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Original Article

Microbiology Section

Antimicrobial and Phytochemical Evaluation of *Nyctanthes arbor-tristis* Linn. Leaf Extracts in Various Solvents against Pathogenic Microbial Strains: An In-vitro Study

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ABSTRACT

Introduction: Antimicrobial Resistance (AMR) poses a growing global health challenge by reducing the effectiveness of conventional antibiotics against a wide range of pathogenic microorganisms. In this context, medicinal plants are increasingly being explored as alternative sources of bioactive compounds with antimicrobial potential. *Nyctanthes arbor-tristis* Linn., a traditionally used medicinal plant, holds promise due to its diverse phytochemical composition.

Aim: To evaluate the in-vitro antimicrobial activity and phytochemical profile of *N. arbor-tristis* leaf extracts obtained using various solvents and to assess their efficacy against selected pathogenic microbial strains.

Materials and Methods: This in-vitro study was conducted at the Shri Guru Ram Rai (SGRR) University campus, Dehradun, Uttarakhand, India, between June 2021 and September 2024. The study investigated the antimicrobial properties, chemical constituents and qualitative phytochemical composition of *N. arbor-tristis* extracts. Leaves were subjected to Soxhlet extraction using five solvents: water, methanol, acetone, chloroform and ethyl acetate. Antimicrobial activity was tested against *Salmonella enterica* serovar Newport {Microbial Type Culture Collection (MTCC) 3225}, *Escherichia coli* (MTCC 723), *Staphylococcus aureus* subsp. *aureus* (MTCC 740), and *Candida albicans* (MTCC 3017) using the well diffusion method. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were also determined at different concentrations. Phytochemical screening was performed to detect secondary metabolites. Following the antimicrobial susceptibility tests, the MIC was determined to identify the lowest concentration inhibiting microbial growth. Data were presented in tables and graphs, with p-values calculated using appropriate statistical tests. A p-value of <0.05 was considered statistically significant.

Results: Methanolic extracts exhibited the highest antimicrobial activity across all tested pathogens, with the largest Zones Of Inhibition (ZOI) observed against *Salmonella* Newport (23.6±0.57 mm at 0.5 mg/100 µL and 24.6±0.57 mm at 1 mg/100 µL). Aqueous extracts showed the highest yield but were the least effective, particularly against *Candida albicans*, which showed no inhibition zone. Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, glycosides, steroids, terpenoids and saponins, while anthraquinones and phlobatannins were absent. Methanol and acetone extracts demonstrated the richest phytochemical diversity.

Conclusion: The study demonstrated that *N. arbor-tristis* methanolic leaf extract possesses significant antimicrobial activity, correlating with its rich phytochemical content. These findings support the potential of this plant as a natural source of antimicrobial agents, particularly in the context of rising antibiotic resistance.

Keywords: Bacterial strain, Fungal strain, Minimum inhibitory concentration, Phytochemical

INTRODUCTION

The escalating threat of AMR continues to pose a critical global health concern, reducing the effectiveness of commonly used antibiotics against an increasing number of pathogenic microorganisms [1]. In response, researchers have turned their attention to natural alternatives, particularly plant-based compounds, which contain a wide array of bioactive constituents. These phytochemicals, abundantly found in medicinal plants, have shown potential as sources of new antimicrobial agents with broad-spectrum activity [2]. One such plant is *Nyctanthes arbor-tristis* Linn., also known as night-flowering jasmine or Parijat, which has been traditionally used in systems like Ayurveda for the treatment of fever, skin ailments and digestive disorders [3].

Although the therapeutic properties of *N. arbor-tristis* have been documented, detailed investigations comparing the antimicrobial effects of its leaf extracts using different solvents remain limited. In particular, the influence of solvent polarity on the antimicrobial activity of these extracts against key pathogens such as *Salmonella* typhi, *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* has not been adequately explored [4].

To address this gap, the present study employed Soxhlet extraction with five solvents—aqueous, methanol, acetone, chloroform and ethyl acetate—selected based on their varying polarity, to maximise the extraction of diverse phytochemical compounds.

The potential mechanisms of action of *N. arbor-tristis* include its antioxidant and anti-inflammatory properties. Its extracts demonstrate significant antioxidant activity, neutralising free radicals and reducing oxidative stress. Phenolic compounds, in particular, contribute to this effect by modulating inflammatory mediators and enzymes such as Cyclooxygenase (COX) and Lipoxygenase (LOX) [5]. The plant also demonstrates hepatoprotective effects, particularly in protecting against liver damage induced by toxins such as CCl4. Furthermore, *N. arbor-tristis* has been shown to modulate immune responses by enhancing macrophage activity and influencing cytokine production. This immunomodulatory effect may strengthen the body's defenses against infections and disease [6].

