In-Vitro Anti-Microbial Potential of Indigenous Medicinal Plant *Pepper ceba* Extracts Against Some Human Pathogenic Bacterial Strains.

Abstract:

The increasing prevalence of antimicrobial resistance among human pathogenic bacteria has emerged as a serious global health challenge, reducing the effectiveness of conventional antimicrobial therapies and creating an urgent need for the discovery of novel and effective antimicrobial agents. Indigenous medicinal plants have long been used in traditional systems of medicine for the treatment and management of various infectious diseases and are considered valuable sources of biologically active phytochemicals. The present study, entitled "*In-Vitro* Anti-Microbial Potential of Indigenous Medicinal Plant *Piper ceba* Extracts Against Some Human Pathogenic Bacterial Strains," was conducted to investigate the *In-Vitro* antimicrobial potential of *Piper ceba* extracts against selected human pathogenic bacteria. The study aimed to evaluate the antibacterial activity of different concentrations of ethanolic extracts of *Piper ceba* against four clinically important bacterial strains, namely *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Klebsiella pneumoniae*.

The plant material was subjected to ethanolic extraction, and the resulting extract was evaluated at four different concentrations, namely 25%, 50%, 75%, and 100%, to determine the concentration-dependent antibacterial effects. The antimicrobial activity was assessed by measuring the diameter of the zone of inhibition (ZOI) produced against the selected bacterial strains and expressed in millimetres (mm). Azithromycin (ATH, 15 µg) was used as the standard antibiotic for comparison. The findings demonstrated that the ethanolic extract of *Piper ceba* exhibited variable antibacterial activity against all four tested bacterial pathogens, with the magnitude of inhibition generally increasing with increasing extract concentration.

Against *E. coli*, the 25% ethanolic extract showed no detectable inhibitory activity, whereas the 50%, 75%, and 100% extracts produced inhibition zones of 7 mm, 12 mm, and 16 mm, respectively. The standard Azithromycin (ATH, 15 µg) disc produced an 8 mm zone of inhibition against *E. coli*. The results indicated that the 100% ethanolic extract exhibited greater inhibition

than the standard antibiotic under the experimental conditions. Against *S. aureus*, the 25% extract showed no detectable inhibition, while the 50%, 75%, and 100% extracts produced inhibition zones of 8 mm, 13 mm, and 18 mm, respectively. In contrast, the Azithromycin disc did not produce a detectable zone of inhibition against *S. aureus* in the present experiment.

The ethanolic extract also demonstrated concentration-dependent activity against *S. pyogenes*. The 25% and 50% extracts showed no detectable inhibition, whereas the 75% and 100% extracts produced inhibition zones of 10 mm and 14 mm, respectively. No detectable zone of inhibition was observed with the Azithromycin disc against *S. pyogenes*. Similarly, the antibacterial activity against *K. pneumoniae* was observed only at higher extract concentrations. The 25% and 50% extracts showed no detectable inhibition, while the 75% and 100% extracts produced inhibition zones of 14 mm and 18 mm, respectively. The standard Azithromycin disc also showed no detectable zone of inhibition against *K. pneumoniae* under the experimental conditions.

Overall, the 100% ethanolic extract exhibited the strongest antibacterial activity among all tested concentrations, producing inhibition zones of 16 mm against *E. coli*, 18 mm against *S. aureus*, 14 mm against *S. pyogenes*, and 18 mm against *K. pneumoniae*. The results indicate that *S. aureus* and *K. pneumoniae* were the most susceptible bacterial strains to the 100% ethanolic extract, followed by *E. coli* and *S. pyogenes*. The progressive increase in the zone of inhibition with increasing extract concentration suggests a concentration-dependent antibacterial effect and indicates that the active antimicrobial constituents may be present at higher effective concentrations in the 100% ethanolic extract.

The observed antibacterial activity of *Piper ceba* may be associated with the presence of biologically active phytochemicals and secondary metabolites capable of interfering with essential bacterial cellular processes. These compounds may exert their effects through mechanisms such as disruption of bacterial cell membranes, inhibition of essential enzymes, interference with protein synthesis, or alteration of nucleic acid metabolism. However, the precise mechanisms responsible for the antibacterial activity require further investigation.

In conclusion, the present study demonstrates that ethanolic extracts of the indigenous medicinal plant *Piper ceba* possess promising *In-Vitro* antibacterial activity against selected human pathogenic bacterial strains. The highest antibacterial activity was observed with the 100%

ethanolic extract, suggesting that higher concentrations of the extract may provide greater inhibitory effects. These findings provide preliminary scientific evidence supporting the potential of *Piper ceba* as a natural source of antimicrobial agents. Further investigations involving phytochemical characterisation, determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), toxicity assessment, antimicrobial mechanism studies, and in-vivo evaluation are recommended to establish the therapeutic efficacy and safety of *Piper ceba*. The findings may contribute to future research aimed at identifying novel plant-derived antimicrobial compounds that could potentially serve as alternative or complementary agents in the management of bacterial infections.

Keywords:*Piper ceba*; Indigenous medicinal plant; Ethanolic extract; Antimicrobial activity; Antibacterial activity; Human pathogenic bacteria; *Escherichia coli*; *Staphylococcus aureus*; *Streptococcus pyogenes*; *Klebsiella pneumoniae*; Zone of inhibition; Azithromycin; Antimicrobial resistance.

