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Exploring *Nyctanthes arbor-tristis* Stem Extract as a Natural Antimicrobial Agent: *In vitro* Evidence and Phytochemical Insights

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Keywords

Antimicrobial studies, Disc diffusion method, Zones of inhibition, Phytochemical screening, Nyctanthes arbor-tristis stem

Abstract

The global escalation of antimicrobial resistance necessitates the discovery of novel therapeutic agents, particularly from natural sources. This study evaluates the in vitro antimicrobial activity and phytochemical screening of the vacuum-dried ethanolic extract of *Nyctanthes arbor-tristis*stem (EENAS) using the disc diffusion method. EENAS demonstrated measurable antibacterial activity against Serratia marcescens (ATCC 174; 7 mm inhibition zone) and Enterococcus faecalis (ATCC 29212; 10 mm), and antifungal activity against Candida albicans (ATCC 10231;0 mm) and Aspergillus niger (ATCC 16404; 7 mm). Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and terpenoids classes of metabolites commonly associated with antimicrobial action. These findings indicate that provide scientific support for the traditional medicinal use of N. arbor-tristis stem extract possesses moderate antimicrobial potential and further investigation, including broader microbial screening and isolation of bioactive constituents.

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1. Introduction

WHO estimates that 80 % of the world population use plants of their active components as folk medicine in traditional therapy. Overall, bacteria possess the genetic capability to spread and gain resistance to synthetic medicine as the therapeutic agent applied. Natural extracts of different higher plants have the potential to serve as a new source of antimicrobial agent with potentially new mechanisms of action. *Nyctanthes arbor-tristis*Linn is of the family Oleaceae, the popular name of which is Night Jasmine (English) or Harsingar (Hindi) since the flowers have a very strong and pleasant smell all through the night [1]. *Nyctanthes* is a generic name, which is formed out of two Greek words; Nykhta (Night) and anther (flower). It begins to shed the flowers past midnight and by the day break, the plant

looks drab. One of such medicinal plants is *Nyctanthes arbortristis* Linn which is commonly referred to as Parijata. It is a popular wild hardy large shrub or small tree. It is also indigenous to India and spreads wild in the sub-Himalayan areas and further southwards to Godavari, Lalitpur Nepal [2]. The phytochemicals in this herbal plant have been reported to comprise flavonoid, glycoside, oleanolic acid, essential oils, tannic acid, carotene, lupeol, glucose and benzoic acid. The plants serve as traditional medicine to women who are related with issues including provoking menstruation, to treat scabies and other skin diseases as well as hair tonic. Therefore, ethanolic extract of the stem of *Nyctanthes arbour-tristis* dried in the vacuum were tested in the current study to determine the level of their antimicrobial and antifungal properties towards Disc diffusion methodology. The antimicrobial properties of medicinal plants represent the microorganisms that are increasingly reported all over the planet. The plants are abundant sources of a broad spectrum of secondary metabolites that include glycosides, flavonoids, tannins and terpenoids to name a few that have been reported to be antimicrobials [3].

Figure 1: Plant of *Nyctanthes arbor-tristis*.

2. Materials and Methods

2.1. Plant Material

The stem material of *Nyctanthes arbor-tristis*was procured from the IFTM University Campus, situated adjacent to Lodhipur Rajput village in Moradabad, Uttar Pradesh, India. Plant specimen verification was conducted by Botanist affiliated with the Department of Botany at IFTM University, Moradabad, Uttar Pradesh. A reference specimen (Ref. No.2025/SOS/BOT/220) was deposited in the herbarium collection for future reference purposes.

2.2. Preparation of Extract

The air-dried stem material of *Nyctanthes arbor-tristis*was coarsely powder passed through a 40-mesh sieve, they were successively extracted using the different solvents increasing polarity order as Petroleum ether 60-80°C, Ethyl acetate, Ethanol and Aqueous by soxhlet apparatus at 45-50°C. The resulting extracts filtration, and the filtrate was subsequently distilled at reduced temperatures (25-30°C) before final concentration under vacuum conditions to obtain the dried different extracts of *Nyctanthes arbor-tristis*stem [4].

2.3. Phytochemical Screening of Stem Extract

The presence or absence of several bioactive components including anthraquinone glycosides, saponin glycoside, alkaloids, tannins, terpenoids, flavonoids, and steroids were determined by using standard procedures [5]. The tests and indicative reactions of each compound are shown in the following form (Table 1).

