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Phytochemical Profiling and Antibacterial Activity of *Curcuma amada*, *Piper betle*, and *Piper nigrum* Against Pathogenic Bacteria

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Abstract

The rapid emergence of antimicrobial resistance (AMR) poses a major global health challenge, necessitating the exploration of alternative antimicrobial agents from natural sources. This study investigates the phytochemical composition and antibacterial efficacy of three traditionally used medicinal plants *Curcuma amada*, *Piper betle*, and *Piper nigrum*. Methanol and acetone extracts were prepared and evaluated against *Escherichia coli*, *Staphylococcus* spp., and *Bacillus* spp. using agar diffusion assays. Phytochemical screening revealed the presence of alkaloids, saponins, tannins, and flavonoids, while terpenoids and reducing sugars varied across extracts (L232). Antibacterial activity showed that methanol extracts exhibited stronger inhibition against *Bacillus* spp., whereas acetone extracts of *C. amada* demonstrated maximum inhibition against *E. coli* (L240). These findings support the potential of these plants as eco-friendly antibacterial agents and highlight their relevance in combating antibiotic-resistant pathogens.

Keywords

Antibacterial activity, phytochemicals, medicinal plants, antimicrobial resistance, *Curcuma amada*, *Piper betle*, *Piper nigrum*

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1. INTRODUCTION

Medicinal plants have long been recognized as an essential source of therapeutic agents and continue to play a significant role in primary healthcare systems worldwide. It is estimated that nearly 80% of the population in developing countries relies on plant-based traditional medicine for treatment and disease prevention (Kalpana & Kodukkur, 2011). These plants are rich in secondary metabolites such as phenolic compounds, flavonoids, tannins, and alkaloids, which exhibit diverse pharmacological activities including antioxidant, anti-inflammatory, and antimicrobial effects (Hamid et al., 2010; Cowan, 1999). The growing interest in natural products stems from their therapeutic efficacy, safety profile, and reduced environmental impact compared with synthetic drugs.

In recent years, antimicrobial resistance (AMR) has emerged as one of the most pressing global public health challenges. The extensive misuse and overuse of antibiotics, especially in developing countries, have significantly contributed to the emergence of resistant microbial strains, thereby limiting the effectiveness of conventional antimicrobial therapies (Vila & Pal, 2010; Soothill et al., 2013). Pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* are increasingly showing resistance to commonly used antibiotics, posing

serious threats to infection control and disease management (Kluytmans, 2010; Aiello et al., 2003). Consequently, there is an urgent need to identify alternative antimicrobial agents, particularly those derived from natural sources.

Hand hygiene is widely recognized as one of the most effective strategies for preventing the transmission of infectious diseases. Pathogenics are a major vector for the spread of pathogenic microorganisms, carrying both resident and transient microflora that can cause infections (Simonne, 2011). Poor hygiene practices contribute significantly to the spread of diarrheal diseases and other communicable infections, especially in regions with inadequate sanitation facilities (Fewtrell et al., 2005). Although modern hand sanitizers and antiseptic agents such as alcohol-based solutions are widely used, their prolonged usage may lead to skin irritation and reduced effectiveness, necessitating the exploration of safer and more sustainable alternatives (Larson et al., 2006).

Traditionally, a variety of plant-based materials have been used as natural cleansing agents due to their antimicrobial and detergent properties. Many of these plants contain saponins and other bioactive compounds that contribute to their cleansing action (Spitz, 2004; Chen et al., 2010). Among them, *Curcuma amada* (mango ginger), *Piper betle* (betel leaf), and *Piper nigrum* (black pepper) are widely used in traditional medicine systems across Asia. *Piper betle* leaves have been extensively studied for their antibacterial and antifungal properties, attributed to the presence of bioactive compounds such as hydroxychavicol, eugenol, and chavicol (Fazal et al., 2014; Chowdhury et al., 2020). Similarly, *Curcuma amada* contains essential oils and phytochemicals with significant anti-inflammatory and antimicrobial activities (Bhattacharya & Chakraborty, 2015; Jayasree et al., 2020). *Piper nigrum* is known for its major bioactive compound piperine, which exhibits antimicrobial and therapeutic properties (Nikolić et al., 2015).

