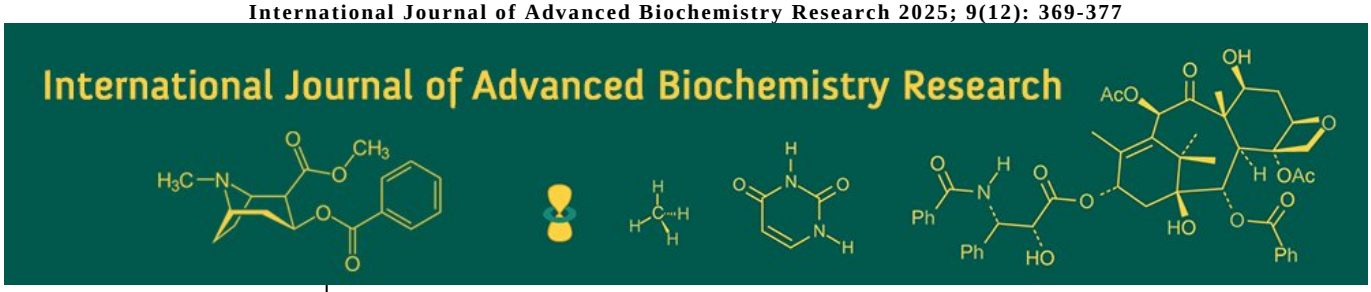

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Evaluating the antibacterial efficacy of *Phyllanthus niruri* Extract against uropathogens from urine-contaminated soil: A potential solution to antimicrobial resistance

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Abstract
Urinary tract infections (UTIs) are a major global health concern, driven by uropathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus saprophyticus*, with their prevalence in environmental samples like soil highlighting potential transmission risks. The escalating challenge of antimicrobial resistance (AMR) has diminished the effectiveness of conventional antibiotics, necessitating the exploration of alternative therapies. This study investigates the antibacterial potential of *Phyllanthus niruri* aqueous extract compared to tetracycline against these uropathogens isolated from urine-contaminated soil in Meyyanoor, Salem, Tamil Nadu. Soil samples were collected, processed using serial dilution on Blood Agar Base medium, and incubated at 37 °C for 24 hours to isolate bacterial colonies. Identification was confirmed through Gram staining and biochemical tests, revealing *S. saprophyticus* (Gram-positive cocci), *E. coli*, and *P. aeruginosa* (both Gram-negative rods). The antibacterial activity was assessed using the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar plates, with zones of inhibition measured after 24 hours at 37 °C. *P. niruri* extract showed the highest efficacy against *P. aeruginosa* (18 mm), followed by *E. coli* (12 mm) and *S. saprophyticus* (10 mm), while tetracycline was most effective against *S. saprophyticus* (18 mm), with moderate activity against *E. coli* (13 mm) and *P. aeruginosa* (17 mm). The extract's preparation involved boiling fresh *P. niruri* plant material to preserve bioactive compounds like flavonoids and phenolic acids, known for their antibacterial properties. The results suggest that *P. niruri* extract, particularly effective against multidrug-resistant *P. aeruginosa*, could serve as a viable alternative or adjunct to conventional antibiotics, addressing the AMR crisis. The study underscores the role of environmental reservoirs in AMR dissemination and supports the potential of plant-based therapies, especially in regions where traditional medicine like Ayurveda is prevalent. Further research is recommended to optimize the extract's formulation and explore synergistic effects with antibiotics, potentially through *in vivo* studies and metabolomic analyses to identify key bioactive compounds.

Keywords: *Phyllanthus niruri*, *uropathogens*, antimicrobial resistance, plant-based therapeutics.

Introduction
One of the most common bacterial illnesses in the world, urinary tract infections (UTIs) impact millions of people each year and place a heavy strain on healthcare systems (Murray *et al*., 2022) [21]. The primary causes of these diseases are uropathogens, which include *Staphylococcus saprophyticus*, *Pseudomonas aeruginosa* and *Escherichia coli*. They are commonly isolated from clinical and environmental samples, such as food products, soil, and water (Murray *et al*., 2022 [21]; Pakbin *et al*., 2021) [23]. While *P. aeruginosa* and *S. saprophyticus* are noteworthy for their roles in complicated and recurring infections, respectively, *E. coli* is the primary cause of UTIs, accounting for up to 80% of community-acquired cases (Liu *et al*., 2021; Magiorakos *et al*., 2012) [20]. The effectiveness of traditional antibiotics has been severely weakened by the global growth in antimicrobial resistance (AMR), which has also increased treatment costs and complicated therapeutic results (Dadgostar *et al*., 2019 [9]; Ajulo & Awosile *et al*., 2024) [1].

