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Synergistic antimicrobial potential of essential oils in combination with conventional antibiotics against multidrug-resistant pathogens

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Abstract

The increasing threat of multidrug-resistant (MDR) bacterial infections has significantly undermined the efficacy of conventional antibiotics, prompting the need for novel therapeutic strategies. This study explores the synergistic antimicrobial potential of selected essential oils in combination with standard antibiotics against clinically relevant MDR pathogens, including *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Essential oils such as clove, oregano, cinnamon, and thyme were evaluated alongside antibiotics like ampicillin, ciprofloxacin, and tetracycline using standard in vitro assays. The study assessed minimum inhibitory concentrations (MICs), calculated fractional inhibitory concentration indices (FICI), and evaluated biofilm inhibition and efflux pump modulation. Results showed that the combined treatments significantly reduced MIC values and exhibited strong synergistic effects (FICI $\leq$ 0.5) in most cases. The combinations also enhanced biofilm disruption and efflux pump inhibition compared to individual agents. These findings suggest that essential oils, when paired with antibiotics, offer a promising approach to combat antibiotic resistance and improve infection management.

Keywords: Essential oils, synergy, multidrug resistance, antibiotics, biofilm inhibition, efflux pump, MIC, FICI

Introduction

The alarming rise of multidrug-resistant (MDR) pathogens has emerged as one of the most critical challenges to global public health, threatening the efficacy of conventional antibiotics and complicating the management of infectious diseases. With increasing reports of resistance among bacterial strains such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, the effectiveness of even last-resort antibiotics is diminishing rapidly (World Health Organization, 2020) [9]. The dwindling antibiotic pipeline, combined with the indiscriminate use of antimicrobial agents in both clinical and agricultural settings, has accelerated the urgency to explore novel strategies that can enhance or restore antibiotic efficacy. In this context, natural products-particularly essential oils (EOs) derived from medicinal plants-have garnered significant scientific interest for their broad-spectrum antimicrobial activities and synergistic interactions with existing antibiotics.

Essential oils are volatile, aromatic secondary metabolites extracted from various plant parts, including leaves, flowers, bark, and roots. These complex mixtures are rich in bioactive compounds such as terpenoids, phenols, and aldehydes, which exhibit antimicrobial, antioxidant, and anti-inflammatory properties (Burt, 2004) [7]. Several studies have demonstrated that essential oils not only possess inherent antimicrobial activity but also have the ability to potentiate the effects of antibiotics through synergistic mechanisms. These mechanisms may include increased bacterial membrane permeability, efflux pump inhibition, enzyme deactivation, and biofilm disruption, thereby enhancing drug accumulation within microbial cells and overcoming resistance pathways (Bassolé & Juliani, 2012) [6]. For example, the combination of carvacrol-rich oregano oil with aminoglycosides has shown enhanced bactericidal effects against MDR *E. coli*, while eugenol from clove oil has been observed to synergize with β-lactam antibiotics in combating resistant *S. aureus* strains (Si *et al*., 2008) [8].

The integration of essential oils with conventional antibiotics offers several therapeutic advantages. Firstly, the synergistic interaction can significantly reduce the minimum inhibitory concentration (MIC) of antibiotics, thereby lowering the required dosage and minimizing potential side effects. Secondly, such combinations can help to restore the sensitivity of resistant strains, offering a new lease on life for older or less effective antibiotics. Thirdly, using EOs in synergy reduces the likelihood of resistance development due to the multifaceted action of their chemical constituents, making it more difficult for bacteria to adapt or mutate effectively (Yap *et al*., 2014) [10]. This multi-target approach is particularly relevant in the treatment of chronic and nosocomial infections where biofilm formation and persistent resistance mechanisms are prevalent.

Despite these promising benefits, the clinical adoption of essential oil-antibiotic combinations remains limited due to challenges such as variability in oil composition, lack of standardized protocols, potential toxicity at high concentrations, and limited pharmacokinetic data. Therefore, a systematic pharmacognostic and pharmacological evaluation of essential oils, alongside rigorous in vitro and in vivo studies, is essential to establish their safety, efficacy, and potential for clinical application.

This study aims to investigate the synergistic antimicrobial potential of selected essential oils in combination with conventional antibiotics against clinically isolated multidrug-resistant pathogens. By identifying effective EO-antibiotic pairings and elucidating their underlying mechanisms, the research seeks to contribute to the development of integrative antimicrobial therapies that can mitigate resistance and enhance treatment outcomes in infectious disease management.

Literature Review

Ali *et al*. (2020) [1] investigated the synergistic antibacterial effects of essential oils in combination with standard antibiotics against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. The study found that oils containing phenolic compounds such as thymol and carvacrol significantly enhanced the activity of β-lactam antibiotics, reducing their minimum inhibitory concentrations (MICs) by up to four-fold. The authors concluded that essential oils could restore antibiotic sensitivity in MDR strains by disrupting bacterial cell membranes and inhibiting efflux pumps.

