

Comparative Antibacterial Efficacy of *Piper Nigrum* L. and *Curcuma longa* L. against *Escherichia Coli* and *Staphylococcus aureus*

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Abstract – The present comparative analysis evaluates the antibacterial potential of two medicinal plant species, *Piper nigrum* L. (black pepper) and *Curcuma longa* L. (turmeric), against clinically significant bacterial pathogens *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). Both studies were designed to explore plant-based alternatives to conventional antibiotics in response to the increasing global threat of antimicrobial resistance. In both dissertations, plant extracts were prepared using aqueous and ethanolic solvents and assessed using standard microbiological techniques such as the agar disc diffusion method for determining zones of inhibition and the broth dilution method for Minimum Inhibitory Concentration (MIC). The results from both studies demonstrated that plant extracts possess measurable antibacterial activity against both tested organisms, although the degree of effectiveness varied depending on the plant and solvent used. In the *Piper nigrum* L. study, both extracts showed antibacterial activity, with aqueous extracts producing larger zones of inhibition, while ethanolic extracts showed stronger MIC performance, indicating higher potency at lower concentrations. Similarly, the *Curcuma longa* L. study also revealed significant antibacterial effects, with aqueous extracts showing slightly higher inhibition zones, whereas ethanolic extracts demonstrated better performance in MIC analysis. Overall, both medicinal plants exhibited broad-spectrum antibacterial activity against Gram-positive and Gram-negative bacteria. However, differences in solvent efficiency and phytochemical composition influenced their antibacterial strength. The findings collectively suggest that both *Piper nigrum* L. and *Curcuma longa* L. contain bioactive compounds with promising antimicrobial properties, supporting their potential use as natural alternatives or complementary agents to conventional antibiotics in the treatment of bacterial infections.

Key Words: *Piper nigrum* L., *Curcuma longa* L., antibacterial activity, *Escherichia coli*, *Staphylococcus aureus*, MIC, phytochemical

1. INTRODUCTION

Infectious diseases caused by pathogenic bacteria remain a major global health concern and are responsible for significant morbidity and mortality worldwide. Among the various bacterial pathogens, *Escherichia coli* and *Staphylococcus aureus* are commonly associated with several human infections ranging from minor skin infections to severe systemic diseases. *E. coli*, a Gram-negative bacterium, is known to cause urinary tract infections, diarrhea, gastroenteritis, and septicemia, whereas *S. aureus*, a Gram-positive bacterium, is responsible for wound infections, food poisoning, pneumonia, and bloodstream infections. The increasing emergence of antimicrobial resistance among these pathogens has reduced the effectiveness of conventional antibiotics and created an urgent need for alternative antimicrobial agents. Medicinal plants have been used since ancient times in traditional systems of medicine such as Ayurveda, Siddha, and Unani for the treatment of infectious diseases. These plants contain various bioactive phytochemicals including alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, and essential oils that exhibit antimicrobial, antioxidant, and anti-inflammatory properties. Among medicinal plants, *Curcuma longa* L. (turmeric) and *Piper nigrum* L. (black pepper) are widely recognized for their therapeutic and antimicrobial properties. Turmeric belongs to the family Zingiberaceae and contains curcumin as its major bioactive compound, which possesses strong antibacterial, antioxidant, and anti-inflammatory activities. Traditionally, turmeric has been used for wound healing, skin diseases, digestive disorders, and infections. Similarly, black pepper, belonging to the family Piperaceae, contains piperine and essential oils

that exhibit antimicrobial and medicinal properties. Black pepper has been traditionally used for respiratory infections, digestive ailments, and inflammatory conditions. Recent scientific studies have demonstrated that extracts of both turmeric and black pepper exhibit antibacterial activity against several pathogenic microorganisms. The antibacterial action of these medicinal plants is mainly attributed to their ability to disrupt bacterial cell membranes, inhibit enzyme activity, interfere with protein synthesis, and alter bacterial metabolic pathways. The efficiency of extraction solvents also plays an important role in determining antibacterial activity, as different solvents extract different concentrations of phytochemicals. Therefore, the present study was undertaken to comparatively evaluate the antibacterial activities of aqueous and ethanolic extracts of *Curcuma longa* and *Piper nigrum* against *Escherichia coli* and *Staphylococcus aureus* and to identify the extract showing better antibacterial potential.

2. OBJECTIVE OF THE STUDY

To evaluate and compare the antibacterial activities of *Curcuma longa* and *Piper nigrum* extracts against *Escherichia coli* and *Staphylococcus aureus*.

