacterial activity. The zone of inhibition increased with increasing concentration of the extracts, indicating a dose-dependent response. The ethanol extract produced a larger inhibition zone at all tested concentrations, suggesting that ethanol is a more effective solvent for extracting bioactive compounds responsible for antibacterial activity.

**Table 1.**

**Zone of inhibition (mm) exhibited by ethanol and methanol extracts of *Nyctanthes arbor-tristis* leaves and standard antibiotic against *Xanthomonas campestris* at different concentrations (µg/ml). Data are presented as mean ± standard deviation.**

Concentration of extract (µg/ml)	Methanolic extract mean zone of inhibition (mm)	Ethanolic extract Mean zone of inhibition (mm)	Amikacin(control) mean zone of inhibition (mm)
200	14 ± 0.4	18 ± 0.5	14.24 ± 0.32
100	12 ± 0.3	15 ± 0.3	
50	9 ± 0.2	12 ± 0.2	

*(Value expressed as mean ± Standard deviation)*

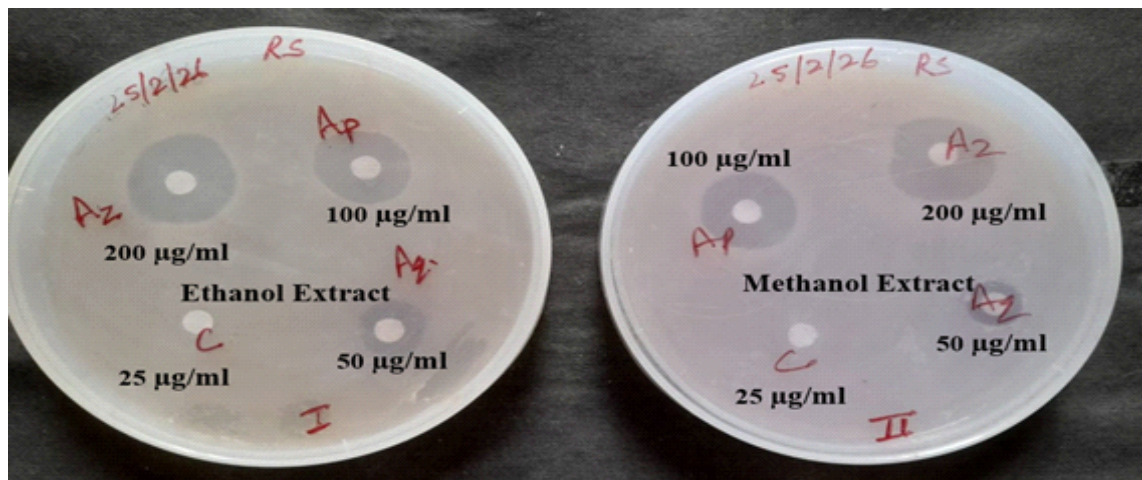


Figure: Showing the inhibition zone against the selected bacteria along with the different concentrations of *Nyctanthes arbor-tristis* leaves

Table 2.

Qualitative phytochemical profile of *Nyctanthes arbor-tristis* leaf extracts indicating the presence (+) or absence (-) of selected secondary metabolites.

Phytochemical	Presence (+/-)
Anthraquinones	+
Terpenoids	+
Saponins	+
Flavonoids	+
Glycosides	+