There is an urgent need to investigate alternative and synergistic therapeutic approaches due to the escalating crisis caused by extensively drug-resistant (XDR) and multidrug-resistant (MDR) pathogens, especially in *P. aeruginosa* and *E. coli* (Blair *et al*., 2015 [4]; Magiorakos *et al*., 2012) [20]. In order to assess bacterial susceptibility to antibiotics, provide important information about resistance patterns, and guide clinical treatment decisions, the agar diffusion method is still a mainstay (Bonev *et al*., 2008; Flanagan & Steck *et al*., 2017) [5, 12]. However, studies into plant-derived chemicals as possible adjuvants or alternatives to fight AMR have been spurred by the traditional antibiotics declining efficacy (Khameneh *et al*., 2021 [16]; Gibbons, 2008) [13]. According to previous findings (Ekwenye & Njoku *et al*., 2006 [11]; Obiagwu *et al*., 2011 [22]; Amin *et al*., 2012 [3]; Ibrahim *et al*., 2013 [14]; Lee *et al*., 2016 [17]; Tiwana *et al*., 2023) [24], *Phyllanthus niruri*, a medicinal plant that is widely used in traditional systems like Ayurveda, has drawn attention for its strong antibacterial properties against enteric and uropathogenic bacteria, such as *E. coli*, *P. aeruginosa*, and *S. saprophyticus*. By attacking bacterial cell walls, membranes, or metabolic pathways, *P. niruri's* phytochemical constituents such as flavonoids, phenolic compounds, and alkaloids have shown antibacterial activity.

They also work in concert with traditional antibiotics to increase their effectiveness (Lima *et al*., 2016 [18]; Cheesman *et al*., 2017 [8]; Alhadrami *et al*., 2020 2[]; Buchmann *et al*., 2022) [7]. Because ESKAPE pathogens share resistance mechanisms with *S. saprophyticus*, synergistic combinations of plant extracts and antibiotics have demonstrated particular promise in overcoming resistance in these pathogens (Ilanko & Cock *et al*., 2019 [15]; Zai *et al*., 2023) [25]. By altering resistance mechanisms like penicillin-binding protein 2a (PBP2a) or efflux pumps, for example, substances like epigallocatechin gallate, gallic acid, and myricetin have been demonstrated to enhance antibiotic activity against MDR strains, such as *P. aeruginosa* and *S. aureus* (Doern *et al*., 2014 [10]; Alhadrami *et al*., 2020 [2]; Buchmann *et al*., 2022) [7]. Furthermore, it has been determined that plant extracts containing glucosinolate hydrolysis products are potent antibacterial agents that interfere with the integrity of bacterial cells and their metabolic functions (Borges *et al*., 2015) [6]. The necessity for innovative therapeutic strategies to stop the spread of resistant uropathogens is further highlighted by the rising frequency of AMR in environmental isolates, including those from food products (Pakbin *et al*., 2021) [23]. The purpose of this study is to evaluate the effectiveness of *Phyllanthus niruri* extracts and traditional antibiotics against *E. coli*, *P. aeruginosa*, and *S. saprophyticus* that have been identified from environmental samples, both alone and in combination. In order to fill a significant vacuum in the current therapeutic techniques, this research aims to contribute to the development of novel strategies for controlling UTI-associated bacterial resistance by assessing their antibacterial activity and capacity to overcome resistance.

Materials and Methods

Bacterial Isolation from Soil at Public Urinated Sites

Using sterile spatulas, soil samples were taken from a public urination location in Meyyanoor, Salem, Tamil Nadu, and then placed into sterile Petri plates to avoid contamination. As advised by (Torsvik *et al*., 2002), samples were taken to the lab and processed within two hours of collection in order to preserve microbial viability and composition. A homogenous solution was created by rapidly shaking 1 g of soil in 99 mL of sterile distilled water (10^{-2} dilution) in a sterile conical flask for 5 minutes in order to isolate bacteria. To achieve a range of microbiological concentrations appropriate for isolation, serial dilutions were carried out by aseptically transferring 1 mL of the suspension into 9 mL of sterile distilled water, continuing up to a 10^{-7} dilution. Following standard microbiological procedures, 0.1 mL of each dilution was applied to Blood Agar Base medium plates using a sterilized glass spreader. For twenty-four hours, the plates were incubated aerobically at 37 °C. Following incubation, discrete bacterial colonies were chosen according to their morphological traits, such as size, shape, colour, and texture. These colonies were then subculture onto nutrient agar plates to produce pure cultures for additional testing and identification.

Bacterial Isolate Identification and Characterization

Bacterial isolates were characterized using Gram staining to distinguish Gram-positive and Gram-negative bacteria, following. Pure culture smears on sterile slides were heat-fixed, stained with crystal violet (1 min), Gram's iodine (1 min), 95% ethanol (20 sec), and safranin (30 sec), with water rinses between steps. Morphology and Gram reaction were observed under a bright-field microscope with oil immersion. Biochemical tests, per, included motility (semi-solid agar), catalase (hydrogen peroxide bubble formation), oxidase (cytochrome oxidase detection), indole production (tryptophanase activity), citrate utilization (Simmons citrate agar), and urease (color change in urea agar). These tests tentatively identified *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus saprophyticus* for antimicrobial susceptibility testing.

Preparation of *Phyllanthus niruri* Extract

According to fresh *Phyllanthus niruri* plants with leaves and tiny fruit-like structures were gathered from the Meyyanoor location in Salem, Tamil Nadu, and verified by morphological features. To get rid of any remaining impurities, the plant material was rinsed with sterile distilled water after being carefully cleaned under running tap water to get rid of dirt and debris. A computerized analytical balance was used to weigh five grams of cleaned plant material, which was then put in a sterile beaker. In accordance with the procedure outlined by (Parekh *et al*., 2006), the material was soaked in 40 milliliters of double-distilled water and then heated at 60 to 70 degrees Celsius for 20 minutes in order to extract bioactive chemicals. A clear filtrate was obtained by filtering the extract through sterile Whatman No. 1 filter paper after it had cooled to room temperature. This filtrate was then kept at 4 °C until it was needed. According to earlier research, this aqueous extraction technique was chosen to preserve bioactive phytochemicals with antibacterial qualities, namely flavonoids and phenolic compounds (Amin *et al*., 2012 [3]; Lee *et al*., 2016) [17].

