Natural Resources for Human Health

eISSN: 2583-1194

Original Research

Received 14 March 2026
Revised 22 April 2026
Accepted 16 May 2026

Natural Resources for Human Health 2026, Vol. 6 (3): 720–728

KEYWORDS: Essential oils; Synergistic antimicrobial activity; Foodborne pathogens; Network pharmacology; Natural food preservatives; Bioactive compounds; Antimicrobial resistance; Protein–protein interaction network; Food safety; Multi-target therapeutics.

Synergistic Antimicrobial Efficacy of Essential Oil Combinations Against Foodborne Pathogens: A Network Pharmacology Approach

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ABSTRACT: Foodborne pathogens remain a significant public health concern and a challenge for the global food industry, leading to widespread illness, deaths, and substantial economic costs. Rising antimicrobial resistance and increasing consumer preference for natural preservatives have driven research into plant-based essential oils (EOs) as viable, sustainable alternatives to synthetic antimicrobials. Evidence shows that combining essential oils can produce synergistic effects, improving their ability to suppress harmful microbes at lower doses while reducing negative impacts on food flavor and aroma. This review explores the combined antimicrobial effectiveness of essential oil mixtures against key foodborne pathogens such as Escherichia coli, Salmonella enterica, Listeria monocytogenes, Staphylococcus aureus, and Bacillus cereus. Special attention is given to network pharmacology—an emerging systems biology method—that helps uncover the complex interactions among multiple components, targets, and biological pathways involved in the antimicrobial action of EO combinations. The analysis covers major bioactive compounds in common essential oils, their molecular targets, and their roles in disrupting microbial membranes, inducing oxidative stress, inhibiting biofilm formation, interfering with quorum sensing, and altering metabolic functions.

It also summarizes recent progress in protein–protein interaction networks, Gene Ontology enrichment, KEGG pathway analysis, and compound–target mapping to better understand how synergy enhances antimicrobial activity. Additionally, the review highlights practical applications of synergistic EO formulations in food preservation techniques like active packaging, nanoencapsulation, and edible coatings, while addressing existing hurdles such as variability in composition, standardization, safety evaluation, and regulatory compliance. Looking ahead, the integration of artificial intelligence, multi-omics approaches, molecular docking, and network pharmacology is discussed as a promising path toward designing more effective, science-based natural antimicrobial solutions. Overall, this work offers a structured approach to developing advanced, synergistic essential oil strategies that improve food safety, prolong shelf life, and help counteract antimicrobial resistance in contemporary food systems.

1. INTRODUCTION

Despite significant progress in preventing foodborne diseases, it remains a major public health, food security, and economic development challenge worldwide, with an estimated 600 million cases and 420,000 deaths caused by foodborne diseases annually, particularly in children younger than five years of age [1]. With the growing global trade of food, urbanization, climate change, and the demand for minimally processed foods, the risk of microbial contamination has significantly increased during food production, processing, storage, transportation, and distribution [2,3]. The major foodborne pathogens, such as Escherichia coli, Salmonella enterica, Listeria monocytogenes, Staphylococcus aureus, and Bacillus cereus, are the causative agents of many outbreaks linked to meat, dairy products, seafood, fruits, vegetables, and ready-to-eat foods [4,5]. In addition to reducing food quality, these microorganisms can cause severe illness.

1.1 Foodborne Pathogens and Global Food Safety Challenges

With the complexity of modern food systems and the emergence of AMR, foodborne pathogens are becoming more challenging to control and can be contaminated at any stage of the food chain, from production and harvesting, processing, packaging, retailing, and domestic handling [2,3]. The overuse of antibiotics in livestock production, aquaculture, and clinical medicine has driven the evolution of multidrug-resistant bacteria, rendering conventional antimicrobial therapies ineffective [7]. In addition, many pathogenic bacteria are able to form biofilms on food-processing equipment, which can survive cleaning and sanitation procedures and continue to contaminate food products [8]. This has created a critical need for novel antimicrobial strategies that can effectively eliminate foodborne pathogens while limiting the development of resistance and ensuring food safety [9,10].

