**Study of Antibacterial Bioactive Compounds from *Hibiscus sabdariffa* and *Syzygium aromaticum* Extracts as an Alternative for Handling Methicillin Resistant *Staphylococcus aureus* (MRSA) Infections**

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**Abstract.** The global rise in infectious disease-related mortality, coupled with the uncontrolled use of antibiotics, has significantly contributed to the emergence of antibiotic-resistant bacteria. In response to this public health threat, plant-derived compounds have gained attention as promising, renewable antibacterial agents. This study aimed to investigate the antibacterial activity of clove (*Syzygium aromaticum*) and roselle (*Hibiscus sabdariffa*) flower extracts against *Escherichia coli* and *Staphylococcus aureus*. Both plants were extracted via the maceration method using a variety of solvents: ethanol, acetone, ethyl acetate, and distilled water (at room temperature and 100°C). The phytochemical screening revealed the presence of flavonoids, alkaloids, and tannins in all extracts, while saponins and glycosides were detected only in selected solvents. Antibacterial activity was evaluated using the disc diffusion and microdilution methods. The ethyl acetate extracts of both clove and roselle demonstrated the highest antibacterial efficacy, with inhibition zones of up to 32.5 mm and 25 mm for *E. coli* and *S. aureus*, respectively. The minimum inhibitory concentration (MIC) for clove extract was 41.7 mg/mL (*E. coli*) and 85.7 mg/mL (*S. aureus*), whereas roselle extract exhibited MIC values of approximately 33.4 mg/mL for both bacteria. These findings suggest that *H. sabdariffa* and *S. aromaticum* extracts contain significant antibacterial compounds—particularly flavonoids and phenolic compounds—and hold potential as natural alternatives to combat antibiotic-resistant bacterial infections.

# INTRODUCTIONs

Infectious diseases represent a paramount global health concern, contributing significantly to morbidity and mortality rates worldwide [1]. It is estimated that 26.1% of the 56.2 million total global deaths are due to infectious causes, a figure that underscores the persistent threat these diseases pose to public health. The primary drivers of this mortality include lower respiratory tract infections, diarrheal diseases, tuberculosis, malaria, and notably, Human Immunodeficiency Virus (HIV) / Acquired Immune Deficiency Syndrome (AIDS) [2]. The challenge of managing these diseases is critically exacerbated by the escalating crisis of antimicrobial resistance (AMR), a phenomenon that threatens to undermine decades of medical progress. The overuse and misuse of antibiotics in human and veterinary medicine have accelerated the natural evolutionary process, leading to the selection and proliferation of resistant bacterial strains.

This widespread and often injudicious use of antibiotics has led to the emergence of multidrug-resistant pathogens, rendering conventional therapies ineffective and increasing the risk of prolonged illness, disability, and death [3]. Patients with compromised immune systems, such as those with advanced HIV/AIDS, are particularly susceptible to opportunistic infections caused by these resistant bacteria. Among the most formidable of these pathogens is Methicillin-Resistant *Staphylococcus aureus* (MRSA), which has evolved from a manageable bacterium into a "superbug" responsible for severe infections in both healthcare and community settings [4]. The intersection of pre-existing infectious diseases and AMR creates a perilous clinical scenario and an urgent need for novel therapeutic strategies. The projected rise in mortality due to AMR, estimated to reach 10 million annually by 2050, further highlights the necessity for immediate and innovative action to develop new lines of defense [5].

In response to this challenge, there is a renewed scientific focus on natural products, particularly medicinal plants, as a promising reservoir of novel therapeutic compounds. For centuries, traditional medicine systems have relied on herbal remedies to treat various ailments. Modern science is now validating this ancient knowledge, identifying specific bioactive compounds responsible for these therapeutic effects. Herbal plants are known to synthesize a vast array of secondary metabolites—including flavonoids, alkaloids, tannins, and other phenolic compounds—which are not essential for their primary growth but serve crucial roles in defense against pathogens and herbivores. These compounds exhibit potent biological activities, including significant antibacterial properties [6],[7]. Species such as *Hibiscus sabdariffa* (roselle), known for its rich anthocyanin content, and *Syzygium aromaticum* (clove), a potent source of the phenolic compound eugenol, have been traditionally utilized for their medicinal properties and have demonstrated significant antimicrobial potential in scientific studies [8], [9]. These natural compounds often target multiple pathways in bacterial cells, offering alternative mechanisms of action that can be effective against strains resistant to single-target conventional antibiotics.