1.0. Introduction

Infectious diseases caused by pathogenic microorganisms remain a major challenge to public health worldwide. Bacterial infections are particularly important because they contribute substantially to morbidity, mortality, hospitalisation, and healthcare expenditure. Although the discovery and widespread use of antibiotics have transformed the treatment of bacterial infections, the increasing emergence and dissemination of antimicrobial resistance (AMR) have threatened the effectiveness of many conventional antimicrobial agents. AMR occurs when microorganisms acquire the ability to survive exposure to medicines that were previously effective against them, making infections increasingly difficult to treat (World Health Organization [WHO], 2024). The inappropriate and excessive use of antimicrobial drugs, inadequate infection prevention and control, and the spread of resistant organisms have accelerated this problem. Consequently, the identification of new antimicrobial agents from alternative sources has become an important priority in biomedical and pharmaceutical research (WHO, 2024).

Medicinal plants represent one of the most important natural resources for the discovery of biologically active compounds. For centuries, indigenous communities have relied on medicinal plants for the treatment of infectious diseases and other health disorders. The therapeutic properties of plants are generally associated with their secondary metabolites, including alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, saponins, glycosides, steroids, and essential oils (Cowan, 1999; Harborne, 1998). These compounds may possess antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, and other pharmacological activities. Plant-derived compounds may also act through mechanisms that differ from those of conventional antibiotics, making medicinal plants a valuable source for the exploration of new antimicrobial molecules (Abreu et al., 2012; Gibbons, 2008).

The antimicrobial activity of medicinal plants is influenced by several factors, including plant species, geographical origin, plant part, stage of harvesting, extraction method, solvent used, concentration of the extract, and susceptibility of the test microorganism (Ríos & Recio, 2005; Tiwari et al., 2011). The selection of an appropriate extraction solvent is particularly important because the polarity of the solvent determines which phytochemicals are extracted from plant material. Ethanol is frequently used in medicinal plant research because it can extract a broad spectrum of polar and moderately non-polar phytoconstituents and is suitable for laboratory investigations (Tiwari et al., 2011). Therefore, ethanolic extraction provides an appropriate approach for preliminary investigation of the antimicrobial potential of medicinal plants.

The genus Piper comprises numerous species distributed mainly in tropical and subtropical regions and has considerable ethnomedicinal and pharmacological importance. Plants belonging to the genus Piper have been investigated for diverse biological properties, including antimicrobial, antioxidant, anti-inflammatory, insecticidal, and cytotoxic activities. The biological effects of Piper species have been associated with various classes of secondary metabolites, including alkaloids, amides, flavonoids, phenylpropanoids, terpenoids, and other phenolic constituents (Saleem et al., 2010; Mgbeahuruike et al., 2017). *Piper ceba* is a medicinal plant belonging to the family Piperaceae and the genus Piper, a diverse group of flowering plants predominantly distributed in tropical and subtropical regions. The genus Piper comprises numerous species of medicinal, aromatic, and economically important plants. Several species belonging to this genus have been traditionally used in different systems of medicine and are

recognised for their diverse phytochemical constituents and biological activities (Cowan, 1999; Saleem et al., 2010).The plant is generally described as a herbaceous or semi-woody plant, depending on its growth habit and environmental conditions. Members of the genus Piper commonly possess simple, entire leaves arranged alternately along the stem. The leaves are generally green, with a distinct petiole and prominent venation. Leaf shape and size may vary among different species within the genus. The stem is generally cylindrical and may be soft, herbaceous, or somewhat woody, with branching commonly occurring at the nodes. These morphological characteristics are important for the preliminary identification of Piper species, although definitive identification should be performed by a qualified taxonomist (Jaramillo & Manos, 2001).The flowers of Piper species are generally small and inconspicuous, occurring in dense spike-like or cylindrical inflorescences. Individual flowers are usually very small and lack conspicuous petals. The inflorescences may arise opposite the leaves or from the nodes, depending on the species. Following flowering, small fruits may develop and are generally aggregated along the inflorescence axis. The fruits typically contain a single seed, and their morphological characteristics may vary among species of the genus (Jaramillo & Manos, 2001).The roots and aerial parts of Piper species are known to contain diverse groups of secondary metabolites, including alkaloids, amides, flavonoids, phenolic compounds, terpenoids, tannins, and other phytochemicals. These compounds have been associated with various biological activities, including antimicrobial, antioxidant, anti-inflammatory, insecticidal, and other pharmacological effects (Cowan, 1999; Saleem et al., 2010). The phytochemical composition of individual Piper species may vary according to species, geographical distribution, environmental conditions, plant part, maturity, and extraction method (Tiwari et al., 2011).

Taxonomic Classification

Kingdom: Plantae

Order: Piperales

Family: Piperaceae

Genus: Piper

Species: *Piper ceba*

Figure 1.1. ***Piper ceba***

Figure 1.2. ***Piper ceba steams***

Figure 1.3. ***Piper ceba roots***

The botanical and taxonomic characteristics of *Piper ceba* provide an important foundation for its scientific investigation as a medicinal plant. Its placement within the genus Piper, together with the known phytochemical diversity and biological activities reported for members of this genus, supports the rationale for investigating its potential antimicrobial properties. However, species-specific phytochemical and pharmacological investigations are necessary to establish the precise medicinal value of *Piper ceba* and to identify the compounds responsible for its observed antibacterial effects (Abreu et al., 2012; Ríos & Recio, 2005).

