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Phytochemical constituents, antioxidant and antibacterial activity of the Philippine Sampa-sampalukan (*Phyllanthus niruri*) leaf extract against selected human bacterial pathogens

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Abstract

The Philippine sampa-sampalukan (*Phyllanthus niruri*) is an underexplored plant with significant ethnopharmacological potential. This study examined the phytochemical constituents, antioxidant, and antimicrobial activity of its leaf ethanolic extract, collected from the botanical garden of Central Philippine University, Iloilo, Philippines. Qualitative analysis identified various bioactive compounds, including alkaloids, tannins, saponins, proteins, phenols, flavonoids, glycosides, carbohydrates, and terpenoids. Quantitatively, the extract contained 258.07±4.13 mg gallic acid equivalent (GAE)/g of total phenolics and 76.10±0.47 mg quercetin equivalent (QE)/g of flavonoids. The 1,1-diphenyl-1-picrylhydrazyl (DPPH) assay confirmed concentration-dependent antioxidant activity. The extract also exhibited strong antibacterial effects against *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *Streptococcus agalactiae*, *Edwardsiella tarda*, *Salmonella typhi, Escherichia coli,* and *Pseudomonas aeruginosa*, with significant inhibition at 100 mg/mL. This study underscores *P. niruri*'s potent bioactive compounds and their potential for therapeutic applications.

Keywords: *Phyllanthus niruri*, antimicrobial activity, antioxidant, phytochemical compounds, sampa-sampalukan, MRSA, *Staphylococcus aureus*, *Salmonella typhi*

1. Introduction

The Philippines, known for its diverse flora, is home to numerous plant species believed to have medicinal properties. However, many of these traditional claims still require scientific validation. The use of herbal remedies predates the Spanish colonization, although formal documentation began only during the Spanish era, with practitioners known as mediquillos or herbolarios—terms referring to traditional healers and herbalists (Quisumbing, 1978). In

1997, the government enacted Republic Act 8423, or the "Traditional and Alternative Medicine Act," which established the Philippine Institute of Traditional and Alternative Health Care. This institution aims to promote traditional healthcare by encouraging scientific research, validating the safety and efficacy of alternative treatments, and aligning these practices with national healthcare standards (Philippine Congress, 1997).

Modern pharmaceuticals often trace their origins to ancient herbal traditions, with many drugs derived from plants. These traditional medicinal practices, developed over centuries through observation and belief, laid the groundwork for modern pharmacology. Medicinal plants have long been used to treat diseases due to their therapeutic components, many of which exhibit antifungal, antibacterial, and antiprotozoal properties that can be applied topically or systemically (Cowan, 1999). Some plants, rich in volatile oils, polyphenols, and alkaloids, remain popular in folk medicine, while others have been developed into standardized products known as phytomedicines (Kaushik *and* Goyal, 2008). In the latter half of the 20th century, the rising popularity of traditional medicine coincided with the emergence of antimicrobial resistance, prompting researchers to explore plant-based treatments as alternatives. Plant-derived antimicrobials offer significant therapeutic potential by effectively treating infections while reducing the side effects commonly associated with synthetic drugs (Cowan, 1999).

One plant of particular interest is *Phyllanthus niruri*, locally known as "Sampa-sampalukan" in the Philippines. This herbaceous plant thrives in tropical regions and has been commonly used in traditional medicine for treating liver disorders, viral infections, and urinary tract conditions (Kaur *et al.*, 2017). In rural areas of the Philippines, where access to modern healthcare may be limited, *P. niruri* serves as a practical, cost-effective alternative to synthetic drugs. Its traditional applications include managing hepatitis, a prevalent health concern, as well as dissolving kidney stones and treating urinary tract infections—both common ailments in the country (Kaur *et al.*, 2017).