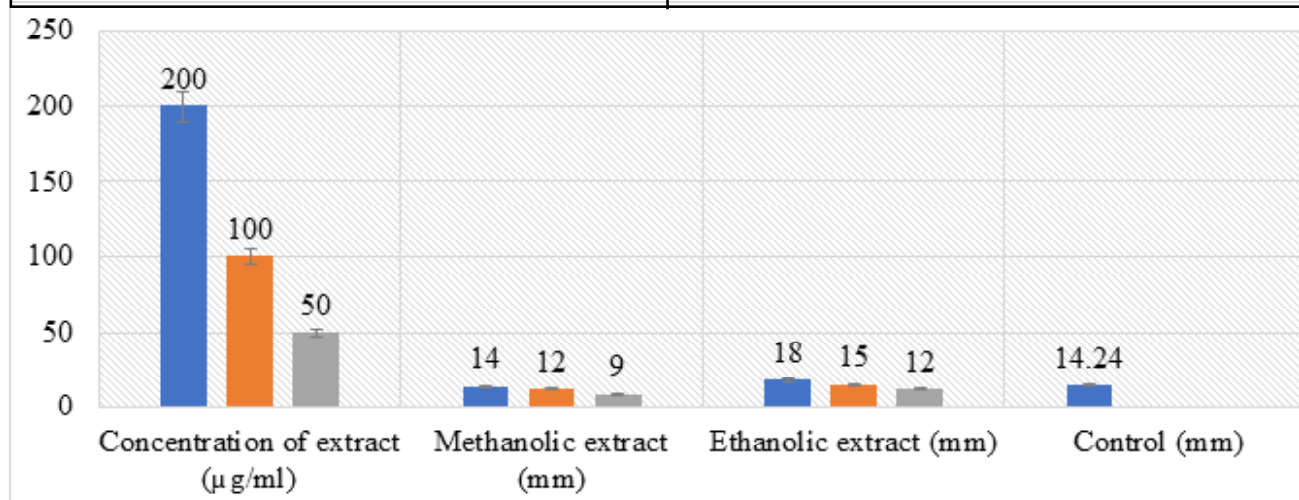


Figure 2. Comparative analysis of antibacterial efficacy of ethanol extract, methanol extract, and standard antibiotic against *Xanthomonas campestris* at varying concentrations (µg/ml), presented as a bar chart of inhibition zones.

## Discussion:

The present study evaluated the antibacterial potential of *Nyctanthes arbor-tristis* leaf extracts against *Xanthomonas campestris*, and the findings clearly demonstrate significant inhibitory activity. As shown in Table 1, both ethanol and methanol extracts exhibited antibacterial effects at all tested concentrations; however, the ethanol extract consistently produced larger zones of inhibition compared to the methanol extract. At the highest concentration (200 µg/ml), the ethanol extract showed the maximum inhibition, indicating its superior efficacy in extracting active antimicrobial constituents.

The dose-dependent increase in antibacterial activity is further illustrated in Figure 1, where a steady rise in the zone of inhibition is observed with increasing extract concentration. This trend confirms that the effectiveness of the plant extract is concentration-dependent. Additionally, comparison with the standard antibiotic, also depicted in Figure 1, shows that while the plant extracts are slightly less potent, they still demonstrate considerable antibacterial activity, supporting their potential as alternative therapeutic agents.

The comparative effectiveness of the extracts is more clearly visualized in Figure 2, where the bar chart highlights the higher antibacterial activity of the ethanol extract over the methanol extract across all concentrations. This difference may be attributed to the polarity of ethanol, which enhances the extraction of bioactive compounds such as flavonoids, phenols, and glycosides known for their antimicrobial properties.

Furthermore, the phytochemical screening results presented in Table 2 confirm the presence of important secondary metabolites including flavonoids, terpenoids, saponins, glycosides, and anthraquinones. These compounds are well known for their antibacterial mechanisms, such as disruption of microbial cell membranes, inhibition of enzymatic activity, and interference with nucleic acid synthesis. The synergistic action of these

phytochemicals likely contributes to the observed antibacterial effects.

However, certain limitations should be considered while interpreting these findings. This study is limited to in vitro evaluation using a single bacterial strain. The absence of in vivo studies and detailed compound isolation restricts a comprehensive understanding of its therapeutic potential. Additionally, environmental and geographical variations in plant composition were not extensively analyzed, which may influence the consistency of the results.

Overall, the results align with previous research supporting the antimicrobial potential of medicinal plants. The observed activity of *Nyctanthes arbor-tristis* reinforces its traditional use and highlights its promise as a source of natural antibacterial agents. Further investigations are necessary to isolate active constituents, explore their mechanisms, and validate their efficacy through advanced studies.

## Conclusion:

The present study demonstrates that *Nyctanthes arbor-tristis* leaf extracts possess significant antibacterial activity against *Xanthomonas campestris*. Among the tested solvents, the ethanol extract showed superior inhibitory effects compared to the methanol extract, indicating its higher efficiency in extracting active phytoconstituents. The antibacterial activity was found to be concentration-dependent, with increased inhibition observed at higher concentrations.

