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Isolation and Identification of Bacteria from Human Clinical Samples and Evaluation of their Susceptibility to Plant Extracts

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ABSTRACT

The increasing global burden of antimicrobial resistance (AMR) poses a significant challenge to the effective management of infectious diseases, as pathogenic microorganisms continue to develop resistance to commonly used antimicrobial agents, resulting in increased morbidity and mortality. The present investigation was undertaken to isolate and identify bacterial pathogens from selected clinical samples, including urine, stool, throat swabs, and pus/wound swabs, and to evaluate their antimicrobial susceptibility patterns against *Guduchi* (*Tinospora cordifolia*) plant extract. Clinical samples were processed using standard microbiological procedures. The isolated bacteria were cultured on Nutrient Agar (NA), MacConkey Agar (MA), and Blood Agar (BA), followed by identification using conventional microbiological and biochemical techniques. Antimicrobial susceptibility testing (AST) was performed using the disc diffusion method in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI). The antibacterial activity of the *Guduchi* plant extract was assessed against the bacterial isolates obtained from the clinical specimens. The bacterial isolates recovered from the clinical samples included *Salmonella Typhi*, *Bacillus* spp., *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecium*, *Staphylococcus saprophyticus*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The findings demonstrated that *Guduchi* plant extract exhibited antibacterial activity against *Salmonella Typhi*. These findings suggest that *T. cordifolia* possesses potential antibacterial properties and may warrant further investigation as a source of plant-derived antimicrobial compounds, particularly in the context of the growing threat of antimicrobial resistance. Further studies involving standardized extracts, phytochemical characterization, minimum inhibitory concentration (MIC) determination, and broader panels of clinically relevant bacterial pathogens are recommended to establish its therapeutic potential.

Keywords

Antimicrobial resistance; AST- Antibiotic sensitivity test; ESBL- extended spectrum- β -lactamase *enterobacteriaceae*

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Introduction

Antimicrobial resistance (AMR) has emerged as a major global public health challenge, threatening the effective prevention and treatment of infectious diseases. Antimicrobial agents, including antibiotics, antivirals, antifungals, and antiparasitic drugs, are essential for treating infections in humans and animals. However, the emergence and dissemination of resistant microorganisms have progressively reduced the effectiveness of several therapeutic agents. AMR occurs when microorganisms acquire or develop mechanisms that enable them to survive or multiply despite exposure to antimicrobial agents that would ordinarily inhibit their growth or eliminate them. Resistance may arise through genetic mutations, horizontal gene transfer, and the selection and dissemination of resistant microbial populations under antimicrobial pressure. Consequently, resistant infections can lead to treatment failure, prolonged hospitalization, increased healthcare expenditure, and elevated mortality (World Health Organization [WHO], 2023).

The global burden of bacterial AMR is substantial. Murray *et al.*, (2022) estimated that bacterial AMR was directly responsible for approximately 1.27 million deaths and associated with approximately 4.95 million deaths worldwide in 2019. These findings demonstrate the importance of strengthening infection prevention, antimicrobial stewardship, surveillance, and research into alternative antimicrobial agents. Clinically important bacterial infections, including urinary tract infections, bloodstream infections, wound infections, pneumonia, and gastrointestinal infections, can become difficult to manage when the causative organisms exhibit resistance to commonly prescribed antibiotics. The emergence of multidrug-resistant (MDR) bacteria is particularly concerning because resistance to multiple antimicrobial classes can restrict the therapeutic options available for treating infections.

Among clinically significant resistant bacteria, the ESKAPE pathogens are recognized for their association with healthcare-associated infections and their capacity to develop resistance to multiple antimicrobial agents. The ESKAPE group comprises *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species. These organisms include both Gram-positive and Gram-negative bacteria and are associated with infections that may be difficult to treat,

particularly in vulnerable patients and healthcare settings. Their clinical importance is further increased by the emergence of specific resistance phenotypes, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and extended-spectrum β -lactamase (ESBL)-producing Enterobacterales. The continued spread of these resistant pathogens emphasizes the need for reliable bacterial identification, antimicrobial susceptibility testing, and the investigation of additional sources of antimicrobial compounds (Rice, 2008).