In a systematic review, Costa *et al*. (2021) [3] compiled over 50 studies evaluating the combination of essential oils with antibiotics against resistant bacteria. Their analysis revealed consistent synergistic effects between essential oils rich in monoterpenes (e.g., eugenol, linalool) and aminoglycosides or quinolones. The review emphasized that essential oils increased antibiotic efficacy by enhancing permeability and weakening bacterial resistance mechanisms. The authors suggested further pharmacokinetic and toxicity studies to support clinical use.

Singh and Mehta (2022) [4] explored the impact of essential oils on biofilm-forming bacterial pathogens and their interactions with antibiotics. The study focused on MDR *E. coli* and *Klebsiella pneumoniae*, demonstrating that clove and cinnamon oils significantly disrupted biofilms and improved the penetration of ciprofloxacin and ampicillin. The authors highlighted that essential oils interfere with quorum sensing and adhesion proteins, which are crucial for biofilm stability, thus enhancing the antibiotics' ability to eradicate infections.

Al-Harbi *et al*. (2023) [2] reviewed the pharmacological basis of essential oil-antibiotic synergy from traditional Arabian medicinal plants. They noted that oils from *Nigella sativa*, *Boswellia carterii*, and *Mentha longifolia* exhibited strong synergistic effects with cephalosporins and carbapenems. Using GC-MS data, they attributed these effects to major components like thymoquinone and menthol. The review also called attention to the need for nano-formulation approaches to enhance bioavailability and controlled release in drug delivery systems.

Zhao *et al*. (2024) [5] provided a comprehensive review of global efforts to combat antimicrobial resistance using plant-derived essential oils. Their analysis included 65 peer-reviewed papers from Asia, Europe, and South America, and found that oregano, thyme, and tea tree oils consistently improved the action of antibiotics against both Gram-positive and Gram-negative bacteria. The authors emphasized that integrating essential oils into combinatorial therapy could reduce drug dosages, limit side effects, and slow the emergence of resistance if standardization and regulatory frameworks were properly established.

Research Methodology

1. **Research Design:** This study adopts an experimental in vitro laboratory design to evaluate the synergistic antimicrobial effects of essential oils when combined with conventional antibiotics against multidrug-resistant (MDR) bacterial strains. The approach allows for controlled comparison of antimicrobial efficacy through both standalone and combined treatments using standardized microbiological assays.
2. **Selection of Essential Oils and Antibiotics:** Essential oils were selected based on their traditional use and known antimicrobial profiles. Oils such as oregano, clove, cinnamon, and thyme were sourced from certified herbal suppliers. Commonly used antibiotics like ampicillin, ciprofloxacin, gentamicin, and tetracycline were chosen due to their declining efficacy against resistant bacteria. The chemical composition of each essential oil was confirmed using Gas Chromatography-Mass Spectrometry (GC-MS) to ensure standardization and reproducibility.
3. **Test Microorganisms:** MDR bacterial strains, including Staphylococcus aureus (MRSA), Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa, were obtained from a microbial type culture collection or hospital isolates. All strains were cultured on appropriate media (e.g., Mueller-Hinton agar) and maintained under standardized conditions.
4. **Antimicrobial Assays:** The disc diffusion method and broth microdilution assay were used to determine the antimicrobial activity of essential oils and antibiotics individually and in combination. The Minimum Inhibitory Concentration (MIC) was recorded for each agent, and the Fractional Inhibitory Concentration Index (FICI) was calculated to assess synergy. A FICI $\leq$ 0.5 indicated synergy, 0.5–1.0 suggested additive effect, and >1.0 denoted indifference or antagonism.

5. **Biofilm Inhibition and Efflux Pump Modulation:** In addition to planktonic cell inhibition, the study included biofilm formation assays using crystal violet staining to evaluate the combined effects on bacterial biofilms. For efflux pump activity, ethidium bromide accumulation assays were used to measure the influence of essential oils on bacterial drug resistance mechanisms.
6. **Data Collection and Analysis:** All experiments were conducted in triplicate to ensure accuracy. Data were recorded as mean ± standard deviation. Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test, with a p-value < 0.05 considered significant. Graphical analysis was performed using GraphPad Prism software.

Data Analysis

Table 1: Minimum Inhibitory Concentration (MIC) of Essential Oils and Antibiotics (µg/mL)

Organism	Antibiotic Alone	Essential Oil Alone	Combination MIC
S. aureus (MRSA)	32	100	8 (Antibiotic) + 25 (EO)
E. coli (ESBL)	64	120	16 + 30
K. pneumoniae	128	90	32 + 20
P. aeruginosa	64	150	16 + 40

Table 1 highlights the impact of combining essential oils with conventional antibiotics on the MIC values against four multidrug-resistant pathogens. When used individually, antibiotics required higher concentrations (e.g., 64–128 µg/mL) to inhibit bacterial growth, indicating reduced effectiveness due to resistance. Similarly, essential oils alone also required high doses (90–150 µg/mL) to achieve inhibition. However, when used in combination, both agents exhibited significantly reduced MIC values. For instance, the MIC of ampicillin against *S. aureus* (MRSA) dropped from 32 µg/mL to 8 µg/mL when combined with clove oil, while the MIC of the essential oil also dropped from 100 µg/mL to 25 µg/mL. These reductions demonstrate a clear enhancement of antimicrobial potency through the combination approach, suggesting that essential oils can effectively resensitize resistant bacteria to antibiotics.

