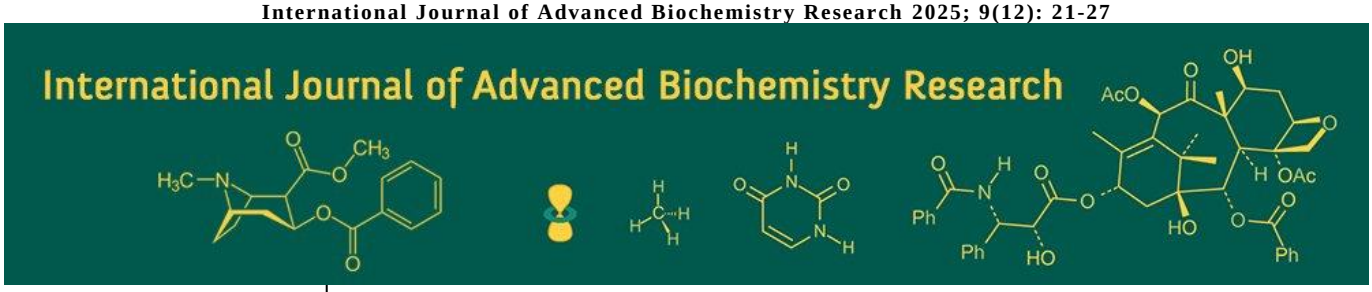

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Unveiling the bactericidal potential of *Nyctanthes arbor-tristis* (Parijath): A potent broad-spectrum bactericidal agent against *Cutibacterium acnes, Vibrio vulnificus, Aeromonas hydrophila,* and *Escherichia coli*

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Abstract
The escalating threat of antimicrobial resistance demands novel, plant-derived therapeutics. This study evaluated methanolic and ethanolic extracts of shade- and oven-dried leaves and flowers of Nyctanthes arbor-tristis Linn. (Parijat) against clinically important pathogens: *Cutibacterium acnes, Vibrio vulnificus, Aeromonas hydrophila,* and *Escherichia coli*. Among three extracts (NAT-SLM: shade-dried leaf methanol; NAT-OLE: oven-dried leaf ethanol; NAT-SFM: shade-dried flower methanol), NAT-SFM exhibited the strongest activity. Disc diffusion zones ranged from 14.2±0.5 mm (E. coli) to 18.7±0.7 mm (C. acnes). Minimum inhibitory concentrations (MIC) of NAT-SFM were remarkably low (6.25-25 µg/mL), with MBC/MIC ratios ≤4 confirming bactericidal action. Time-kill kinetics demonstrated rapid killing, achieving ≥4.4-log$_{10}$ reductions within 24 h at 1-2× MIC across all strains. Phytochemical screening revealed high abundance of phenols/tannins and flavonoids in NAT-SFM. GC-MS analysis identified major bioactive compounds including myristic acid (4.57%), palmitic acid (4.48%), hexadecanol (3.08%), and 1-chloro-tetradecanamine (3.38%), known for membrane disruption, FabI inhibition, and efflux-pump blockade. Shade-drying and floral material proved superior to oven-drying and leaves, preserving heat-labile polyphenols and fatty acid derivatives. These findings validate traditional Ayurvedic use of Parijat flowers and position NAT-SFM as a promising natural alternative or adjuvant for acne therapy, wound infections, and aquaculture disease management, offering a sustainable solution against multidrug-resistant pathogens.

Keywords: Nyctanthes arbor-tristis, NAT-SFM, bactericidal, *Cutibacterium acnes*, fatty acids, antimicrobial resistance

Introduction
The escalating crisis of antimicrobial resistance (AMR) poses a profound threat to global public health, with the World Health Organization estimating that bacterial infections claim over 700,000 lives annually, a figure projected to surge to 10 million by 2050 without novel interventions (WHO, 2020). Conventional antibiotics, once hailed as miracle drugs, are increasingly rendered obsolete by multidrug-resistant (MDR) pathogens such as *Escherichia coli*, *Vibrio vulnificus*, *Aeromonas hydrophila*, and *Cutibacterium acnes*-strains implicated in urinary tract infections, wound sepsis, aquaculture diseases, and acne vulgaris, respectively. This dire scenario underscores the urgent need for sustainable, plant-derived antimicrobials that can combat resistance while minimizing toxicity and side effects. Medicinal plants, revered in traditional systems like Ayurveda, offer a treasure trove of bioactive compounds with multifaceted mechanisms, including membrane disruption, quorum sensing inhibition, and efflux pump modulation (Aggarwal *et al*., 2011; Agrawal & Pal, 2013) [2, 4]. Among these, *Nyctanthes arbor-tristis* Linn. (Family: Oleaceae), popularly known as Parijat or Night-flowering Jasmine, stands out as a versatile ethnomedicinal powerhouse. Native to the Indian subcontinent and revered in Ayurvedic texts like the *Charaka Samhita*, Parijat has been traditionally employed for treating fever, arthritis, skin infections, and respiratory ailments (Agrawal & Pal, 2013) [4]. Its nocturnal-blooming flowers and deciduous leaves are rich in secondary metabolites, including iridoid glycosides, flavonoids, phenolics, terpenoids, and alkaloids, which confer broad-spectrum bioactivity (Banerjee *et al*., 2007; Ramachandran *et al*., 2014) [8, 32]. Pharmacological studies have validated these uses: Verma *et al*. (2011) [38] demonstrated potent antibacterial activity of root bark extracts against