Antimicrobial resistance is not confined to hospital environments. Resistant bacterial strains can also cause community-acquired infections, creating challenges for empirical treatment and routine clinical management. In India, antimicrobial resistance represents an important public health concern because of the combined effects of antimicrobial consumption, inappropriate prescribing, self-medication, limited access to diagnostic testing in some settings, and the transmission of resistant organisms. National surveillance and systematic investigation of clinical bacterial isolates are therefore important for understanding local resistance patterns and supporting appropriate antimicrobial use. The susceptibility of a bacterial isolate to a particular antibiotic cannot be reliably predicted from its species identity alone; consequently, laboratory-based identification and susceptibility testing remain important components of evidence-based infection management.

In addition to investigating resistance to conventional antibiotics, scientific research has increasingly examined medicinal plants as potential sources of antimicrobial compounds. Plants synthesize a wide range of secondary metabolites that contribute to their ecological interactions and defence against microorganisms. These metabolites include phenolic compounds, flavonoids, tannins, alkaloids, terpenoids, saponins, and other phytochemicals that may exhibit biological activity. Certain plant-derived compounds have demonstrated antibacterial effects under laboratory conditions, supporting continued investigation of their chemical composition and antimicrobial properties (Cushnie & Lamb, 2011).

The antibacterial activity of plant-derived compounds may involve several mechanisms, including disruption of bacterial membrane integrity, alteration of membrane permeability, inhibition of essential enzymes, interference with energy metabolism, and disruption of cellular processes. However, these mechanisms vary

according to the chemical structure of the compound, its concentration, and the bacterial species under investigation. Furthermore, plant extracts are chemically complex mixtures, and their activity may be influenced by plant species, geographical origin, maturity, plant part, extraction solvent, extraction procedure, storage conditions, and the concentration of bioactive constituents. Therefore, the antimicrobial properties of individual medicinal plants cannot be assumed to be uniform or effective against all bacterial pathogens (Cushnie & Lamb, 2011; Nazzaro *et al.*, 2013).

The evaluation of plant extracts against clinically isolated bacteria is particularly relevant because clinical isolates may differ in their antimicrobial susceptibility profiles and resistance characteristics. Investigating these isolates can provide preliminary information about whether selected plant extracts inhibit the growth of bacteria recovered from clinical specimens. Nevertheless, observed antibacterial activity *in vitro* should not be interpreted as proof of clinical efficacy. The identification of active constituents, determination of effective concentrations, assessment of toxicity, evaluation of pharmacological properties, and confirmation of safety and efficacy are necessary before any potential therapeutic application can be established.

The isolation and identification of bacteria from human clinical samples constitute fundamental steps in clinical microbiology. Specimens such as urine, pus, wound swabs, and other appropriately collected clinical materials may yield bacterial isolates associated with infectious conditions. The selection of suitable culture media, incubation conditions, colony characteristics, staining procedures, and biochemical tests supports the presumptive and confirmatory identification of bacterial isolates. Accurate identification is important for characterizing the bacterial diversity represented in a study and interpreting the results of subsequent antimicrobial testing. However, bacterial recovery from a clinical specimen must be interpreted in relation to specimen type, collection quality, clinical context, and the possibility of contamination or colonization.

Antimicrobial susceptibility testing provides a standardized approach for examining the response of bacterial isolates to selected antimicrobial agents. The disc diffusion method is widely used to assess antibacterial activity by measuring the diameter of the zone of inhibition surrounding a disc containing an antimicrobial substance. For conventional antibiotics,

zone diameters may be interpreted using the relevant Clinical and Laboratory Standards Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria, provided that the specified organism, antibiotic, medium, inoculum, incubation conditions, and quality-control requirements are met. In contrast, plant extracts do not generally have established clinical breakpoints comparable to those available for approved antibiotics. Consequently, their inhibition zones should be reported as experimental measurements and evaluated using appropriate positive controls, solvent controls, standardized extract concentrations, and biological replicates. The methodological limitations of diffusion-based assays, particularly the influence of compound solubility and diffusion through agar, must also be considered when comparing the activity of different extracts (Balouiri *et al.*, 2016).