The antimicrobial activity of plant extracts is largely attributed to the presence of phytochemicals that act through multiple mechanisms, including disruption of cellular membranes, inhibition of enzyme activity, and interference with microbial metabolism (Cowan, 1999). The efficiency of these compounds depends on factors such as extraction method, solvent type, and plant origin. Organic solvents such as methanol and acetone have been reported to be highly effective in extracting bioactive antimicrobial compounds compared with aqueous solvents (Tiwari et al., 2011; Eloff, 1999). These solvents can dissolve both polar and non-polar compounds, thereby enhancing the recovery of phytochemicals with antibacterial properties.

Despite extensive traditional usage, scientific validation of the antibacterial efficacy of these plants against clinically relevant pathogens remains limited. Furthermore, there is a lack of comparative studies evaluating the phytochemical composition and antimicrobial effectiveness of different solvent extracts. Therefore, the present study aims to investigate the phytochemical characteristics and antibacterial activity of *Curcuma amada*, *Piper betle*, and *Piper nigrum* extracts against selected pathogenic bacteria such as *E. coli*, *Staphylococcus* spp., and *Bacillus* spp. By integrating traditional knowledge with modern scientific approaches, this research seeks to contribute to the development of safe, effective, and environmentally sustainable antimicrobial agents for improving public health outcomes.

2. MATERIALS AND METHODS

2.1 Study Area and Duration

The study was conducted between February and April 2026 using plant materials collected from local markets in Kavali, Andhra Pradesh, India. The selection of plant samples was based on ethnobotanical information regarding their traditional use for antibacterial and cleansing purposes (Kumbi, 2010; Suleman & Alemu, 2012).

2.2 Plant Materials and Collection

Three medicinal plants *Curcuma amada* Roxb. (rhizome), *Piper betle* L. (leaves), and *Piper nigrum* L. (seeds) were selected for this study due to their well-documented use in traditional medicine systems. The plant materials were collected from local sources and were carefully inspected to ensure that they were healthy and free from visible infections or contaminants (Cannell, 1998).

2.3 Sample Preparation

Collected plant materials were thoroughly washed to remove dust and other foreign particles and then shade-dried at room temperature for 10–20 days to prevent degradation of bioactive compounds. The dried materials were ground into fine powder using a mechanical grinder and sieved through a 2.5 mm mesh to ensure uniform particle size. The powdered samples were stored in airtight containers at 4 °C until further analysis to preserve phytochemical integrity (Cannell, 1998).

2.4 Extraction of Plant Materials

Extraction of bioactive compounds was carried out using methanol and acetone solvents through maceration. Approximately 100 g of powdered plant material was subjected to extraction with 100 mL of solvent and agitated continuously for 72 hours at 300

rpm using an orbital shaker. After extraction, the mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated by solvent evaporation at room temperature. The concentrated extracts were dried in a desiccator and stored at temperatures below 4 °C for further experimental analysis (Tiwari et al., 2011). Methanol and acetone were selected due to their intermediate polarity, low toxicity, and efficiency in extracting a wide range of bioactive compounds. Previous studies have demonstrated that these solvents can extract both hydrophilic and hydrophobic phytochemicals effectively, thereby enhancing antimicrobial activity (Eloff, 1999; Das et al., 2010).

Figure 1. Extraction Methodology

2.5 Phytochemical Screening

Qualitative phytochemical analysis of the plant extracts was conducted using standard protocols to detect the presence of major bioactive compounds such as alkaloids, flavonoids, tannins, saponins, terpenoids, and reducing sugars. The tests were performed using established reagents including Mayer's reagent, Wagner's reagent, Hager's reagent, Molisch's reagent, Benedict's reagent, and Fehling's reagent (Vishnoi, 2000; Roopashree et al., 2008). Phytochemical compounds identified in this study are known to exhibit antimicrobial activity through various mechanisms such as inhibition of microbial enzymes, disruption of cell membranes, and interference with metabolic processes (Harborne, 1998).

2.6 Thin Layer Chromatography (TLC) Analysis

Thin Layer Chromatography (TLC) was employed to separate and identify bioactive compounds present in the plant extracts. The analysis was conducted using a solvent system consisting of chloroform and methanol in a ratio of 95:5. Silica gel-coated glass plates (20 × 10 cm, thickness 0.5 mm) were used as the stationary phase. The extracts were applied onto the TLC plates, which were then placed in a chromatography chamber containing the solvent system. The solvent was allowed to ascend the plate by capillary action until it reached three-fourths of the plate length. After drying, the spots were visualized under ultraviolet (UV) light. The retention factor (Rf) values were calculated using the formula:

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Rf values were compared with standard values to identify phytochemical constituents present in the extracts (Harborne, 1998).