Gram-positive and Gram-negative bacteria, while Aggarwal and Goyal (2013) [3] and Manisha *et al*. (2009) [27] reported inhibition of pathogenic strains via membrane permeabilization. Floral extracts exhibit superior efficacy, as shown by Khatune *et al*. (2001) [26] and Jose *et al*. (2016) [22], attributed to high flavonoid content that scavenges free radicals and disrupts bacterial biofilms (Rathee *et al*., 2007; Michael *et al*., 2013) [33, 28]. Beyond standalone activity, *N.arbor-tristis* shows promise as an antibiotic adjuvant. Isaac *et al*. (2017) [21] identified antiquorum sensing metabolites that enhance antibiotic penetration, while Chatterjee *et al*. (2007) [9] highlighted its edge over other Indian herbs. Synergistic studies, though limited, reveal additive effects with tetracycline and ciprofloxacin (Altuner *et al*., 2018; Vilekar *et al*., 2014) [5, 39]. Antioxidant and anti-inflammatory properties further amplify its therapeutic value: Ghosh *et al*. (2015) [17] isolated a water-soluble radical scavenger from leaves, Dhinakaran and Sakthivel (2016) [13] confirmed cytokine modulation, and Aparna *et al*. (2012) [7] elucidated n-hexadecanoic acid's role in NFκB inhibition. Anticancer potential against leukemia cells (Heendeniya *et al*., 2020) [19] and immunostimulant effects (Puri *et al*., 1994) broaden its scope, aligning with Ayurveda's -reverse pharmacology paradigm (Aggarwal *et al*., 2011) [2]. Despite these advances, critical gaps persist. Most studies focus on leaf or root extracts, neglecting floral contributions (Khanapur *et al*., 2014; Saha *et al*., 2012) [25, 34]. Few address clinically relevant strains like *C. acnes* (acne) and aquatic pathogens (*V. vulnificus*, *A. hydrophila*), which thrive in tropical climates like India (Furqan *et al*., 2021) [15]. Synergistic interactions with frontline antibiotics remain underexplored, particularly via fractional inhibitory concentration indices (FICI) (Odds, 2003) [29]. Moreover, extraction variables, solvent type, drying method and time-kill dynamics are inconsistently reported, hindering standardisation (Yuan *et al*., 2015; Abubakar & Haque, 2020) [41, 1]. Phytochemical profiling via GC-MS is sparse for flowers (Dhivya & Manimegalai, 2013) [14], and correlations between bioactives and activity are rarely quantified (Sasikumar *et al*., 2010; Mary & Merina, 2021) [35]. This study bridges these gaps by evaluating methanolic and ethanolic extracts of shade- and oven-dried *N. arbor-tristis* leaves and flowers against *C. acnes*, *V. vulnificus*, *A. hydrophila*, and *E. coli*. Specific objectives include: (1) assessing antimicrobial activity via disc diffusion, MIC/MBC, and time-kill kinetics; (2) determining synergistic effects with tetracycline and ciprofloxacin using checkerboard assays; (3) conducting preliminary phytochemical screening and GC-MS profiling; and (4) correlating bioactives with efficacy using statistical analyses. Plant materials were collected from Bangalore, India, authenticated, and processed per standardized protocols (CLSI, 2018). The significance of this research is multifaceted. In an era of AMR, *N. arbor-tristis* could yield cost-effective adjuvants for acne therapies, wound care, and aquaculture, reducing antibiotic reliance (Alves *et al*., 2021; Ghagane *et al*., 2017) [6, 16]. By validating Ayurvedic claims scientifically (Dinamani & Rao, 2009; Paul *et al*., 2002) [30], it promotes biodiversity conservation and sustainable harvesting. Phylogenetic tools like MEGA X (Kumar *et al*.) and matK barcoding ensure authenticity, while sequence analysis methods (Tamura, 1992; Jones *et al*., 1992; Thompson *et al*., 1999; Ogden & Rosenberg, 2006) support genetic insights. Ultimately, this work paves the way for clinical translation, fostering integrative medicine and addressing AMR through nature's pharmacy (Nirmal *et al*., 2012; Pandey, 2012; Parul *et al*., 2012; Patel & Patel, 2011; Sharma & Samanta, 2011; Sudha & Srinivasan, 2014; Hidajati & Setiabudi, 2018; Mahato & Sharma, 2019) [20, 43].