Although medicinal plants have been widely investigated for their antimicrobial properties, their activity varies considerably across plant species, extraction methods, bacterial strains, and experimental conditions. Furthermore, the detection of an inhibition zone alone does not establish the minimum inhibitory concentration, bactericidal activity, mechanism of action, or clinical usefulness of a plant extract. Complementary approaches, such as broth microdilution for determining the minimum inhibitory concentration and, where appropriate, minimum bactericidal concentration testing, can provide additional quantitative evidence of antibacterial activity. These methods can help distinguish preliminary growth inhibition from more detailed measures of antimicrobial potency (Balouiri *et al.*, 2016).

Against this background, the present study aims to isolate and identify bacterial species from selected human clinical samples and evaluate the *in vitro* antibacterial activity of selected medicinal plant extracts against the recovered bacterial isolates. The bacterial isolates are characterized using standard bacteriological and biochemical techniques, while their responses to the selected plant extracts are assessed using the disc diffusion method under defined experimental conditions. Where conventional antibiotics are included, their susceptibility patterns are evaluated using applicable standardized interpretive criteria. The study is intended to characterize the bacterial isolates obtained from the investigated clinical specimens and generate preliminary experimental evidence regarding the antibacterial activity of the selected plant extracts. The findings may provide a basis for further phytochemical characterization and

quantitative evaluation of plant-derived antibacterial compounds, contributing to the broader investigation of potential sources of antimicrobial agents in the context of increasing antimicrobial resistance.

Materials and Methods

Laboratory Materials and Reagents

The materials used in the study included culture media, sterile Petri plates, test tubes, conical flasks, beakers, glass slides, sterile cotton swabs, inoculating loops, L-shaped glass spreaders, sterile forceps, filter paper, and other routine microbiological laboratory equipment. The culture media and biochemical reagents were prepared and used according to the manufacturers' instructions and established microbiological procedures. Appropriate aseptic techniques were followed during specimen processing, bacterial isolation, biochemical identification, and antimicrobial activity testing to minimize contamination and ensure the reliability of experimental observations (Tille, 2022).

Collection and Transportation of Clinical Samples

Human clinical specimens were obtained from routine diagnostic laboratories for the isolation and identification of bacterial isolates. The specimens comprised urine samples, pus or wound swabs, throat swabs, and stool samples. According to the recorded sample distribution, 40 urine samples, 25 pus or wound swabs, 19 throat swabs, and 15 stool samples were collected. These categories accounted for 99 specimens; therefore, the final sample number must be confirmed against the original laboratory records before publication. Specimens were collected using appropriate sterile containers or swabs and handled aseptically to minimize contamination. Urine specimens were collected as clean-catch midstream samples in sterile, leak-proof containers after appropriate perineal cleansing. Throat swabs were obtained from the tonsillar pillars and posterior pharyngeal wall while avoiding contact with the tongue and other oral surfaces. Pus and wound specimens were collected from the affected site using sterile swabs or appropriate specimen collection devices, with care taken to minimize contamination from surrounding skin. Stool specimens were collected in sterile, wide-mouthed, leak-proof containers without contamination by urine or other materials.

Following collection, specimens were appropriately labelled and transported promptly to the microbiology laboratory under conditions suitable for the specimen type. When immediate processing was not possible, storage and transport conditions were selected according to established specimen-handling recommendations. Specimen identity, source, collection date, and relevant laboratory details were documented before processing. Standardized collection and transport procedures are important for maintaining specimen integrity and minimizing the recovery of contaminating microorganisms (Tille, 2022).

Isolation and Purification of Bacterial Isolates

Clinical specimens were processed aseptically using appropriate bacteriological culture procedures. For specimens processed by direct plating, an aliquot of 0.1 mL was transferred onto solidified nutrient agar and distributed evenly across the agar surface using a sterile L-shaped glass spreader. Specimens collected using swabs were inoculated using an appropriate streaking technique. The actual inoculation procedure used for each specimen type should be reported in accordance with the laboratory records.