In the present study, *Piper ceba* was selected as an indigenous medicinal plant for the investigation of its potential antibacterial activity. The plant material was subjected to ethanolic extraction, and different concentrations of the extract were evaluated against selected human pathogenic bacterial strains. The selection of ethanol as an extraction solvent was based on its ability to extract a broad range of biologically active phytochemicals from plant materials (Tiwari et al., 2011). The resulting extract was investigated for its antibacterial potential against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Klebsiella pneumoniae*. Accurate botanical identification is essential in medicinal plant research because variations in

species identity can result in significant differences in phytochemical composition and biological activity. Therefore, the taxonomic identity of *Piper ceba* used in the present study should ideally be confirmed by a qualified taxonomist, and a representative voucher specimen should be deposited in a recognised herbarium for future reference and scientific reproducibility (Jarić et al., 2015). Indigenous medicinal plants are of particular importance in countries where traditional medicine remains an integral component of healthcare. Scientific investigation of such plants may contribute to the validation of traditional knowledge while also providing opportunities for the identification of novel bioactive compounds. However, traditional use alone does not establish antimicrobial efficacy or clinical safety; therefore, systematic laboratory evaluation is essential before therapeutic claims can be supported (Cowan, 1999; Ríos & Recio, 2005).

The present investigation evaluated the antibacterial activity of ethanolic *Piper ceba* extracts against four human pathogenic bacterial strains: *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Klebsiella pneumoniae*. These organisms represent important Gram-negative and Gram-positive bacterial pathogens associated with a wide range of human infections. *E. coli* is a Gram-negative bacterium that forms part of the normal intestinal microbiota but includes pathogenic strains capable of causing urinary tract infections, gastrointestinal disease, bloodstream infections, and other clinical conditions. *K. pneumoniae* is another clinically significant Gram-negative pathogen associated with respiratory, urinary tract, bloodstream, and healthcare-associated infections. The increasing occurrence of antimicrobial-resistant strains of these organisms has complicated therapeutic management and increased the importance of identifying alternative antimicrobial resources (Paczosa & Mecsas, 2016; Wyres & Holt, 2016).

Among the Gram-positive organisms investigated, *S. aureus* is a major human pathogen capable of causing skin and soft-tissue infections, wound infections, pneumonia, bacteraemia, endocarditis, and other invasive diseases. The emergence of resistant *S. aureus* strains has created a substantial clinical challenge and has stimulated continuing research into alternative antimicrobial compounds (Lowy, 1998; Tong et al., 2015). *S. pyogenes* is also an important human pathogen responsible for infections such as pharyngitis, scarlet fever, and skin and soft-tissue infections, as well as potentially severe invasive diseases and post-infectious complications (Carapetis et al., 2005; Walker et al., 2014). The inclusion of these organisms in

the present study therefore provides an opportunity to evaluate the activity of *Piper ceba* extract against bacteria with different structural and pathogenic characteristics.

The antibacterial activity of medicinal plant extracts can be initially evaluated using *In-Vitro* methods such as agar diffusion and broth dilution techniques. Agar diffusion methods are commonly employed for preliminary antimicrobial screening because they provide a relatively simple means of observing bacterial growth inhibition. The diameter of the zone of inhibition surrounding the test substance can be measured and used as an indicator of antimicrobial activity. However, zone diameter is influenced by several factors, including the concentration and diffusion characteristics of active compounds, molecular size, solubility, agar composition, inoculum density, incubation conditions, and experimental methodology (Balouiri et al., 2016). Consequently, results obtained from plant extracts should be interpreted cautiously and under standardised experimental conditions.

The observed antibacterial activity of *Piper ceba* may be related to the presence of bioactive phytochemicals capable of affecting bacterial growth through multiple mechanisms. Plant-derived phenolic compounds, flavonoids, alkaloids, terpenoids, and other secondary metabolites may disrupt bacterial membranes, alter membrane permeability, inhibit enzymes, interfere with nucleic acid synthesis, or affect essential metabolic pathways (Cushnie & Lamb, 2005; Daglia, 2012; Simões et al., 2009). The combined or synergistic action of multiple constituents within a crude plant extract may also contribute to its overall antimicrobial effect (Hemaiswarya et al., 2008). Nevertheless, the precise compounds responsible for the antibacterial activity of *Piper ceba* remain to be established through phytochemical and pharmacological investigations.

Therefore, the present study was designed to investigate the *In-Vitro* antimicrobial potential of indigenous medicinal plant *Piper ceba* extracts against selected human pathogenic bacterial strains. The study aims to evaluate the antibacterial effects of different concentrations of ethanolic *Piper ceba* extract, determine the zones of inhibition against selected bacterial pathogens, assess the concentration-dependent nature of the activity, and compare the observed effects with Azithromycin as a reference antimicrobial agent.

2.0. Objectives of the study

2.1. General Objective

The main aim of this study is to evaluate the *In-Vitro* antimicrobial potential of ethanolic extracts of the indigenous medicinal plant *Piper ceba* against selected human pathogenic bacterial strains.

2.2. Specific Objectives

- To prepare ethanolic extracts of *Piper ceba* for antimicrobial investigation.
- To evaluate the antibacterial activity of 25%, 50%, 75%, and 100% concentrations of *Piper ceba* ethanolic extract.
- To determine the antibacterial activity of the extracts against *E. coli*, *S. aureus*, *S. pyogenes*, and *K. pneumoniae*.
- To investigate the concentration-dependent antibacterial activity of *Piper ceba* ethanolic extract.
- To compare the observed antibacterial activity of the plant extract with Azithromycin (ATH, 15 µg) as a standard antibiotic.

Below is a methodology section written in an academic article style, based on the experimental details you provided. I have used APA-style in-text citations and authoritative sources, particularly WHO and CLSI guidance. Because you did not provide the exact plant collection location, plant part, extraction duration, bacterial strain numbers, number of replicates, or statistical method, I have marked those details for you to insert rather than inventing them.

