ORIGINAL ARTICLE

Evaluation of Antimicrobial Property and Phytochemicals of Local *Phyllanthus niruri* Plant

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ABSTRACT

Introduction: Employing natural plants as antibacterial agents is one strategy for combating the challenges of drug-resistant microorganisms. This research examines the antibacterial activity of methanolic *Phyllanthus niruri* (*P. niruri*) plant extract against *Propionibacterium acnes* (*P. acnes*), *Staphylococcus epidermidis* (*S. epidermidis*), *Streptococcus mutans* (*S. mutans*), *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), as well as its phytochemical components. **Materials and methods:** The antibacterial activity was evaluated utilising agar well diffusion, broth microdilution, and the streaking technique. The phytochemical compounds were identified via qualitative testing. **Results:** Antimicrobial susceptibility testing (AST) revealed that the mean inhibition zone ranged between 48.00 mm and 9.33 mm. *P. acnes* had the highest level of inhibition (48.00±1.00 mm), whereas *E. coli* had the lowest (9.33±0.58 mm). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) suggested a concentration range between 1.95 mg/ml and 125 mg/ml. The extract's antibacterial activity indicated that gram-positive bacteria were more sensitive than gram-negative bacteria. Additionally, the plant extract contained alkaloids, flavonoids, glycosides, phenolics, tannins, saponins, and steroids. **Conclusion:** The findings of this investigation indicate that *P. niruri* extract might serve as a source of antibacterial agent in the development of alternative antibiotic medication.
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Keywords: *Phyllanthus niruri*, Antibacterial activity, Phytochemical compounds, Plant extract, Methanolic

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INTRODUCTION

Pathogenic bacterial infections represent a global public health threat, underscoring the critical need for effective interventions. Recent studies have shown that the rise of drug-resistant bacterial strains has resulted in the fatalities of approximately 4.95 million people in 204 countries, including 1.27 million deaths directly related to bacterial antibacterial resistance (1). The majority of fatalities attributed to bacterial antibacterial resistance were observed in low- and middle-income countries, whereas high-income countries reported relatively fewer cases (2). Insufficient testing coverage and weak laboratory capacity are contributing factors to the management of drug-resistant bacterial strains. According to recent research, the region of western sub-Saharan Africa reported a high mortality rate of 27.3 deaths per 100,000 individuals, while the mortality rate in Australasia was the lowest at 6.5 deaths per 100,000 people (1). The World Health Organisation (WHO) reported that significant amounts of resistance are found in bacteria, including *K. pneumoniae* and *Acinetobacter spp.*, which frequently cause bloodstream infections in healthcare facilities (3). In Malaysia, the most prevalent drug-resistant bacterial strain cases are associated with urinary tract infections caused by *E. coli* (4).

In a recent clinical investigation, it was discovered that *K. pneumoniae* is resistant to aminoglycosides, ciprofloxacin, cotrimoxazole, carbapenem, piperacillin, and tazobactam (5). The finding is consistent with a study performed in Spain involving four hospitals, in which *K. pneumoniae* was found to be resistant to amoxicillin, ciprofloxacin, piperacillin, tazobactam, ceftazidime, and fosfomycin. The bacterial strains isolated from urine samples exhibit significantly higher levels of resistance compared to those obtained from blood and respiratory systems (6). The presented data unambiguously indicate a decline in commercial antibiotics' efficacy to treat bacterial infections. In response to a growing number of drug-resistant bacterial strains, pharmaceutical

prescription research based on plant extracts has become increasingly popular and is spreading rapidly.

Malaysia is a nation that possesses a wealth of botanical resources, which encompasses various herbs such as *Orthosiphon aristatus*, *Coriandrum sativum, Labisia pumila,* and *Cymbopogon nardus*. The utilisation of herbs is restricted to culinary and medicinal purposes in traditional practices, lacking the development of these herbs into clinical therapies. The *Phyllanthus niruri* plant belongs to the *Phyllanthaceae* family and is commonly referred to as *dukung anak* in local parlance. The botanical specimen is classified as a weed. It exhibits a propensity for flourishing in soil with high moisture content, predominantly prevalent in tropical and subtropical climate regions, such as India, China, Africa, and Southeast Asia (7). This plant has the potential to attain a height of 30 to 40 cm through the growth of its herbaceous branches. The leaves exhibit several distinguishing features, including their diminutive size, green colouration, sub-sessile nature, closely packed arrangement, elliptical-oval shape, obtuse apex, short petioles, stipules, and alternate disposition along the stem. The fruits connected with the leaves possess capsules that are tiny globose depressed and additional capsules that are smooth with 2 to 3 mm in diameter. The botanical specimen in question features a verdant cylindrical structure, characterised by transverse appendages that are interconnected through a branching system and accompanied by subterranean solid roots (8).