In addition, the plant has been investigated for its anticancer properties, with studies indicating that it induces apoptosis, or programmed cell death, in cancer cells. By altering apoptotic pathways and regulating cell cycle processes, it exerts cytotoxic effects, suggesting a promising role in cancer treatment [7].

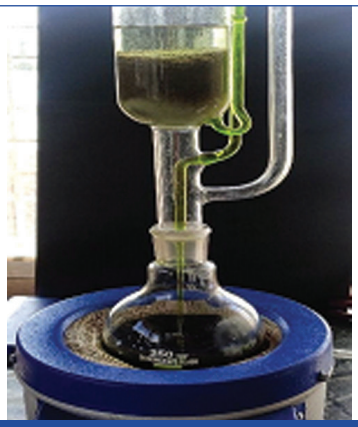
The present study aimed to provide a systematic evaluation of the antimicrobial properties of *Nyctanthes arbor-tristis* leaf extracts against selected pathogenic microorganisms, while also establishing a link between their biological activity and phytochemical composition. Using Soxhlet extraction with a range of solvent systems, the findings contribute to the ongoing search for effective plant-based antimicrobials and support the potential of *N. arbor-tristis* as a source of novel therapeutic agents in response to the growing challenge of AMR.

MATERIALS AND METHODS

This in-vitro study was conducted to investigate the antimicrobial properties, chemical constituents, and qualitative phytochemical composition of *N. arbor-tristis* leaf extracts. The research was carried out at the SGRR University campus in Dehradun, Uttarakhand, India, with experimental work commencing in June 2021 and concluding in September 2024.

This study did not involve any experiments on human participants or animals. All procedures followed appropriate scientific protocols and posed no risk or harm to human populations. The research was entirely self-funded, with no external financial support. All data were thoroughly verified using standardised tools and validated scientific methods before inclusion in the manuscript.

Plant material collection and identification: Fresh leaves of *N. arbor-tristis* were collected from the SGRR University campus in Dehradun, Uttarakhand, India. The plant material was identified and authenticated by the Botanical Survey of India (BSI), Dehradun. The leaves were shade-dried, powdered and stored in airtight plastic bags until further use.



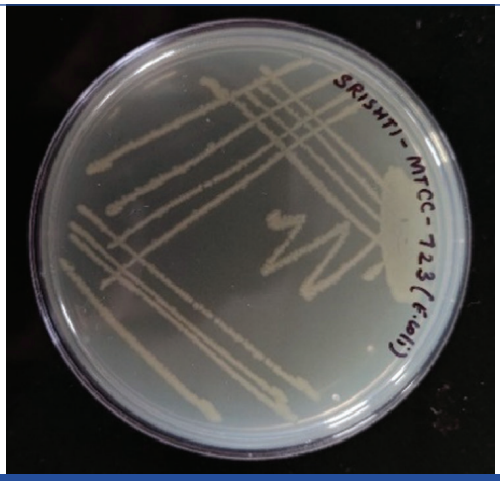
[Table/Fig-1]: Soxhlet apparatus used for the extraction of *Nyctanches arbor-tritis* leaf samples with different solvents.

Preparation of leaves extract: Extraction was performed using a Soxhlet apparatus [Table/Fig-1]. Methanol, chloroform, acetone, ethyl acetate and distilled water were used as solvents.

For each extraction, 25 g of leaf powder was placed in a thimble and extracted for 48 hours at an optimal temperature using 250 mL of the respective solvent in a round-bottom flask. The resulting filtrates were concentrated using a rotary evaporator. The crude extracts were stored in sealed bottles at 4°C until further use.

Chemicals and reagents used: Pure analytical-grade chemicals and solvents were used, including DMSO (dimethyl sulfoxide), chloroform, methanol, acetone, ethyl acetate and ethanol. Nutrient Agar Media (NAM), Yeast Extract Mannitol Agar (YEM) and broth were obtained from HiMedia Laboratories (Mumbai, India).

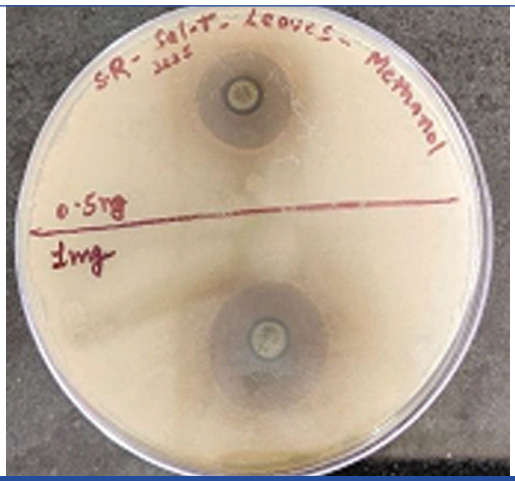
Screening for antimicrobial action: The antimicrobial sensitivity of all pathogenic strains was assessed using the agar well diffusion method against various *N. arbor-tristis* extracts [8]. The bacterial strains were revived from MTCC ampoules and streaked on nutrient agar plates for isolation [Table/Fig-2].