2.3.1. Anthraquinone Glycoside (Borntrager Test)

10 mL of benzene was added to powdered plant extract and filtered. Further, 5 mL of 10% ammonium

hydroxide (NH_4OH) solution was introduced into the filtrate. The appearance of a pink, crimson, or violet coloration in the lower ammoniacal layer was the indication of the presence of anthraquinones [6].

2.3.2. Saponin Glycoside (Foam Test)

A dilute of the extract (approximately 0.5-1 mL) is added to 10 mL of the distilled water in a test tube and shaken vigorously in 30 seconds. The mixture is then left to rest, for 10-15 minutes. The saponin glycosides are realized by formation of a stable and persistent honeycomb-like froth (1-2 cm in height) [7].

2.3.3. Alkaloids (Wagner Test)

The extract is acidified with dilute hydrochloric acid in a small amount and filtered. The reagent used by Wagner (iodine-potassium iodide solution) is dropwise added to the filtration. The alkaloids are confirmed by the forming of a reddish-brown or precipitate [8].

2.3.4. Tannins (Ferric Chloride Test)

A little amount of the extract is mixed with distilled water, and a few drops of ferric chloride 5% solution are added. A blue black or a greenish black color will form, which means there are tannins [9].

2.3.5. Terpenoids (Liebermann-Burchard Test)

A little amount of the extract is combined with 2 mL of chloroform; 1 mL of acetic anhydride is added. Then concentrated sulfuric acid is added cautiously at the side of the test tube. The development of reddish-brown, blue-green or violet coloration at the interface proves the existence of terpenoids [10].

2.3.6. Flavonoids (Shinoda Test)

1 mL of 10% lead acetate solution was poured into 10 mL of the leaf extract and the solution shaken. The development of a yellow-colored product is an indication that flavonoids are present [11].

2.3.7. Steroids (Salkowski Test)

2 mL of the extract is combined with 2 mL of chloroform and 2 mL of concentrated sulfuric acid are then added to the side of the test tube. A red, reddish-brown or golden yellow coloration of the chloroform layer is an indication of steroids [12].

2.4. Antimicrobial and Antifungal Activities

The antimicrobial and antifungal properties of the ethanolic extract derived from *Nyctanthes arbor-tristis* stem were evaluated through microbiological investigations employing two bacterial species: the gram-negative organism *Serraatia marcescens* ATCC174 and the gram-positive organism *Enterococus faecalis* ATCC 29212, alongside two fungal species comprising *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC16404. The evaluation of antimicrobial and antifungal efficacy was conducted utilizing the Zone Inhibition assay through the disc well diffusion technique [13].

2.4.1. Preparation of the Solutions Extract

Precisely measured 5 mg quantities of ethanolic *Nyctanthes arbor-tristis* stem extract was placed into separate 10 ml volumetric flasks. The extracts underwent dissolution in DMSO, with each flask's volume subsequently adjusted to 10 ml using DMSO. Stock solutions with concentrations of 5 mg/ml were thereby prepared [14]. Subsequently, 20 ml aliquots from these stock solutions were obtained and subjected to sterilization on blank discs. Consequently, the final concentration range of the solution applied to each disc varied from 0 to 5000 µg/disc across the petri dishes [15].

2.4.2. Preparation of Nutrient Agar Media (2%, pH 6.8±0.2) for Antibacterial Activity

Approximately 5 gm of beef extract, 5 gm of peptone, and 2.5 gm of sodium chloride were dissolved in 400 ml of distilled water within a 500 ml volumetric flask and heated [16]. Subsequently, 10 gm of agar was dissolved in 50 ml of heated distilled water. Both solutions were combined, and the final volume in the volumetric flask was adjusted to 500 ml using warm distilled water. The prepared nutrient agar medium underwent sterilization via autoclave at 121°C under 15 lb/inch² pressure for a duration of 15 minutes [17].

2.4.3. Preparation of Sabouraud Dextrose Agar Media (2%, pH 6.8±0.2) for Antifungal Activity

For the preparation of Sabouraud dextrose agar medium, precisely weigh 20g of dextrose monohydrate and 5g of peptone, then dissolve these components in 400ml of distilled water within a 500ml volumetric flask under gentle heating. Separately, dissolve 7.5g of agar in 50ml of heated distilled water. Subsequently, combine both solutions and adjust the total volume to 500ml using warm distilled water in the volumetric flask. The prepared medium underwent sterilization via autoclave treatment at 121°C under 15lbs/inch²pressure for a duration of 15 minutes [18].