The application of the *P. niruri* plant in traditional medicine has been a longstanding practise spanning generations. The functionality of the plant is contingent upon its location. The plant is commonly utilised for medicinal purposes in the treatment of renal calculi, asthma, bronchitis, and cough (9,10). Additionally, it has been used as a hematinic for liver function restoration and to enhance the body's immune system (11). According to recent research, *P. niruri* has demonstrated its potential as an antidiabetic agent. The study revealed that the water extract of *P. niruri* comprises corilagin and repandusinic, which function as inhibitors of α-glucosidase (12). The molecules are known to negatively impact carbohydrate absorption by competitively inhibiting enzymes responsible for converting complex non-absorbable carbohydrates into simple absorbable carbohydrates, thereby leading to a decrease in blood glucose concentration. Another study demonstrated the advantageous effects of *P. niruri* extract as an antioxidant. The phenolic compound present in *P. niruri* is known to significantly impact the scavenging and neutralisation of free radicals. In contrast, *P. niruri* exhibits antimicrobial properties . Recent research has revealed that trimetallic nanoparticles of Ag-Cu-Co, synthesized from *P. niruri* extract induce cellular demise by engaging in interactions between the cationic metal ions of the nanoparticles and the anionic bacterial cell walls and proteins. Furthermore, these metal ions impede bacterial proliferation as they interact with DNA (13). The hydroalcoholic extract of *P. niruri* leaf was also found to be effective in preventing the growth of *Candida sp.* Simultaneously, it does not exhibit cytotoxicity towards human cells (14).

The emergence of resistant strains of microorganisms against existing antibiotics is becoming a great concern nowadays, resulting in more antibiotics becoming ineffective against the organisms. The antibacterial efficacy of *P. niruri* extract has been extensively documented, albeit with limited focus on drug-resistant bacterial strains. Conversely, a prior investigation exclusively employed *P. niruri* leaves. Consequently, this research endeavors to assess the antimicrobial potential of *P. niruri* plant against bacterial strains resistant to conventional antibiotics, presenting it as a viable alternative to synthetic counterparts. Notably, this study comprehensively utilized entire parts of the plant rather than isolating the leaves, thus optimizing resource utilization. The primary goal of this research is to examine the phytochemical composition of the methanolic extract derived from the *P. niruri* plant. Additionally, the study aims to evaluate the antimicrobial efficacy of the plant against a selected group of pathogens. The findings of this investigation may serve as a basis of reference for the commercialisation of natural products within the pharmaceutical sector.

MATERIALS AND METHODS

The primary material utilized in this study was the entire part of *P. niruri* plant including the roots, stems, leaves and fruits. Extraction was carried out using absolute methanol (Merck, Germany), followed by dilution with Dimethyl sulfoxide (DMSO) (Amresco, USA). Moreover, Ammonia (Merck, Germany), Benzene (Merck, Germany), Chloroform (Merck, Germany), Fehling A and Fehling B reagents (R&M Chemicals, Malaysia), Ferric Chloride (MP Biomedicals, USA), Sulfuric Acid (SPL Life Science, Korea), and Wagner's Reagent (R&M Chemicals, Malaysia) were employed in conducting phytochemical tests on the *P. niruri* extract. 5% of sheep blood agar (Oxoid, England), Mueller-Hinton Broth (MHB) (Oxoid, England), Mueller-Hinton Agar (MHA) (Oxoid, England) was used for bacterial growth. To evaluate their effects on the specified organisms, Amikacin (Oxoid, England), Chloramphenicol (Oxoid, England), and Gentamicin (Oxoid, England) served as positive controls.

Preparation of *P. niruri* Plant Sample

P. niruri was collected from Haji Nursery in Batu Caves, Selangor, Malaysia. The plant was identified using plant identifier application. A healthy plant with upright growth, open rather than coiled development, and consistent colour was selected. The plant's surface was wiped dry using tissue paper. It plant was put in a clear

zip-lock bag and brought to the lab at room temperature. The *P. niruri* plant was cleaned and dried with distilled water to eliminate dust. It was dried at 65 °C for 48 hours and powdered using a mechanical grinder. The sample was weighed and stored in a Schott bottle in a 4 °C chiller.