1. To prepare aqueous and ethanolic extracts of turmeric and black pepper.
2. To evaluate antibacterial activity using disc diffusion method.
3. To determine Minimum Inhibitory Concentration (MIC).
4. To compare antibacterial efficacy of aqueous and ethanolic extracts.
5. To identify the extract showing better antibacterial activity.

3. REVIEW OF LITERATURE

Medicinal plants have been used since ancient times for the treatment of infectious diseases because they contain several bioactive compounds with therapeutic properties. Due to the rapid increase in antibiotic resistance, researchers are now focusing on plant-based antibacterial agents as safer and effective alternatives to synthetic antibiotics. Plants contain phytochemicals such as alkaloids, flavonoids, tannins, phenolics, terpenoids, and essential oils that inhibit bacterial growth by damaging the cell membrane and interfering with metabolic activities.

Black Pepper is one of the most important medicinal spices studied for antibacterial activity. It contains bioactive compounds such as piperine, chavicine, piperidine, flavonoids, and essential oils including β -caryophyllene and limonene. These compounds show strong antimicrobial and antioxidant properties. Several studies reported that black pepper extracts are effective against pathogenic bacteria like *Escherichia coli* and *Staphylococcus aureus*. The antibacterial activity occurs through disruption of bacterial cell membranes and inhibition of enzyme activity.

Similarly, Turmeric is widely known for its medicinal and antibacterial properties. Turmeric contains curcumin, turmerone, zingiberene, and other curcuminoids that possess antimicrobial, antioxidant, and anti-inflammatory activities. Curcumin is considered the major active compound responsible for antibacterial action. Studies showed that turmeric extracts inhibit the growth of both Gram-positive and Gram-negative bacteria by altering membrane permeability and inactivating cellular enzymes.

Many researchers have previously worked on the antibacterial activities of black pepper and turmeric. Cowan (1999), Srinivasan (2007), Meghwal & Goswami (2013), Gorgani et al. (2017), and Karsha & Lakshmi (2010) studied the antimicrobial properties of black pepper and reported its effectiveness against several human pathogens.

Similarly, Chandarana et al. (2005), Odhav et al. (2002), Moreno et al. (2006), Chattopadhyay et al. (2004), and Moghadamtousi et al. (2014) investigated the antibacterial activity of turmeric and confirmed the antimicrobial potential of curcumin and turmeric extracts against pathogenic bacteria.

4. MATERIALS AND METHODS

4.1 Collection of Plant Materials

Dried seeds of *Piper nigrum* (Black pepper) and *Curcuma longa* (Turmeric) were procured from local spice markets of Brahmapur, Odisha. The plant materials were cleaned, shade dried, and ground into fine powder using a mechanical grinder.

4.2 Preparation of Plant Extracts

Aqueous and ethanolic extracts of black pepper and turmeric were prepared using solvent extraction method. About 10 g of powdered plant material was mixed with 100 mL of distilled water and ethanol separately in a ratio of 1:10 (w/v). The mixtures were kept under continuous shaking for 72 hours at room temperature.

The extracts were filtered using Whatman No.1 filter paper and concentrated using rotary evaporation. The concentrated extracts were stored at 4°C until further experimental analysis.

4.3 Test microorganisms

The bacterial strains used in the present study were *Escherichia coli* (MTCC-443) and *Staphylococcus aureus* (MTCC-1430). Pure cultures were obtained from the Regional Institute of Biotechnology (RIB), Brahmapur, and sub-cultured onto solidified nutrient agar medium using a sterile inoculation loop and incubated at optimum temperature for growth. The cultures were sub-cultured every 2–3 days to maintain sterility and active growth.

4.4 Preparation of Culture Media

Nutrient agar and nutrient broth media were prepared and sterilized by autoclaving at 121°C for 15 minutes. The sterilized media were cooled and poured aseptically into sterile Petri plates for antibacterial assay.

4.5 Preparation of Bacterial Inoculum

Fresh bacterial inoculum was prepared by inoculating bacterial colonies into sterile nutrient broth followed by incubation at 37°C for 18–24 hours. The turbidity of bacterial suspension was adjusted to match McFarland standard for uniform inoculation.