2.4.4. Disc Diffusion Method

This approach involved preparing culture media that underwent sterilization at 121°C under 15 lbs/inch² pressure for a 15-minute duration. Following sterilization, the media were dispensed into plates and permitted to achieve solidification. Microbial

suspensions were subsequently distributed across the media surface using a sterilized, curved glass rod. The prepared nutrient agar medium was transferred to pre-sterilized Petri dishes where microorganism cultivation occurred [19-21]. Discs measuring 7 mm in diameter were saturated with varying concentrations ranging from 0 to 5000 µg/disc of ethanolic *Nyctanthes arbor-tristis* stem extract solution, while standard reference discs containing Ciprofloxacin (10 µg/disc) for antibacterial assessment and Fluconazole (500 µg/disc) for antifungal evaluation were positioned centrally on each plate. The experimental plates remained at ambient temperature for one hour to facilitate compound diffusion before being incubated at 37±0.5°C for 24 hours to assess antibacterial properties and 48 hours for antifungal properties, respectively. Inhibition zone diameters were recorded in millimetres and subjected to comparative analysis against standard pharmaceutical agents [22-23].

2.4.5. Preparation of Inoculums

The vials containing lactose dilution (dehydrated powder) of microbial inocula comprising two bacterial species; gram-negative *Serratia marcescens* and gram-positive *Enterococcus faecalis*, along with two fungal species *Candida albicans* and *Aspergillus niger*, were individually opened under aseptic conditions using a sterile scalpel blade within conical flasks containing 100 ml of nutrient broth. These flasks underwent incubation for 24 hours at 37°C within a BOD incubator. Following the 24-hour incubation period, a turbid suspension was achieved [24-26].

3. Result and Discussion

3.1. Qualitative Screening *Nyctanthes arbor-tristis* Stem Extract

An initial qualitative phytochemical screening of *Nyctanthes arbor tristis* stem extract collected using different solvents according to non-polar to polar solvents which indicated that the extract contained a number of secondary metabolites. The analysis showed the presence of bioactive compounds to include such as tannins, glycosides, saponins, alkaloids, steroids, terpenoids, and flavonoids in ethanolic solvent extract as well as some phytoconstituents present in different solvents (Table 1). These phytoconstituents are also reported to have added therapeutic value for the various pharmacological effects which have been expressed by plants such as antimicrobial, anti-inflammatory and antioxidant activity.

Antimicrobial investigations conducted using triplicate plates for each bacterial and fungal species demonstrated that the ethanolic extract of *Nyctanthes arbor-tristis* stem exhibited notable antibacterial properties. The inhibition zone measured 07 mm against *Serratia marcescens*, while enhanced activity of 10 mm was observed against *Enterococcus faecalis*, in comparison to the reference antibiotic Ciprofloxacin which showed 14.67 mm and 28.67 mm respectively (Table 2,3 and figure 2,3,4,5). Conversely, the extract demonstrated reduced efficacy against fungal species, with *Candida albicans* showing no inhibition (0 mm) and *Aspergillus niger* displaying 07 mm inhibition zones, contrasted with the standard antifungal fluconazole which exhibited 27.67 mm and 21.33 mm respectively (table 4, 5 and figure 6,7,8,9), as determined through inhibition zone analysis. The potential antimicrobial mechanisms encompass three primary pathways: disruption of cell wall biosynthesis, irreversible interaction with DNA nucleotide bases, and compromise of cellular membrane integrity.

Table 1: Phytochemical analysis of *Nyctanthes arbor-tristis* stem extract.

Bioactive compounds	Phytochemical tests	Petroleum ether extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
Anthraquinone Glycosides	Borntrager test	–	+	+	+
Saponins Glycoside	Foam test	–	+	+	+
Alkaloids	Wagner test	–	–	+	+
Tannins	Ferric chloride test	–	–	+	–
Terpenoid	Liebermann-Burchard test	–	+	+	+
Flavonoids	Shinoda test	+	+	+	–
Steroids	Salkowski test	+	–	–	–

Note: *(+) indicates presence and (–) indicates absence of the respective phytochemical constituents.*

3.2 Antibacterial Activity of Ethanolic *Nyctanthes arbor-tristis* Stem Extract (EENATS)

The antibacterial activity of the ethanolic extract of *Nyctanthes arbor-tristis* stem (EENATS) against *Serratia marcescens* is presented in Table 2. The extract showed no detectable antibacterial activity at concentrations of 312.5, 625, 1250, and 2500 µg/disk, with no zones of inhibition observed. At the highest concentration (5000 µg/disk), EENATS exhibited a weak antibacterial effect, producing a consistent inhibition zone of 7 mm across all three replicates. In contrast, the standard control produced a mean inhibition zone of 14.67 mm, indicating significantly higher antibacterial efficacy. Figure 2 illustrates the comparative zones of inhibition of EENATS and the standard drug, while Figure 3 graphically represents the concentration-dependent antibacterial activity of EENATS against *Serratia marcescens*.

