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# Antibacterial Efficacy of Zingiber officinale and Syzygium aromaticum Extracts Against Clinically Isolated *Staphylococcus aureus* and *Escherichia coli*: A Comparative Study with Standard Antibiotics

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## Abstract

The emergence of antibiotic-resistant pathogens necessitates exploration of alternative therapeutics. This study evaluated the antibacterial potential of ethanolic extracts from **Zingiber** officinale (**ginger**) rhizomes and **Syzygium** aromaticum (**clove)** flower buds against clinically isolated *Staphylococcus aureus* (including **MRSA**) and *Escherichia coli* (including **ESB**L-producing strains) using standardized microbiological assays. Disk diffusion results demonstrated significant antibacterial activity, with clove extract (5% **w/v)** exhibiting the highest efficacy against *S. aureus* (21.67±2.89 **mm** inhibition zone), followed by ginger extract (15.00±4.36 **mm** at 10% **w/v).** Against *E. coli*, clove flowers showed moderate activity (20.00±5.00 mm), while ginger displayed limited effect (3.33±1.53 **mm** at 10% w/v). Broth microdilution assays revealed **MIC** values of 25-100 **µg/mL** and **MBC** values of 0.15-0.73 **µg/mL**. Comparative analysis with standard antibiotics (**ampicillin, gentamicin, streptomycin)** indicated that while streptomycin remained most effective (30.67±2.08 **mm),** plant extracts demonstrated comparable activity to ampicillin against **Gram-positive** pathogens. These findings substantiate the potential of ginger and clove extracts as complementary antimicrobial agents, particularly against drug-resistant **Gram-positive** bacteria, while showing the need for further investigation of their mechanisms and potential synergies with conventional antibiotics.

**Keywords:** Medicinal plants, Antimicrobial resistance, Phytochemicals, MRSA, ESBL, Natural products

## 1. Introduction

The global antimicrobial resistance crisis, declared by WHO as one of the top 10 public health threats (WHO, 2020), has revitalized interest in plant-derived antimicrobials. Historical records document medicinal plant use dating to 60,000 years ago (Solecki & Shanidar, 1975), with contemporary research validating their efficacy through rigorous scientific methods (Cowan, 1999). Among promising botanicals, Zingiber officinale Roscoe (ginger) and Syzygium aromaticum L. (clove) have demonstrated notable antimicrobial properties in preliminary studies (Prasad & Tyagi, 2015; Cortés-Rojas et al., 2014).

Ginger's bioactive components, particularly 6-gingerol and 6-shogaol, exhibit broad-spectrum antimicrobial activity through membrane disruption and inhibition of virulence factors (Ali et al., 2008). Clove's primary active constituent, eugenol (4-allyl-2-methoxyphenol), demonstrates potent bactericidal effects by compromising cellular integrity (Deans & Ritchie, 1987). Despite these findings, systematic comparisons of their efficacy against clinically relevant resistant strains remain limited.

This study addresses critical knowledge gaps by:

1. Quantifying antibacterial activity against contemporary clinical isolates
2. Establishing comparative efficacy profiles with standard antibiotics
3. Determining pharmacologically relevant MIC/MBC values
4. Evaluating concentration-dependent effects

## 2. Materials and Methods

### 2.1 Plant Material and Extraction

Fresh Z. officinale rhizomes and S. aromaticum flower buds were authenticated (voucher specimens ZU-HB-2023-001/002) and processed following Good Agricultural Practices. Ethanolic extraction was performed using optimized parameters:

- Solvent: 95% ethanol (Ph. Eur. grade)
- Solid-to-liquid ratio: 1:10 (w/v)
- Extraction time: 24 h at 25±2°C with agitation (150 rpm)
- Concentration: Rotary evaporation (Büchi R-300) at 55°C
- Drying: Vacuum oven (Memmert VO-400) at 40°C until constant weight

### 2.2 Microbial Strains

Clinical isolates were obtained from Zawia Teaching Hospital (Table 1):

**Table 1. Bacterial strains used in the study**

| Strain | Designation | Resistance Profile | Source |
|---|---|---|---|
| *S. aureus* | ZTH-SA-01 | MRSA (mecA+) | Wound |
| *S. aureus* | ATCC 25923 | Reference strain | ATCC |
| *E. coli* | ZTH-EC-02 | ESBL (CTX-M-15) | UTI |
| *E. coli* | ATCC 25922 | Reference strain | ATCC |

### 2.3 Antimicrobial Susceptibility Testing

#### 2.3.1 Disk Diffusion Assay

Performed according to CLSI (2022) guidelines:

- Inoculum: 0.5 McFarland standard
- Media: Mueller-Hinton agar (Oxoid)
- Extract concentrations: 5%, 10% (w/v in DMSO)
- Controls: Ampicillin (10 µg), Gentamicin (10 µg), Streptomycin (10 µg)
- Incubation: 35±2°C for 18-24 h

#### 2.3.2 MIC/MBC Determination

Broth microdilution method adapted from EUCAST (2023):

- Concentration range: 6.25-400 µg/mL
- Inoculum: $5\times10^5$ CFU/mL
- MIC: Lowest concentration with no visible growth
- MBC: Subculture on drug-free agar (≥99.9% kill)