3.0. Materials and Methods

3.1. Study Design

The present study was designed as an *in-vitro* experimental investigation to evaluate the antibacterial potential of ethanolic extracts of the indigenous medicinal plant *Piper ceba* against selected human pathogenic bacterial strains. Four concentrations of the ethanolic extract (25%, 50%, 75%, and 100%) were evaluated against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Klebsiella pneumoniae*. Azithromycin (ATH, 15 µg) was used as the standard antibiotic for comparative assessment. The antibacterial activity of the plant extracts was determined by measuring the diameter of the zones of inhibition (ZOIs) produced around the

test samples. The antimicrobial susceptibility testing procedures were conducted using standardised microbiological principles. The disk-diffusion approach is a widely used method for assessing antimicrobial activity, although interpretation of susceptibility categories requires validated standards appropriate to the organism, antimicrobial agent, and test method (Balouiri et al., 2016; Clinical and Laboratory Standards Institute, 2024). WHO laboratory guidance similarly emphasises standardisation of inoculum preparation, culture media, antimicrobial disks, incubation conditions, and measurement procedures to ensure reproducibility of antimicrobial susceptibility testing (WHO, 2003).

3.2. Collection and Identification of Plant Material

The plant material of *Piper ceba* was collected from Birat,Batiaghata, Khulna district**,** Bangladesh during month of January 2026. The plant was initially identified based on its morphological characteristics and subsequently authenticated by the pharmacognosy department of Hamdard University Bangladesh.

3.3. Preparation of Plant Material

The collected *Piper ceba* material, specifically the roots, was separated from unwanted foreign materials and carefully washed with clean water to remove adhering soil and other contaminants. The material was then dried under shade drying until a constant weight was obtained. The dried material was subsequently pulverised into a coarse powder using a clean laboratory grinder. The powdered plant material was stored in a clean, dry, airtight container until extraction because controlled drying and appropriate storage conditions is important because excessive heat, moisture, light, and prolonged storage may affect the stability of phytochemicals and consequently influence the biological activity of plant extracts (Tiwari et al., 2011).

3.4. Preparation of Ethanolic Extract

The powdered plant material was extracted using ethanol as the extraction solvent. 100 grams of powdered plant material was subjected to extraction using macerationwith ethanol for 72 hours. The extraction procedure was performed according to the laboratory protocol used for the present investigation.Following extraction, the mixture was filtered through Whatman Filter Paper No. 1 to remove insoluble plant residues. The resulting filtrate was concentrated using

water bathat 40 degrees centigrade to obtain the concentrated ethanolic extract. The extract was stored in a suitable airtight container at 4degrees centigrade until further analysis.

3.5. Preparation of Extract Concentrations

The concentrated ethanolic extract of *Piper ceba* was used to prepare four test concentrations: 25%, 50%, 75%, and 100%. The required concentrations were prepared using the Dimethyl sulfoxide (DMSO) in the experimental protocol. The prepared extract solutions were labelled appropriately and maintained under suitable conditions until antimicrobial testing.The use of different concentrations enabled assessment of the concentration-dependent antibacterial activity of the plant extract (Balouiri et al., 2016).

3.6. Test Bacterial Strains

Four human pathogenic bacterial strains were selected for evaluation of the antibacterial activity of Piper ceba ethanolic extract. The organisms investigated were*Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Klebsiella pneumoniae*. The bacterial strains were obtained from microbiology laboratory of Authentic Diagnostic Center, Dhaka and maintained according to standard microbiological procedures. The selected organisms represented both Gram-negative and Gram-positive bacteria, allowing the antibacterial activity of the plant extract to be assessed against organisms with different cellular structures and physiological characteristics.

3.7. Preparation of Bacterial Inoculum

Pure cultures of the test organisms were used for preparation of bacterial inoculum. Fresh colonies from appropriate culture media were transferred aseptically into sterile physiological saline. The turbidity of each bacterial suspension was adjusted to approximately 0.5 McFarland standard, corresponding to an inoculum of approximately 1-2 × 10^8 CFU/mL, as appropriate for standardised disk-diffusion testing (WHO, 2022).

3.8. Antibacterial Activity Assay

The antibacterial activity of the *Piper ceba* ethanolic extracts was evaluated using an agar diffusion method. Appropriate sterile agar plates were prepared using Mueller–Hinton

agaraccording to the manufacturer's instructions and standard microbiological procedures.The standardised bacterial suspension was inoculated uniformly onto the surface of the agar medium using a sterile technique to produce an even bacterial lawn. The prepared extract concentrations (25%, 50%, 75%, and 100%) were then applied to the inoculated agar plates using sterile blank paper disks. Azithromycin (ATH, 15 µg) was included as the standard antibiotic control. Dimethyl sulfoxide (DMSO)was used as negative controls containing the extraction vehicleto determine whether the solvent itself contributed to any observed antibacterial effect.The inoculated plates were incubated under 37^{o}C for the all bacterial stains. (CLSI, 2024; WHO, 2003).

3.9. Measurement of Zone of Inhibition

Following incubation, the plates were examined for visible bacterial growth inhibition surrounding the extract samples and antibiotic control. The diameter of each zone of inhibition was measured in millimetres using a calibrated ruler or measuring device. Where no visible zone of inhibition was observed, the result was recorded as resistant in experimental records.The antibacterial activity was evaluated by comparing the inhibition zones produced by the different concentrations of *Piper ceba* extract. The results were recorded systematically for each bacterial strain and concentration. The inhibition zone measurements were subsequently used to compare the relative antibacterial activity of the different extract concentrations.