The medicinal value of *P. niruri* lies in its rich phytochemical profile, which includes lignans, tannins, coumarins, terpenes, flavonoids, alkaloids, saponins, and phenylpropanoids (Bagalkotkar *et al.*, 2006; Colpo *et al.*, 2014; Kaur *et al.*, 2017). These compounds provide multiple therapeutic benefits, with antioxidant properties being particularly noteworthy. By neutralizing harmful free radicals, the plant's antioxidants protect cells from oxidative damage, reducing the risk of chronic diseases (Colpo *et al.*, 2014). Moreover, *P. niruri* exhibits broad-spectrum antibacterial activity. Extracts from its leaves—whether aqueous, alcoholic, or methanolic—have demonstrated effectiveness against various bacterial strains, including both Gram-positive and Gram-negative pathogens such as *Bacillus pumilus*, *Escherichia coli*, *Vibrio cholerae*, and *Salmonella typhi* (Chandana *et al.*, 2020). This antimicrobial activity makes it a promising candidate for combating drug-resistant bacteria, which pose an increasing threat globally and within the Philippines.

While previous studies have examined *P. niruri*'s antioxidant and antibacterial properties, research on its efficacy against human pathogenic bacteria, especially emerging drug-resistant strains in the Philippines, remains limited. This gap in the literature underscores the importance of conducting comprehensive studies on the antimicrobial properties of *P. niruri*, particularly given its indigenous status in the Philippines. To bridge this gap, the current study examined the phytochemical constituents, antioxidant potential, and antibacterial activity of the *P. niruri* leaf ethanolic extract against selected human bacterial pathogens, including those implicated in zoonotic diseases. Resolute results from this study will not only deepen our understanding of *P. niruri*'s therapeutic potential but also lay the foundation for future research aimed at isolating novel bioactive compounds and developing effective treatments for infectious diseases. Additionally, the results of the current study will undoubtedly play a crucial role in strengthening both national and global efforts aimed at combating drug resistance, reinforcing the Philippines' healthcare system by providing accessible and effective treatments derived from local flora.

2. Materials and Methods

Collection of plant material

The *P. niruri* leaves were collected from the botanical garden of Central Philippine University, Iloilo (10° 43' 47.08" N, 122° 32' 58.32" E) in January 2024 at daytime with ambient temperature ranging from 29-32°C. The plant samples collected were authenticated by a botanist at the Life Sciences Department of Central Philippine University. Only those with green and undamaged leaves were considered for use. The *P. ninuri* leaf extract was prepared by washing leaf samples with tap water twice and once with distilled water, and subsequently dried in a drying oven at 45°C for 24 to 36 hours following a modified method adapted from Pakingking *et al.* (2022). The dried samples were homogenized and ground into small pieces using a mixer grinder. The powdered samples were collected in sterile amber bottles and stored at -20°C until used (Pakingking *et al.*, 2022).

Preparation of *P. niruri* extract

Using a modified method adapted from Pakingking *et al.* (2022), we extracted the bioactive compounds from powdered *P. niruri* leaves with ethanol. We soaked 150 grams of dried plant powder in 450 mL of 80% ethanol for 72 hours at room temperature (28ºC) with a 1:3 dilution ratio. After filtering the extract through sterile Whatman filter paper No. 1, we repeated the extraction for another 72 hours. The combined filtrates were concentrated with a rotary evaporator at 45°C under reduced pressure and stored at -20°C until needed for various analyses.

Qualitative Analysis

Phytochemical Testing

The *P. niruri* leaf ethanolic extract underwent a series of assays to identify its phytochemical compounds, following standard procedures from Harborne (1998), Evans (2009), and Silva *et al.* (2017). The presence of alkaloids was tested using Mayer's and Wagner's tests. Flavonoids were detected with the Shinoda test, glycosides with the Keller-Killiani test, and phenols and tannins with the ferric chloride test. Saponins were examined using the foam test, proteins with the Biuret test, carbohydrates with Molisch's test, and terpenoids with the Salkowski test.

Quantitative Analysis

Total phenolic content

The total soluble phenolic compounds in the *P. niruri* extract were quantified following Singleton's method (Singleton *et al.*, 1999) as detailed by Stankovic *et al.* (2011). The Folin-Ciocalteu reagent, with gallic acid as the reference standard, was used for this analysis. The extract, dissolved in ethanol at a concentration of 1 mg/mL, was combined with 2.5 mL of 10-fold diluted Folin-Ciocalteu reagent, 2 mL of 7.5% $NaHCO_3$, and 0.5 mL of the ethanolic extract. The mixture was allowed to stand for 45 minutes at 45°C before measuring the absorbance at 765 nm using a spectrophotometer. The total phenolic content was calculated from a gallic acid standard curve and expressed as milligrams of gallic acid equivalent per gram (mg GAE/g) of extract.