Table 2: Antibacterial activity of bacterial strain *Serraatia marcescens* of ethanolic extract *Nyctanthes arbor-tristis* stem (EENATS).

EENATS (µg/disk)	Plate A (mm)	Plate B (mm)	Plate C (mm)	Average Zone of Inhibition (mm)
Control (10)	14	14	16	14.67
0	0	0	0	0.00
312.5	0	0	0	0.00
625	0	0	0	0.00
1250	0	0	0	0.00
2500	0	0	0	0.00
5000	7	7	7	7.00

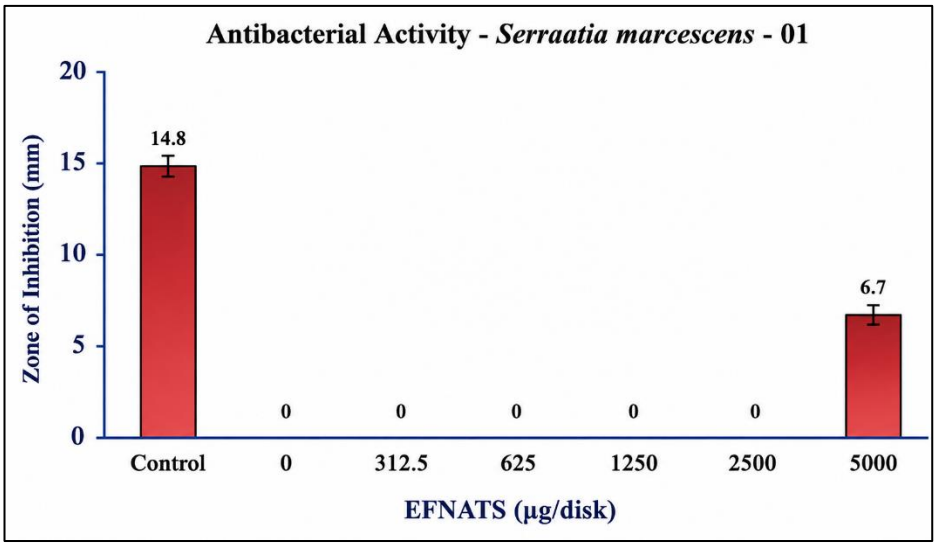

Figure 2: Zone of Inhibition EENATS and Standard drug on *Serraatia marcescens*.

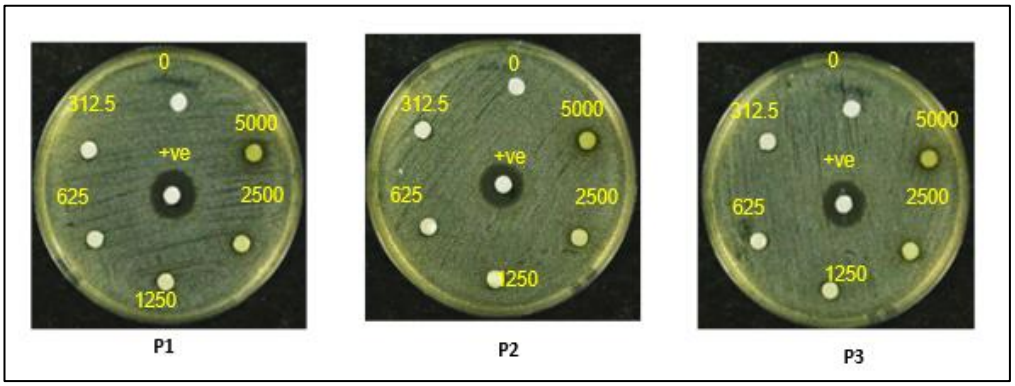

Figure 3: Antibacterial activity of EENATS in the *Serraatia marcescens*.

3.2.2 Antibacterial Activity Against *Enterococcus faecalis*

The antibacterial activity of the ethanolic extract of *Nyctanthes arbor-tristis* stem (EENATS) is summarized in Table 3. The extract exhibited concentration-dependent antibacterial activity

against the test bacterial strain. No inhibition was observed in the negative control, whereas measurable inhibition zones were detected at all tested concentrations from 312.5 to 5000 µg/disk. The inhibition zone increased from 8.00 mm at 312.5 µg/disk to a maximum of 10.00 mm at 5000 µg/disk, indicating enhanced antibacterial activity with increasing extract concentration. However, the standard control showed a substantially higher mean inhibition zone of 28.67 mm. Figure 4 illustrates the comparative zones of inhibition produced by EENATS and the standard drug, while Figure 5 presents the concentration-dependent antibacterial activity of EENATS graphically.