[Table/Fig-2]: MTCC bacterial culture revived from ampule and streaked on Petri plate.

After the strains were suspended in Nutrient Agar (NA) and Yeast Extract Mannitol (YEM) Agar and incubated at 37ºC for bacterial growth and 33ºC for fungal growth—18 hours for bacteria and 48 hours for fungi, as per the MTCC guidebook—the cell density of each strain to be examined was adjusted to 10^5 CFU/mL using the McFarland Turbidity standards [9]. NA and YEM plates were treated with a suspension of 20 µL of each bacterial and fungal isolate to produce a uniform microbial lawn. New media plates were then pierced with 9 mm wells.

A volume of 100 µL of each extract was immediately added to the wells at doses of 0.5 mg/100 µL and 1 mg/100 µL. The ZOI was determined following an 18-hour incubation period at 37°C for bacteria and a 48-hour incubation period at 33°C for fungi. The antimicrobial effect of menthol extract against *S. typhi* is shown in [Table/Fig-3]. The positive controls were the commercial antibiotic chloramphenicol for bacteria and fluconazole for fungi. Several zones of inhibition were observed at the conclusion of this triple test.



[Table/Fig-3]: Zone Of Inhibition (ZOI) of MTCC 3225 (*S. Typhi*) by methanol leaf extract on petri plate.

MIC testing by broth dilution: The MIC of all crude extracts against three bacterial strains and one fungal strain was determined using a macro broth dilution experiment. Extracts were serially diluted in a 2-fold range in well plates using nutrient broth and yeast extract mannitol broth, based on the results of the agar well diffusion assay. The same positive and negative controls were used. After inoculating 20 µL of freshly prepared microbial suspension (5×10^5 CFU/mL), the plates were incubated at 37°C for 24 hours. MIC was defined as the lowest concentration at which no growth was observed in the plant extract [10,11].

Determination of MBC: A volume of 20 µL was taken from the broth and plated on NA plates, which were then incubated at 37°C for 24 hours. The bactericidal concentration, or MBC, was defined as the lowest concentration at which no bacterial growth occurred. MIC and MBC values were calculated as the mean of three independent tests. The MIC index—the ratio between MBC and MIC—was used to classify extracts as bacteriostatic (>4) or bactericidal (<4) [12].

Qualitative analysis of the leaves extracts: Qualitative tests were conducted to determine the presence or absence of phytochemicals, including alkaloids, saponins, tannins, flavonoids, anthraquinones, glycosides, steroids, phlobatannins and terpenoids, using standard procedures.

The following active compounds were identified in the plant extracts, along with their respective testing methods [Table/Fig-4]:

Alkaloids (Hager's Test): A few drops of Hager's reagent (saturated picric acid solution) were added to 2 mL of the plant extract. The presence of alkaloids was indicated by the formation of a bright yellow precipitate [13].

Flavonoids (Lead Acetate Test): Ten milliliters of leaf extract were mixed with a few drops of 1 mL lead acetate solution. The presence of flavonoids was indicated by the yellow appearance of the precipitate [14].

Anthraquinones: Powdered extracts were shaken with 10 mL of benzene. The solution was filtered, and 5 mL of 10% NH_4OH solution was added to the filtrate. The presence of anthraquinones was indicated by a pink, crimson, or violet hue in the ammoniacal (lower) phase [15].

Tannins (ferric test): Powdered extract was mixed with 10 mL of hot distilled water, filtered and ferric chloride was added to the filtrate. The formation of a blue-black, blue-green, or green precipitate indicated the presence of tannins [15].

Steroids (Salkowski test): The crude extract was thoroughly mixed with chloroform and a few drops of concentrated H_2SO_4 and left to stand. The appearance of a crimson lower layer indicated the presence of steroids [15].

Phlobatannins (HCl test): Phlobatannins were detected by boiling an aqueous extract with 1% hydrochloric acid, which resulted in the formation of a crimson precipitate [15].

Glycosides (bromine water test): A few milliliters of bromine water were added to the plant extract. The presence of glycosides was indicated by the formation of a yellow precipitate [16].