The inoculated culture plates were incubated under the conditions specified in the study protocol. According to the recorded procedure, plates were incubated aerobically at 37°C for 24 hours. Following incubation, bacterial growth was examined for colony characteristics, including size, shape, pigmentation, margin, elevation, surface appearance, and consistency. Colonies exhibiting distinct morphological characteristics were selected for further purification.

Individual colonies were subcultured onto fresh agar media using the streak-plate technique to obtain isolated colonies. Repeated subculturing was performed when necessary until cultures exhibiting consistent colony morphology were obtained. Purified isolates were subsequently used for staining, biochemical identification, and antimicrobial susceptibility testing. The isolates were maintained on suitable culture media under appropriate storage conditions until further analysis.

Because different bacterial species have different nutritional and growth requirements, nutrient agar alone may not recover or differentiate all clinically significant

pathogens. Accordingly, specimen-specific media and incubation conditions should be included in the final methodology if they were used during the investigation (Tille, 2022).

Preliminary Identification of Bacterial Isolates

The purified bacterial isolates were subjected to preliminary characterization based on colony morphology and their growth characteristics on differential culture media. Blood agar was used to assess haemolytic reactions where appropriate, while MacConkey agar was used to differentiate lactose-fermenting from non-lactose-fermenting Gram-negative bacteria.

On MacConkey agar, lactose-fermenting colonies generally exhibit pink to red coloration, whereas non-lactose-fermenting colonies typically remain colourless or pale. On blood agar, haemolysis was recorded according to the appearance of the agar surrounding the colonies. Haemolytic reactions were documented as alpha, beta, or gamma, as appropriate, rather than being used alone to establish bacterial identity.

These observations were considered preliminary identification characteristics and were interpreted in conjunction with the results of Gram staining and biochemical tests. Growth on these media and the resulting colony characteristics were used to guide the selection of subsequent identification procedures (Tille, 2022).

Biochemical Identification of Bacterial Isolates

Biochemical tests were performed on purified bacterial isolates to support their identification. The tests included indole production, methyl red, Voges–Proskauer, citrate utilization, coagulase production, and oxidase activity, as applicable to the presumptive identity of each isolate.

Biochemical identification of bacterial isolates was performed using standard procedures. The indole test was conducted using tryptophan-containing broth, with a red surface layer following the addition of indole-detection reagent indicating a positive result. In the methyl red (MR) test, a stable red colour indicated a positive reaction, whereas yellow coloration indicated a negative result. The Voges–Proskauer (VP) test was interpreted as positive when the characteristic red colour developed after the addition of appropriate reagents. For the citrate

utilization test, growth accompanied by a colour change from green to blue on Simmons citrate agar indicated a positive reaction. In the oxidase test, the development of a rapid purple or dark-blue colour within the specified reading time indicated a positive result. Coagulase activity was assessed using the validated method employed in the study, with clot formation or characteristic agglutination interpreted according to the selected procedure. All biochemical reactions were recorded and interpreted using standard microbiological criteria (Tille, 2022).

The biochemical reactions were interpreted collectively alongside colony morphology, Gram-staining characteristics, and growth on differential media. Bacterial identities were assigned only to the level supported by the identification procedures employed.

Collection and Preparation of Medicinal Plant Material

Leaves of the selected medicinal plants were collected and cleaned to remove visible dust and adhering debris. The plant material was allowed to dry in the shade in a clean, well-ventilated environment to minimize direct sunlight exposure and potential degradation of light-sensitive phytochemicals. The dried leaves were ground into a fine powder and stored in a clean, dry, appropriately labelled container until extraction.

For reproducibility, the scientific name of each plant, plant part used, collection location, collection period, authentication method, and voucher specimen number, where available, should be documented.

The drying conditions, duration, and storage period should also be recorded. Botanical authentication is important because plant misidentification can compromise the reproducibility and interpretation of phytochemical and antimicrobial investigations (Eloff, 1998; Nazzaro *et al.*, 2013).

Preparation of Ethanolic Plant Extracts

An ethanolic extraction procedure was used to obtain crude extracts from the dried plant material. Briefly, 10 g of powdered leaf material was mixed with 100 mL of ethanol, corresponding to a plant-material-to-solvent ratio of 1:10 (w/v). The mixture was subjected to the extraction procedure documented in the laboratory records.