4.6 Preparation of Antibiotic Discs

Sterile paper discs were prepared from thick chromatography paper and sterilized using 70% ethanol followed by ultraviolet (UV) irradiation inside a laminar airflow chamber. Uniform circular discs were punched out aseptically using a sterile paper puncher. Crude extracts of black pepper and turmeric were prepared at a stock concentration of 100 mg/ mL. Working concentrations of 10 mg/ mL and 5 mg/ mL were prepared separately for both aqueous and ethanolic extracts using sterile distilled water and ethanol respectively. The sterile paper discs were immersed in the prepared extract solutions for approximately 15 minutes to ensure proper impregnation. After soaking, the discs were removed aseptically using sterile forceps and dried inside the laminar airflow chamber before use in antibacterial assay.

4.7 Antibacterial activity by Disc Diffusion Method

The antibacterial activity of aqueous and ethanolic extracts of *Piper nigrum* (Black pepper) and *Curcuma longa* (Turmeric) was evaluated using disc diffusion assay by pour plate technique. Sterile molten nutrient agar medium was inoculated with bacterial cultures and poured aseptically into sterile Petri plates. After

solidification of the medium, sterile filter paper discs impregnated with plant extracts were carefully placed on the agar surface using sterile forceps. Standard antibiotic discs were used as positive control, while solvent-treated discs served as negative control. The plates were incubated at 37°C for 24 hours, and antibacterial activity was determined by measuring the diameter of zones of inhibition.

4.8 Determination of Minimum Inhibitory Concentration (MIC)

The MIC of plant extracts was determined using standard serial broth dilution method. The first test tube contained 10 mL sterile nutrient broth, while subsequent tubes contained 9 mL broth. Stock extract (100 mg/ mL) was diluted to prepare 10 mg/ mL working solution. Serial two-fold dilutions were carried out to obtain concentrations of 10 mg/ mL, 5 mg/ mL, 2.5 mg/ mL, 1.25 mg/ mL, 0.625 mg/ mL, and 0.312 mg/ mL. Each tube was inoculated with standardized bacterial suspension and incubated at 37°C for 24 hours. Following incubation, bacterial growth was assessed by measuring optical density (OD) at 600nm using a spectrophotometer. The MIC was considered as the lowest concentration showing no visible growth or significant reduction in turbidity compared to the control.

4.9 Gas Liquid Chromatography (GLC) Analysis

Analysis Gas Liquid Chromatography (GLC) analysis was performed to identify the major phytochemical constituents present in black pepper and turmeric extracts. The chromatographic peaks obtained were analyzed to identify major bioactive compounds responsible for antibacterial activity, including piperine in black pepper and curcuminoids in turmeric.

5. RESULT AND DISCUSSION

5.1 Zone of Inhibition against *Escherichia coli*

In the disc diffusion assay, both medicinal plants exhibited antibacterial activity against the tested bacterial strains; however, *Piper nigrum* demonstrated comparatively higher antibacterial efficacy than *Curcuma longa*. Against *E. coli*, the aqueous extract of *Piper nigrum* showed a maximum zone of inhibition of 0.9cm, whereas *Curcuma longa* showed only 0.5cm at the same concentration.

Table 1: Zone of Inhibition of aqueous extracts against *Escherichia coli*

Concentration	Black pepper extract (Aqueous)	Turmeric extract (Aqueous)
5mg	0.8mm	1.1mm
10mg	0.9mm	1.3mm
Vancomycin	1.3mm	1.3mm

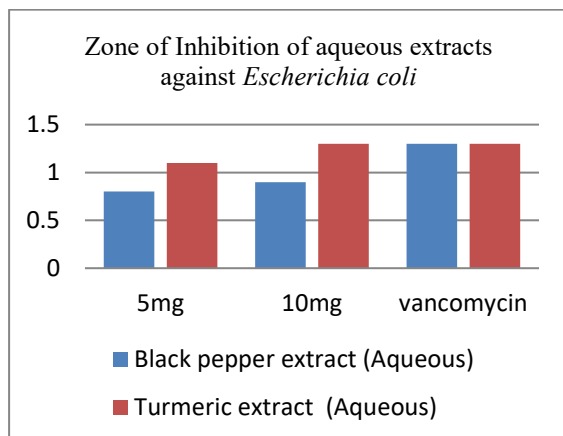


Fig.- 1



Fig.- 2

Fig.1: Zone of Inhibition of aqueous extract of Black pepper against *Escherichia coli*

Fig.2: Zone of Inhibition of aqueous extract of Turmeric against *Escherichia coli*

Fig.3: Zone of Inhibition of ethanolic extract of Black pepper against *Escherichia coli*

Fig.- 4

Fig.4: Zone of Inhibition of ethanolic extract of Turmeric against *Escherichia coli*

5.2 Zone of Inhibition against *Staphylococcus aureus*

Similarly, against *S. aureus*, *Piper nigrum* exhibited a maximum inhibition zone of 1.0 cm compared to 0.6 cm observed in *Curcuma longa*. These findings indicate that black pepper possessed stronger antibacterial potential against both Gram-positive and Gram-negative bacteria.