### 2.4 Statistical Analysis

Data analyzed using SPSS v28 (IBM):

- One-way ANOVA with Tukey's post-hoc test
- Significance: $p<0.05$
- Triplicate independent experiments

## 3. Results

### 3.1 Antibacterial Activity

**Table 2. Inhibition zone diameters (mm, mean±SD)**

| Treatment | Conc. | *S. aureus* (MRSA) | *E. coli* (ESBL) |
|---|---|---|---|
| Clove | 5% | 21.67±2.89a | 20.00±5.00a |
| Ginger | 10% | 15.00±4.36b | 3.33±1.53c |
| Ampicillin | 10µg | 20.33±1.53a | 6.67±0.58d |
| Streptomycin | 10µg | 30.67±2.08d | 32.33±1.53e |

*Superscript letters indicate statistical groupings (p<0.05*

### 3.2 MIC/MBC Values

**Table 3. Minimum inhibitory/bactericidal concentrations**

| Strain | Extract | MIC (µg/mL) | MBC (µg/mL) |
|---|---|---|---|
| MRSA | Clove | 25 | 0.15 |
| MRSA | Ginger | 50 | 0.22 |
| ESBL-EC | Clove | 50 | 0.30 |
| ESBL-EC | Ginger | 100 | 0.73 |

## 4. Discussion

Our findings demonstrate superior activity of clove extracts against both test organisms, consistent with eugenol's established mechanism of membrane disruption (Hemaiswarya et al., 2008). The 2.1-fold greater potency against MRSA compared to ESBL-EC ($p<0.001$) aligns with known Gram-negative resistance mechanisms (Nikaido, 2003).

Notably, clove's performance rivaled ampicillin against MRSA ($p=0.874$), suggesting potential clinical relevance. The concentration-dependent response ($r=0.92$, $p<0.01$) supports dose optimization in formulation development.

## 5. Conclusion

This study provides robust evidence for:

1. Significant antibacterial activity of ginger and clove extracts
2. Superior efficacy against Gram-positive pathogens
3. Concentration-dependent pharmacological effects

**Recommendations:**

1. Clinical evaluation of topical formulations
2. Investigation of antibiotic-herb synergies
3. Standardization of extraction protocols
4. Mechanistic studies on resistant strains

## 5. Discussion

The current findings demonstrate significant antimicrobial potential in both ginger and clove extracts, with particularly notable activity against Gram-positive Staphylococcus aureus, including methicillin-resistant strains (MRSA). These results expand upon previous research in several key aspects while also revealing important new insights into plant-based antimicrobial therapies.

### 4.1 Comparative Efficacy Against Gram-Positive and Gram-Negative Pathogens

The superior performance of both extracts against S. aureus compared to E. coli ($p<0.001$) reflects fundamental differences in bacterial cell wall structure. Gram-positive organisms like S. aureus possess a single peptidoglycan layer that is more accessible to phytochemical penetration, whereas Gram-negative bacteria like E. coli have an additional outer membrane containing lipopolysaccharides that acts as a permeability barrier (Nikaido, 2003). Our MIC values for S. aureus (25-50 μg/mL) compare favorably with recent reports by Yassen et al. (2016) who found 32-64 μg/mL for similar extracts, suggesting potential batch-to-batch variability in active compound concentrations.

The limited efficacy against ESBL-producing E. coli (MICs 50-100 μg/mL) likely involves multiple resistance mechanisms:

1. **Membrane impermeability**: The outer membrane's porin channels restrict compound entry (Pagès et al., 2008)
2. **Efflux pumps**: RND-type transporters actively expel antimicrobials (Li et al., 2015)
3. **Enzymatic modification**: β-lactamases may degrade certain phytochemicals (Bush & Jacoby, 2010)

### 4.2 Concentration-Dependent Effects and Pharmacological Implications

The clear dose-response relationship ($r=0.92$, $p<0.01$) has important therapeutic implications. At 10% concentration, ginger extract achieved inhibition zones comparable to ampicillin against MRSA (15.00±4.36 mm vs 20.33±1.53 mm), suggesting potential clinical utility at higher doses. However, the nonlinear increase in activity between 5-10% concentrations indicate possible saturation kinetics, warranting further pharmacokinetic studies.

### 4.3 Mechanistic Considerations

Clove's superior performance likely stems from eugenol's dual mechanism:

1. **Membrane disruption**: Hydroxyl group interaction with phospholipids (Gill & Holley, 2006)
2. **Protein denaturation**: Phenolic compounds binding to essential enzymes (Marchese et al., 2017)

Ginger's moderate activity may involve:

1. **Gingerols inhibiting FtsZ** - a key bacterial division protein (Domadia et al., 2007)
2. **Shogaols disrupting biofilms** (Karuppiah & Mustaffa, 2013)

### 4.4 Comparison with Conventional Antibiotics

While streptomycin remained most potent (30.67±2.08 mm), the plant extracts' performance against MRSA was remarkable:

- Clove (5%): 91.3% of streptomycin's efficacy
- Ginger (10%): 63.4% of streptomycin's efficacy

Notably, clove extract surpassed ampicillin against ESBL-E. coli (20.00±5.00 mm vs 6.67±0.58 mm), suggesting possible synergy or novel mechanisms bypassing β-lactam resistance.

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