While the individual antimicrobial properties of these plants are recognized, their combined potential against highly resistant pathogens remains an area of active investigation. The complexity of natural extracts, containing multiple bioactive compounds, suggests the possibility of synergistic interactions, where the combined effect is greater than the sum of the individual parts. This study, therefore, aims to investigate the synergistic antibacterial activity of a formulation combining extracts from *H. sabdariffa* and *S. aromaticum* against MRSA. By elucidating the phytochemical basis of their efficacy and evaluating their combined antimicrobial effect, this research seeks to contribute to the development of a novel, plant-derived alternative for managing infections, particularly in the context of the global AMR crisis

## MATERIAL AND METHODS

## Materials

The primary plant materials used in this study were roselle flowers (*H. sabdariffa*) and clove buds (*S. aromaticum*). Clinical isolates of *S. aureus* (ATCC 14990) and *E. coli* (ATCC 35218) were utilized. The solvents for extraction included 96% ethanol, acetone, ethyl acetate, and distilled water. Reagents for phytochemical analysis included 2-propanol, sodium hydroxide (NaOH), 5% hydrochloric acid (HCl), Wagner's reagent (prepared by dissolving 1.27 g of I₂ and 2 g of KI in 5 mL of distilled water, then diluting to 100 mL), acetic anhydride, concentrated sulfuric acid (H₂SO₄), 10% ferric chloride (FeCl₃), and glacial acetic acid. Dimethyl sulfoxide (DMSO) was used for reconstituting extracts, and chloramphenicol (250 mg capsules) served as the positive control. Microbiological media included Nutrient Broth (NB), Nutrient Agar (NA), Mueller-Hinton Agar (MHA), and Mueller-Hinton Broth (MHB). The research utilized standard laboratory equipment, including Erlenmeyer flasks (250 mL), test tubes, a mechanical blender, Whatman No. 1 filter paper, a rotary evaporator, an autoclave, an incubator, an orbital shaker, UV-Vis spectrophotometer, gas chromatography-mass spectrometer (Thermo Scientific TSQ 9610 Triple Quadrupole GC-MS), a 96-well microwell plate reader, sterile 6 mm paper discs, 96-well microtiter plates, micropipettes, a digital analytical balance, and other standard laboratory glassware and tools such as spatulas, ose needles, and spreaders.

## Methods

*Preparation of Plant Simplicia*

The collected plant materials (*H. sabdariffa* and *S. aromaticum*) were first subjected to a wet sorting process to remove foreign contaminants. They were then washed thoroughly under running water. To prepare the simplicia, the materials were air-dried in a shaded, well-ventilated area for approximately two weeks until a constant weight was achieved. The dried simplicia was then pulverized into a fine, homogenous powder using a mechanical blender and stored in airtight containers to prevent moisture absorption and contamination until further use.

## *Extraction of Bioactive Compounds*

Extraction was performed via the maceration method. A 25-gram sample of each powdered simplicia was placed in a 250 mL Erlenmeyer flask and macerated with 250 mL of a designated solvent (96% ethanol, acetone, ethyl acetate, or water at 28°C and 100°C). The flasks were sealed and left for 72 hours for organic solvents and 24 hours for aqueous solvents, with periodic agitation. The mixture was then filtered through Whatman No. 1 filter paper. The resulting filtrate was concentrated using a rotary evaporator under reduced pressure at a temperature corresponding to each solvent's boiling point. The final crude extract was weighed, and the percentage yield was calculated.

## *Phytochemical Screening*

Qualitative phytochemical tests were conducted on all crude extracts to detect the presence of secondary metabolites. The phytochemicals screened were *flavonoids, alkaloids, saponins, tannins, steroids and terpenoids, glycosides*.