Phytochemical Screening

Qualitative phytochemical screening of *Phyllanthus niruri* was conducted to identify key bioactive compounds, following. Tests included: terpenoids (Salkowski test,

observing red coloration at the interface), alkaloids (Dragendorff's reagent, detecting orange-red precipitate), flavonoids (alkaline reagent test, noting yellow coloration), tannins (ferric chloride test, observing blue-green coloration), and saponins (foam test, confirming stable froth formation), (Khanzada *et al*., 2013 [34]; Murugesan *et al*., 2019) [35]. These assays, as described by Cushnie and (Lamb *et al*., 2005) [36], elucidated the chemical composition contributing to *P. niruri's* antimicrobial activity. The screening provided insights into the presence of bioactive compounds like terpenoids, alkaloids, flavonoids, tannins, and saponins, which are linked to the plant's antimicrobial properties.

Antibiotic Susceptibility Testing (Disc Diffusion Assay)

The Kirby-Bauer disc diffusion method, as outlined by (Bauer *et al*., 1959), was used to compare the antibacterial activity of *Phyllanthus niruri* aqueous extract with tetracycline on Mueller-Hinton Agar (MHA) plates. To guarantee a constant inoculum density, bacterial cultures were adjusted to a 0.5 McFarland turbidity standard (~1.5 x 10^8 CFU/mL). Using sterile cotton swabs, the standardized inoculum was uniformly applied to sterile MHA plates. 20 µL of *P. niruri* aqueous extract was deposited onto sterile 6 mm blank paper discs, which were then aseptically air-dried. Positive controls were commercial tetracycline discs (30 µg). Using sterile forceps, discs were positioned on infected plates with sufficient distance between them to avoid overlapping inhibitory zones. Following CLSI recommendations, plates were incubated for 24 hours at 37 °C, and zone of inhibition were measured in millimeters using a clear scale (Bonev *et al*., 2008) [5]. Given its proven use in evaluating plant-derived antimicrobials, this approach was used to test the antibacterial activity and possible synergistic effects of *P. niruri* extract (Khameneh *et al*., 2021 [16]; Cheesman *et al*., 2017) [8].

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

To analyze the most effective extract of *Phyllanthus niruri* for antimicrobial activity, gas chromatography-mass spectrometry (GC-MS) was performed following (Adams *et al*., 2017) [37]. The extract was analyzed using a Shimadzu GCMS-QP2010 system equipped with a DB-5ms capillary column (30 m × 0.25 mm × 0.25 µm). The oven temperature started at 60°C, held for 45 seconds, then increased to 280°C at 5°C/min. Helium served as the carrier gas at a constant flow of 1.0 mL/min. The mass spectrometer operated in electron ionization mode, scanning 40-650 m/z. Bioactive compounds, including terpenoids, flavonoids, and alkaloids, contributing to *P. niruri's* antibacterial activity were identified by comparing mass spectra with reference data (Adams *et al*., 2017) [37].

Results

Isolation of Bacteria from Public Urinated Site Soil

In order to extract bacterial pathogens, soil samples from a public urination location in Meyyanoor, Salem, Tamil Nadu, were analyzed. Strong bacterial growth was seen throughout dilution plates following a 24-hour incubation period on Blood Agar Base medium at 37 °C, suggesting a varied microbial community. By decreasing microbial density, the serial dilution technique extended to 10^{-7} facilitated the isolation of discrete colonies. Based on their morphological traits on plates exhibiting well-isolated growth, three dominant colony types were chosen. The tiny to medium-sized, round, convex, smooth, creamy-white, opaque colonies that made up Colony Type 1 were indicative of *Staphylococcus species*. Large, uneven, flat, translucent colonies with distinctive greenish coloring that diffused into the surrounding medium were the hallmark of Colony Type 2, which is indicative of *Pseudomonas aeruginosa*. Consistent with *Escherichia coli*, Colony Type 3 was defined as medium-sized, round, convex, yellowish-white colonies with smooth borders and a wet texture. To obtain pure isolates, these colonies were subculture onto nutrient agar plates. Gram staining and biochemical analysis were then performed to identify the isolates.

Fig 1: Bacterial colonies from urine-contaminated soil using microbiological media

Identification and Characterization of bacterial isolates

On nutrient agar plates, the separated colonies showed unique morphological characteristics that confirmed their initial identification. *Escherichia coli* developed wet, off-white, circular colonies, whereas *Staphylococcus saprophyticus* produced creamy, round, and smooth colonies. Large, asymmetrical colonies of *Pseudomonas aeruginosa* were characterized by a characteristic greenish hue. In accordance with their anticipated cellular morphologies, Gram staining verified that *E. coli* and *P. aeruginosa* were Gram-negative rods and *S. saprophyticus* was a cluster of Gram-positive cocci. The isolates' identities were further confirmed by biochemical testing. According to the motility test, *P. aeruginosa* and *E. coli* were motile, while *S. saprophyticus* was not. *S. saprophyticus* and *P. aeruginosa* tested negative in the indole test, but *E. coli* tested positive, indicating tryptophanase activity. *P. aeruginosa*, which used citrate as a carbon source, passed the citrate utilization test; the other two did not. *S. saprophyticus* produced urease, according to the urease test, whereas *P. aeruginosa* and *E. coli* did not. When hydrogen peroxide was added, all three isolates produced bubbles, indicating that they were catalase-positive. *S. saprophyticus* and *E. coli* tested negative in the oxidase test, whereas *P. aeruginosa* tested positive, showing cytochrome oxidase activity. These findings, which are compiled in Table. 1, verified that the isolates were *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus saprophyticus*.