Extraction Process of *P. niruri* Plant
100 g of plant powder was soaked in 1000 ml of 100% methanol and covered with aluminium foil to reduce solvent evaporation. The sample was left for seven days at room temperature with 110 rpm platform shaker shaking. After seven days, the solution was filtered and transferred to a flask. A crude extract was yielded using a rotating evaporator at 40 to 45 °C and 250 mbar pressure. The extract was collected halfway through the process and evaporated in the fume hood. The crude extract was placed in a smaller Schott container and chilled to 4 °C. The Schott bottle was weighed before and after filling the extract plant to quantify the extract yield.

Preparation of Concentrated *P. niruri* Plant Extract
About 1000 mg/ml of *P. niruri* extract was used as the working solution. 10 g of the plant extract was combined with 10 ml of 10% DMSO to make a 1000 mg/ml solution.

Qualitative Phytochemical Analysis of *P. niruri* Extract
A preliminary phytochemical screening test was conducted on 1000 mg/ml of methanolic extract of *P. niruri*. The test was done to determine the phytochemical compound in the extract using previous studies' standard method with slight modification (15,16). The visual colour change reaction was used as the basis for the qualitative analysis in response to the presence of particular phytochemical components.

Wagner's Test for Alkaloids
2 ml of chloroform, 1 ml of *P. niruri* extract, and a few drops of Wagner's reagent were added to a test tube and mixed well. The reddish-brown precipitate's appearance suggested the presence of alkaloids.

Borntrager's Test for Anthraquinones
1 ml of *P. niruri* extract and 0.05 ml of benzene were added to a test tube. The mixture was then shaken gently and filtered using filter paper. About 2.5 ml of 10% ammonia was added to the filtrate and mixed well. The appearance of pink, red, or violet indicated the presence of anthraquinones.

Diluted Ammonia Test for Flavonoids
A few drops of 1% ammonia were added to 2 ml of *P. niruri* extract and mixed well. The production of a yellow or red colour showed the presence of flavonoids.

Glycosides Test for Glycosides
Equal amounts of Fehling A and Fehling B were added to 2 ml of plant extract and mixed well. The solution was then heated until homogeneous. The production of brick-red precipitate indicated the presence of glycosides.

Ferric Chloride Test for Phenolics
2 ml of *P. niruri* extract was added to 3 ml of distilled water. A few drops of 10% ferric chloride were introduced into the mixture. The solution was mixed well and observed. Phenolics could be identified by deep blue or blue - black appearance.

Ferric Chloride Test for Tannins
A few drops of 5% ferric chloride were added to 1 ml of *P. niruri* extract. The mixture was mixed well and observed. Brownish green or blue-black appearance indicated the presence of tannins.

Foam Test for Saponins
3 ml of distilled water was added to 2 ml of *P. niruri* extract. The mixture was then shaken vigorously to obtain persistent foam. The development of a foamy layer indicated the presence of saponins.

Liebermann-Burchard's Test for Steroids
2 ml of chloroform was dissolved with 2 ml of *P. niruri* extract. A few drops of sulphuric acid were added along the sides of the test tube. Red colour on the top layer of the mixture and yellow colour on the sulphuric acid layer were observed.

Bacterial Culture and Identification
P. acnes (ATCC 6919), *S. epidermidis* (ATCC 12228), *S. mutans* (ATCC 25175), *E. coli* (ATCC 259220, and *K. pneumoniae* (ATCC 13883) were streaked on 5% of sheep blood agar to form a single colony. The organisms were incubated at 37 °C for 24 hours, with the exception of *P. acnes*, which was set anaerobically for 168 hours. Single colonies of each selected bacteria were mixed with saline water on a glass slide to perform gram staining. The slides were treated with crystal violet, gram's iodine, decolouriser, and safranin. The bacteria morphology was viewed usi

P. acnes was tested on catalase, indole, and carbohydrate fermentation tests. The catalase and antibiotic susceptibility tests were performed on *S. epidermidis* and *S. mutans*. Both bacterial species were treated with antibiotics, novobiocin, and optochin, respectively. A coagulase test was performed on *S. epidermidis* to differentiate between coagulase-negative *Staphylococci* and *Staphylococcus aureus*. Both *E. coli* and *K. pneumoniae* were tested on citrate, motility, TSI, urease, indole, MR, VP, and oxidase tests. Every reaction was read and recorded.