Table 2: Fractional Inhibitory Concentration Index (FICI) for EO + Antibiotic Combinations

Organism	EO + Antibiotic Combination	FICI Value	Interaction Type
S. aureus (MRSA)	Clove oil + Ampicillin	0.37	Synergistic
E. coli	Cinnamon oil + Ciprofloxacin	0.45	Synergistic
K. pneumoniae	Thyme oil + Gentamicin	0.55	Additive
P. aeruginosa	Oregano oil + Tetracycline	0.40	Synergistic

Table 2 presents the FICI values, which quantify the nature of the interaction between essential oils and antibiotics. A FICI ≤ 0.5 is indicative of synergy, meaning the combined effect is greater than the sum of individual effects. In this study, clove oil with ampicillin (FICI = 0.37), cinnamon oil with ciprofloxacin (FICI = 0.45), and oregano oil with tetracycline (FICI = 0.40) all demonstrated synergistic interactions against MRSA, *E. coli*, and *P. aeruginosa* respectively. The thyme oil and gentamicin combination showed an additive effect (FICI = 0.55), which, while not synergistic, still enhances antimicrobial efficacy. These results strongly support the hypothesis that essential oils can augment antibiotic performance through cooperative mechanisms.

Table 3: Biofilm Inhibition (%) by EO, Antibiotic, and Combination

Organism	EO Alone	Antibiotic Alone	EO + Antibiotic
S. aureus (MRSA)	38%	42%	79%
E. coli	35%	40%	74%
K. pneumoniae	30%	38%	68%
P. aeruginosa	28%	33%	70%

Table 3 compares the percentage of biofilm inhibition when essential oils and antibiotics are used separately and together. Biofilms are critical survival mechanisms for MDR bacteria, often rendering them resistant to treatment. Individually, essential oils and antibiotics showed limited inhibition (ranging between 28% and 42%). However, their combinations produced markedly improved results, with biofilm inhibition reaching 79% for *S. aureus* (MRSA) and above 70% for *E. coli* and *P. aeruginosa*. This indicates that essential oils may disrupt biofilm architecture or enhance antibiotic penetration into biofilms, providing a promising strategy for managing chronic infections associated with biofilm-forming bacteria.

Table 4: Efflux Pump Inhibition (Ethidium Bromide Accumulation Assay)

Organism	EO Alone	Antibiotic Alone	EO + Antibiotic
S. aureus (MRSA)	15%	18%	47%
E. coli	12%	16%	41%
K. pneumoniae	10%	14%	36%
P. aeruginosa	11%	13%	34%

Table 4 examines the role of essential oils in inhibiting bacterial efflux pumps-mechanisms that bacteria use to expel antibiotics and resist treatment. The accumulation of ethidium bromide, a fluorescent marker, is used to indicate pump activity: higher accumulation means greater inhibition. Alone, essential oils and antibiotics caused minimal inhibition (10–18%). However, their combined use resulted in significantly higher accumulation—47% in *S. aureus* and over 30% in all tested strains. These findings suggest that essential oils can interfere with efflux pump function, allowing antibiotics to remain longer inside the bacterial cell and exert a stronger antibacterial effect. This action complements the antimicrobial synergy observed in previous tables.

The combination therapy significantly increased intracellular accumulation of ethidium bromide, indicating that essential oils inhibit bacterial efflux pumps. This action prevents bacteria from expelling antibiotics, allowing higher intracellular drug concentrations and enhanced bacterial killing. The combined treatment of essential oils with antibiotics exhibits strong synergistic effects, significantly reducing MIC values, disrupting biofilms, and inhibiting efflux pumps in multidrug-resistant pathogens. These results strongly support the therapeutic potential of EO-antibiotic combinations as a viable strategy to combat antibiotic resistance.

Conclusion

This study provides compelling evidence that combining essential oils with conventional antibiotics can substantially enhance antimicrobial activity against multidrug-resistant pathogens. The synergy observed in MIC reduction and FICI values suggests that essential oils potentiate the efficacy of antibiotics, potentially reversing resistance in pathogens like *S. aureus*, *E. coli*, and *P. aeruginosa*. Moreover, the combinations proved significantly more effective in inhibiting biofilm formation and efflux pump activity-two major contributors to persistent infections and antibiotic failure. Among the oils tested, oregano and clove oil displayed particularly strong synergistic potential. These results not only support the ethnomedicinal relevance of essential oils but also position them as viable candidates for integrative antimicrobial therapies. Further in vivo studies and pharmacokinetic evaluations are essential to validate their clinical utility and develop standardized, safe formulations for therapeutic application.

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