Table 3: Antibacterial activity of bacterial strain *Enterococus faecalis* of ethanolic extract *Nyctanthes arbor-tristis* stem (EENATS)

EENATS (µg/disk)	Plate A (mm)	Plate B (mm)	Plate C (mm)	Average Zone of Inhibition (mm)
Control (10)	28	29	29	28.67
0	0	0	0	0.00
312.5	8	8	8	8.00
625	8	9	8	8.33
1250	9	10	9	9.33
2500	9	9	9	9.00
5000	10	10	10	10.00

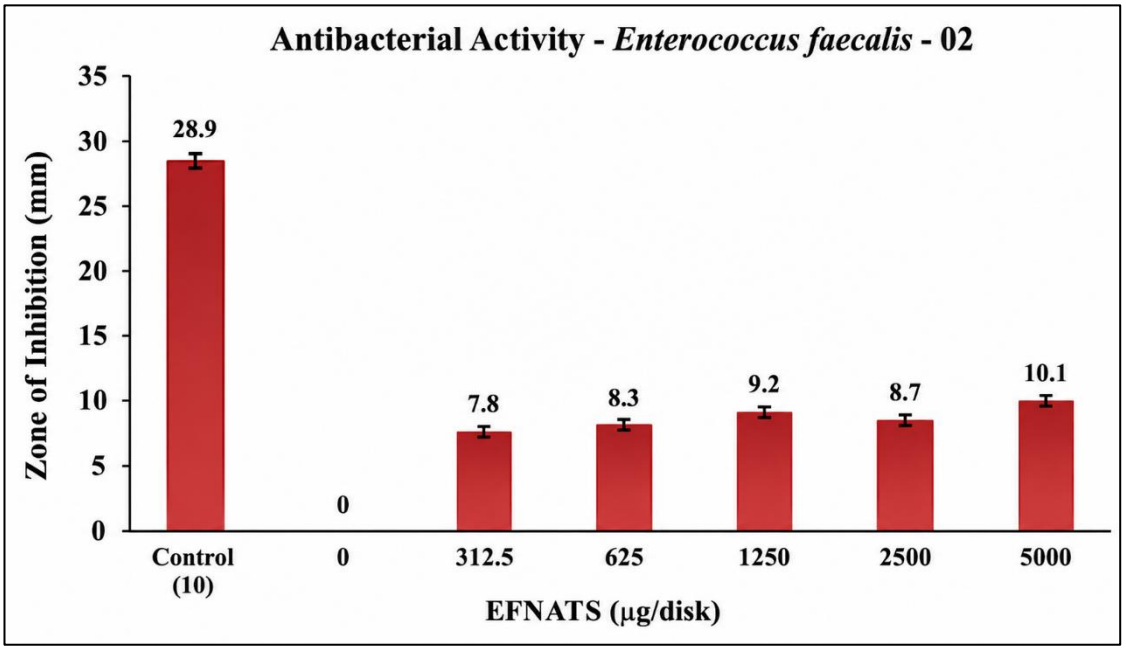

Figure 4: Zone of Inhibition EENATS and Standard drug on *Enterococus faecalis*.