Antimicrobial Susceptibility Testing (AST)
The methodology of antimicrobial testing was adopted from the Clinical and Laboratory Standard Institute (CLSI) with slight modifications. Each organism was

introduced into MHB with three to five colonies, and the bacteria were grown at 37 °C for a specified time based on the organism's log phase. Turbidity was adjusted to 0.5 McFarland. A sterile cotton swab was dipped into the prepared bacterial culture and lawn on MHA. Two wells were made in the agar using a sterile borer. Each well contained 50 µl of 1000 mg/ml plant extract and 50 µl of 10% DMSO as a negative control. Additionally, an antibiotic disc was laid on the lawn agar. Chloramphenicol (30 µg) was used as a control positive for *P. acnes, S. epidermidis,* and *S. mutans*, gentamicin (10 µg) for *E. coli*, and amikacin (30 µg) for *K. pneumoniae*. The well-diffusion testing was performed in triplicate to determine the average inhibition zone of the plant extract. The bacteria were incubated at 37 °C for 24 hours except for *S. mutans* for 48 hours and *P. acnes*, anaerobically, for 168 hours. The inhibition zone on the agar was observed, measured, and recorded after the incubation period (17).

Minimum Inhibitory Concentration (MIC) Determination

Each organism was introduced into MHB with three to five colonies. The bacteria were then grown at 37 °C for a log phase-specific period. The suspension's turbidity was adjusted to 0.5 McFarland and a 1:100 suspension of diluted bacterial culture was made. MIC testing was done using a microtiter plate of 96 wells. Approximately 1000 mg/ml of *P. niruri* plant extract was diluted two-fold. 50 µl of diluted plant extract was pipetted into wells 1 to 10 in decreasing concentration order. 50 µl of microbial suspension was added to wells 1 to 10 for a final inoculum concentration of $5x10^5$ CFU/ml. The last two wells were filled with sterilised MHB. 50 µl of bacterial suspension was added into wells 1 to 11 and 10% DMSO into well 12 as the negative growth control. All bacterial species were incubated at 37 °C for 24 hours, except for *S. mutans* and *P. acnes*, which were incubated at 37 °C for 48 hours aerobically and at 168 hours anaerobically. The bacterial growth was observed by the turbidity and pallet formation at the bottom of the well. MIC was determined at the last clear well, next to a turbid well (17).

Minimum Bactericidal Concentration (MBC) Determination

The aliquot of the well possessing equal to or higher than the MIC concentration and controls was cultured on MHA. The media was then incubated at 37 °C for 24 hours except for *S. mutans* for 48 hours and *P. acnes*, anaerobically, for 168 hours. The test was performed in triplicate. The growth of bacteria was observed after the incubation time. The MBC was determined and recorded based on the lowest leaf extract concentration, inhibiting 99.9% of bacterial growth (17).

Statistical Data Analysis

The AST result was analysed using an independent t-test. The Statistical Package for the Social Sciences (SPSS) software version 2 was used to analyse the data.

RESULTS

The application of 1000 ml of absolute methanol in the extraction process of 100g of *P. niruri* plant powder led to the production of a 9.13 g crude extract. This extract exhibited distinct attributes, including a dark brown pigment, a viscid texture, and a lustrous surface.

Table I: Qualitative phytochemical analysis results for *P. niruri* plant extract.

Phytochemical test	Active compound	Result
Wagner's Test	Alkaloids	+
Borntrager's Test	Anthraquinones	-
Diluted Ammonia Test	Flavonoids	+
Glycosides Test	Glycosides	+
Ferric Chloride Test	Phenolics	+
Ferric Chloride Test	Tannins	+
Foam Test	Saponins	+
Liebermann-Burchard's Test	Steroids	+

(+) present, (-) absent

Table I shows the phytochemical examination results of the *P. niruri* methanolic extract, which demonstrated the presence of alkaloids, flavonoids, glycosides, phenolics, saponins, steroids, and tannins.

Figure 1 illustrates the AST results of each organism. Plant extract samples as well as positive and negative controls were tested on the same plate. Clear areas were observed around the well and disc, indicating no bacterial growth. The area was known as the inhibition zone. Its diameter was measured across the well or disc's centre from one endpoint to another. The standard size of the well and disk was 6 mm. Therefore, although no inhibition was seen, the measurement was 6 mm considering the size of the well.