Phytochemical screening confirmed the presence of important bioactive compounds such as flavonoids, terpenoids, saponins, glycosides, and anthraquinones, which are known to contribute to antimicrobial properties. These compounds likely act through disruption of microbial cell membranes, inhibition of enzymatic activity, and interference with cellular metabolism. Overall, the findings support the traditional use of *Nyctanthes arbor-tristis* as a medicinal plant and highlight its potential as a natural source of antibacterial agents.

## References:

1. Bhatnagar, D., & McCormick, S. P. (1988). Inhibitory effects of neem extracts on fungal toxins. *Journal of the American Oil Chemists' Society*, *65*, 1166–1168.
2. Harborne, J. B. (1973). *Phytochemical methods: A guide to modern techniques of plant analysis*. London: Chapman & Hall.
3. Kala, C. P. (2017). Traditional healthcare systems and herbal medicines. *European Journal of Environment and Public Health*, *1*, 1–6. <https://doi.org/10.20897/ejeph.201703>
4. Khan, M. S., & Ahmad, I. (2019). Herbal medicine: Current trends and future prospects. *New Look to Phytomedicine*, *1*, 3–13.
4. Kirtikar, K. R., & Basu, B. D. (1935). *Indian medicinal plants*. Allahabad: Lalit Mohan Basu.
5. Lemonnier, N., Zhou, G. B., Prasher, B., Mukerji, M., Chen, Z., Brahmachari, S. K., Noble, D., Auffray, C., & Sagner, M. (2017). Traditional knowledge-based medicine: A review of history, principles, and relevance in the present context of P4 systems medicine. *Progress in Preventive Medicine*, *2*, e0011.
6. Nadkarni, A. K. (1982). *Indian materia medica* (Vol. 1, 3rd ed.). Mumbai: Popular Prakashan.
7. Nielsen, P. V., & Rios, R. (2000). Inhibition of fungal growth by plant compounds. *International Journal of Food Microbiology*, *60*, 219–229.
8. Parekh, S., & Soni, A. (2020). *Nyctanthes arbor-tristis*: Comprehensive review on its pharmacological, antioxidant, and anticancer activities. *Journal of Applied Biology & Biotechnology*, *8*, 95–104. <https://doi.org/10.7324/JABB.2020.80116>
9. Reddy, P. S., Jamil, K., & Madhusudhan, P. (2001). Antibacterial activity of plant isolates. *Pharmaceutical Biology*, *39*, 236–238.
10. Riaz, M., Khalid, R., Afzal, M., Anjum, F., Fatima, H., Zia, S., Rasool, G., Egbuna, C., Mtewa, A. G., Uche, C. Z., & Aslam, M. A. (2023). Phytobioactive compounds as therapeutic agents for human diseases: A review. *Food Science & Nutrition*, *11*, 2500–2529.
11. Runyoro, D., Matee, M., Olipa, N., Joseph, C., & Mbwambo, H. (2006). Screening of medicinal plants for antifungal activity. *BMC Complementary and Alternative Medicine*, *6*, 11.
12. Shahidi, B. H. (2004). Antimicrobial properties of medicinal plants. *Asian Journal of Plant Sciences*, *3*, 82–86.
13. Singh, B., Bhat, T. K., & Sharma, O. P. (2003). Potential therapeutic applications of plant secondary metabolites. *Journal of Agricultural and Food Chemistry*, *51*, 5579–5597.
14. Singhal, R. (2005). Medicinal plants and primary health care: The role of gender. *Journal of Health Management*, *7*, 277–293. <https://doi.org/10.1177/097206340500700208>
15. Terzi, V., Tumino, G., Stanca, A. M., & Morcia, C. (2014). Control of fungal infections in cereals. *Journal of Cereal Science*, *59*, 284–293.
16. Thapa, P., & Kumar, A. (2024). Phytochemicals and pharmacological properties of *Nyctanthes arbor-tristis*: A review. *International Journal of Pharmaceutical Sciences and Medicine*, *9*, 1–10.
17. Thomas, V., Nair, S. N. V., Ved, D. K., & Shankar, D. (2020). Controversial identities of medicinal plants in classical Ayurveda literature. *Journal of Ayurveda and Integrative Medicine*, *11*, 565–572.
18. Vijayalakshmi, R., & Ravindran, R. (2012). Preliminary comparative phytochemical screening of root extracts. *Asian Journal of Plant Science and Research*, *2*, 581–587.