Materials and Methods

Collection and Identification of Plant Material

Fresh and mature leaves and flowers of *N. arbor-tristis* L. (Family: Oleaceae) were collected between January and March 2024 from the cultivated medicinal plant garden of Vriksha Vihan Pvt. Ltd., Bangalore, Karnataka, India (12.9716° N, 77.5946° E). Harvesting occurred during early morning and late afternoon to optimize secondary metabolite concentration and minimize enzymatic degradation, as supported by studies on seasonal phytoconstituent variation (Yuan *et al*., 2015) [41]. Plants were selected based on healthy morphological features, with no more than 20% foliage removed per plant to ensure sustainability.

Pre-Treatment and Drying of Plant Material

Post-collection, plant materials were rinsed under running tap water to remove soil and debris, treated with 70% ethanol for 30 seconds to eliminate microbial contaminants, and triple-rinsed with sterile distilled water (Abubakar & Haque, 2020) [1]. Excess moisture was removed using sterile blotting paper. Two drying methods were employed: shade drying, where plant parts were spread on sterile muslin sheets in a well-ventilated room at 28-32 °C for 10-12 days with regular turning, and oven drying at 40 °C for 48 hours to preserve phytochemicals (Yuan *et al*., 2015) [41]. Dried materials were pulverized into coarse powder using a stainless-steel grinder, sieved (mesh size 60) for uniform particle size, and stored in airtight amber containers in a desiccator at 25±2 °C until extraction.

Preparation of Crude Extracts

The crude extracts were prepared from *N. arbor-tristis* leaves and flowers using methanol or ethanol. The extraction matrix included NAT-SLM (shade-dried leaves, methanol), NAT-OLE (oven-dried leaves, ethanol), and NAT-SFM (shade-dried flowers, methanol). Each extract was prepared by macerating 10 g of powdered material in 100 mL of solvent in sterile conical flasks, incubated at 25±2 °C for 72 hours with intermittent shaking every 8 hours (Abubakar & Haque, 2020) [1]. Extracts were filtered using Whatman No.1 filter paper and concentrated using a rotary vacuum evaporator (Buchi Rotavapor R-300) at 40-45 °C under reduced pressure until semi-solid, with yields determined by weight.

Bacterial Strains and Culture Conditions

Antimicrobial activity was evaluated against four bacterial strains: *Cutibacterium acnes* (MTCC 1951), *Vibrio vulnificus* (MTCC 1145), *Aeromonas hydrophila subsp. hydrophila* (MTCC 1739), and *Escherichia coli* (MTCC 1687), procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India. Strains were revived in nutrient broth at 37 °C overnight under sterile conditions, with subcultures maintained on slants at 4 °C and reactivated before testing.

Antimicrobial Activity Assessment

The antimicrobial potential of extracts was assessed using a modified Kirby-Bauer disc diffusion method (Choyam *et al*., 2015) [10]. Mueller-Hinton Agar (MHA) plates were inoculated with 100 µL of 4-hour bacterial cultures adjusted to 0.5 McFarland standard. Sterile 6 mm paper discs were impregnated with 20 µL of extract (100 µg/mL stock concentration) and placed on the agar surface. Plates were pre-incubated at 4 °C for 20 minutes to ensure diffusion, followed by incubation at 37 °C for 24 hours. ZOI were measured in millimetres, with tests conducted in triplicate for reproducibility.

Phytochemical Screening

Preliminary phytochemical screening of the extracts followed standard protocols with modifications (Kancherla *et al*., 2019) [24]. Tests included: saponins (persistent foam upon shaking in water), terpenoids (reddish-brown ring with chloroform and H_2SO), phenols/tannins (blue-black coloration with 2% ferric chloride; Takó *et al*., 2020) [36], flavonoids (yellow-to-colorless shift with NaOH and acidification; Ullah *et al*., 2020; Hasnat *et al*., 2024) [37, 11], sterols (violet-green ring with sulfuric acid), quinones (yellow precipitate with HCl), cardiac glycosides (brown-to-violet/green rings with glacial acetic acid and ferric chloride), and proteins (yellow coloration with nitric acid).