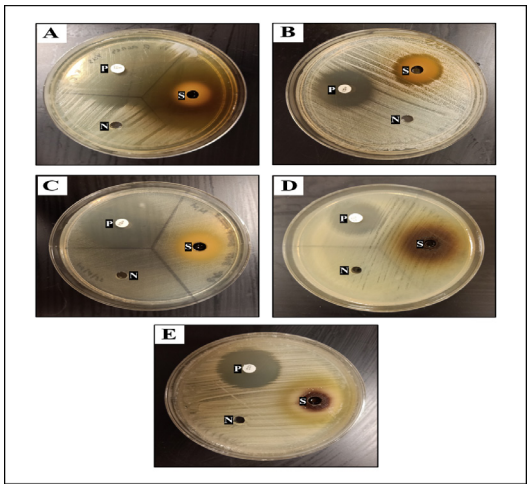

Figure 1: The zone of inhibition indicating antimicrobial activity of P. niruri plant extract.
A = test on *P. acnes*, B = test on *S. epidermidis*, C = test on *S. mutans*, D = test on *E. coli*, E = test on *K. pneumoniae*, N = negative control, P = positive control, S = extract sample.

Table II: The inhibition zone of *P. niruri* plant extracts against selected bacteria.

Variables	Mean inhibition zone of triplicate test ± SD (mm)				
	P. acnes	*S. epidermidis*	*S. mutans*	*E. coli*	*K. pneumoniae*
Plant extract (1000 mg/dl)	48.00±1.00	21.00±1.00	23.00±1.00	9.33±0.58	13.33±1.53
Chloramphenicol[a] (30 µg)	50.33±1.53	24.33±0.58	37.00±2.65	-	-
Gentamicin[a] (10 µg)	-	-	-	23.33±0.58	-
Amikacin[a] (30 µg)	-	-	-	-	27.00±1.00
10% DMSO[b]	6.00*±0.00	6.00*±0.00	6.00*±0.00	6.00*±0.00	6.00*±0.00

(a) positive control, (b) negative control, (SD) standard deviation, (*) diameter of well, (-) not tested

The well diffusion technique was used to evaluate the antimicrobial activity of the methanolic *P. niruri* plant extract against *P. acnes, S. epidermidis, S. mutans, E. coli,* and *K. pneumoniae*. This test was done in triplicate. Table II shows the mean inhibition zone of the selected organism against the plant crude extract. The results clearly showed that the crude extract was susceptible to all selected microorganisms. *P. acnes* demonstrated the largest inhibition zone with an average size of 48.00±1.00 mm. The size of inhibition zones decreased, followed by *S. mutans, S. epidermidis, K. pneumoniae*, and *E. coli*, with the mean inhibition zones of 23.00±1.00 mm, 21.00±1.00 mm, 13.33±1.53 mm, and 9.33±0.58 mm, respectively.

Table III: MIC and MBC values of methanolic *P. niruri* plant extract.

Selected bacteria	Concentration of plant extract (mg/ml)		MBC/MIC ratio
	MIC	MBC	
P. acnes	1.95	1.95	1
S. epidermidis	15.63	15.63	1
S. mutans	1.95	1.95	1
E. coli	125.00	125.00	1
K. pneumoniae	62.00	125.00	2

Table III displays the mean of MIC and MBC values against the five selected microorganisms. Based on the findings, the MIC range for the methanolic plant extract of *P. niruri* against the selected organism was between 125 mg/ml and 1.95 mg/ml. The MIC value for *P. acnes* and *S. mutans* was the lowest at 1.95 mg/ml. Whereas, increasing MIC values were recorded by *S. epidermidis, K. pneumonia,* and *E. coli* with 15.63 mg/ml, 62.00 mg/ml, and 125.00 mg/ml, respectively. The MBC results were almost similar to the MIC results. The MBC of the crude extract against the investigated organisms ranged between 125 mg/ml and 1.95 mg/ml. Meanwhile, the MIC and MBC values of *K. pneumoniae* were distinct; the bacteria were inhibited at 62.00 mg/ml but killed at 125.00 mg/ml. The concentration showed in MBC was the lowest that this plant could kill the bacteria in vitro. This served as the baseline data that could be used before manipulating the concentration, which was suitable for the animal model.