2.7 Microorganisms and Culture Conditions

The bacterial strains used in this study included *Escherichia coli*, *Staphylococcus* spp., and *Bacillus* spp., which were obtained from the Department of Zoology, Vikrama Simhapuri University College. These bacteria were selected due to their clinical relevance and association with hand-transmitted infections. The bacterial cultures were grown in Luria Broth (LB) medium containing tryptone (10 g), yeast extract (5 g), and sodium chloride (10 g) per liter of distilled water. For solid media, agar was added at a concentration of 1.5%. The media were sterilized by autoclaving at 121–124 °C for 15 minutes (Sambrook & Russell, 2001; Gerhardt et al., 1994).

2.8 Antibacterial Activity Assay

The antibacterial activity of plant extracts was evaluated using the agar well diffusion method. Sterile nutrient agar plates were inoculated with bacterial cultures, and wells were created in the agar medium. Different concentrations of plant extracts (10–50 µL) were introduced into the wells, and the plates were incubated under appropriate conditions. The zones of inhibition surrounding the wells were measured in millimeters as an indicator of antibacterial activity. All experiments were

performed in replicates to ensure accuracy and reproducibility of results (Das et al., 2010).

2.9 Quality Control

All experimental procedures were carried out following standard laboratory protocols. The quality of culture media, reagents, and solvents was carefully monitored. Control experiments were performed using standard antimicrobial agents to validate the results. Parameters such as temperature, incubation time, and sterility conditions were strictly maintained to ensure reliability and reproducibility of the data (Das et al., 2010).

3. RESULTS

3.1 Extraction Yield of Plant Materials

The extraction yields of *Curcuma amada*, *Piper betle*, and *Piper nigrum* varied depending on the plant species and solvent used. Methanol extracts generally produced higher yields compared to acetone extracts, confirming the efficiency of methanol as a solvent for extracting a broad range of phytochemicals. Among the three plant species, *Curcuma amada* exhibited a relatively high extraction yield (13.92 g in methanol and 14.10 g in acetone), indicating the presence of a significant amount of soluble bioactive compounds. In contrast, *Piper betle* showed the lowest yield in acetone extraction (9.44 g), suggesting differences in phytochemical solubility and composition among plant species (Fig. 2). The variation in extraction yield can be attributed to differences in plant morphology, chemical composition, and solvent polarity. These findings align with previous reports indicating that solvents of intermediate polarity are more effective in extracting antimicrobial compounds.

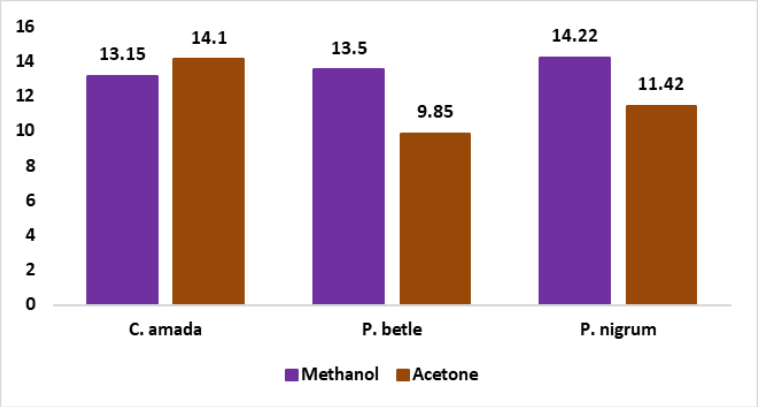


Figure 2. Graphical representation of extract yield of the plants

3.2 Phytochemical Screening

Qualitative phytochemical analysis revealed the presence of key bioactive compounds across all plant extracts. Methanol and acetone extracts of *Curcuma amada*, *Piper betle*, and *Piper nigrum* tested positive for: alkaloids, saponins, tannins, flavonoids. The presence of these compounds indicates the potential antimicrobial activity of the extracts. Methanol extracts, in particular, showed a higher concentration of phytochemicals compared to acetone extracts, suggesting that methanol is more effective in extracting biologically active compounds. Reducing sugars were absent in acetone extracts of *Curcuma amada*, while terpenoids were detected in relatively low concentrations across all samples. This variation indicates that the type of solvent significantly influences the extraction efficiency of specific phytochemicals. These phytochemical constituents are widely reported to possess antimicrobial properties, including the ability to disrupt bacterial cell membranes and inhibit metabolic processes, thereby contributing to the observed antibacterial activity.