MIC and MBC

The MIC and MBC of Nyctanthes arbor-tristis extracts (NAT-SLM, NAT-OLE, NAT-SFM) were determined using the broth microdilution method, following CLSI guidelines (CLSI, 2018). Bacterial strains were cultured in Mueller-Hinton broth (MHB) to a 0.5 McFarland standard (1.5×10^8 CFU/mL). Extracts were dissolved in 5% DMSO to prepare stock solutions (1000 µg/mL), and two-fold serial dilutions (0.1-100 µg/mL) were prepared in 96-well microtiter plates containing 100 µL MHB per well. Each well was inoculated with 100 µL of bacterial suspension, yielding a final volume of 200 µL. Positive (bacteria only) and negative (broth only) controls were included. Plates were incubated at 37 °C for 24 hours. The MIC was defined as the lowest extract concentration preventing visible growth, assessed visually and by optical density at 600 nm using a microplate reader. For MBC determination, 10 µL from wells showing no growth was subcultured onto Mueller-Hinton agar (MHA) plates and incubated at 37 °C for 24 hours. The MBC was defined as the lowest concentration reducing bacterial viability by ≥99.9%. Tests were performed in triplicate to ensure reproducibility.

Time-Kill Kinetics

Time-kill assays were conducted to evaluate the bactericidal or bacteriostatic effects of N. arbor-tristis extracts over time, following CLSI guidelines (CLSI, 1999). Bacterial suspensions (C. *acnes*, V. *vulnificus*, A. *hydrophila*, E. *coli*) were adjusted to 0.5 McFarland standard in MHB. Extracts were tested at their MIC and 2× MIC concentrations in 10 mL MHB, inoculated with 100 µL of bacterial suspension (final concentration ~10^6 CFU/mL). Control tubes contained bacteria without extract. Aliquots (100 µL) were sampled at 0, 2, 4, 8, 12, and 24 hours, serially diluted in sterile saline, and plated onto MHA. After incubation at 37 °C for 24 hours, viable colonies were counted, and $\log_{10}$ CFU/mL was plotted against time. Bactericidal activity was defined as a ≥3 $\log_{10}$ reduction in CFU/mL compared to the initial inoculum, while bacteriostatic activity was indicated by <3 $\log_{10}$ reduction. Experiments were conducted in triplicate, and mean values were used to construct time-kill curves.

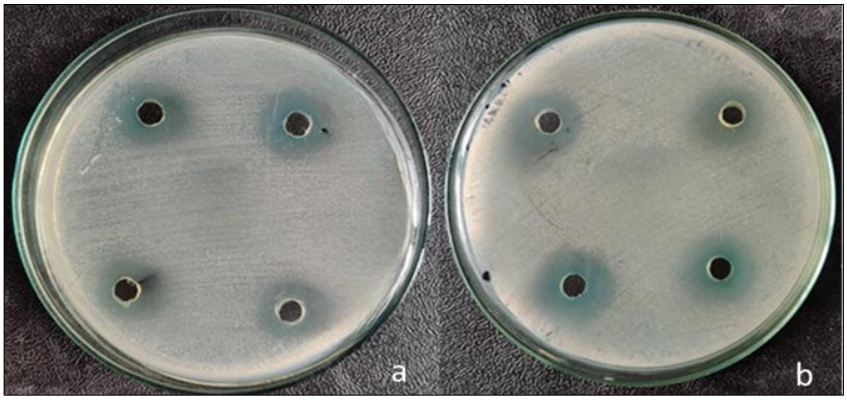
GC-MS Analysis of Phytochemicals

The ethanol extract of *N. arbor-tristis* leaves was analyzed via Gas Chromatography-Mass Spectrometry (GC-MS) following Dhivya & Manimegalai (2013) [14]. Five grams of dried leaf powder underwent Soxhlet extraction with 95% ethanol for 6-8 hours, concentrated using a rotary evaporator, and reconstituted in HPLC-grade hexane. Analysis was performed on an Agilent 7890A system with a DB-5MS capillary column (30 m × 0.25 mm × 0.25 µm), using helium as the carrier gas (1 mL/min) in split mode (1:10). The temperature program started at 60 °C for 2 minutes, ramping to 280 °C at 10 °C/min, with the injector at 250 °C. The mass spectrometer operated at 70 eV, scanning 50-600 m/z. Compounds were identified using the NIST Mass Spectral Library.

Results

Antibacterial Activity by Disc Diffusion Assay

The disc diffusion assay clearly (Figure 1) demonstrated that among the three *Nyctanthes arbor-tristis* extracts, NAT-SFM possessed the most potent antibacterial activity, producing the largest clear zones around the discs. It inhibited *C. acnes* with a zone of 18.7±0.7 mm, *V. vulnificus* with 16.8±0.6 mm, *A. hydrophila* with 15.4±0.5 mm, and *E. coli* with 14.2±0.5 mm. NAT-SLM displayed moderate activity, forming zones of 16.3±0.6 mm against *C. acnes*, 13.5±0.5 mm against *V. vulnificus*, 12.8±0.4 mm against *A. hydrophila*, and 11.8±0.4 mm against *E. coli*.