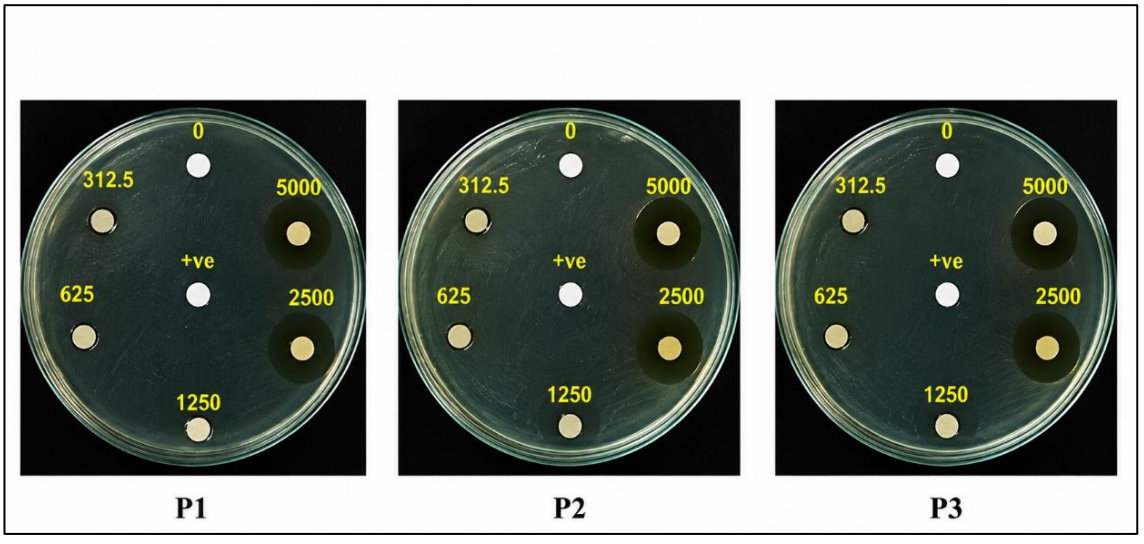

Figure 5: Antibacterial Activity of EENATS in the *Enterococus faecalis*.

3.3 Antifungal Activity of Ethanolic *Nyctanthes arbor-tristis* Stem Extract (EENATS)

3.3.1 Antifungal Activity Against *Candida albicans*

The antifungal activity of the ethanolic extract of *Nyctanthes arbor-tristis* stem (EENATS) against *Candida albicans* is presented in Table 4. The extract did not exhibit any antifungal activity at the tested concentrations ranging from 312.5 to 5000 µg/disk, as no zones of inhibition were observed in any of the replicates. In contrast, the standard control produced a mean inhibition zone of 27.67 mm, confirming the susceptibility of the fungal strain to the standard antifungal drug. Figure 6 compares the zones of inhibition produced by EENATS and the standard drug, while Figure 7 graphically illustrates the absence of concentration-dependent antifungal activity of EENATS against *Candida albicans*.

Table 4: Antifungal activity of fungal strain *Candida albicans* of ethanolic extract *Nyctanthes arbor-tristis* stem (EENATS)

EENATS(µg/disk)	Plate A	Plate B	Plate C	Average
Control (500)	27	28	28	27.6667
0	0	0	0	0
312.5	0	0	0	0
625	0	0	0	0
1250	0	0	0	0
2500	0	0	0	0
5000	0	0	0	0