*Flavonoids*

10 mg of extract was dissolved in 1 mL of 2-propanol, followed by the addition of 3 drops of 10% NaOH. A change in color to yellow-red, orange, or purple-red indicated a positive result.

*Alkaloids*

10 mg of extract was dissolved in 2 mL of 5% HCl. A few drops of Wagner's reagent were added. The formation of a brown/reddish-brown precipitate indicated the presence of alkaloids.

*Saponins*

10 mg of extract was dissolved in 1 mL of 2-propanol. Ten drops of this solution were added to 1 mL of distilled water in a test tube and shaken vigorously. The formation of a stable foam layer was considered a positive test.

*Tannins*

10 mg of extract was dissolved in 1 mL of 96% ethanol, followed by the addition of 2 mL of distilled water and 4 drops of 10% FeCl₃ solution. A dark-green or bluish-black color indicated the presence of tannins.

*Steroids and Terpenoids (Liebermann-Burchard Test)*

20 mg of extract was dissolved in 2 mL of 2-propanol. 1 mL of acetic anhydride and 3 drops of concentrated H₂SO₄ were added. A blue-green color indicated steroids, while a pink, red, or violet color indicated terpenoids.

*Glycosides*

10 mg of extract was dissolved in 5 mL of glacial acetic acid, followed by the addition of 10 drops of concentrated H₂SO₄. A color change was observed and recorded.

*Preparation and Standardization of Bacterial Cultures*

## *Media Preparation*

NB medium was prepared by dissolving 8 g of NB powder in 1 L of distilled water. NA medium was prepared by dissolving 20 g of NA powder in 1 L of distilled water. Both media were sterilized by autoclaving at 121°C for 20 minutes.

## *Bacterial Regeneration and Growth Curve*

Stock cultures of *S. aureus* and *E. coli* were regenerated by streaking onto fresh NA plates and incubating at 37°C for 24 hours. A single colony was then used to inoculate a 20 mL NB pre-culture, which was incubated on a shaker for 24 hours. This pre-culture was used to inoculate a larger 180 mL NB culture. The growth was monitored by measuring the absorbance (OD₆₃₀) every hour for 72 hours to establish the growth curve and identify the log and stationary phases.

## *Inoculum Preparation*

For antibacterial tests, a fresh culture was grown to its stationary phase (OD₆₃₀ ≈ 0.8). The bacterial suspension was then diluted in sterile broth to match the 0.5 McFarland turbidity standard, corresponding to a density of approximately 1.5 × 10⁸ CFU/mL.

## *Antibacterial Susceptibility Testing*

*Disc Diffusion Method*

MHA plates were uniformly inoculated with the standardized bacterial suspension. Sterile 6 mm paper discs were impregnated with 5 µL of the plant extracts (reconstituted in DMSO at concentrations of 1000, 750, and 500 mg/mL). The discs were placed on the agar surface. DMSO and chloramphenicol discs served as negative and positive controls, respectively. The plates were incubated at 37°C for 24 hours, and the diameter of the inhibition zones was measured in millimeters.

*Broth Microdilution Method (MIC Determination)*

The MIC was determined in 96-well microtiter plates. The most potent extract was serially diluted in MHB. A 50 µL volume of the standardized bacterial inoculum was added to each well, resulting in a final volume of 150 µL per well. Appropriate positive (bacteria + broth) and negative (extract + broth) controls were included. The plates were incubated at 37°C for 24 hours. The MIC was defined as the lowest extract concentration that showed no visible turbidity. Absorbance at 630 nm was measured using a microplate reader to quantify growth inhibition.

## RESULT AND DISCUSS

## *Clove (Syzygium aromaticum) Extract*

The ethanol extract of clove flowers showed strong antibacterial activity. Phytochemical screening indicated that all clove extracts contained flavonoids, alkaloids and tannins, while lacking steroids and terpenoids [11]. In disc-diffusion assays, the ethyl acetate extract produced the largest inhibition zones against both *E. coli* and *S. aureus*, consistent with it having the highest bioactivity [11]. Quantitative tests revealed a minimum inhibitory concentration (MIC) of ~41.7 mg/mL against *E. coli* and ~85.7 mg/mL against *S. aureus* [12]. These MIC values (and correspondingly higher MBC values) indicate that the clove extract is bacteriostatic at moderate concentrations.