Table 2: Zone of Inhibition of ethanolic extracts against *Escherichia coli*

Concentration	Black pepper extract (Ethanol)	Turmeric extract (Ethanol)
5mg	0.5mm	0.3mm

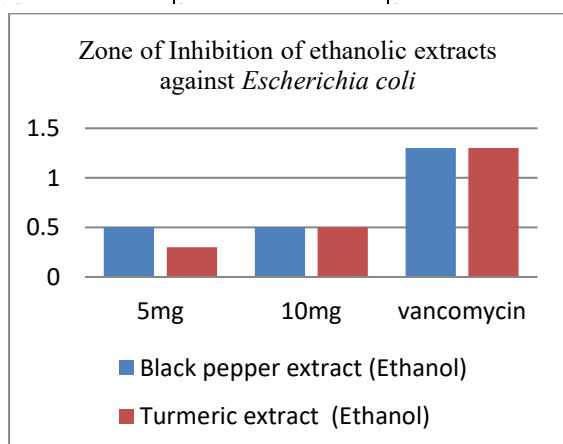


Table 3: Zone of Inhibition of aqueous extracts against *Staphylococcus aureus*

Concentration	Black pepper extract (Aqueous)	Turmeric extract (Aqueous)
5mg	0.8mm	0.3mm
10mg	1mm	0.6mm
Vancomycin	1.4mm	1.4mm

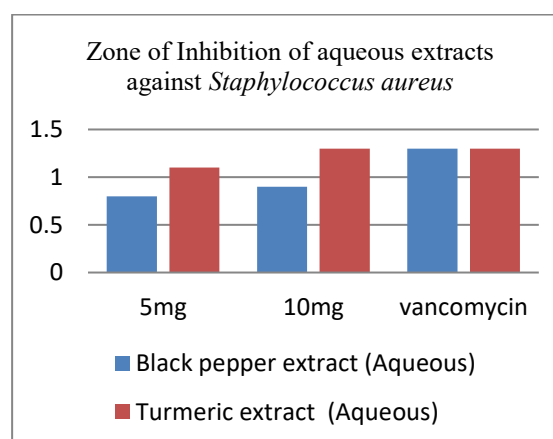


Table 4: Zone of Inhibition of ethanolic extracts against *Staphylococcus aureus*

Concentration	Black pepper extract (Ethanol)	Turmeric extract (Ethanol)
5mg	0.5	0.4
10mg	0.5	0.5
Vancomycin	1.4	1.4

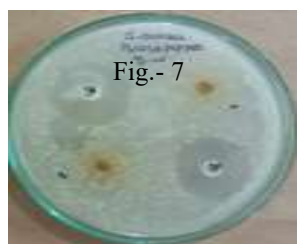
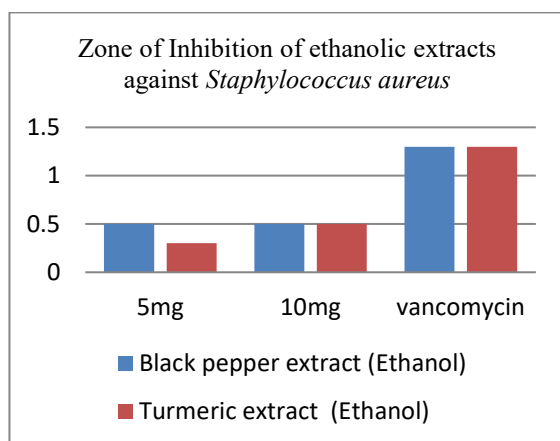


Fig 5: Zone of Inhibition of aqueous extract of Black pepper against *Staphylococcus aureus*

Fig 6: Zone of Inhibition of aqueous extract of Turmeric against *Staphylococcus aureus*

Fig 7: Zone of inhibition of ethanolic extract of Black pepper against *Staphylococcus aureus*

Fig 8: Zone of Inhibition of ethanolic extract of Turmeric against *Staphylococcus aureus*

Comparative evaluation demonstrated that *Piper nigrum* L exhibited stronger antibacterial activity than *Curcuma longa* L. against both *Escherichia coli* and *Staphylococcus aureus*. Ethanolic extracts of both medicinal plants showed comparatively greater antibacterial efficacy than aqueous extracts, indicating better extraction of bioactive phytochemical constituents in ethanol. The antibacterial activity of all extracts increased with increasing concentration from 5 mg/mL to 10 mg/mL. Among the tested bacterial strains, *Escherichia coli* showed comparatively higher susceptibility to the plant extracts than *Staphylococcus aureus*.