Total flavonoid content

The flavonoid content in *P. niruri* was assessed using the Dowd method (Arvouet-Grand *et al.*, 1994). A 1 mL sample of the extract solution (ranging from 25 to 200 μg/mL) or quercetin (25 to 200 μg/mL) was mixed with 0.2 mL of 10% aluminium chloride solution in methanol, 0.2 mL of 1 M potassium acetate, and 5.6 mL of distilled water. The mixture was incubated at room temperature for 30 minutes, and then the absorbance was measured at 415 nm against a blank. The flavonoid content was reported as milligrams of quercetin equivalents per gram (mg QE/g) of dry extract.

1,1-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The ability of *P. niruri* extract to neutralize 1,1-diphenyl-1-picrylhydrazyl (DPPH) free radicals was evaluated following the method of Cho *et al.* (2010), with some modifications. Stock solutions of the extracts were prepared in ethanol at concentrations of 2.5, 2.0, 1.0, 1.5, 0.75, 0.50, and 0.25 mg/mL. A 100 μL aliquot of each solution was mixed with 100 μL of 0.1 mM DPPH in ethanol. The mixture was incubated in the dark at room temperature (28°C) for 30 minutes, after which absorbance was measured at 515 nm using a microplate reader. Ethanol served as the blank, while 100 μL of ethanol mixed with 100 μL of DPPH was used as the control. Ascorbic acid (20 μg/mL) was included as a positive reference. The percentage of DPPH scavenged was calculated using the formula:

$$\text{DPPH Scavenging activity (\%)} = ([A_{Con} - A_{Test}]/A_{Con}) \times 100$$

where A_{Con} is the absorbance of the control and A_{Test} is the absorbance of the sample. The half-maximal inhibitory concentration (IC_{50}) was determined through linear regression analysis, with results averaged from three determinations.

Antibacterial Assay

Bacterial strains

The bacterial strains used in the assay included two *Staphylococcus aureus* strains (ATCC 25923 and BIOTECH 1582), *Streptococcus agalactiae* (TMD10206) (Pakingking *et al.*, 2022), methicillin-resistant *S. aureus* (RMC0224), *Edwardsiella tarda* (TK2014) (Pakingking and Nguyen, 2022), *Escherichia coli* (BIOTECH 1634), *Pseudomonas aeruginosa* strains (ATCC 27853 and BIOTECH 1335), and *Salmonella typhi* (RMC 0324) (see Table 1). These microorganisms were maintained in trypticase soy broth (TSB; Merck) with 15% glycerol at -80°C (Pakingking *et al.*, 2022).

Agar Well Diffusion Method

The antibacterial activity of the *P. niruri* ethanolic extract was assessed using a modified agar well diffusion method adapted from Mattana *et al.* (2010) and described by Pakingking *et al.* (2022). The extract was dissolved in sterile normal saline solution (NSS) to create a 1000 mg/mL concentration and then sterilized using a 0.45 μm membrane filter (Millipore). The tests were performed in triplicate with various concentrations of the extract diluted in NSS. Amoxicillin (0.025 mg/mL) served as the standard antimicrobial agent.

For the assay, bacterial isolates were cultured following a method described by Pakingking *et al.* (2015). Bacterial isolates were cultured on tryptic soy agar (TSA; Merck) plates and incubated at 37°C for 18–24 hours. Three to five colonies were then collected, suspended in sterile normal saline solution (NSS), and adjusted to match the 0.5 McFarland standard, achieving a concentration of approximately 1×10^8 CFU/mL. Bacterial suspensions were then evenly spread on Mueller-Hinton agar (MHA; Merck) plates and punctured with 7 mm wells using a sterile cork borer. Each well was filled with 100 µL of *P. niruri* extract at concentrations ranging from 1.562 to 100 mg/mL. Wells containing 100 µL of amoxicillin (0.025 mg/mL) and NSS served as positive and negative controls, respectively. The plates were incubated at 37°C for 24 hours, after which the zones of inhibition around the wells were measured. Clear zones around the wells indicated antibacterial activity.