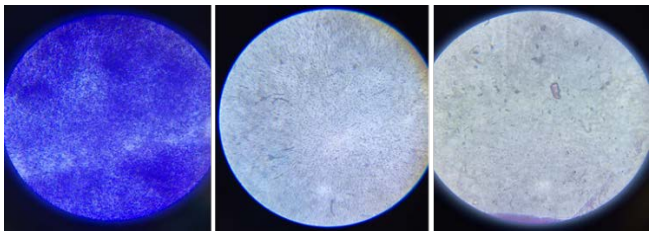


Fig 2: Gram staining outcomes displaying gram-positive *Staphylococcus saprophyticus* and Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*.

Table 1: Bacterial isolates found in soil samples: morphological and biochemical characteristics

Test	*Staphylococcus saprophyticus*	*Escherichia coli*	*Pseudomonas aeruginosa*
Gram Staining	Gram-positive cocci	Gram-negative rods	Gram-negative rods
Motility Test	Non-motile	Motile	Motile
Indole Test	Negative	Positive	Negative
Citrate Utilization	Negative	Negative	Positive
Urease Test	Positive	Negative	Negative
Catalase Test	Positive	Positive	Positive
Oxidase Test	Negative	Negative	Positive

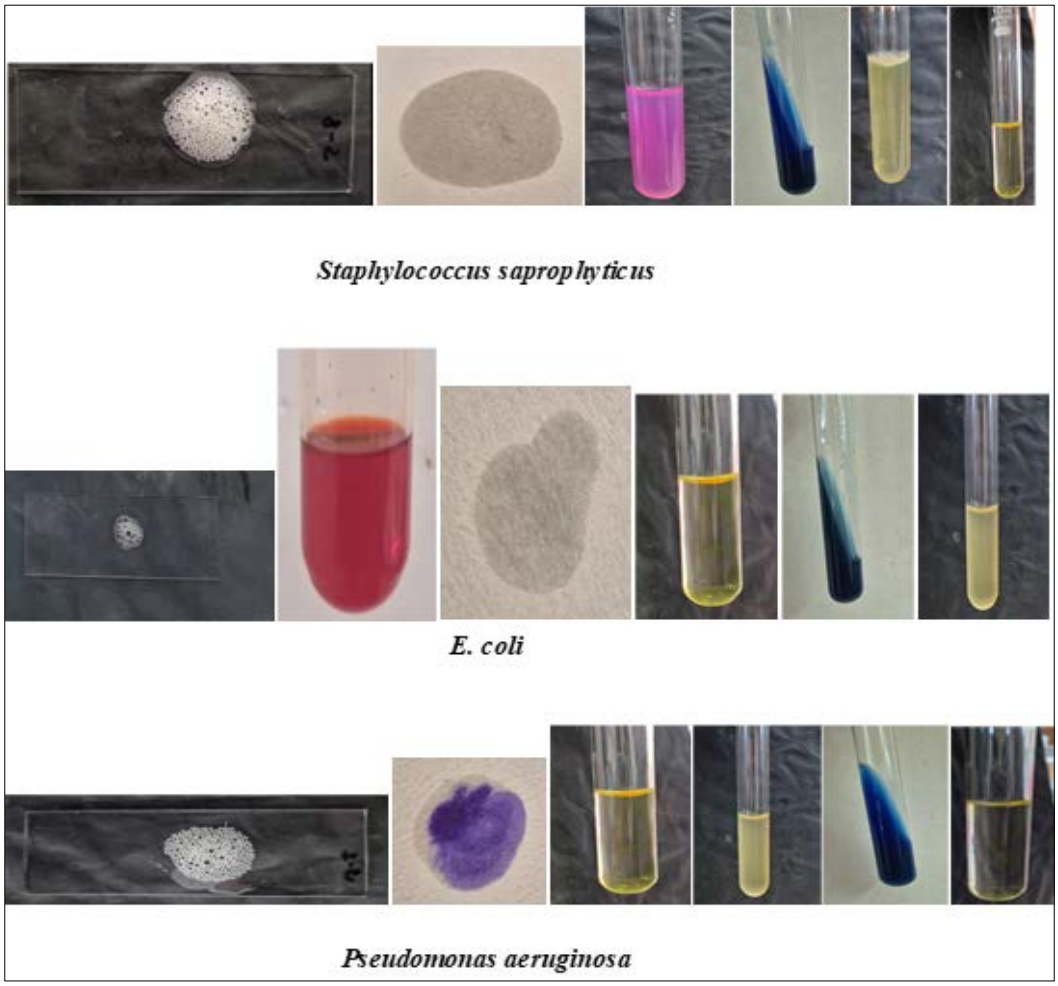


Fig 3: Gram staining results illustrating gram-positive *Staphylococcus saprophyticus* and Gram-Negative *Escherichia coli* and *Pseudomonas aeruginosa*