Following extraction, the mixture should be filtered to separate the liquid extract from the plant residue if this step was performed. The ethanolic filtrate was then concentrated by an appropriate solvent-removal procedure. If a water bath was used at 40°C, the actual duration and method of solvent removal should be reported. The resulting crude extract was collected and stored in a suitable, tightly closed container under conditions appropriate for its stability.

The mass of the recovered extract, percentage extraction yield, final stock concentration, and storage conditions should be recorded whenever these measurements are available. Extraction yield can be calculated as:

Extraction yield (%) = (Mass of dried extract / Mass of dried plant material) × 100.

For preparation of the working solution, 1 mL of plant extract was mixed with 9 mL of distilled water, as documented in the original procedure. This dilution represents a 1:10 volumetric dilution of the starting extract solution; however, the final mass concentration cannot be established unless the concentration of the starting extract is known.

The solubility and stability of the extract in the selected diluent should be considered. If ethanol or another solvent remained in the final preparation, an appropriate solvent control should be included to distinguish the antibacterial effect of the plant extract from that of the solvent. The extraction solvent, extraction duration, filtration procedure, concentration method, and final extract concentration are important methodological variables in plant-based antimicrobial research (Eloff, 1998; Balouiri *et al.*, 2016).

Preparation of Plant-Extract-Impregnated Discs

Sterile discs prepared from suitable filter paper were used for the disc diffusion assay. An aliquot of 100 µL of the prepared plant extract was applied to each disc. According to the recorded procedure, additional aliquots were applied two or three times, with the discs allowed to dry between applications.

The final volume applied per disc must be clarified in the study records because repeated applications of 100 µL may produce a total loading of 200–300 µL or more, depending on the number of applications. The concentration of extract, total volume per disc, and

extract mass per disc should be reported wherever possible. The discs were allowed to dry under appropriate conditions before use, while avoiding contamination and excessive loss of volatile or unstable constituents.

Negative-control discs containing the same solvent or diluent without plant extract were required to evaluate solvent-associated effects. A suitable positive control was also necessary to confirm that the assay could detect antibacterial activity under the test conditions. These controls are important when evaluating crude plant extracts because solvent properties, compound solubility, and agar diffusion can influence the measured inhibition zones (Balouiri *et al.*, 2016).

Antibiotic Susceptibility Testing of Bacterial Isolates

The susceptibility of bacterial isolates to selected conventional antibiotics was assessed using the Kirby–Bauer disc diffusion method, where applicable. For standardized antibiotic susceptibility testing, a pure culture of the test organism was used to prepare a bacterial suspension adjusted to the prescribed inoculum density. The suspension was inoculated uniformly onto Mueller–Hinton agar using a sterile swab to produce a confluent bacterial lawn.

Commercially prepared antibiotic discs of specified antimicrobial agents and potencies were placed on the agar surface using sterile forceps or a suitable disc dispenser. The plates were incubated under the conditions specified for the organism and antibiotic combination. After incubation, the diameters of the inhibition zones were measured in millimetres.

The observed zone diameters were interpreted using the applicable Clinical and Laboratory Standards Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria, where available and appropriate for the organism and antimicrobial agent tested. Isolates were classified as susceptible, intermediate or susceptible with increased exposure, or resistant according to the selected standard and edition.

The use of nutrient agar, an unstandardized inoculum, or unspecified antibiotic disc potencies would limit the validity of categorical susceptibility interpretations. Therefore, the final manuscript should report the actual

medium, inoculum standard, antibiotic names and disc potencies, incubation conditions, interpretive standard and edition, and quality-control procedures used. Standardized methods are essential for reproducible and clinically interpretable antibiotic susceptibility results (Clinical and Laboratory Standards Institute [CLSI], 2024).

Evaluation of the Antibacterial Activity of Plant Extracts

The antibacterial activity of the prepared medicinal plant extracts was assessed using the disc diffusion method. A standardized suspension of each purified bacterial isolate was used to prepare a uniform bacterial lawn on the selected agar medium.

Plant-extract-impregnated discs were placed on the inoculated agar surface using sterile forceps, with adequate spacing to prevent overlapping inhibition zones.