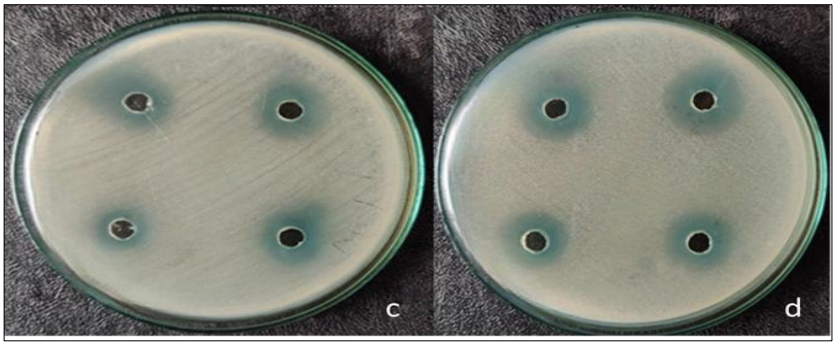


Fig 1: Zones of Inhibition (mm) of *N. arbor-tristis* Extracts against all four Bacterial Strains. a-C. *acnes* (MTCC 1951),b-V. *vulnificus* (MTCC 1145),c-A. *hydrophila*, d-E. *coli.*

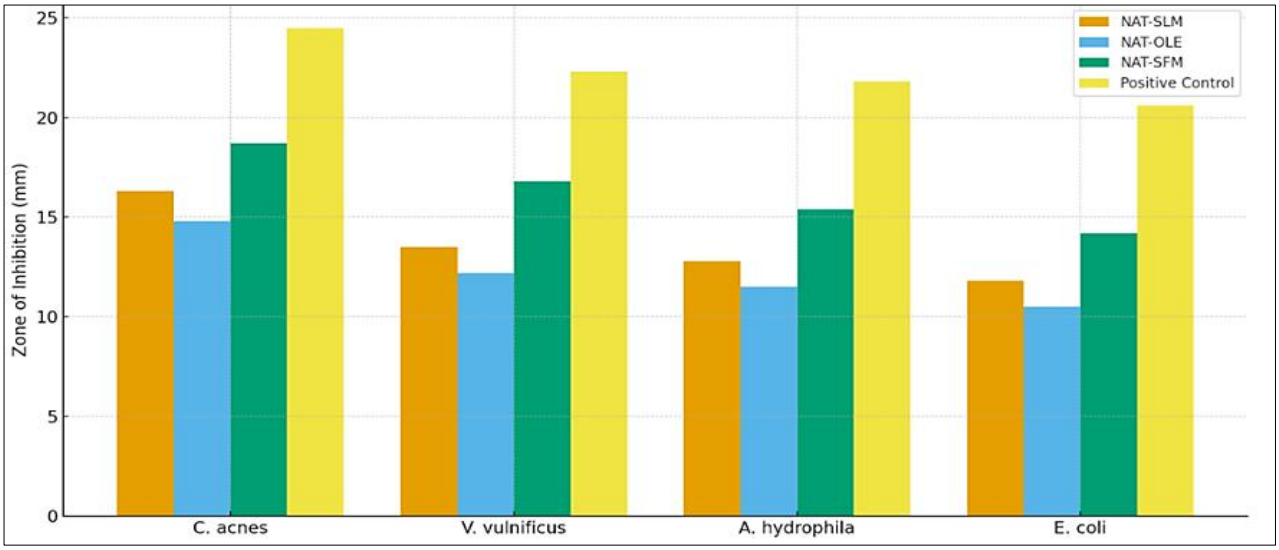


Fig 2: Zones of Inhibition of *N. arbor-tristis* Extracts against Bacterial Strains

In contrast, NAT-OLE exhibited the weakest diffusion-based inhibition, with zones ranging from 10.5±0.3 mm (E. coli) to 14.8±0.5 mm (C. acnes) (Figure 2). The positive control antibiotics (tetracycline for C. acnes and ciprofloxacin for the Gram-negative strains) produced significantly larger zones (20.6-24.5 mm), confirming the validity of the assay and the susceptibility of all tested strains.

Killing Kinetics and Bactericidal Activity

The 24-hour time-kill study revealed (Figure 3) pronounced bactericidal effects of the extracts, with NAT-SFM emerging as the fastest and most effective killer across all pathogens. Starting from an inoculum of 6.0 $\log_{10}$ CFU/mL, NAT-SFM reduced *C. acnes* to 1.5 log (4.5-log reduction), *V. vulnificus* to 2.0 log (4.7-log reduction), *A. hydrophila* to 1.9 log (4.9-log reduction), and *E. coli* to 2.5 log (4.4-log reduction) within 24 hours.