Statistical Analysis

All experiments were performed in triplicate. To compare mean values across different treatments, one-way ANOVA was used. Significant differences between bacterial isolates for each treatment or *P. niruri* extract concentration were assessed using the Duncan test ($P < 0.05$).

3. Results

Yield of *P. niruri* extract

In the current study, 17.6 grams of crude *P. niruri* ethanolic extract were obtained, resulting in a yield of 11.73%. This yield is higher compared with the yield obtained by Sijuade (2016), who reported a yield of approximately 7.2% using 95% methanol (900 mL) as the extraction solvent from 510 grams of powdered *P. niruri*.

Qualitative Analysis

Phytochemical screening

As shown in Table 1, the qualitative phytochemical analysis of different aliquot samples of the *P. niruri*ethanolic extract identified a range of bioactive compounds. These include alkaloids, tannins, saponins, proteins, phenols, flavonoids, glycosides, carbohydrates, and terpenoids.

Table 1. Qualitative phytochemical screening of the *Phyllanthus niruri* ethanolic extract

Phytochemical compound	Test	Result		
		Aliquot 1	Aliquot 2	Aliquot 3
Alkaloids	Mayer's and Wagner's Test	+	+	+
Tannins	Ferric Chloride Test	+	+	+
Saponins	Foam Test	+	+	+
Proteins	Biuret Test	+	+	+
Phenols	Ferric Chloride Test	+	+	+
Flavonoids	Shinoda Test	+	+	+
Glycosides	Keller Killiani Test	+	+	+
Carbohydrates	Molisch's test	+	+	+
Terpenoids	Salkowski Test	+	+	+

(+) = present

Quantitative Analysis

Total phenolic content

As presented in Table 2, the mean total phenolic content in the three aliquots of *P. niruri* ethanolic extract was 258.07±4.13 mg GAE/g.

Total flavonoid content

The total flavonoid content of the *P. niruri* ethanolic extract was determined using quercetin as the standard and expressed as milligrams of quercetin equivalents per gram (mg QE/g). The average flavonoid content across the three aliquots of the extract was 76.10±0.47 mg QE/g (Table 2).

Table 2. Total phenolic and flavonoid contents of the *Phyllanthus niruri* ethanolic extract

Test	Result*
Total phenolic content (mg of gallic acid equivalent per gram [mg GAE/ g] of extract)	258.07±4.13
Total flavonoid content (mg of quercetin equivalent per gram [mg QE/g] of extract)	76.10±0.47

*Data are presented as Mean ± SD of 3 aliquot samples

DPPH radical scavenging activity

Fig. 1 shows the DPPH radical scavenging activity of the *P. niruri* ethanolic extract, which demonstrated a concentration-dependent increase in its scavenging capacity, with values ranging from 0.25 to 2.5 mg/mL. The scavenging activity increased from 21.7±1.53% at the lowest concentration (0.25 mg/mL) to 89.6±1.04% at the highest concentration (2.5 mg/mL). The IC_{50} value for the extract was determined to be 0.9 mg/mL. Additionally, the scavenging performance of the *P. niruri* extract was similar to that of the positive control, ascorbic acid.

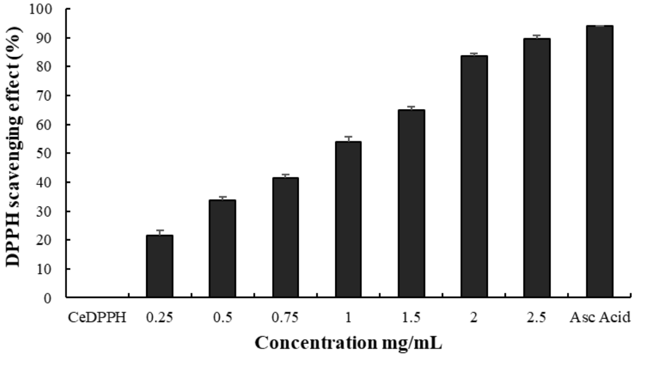

Figure 1. DPPH (1,1-Diphenyl-1-picrylhydrazyl) radical scavenging activity of the *Phyllanthus niruri* ethanolic extract. Ethanol + DPPH (CeDPPH) and ascorbic acid (Asc) were used as negative and positive controls, respectively.