The inoculated plates were incubated under the conditions specified in the study protocol. Following incubation, the plates were examined for clear zones surrounding the discs. The diameter of each inhibition zone, including the disc diameter where required by the measurement convention, was measured in millimetres and recorded for each plant extract and bacterial isolate. Solvent-control and positive-control results were recorded alongside the test results.

The antibacterial activity of the plant extracts was compared descriptively using the measured inhibition zones, provided that the extract concentrations, disc loading, inoculum density, culture medium, and incubation conditions were comparable. Larger inhibition zones may indicate greater growth inhibition under the specific assay conditions, but they do not necessarily establish greater intrinsic potency because diffusion and solubility differ among compounds.

Unlike conventional antibiotics, crude plant extracts do not generally have validated clinical susceptibility breakpoints. Consequently, their results were interpreted as in vitro antibacterial activity rather than as susceptible, intermediate, or resistant categories. The disc diffusion assay provides preliminary evidence of growth inhibition; quantitative methods such as broth microdilution are required when determination of the minimum inhibitory concentration is an objective of the investigation (Balouri *et al.*, 2016).

Analysis of Experimental Results

The results of bacterial isolation, preliminary identification, biochemical testing, antibiotic susceptibility testing, and plant-extract screening were recorded systematically. The number and types of clinical specimens processed, the number of bacterial isolates recovered, and the identification results were summarized using descriptive statistics.

For plant-extract testing, inhibition-zone diameters were recorded in millimetres for each bacterial isolate and extract. If replicate measurements were performed, results should be presented as the mean and standard deviation, together with the number of independent replicates.

Where appropriate, comparisons among plant extracts or bacterial groups should be conducted using statistical tests selected according to the study design, data distribution, and independence of observations. Statistical significance, if assessed, should be reported with the test used, sample size, and significance threshold.

Results and Discussion

Isolation and Identification of Bacterial Isolates from Human Clinical Samples

A total of 100 human clinical samples, comprising urine, pus/wound swabs, stool, and throat swabs, were collected and processed for the isolation and identification of bacterial pathogens using standard microbiological procedures. Based on cultural, morphological, Gram-staining, and biochemical characteristics, both Gram-positive and Gram-negative bacterial isolates were recovered from the clinical specimens.

The bacterial isolates identified in the present investigation included *Salmonella* spp., *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Enterococcus* spp., *Streptococcus pneumoniae*, and *Paenibacillus alvei*. The isolates exhibited distinct cultural characteristics on different bacteriological media, including nutrient agar, MacConkey agar, and blood agar. These cultural characteristics, in combination with Gram staining and biochemical reactions, facilitated the presumptive identification of the isolates.

Table.1 Biochemical Characteristics of Bacterial Isolates

S. No.	Bacterial Isolate	Indole	MR	VP	Citrate	Gram Staining	Oxidase
1	<i>Pseudomonas aeruginosa</i>	–	–	–	+	Gram-negative rod	+
2	<i>Staphylococcus aureus</i>	+	–	–	+	Gram-positive cocci	–
3	<i>Staphylococcus saprophyticus</i>	–	–	–	+	Gram-positive cocci	–
4	<i>Salmonella</i> spp.	–	+	–	–	Gram-negative rod	–
5	<i>Proteus mirabilis</i>	–	+	+	+	Gram-negative rod	–
6	<i>Klebsiella pneumoniae</i>	–	+	–	+	Gram-negative rod	–
7	<i>Escherichia coli</i>	+	+	–	–	Gram-negative rod	–
8	<i>Streptococcus pneumoniae</i>	–	+	–	+	Gram-positive cocci	–
9	<i>Enterococcus</i> spp.	–	+	–	–	Gram-positive cocci	–
10	<i>Paenibacillus alvei</i>	+	–	+	+	Gram-positive cocci*	–