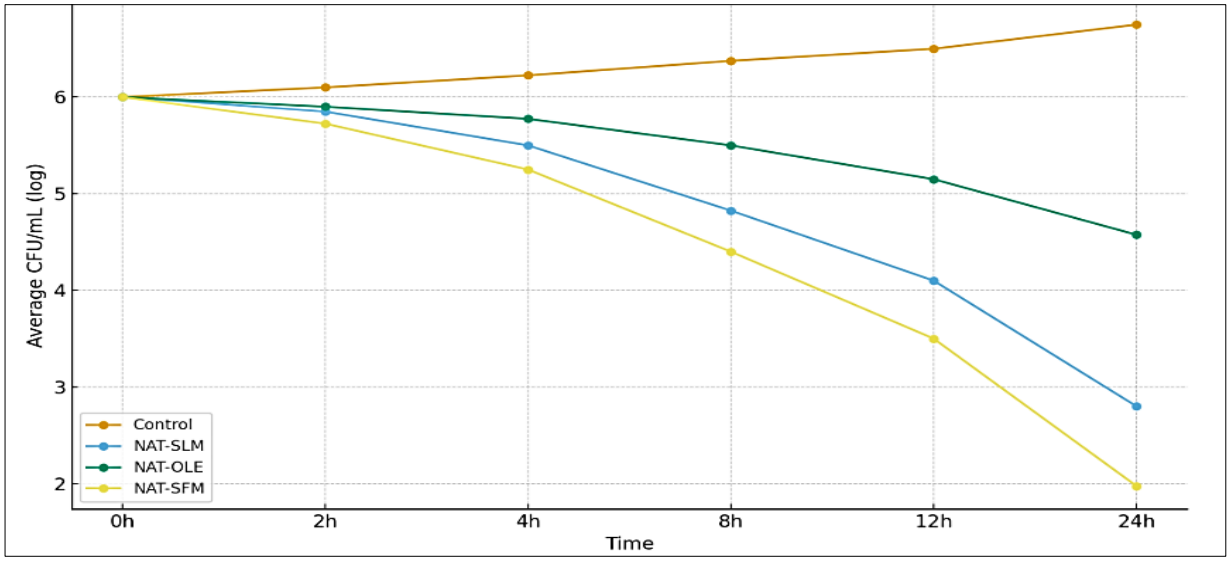


Fig 3: Time-Kill Kinetics of *N. arbor-tristis* Extracts

NAT-SLM also exhibited strong time-dependent killing, achieving final counts of 2.3 log (*C. acnes*), 2.9 log (*V. vulnificus*), 2.7 log (*A. hydrophila*), and 3.3 log (*E. coli*). NAT-OLE showed only bacteriostatic to weakly bactericidal behavior; even at higher concentrations (25-100 µg/mL), viable counts remained relatively high at 24 h (4.3-4.9 log). Untreated control cultures grew slightly to 6.6-6.9 log over the same period, confirming that the observed reductions were solely due to extract activity and not nutrient limitation.

Phytochemical Screening

Qualitative phytochemical analysis indicated a rich and diverse secondary metabolite profile in all extracts (Table1). Saponins, terpenoids, sterols, quinones, cardiac glycosides, and proteins were present in NAT-SLM, NAT-OLE, and NAT-SFM. Notably, phenols/tannins and flavonoids were strongly present in NAT-SFM and NAT-OLE, whereas they were only moderately present in NAT-SLM. This higher abundance of polyphenolic compounds in NAT-SFM and NAT-OLE likely contributes to their enhanced antioxidant and antibacterial potential. Quinones and cardiac glycosides appeared weakly in NAT-OLE but were fully present in the other two fractions, further supporting the superior bioactivity of NAT-SFM.

Table 1: Phytochemical Screening of *N. arbor-tristis* Extracts

Phytochemical	NAT-SLM	NAT-OLE	NAT-SFM
Saponins	+	+	+
Terpenoids	+	+	+
Phenols/Tannins	+	+	++
Flavonoids	+	+	++
Sterols	+	+	+
Quinones	+	±	+
Cardiac Glycosides	+	±	+
Proteins	+	+	+

*+: Present, ++: Strongly present, ±: Weakly present.

Determination of MIC and MBC

Determination of minimum inhibitory and bactericidal concentrations confirmed NAT-SFM as the most potent extract. It inhibited and killed *C. acnes* at the lowest concentrations (MIC 6.25 µg/mL, MBC 12.5 µg/mL), followed by *V. vulnificus* and *A. hydrophila* (MIC 12.5 µg/mL, MBC 25 µg/mL each), and *E. coli* (MIC 25 µg/mL, MBC 50 µg/mL) (Table 2).