Antibacterial activity

To assess the antibacterial activity of *P. niruri* ethanolic extract, the agar well diffusion method was used. As shown in Table 3, amoxicillin demonstrated strong activity against all tested Gram-positive bacteria, with inhibition zones ranging from 27.7±0.6 mm for S. aureus BIOTECH 1582 to 30.7±1.2 mm for *S. aureus* ATCC 25923. It also showed effectiveness against most Gram-negative bacteria, except for *E. coli* and both strains of *P. aeruginosa*, where no inhibition occurred. The negative control (NSS) produced no inhibition in any bacteria tested.

The extract displayed significant antibacterial activity against both strains of *S. aureus*, with inhibition zones at 100 mg/mL similar to amoxicillin. A clear concentration-dependent decrease in activity was observed, with lower concentrations leading to reduced inhibition zones. *S. aureus* ATCC 25923 was slightly more sensitive than BIOTECH 1582 at lower extract concentrations. The extract also had moderate activity against MRSA, with the highest inhibition zone at 100 mg/mL (22.3±0.6 mm), though it was less effective below 50 mg/mL. Additionally, the extract was effective against *S. agalactiae*, with inhibition zones similar to those of *S. aureus* at higher concentrations.

Among Gram-negative bacteria, it showed strong activity against *E. tarda* at 100 mg/mL, but this decreased significantly below 12.5 mg/mL, with no inhibition at 3.13 mg/mL or lower. The extract was moderately effective against *E. coli* at 100 mg/mL but less so at lower concentrations. Both strains of *P. aeruginosa* showed moderate sensitivity to the extract at higher concentrations. Finally, *S. typhi* was highly sensitive to the extract, with inhibition zones similar to *S. aureus* and *S. agalactiae* at 100 mg/mL, though the activity decreased as the concentration was lowered.

Table 3. Antimicrobial activity of *Phyllanthus niruri* ethanolic extract against selected human bacterial pathogens

Microorganism	Zone (mm ± SD)								
	Amox.[1] 0.025 mg/mL	NSS[2]	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	3.13 mg/mL	1.56 mg/mL
Gram-Positive									
Staphylococcus aureus ATCC 25923	30.7±1.2	–	29.3±0.6[a]	27.3±0.6[ab]	24.7±0.6[a]	21.3±0.6[a]	19.3±0.6[a]	16.7±0.6[a]	14.3±0.6[a]
Staphylococcus aureus BIOTECH 1582	27.7 ±0.6	–	28.3±1.2[a]	25.7±0.6[c]	24.3±0.0[a]	21.7±0.6[a]	19.7±0.6[a]	15.7±0.6[b]	–
Methicillin resistant *S. aureus* RMC0224	–	–	22.3±0.6[c]	18.3±0.6[e]	–	–	–	–	–
Streptococcus agalactiae TMD10206	29.7±0.6	–	28.3±0.6[a]	25.7±0.6[c]	22.3±0.6[b]	19.7±0.6[b]	17.7±0.6[b]	14.7±0.6[c]	–
Gram-negative									
Edwardsiella tarda TK2014	29.3±0.6	–	29.0±1.0[a]	27.7±0.6[a]	24.3±0.6[a]	22.3±0.6[a]	14.7±0.6[c]	–	–
Escherichia coli BIOTECH 1634	–	–	23.3±0.6[bc]	19.0±1.0[de]	17.3±0.6[c]	14.0±1.0[c]	11.3±1.2[d]	–	–
Pseudomonas aeruginosa ATCC 27853	–	–	23.7±0.6[b]	20.0±0.0[d]	18.0±0.0[c]	14.7±0.6[c]	12.3±0.6[d]	–	–
P. aeruginosa BIOTECH 1335	–	–	23.3±0.6[bc]	19.7±0.6[d]	17.7±0.6[c]	14.7±0.6[c]	12.0±0.0[d]	–	–
Salmonella typhi RMC 0324	29.3±0.6	–	28.3±0.6[a]	26.3±1.2[bc]	24.3±0.6[a]	21.3±0.6[a]	19.3±0.0[a]	16.7±0.6[a]	14.3±0.6[a]