Table.2 Antimicrobial Susceptibility Profile of Selected Clinical Isolates

Bacterial isolate	Antibiotic	Observed response
<i>Pseudomonas aeruginosa</i>	Vancomycin	Resistant
<i>Pseudomonas aeruginosa</i>	Amikacin	Resistant
<i>Pseudomonas aeruginosa</i>	Nitrofurantoin	Resistant
<i>Pseudomonas aeruginosa</i>	Ciprofloxacin	Resistant
<i>Pseudomonas aeruginosa</i>	Piperacillin	Resistant
<i>Staphylococcus aureus</i>	Azithromycin	Resistant
<i>Staphylococcus aureus</i>	Cefepime	Resistant
<i>Staphylococcus aureus</i>	Tetracycline	Resistant
<i>Staphylococcus aureus</i>	Linezolid	Resistant
<i>Salmonella</i> spp.	Meropenem	Resistant
<i>Salmonella</i> spp.	Other tested antibiotics	Susceptible, as reported

Table.3 Antibacterial Activity of *Tinospora cordifolia* and *Achyranthes aspera* Extracts

S. No.	Plant extract	Botanical name	Isolate bacteria	Zone of inhibition (mm)
1	Guduchi	<i>Tinospora cordifolia</i>	<i>Salmonella</i> spp.	20
2	Guduchi	<i>Tinospora cordifolia</i>	<i>Escherichia coli</i>	13
3	Nayuruvi	<i>Achyranthes aspera</i>	<i>Escherichia coli</i>	11
4	Nayuruvi	<i>Achyranthes aspera</i>	<i>Bacillus</i> spp.	9

Table.4 Comparative Antibacterial Activity of the Two Plant Extracts

Extract	Isolate bacteria	Zone of inhibition (mm)
<i>T. cordifolia</i>	<i>Salmonella</i> spp.	20
<i>T. cordifolia</i>	<i>E. coli</i>	13
<i>A. aspera</i>	<i>E. coli</i>	11
<i>A. aspera</i>	<i>Bacillus</i> spp.	9

Antimicrobial Susceptibility Testing of Bacterial Isolates

Antimicrobial susceptibility testing (AST) was performed on selected bacterial isolates to assess their responses to the antibiotics included in the experimental panel. The results demonstrated considerable variation in antimicrobial susceptibility among the isolates.

Pseudomonas aeruginosa exhibited resistance to several of the antibiotics tested, including vancomycin, amikacin, nitrofurantoin, ciprofloxacin, and piperacillin, according to the experimental observations. The apparent multidrug-resistant phenotype of this isolate is of particular microbiological importance because antimicrobial resistance in *P. aeruginosa* can substantially complicate the treatment of infections. However, classification as multidrug-resistant should be made according to an established AST standard and the defined antimicrobial classes represented in the test panel.

Among the Gram-positive isolates, *Staphylococcus aureus* demonstrated resistance to azithromycin, cefepime, tetracycline, and linezolid in the present study. The observed resistance pattern indicates reduced susceptibility to multiple antimicrobial agents. Because resistance profiles may vary considerably among strains, these findings should be interpreted specifically for the isolate tested and should not be generalized to the species as a whole. The tested *Salmonella* isolate was susceptible to most of the antibiotics evaluated but exhibited resistance to meropenem.

This finding is noteworthy because carbapenems such as meropenem are important therapeutic agents for serious infections caused by susceptible Gram-negative bacteria. Nevertheless, the significance of the observed phenotype should be confirmed using the applicable CLSI or EUCAST interpretive criteria and appropriate quality-control procedures.

Overall, the AST results demonstrate that the clinical isolates possessed heterogeneous antimicrobial susceptibility profiles, emphasizing the importance of laboratory-based susceptibility testing for appropriate antimicrobial selection. The observed resistance patterns may also reflect the selective pressure associated with antimicrobial exposure; however, mechanisms of resistance cannot be inferred solely from phenotypic AST results without appropriate confirmatory testing.

Antibacterial Activity of *Tinospora cordifolia* and *Achyranthes aspera* Extracts

The antibacterial activities of Guduchi (*Tinospora cordifolia*) and Nayuruvi (*Achyranthes aspera*) extracts were evaluated against selected bacterial isolates using the zone-of-inhibition assay. The extracts demonstrated variable inhibitory activity against the tested organisms, indicating that their antibacterial effects were dependent on the bacterial species and the plant extract evaluated.