3.10. Experimental Controls

Azithromycin (ATH, 15 µg) was used as the positive control and standard antibiotic for comparative evaluation. A negative control containing the extraction vehicle DMSO, was used to assess any potential antibacterial effect attributable to the solvent rather than the plant extract.Appropriate quality-control procedures were followed during the antimicrobial testing process. Standardised antimicrobial susceptibility methods emphasise the importance of appropriate controls and reproducible laboratory conditions when generating antimicrobial susceptibility data (CLSI, 2024; WHO, 2003).

3.11. Data Recording and Analysis

The antibacterial activity was expressed as the diameter of the zone of inhibition in millimetres (mm). The results obtained for the 25%, 50%, 75%, and 100% ethanolic extracts were tabulated for each test organism and compared with the Azithromycin (ATH, 15 µg) control.The primary outcome measure was the diameter of the inhibition zone produced by each extract concentration. The antibacterial response was evaluated descriptively to determine the concentration-dependent pattern of activity and to identify the bacterial strains that demonstrated the greatest apparent susceptibility to the *Piper ceba* extract.

3.12. Ethical and Biosafety Considerations

All microbiological procedures were conducted in an appropriate laboratory environment using standard aseptic techniques and institutional biosafety procedures. The handling, cultivation, and disposal of bacterial cultures were performed in accordance with applicable institutional laboratory safety requirements. Appropriate personal protective equipment was used during microbiological work, and contaminated materials were decontaminated and disposed of according to established laboratory procedures.

4.0. RESULTS AND DISCUSTION

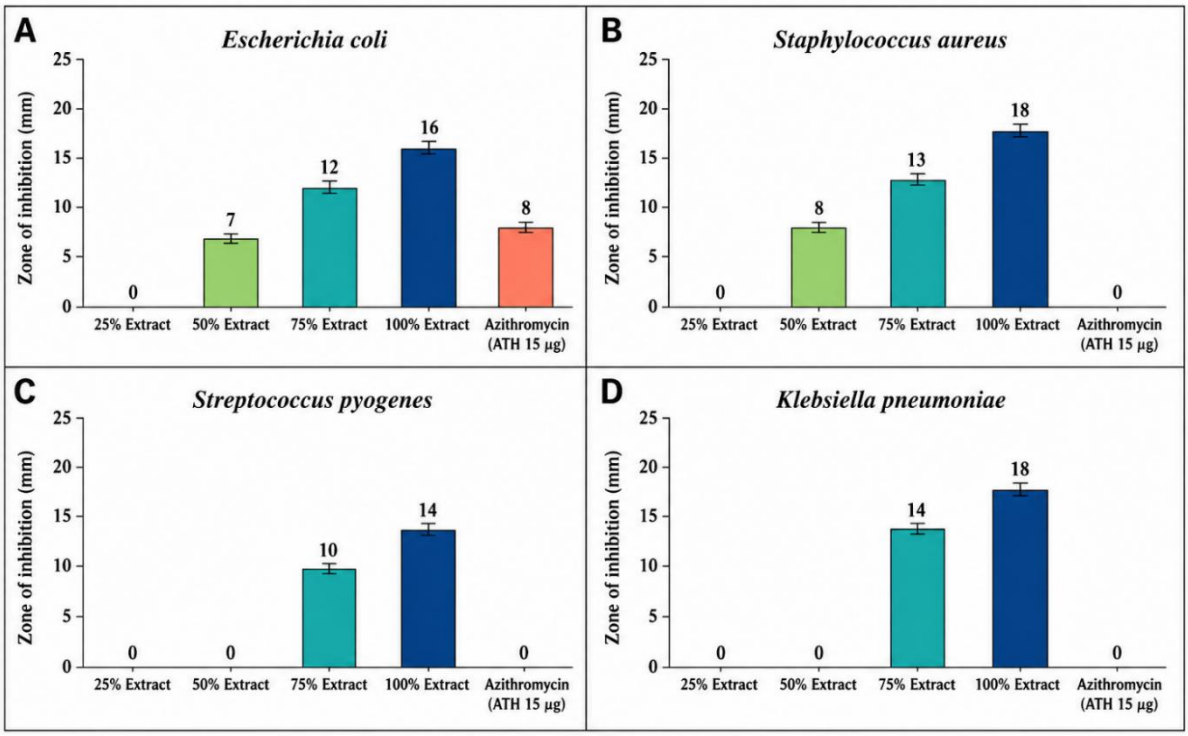
4.1. Result of ethanolic extract of *Pepper ceba*

The present study evaluated the *In-Vitro* antibacterial activity of different concentrations (25%, 50%, 75%, and 100%) of ethanolic extract of *Piper ceba* against four human pathogenic bacterial strains, namely *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Klebsiella pneumoniae*. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition (ZOI) in millimetres (mm). Azithromycin (ATH, 15 µg) was used as the standard antibiotic for comparative evaluation.The results demonstrated a generally concentration-dependent antibacterial effect of the *Piper ceba* ethanolic extract. Against *E. coli*, the 25% extract showed no detectable inhibition, whereas the 50%, 75%, and 100% extracts produced inhibition zones of 7, 12, and 16 mm, respectively. The standard Azithromycin disc produced an 8 mm zone of inhibition. Against *S. aureus*, no detectable activity was observed with the 25% extract, while the 50%, 75%, and 100% extracts produced zones of 8, 13, and 18 mm, respectively. No detectable inhibition was observed with the Azithromycin disc against *S. aureus*

under the experimental conditions.Similarly, the extract demonstrated increasing antibacterial activity against *S. pyogenes* with increasing concentration. The 25% and 50% extracts showed no detectable inhibition, while the 75% and 100% extracts produced zones of 10 and 14 mm, respectively. Azithromycin showed no detectable zone of inhibition against this organism. Against *K. pneumoniae*, the 25% and 50% extracts showed no detectable antibacterial activity, whereas the 75% and 100% extracts produced inhibition zones of 14 and 18 mm, respectively.Overall, the 100% ethanolic extract exhibited the highest antibacterial activity, with the maximum inhibition zone of 18 mm observed against both *S. aureus* and *K. pneumoniae*.