[1]Amoxicillin. [2]Normal saline solution. (–) no zone of inhibition. Data are presented as Mean ± SD. Values with different superscripts a, b, c, d, e within each column are significantly different as determined by Duncan test ($P < 0.05$)

4. Discussion

The rise of multidrug-resistant (MDR) bacteria presents a significant global health crisis, requiring continuous monitoring and placing immense pressure on the scientific community to discover effective solutions (Lai *et al.*, 2014). The diminishing efficacy and heightened toxicity of synthetic drugs have exacerbated this issue, leading researchers to explore natural alternatives (Abellera *et al.*, 2019; Valle *et al.*, 2015). *P. niruri*, locally known as sampa-sampalukan, has demonstrated antimicrobial properties effective against bacterial infections responsible for conditions such as diarrhea, dysentery, tuberculosis, cough, and vaginitis (Amin *et al.*, 2012; Bagalkotkar *et al.*, 2006; Campos *and* Schor, 1999). However, data on its efficacy against human pathogenic bacteria, particularly multidrug-resistant strains and those implicated in zoonotic diseases, remain scarce or underexplored in the Philippines. This study addressed this gap by examining the phytochemical composition, antioxidant capacity, and antibacterial activity of the ethanolic extract from *P. niruri*.

The 11.73% yield obtained from the ethanolic extract of *P. niruri* in this study is significant, as it exceeded previously reported yields with other solvents. For instance, Sijuade (2016) reported a yield of approximately 7.2% using 95% methanol, which is notably lower than what was achieved in this study. Several factors could explain this discrepancy, including differences in solvent polarity, extraction techniques, plant material preparation, or variations in the plant's chemical composition due to regional factors. Ethanol's ability to extract both polar and non-polar compounds likely contributed to the higher yield observed. This finding suggests that ethanolic extraction may be more effective for isolating bioactive compounds from *P. niruri*, making it a favorable method for studies focused on phytochemical analysis and biological activity (Azmir *et al.*, 2013).

Qualitative phytochemical analysis of *P. niruri* revealed the presence of diverse bioactive compounds, including alkaloids, tannins, saponins, proteins, phenols, flavonoids, glycosides, carbohydrates, and terpenoids (Table 1). These compounds are well known for their diverse biological activities and contribute to the plant's traditional medicinal applications. Notably, our findings are consistent with previous studies (Buba *et al.*, 2023; Rusmana *et al.*, 2017); however, there are some discrepancies. Buba *et al.* (2023) did not detect tannins in their analysis, while Rusmana *et al.* (2017) failed to identify alkaloids and terpenoids. Such variations could be attributed to differences in plant species, the geographical origin of the plant samples, seasonal changes, or the extraction techniques employed. These factors can greatly influence the chemical profile of a plant, leading to differing results across studies (Bedona *et al.*, 2024).

The bioactive compounds identified in the ethanolic extract of *P. niruri* play crucial roles in its therapeutic potential. Alkaloids, for example, are well-documented for their antimicrobial and anticancer activities, while phenols and flavonoids are renowned for their potent antioxidant properties (Doughari *et al.*, 2007; Gutiérrez-Grijalva *et al.*, 2020; Thawabteh *et al.*, 2019). These antioxidants help scavenge free radicals, reducing oxidative stress, which is a key factor in aging and various chronic diseases. Additionally, the presence of saponins and tannins in the extract further highlights the medicinal value of *P. niruri*. Saponins are known for their immune-modulatory properties, contributing to enhanced immune system function and defense against infections. They are also recognized for their role in cholesterol regulation. Tannins, on the other hand, possess antimicrobial and astringent properties, which support wound healing and infection prevention (Edeoga *et al.*, 2005).