Guduchi extract exhibited a 20 mm zone of inhibition against the tested *Salmonella* isolate and a 13 mm zone of inhibition against *Escherichia coli*. Among the organisms evaluated with Guduchi, the largest inhibition zone was therefore observed against *Salmonella*. This finding indicates comparatively greater in-vitro growth inhibition of the tested *Salmonella* isolate by the Guduchi extract under the experimental conditions.

Nayuruvi extract produced an 11 mm zone of inhibition against *E. coli* and a 9 mm zone of inhibition against *Bacillus* spp. The observed inhibition zones were smaller than the maximum zone recorded for Guduchi against *Salmonella*, suggesting variation in the inhibitory response among the tested extract-bacterium combinations.

The differences in antibacterial activity observed between the two plant extracts may be associated with differences in their phytochemical composition, concentration of bioactive constituents, extraction efficiency, solvent polarity, and susceptibility of the bacterial species. Medicinal plants such as *T. cordifolia* and *A. aspera* contain diverse classes of secondary metabolites, including phenolic compounds, flavonoids, alkaloids, terpenoids, tannins, and other constituents that have been investigated for potential antimicrobial activity. However, the contribution of individual compounds cannot be established from an inhibition-zone assay alone.

The comparatively larger inhibition zone produced by Guduchi against *Salmonella* suggests that the extract had stronger in-vitro inhibitory activity against the particular isolate tested under the conditions of the present experiment. This should not, however, be interpreted as evidence of clinical efficacy or superiority as a therapeutic agent. Similarly, the smaller inhibition zones observed with Nayuruvi indicate measurable antibacterial activity but do not by themselves establish

its potency, mechanism of action, or therapeutic applicability.

The present investigation demonstrated the successful isolation of diverse bacterial species from human clinical specimens using conventional microbiological techniques. The isolates included both Gram-positive and Gram-negative organisms with distinct cultural, staining, and biochemical characteristics. The differential growth and colony morphology observed on nutrient agar, MacConkey agar, and blood agar provided useful phenotypic characteristics for the preliminary identification of the bacterial isolates.

The antimicrobial susceptibility results revealed species- and isolate-dependent variations in resistance and susceptibility patterns. In particular, the resistance observed among the tested *P. aeruginosa* and *S. aureus* isolates highlights the importance of routine antimicrobial susceptibility testing in clinical microbiology. Such phenotypic resistance findings should be interpreted according to standardized guidelines and, where appropriate, supplemented by molecular or phenotypic tests to determine specific resistance mechanisms.

The plant extracts investigated in this study also demonstrated variable antibacterial activity. Guduchi extract showed its highest observed inhibition against *Salmonella* (20 mm), followed by *E. coli* (13 mm), whereas Nayuruvi extract produced inhibition zones of 11 mm against *E. coli* and 9 mm against *Bacillus* spp. These findings indicate that both plant extracts possess measurable in-vitro antibacterial activity, although the magnitude of inhibition varied according to the bacterial species and extract.

The antibacterial activity of these medicinal plants may be attributable to the presence of bioactive phytochemicals capable of interfering with bacterial cellular processes, membrane integrity, enzyme activity, or other essential physiological functions. Nevertheless, the precise mechanisms responsible for the observed inhibition require further investigation through phytochemical characterization, determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), and appropriate mechanistic studies.

In conclusion, the findings demonstrate that clinical bacterial isolates exhibit diverse antimicrobial

susceptibility patterns and that extracts of *Tinospora cordifolia* and *Achyranthes aspera* possess variable in-vitro antibacterial activity. Guduchi demonstrated the highest recorded inhibition zone against the tested *Salmonella* isolate, while Nayuruvi exhibited comparatively lower but detectable inhibitory activity against the organisms examined. These observations support the potential of the investigated medicinal plants as sources of antibacterial phytochemicals. Further studies involving standardized extract preparation, phytochemical profiling, MIC/MBC determination, toxicity assessment, and identification of active compounds are necessary to substantiate their antibacterial potential.

Author Contributions

S. Aruljothy: Conceived the original idea and designed the model and wrote the manuscript. Dr. M. Prakash: Designed the model and the computational framework and analysed the data.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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