3.3 Thin Layer Chromatography (TLC) Analysis

Thin Layer Chromatography (TLC) analysis confirmed the presence of multiple phytochemical compounds in both methanol and acetone extracts. The Rf values obtained from TLC indicated the separation and identification of compounds such as flavonoids, alkaloids, tannins, terpenoids, and saponins (Fig. 3). For *Curcuma amada*, Rf values of 0.85 (methanol) and 0.80 (acetone) corresponded to flavonoids, while alkaloids were detected at lower Rf values (0.41 and 0.36). Similarly, *Piper betle* extracts showed prominent flavonoid and alkaloid peaks, while *Piper nigrum* extracts mainly exhibited alkaloids and saponins. The TLC results demonstrated that methanol extracts consistently showed a higher diversity of phytochemicals compared to acetone extracts. This indicates that methanol is a more effective solvent for extracting a wider range of bioactive compounds from plant materials.

Figure 3. Shows the TLC of Plant extracts (methanol and acetone) under UV light

3.4 Antibacterial Activity of Plant Extracts

The antibacterial activity of the plant extracts was evaluated against Escherichia coli, Staphylococcus spp., and Bacillus spp. using the agar well diffusion method. All extracts exhibited measurable antibacterial activity, with variations observed depending on the plant species, solvent type, and concentration (Fig. 4 A&B).

Methanol extracts of all three plants demonstrated significant antibacterial activity: Piper nigrum exhibited the highest inhibition against Bacillus spp.; Curcuma amada and Piper betle showed strong activity against Staphylococcus spp.; Moderate activity was observed against E. coli across all extracts. The inhibition zones increased with increasing concentration (10–50 µL), indicating a dose-dependent antibacterial effect (Fig. 5). Acetone extracts also showed antibacterial activity, though variations were more pronounced: Curcuma amada demonstrated the strongest inhibition against E. coli, with the highest zone of inhibition among all samples; Piper betle showed moderate antibacterial activity across all pathogens; Piper nigrum exhibited the lowest antibacterial activity compared to other plants (Fig. 6). These results highlight the influence of solvent type on antibacterial efficacy and suggest that certain compounds responsible for antibacterial activity are more effectively extracted by acetone in specific plant species.

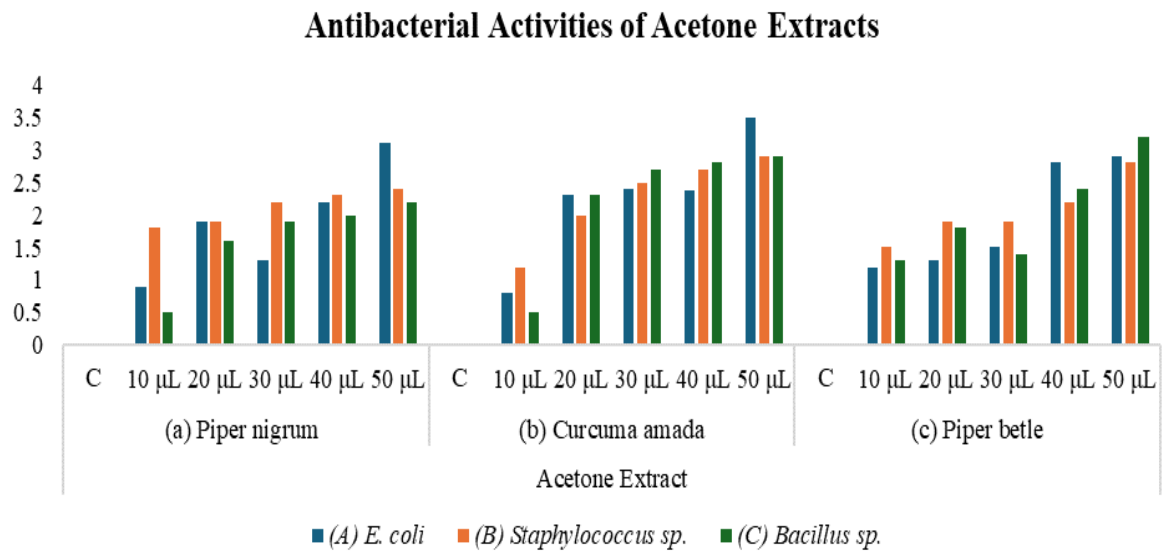
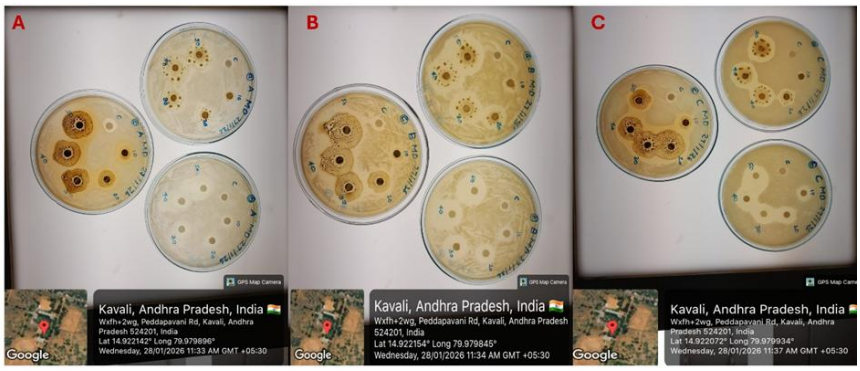