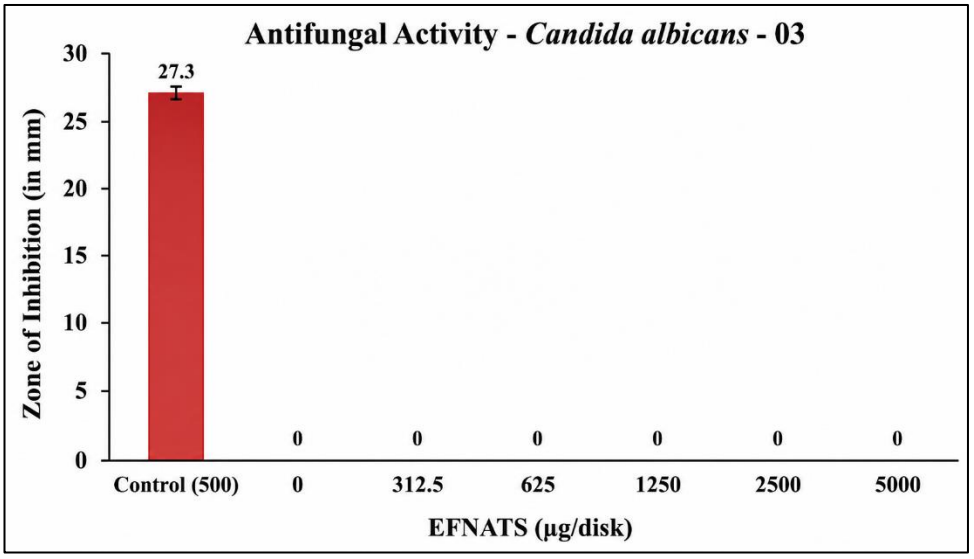

Figure 6: Zone of Inhibition EENATS and Standard drug on *Candida albicans*

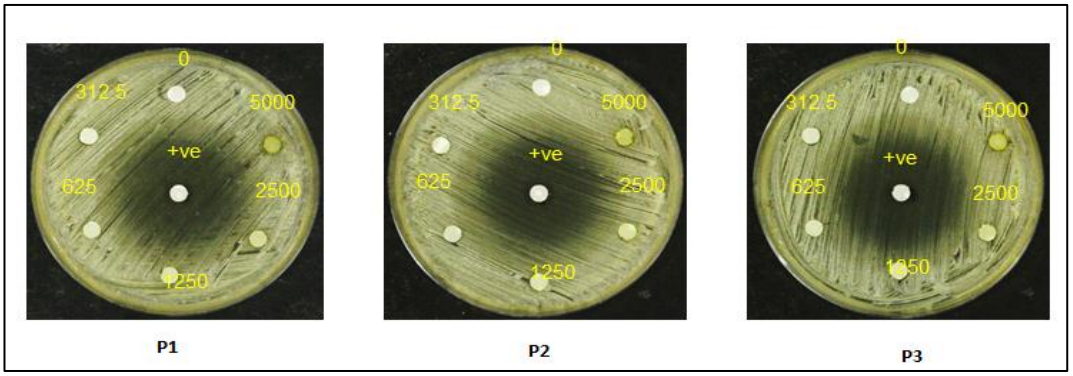

Figure 7: Antifungal Activity of EENATS in the *Candida albicans*

3.3.2 Antifungal Activity Against *Aspergillus niger*

The antifungal activity of the ethanolic extract of *Nyctanthes arbor-tristis* stem (EENATS) against *Aspergillus niger* is presented in Table 5. The extract exhibited mild antifungal activity, producing a 6 mm zone of inhibition at 312.5 µg/disk. The inhibition zone increased to 7 mm at 625 µg/disk and remained

constant up to 5000 µg/disk, indicating no further enhancement in antifungal efficacy at higher concentrations. In comparison, the standard control produced a mean inhibition zone of 21.33 mm, demonstrating considerably stronger antifungal activity. Figure 8 compares the zones of inhibition produced by EENATS and the standard drug, while Figure 9 graphically illustrates the concentration-dependent antifungal activity of EENATS against *Aspergillus niger*.

Table 5: Antifungal activity of fungal strain *Aspergillus niger* of ethanolic extract *Nyctanthes arbor-tristis* stem (EENATS).

EENATS (µg/disk)	Plate A	Plate B	Plate C	Average
Control (500)	21	22	21	21.3333
0	0	0	0	0
312.5	6	6	6	6
625	7	7	7	7
1250	7	7	7	7
2500	7	7	7	7
5000	7	7	7	7

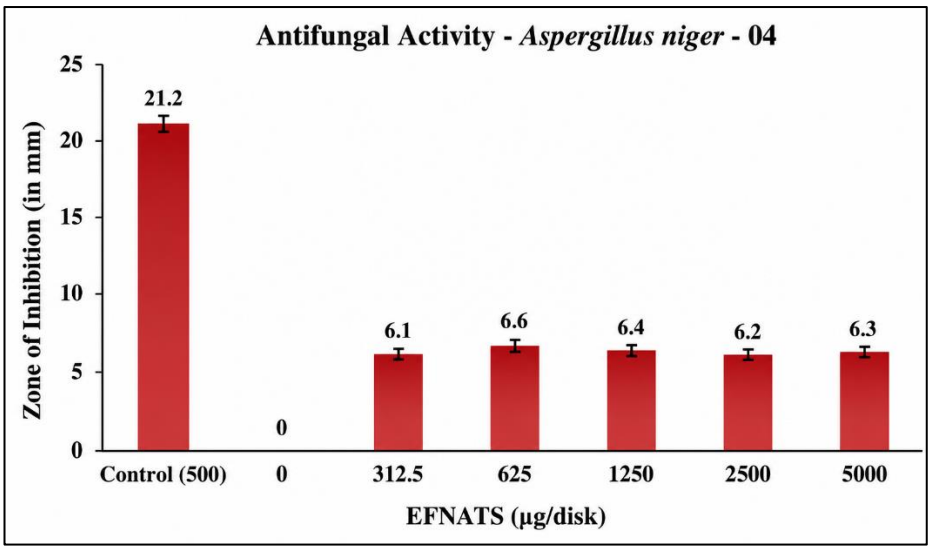

Figure 8: Zone of Inhibition EENATS and Standard drug on *Aspergillus niger*.