This phytochemical profile emphasizes the therapeutic potential of *P. niruri*, reinforcing its role in traditional medicine. These compounds have been associated with various biological activities, including antioxidant, antimicrobial, and anti-inflammatory effects, which are consistent with the plant's use in treating various ailments. For instance, *P. niruri* has been traditionally used in the treatment of liver disorders, kidney stones, and diabetes, all of which may be related to its rich phytochemical composition. Moreover, the presence of flavonoids and phenols aligns with its known anti-inflammatory properties, which are essential for managing inflammatory diseases and conditions. The consistency of these results with earlier studies (Bagalkotkar *et al.*, 2006) further supports the reliability of the phytochemical screening methods used and underscores the broad applicability of *P. niruri* as a source of bioactive compounds. As scientific interest in natural products grows, the comprehensive phytochemical profile of *P. niruri* offers a promising foundation for developing therapeutic agents, particularly for combating oxidative stress-related diseases, microbial infections, and inflammation.

The total phenolic content of 258.07±4.13 mg GAE/g and total flavonoid content of 76.10±0.47 mg QE/g observed in this study underscore *P. niruri* as a rich source of antioxidants. Phenolic compounds, known for their ability to neutralize free radicals, play an essential role in protecting cells from oxidative stress by preventing cellular damage and reducing the risk of chronic diseases (Dai *and* Mumper, 2010). Phenolics achieve this by donating hydrogen atoms or electrons to free radicals, effectively inhibiting their harmful effects on biological systems. This antioxidant capacity is particularly important in addressing conditions associated with oxidative stress, including aging, cancer, and cardiovascular diseases (Gulcin, 2020).

Flavonoids, a diverse group of secondary metabolites, are also recognized for their potent antioxidant and anti-inflammatory properties (Kumar *and* Pandey, 2013). These compounds not only scavenge free radicals but also modulate signaling pathways involved in inflammation and immune responses. Their high concentration in *P. niruri* adds to the plant's potential to mitigate oxidative damage and inflammation, which are critical factors in the development of various chronic diseases, such as neurodegenerative disorders, diabetes, and inflammatory conditions (Akinmoladun *et al.*, 2007). By stabilizing reactive oxygen species (ROS), flavonoids contribute to maintaining cellular homeostasis and protecting tissues from oxidative damage (Kumar *and* Pandey, 2013).

The ethanolic extract of *P. niruri* exhibited remarkable antioxidant activity, as shown by its ability to scavenge DPPH radicals in a concentration-dependent manner. At 2.5 mg/mL, it achieved 89.6% inhibition, with an IC_{50} value of 0.9 mg/mL, highlighting its potency in neutralizing free radicals, which are major contributors to oxidative stress and cellular damage. This antioxidant capacity is largely due to its high concentrations of phenolic and flavonoid compounds, which are known for their role in combating oxidative stress-related diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions. These bioactive compounds not only disrupt harmful oxidative reactions but also protect critical cellular components, such as lipids, proteins, and DNA, from damage.

Studies, such as those by Silva *et al.* (2017) and Singh *et al.* (2016), have consistently supported these findings, demonstrating that *P. niruri*'s phenolics and flavonoids contribute significantly to its antioxidant and anti-inflammatory effects. Furthermore, the synergistic action of these compounds enhances the overall antioxidant capacity of the extract, making it an even more effective natural remedy. This synergy emphasizes the importance of preserving a wide range of bioactive compounds during extraction to maximize therapeutic benefits. Given its strong antioxidant activity and protective properties, *P. niruri* holds promise for developing natural therapies aimed at managing oxidative stress and its associated health conditions. The consistency of these findings across multiple studies also validates its traditional use in treating diseases linked to oxidative stress, making it a valuable candidate for further research and potential clinical applications.

Our current study revealed that *P. niruri* ethanolic extract is particularly potent against Gram-positive bacteria, especially *S. aureus*, including methicillin-resistant strain (MRSA). The extract produced a notable inhibition zone of 22.3±0.6 mm at 100 mg/mL against MRSA, highlighting its potential as a complementary treatment for drug-resistant infections. This highlights *P. niruri* as a promising candidate in the fight against antibiotic-resistant bacteria, a global health issue exacerbated by the rise of antimicrobial resistance (Giedraitienė *et al.*, 2011; Huemer *et al.*, 2020). The effectiveness of *P. niruri* against *S. aureus* strains is consistent with earlier studies. For example, Kumar *et al.* (2023) and Ibrahim *et al.* (2013) also reported antibacterial efficacy of *P. niruri* extracts. Notably, our study demonstrated higher inhibition zones (29.3±0.6 mm and 28.3±1.2 mm for strains ATCC 25923 and BIOTECH 1582, respectively), which surpass previous reports of 15.0±0.3 mm (Ibrahim *et al.*, 2013) and 7.33 ±1.20 mm (Singh *et al.*, 2016) for *P. niruri* methanolic and ethanolic extracts, respectively.