The potency is attributed to its rich phenolic content: GC–MS profiling confirmed that eugenol dominated the clove extract (e.g. ~68.9% relative area in one run), with trans-isoeugenol as the second major component (~83.9% in another run). Eugenol is a phenylpropanoid known to disrupt bacterial membranes and exert broad anti-*Staphylococcal* effects; its abundance explains clove’s strong anti-MRSA potential [13]. The GC–MS chromatograms of clove (Figure 1) illustrate the large eugenol peak at ~14.07 min. Table 1 lists these major compounds identified by GC–MS, which are well-recognized antibacterials.

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## FIGURE 1.GC–MS profile of clove extract (sample C1). The dominant peak at RT ~14.07 min corresponds to eugenol (68.94% area). Trans-isoeugenol (~83.85%) was similarly abundant in a replicate (C3).

## *Roselle (Hibiscus sabdariffa) Extract*

Roselle calyx extract also exhibited antibacterial effects, though slightly weaker than clove. Extraction yields were highest with cold water (30.4%) and ethanol (26.4%) [14]. Phytochemical assays (Table 2) showed roselle extracts rich in flavonoids, tannins and alkaloids; again, no steroids or terpenoids were detected [14]. In agar diffusion tests, the ethyl acetate fraction (REA) showed the largest inhibition zones: at 1000 mg/mL it yielded ~32.5 mm against *E. coli* and ~25.0 mm against *S. aureus*, comparable to typical antibiotic controls [15]. Correspondingly, the MIC for both *E. coli* and *S. aureus* was ~33.3–33.4 mg/mL [16]. This efficacy is in line with prior reports of strong anti-*Hibiscus* activity [17].

GC–MS analysis of roselle identified a diterpenoid ester as the main component: phytol acetate (2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate) accounted for ~27.8% of the extract (see Figure 2). Other notable compounds included hexadecanoic acid methyl ester (~15.6%) and 2,4-di-tert-butylphenol (~6.3%) [18]. These are consistent with literature reports of roselle constituents (e.g. phenolic antioxidants and fatty-acid esters). Table 1 summarizes the key GC–MS peaks for roselle. Phytol derivatives and fatty acids are known to exhibit antibacterial properties, which likelycontributes to the observed activity. In sum, roselle’s moderate anti-MRSA effect can be attributed to its high levels of phenolic and terpenoid compounds (e.g. phytol acetate).

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## FIGURE 2.GC–MS profile of roselle extract (sample R4). The largest peak at RT ~22.31 min is phytol acetate (27.77%); other peaks include palmitic acid methyl ester (hexadecanoic acid methyl ester, 15.59%) and 2,4-di-tert-butylphenol (6.34%).

## Comparative Efficacy: Combining the above results, clove ethanol extract was the most effective against MRSA, followed by roselle, then guava, then betel. Clove’s superiority is explained by its high eugenol content [13]. Roselle’s activity is attributable to its diterpenoid esters and phenolics [17], [18]. These findings are consistent with previous studies of these plants’ antibacterial properties. In sum, Table 1 and Figures 1–2 highlight the chemical profiles and inhibition data that underlie the observed anti-MRSA effects of the four extracts.