Saponins: One milliliter of plant extract was boiled with 10 mL of distilled water, then filtered through Whatman filter paper. Two milliliters of distilled water were mixed with 5 mL of the filtrate, and the mixture was shaken vigorously. The presence of saponins was indicated by the formation of a stable, persistent foam [17].

Terpenoids (Salkowski test): The extract was shaken with concentrated sulfuric acid. The presence of a golden yellow layer at the bottom of the filtrate indicated possible terpenoids [18]. Phytochemical screening of the leaf extracts confirmed the presence of several key secondary metabolites, including alkaloids, flavonoids, glycosides, steroids, tannins, terpenoids, and saponins. However, anthraquinones and phlobatannins were absent in all extracts. Among the solvents tested, methanol and acetone produced extracts with the richest and most diverse phytochemical profiles, which may explain their comparatively stronger antimicrobial activity.

STATISTICAL ANALYSIS

The results of this study were expressed as mean±standard deviation. One-way ANOVA was performed to determine statistical significance, with p-value <0.05 considered significant, using Excel's Data Analysis Toolpak.

RESULTS

The study found that the yield of *Nyctanthes arbor-tristis* leaf extracts varied across different solvents, likely due to differences in solvent polarity used in the Soxhlet extraction process [Table/Fig-5]. The aqueous extract produced the highest yield, followed by acetone, while ethyl acetate produced the lowest yield. These differences in extraction efficiency are likely attributable to the varying solubility of phytochemicals in solvents of differing polarity.

The antimicrobial potential of the extracts was evaluated using the well diffusion method, complemented by MIC and MBC assays. Each extract was tested at two concentrations: 0.5 mg/100 µL and 1 mg/100 µL. Chloramphenicol was used as the positive control for bacterial cultures. The highest ZOI were measured as follows: 0.5 mg/100 µL—32 mm for *Salmonella* Newport (MTCC 3225), *Escherichia coli* (MTCC 723), and *Staphylococcus aureus* subsp. *aureus* (MTCC 740); 1 mg/100 µL—36 mm for *E. coli* (MTCC 723). Fluconazole was used as the positive control for fungal cultures, with the highest zones measured as 34 mm at 0.5 mg/100 µL and for *Candida albicans* (MTCC 3017) at 1 mg/100 µL is 37 mm [Table/Fig-6].

Maximum ZOI against all pathogenic strains were observed with the methanol extract of *N. arbor-tristis* leaves, whereas the minimum ZOI was recorded with the aqueous extract [Table/Fig-7]. At a concentration of 0.5 mg/100 µL of methanol extract, MTCC 3225 (*Salmonella* Newport) exhibited the highest ZOI (23.6±0.57 mm), followed by MTCC 723 (*Escherichia coli*) (21.6±1.52 mm), MTCC 740 (*Staphylococcus aureus* subsp. *aureus*) (19.6±0.57 mm), and MTCC 3017 (*Candida albicans*) with the lowest ZOI (14±1 mm).

At a concentration of 1 mg/100 µL, the methanol extract showed the highest ZOI against MTCC 3225 (24.6±0.57 mm), followed by MTCC 723 (22.6±0.57 mm), and the lowest ZOI against MTCC 3017 (19±0 mm) and MTCC 740 (22.6±0.57 mm) [Table/Fig-8-11].

The aqueous extract (0.5 mg/100 µL) was highly effective against MTCC 3225 (20±0 mm), moderately effective against MTCC 740 and MTCC 723 (16±0 mm and 15±0 mm, respectively), and ineffective against MTCC 3017, which showed no zone of inhibition. At 1 mg/100 µL, the aqueous extract was most effective against

Phytochemicals	Phytochemical tests	Extracts Ethyl acetate	Chloroform	Acetone	Methanol	Aqueous
Alkaloids	Hager's test	+	+	-	+	+
Flavonoids	Lead acetate test	+	+	+	+	-
Anthraquinones	-	-	-	-	-	-
Glycosides	Bromine water test	+	+	+	+	+
Tannins	Ferric chloride test	+	+	+	+	+
Steroids	Salkowski test	+	+	+	+	+
Saponins	Foam test	+	+	+	+	+
Pholobatannins	HCL test	-	-	-	-	-
Terpenoids	Salkowski test	+	+	+	+	+

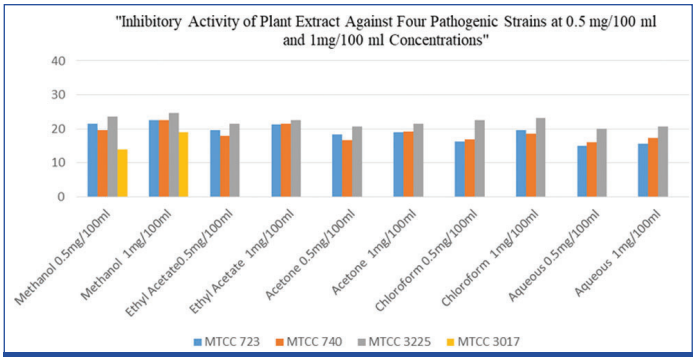
[Table/Fig-4]: Comprehensive phytochemical analysis of leaf extracts from *Nyctanthes arbor-tristis* for the presence of various phytochemicals.