Table 4.1. Zone of inhibition produced bydifferent concentration of ethanolic extract of *Pepper ceba*against*Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes* and*Klebsiella pneumoniae*

Test microorganism	25% extract	50% extract	75% extract	100% extract	Azithromycin (ATH 15 µg)
Escherichia coli	Resistant	7 mm	12 mm	16 mm	8 mm
Staphylococcus aureus	Resistant	8 mm	13 mm	18 mm	Resistant
Streptococcus pyogenes	Resistant	Resistant	10 mm	14 mm	Resistant
Klebsiella pneumoniae	Resistant	Resistant	14 mm	18 mm	Resistant



Graph 4.1. Zone of inhibition produced by different concentration of ethanolic extract of *Pepper ceba*against*Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Klebsiella pneumoniae*

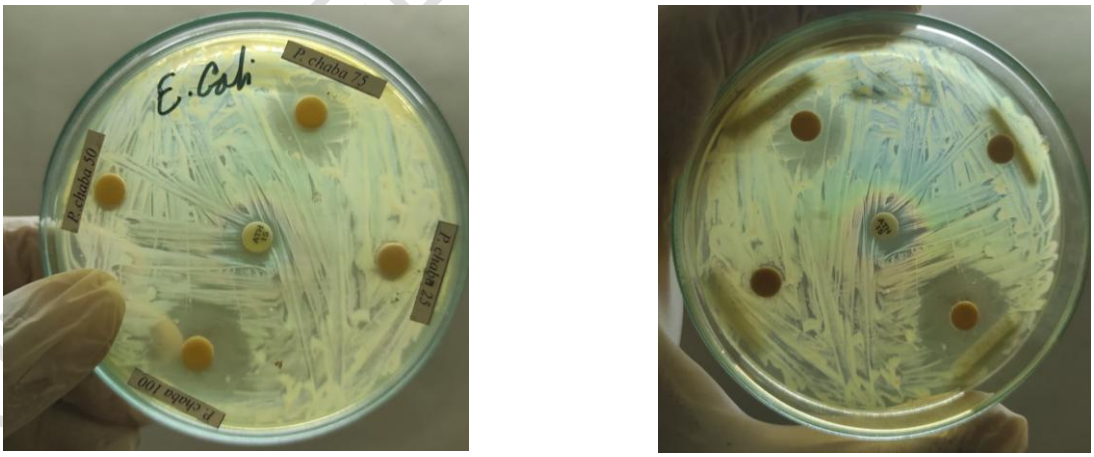


Figure 4.1. Zone of inhibition produced by 25%, 50%, 75% and 100% ethanolic extract of *Pepper ceba* against *Escherichia coli* show resistance, 07 mm, 12 mm and 16 mm where standard antibiotic disk of Azithromycin (ATH 15 µg) produce 8 mm zone of inhibition against *Escherichia coli*.

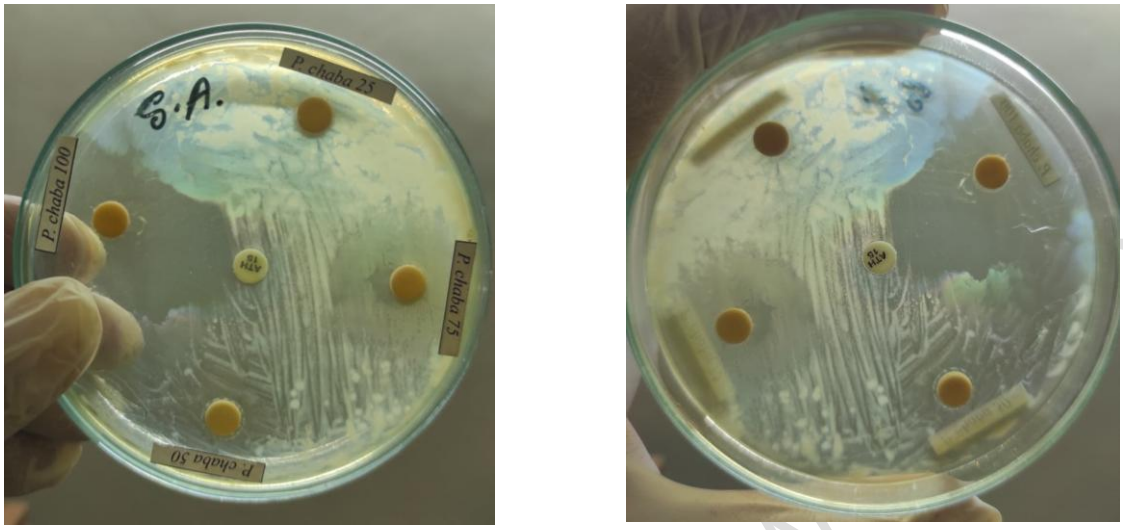


Figure 4.2. Zone of inhibition produced by 25%, 50%, 75% and 100% ethanolic extract of *Pepper ceba* against *Staphylococcus aureus* show resistance, 8 mm, 13 mm and 18 mm where standard antibiotic disk of Azithromycin (ATH 15 µg) produces no zone of inhibition against *Staphylococcus aureus.*