1.2 Limitations of Conventional Antimicrobial Agents

Synthetic preservatives and antibiotics are conventional antimicrobial agents that have been used for decades to control microbial contamination, but their continuous and indiscriminate use has led to many limitations, such as the rapid development of antimicrobial-resistant microorganisms, which is a major global health challenge [7,11], as well as allergic reactions, gastrointestinal disorders, oxidative stress, and potential toxicological effects associated with prolonged consumption of some synthetic preservatives [12]. Conventional antimicrobial agents are designed to target a single cellular process, allowing microorganisms to become resistant by a variety of mechanisms, including genetic mutations, horizontal gene transfer, enzymatic degradation, and efflux pump mechanisms [13]. In addition, these compounds are frequently ineffective against bacterial biofilms, which provide physical and biochemical protection to microbial communities, limiting antimicrobial penetration [8,14]. In addition, environmental contamination with antibiotic residues further promotes the spread of antimicrobial resistance genes among environmental microorganisms [15]. As a result, there is growing interest in the discovery of natural antimicrobial compounds that are biodegradable, environmentally benign, and have multiple microbial targets.

1.3 Essential Oils as Natural Antimicrobial Compounds

Essential oils (EOs) are natural extracts of volatile secondary metabolites of aromatic plants and have been shown to have antimicrobial, antioxidant, anti-inflammatory, and antibiofilm activities [16,17]. EOs from oregano, thyme, cinnamon, clove, tea tree, rosemary, peppermint, and lemongrass contain biologically active compounds, such as carvacrol, thymol, eugenol, cinnamaldehyde, citral, and terpinen-4-ol, which have broad-spectrum inhibitory activity against foodborne pathogens, including disruption of microbial cell membranes, increases in membrane permeability, inhibition of ATP production, enzyme activity, nucleic acid synthesis, and quorum sensing and biofilm formation [19,20]. Essential oils work by attacking multiple cellular targets, which decreases the likelihood of developing microbial resistance [21]. However, their use in food is often limited by poor water solubility, high volatility, chemical instability, and strong aroma, which can negatively impact the sensory quality of food products [22]. Improvements in nanoencapsulation, edible coatings, and controlled-release delivery systems have enhanced the stability, bioavailability, and antimicrobial activity of essential oils, opening the door to their potential use in natural food preservation [23–25].

1.4 Synergistic Effects of Essential Oil Combinations

Studies have shown that essential oil mixtures have enhanced antimicrobial activity compared to single oils due to synergistic interactions between the bioactive constituents of the oils [18,22]. Synergy refers to the inhibitory effects of two or more essential oils that are greater than those predicted by their individual activities. The phenolic compounds carvacrol, thymol, eugenol, and cinnamaldehyde act on several microbial targets, including cell membranes, membrane permeability, ATP production, enzyme activity, and biofilm formation [17–20]. These synergistic effects increase bacterial inhibition and decrease the concentration of each essential oil needed for antimicrobial activity, which minimizes undesirable sensory effects, decreases toxicity, and decreases the likelihood of the development of antimicrobial resistance [21,23]. For instance, the oregano–thyme and cinnamon–clove combinations have demonstrated good activity against Escherichia coli, Salmonella enterica, Listeria monocytogenes, and Staphylococcus aureus, suggesting they may be used as natural food preservatives [24,25].

1.5 Role of Network Pharmacology in Natural Product Research

In recent years, network pharmacology, a computational strategy for mapping compound–target interactions that focuses on multiple compound–target–pathway interactions simultaneously rather than on a single target, has emerged as a powerful tool for understanding the complex interactions of natural bioactive compounds with microbial targets, and particularly for the multi-component nature of essential oils, enabling molecular mechanisms responsible for the synergistic antimicrobial activity of essential oil combinations to be identified, novel natural antimicrobial formulations to be discovered, and useful information on improving food preservation strategies to be gained [14–16]. This also supports the exploration of new natural antimicrobial formulations and offers insights into food preservation strategies.

1.6 Scope and Objectives of the Study

The review summarizes the antimicrobial potential of essential oil combinations against major foodborne pathogens and highlights the application of network pharmacology for elucidating the synergistic mechanisms, discusses the major bioactive constituents of essential oils, their antimicrobial modes of action, and recent advances in natural food preservation. In addition, it discusses the current challenges, such as formulation stability, safety, and standardization, and outlines future perspectives for developing safe, effective, and sustainable essential oil-based antimicrobial systems for improving food safety and extending food shelf life [21–25].