5.3 Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) analysis demonstrated that ethanolic extracts of both black pepper and turmeric exhibited greater bacterial growth inhibition compared to aqueous extracts. Optical density (OD) values measured at 600 nm showed reduced bacterial growth with increasing extract concentration. *Moringa* extracts exhibited lower optical density values than bitter melon extracts against both *Escherichia coli* and *Staphylococcus aureus*, indicating comparatively stronger antibacterial efficacy. The inhibitory effect was concentration dependent, with higher extract concentrations showing greater bacterial growth suppression.

Table 5: MIC of Black pepper and Turmeric in aqueous extracts on *Escherichia coli*

Concentration	Black pepper extract (Aqueous)	Turmeric extract (Aqueous)
10mg	0.34	0.62
5mg	0.36	0.66
2.5mg	0.42	0.76
1.25mg	0.47	0.77
0.62mg	0.56	0.78
0.31mg	0.77	0.94

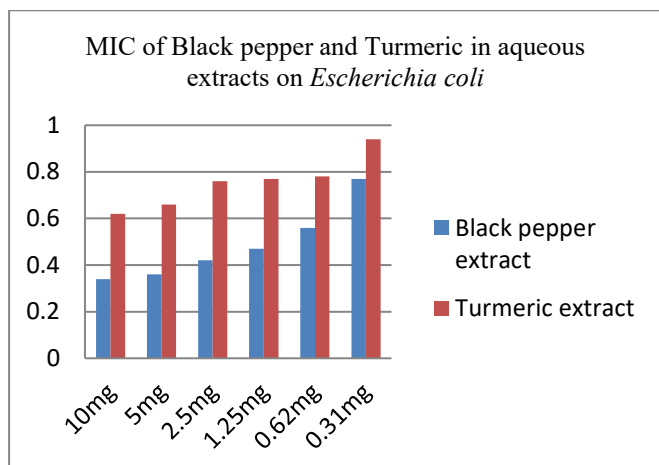


Table 6: MIC of Black pepper and Turmeric in ethanolic extracts on *Escherichia coli*

Concentration	Black pepper extract (Ethanol)	Turmeric extract (Ethanol)
10mg	0.34	0.53
5mg	0.36	0.62
2.5mg	0.42	0.66
1.25mg	0.43	0.78
0.62mg	0.48	0.81
0.31mg	0.77	0.94

0.31mg	0.90	0.98
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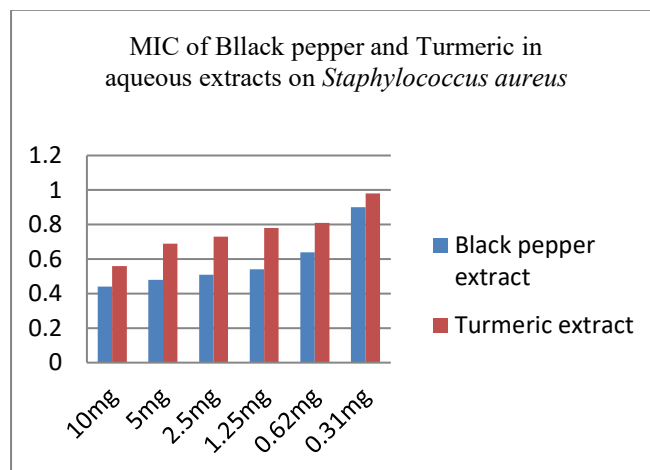


Fig. 9: GLC Analysis of Black pepper extract

Table 8: MIC of Black pepper and Turmeric in ethanolic extracts on *Staphylococcus aureus*

Concentration	Black pepper extract (Ethanol)	Turmeric extract (Ethanol)
10mg	0.37	0.42
5mg	0.44	0.51
2.5mg	0.69	0.65
1.25mg	0.73	0.82
0.62mg	0.88	0.86
0.31mg	0.94	0.88