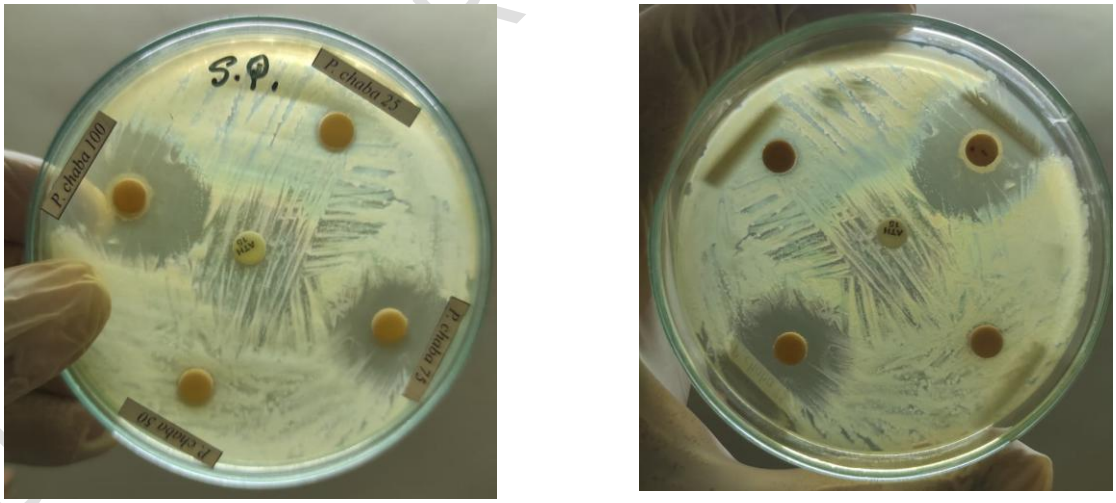


Figure 4.3. Zone of inhibition produced by 25%, 50%, 75% and 100% ethanolic extract of *Pepper ceba* against *Strephylococcus pyogenes* show resistance, resistance, 10 mm and 14 mm where standard antibiotic disk of Azithromycin (ATH 15 µg) produces no zone of inhibition against *Strephylococcus pyogenes*

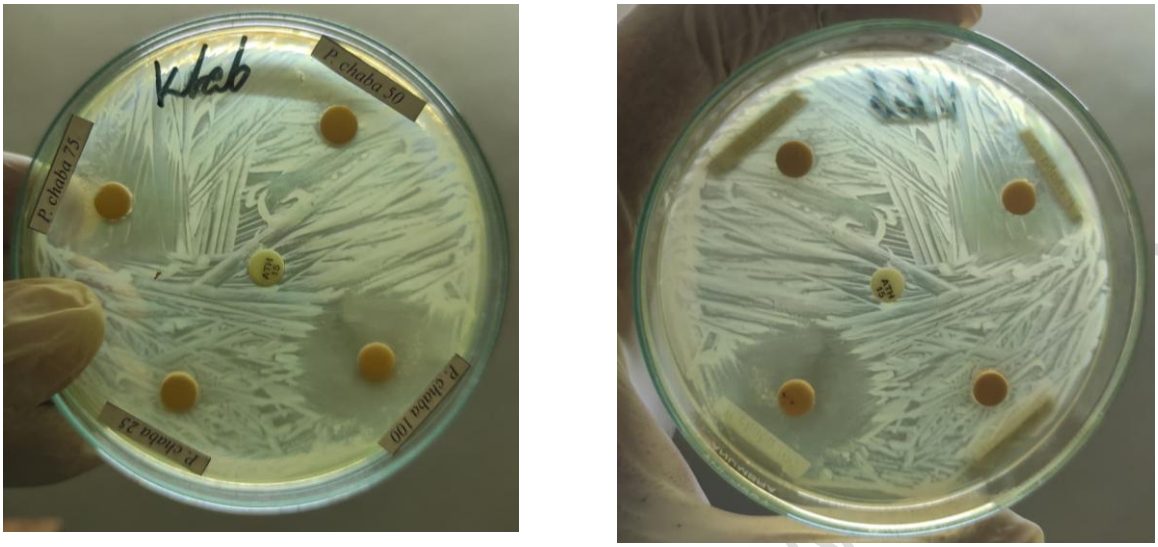


Figure 4.4. Zone of inhibition produced by 25%, 50%, 75% and 100% ethanolic extract of *Pepper ceba* against *Klebsiella pneumoniae* show Resistance, Resistance, 14 mm and 18 mm where standard antibiotic disk of Azithromycin (ATH 15 µg) produces no zone of inhibition against *Klebsiella pneumoniae*

4.2. Discussion

The present study investigated the *In-Vitro* antibacterial potential of ethanolic extracts of *Piper ceba* at concentrations of 25%, 50%, 75%, and 100% against four human pathogenic bacterial strains, namely *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Klebsiella pneumoniae*. The findings demonstrated that the antibacterial activity of the extract generally increased with increasing concentration. This concentration-dependent pattern is an important observation because it suggests that the biologically active constituents responsible for antibacterial activity may be present in greater effective concentrations in the higher-concentration extracts. Similar concentration-dependent antimicrobial effects have been reported in studies investigating medicinal plant extracts (Cowan, 1999; Ríos & Recio, 2005; Balouiri et al., 2016).