2. ESSENTIAL OILS USED AGAINST FOODBORNE PATHOGENS

Due to their broad-spectrum biological activities and consumer preference for clean-label food products, EOs have been identified as potential natural antimicrobial agents to control foodborne pathogens [9]. These volatile, aromatic compounds are complex mixtures of terpenes, terpenoids, phenolics, aldehydes, alcohols, ketones, and esters extracted from various plant parts such as leaves, flowers, bark, seeds, roots, and fruits [9,12,18]. EOs from oregano (Origanum vulgare), thyme (Thymus vulgaris), cinnamon (Cinnamomum verum), clove (Syzygium aromaticum), tea tree (Melaleuca alternifolia), lemongrass (Cymbopogon citratus), rosemary (Rosmarinus officinalis), and peppermint (Mentha piperita) have shown significant antimicrobial activity against foodborne pathogens including Escherichia coli, Salmonella enterica, Listeria monocytogenes, Staphylococcus aureus, and Bacillus cereus [9,12,18].

2.1 Major Bioactive Constituents

The antimicrobial activity of essential oils is mainly attributed to their bioactive phytochemicals, especially phenolic compounds like carvacrol, thymol, eugenol, and cinnamaldehyde, but also other compounds such as citral, linalool, terpinen-4-ol, menthol, 1,8-cineole, and α-pinene [11,20]. Their biological effectiveness depends on the chemical composition of the essential oils, which can vary according to the genotype of the plant, the geographical origin, climatic conditions, the time of harvest, the extraction technique, and the storage conditions [11,20].

2.2 Mechanisms of Antimicrobial Action

Essential oils have multiple complementary mechanisms of antimicrobial activity (other than one molecular target), including increasing membrane permeability and causing leakage of ions, proteins, ATP, and nucleic acids, inhibiting key metabolic enzymes, disrupting energy production, inducing oxidative stress, interfering with DNA and protein synthesis, suppressing quorum sensing, and inhibiting biofilm formation, all of which minimize the potential for microbial resistance compared with traditional antibiotics [10,13,21].

2.3 Factors Affecting Antimicrobial Activity

The antimicrobial activity of essential oils is affected by many factors, including intrinsic factors (e.g., chemical composition, extraction method, concentration, storage temperature, pH, water activity, and food matrix components, such as fats and proteins) and extrinsic factors (e.g., variation in chemical composition, extraction method, concentration, storage temperature, pH, water activity, and food matrix components, such as fats and proteins) [12,13]. In addition, essential oil efficacy is affected by differences in the chemical composition of microbial cell walls, the physiological state of the target microorganism, and the ability of pathogens to form biofilms. Gram-negative bacteria in general are more resistant than Gram-positive bacteria because of the outer membrane that restricts the access of hydrophobic compounds. To enhance essential oil stability, solubility, and controlled release in food systems, various advanced delivery systems, such as nanoemulsions, liposomes, and biodegradable edible coatings, have been developed [22–24].

2.4 Safety and Toxicological Considerations

While essential oils are generally recognized as safe (GRAS) when used within recommended limits, safety is dosage dependent and is influenced by the chemical composition, route of exposure, and duration of use. High concentrations may cause cytotoxicity, allergic reactions, mucosal irritation, or undesirable sensory changes in food products; therefore, essential oils require extensive toxicological evaluation, standardized quality control, and regulatory compliance before commercial application [23–25]. However, recent studies indicate that encapsulation technologies and synergistic combinations may allow for lower dosages to be used with equivalent antimicrobial efficacy and that this will enhance both safety and consumer acceptability [23–25].

Table 1. Major Essential Oils, Their Principal Bioactive Compounds, and Target Foodborne Pathogens

Essential Oil	Major Compound	Target Pathogens	Reported Mechanism
Oregano	Carvacrol	*E. coli*, *Salmonella*	Membrane disruption
Thyme	Thymol	*Listeria monocytogenes*	Cell leakage
Cinnamon	Cinnamaldehyde	*Staphylococcus aureus*	Protein inhibition
Clove	Eugenol	*Bacillus cereus*	Cell membrane damage
Tea Tree	Terpinen-4-ol	Mixed bacteria	Oxidative stress

3. SYNERGISTIC ESSENTIAL OIL COMBINATIONS

In fact, combining two or more essential oils has been proposed as a way to enhance antimicrobial activity against foodborne pathogens, and synergistic essential oil combinations have been shown to improve bacterial inhibition by targeting multiple cellular pathways at lower concentrations of individual oils while reducing toxicity and minimizing undesirable sensory effects in food products [18-21].