Table IV: Independent t-test comparing plant extract inhibition zone to the antibiotic standard.

Selected organism	Mean difference (95% CI*)	t-test (df)	*P*-value**
P. acnes	-2.33(-5.26, 0.59)	-2.21 (4)	0.091
S. epidermidis	-3.33(-5.18, -1.48)	-5.00 (4)	0.007
S. mutans	-14.0(-18.53, -9.47)	-8.57 (4)	0.001
E. coli	-14.00(-15.31, -12.69)	-26.70 (4)	<0.001
K. pneumoniae	-13.67(-16.79, -10.74)	-12.97 (4)	<0.001

*95% Confidence Interval, ***P*-value <0.05, (df) degree of freedom

An independent t-test was applied to the mean diameter of the inhibitory zone. This test determined the significance of the difference between the inhibition diameter of the *P. niruri* plant extract in methanol and the corresponding antibiotic for each studied bacterium. The significance level was determined using a *P*-value of 0.05 and a confidence range of 95%. The statistics shown in Table IV indicate that the *P*-value for each organism examined is less than 0.05, except for *P. acnes* (0.091). Therefore, the t-test demonstrated that the difference between the inhibitory zone diameter of methanolic *P. niruri* plant extract and the respective antibiotics on *P. acnes* was not statistically significant. On the other hand, the t-test results from the other four organisms studied were statistically significant.

DISCUSSION

P. acnes, S. epidermidis, S. mutans, E. coli, and *K. pneumoniae* were the bacteria found to induce wound infection (18–22). Previous studies revealed that these bacteria are resistant to various synthetic medicines. Erythromycin, azithromycin, clarithromycin, and clindamycin are all impervious to *P. acnes* (23). Penicillin, tetracycline, erythromycin, cefazolin, and trimethoprim-sulfamethoxazole are resistance in *S. epidermidis* (24). *S. mutans* is resistant to ampicillin, zithromax, ceftriaxone, and vancomycin (25). Ampicillin, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim are impervious to *E. coli* (26). In contrast, *K. pneumoniae* is resistant to ampicillin, cefazolin, and cefuroxime (27). The emergence of antibiotic-resistant bacterium species is bringing concern to the medical establishment. In the interest of solving this issue, the

development of antibiotics derived from natural sources should be further explored. Additionally, *P. niruri*, a common tropical shrub found along the coast, is helpful in managing a variety of diseases, including renal stones (11).

The crude extract required the utilisation of the entire *P. niruri* plant in its preparation. Due to the fact that some parts of the plant contain a greater concentration of water, the temperature must be slightly high in order to dry the plant thoroughly. Conversely, the risk of fungus contamination increases if the plant is exposed to low temperatures for too long. Prior research demonstrated that extended period of drying and elevated temperatures can enhance the production of phenolic chemicals. The plant's cellular structure is degraded, and certain bound phenolic compounds are released as a result of the high temperature (28). Moreover, subjecting the plant to elevated temperatures leads to alterations in the composition of polyphenols, resulting in an increased production of phenolic compounds.

The *P. niruri* plant extract was prepared using a standard extraction technique called maceration. In principle, the solvent should be able to penetrate the dense structure of the plant material and dissolve its substance. The substance then diffuses out of the solid matrix, yielding the pure extract (29). The quantity of crude extract output is impacted by several variables, including sample composition, extraction duration, temperature, pH, solvent polarity, particulate size, and solvent polarity (30). Small particulate diameters and high temperatures may improve the extraction rate due to increased liquid entry and chemical dispersion. Within a specific interval of time, the extraction's effectiveness increases in direct proportion to the length of time spent performing the extraction (30). Therefore, within the scope of this investigation, the *P. niruri* plant powder underwent maceration with absolute methanol over an extended duration of 7 consecutive days, consequently yielding a substantial amount of crude extract.

The present investigation revealed that the yield of *P. niruri* plant extract achieved by the utilisation of pure methanol corresponded to 9.3%. The selection of methanol as the solvent for the extraction of *P. niruri* plant in this research was based on its notable polarity attributes. The augmentation in the solvent's polarity correlated with the enhanced production of the unrefined extract. Moreover, previous investigations into the methanolic extract derived from the leaves of *P. niruri* have exhibited favorable outcomes in terms of both the crude extract yield and its efficacy against the tested bacteria (31). Besides, the use of methanol as a solvent for extracting jalapeño peppers, *Moringa oleifera, Azadirachta indica*, and *Lepidium sativum* has demonstrated superior outcomes than other solvents (32,33).This indicates that methanol is the most effective solvent for plant extraction.