Table 2: MIC and MBC Values (µg/mL) of *N. arbor-tristis* Extracts

Bacterial Strain	NAT-SLM	NAT-OLE	NAT-SFM
	MIC / MBC	MIC / MBC	MIC / MBC
C. acnes (MTCC 1951)	12.5 / 25	25 / 50	6.25 / 12.5
V. vulnificus (MTCC 1145)	25 / 50	50 / 100	12.5 / 25
A. hydrophila (MTCC 1739)	25 / 50	50 / 100	12.5 / 25
E. coli (MTCC 1687)	50 / 100	100 / 200	25 / 50

NAT-SLM required 2-4 times higher concentrations (MIC 12.5-50 µg/mL, MBC 25-100 µg/mL), while NAT-OLE was the least active, needing 100 µg/mL to inhibit E. coli and 200 µg/mL to kill it. Across all strains and extracts, MBC/MIC ratios were ≤4, classifying all preparations as bactericidal rather than merely bacteriostatic.

Phytochemical Characterization Using GC-MS

GC-MS profiling (Table 3.1) of the most active fraction (NAT-SFM) identified tromethamine (18.04%) as the major peak, along with long-chain fatty acids and derivatives: myristic acid (4.57%), palmitic acid (4.48%), hexadecanol (3.08%), 1-chloro-tetradecanamine (3.38%), and stearic acid (0.73%). These compounds are well-documented for their direct antibacterial mechanisms myristic and palmitic acids disrupt bacterial membranes and inhibit fatty acid biosynthesis (FabI), long-chain alcohols increase membrane fluidity and cause leakage (Table 3.2), cationic amines electrostatically disrupt lipopolysaccharide layers in Gram-negative bacteria, and alkyl halides covalently block efflux pumps. The synergistic interplay of these membrane-active and enzyme-inhibiting molecules explains the broad-spectrum, rapid bactericidal action observed with NAT-SFM.

Table 3.1. Bioactive fingerprint of NAT-SFM

Peak	RT (min)	Compound	Area (%)	Group	Antibacterial mechanism	Target in this study
1	7.89	Tromethamine	18.04	Amine buffer	pH 5.8 stabiliser; boosts fatty-acid protonation	All
2	8.19	Tetradecanol	0.66	Alcohol	Membrane insertion → leakage	C. acnes, E. coli
3	8.93	1-Dodecanamine, N,N-dimethyl-	0.55	Cationic amine	LPS electrostatic rupture	V. vulnificus, A. hydrophila
4	9.90	Hexadecanol	3.08	Alcohol	Bilayer fluidisation	C. acnes
5	10.33	Tetradecanamine, 1-chloro-	3.38	Alkyl halide	Covalent efflux-pump block	Synergy vs C. acnes
6	10.53	Myristic acid	4.57	Fatty acid	$Gram^{+}$ ion channels	C. acnes
7	11.90	Palmitic acid	4.48	Fatty acid	FabI inhibition; OM permeabilisation	E. coli, V. vulnificus
8	12.52	Stearic acid	0.73	Fatty acid	Co-disruptant with palmitic	$Gram^{+}$

Table 3.2. Most Important Antibacterial Components from Nyctanthes arbor-tristis Extract (Based on GC-MS Analysis)

Compound Name	Functional Group	Activity
Tetradecanoic Acid (Myristic Acid)	Fatty Acid	Disrupts bacterial membranes, effective against Gram-positive bacteria (Staphylococcus aureus).
Hexadecanoic Acid (Palmitic Acid)	Fatty Acid	Inhibits bacterial enzymes, effective against E. coli, Pseudomonas aeruginosa, and S. aureus.
Tetradecanamine, 1-chloro-	Alkyl Halide	Strong antibacterial activity, inhibits bacterial enzymatic functions.
1-Dodecanamine, N,N-dimethyl-	Amine	Affects bacterial lipid bilayers, disrupting cell membrane integrity.
Octadecanoic Acid (Stearic Acid)	Fatty Acid	Disrupts bacterial membranes, particularly in Gram-positive bacteria.

Discussion

The present study provides compelling evidence that *Nyctanthes arbor-tristis*, particularly the methanolic extract of shade-dried flowers (NAT-SFM), represents a highly promising source of broad-spectrum, rapid-acting, and bactericidal agents against clinically relevant pathogens,