Figure 4. Antibacterial activities of methanol extracts (A) and acetone extracts (B) of the selected plants.

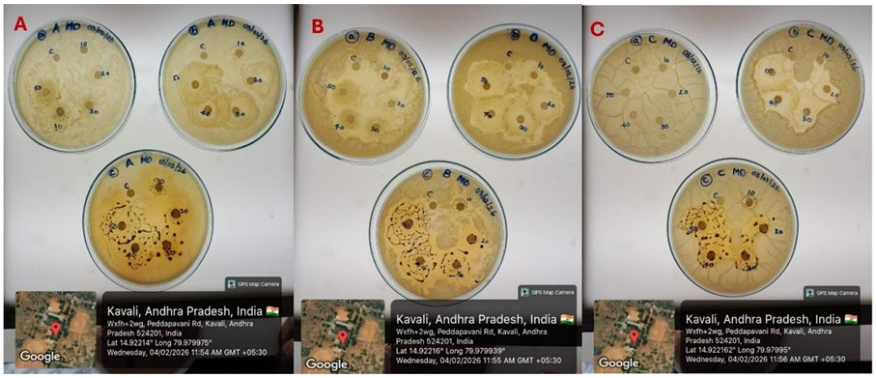
A comparative analysis of the results revealed that both methanol and acetone extracts were effective against Gram-positive (*Staphylococcus* spp., *Bacillus* spp.) and Gram-negative (*E. coli*) bacteria. However, the degree of inhibition varied: Methanol extracts generally showed stronger activity against Gram-positive bacteria; Acetone extracts showed enhanced activity against *E. coli; Piper nigrum* consistently exhibited lower antibacterial activity than *Curcuma amada* and *Piper betle.* The differences in antibacterial activity can be attributed to variations in phytochemical composition and concentration among the plant extracts. Bioactive compounds such as tannins, flavonoids, and alkaloids are known to contribute to antimicrobial activity through mechanisms such as enzyme inhibition and membrane disruption.



A - *E. coli*
B - *Staphylococcus* sp.
C - *Bacillus* sp.

(a) – Curcuma amada
(b) – Piper betle
(c) – Piper nigrum

Figure 5. Methanol extracts for antimicrobial activities by Disc method



A - *E. coli*
B - *Staphylococcus* sp.
C - *Bacillus* sp.

(a) – Curcuma amada
(b) – Piper betle
(c) – Piper nigrum

Figure 6. Acetone extracts for antimicrobial activities by Disc method

4. DISCUSSION

The present study demonstrates that the selected medicinal plants *Curcuma amada*, *Piper betle*, and *Piper nigrum* possess significant antibacterial activity, which can be attributed to their phytochemical composition. The findings provide scientific validation for the traditional use of these plants as natural antimicrobial agents and cleansing materials in rural and ethnomedicinal practices (Kumbi, 2010; Suleman & Alemu, 2012).

The variation observed in extraction yields between methanol and acetone solvents highlights the importance of solvent polarity in extracting bioactive compounds. Methanol extracts generally produced higher yields and exhibited richer phytochemical profiles compared to acetone extracts. This observation is consistent with previous studies, which reported that organic solvents of intermediate polarity are more efficient in extracting antimicrobial constituents (Tiwari et al., 2011; Eloff, 1999).