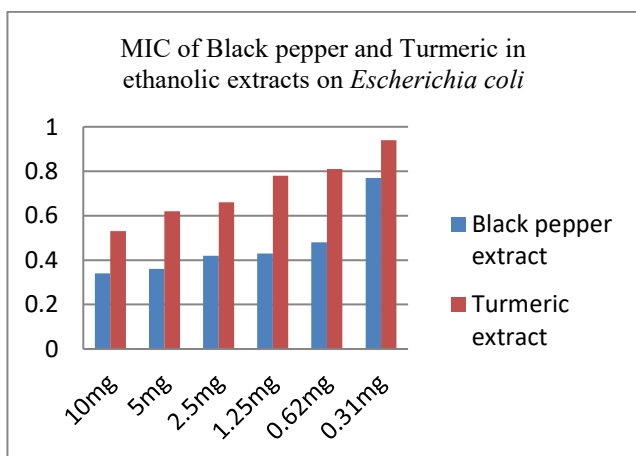
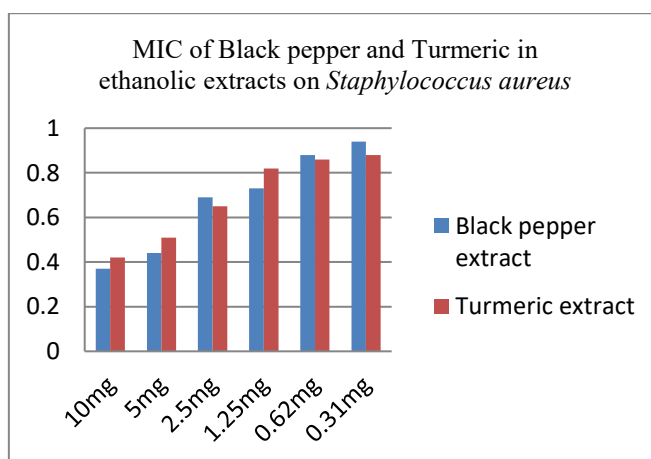


Table 7: MIC of Black pepper and Turmeric in aqueous extracts on *Staphylococcus aureus*

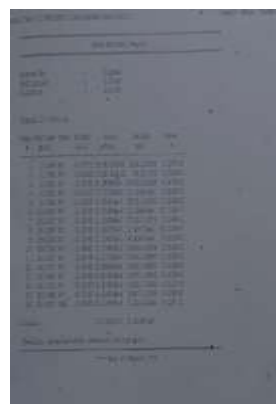
Concentration	Black pepper extract (Aqueous)	Turmeric extract (Aqueous)
10mg	0.44	0.56
5mg	0.48	0.69
2.5mg	0.51	0.73
1.25mg	0.54	0.78
0.62mg	0.64	0.81



5.4 Gas Liquid Chromatography (GLC) Analysis

The Gas Liquid Chromatography (GLC) analysis of *Piper nigrum* and *Curcuma longa* revealed the presence of important bioactive compounds responsible for their antibacterial activity. *Piper nigrum* showed a major peak

corresponding to Quercetin with an area percentage of about 36.03%, whereas *Curcuma longa* exhibited a dominant peak of ar-turmerone with a higher area percentage of nearly 65.30%. Comparative analysis indicated that turmeric contained a higher concentration of volatile bioactive compounds, while black pepper possessed flavonoid-rich constituents with strong antimicrobial properties. The GLC findings suggest that both medicinal plants contain phytochemicals capable of inhibiting bacterial growth and contributing to their broad-spectrum antibacterial activity.



6. CONCLUSIONS

The present study successfully demonstrated the antibacterial activity of aqueous and ethanolic extracts of *Piper nigrum* and *Curcuma longa* against *Escherichia coli* and *Staphylococcus aureus*. Both medicinal plants exhibited inhibitory effects against Gram-positive and Gram-negative bacteria, although differences were observed depending on the type of solvent and bacterial strain. The aqueous extracts produced larger zones of inhibition, whereas ethanolic extracts exhibited greater antibacterial potency in MIC analysis. *Piper nigrum* showed relatively stronger antibacterial activity than *Curcuma longa*, which may be associated with the

presence of piperine and other potent phytochemicals. Similarly, curcumin and related compounds present in turmeric contributed significantly to its antibacterial activity. The study findings indicate that both *Piper nigrum* and *Curcuma longa* possess promising broad-spectrum antibacterial properties and may serve as potential natural alternatives or complementary therapies to conventional antibiotics. Further investigations involving purification of active compounds, molecular characterization, toxicity studies, and in vivo evaluation are recommended to validate their pharmaceutical and clinical applications in combating antimicrobial resistance.

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