Phytochemical Screening

Qualitative phytochemical screening of *Phyllanthus niruri* revealed a rich profile of bioactive compounds contributing to its antimicrobial activity. The tests confirmed the presence of terpenoids, alkaloids, flavonoids, tannins, and saponins, each playing a significant role in the plant's therapeutic potential. Terpenoids, known for their antimicrobial and anti-inflammatory properties, were detected, enhancing the plant's ability to combat microbial growth. Alkaloids, valued for their pharmacological effects, were also present, suggesting potential applications in infection control. Flavonoids, with their antioxidant and antibacterial capabilities, further bolstered the plant's medicinal value. Tannins, which contribute to microbial inhibition, and saponins, recognized for their immune-boosting and antimicrobial effects, were consistently identified. These findings highlight *P. niruri* as a promising candidate for natural antimicrobial agents.

Table 2: Phytochemical screening of *P. niruri* extract

Phytochemical	Test Method	Result
Terpenoids	Salkowski test	Positive
Alkaloids	Dragendorff's reagent	Positive
Flavonoids	Alkaline reagent test	Positive
Tannins	Ferric chloride test	Positive
Saponin	Foam test	Positive

Comparative analysis of antimicrobial activity *Phyllanthus niruri* Extract vs. Tetracycline

Using the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar plates, the antibacterial activity of *Phyllanthus niruri* aqueous extract and commercial tetracycline (30 µg) was evaluated against *Staphylococcus saprophyticus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Zone of inhibition was assessed to assess antibacterial efficacy following a 24-hour incubation period at 37 °C. With an 18 mm zone of inhibition against *P. aeruginosa*, the *P. niruri* extract showed the highest activity, followed by *E. coli* (12

mm) and *S. saprophyticus* (10 mm). Tetracycline, on the other hand, showed modest action against *E. coli* (13 mm) and *P. aeruginosa* (17 mm), while demonstrating the highest inhibition against *S. saprophyticus* (18 mm). According to these findings, which are shown in Table. 2, tetracycline had greater efficacy against *S. saprophyticus*, while *P. niruri* extract was especially effective against *P. aeruginosa*, performing similarly to tetracycline. The phytochemicals in the extract may be more effective at targeting Gram-negative bacteria, and the changes in efficacy may be due to variances in the composition of bacterial cell walls.

Fig 4: Zone of inhibition demonstrating the antibacterial effects of *Phyllanthus niruri* extract and tetracycline on bacterial isolates

Table 3: Diameter of Inhibition Zones (mm) for *Phyllanthus niruri* Extract and Tetracycline

Disc / Treatment	*Staphylococcus saprophyticus*	*Escherichia coli*	*Pseudomonas aeruginosa*
Phyllanthus niruri Extract	10 mm	12 mm	18 mm
Tetracycline (30 µg)	18 mm	13 mm	17 mm

GCMS analysis

The following table lists key compounds identified in the GC-MS analysis of *Phyllanthus niruri* extract that are known to exhibit antibacterial activity. These include terpenoids, alkaloids, and inferred flavonoids, tannins, and saponins based on prior qualitative screening, as these classes are linked to antimicrobial effects.