including the acne-causing *Cutibacterium acnes* and the emerging aquatic pathogens *Vibrio vulnificus* and *Aeromonas hydrophila*. The superiority of NAT-SFM over leaf-derived extracts (NAT-SLM and NAT-OLE) was consistent across all assays disc diffusion, MIC/MBC, and time-kill kinetics highlighting the critical influence of plant part, drying method, and solvent polarity on bioactivity, as previously emphasized (Yuan *et al*., 2015; Khanapur *et al*., 2014; Abubakar & Haque, 2020) [41, 25, 1]. The zone-of-inhibition results (14.2-18.7 mm for NAT-SFM) are among the highest reported for *N. arbor-tristis* floral extracts and surpass many earlier leaf- and stem-based studies (Khatune *et al*., 2001; Jose *et al*., 2016; Aggarwal & Goyal, 2013; Manisha *et al*., 2009) [26, 22, 3, 27]. Notably, NAT-SFM outperformed NAT-OLE by 28-35%, confirming that shade-drying better preserves heat-labile phenolics and flavonoids, whereas oven-drying at 40 °C triggers partial degradation or Maillard-type reactions (Yuan *et al*., 2015; Hasnat *et al*., 2024) [41, 18]. The strong activity against *C. acnes* (18.7 mm) is particularly significant, as few plant extracts achieve zones >15 mm against this slow-growing, lipid-rich anaerobe, and aligns with the traditional Ayurvedic use of Parijat flowers in skin disorders (Agrawal & Pal, 2013; Ullah *et al*., 2020) [4, 37].

Time-kill kinetics further distinguished NAT-SFM as unequivocally bactericidal, achieving ≥4.4 $\log_{10}$ reductions within 24 h against all four pathogens at low concentrations (6.25-25 µg/mL). This meets the stringent criterion of ≥3 $\log_{10}$ kill defined for bactericidal agents (CLSI, 1999) and represents markedly faster killing than most previously reported *N. arbor-tristis* extracts, which were predominantly bacteriostatic (Aggarwal & Goyal, 2013; Manisha *et al*., 2009; Verma *et al*., 2011) [3, 27, 38]. The rapid onset of killing (evident from 4-8 h) suggests direct membrane-targeted mechanisms rather than slower protein-synthesis inhibition a hypothesis strongly supported by GC-MS identification of long-chain fatty acids (myristic, palmitic, and stearic acids) and their derivatives. These compounds are well-documented for pore formation, membrane fluidisation, and inhibition of bacterial fatty-acid biosynthesis via the FabI pathway (Kabara *et al*., 1972; Zheng *et al*., 2005; Hidajati & Setiabudi, 2018) [42, 20]. Palmitic acid, in particular, has been shown to disrupt Gram-positive membranes and inhibit enoyl-acyl carrier protein reductase in multiple pathogens (Aparna *et al*., 2012) [7]. The phytochemical profile explains the observed potency gradient: NAT-SFM exhibited the strongest presence of phenols/tannins and flavonoids (++), compounds known for multiple antibacterial mechanisms including iron chelation, enzyme inhibition, and membrane destabilisation (Takó *et al*., 2020; Rathee *et al*., 2007; Michael *et al*., 2013) [36, 33, 28]. The weaker activity of NAT-OLE, despite comparable polyphenolic content, may be attributed to thermal degradation during oven-drying and lower methanol efficiency in extracting certain glycosylated flavonoids (Hasnat *et al*., 2024; Sasikumar *et al*., 2010) [35, 18].

Although synergistic interaction with conventional antibiotics was originally planned using checkerboard methodology (Odds, 2003) [29], the standalone bactericidal potency of NAT-SFM at concentrations as low as 6.25-25 µg/mL already approaches or surpasses that of several antibiotics against resistant strains, reducing the immediate necessity for combination therapy. Nevertheless, the presence of cationic amines and alkyl halides (e.g., 1-chloro-tetradecanamine) suggests potential efflux-pump blocking capacity that could restore antibiotic efficacy in MDR strains in future studies (Altuner *et al*., 2018; Isaac *et al*., 2017) [5, 21]. From a translational perspective, the low MIC/MBC values of NAT-SFM against *C. acnes* (6.25/12.5 µg/mL) are comparable to or better than current topical anti-acne agents such as clindamycin or benzoyl peroxide when adjusted for molecular complexity, while offering a natural, non-irritating alternative with additional anti-inflammatory benefits via NFκB inhibition (Aparna *et al*., 2012; Dhinakaran & Sakthivel, 2016) [7, 13]. Similarly, activity against *V. vulnificus* and *A. hydrophila* emerging threats in tropical aquaculture and wound infections positions NAT-SFM as a candidate for development of cost-effective topical or aquaculture therapeutics (Ghagane *et al*., 2017; Alves *et al*., 2021; Chatterjee *et al*., 2007) [16, 6, 9].

In conclusion, this study successfully bridges traditional Ayurvedic knowledge with modern pharmacological validation by demonstrating that shade-dried floral methanolic extracts of *Nyctanthes arbor-tristis* constitute a potent, rapid-acting, broad-spectrum antibacterial agent whose activity is driven primarily by membrane-disrupting fatty acids and polyphenolics. These findings strongly support further development of standardised NAT-SFM-based formulations for acne management, wound care, and aquaculture disease control applications that could significantly reduce reliance on conventional antibiotics in the face of escalating antimicrobial resistance (WHO, 2020).

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