The concentration-dependent antibacterial activity against *S. aureus* further emphasizes the extract's potential in treating infections resistant to first-line antibiotics, such as skin infections and foodborne illnesses caused by *S. aureus* (Arfatahery *et al.*, 2015). The extract also displayed strong antibacterial activity against *S. agalactiae*, a pathogen of growing concern due to its zoonotic potential, especially in humans and fish (Li *et al.*, 2020). This finding supports the therapeutic relevance of *P. niruri* in addressing multidrug-resistant *S. agalactiae* strains, which have been linked to infections through contaminated fish (Li *et al.*, 2020).

P. niruri also exhibited significant antibacterial effects against several Gram-negative bacteria, although the activity was generally lower compared to Gram-positive bacteria. This may be attributed to the more complex cell wall structure of Gram-negative bacteria, which often acts as a barrier to bioactive compounds. In particular, the extract demonstrated antibacterial activity against *E. coli*, *P. aeruginosa*, *E. tarda*, and *S. typhi*. At a concentration of 100 mg/mL, substantial inhibition zones were observed, and even at lower concentrations (6.25 mg/mL), antibacterial activity remained evident.

Our current data highlight the potential of *P. niruri* extract as a valuable source of antibacterial compounds, offering a pragmatic strategy for combating multi drug-resistant bacterial pathogens (Parmanik *et al.*, 2022). It is particularly noteworthy that this study is the first to report the antibacterial activity of *P. niruri* ethanolic extract against *E. tarda*. Human infections caused by *E. tarda* typically manifest as gastroenteritis but can progress to severe systemic and potentially life-threatening conditions (Pakingking and Nguyen, 2022). The ability of *P. niruri* to inhibit *E. tarda* makes it a promising candidate for further development as a treatment for infections caused by this pathogen.

A particularly significant finding of this study is the extract's potent activity against MRSA, a major public health threat due to its resistance to β-lactam antibiotics, including amoxicillin. MRSA is a leading cause of severe infections such as bacteremia, endocarditis, and hospital-acquired infections (Turner *et al.*, 2019). The production of β-lactamase enzymes by MRSA strains renders traditional β-lactam antibiotics ineffective, creating an urgent need for new therapeutic strategies. The ability of *P. niruri* to inhibit MRSA growth suggests its potential as an alternative treatment, warranting further investigation.

Our current results concur with those of Valle *et al.* (2015), who also documented the efficacy of *P. niruri* against MRSA. The concentration-dependent response observed in the current study further supports the therapeutic potential of *P. niruri* for treating MRSA infections. Future research should therefore focus on isolating and characterizing the major bioactive components responsible for this activity, to develop more targeted antibacterial therapies. Additionally, further investigation into the antiparasitic properties of *P. niruri*, particularly against protozoan pathogens like *Entamoeba histolytica*, would expand its therapeutic applications (Beup *et al.*, 2024). Understanding the specific mechanisms of action of *P. niruri* compounds will be crucial in developing new treatments for multidrug-resistant bacterial infections and parasitic diseases.

In conclusion, the ethanolic extract of *P. niruri* demonstrated considerable antibacterial activity against various human pathogens, including drug-resistant strains like MRSA. Its broad-spectrum effectiveness, in light of the rising threat of antimicrobial resistance, positions *P. niruri* as a strong candidate for further exploration in developing treatments for bacterial infections. This study underscores the importance of traditional medicinal plants as potential sources of innovative medical solutions. The findings also highlight the extract's promising potential, with its high yield, rich phytochemical content, potent antioxidant activity, and effective antibacterial properties, especially against resistant pathogens like MRSA. These results are pivotal to the ongoing investigation of *P. niruri* for its medicinal applications, particularly in the realm of traditional remedies and the fight against antimicrobial resistance.

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Conflict of interests
The authors affirm that they have no competing interests to disclose.

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