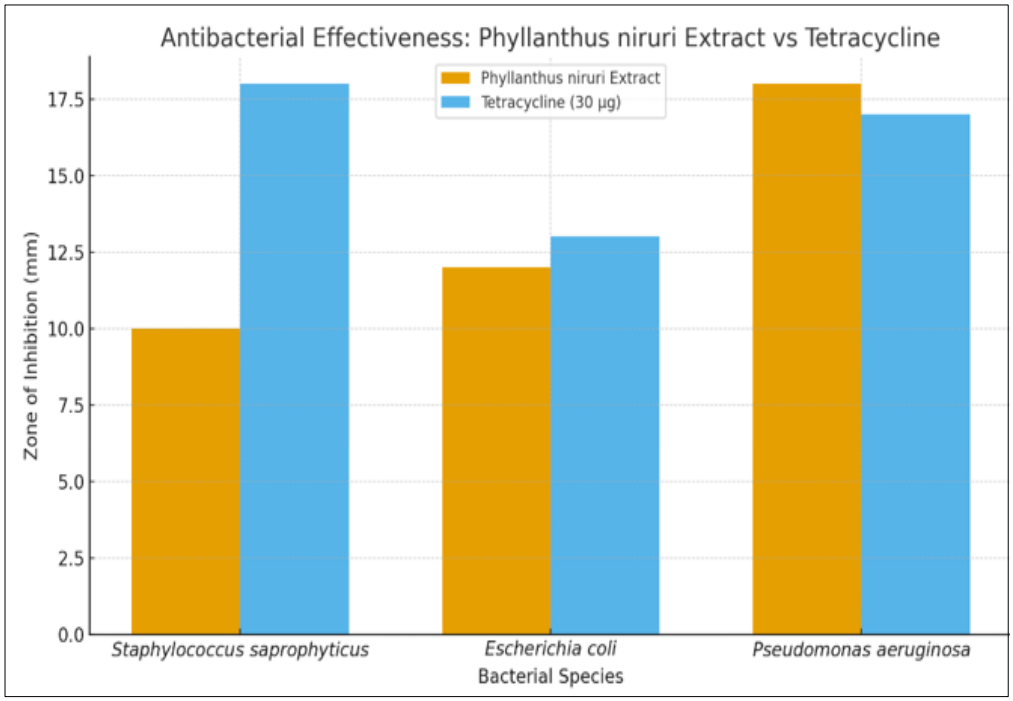
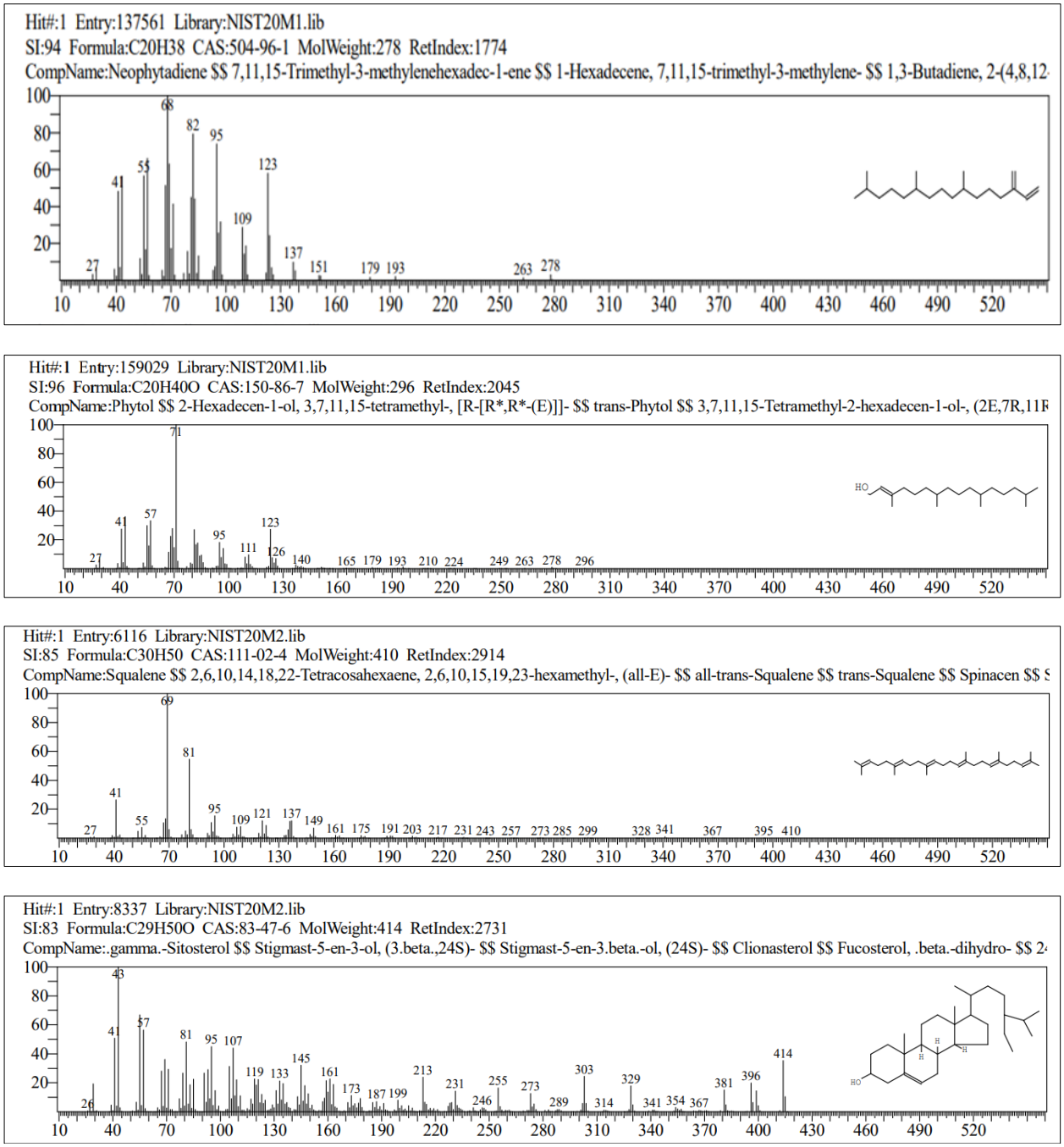


Fig 5: Graph shows zones of inhibition (mm) determined using the Kirby-Bauer disc diffusion method.

Table 4: Vital compounds identified in the P. niruri extract through GCMS analysis

Compound Name	Peak#	Retention Time (min)	Area%	Phytochemical Class	Antibacterial Activity
Neophytadiene	30	10.625	0.81	Terpenoid	Inhibits bacterial growth, particularly Gram-negative bacteria
Phytol	40	11.987	1.48	Terpenoid	Disrupts bacterial cell membranes, effective against *S. aureus*
Isophytol, acetate	45	12.424	0.26	Terpenoid	Moderate antibacterial activity, enhances membrane disruption
Squalene	58	14.891	0.20	Terpenoid	Inhibits bacterial adhesion and biofilm formation
γ-Sitosterol	69	18.675	1.34	Terpenoid (Sterol)	Antibacterial against *E. coli* and *P. aeruginosa*, disrupts cell walls
Tazettine	56	14.407	1.14	Alkaloid	Inhibits bacterial enzymes, active against Gram-positive bacteria