Phytochemical screening revealed the presence of key bioactive compounds such as alkaloids, flavonoids, tannins, and saponins in all plant extracts. These findings are in agreement with earlier reports that identified similar phytochemical constituents in medicinal plants with antimicrobial properties (Doughari et al., 2008; Kamaraj et al., 2012). The variation in phytochemical composition across plant species and solvents may be influenced by environmental factors, geographical conditions, and plant metabolism (Ubani et al., 2012). Furthermore, the TLC analysis confirmed the presence of multiple phytochemicals in both methanol and acetone extracts, supporting the qualitative screening results. The identification of compounds based on Rf values provides additional evidence for the presence of bioactive metabolites responsible for antibacterial activity.

The antibacterial assay results indicated that all plant extracts exhibited activity against both Gram-

positive and Gram-negative bacteria, including *Staphylococcus* spp., *Bacillus* spp., and *Escherichia coli*. This broad-spectrum activity is significant, as Gram-negative bacteria are generally more resistant due to their complex cell wall structure. Methanol extracts exhibited comparatively stronger antibacterial activity against Gram-positive bacteria, particularly *Bacillus* spp., while acetone extracts of *Curcuma amada* demonstrated the highest activity against *E. coli*. These findings suggest that different solvents may extract specific bioactive compounds with selective antibacterial properties. The observed antibacterial activity aligns with previous studies, which reported that acetone and methanol extracts of medicinal plants exhibit higher antimicrobial efficacy compared to aqueous extracts (Das et al., 2010; Bhattacharjee et al., 2013). Similarly, Doughari et al. (2008) reported that acetone extracts of *Senna obtusifolia* showed superior antibacterial activity against both Gram-positive and Gram-negative bacteria. Among the tested plants, *Curcuma amada* and *Piper betle* exhibited higher antibacterial activity compared to *Piper nigrum*. This difference may be due to the higher concentration of flavonoids, tannins, and saponins in these plants, which are known to possess strong antimicrobial properties. Previous research has shown that plant extracts rich in these compounds exhibit enhanced antibacterial effects (Cowan, 1999).

The antimicrobial activity observed in this study can be explained by the presence of phytochemicals acting through multiple mechanisms. Tannins are known to form complexes with bacterial cell wall proteins, leading to membrane disruption and inhibition of microbial growth. Flavonoids can interfere with bacterial enzyme systems and disrupt membrane integrity, while saponins exhibit detergent-like properties, leading to increased cell permeability and eventual cell lysis (Cowan, 1999; Harborne, 1998). Alkaloids have also been reported to interfere with DNA replication and protein synthesis in bacterial cells, contributing to antimicrobial activity. The combined presence of these phytochemicals may result in a synergistic effect, enhancing the overall antibacterial potency of the plant extracts.

The results of the present study are consistent with several earlier investigations that reported the antimicrobial potential of plant extracts used in traditional medicine. For instance, studies on herbal hand sanitizers have demonstrated significant antibacterial activity against pathogenic bacteria, supporting the use of plant-based formulations as alternatives to synthetic sanitizers (Vyas et al., 2011). Similarly, research on *Azadirachta indica* and *Sida cordifolia* extracts in soap formulations showed effective antibacterial activity against both Gram-positive and Gram-negative bacteria (Joy et al., 2012). These findings reinforce the potential application of plant extracts in hygiene products for controlling microbial infections. The variability in antibacterial activity observed in different studies may be due to differences in extraction methods, plant sources, environmental conditions, and experimental procedures. As noted by Das et al. (2010), the lack of standardized methodologies for evaluating antimicrobial activity makes direct comparison between studies challenging.

5. CONCLUSION

This study demonstrates that *Curcuma amada*, *Piper betle*, and *Piper nigrum* possess significant antibacterial activity against *Escherichia coli*, *Staphylococcus* spp., and *Bacillus* spp. The presence of key phytochemicals such as alkaloids, flavonoids, tannins, and saponins is likely responsible for the observed antimicrobial effects. Methanol extracts generally exhibited stronger antibacterial activity, while acetone extracts particularly of *Curcuma amada* showed enhanced activity against *E. coli*. Among the tested plants, *Curcuma amada* and *Piper betle* were more effective compared to *Piper nigrum*. Overall, the results support the potential of these medicinal plants as natural antibacterial agents and validate their traditional use. Further studies focusing on isolation of active compounds and in vivo evaluation are recommended to confirm their therapeutic applications.

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