S. No.	Solvent used	Polarity index	Colour	Yield %
1	Chloroform	4.1	Dark green	23.2
2	Acetone	5.1	Transparent green	34.76
3	Ethyl acetate	4.4	Light green	15.6
4	Methanol	5.1	Dark green	22
5	Aqueous	9	Very dark green	38

[Table/Fig-5]: Polarity index and extraction yield of *Nyctanthes arbor-tristis* leaf extracts.

Pathogen	Strain name	AB/AF	Concentration	
			0.5 mg/ 100 µL	1 mg/ 100 µL
Bacteria	*Salmonella* Newport (MTCC 3225)	Chloramphenicol (Antibiotic)	32	35
Bacteria	*Escherichia coli* (MTCC 723)	Chloramphenicol (Antibiotic)	32	36
Bacteria	*Staphylococcus aureus* Subsp. *aureus* (MTCC 740)	Chloramphenicol (Antibiotic)	32	34
Fungus	*Candida albicans* (MTCC 3017)	Fluconazole (Antifungal)	34	37

[Table/Fig-6]: Susceptibility of pathogenic strains to chloramphenicol and fluconazole at concentrations of 0.5 mg/100 µL and 1 mg/100 µL.



[Table/Fig-7]: Antimicrobial inhibitory activity of *Nyctanthes arbor-tristis* extract against four pathogenic strains.

Solvents	AST (0.5)±SD	p-value (0.5 mg/100)	F value (0.5)	AST (1.0)±SD	p-value (1.0)	F value (1.0)
Methanol	23.6±0.57			24.6±0.57		
EA	21.6±0.57			22.6±0.57		
Acetone	20.6±0.57	0.000036	24.625	21.6±0.57	0.000071	21.2
Chloroform	22.6±0.57			23.3±0.57		
Aqueous	20±0			20.6±0.57		

[Table/Fig-8]: Resistance profile of pathogenic bacteria MTCC 3225 to leaves extract of NAT at concentrations of 0.5 mg/100 µL and 1 mg/100 µL. (Excel's Data Analysis Toolpak) Test applied- One=way ANOVA.

Solvents	AST (0.5)±SD	p-value (0.5)	F value (0.5)	AST (1.0)±SD	p-value (1.0)	F value (1.0)
Methanol	21.6±1.52			22.6±0.57		
E.A	19.6±1.52			21.3±1.15		
Acetone	18.3±1.54	0.0002	16.52	19±0	0.000002	45.3
Chloroform	16.3±0.57			19.6±0.57		
Aqueous	15±0			15.6±0.57		

[Table/Fig-9]: Resistance profile of pathogenic bacteria MTCC 723 to leaves extract of NAT at concentrations of 0.5 mg/100 µL and 1 mg/100 µL.
Test applied- One-way ANOVA

MTCC 3225 (20.6±0.57 mm), followed by MTCC 740 (17.3±0.57 mm) and MTCC 723 (15.6±0.57 mm), while MTCC 3017 remained unaffected. Except for the fungal strain MTCC 3017, all crude extracts demonstrated notable effectiveness against the tested pathogenic microorganisms [Table/Fig-4]. The methanolic extracts

Solvents	AST (0.5)±SD	p-value (0.5)	F value (0.5)	AST (1.0)±SD	p-value (1.0)	F value (1.0)
Methanol	19.6±0.57			22.6±0.57		
EA	18±1			21.6±0.57		
Acetone	16.6±0.57	0.00013	18.3	19.3±0.57	0.000003	43.2
Chloroform	17±0			18.6±0.57		
Aqueous	16±0			17.3±0.57		

[Table/Fig-10]: Resistance profile of pathogenic bacteria MTCC 740 to leaves extract of NAT at concentrations of 0.5 mg/100 µL and 1 mg/100 µL.
Test applied- One-way ANOVA

Solvents	AST (0.5)±SD	p-value (0.5)	F value (0.5)	AST (1.0)±SD	p-value (1.0)	F value (1.0)
Methanol	14±1			19±0		
EA	Null			Null		
Acetone	Null	Null	Null	Null	Null	Null
Chloroform	Null			Null		
Aqueous	Null			Null		

[Table/Fig-11]: Resistance profile of pathogenic fungus MTCC 3017 to leaves extract of NAT at concentrations of 0.5 mg/100 µL and 1 mg/100 µL.
Test applied- One-way ANOVA

consistently demonstrated the strongest antimicrobial activity across all tested microbial strains, with *Salmonella enterica* serovar Newport (MTCC 3225) exhibiting the highest sensitivity. Conversely, the aqueous extract showed minimal effectiveness, particularly against *Candida albicans* (MTCC 3017), underscoring the critical role of solvent selection in determining antimicrobial outcomes.