In the present investigation, the 100% ethanolic extract showed the strongest antibacterial activity, producing inhibition zones of 16 mm against *E. coli*, 18 mm against *S. aureus*, 14 mm against *S. pyogenes*, and 18 mm against *K. pneumoniae*. The highest activity was therefore

observed against *S. aureus* and *K. pneumoniae*. This finding supports previous research demonstrating that plants belonging to the genus *Piper* possess significant antimicrobial potential. Saleem et al. (2010) reported that several natural products derived from *Piper* species exhibit promising antimicrobial activity and may represent useful sources of future antimicrobial compounds. Similarly, Mgbeahuruike et al. (2017) highlighted the diverse biological activities of *Piper* species and attributed many of these effects to their chemically diverse secondary metabolites.

The activity observed against *S. aureus* is particularly noteworthy. In this study, the 100% extract produced an 18 mm inhibition zone, whereas the lower concentrations showed progressively weaker activity. *S. aureus* is an important human pathogen responsible for skin infections, wound infections, pneumonia, bacteraemia, and other diseases (Lowy, 1998; Tong et al., 2015). The susceptibility of this organism to *Piper ceba* extract may indicate the presence of phytochemicals capable of interfering with the bacterial cell envelope or other essential cellular processes. Phenolic compounds and flavonoids, for example, have been reported to exert antibacterial effects through membrane disruption, enzyme inhibition, and interference with nucleic acid synthesis (Cushnie & Lamb, 2005; Daglia, 2012).

The extract also demonstrated notable activity against the Gram-negative bacteria *E. coli* and *K. pneumoniae*. The 100% extract produced inhibition zones of 16 and 18 mm, respectively. However, the lower concentrations showed reduced or no detectable activity, particularly against *K. pneumoniae*. Gram-negative bacteria possess an outer membrane that can restrict the penetration of many antimicrobial substances, making them potentially less susceptible to certain plant-derived compounds (Nazzaro et al., 2013). The observed activity at higher concentrations may therefore indicate that sufficient quantities of active phytochemicals were required to overcome these protective barriers.

Against *S. pyogenes*, measurable antibacterial activity was observed only with the 75% and 100% extracts, producing inhibition zones of 10 and 14 mm, respectively. This result further supports the concentration-dependent pattern observed in the study. Differences in bacterial susceptibility may be related to variations in cell-wall structure, membrane composition,

metabolic characteristics, and resistance mechanisms among bacterial species (Silhavy et al., 2010).

The antibacterial effects observed in this study may be associated with the presence of secondary metabolites in *Piper ceba*. Medicinal plants commonly contain alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, and other bioactive constituents that may act individually or synergistically against bacterial pathogens (Cowan, 1999; Abreu et al., 2012). The use of ethanol as an extraction solvent may have facilitated the extraction of a broad range of biologically active constituents, potentially contributing to the observed antibacterial effects (Tiwari et al., 2011).

Azithromycin (ATH, 15 µg) was used as the standard antibiotic in the present investigation. It produced an 8 mm zone against *E. coli* but no detectable zone against *S. aureus, S. pyogenes*, or *K. pneumoniae* under the experimental conditions. Although the 100% *Piper ceba* extract produced larger inhibition zones than Azithromycin for some organisms, these results should be interpreted cautiously. Crude plant extracts and standardised antibiotic discs differ substantially in chemical composition, concentration, diffusion characteristics, and standardised susceptibility interpretation. Therefore, direct comparison of zone sizes does not establish that the plant extract is clinically more effective than the antibiotic (Balouiri et al., 2016; CLSI, 2024).

Overall, the findings of the present study are consistent with previous reports describing the antimicrobial potential of medicinal plants and *Piper* species. The progressive increase in inhibition zones from lower to higher extract concentrations suggests that *Piper ceba* contains potentially active antibacterial constituents. However, further research involving phytochemical screening, MIC and MBC determination, compound isolation, toxicity studies, and in-vivo evaluation is necessary to confirm the therapeutic significance of these findings.

5.0 CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

The present study investigated the *In-Vitro* antimicrobial potential of ethanolic extracts of the indigenous medicinal plant *Piper ceba* against four human pathogenic bacterial strains:

Escherichia coli,*Staphylococcus aureus*,*Streptococcus pyogenes*,and*Klebsiella pneumoniae*. The findings demonstrated that the extract exhibited concentration-dependent antibacterial activity. The 25% extract showed no detectable inhibition, while higher concentrations demonstrated progressively greater antibacterial effects. The 100% ethanolic extract showed the strongest activity, producing inhibition zones of 16 mm against *E. coli*, 18 mm against *S. aureus*, 14 mm against *S. pyogenes*, and 18 mm against *K. pneumoniae*. These findings indicate that *Piper ceba* contains potentially bioactive antibacterial constituents capable of inhibiting both Gram-positive and Gram-negative bacteria under the experimental conditions. Overall, the study provides preliminary scientific evidence supporting the antimicrobial potential of *Piper ceba* and its possible importance as a natural source of antibacterial compounds.

5.2. Recommendations:

Further studies are recommended to identify the specific phytochemicals responsible for the antibacterial activity of *Piper ceba*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) should be determined using standardised methods. Bioassay-guided fractionation and chromatographic techniques should be employed to isolate active compounds. Additional investigations should evaluate the mechanisms of antibacterial action, toxicity, cytotoxicity, and possible synergistic effects with conventional antibiotics. *In-vivo* studies are also recommended to establish efficacy, safety, pharmacokinetic properties, and therapeutic potential before considering clinical applications.

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7.0. Conflicts of interest

No conflicting interests are stated by the authors

8.0. References

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