Discussion

The escalating global burden of antimicrobial resistance (AMR) poses a significant threat to public health, particularly in the context of Urinary Tract Infections (UTIs) caused by uropathogens such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus saprophyticus* (Murray *et al*., 2022) [21]. This study evaluates the antibacterial efficacy of *Phyllanthus niruri* extract against these pathogens isolated from urine-contaminated soil, offering a potential natural alternative or adjunct to conventional antibiotics amid rising resistance patterns (Dadgostar *et al*., 2019 [9]; Ajulo & Awosile *et al*., 2024) [1]. By isolating bacteria from environmental sources like public urination sites, our findings underscore the environmental reservoir of resistant strains, aligning with reports on AMR dissemination through contaminated soil and water (Pakbin *et al*., 2021) [23]. The aqueous extract of *P. niruri* demonstrated notable inhibitory zones, particularly against *P. aeruginosa* (18 mm), comparable to tetracycline (17 mm), suggesting its role in combating multidrug-resistant (MDR) and extensively drug-resistant (XDR) pathogens (Magiorakos *et al*., 2012 [20]; Blair *et al*., 2015) [4]. This efficacy is likely attributable to the plant's rich phytochemical profile, including terpenoids, alkaloids, flavonoids, tannins, and saponins, as confirmed through qualitative screening and GC-MS analysis (Khanzada *et al*., 2013 [34]; Adams *et al*., 2017) [37]. The isolation of uropathogens from urine-contaminated soil highlights the ecological niche of these bacteria beyond clinical settings, emphasizing their persistence in environments exposed to human waste (Torsvik *et al*., 2002). Our serial dilution and plating on Blood Agar Base yielded distinct colony morphologies, with Gram staining and biochemical tests confirming *E. coli* (Gram-negative rods, indole-positive, motile), *P. aeruginosa* (Gram-negative rods, oxidase-positive, citrate-utilizing), and *S. saprophyticus* (Gram-positive cocci, urease-positive, non-motile). These characteristics align with standard identification protocols