MIC and MBC: MIC is defined as the lowest concentration of an antibiotic or test sample required to inhibit bacterial growth. The MIC and MBC of the control extracts were 0.0156 mg/mL and 0.0312 mg/mL, respectively. For all bacterial strains, the MIC index of the extracts and controls was 2.0. The highest ZOI was observed with methanol extract, followed by ethyl acetate, chloroform, acetone and aqueous extracts. MBC values ranged from 0.0312 to 0.5 mg/mL [Table/Fig-12-15]. The MIC index was used to determine whether an extract was bacteriostatic (>4) or bactericidal (<4).

Qualitative analysis of phyto-constituents in *Nyctanthes arbor-tristis*: Preliminary qualitative phytochemical screening of *N. arbor-tristis* leaf extracts in various solvents revealed the presence of secondary metabolites. Analysis detected tannins, glycosides, saponins, alkaloids, steroids, terpenoids and flavonoids in the extracts.

Extracts (NAT Leaves)	MIC Control (mg/mL)	MIC Extract (mg/mL)	MBC Control (mg/mL)	MBC Extract (mg/mL)	MIC Index (Control)	MBC Index (Extract)
Methanol	0.0156	0.0156	0.0312	0.0312	2	2
EA	0.0156	0.125	0.0312	0.25	2	2
Acetone	0.0156	0.125	0.0312	0.25	2	2
Chloroform	0.0156	0.0625	0.0312	0.125	2	2
Aqueous	0.0156	0.25	0.0312	0.5	2	2

[Table/Fig-12]: NAT extracts: MIC, MBC, and MIC index values against *Salmonella* Newport (MTCC 3225).

Extracts (NAT Leaves)	MIC Control (mg/mL)	MIC Extract (mg/mL)	MBC Control (mg/mL)	MBC Extract (mg/mL)	MIC Index (Control)	MBC Index (Extract)
Methanol	0.0156	0.0625	0.0312	0.125	2	2
EA	0.0156	0.0625	0.0312	0.125	2	2
Acetone	0.0156	0.125	0.0312	0.25	2	2
Chloroform	0.0156	0.125	0.0312	0.25	2	2
Aqueous	0.0156	0.25	0.0312	0.5	2	2

[Table/Fig-13]: NAT extracts: MIC, MBC, and MIC index values against *Escherichia coli* (MTCC 723).

Extracts (NAT Leaves)	MIC Control (mg/mL)	MIC Extract (mg/mL)	MBC Control (mg/mL)	MBC Extract (mg/mL)	MIC Index (Control)	MBC Index (Extract)
Methanol	0.0156	0.0625	0.0312	0.125	2	2
EA	0.0156	0.125	0.0312	0.25	2	2
Acetone	0.0156	0.25	0.0312	0.5	2	2
Chloroform	0.0156	0.125	0.0312	0.25	2	2
Aqueous	0.0156	0.25	0.0312	0.5	2	2

[Table/Fig-14]: NAT extracts: MIC, MBC, and MIC index values against *Staphylococcus aureus* Subsp. *aureus* (MTCC 740).

EXTRACTS (NAT Leaves)	MIC Control (mg/mL)	MIC Extract (mg/mL)	MBC Control (mg/mL)	MBC Extract (mg/mL)	MIC Index (Control)	MBC Index (Extract)
Methanol	0.0156	0.0625	0.0312	0.125	2	2
E.A	null	null	null	null	null	null
Acetone	null	null	null	null	null	null
Chloroform	null	null	null	null	null	null
Aqueous	null	null	null	null	null	null

[Table/Fig-15]: NAT Extracts: MIC, MBC, and MIC Index Values against *Candida albicans* (MTCC 3017).

DISCUSSION

This study investigated the antimicrobial and phytochemical properties of *Nyctanthes arbor-tristis* leaf extracts using various solvents. The findings are consistent with recent research, highlighting the plant's significant antibacterial activity, particularly in methanol extracts, while noting limited antifungal effects.