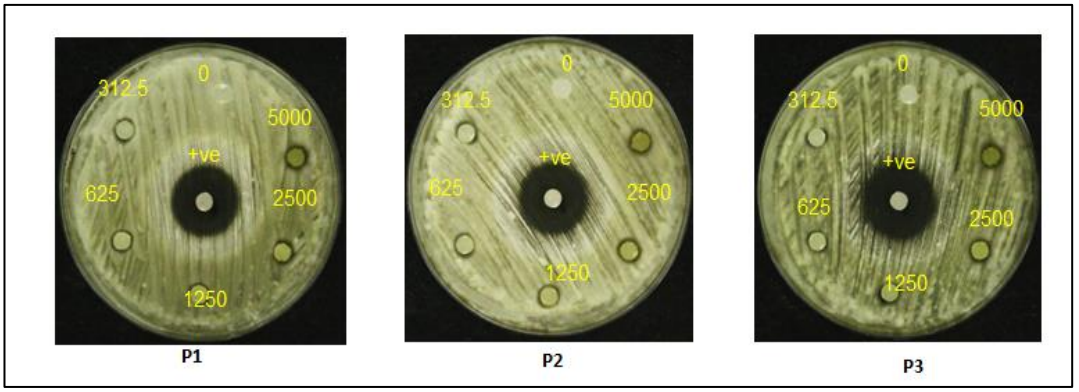

Fig. 9: Antifungal Activity of EENATS in the *Aspergillus niger*

4. Discussion

The present investigation aimed to evaluate the *in vitro* antimicrobial potential of the ethanolic stem extract of *Nyctanthes arbor-tristis* (EENAS) and to correlate the observed bioactivities with its phytochemical composition. Qualitative phytochemical screening confirmed the presence of diverse secondary metabolites alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and Terpenoids each of which is widely documented to contribute to antimicrobial properties through multiple biochemical mechanisms.

The ethanolic extract demonstrated notable antibacterial activity, particularly against *Enterococcus faecalis* (10 mm inhibition zone), a clinically significant Gram-positive pathogen

often associated with antibiotic resistance. The moderate inhibition of *Serratia marcescens* (7 mm), a Gram-negative organism known for its intrinsic resistance mechanisms, suggests the presence of phytochemicals capable of traversing the outer membrane barrier characteristic of Gram-negative bacteria. This dual activity against both Gram-positive and Gram-negative strains underscores the broad-spectrum antibacterial potential of *N. arbor-tristis* stem constituents.

Conversely, the extract exhibited relatively weak antifungal activity, with only mild inhibition against *Aspergillus niger* (7 mm) and negligible action against *Candida albicans*. This discrepancy likely reflects fundamental differences in microbial cell architecture. Fungal cells possess a robust cell wall composed of chitin, glucans, and mannoproteins, which limits the penetration of many phytochemicals. Additionally, fungal organisms exhibit intrinsic resistance mechanisms including efflux transporters, detoxifying enzymes, and membrane sterol alterations that may reduce susceptibility to plant-derived compounds. These factors collectively account for the reduced antifungal efficacy observed in this study. The differential antimicrobial outcomes also highlight the importance of solvent polarity in extracting bioactive metabolites. Ethanol, a polar protic solvent, efficiently solubilizes phenolic acids, flavonoids, glycosides, and other polar constituents known for pronounced antibacterial effects.

The various phytochemical constituents of *N. arbor-tristis* likely play a central role in its antimicrobial efficacy. Alkaloids and flavonoids may inhibit microbial growth by disrupting protein synthesis and enzyme activity, while tannins can induce protein precipitation and impair cell wall integrity. Saponins, known for their surfactant properties, may increase membrane permeability, leading to cell lysis. Terpenoids and steroids may interact with membrane lipids, altering membrane fluidity and contributing to antimicrobial action. Although the antifungal activity was limited, the extract's antibacterial efficacy particularly against *E. faecalis* indicates that *N. arbor-tristis* stems represent a promising source of natural antimicrobial agents.

Conclusion

The findings of this study demonstrate that the ethanolic stem extract of *Nyctanthes arbor-tristis* possesses antimicrobial activity, particularly against *Enterococcus faecalis* and *Serratia marcescens*, highlighting its potential as a source of natural antibacterial agents. The presence of diverse phytochemicals such as alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and terpenoids likely contributes to the observed bioactivity through synergistic mechanisms that disrupt microbial cell integrity and essential metabolic processes. Although the extract exhibited limited antifungal activity, its notable efficacy against both Gram-positive and Gram-negative bacteria positions *N. arbor-tristis* as a promising candidate for further antimicrobial exploration. Overall, the results provide scientific support for the traditional medicinal use of *N. arbor-tristis* and underscore its relevance in the ongoing search for novel plant-based antimicrobial compounds.

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Author Contribution

DS: Conceptualization; **AK:** Supervision; **MS:** Formal analysis; **KS:** Investigation; **MIT:** Data curation; **PA:** Writing – Review & Editing.

Conflict of interests

The authors declare that there is no conflict of interest.

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There is no funding available to conduct this study.

AI Declarations

The authors declare that they used AI language tools (ChatGPT and Grammarly Premium) to enhance this manuscript's linguistic clarity and readability. They carefully reviewed and edited all generated text to ensure accuracy and alignment with the research's intended meaning.

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