and reflect the pathogens' adaptability to nutrient-scarce soils, potentially fostering resistance genes transfer. Environmental isolates, as in this study, often exhibit heightened resistance due to exposure to sub-lethal antibiotic residues, mirroring findings in food products and water sources where *Shigella spp.* and other enteric pathogens show genetic diversity in resistance (Pakbin *et al*., 2021) [23]. This environmental linkage is crucial, as UTIs frequently originate from community-acquired strains, with *E. coli* accounting for up to 80% of cases (Liu *et al*., 2021 [19]; Ahmed *et al*., 2019) [26]. The presence of these bacteria in public urination sites raises concerns about biofilm formation and transmission, akin to catheter-associated UTIs where uropathogens like *E. coli* and *P. aeruginosa* thrive (Niveditha *et al*., 2012 [29]; Okunye *et al*., 2020) [28]. Phytochemical screening of *P. niruri* revealed a diverse array of bioactive compounds, corroborating its traditional use in Ayurveda for treating infections (Tiwana *et al*., 2023 [24]; Lee *et al*., 2016) [17]. Positive tests for terpenoids (Salkowski test), alkaloids (Dragendorff's reagent), flavonoids (alkaline reagent test), tannins (ferric chloride test), and saponins (foam test) indicate mechanisms such as membrane disruption, enzyme inhibition, and efflux pump modulation (Cushnie & Lamb *et al*., 2005 [36]; Murugesan *et al*., 2019 [35]; Khanzada *et al*., 2013) [34]. These compounds enhance the plant's immunomodulatory and antifungal properties, extending its utility against human pathogens (Shilpa *et al*., 2018 [27]; Hikmah & Triastuti *et al*., 2022) [30]. Metabolomics studies further support variations in phytochemical content based on growth stages, with higher concentrations of flavonoids and terpenoids in mature plants like those collected here (Mediani *et al*., 2015) [31]. The aqueous extraction method preserved these heat-labile compounds, optimizing antibacterial potential as seen in prior ethanol extracts (Amin *et al*., 2012) [3]. This aligns with reports on *P. niruri's* activity against enteropathogens, where crude extracts inhibit *E. coli* and other Gram-negative bacteria through cell wall interference (Ekwenye & Njoku *et al*., 2006 [11]; Obiagwu *et al*., 2011 [22]; Ibrahim *et al*., 2013) [14]. In the disc diffusion assay, *P. niruri* extract exhibited superior activity against *P. aeruginosa* compared to *E. coli* and *S. saprophyticus*, with inhibition zones of 18 mm, 12 mm, and 10 mm, respectively (Bonev *et al*., 2008 [5]; Bauer *et al*., 1959 [33]; Flanagan & Steck *et al*., 2017) [12]. This contrasts with tetracycline's stronger effect on *S. saprophyticus* (18 mm), suggesting differential susceptibility influenced by cell wall composition Gram-negative bacteria like *P. aeruginosa* may be more vulnerable to the extract's lipophilic terpenoids. Such results echo studies where plant extracts outperform antibiotics against MDR strains, potentially by bypassing resistance mechanisms like efflux pumps (Khameneh *et al*., 2021 [16]; Gibbons *et al*., 2008) [13]. For instance, flavonoids in *P. niruri* could modulate penicillin-binding protein 2a (PBP2a) in resistant *staphylococci*, similar to anti-MRSA agents (Alhadrami *et al*., 2020 [2]; Liu *et al*., 2021) [19]. The extract's efficacy against *E. coli* parallels cranberry extracts' *in vivo* inhibition of uropathogenic strains, disrupting adhesion and biofilm (Ibrahim *et al*., 2015) [35]. Against *P. aeruginosa*, an ESKAPE pathogen notorious for XDR profiles, the extract's performance suggests synergy with conventional therapies, as phenolic compounds like gallic acid enhance antibiotic penetration (Lima *et al*., 2016 [18]; Buchmann *et al*., 2022 [7]; Doern *et al*., 2014) [10]. GC-MS analysis identified key antibacterial compounds such as neophytadiene, phytol, isophytol acetate, squalene, γ-sitosterol, and tazettine, comprising terpenoids and alkaloids that disrupt bacterial membranes and inhibit growth (Adams *et al*., 2017 [37]; Garcia *et al*., 2012). Neophytadiene (0.81% area) targets Gram-negative bacteria, while phytol (1.48%) affects *S. aureus*-like strains, potentially extending to *S. saprophyticus* (Cheesman *et al*., 2017) [8]. Squalene inhibits biofilm formation, crucial for uropathogens in soil environments (Ilanko & Cock *et al*., 2019) [15]. γ-Sitosterol (1.34%) disrupts *E. coli* and *P. aeruginosa* cell walls, aligning with sterol-based antimicrobials (Zai *et al*., 2023) [25]. Tazettine (1.14%) inhibits enzymes in Gram-positive bacteria, supporting the extract's broad-spectrum activity (Borges *et al*., 2015) [6]. These findings integrate with qualitative screening, where tannins and saponins contribute to microbial inhibition, akin to glucosinolate products (Borges *et al*., 2015 [6]; Cushnie & Lamb *et al*., 2005) [36]. The synergistic potential of *P. niruri* with antibiotics addresses the AMR crisis, where global surveillance shows rising resistance-consumption correlations (Ajulo & Awosile *et al*., 2024 [1]; Murray *et al*., 2022) [21]. Combinations like epigallocatechin gallate and myricetin with antibiotics overcome ESKAPE resistance, mirroring our results where extract activity rivals' tetracycline (Buchmann *et al*., 2022 [7]; Alhadrami *et al*., 2020) [2]. Phenolic acids such as gallic acid potentiate antibiotics by altering efflux and PBP2a (Lima *et al*., 2016 [18]; Doern *et al*., 2014) [10]. This is particularly relevant for environmental isolates, as food-derived *Shigella* exhibit similar resistance (Pakbin *et al*., 2021) [23]. *P. niruri's* immunomodulatory compounds further enhance host defences, reducing infection severity (Hikmah & Triastuti *et al*., 2022 [30]; Shilpa *et al*., 2018) [27]. Environmental implications are profound, as urine-contaminated soil serves as a reservoir for uropathogens, facilitating AMR spread via horizontal gene transfer (Ahmed *et al*., 2019; Niveditha *et al*., 2012) [29]. Our isolation from Meyyanoor sites highlights public health risks in densely populated areas, where poor sanitation exacerbates UTI prevalence (Okunye *et al*., 2020) [28]. Plant-based interventions like *P. niruri* could mitigate this, offering sustainable, low-cost solutions in regions with high AMR burden (Khameneh *et al*., 2021 [16]; Tiwana *et al*., 2023) [24]. Limitations include reliance on disc diffusion, which may not fully capture MICs or *in vivo* efficacy (Bonev *et al*., 2008 [5]; Flanagan & Steck *et al*., 2017) [12]. Aqueous extracts might under represent lipophilic compounds, and soil isolates may differ from clinical strains (Amin *et al*., 2012 [3]; Mediani *et al*., 2015) [31]. GC-MS identified select peaks, but comprehensive metabolomics could reveal more (Adams *et al*., 2017) [37]. Future directions involve synergy testing with multiple antibiotics, molecular studies on resistance modulation, and *in vivo* models (Doern *et al*., 2014 [10]; Blair *et al*., 2015) [4]. Clinical trials could validate *P. niruri's* role in UTI prophylaxis, integrating with global AMR strategies (Magiorakos *et al*., 2012 [20]; Dadgostar *et al*., 2019) [9]. In conclusion, this study positions *P. niruri* as a viable solution to AMR in uropathogens, with its phytochemicals offering targeted efficacy (Lee *et al*., 2016 [17]; Ekwenye & Njoku *et al*., 2006) [11]. By bridging traditional medicine and modern microbiology, it paves the way for eco-friendly therapeutics (Gibbons *et al*., 2008 [13]; Ilanko & Cock *et al*., 2019) [15].

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Conflict of Interest:
Nil

Author's contributions
KP Conceptualized the study, designed the methodology, led the analysis, and drafted the original manuscript. HS and DP contributed to data curation and assisted with manuscript review and editing. JA and RK provided support with the data analysis MJR supervised and oversaw the study. MS provided overall direction and oversight the project.

Declarations
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Not applicable

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Availability of data and materials
The datasets and materials supporting the findings of this study, including original GC-MS data, are available from the corresponding author upon reasonable request for clarification.

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