Methanol extracts exhibited the highest antibacterial efficacy, with a maximum ZOI of 24.6 mm against *Salmonella* Newport (MTCC 3225) at a concentration of 1 mg/100 µL.

The observed antibacterial potency of methanol extracts is likely due to the solvent's ability to extract a wide range of bioactive compounds, including alkaloids, flavonoids and tannins, which were identified in previous phytochemical screenings of *N. arbor-tristis* leaves. These compounds are well-known for their antimicrobial properties, contributing to the observed antibacterial activity.

In contrast, present study observed limited antifungal activity against *Candida albicans* (MTCC 3017), with no significant inhibition in aqueous extracts and minimal activity in methanol extracts. This differs from the findings of [19], who reported significant antifungal effects of *N. arbor-tristis* against *Candida* species using both methanol and aqueous extracts. The discrepancy may be attributed to variations in experimental conditions, including differences in extract concentrations, incubation periods, and fungal strain variations, all of which can influence antifungal efficacy.

Phytochemical analysis revealed the presence of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, glycosides, steroids and terpenoids in the leaf extracts of *N. arbor-tristis*. These compounds are known for various pharmacological activities, including antimicrobial effects. The variation in antimicrobial activity across different solvent extracts may be due to the differing solubility of these compounds in the solvents used, highlighting the importance of solvent selection in the extraction process to obtain extracts with desired bioactivities.

Comparative studies have shown that methanol extracts of *N. arbor-tristis* consistently exhibit strong antibacterial activity. For example, [20] reported significant antibacterial effects of methanol extracts against various bacterial strains, supporting our findings. However, the limited antifungal activity observed in our study aligns with the findings of [21], who noted that *N. arbor-tristis* flower extracts showed moderate antibacterial activity but no significant antifungal effects.

The antibacterial activity of *N. arbor-tristis* leaf extract suggests its potential as an alternative to conventional antibiotics, particularly in the context of growing antibiotic resistance. While the extract showed limited antifungal effects, further refinement of its formulation could improve efficacy against fungal infections. Clinical studies are needed to assess safety and appropriate dosage for human use. Overall, *N. arbor-tristis* may offer a valuable addition to current antimicrobial treatments, particularly for bacterial infections.

This study demonstrates the notable antimicrobial potential of *N. arbor-tristis* leaf extract, showing significant activity against bacterial strains, while exhibiting limited efficacy against fungal pathogens. The promising antibacterial results indicate that further research is necessary to elucidate the mechanisms underlying its antimicrobial properties. Optimisation of extraction techniques and evaluation of different concentrations could enhance the therapeutic utility of the extract. Additionally, the phytochemical analysis conducted in this study identified bioactive compounds that warrant further exploration.

Future investigations should focus on isolating and characterising these compounds, as well as assessing their individual and synergistic effects on microbial targets. Given the extract's partial antifungal activity, refining its formulation or exploring different treatment regimens may improve efficacy. In conclusion, *N. arbor-tristis* holds potential as a natural antimicrobial agent, with future research potentially contributing to the development of novel antibacterial therapies and more effective antifungal interventions.

Limitation(s)

While this study provides valuable insights into the antimicrobial properties of *N. arbor-tristis*, certain limitations must be acknowledged. The antifungal activity was limited, particularly against *Candida albicans*, which could be due to factors such as extract concentration, fungal resistance, or differences in experimental conditions. Future studies should explore the use of different solvents or optimise extraction methods to enhance antifungal activity. Additionally, in-vivo studies are needed to assess the safety and therapeutic efficacy of the extract. Further research focusing on a broader spectrum of pathogens and detailed isolation of active compounds could provide a more comprehensive evaluation of the plant's antimicrobial potential.

CONCLUSION(S)

The present study concluded that the methanol extract of *N. arbor-tristis* exhibited the highest ZOI values among the five solvent extracts tested (aqueous, acetone, chloroform, ethyl acetate and methanol) and was effective in suppressing the growth of pathogenic bacterial strains. The only fungal strain tested, *Candida albicans* (MTCC 3017), showed limited activity, with positive results observed only for the methanol extract. These findings highlight the potential of *N. arbor-tristis* for developing a wide range of herbal pharmacological formulations with a high safety profile containing important bioactive compounds.