Phytochemicals, which are found in plants, are biologically active substances that have been shown to improve health and reduce the risk of developing severe chronic illnesses (34). The present research found that the crude methanolic extract of *P. niruri* plant contained alkaloids, flavonoids, glycosides, phenolics, saponins, steroids, and tannins as stated in Table I. This finding aligns with the results of earlier research on the methanol extraction of *P. niruri* leaves at 95% concentration that identified the presence of carbohydrates, saponins, alkaloids, terpenoids, steroids, flavonoids, phenolics, and tannins; nevertheless, the glycoside content of *P. niruri* leaf extract was nil (35).

Phytochemical substances play a significant role in the antimicrobial action of the methanolic extract of *P. niruri*. Numerous studies conducted in the past have established the antimicrobial properties of phytochemical substances. The mechanism of action of phytochemicals against microbes varies from molecule to molecule group. Previous studies have uncovered multiple action mechanisms of compound alkaloids. These include nucleic acid inhibition and efflux pump disturbance (36,37). The genetic material needed for reproduction and protein synthesis is stored in nucleic acid, making it an indispensable part of bacteria. Alkaloids have been shown to attach to nucleic acid, altering its structure in ways that could be harmful to DNA (37). As a result, the bacteria's biochemical processes will be disrupted, leading to their eventual demise.

In contrast, drug efflux pumps are transmembrane protein complexes found in bacteria that remove antibacterial drugs from the bacterium cell (36). The accumulation of alkaloids and their association with the efflux pump, on the other hand, may prevent the protein from performing its function (37). As a result, the antimicrobial substances have an easier time penetrating the bacterial transmembrane and entering the bacterial cell (38).

According to several studies, flavonoids, glycosides, phenolics, saponins, steroids, and tannins have the same antibacterial effect by damaging the bacterial membrane (39–44). The cellular membranes of bacteria are formed by two layers, each composed of phospholipids and proteins. Microorganisms are protected from extreme environmental circumstances by the semipermeable membrane. Due to the interaction between the phytochemical substance and the cell membrane, the physiological properties of the membrane will be changed. This will have an impact over the permeability of the membrane, subsequently weakening this organism's defence system. Severe damage to the bacterial membrane may obliterate the cell's protective barrier, resulting in both autolysis and osmotic lysis. Numerous intracellular components will also be released, including nucleic acid, proteins, and other biomolecules. The occurrence inadvertently

causes an electrolyte imbalance and disrupts the normal functioning of the bacteria's metabolic processes, ultimately resulting in their death (38).

P. acnes (ATCC 6919), *S. epidermidis* (ATCC 12228), *S. mutans* (ATCC 25175), *E. coli* (ATCC 259220, and *K. pneumoniae* (ATCC 13883) were the five designated bacteria species tested for the antimicrobial activity of the methanolic extract from the *P. niruri* plant. The use of pure bacterial strain is critical for monitoring the accuracy and precision of antimicrobial susceptibility testing. In this investigation, all selected bacteria were susceptible to methanolic *P. niruri* plant extract. The inhibition zone of *P. niruri* plant extract against selected bacteria in Table II indicate that *P. acnes* exhibited the greatest degree of inhibition, as evidenced by the mean zone of inhibition with 48.00±1.00 mm in diameter. The size of the inhibitory zone declined in the following order: *S. mutans* (23.00±1.00 mm), *S. epidermidis* (21.00±1.00 mm), *K. pneumoniae* (13.33±1.53 mm), and *E. coli* (9.33±0.58 mm). The observed inhibitory pattern suggested that the methanolic extract derived from the *P. niruri* plant exhibited minimal impact on gram-negative bacteria, specifically *E. coli* and *K. pneumoniae*. However, it showed significant suppression against gram-positive bacteria, including *P. acnes, S. epidermidis,* and *S. mutans*. The present discovery aligns with a prior study in which the ethanolic extract of *P. niruri* demonstrated significant efficacy against gram-positive bacteria (45). Given the presence of both gram-positive and gram-negative bacteria, it may be inferred that the methanolic *P. niruri* plant extract possesses the capacity to serve as an antibacterial agent exhibiting a wide range of effectiveness.