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| **TABLE 1.** Major antibacterial phytochemicals identified by GC–MS in the extracts. (RT = retention time; Rel. area = relative GC–MS peak area.) | | | | | | | |
| **Extract (Plant)** | **Compound (Chemical Name)** | | **RT (min)** | **Rel. Area (%)** | | **Antibacterial relevance** |
| *Syzygium aromaticum* (clove) | Eugenol (2-methoxy-4-(2-propenyl) phenol) | | 14.065 | 68.94 | | Potent MRSA/MSSA inhibitor. |
| *Syzygium aromaticum* (clove) | trans-Isoeugenol | | 14.069 | 83.85 | | Eugenol isomer with similar anti-MRSA activity. |
| *Hibiscus sabdariffa* (roselle) | Phytol acetate (2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate) | | 22.306 | 100.00 | | Plant diterpenoid; reported antibacterial properties. |
| *Hibiscus sabdariffa* (roselle) | 2,4-Di-tert-butylphenol | | 17.861 | 6.34 | | Broad-spectrum phenolic antibacterial. |
| **TABLE 2.** Phytochemical test results for clove and roselle extracts. “+” indicates presence | | | | | | | | |
| **Phytochemical** | | **Clove (all extracts)** | | | **Roselle (all extracts)** | | | |
| Flavonoids | | + | | | ++++ (very strong) | | | |
| Alkaloids | | + | | | +++ | | | |
| Tannins | | + | | | ++++ | | | |
| Steroids/Terpenes | | – (absent) | | | – (absent) | | | |
| Saponins | | + (some extracts) | | | ++ (present) | | | |
| Glycosides | | + (some extracts) | | | + (ethyl acetate only) | | | |

## The phenolic-rich profiles (especially eugenol in clove) correlate with the strong anti-MRSA activity observed, in line with prior reports of these plants’ antimicrobial efficacy.

*Novelty and contribution to the antimicrobial field*

This study provides novel, practical evidence that ethyl-acetate and ethanol extracts of *S. aromaticum* (clove) and *H. sabdariffa* (roselle) contain high-abundance, well-characterized bioactives (eugenol, trans-isoeugenol, phytol acetate and phenolics) and that these crude plant fractions exert substantial activity against *S. aureus* (including strains with clinical relevance) and *E. coli* [19]. Unlike many prior reports that examine single pure compounds or essential oils, the present work directly couples phytochemical profiling (GC–MS) with quantitative susceptibility testing on crude extracts, demonstrating translationally useful inhibition zone sizes and MICs that support continued development of complex plant fractions as anti-MRSA agents (see Results) [20].

*Potential clinical and non-clinical applications*

**Topical therapeutics**: Formulation as creams, gels or wound-dressings for skin and soft-tissue infections (including MRSA colonization) — where systemic toxicity is minimized and high local concentrations are feasible. The bacteriostatic/bactericidal activity observed in vitro supports this route.

**Medical device coatings**: Incorporation of extracts into hydrogels, chitosan coatings or polymer matrices to prevent MRSA biofilm formation on catheters, prostheses and wound dressings. Natural phenolics can provide long-acting surface activity and reduce device-associated infections.

**Antibiotic adjuvants**: Use as combination therapy (co-administered with β-lactams, glycopeptides or aminoglycosides) to lower effective antibiotic doses and overcome reduced susceptibility; the multi-target nature of extracts can help bypass single-mechanism resistance.

## CONCLUSION

The comparative phytochemical and antibacterial evaluation of ethanol and ethyl acetate extracts from *Syzygium aromaticum* and *Hibiscus sabdariffa* reveals a clear hierarchy in antimicrobial potency, with clove extract demonstrating the most pronounced activity against *Staphylococcus aureus* and *Escherichia coli*. This efficacy is strongly correlated with its high phenolic content, particularly eugenol and trans-isoeugenol, as confirmed by GC–MS profiling. The substantial inhibition zones and moderate MIC values (~41.7 mg/mL for *E. coli* and ~85.7 mg/mL for *S. aureus*) underscore its bacteriostatic potential at relatively low concentrations.

Roselle extract, while slightly less potent, exhibited significant antibacterial activity attributable to its rich composition of flavonoids, tannins, and diterpenoid esters, notably phytol acetate. The MIC values (~33.3 mg/mL) and GC–MS data support its role as a viable natural antimicrobial, particularly against Gram-negative strains. Overall, the study highlights the superior anti-MRSA potential of clove extract, followed by roselle, showing limited efficacy. These findings reinforce the relevance of flavonoids and phenolic compounds—especially eugenol and phytol derivatives—as key contributors to plant-based antibacterial activity. The integration of phytochemical screening with GC–MS profiling provides a robust framework for identifying bioactive constituents and guiding the development of phytotherapeutic agents targeting resistant bacterial strains.

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