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REFERENCES

[1] World Health Organization. Antimicrobial resistance Geneva: WHO; 2020 [cited 2025 Apr 15]. Available from: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.
[2] Gurung S, Bhattarai DR, Shrestha R. Medicinal plants as antimicrobial agents: Recent developments and future perspectives. Front Pharmacol. 2022;13:855233.
[3] Kumar D, Sharma S, Singh M. Ethnomedicinal applications and phytochemical composition of *Nyctanthes arbor-tristis*: A comprehensive review. J Ethnopharmacol. 2021;268:113636.
[4] Gahtori R, Tripathi AH, Chand G, Pande A, Joshi P, Rai RC, et al. Phytochemical screening of *Nyctanthes arbor-tristis* plant extracts and their antioxidant and antibacterial activity analysis. Appl Biochem Biotechnol. 2023;196(1):436-56.

[5] Nag M, Islam M, Biswas S, Alam F, Dasgupta D, Judder M, et al. Anti-inflammatory activity of ethanol leaf extracts of *nyctanthes arbor-tristis*. YMER Digital. 2023;22(11):753-66. Doi: 10.37896/YMER22.11/53.
[6] Akki KS, Akki P, Jawarkar S, Tiwari VK, Chavhan G, Wagh H, et al. Hepatoprotective and antioxidant effect of *Nyctanthes arbor–tristis* leaf fractions against CCl4-induced liver injury in rats. J Adv Zool. 2023;44(S6). Doi: 10.17762/jaz.v44iS6.2158.
[7] Goswami P, Singh V, Koch B. Mitochondria mediated inhibitory effect of *Nyctanthes arbor-tristis* (L.) flower extract against breast adenocarcinoma and T-cell lymphoma: An in vitro and in vivo study. J Ethnopharmacol. 2024;334:118537. Doi: 10.1016/j.jep.2024.118537. Epub 2024 Jul 9. PMID: 38992400.
[8] Sharma A, Patel S. Comparative analysis of antibacterial activity of methanolic & aqueous extracts of *Nyctanthes arbor-tristis* against *Xanthomonas citri* and *Xanthomonas campestris*. Int J Innov Res Technol. 2020;8(1):1338-43.
[9] Sawhney SS, Painuli RM, Chauhan N. Evaluation of bactericidal and anticancer properties of fruits of *Piper longum*. Int J Pharm Sci. 2011;3:282-87.
[10] National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, 5th ed. M7-A5. Wayne (PA): NCCLS; 2000.
[11] Wayne PA. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 11th ed. CLSI standard M07. Wayne (PA): Clinical and Laboratory Standards Institute; 2018.
[12] Radhakrishnan N, Gnanamani A, Mandal AB. A potential antibacterial agent Embelin, a natural benzoquinone extracted from *Embelia ribes*. Biol Med. 2011;3(2):01-07.
[13] Archana P, Samatha T, Mahitha B, Chamundeswari NR. Preliminary phytochemical screening from leaf and seed extracts of *Senna alata* L. Roxb-an ethnomedicinal plant. Int J Pharm Biol Res. 2012;3(3):82-89.
[14] Deshmukh MA, Theng MA. Phytochemical screening, quantitative analysis of primary and secondary metabolites of *Acacia arabica* bark. Int J Curr Pharm Res. 2018;10(2):35-37.
[15] Sharma A, Patel S. Preliminary phytochemical analysis of methanolic and aqueous extract of medicinal plant-*Nyctanthes arbor-tristis* Linn. World J Pharm Pharm Sci. 2016;5(11):1393-401.
[16] Ray S, Chatterjee S, Chakrabarti CS. Antiproliferative activity of allelochemicals present in aqueous extract of *Synedrella nodiflora* (L.) Gaertn. in apical meristems and Wistar rat bone marrow cells. IOSR J Pharm. 2013;3(2):01-10.
[17] Rajani KS, Khandeparker Naik S, Satapathy S, Mishra S. Phytochemical analysis of the leaf extracts of *Nyctanthes arbor-tristis*. Plant Arch. 2021;21(1):1878-81.
[18] Singh V, Kumar R. Study of phytochemical analysis and antioxidant activity of *Allium sativum* of Bundelkhand region. Int J Life Sci Sci Res. 2017;3(6):1451-58.
[19] Singh AK, Sharma J. Evaluating antimicrobial activity of extract from the leaves of Parijat-*Nyctanthes arbortristis* on bacteria and fungi. Int J Novel Res Dev. 2024;9(1):c665-c671. Available from: https://ijnrd.org/papers/IJNRD2401283.pdf.
[20] Peddinti R. Phytochemical, antibacterial and in vitro antioxidant analysis of *Nyctanthes arbor-tristis* leaves. Int J Pharm Sci Invention. 2020;9(1):06-09.
[21] Srinivasan KK, Goomber A, Kumar SS, Thomas AT, Joseph A. Phytochemical, antioxidant and antimicrobial study of flowers of *Nyctanthes arbor-tristis* Linn. Pharmacologyonline. 2011;2:16-21.

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