In this study, a 1000 mg/ml concentration of phytochemicals for AST was used to examine the extract's effectiveness against pure colonies. The crude extracts were subjected to an initial screening for preliminary testing with a concentration of 1000 mg/ml to determine whether or not they were effective against the selected bacteria (46). The extract's concentration, on the other hand, was decreased in both the MIC and MBC to detect the lowest concentration at which the extract was able to inhibit or kill the pathogen.

The MIC finding for the methanolic extract of *P. niruri* plant stated in Table III demonstrated an impressive antibacterial activity against *P. acnes* and *S. mutans* as both organisms exhibited the lowest MIC value (1.95 mg/ml). This indicates that the methanolic extract is effective against both of these bacteria. On the contrary, elevated MIC values were observed for *E. coli* (125 mg/ml) and *K. pneumoniae* (62.00 mg/ml), suggesting that the *P. niruri* extract exhibited limited efficacy as a microbiological inhibitor against gram-negative bacteria. A plant product's bactericidal and bacteriostatic impacts are evaluated based on its MBC/MIC ratio. If the number is less than 4, the antibacterial action is considered bactericidal; if it is greater than 4, it is bacteriostatic (47). The current research demonstrated that the MBC/MIC ratio of the *P. niruri* plant extract on all selected bacteria was less than 4, indicating that the antibacterial activity of the methanolic extract of *P. niruri* displayed a bactericidal impact.

Plant extract from the *P. niruri* species has been shown to be efficient against both gram-positive and gram-negative bacteria by altering their respective membrane structure. Gram-negative bacteria possess three fundamental layers that form their cell wall. Each of these layers performs an essential role in the preservation of these microorganisms. The cell wall of gram-negative bacteria is complicated and comprehensive, consisting of lipopolysaccharides, an inner membrane, and a thin layer of peptidoglycan (48). The outer coating of lipopolysaccharides performs a vital role in the process of regulating the susceptibility of the cell. As a direct consequence of this, antibacterial chemicals and other small molecules are unable to enter bacterium cells in an orderly manner (49). Additionally, gram-negative bacteria have an abundance of efflux pumps, some of which are overexpressed, thus limiting the number of antibacterial chemicals that can accumulate inside the cells (48).

In contrast, the cell wall of gram-positive bacteria comprises a dense and thick peptidoglycan layer that is intimately affixed to the exterior layer of the cell membrane. Due to the straightforward and brittle nature of the cell wall construction of gram-positive bacteria, even the smallest substances are able to penetrate the cells with relative ease (48). As the antibacterial substances cross the cell barrier, the enzymatic system may be changed, resulting in bacterial suppression. Therefore, antibacterial drugs are more effective against gram-positive than gram-negative bacteria (48).

This experiment employed gentamicin (10 µg) as a positive reference for *E. coli* and chloramphenicol (30 µg) for *P. acnes, S. epidermidis*, and *S. mutans*. Additionally, *K. pneumoniae* was treated with amikacin (30 µg). The statistical data analysis in Table IV indicated that there was no significant difference between the inhibitory diameter of the methanolic *P. niruri* plant extract and the positive control on *P. acnes*, as the *P*-value was greater than 0.05 (0.091). According to the findings, the inhibition measurement of *P. acnes* in the sample was virtually identical to that of the corresponding antibiotic (50.33±1.53 mm). In other words, the antibacterial activity of *P. niruri* plant extract against *P. acnes* was nearly identical to that of the positive control.

CONCLUSION

The methanolic extract of the *P. niruri* plant was used to assess the potential of traditional herbs to serve as therapeutic substitutes in contemporary medicine. The

plant's antibacterial effect is attributed to the extract's alkaloids, flavonoids, glycosides, phenolics, saponins, steroids, and tannins. The antimicrobial susceptibility testing revealed that the *P. niruri* extract could inhibit the growth of P*. acnes, S. epidermidis, S. mutans, E. coli,* and *K. pneumoniae*. The extract is more effective against gram-positive bacteria than gram-negative bacteria. The findings from this study indicated that *P. niruri* plant extract has the potential to be employed as an antibacterial agent to combat the emergence of antibiotic-resistant bacteria. However, further research on the *P. niruri* plant should be conducted, including a time-killing assay and the effect of cytotoxicity. In addition, quantitative phytochemical analysis should also be performed on *P. niruri* extract using several solvent extraction methods to establish the highest yield of each phytochemical compound.

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