3.1 Concept of Synergy

Synergy is the concept that the combined antimicrobial effect of two or more essential oils is greater than the sum of their individual effects, and synergistic interactions promote microbial inhibition through complementary mechanisms such as membrane disruption, enzyme inhibition, oxidative stress induction, and biofilm suppression, decreasing the likelihood of antimicrobial resistance [18,19].

3.2 Experimental Methods for Synergy Evaluation

To assess synergistic interactions between essential oils, several laboratory methods are employed, including the checkerboard assay, time-kill assay, disk diffusion test, and broth microdilution method, which measure synergistic, additive, indifferent, or antagonistic antimicrobial effects against target pathogens [22,23].

3.3 Fractional Inhibitory Concentration (FIC) Index

The most commonly used approach to quantify synergy is the Fractional Inhibitory Concentration (FIC) Index, which compares the MIC of each essential oil alone and in combination; an FIC index ≤ 0.5 indicates synergy, 0.5–1.0 indicates an additive effect, 1–4 suggests no interaction, and >4 indicates antagonism [22].

3.4 Reported Synergistic Essential Oil Pairs

Combinations of essential oils have shown increased antimicrobial activity against foodborne pathogens such as Escherichia coli, Salmonella enterica, Listeria monocytogenes, and Staphylococcus aureus, which reduces microbial growth, inhibits biofilm formation, and increases the efficacy of food preservation [23–25].

3.5 Challenges in Combination Formulation

Essential oil combinations present a number of difficulties despite their promising antibacterial potential, such as chemical co mposition fluctuation, low water solubility, volatility, instability during food processing, and potential sensory changes in food For commercial application to be effective, standardization of formulations, dose optimization, sophisticated encapsulating pr ocesses, and regulatory approval are still crucial [21,24,25].

Table 2. Reported Synergistic Essential Oil Combinations and Their Antimicrobial Performance

Combination	Pathogen	Synergy Method	Reported Effect
Oregano + Thyme	*Salmonella enterica*	Checkerboard	Strong synergy
Cinnamon + Clove	*E. coli*	Time-kill assay	Enhanced inhibition
Tea Tree + Lemongrass	*Listeria*	MIC	Moderate synergy
Thyme + Rosemary	*S. aureus*	FICI	Synergistic

4. NETWORK PHARMACOLOGY APPROACH

Network pharmacology combines principles from biology, pharmacology, bioinformatics, and computational methods to explore how multiple compounds interact with various molecular targets and signaling pathways. This approach enables a comprehensive view of how natural products exert their therapeutic effects.

4.2 Compound–Target Identification

The initial step involves pinpointing the primary bioactive components in essential oils and predicting their potential molecular targets using chemical and biological databases. This process helps identify which specific compounds contribute to antimicrobial activity.

4.3 Target Protein Prediction

Once candidate compounds are identified, their likely protein targets are analyzed to assess roles in microbial growth, metabolism, virulence, and survival. This analysis aids in highlighting key proteins that could be inhibited to combat foodborne pathogens effectively.

4.4 Protein–Protein Interaction (PPI) Network Analysis

By mapping interactions among target proteins, PPI network analysis identifies central or "hub" proteins that play critical roles in microbial cellular functions. These hub proteins are considered high-priority targets for antimicrobial strategies.

4.5 Gene Ontology (GO) Enrichment

GO enrichment categorizes predicted target proteins based on biological processes, molecular functions, and cellular locations. This classification sheds light on the biological roles influenced by essential oil components.

4.6 KEGG Pathway Analysis

KEGG pathway mapping reveals metabolic and signaling pathways linked to the target proteins. It clarifies how essential oil compounds interfere with vital microbial processes such as energy production, biofilm development, and pathogenicity.

4.7 Compound–Target–Pathogen Interaction Network

This network integrates data on bioactive compounds, their molecular targets, and foodborne pathogens into a visual model. It supports the identification of key active ingredients, major targets, and possible synergistic effects, guiding the rational design of effective natural antimicrobials.

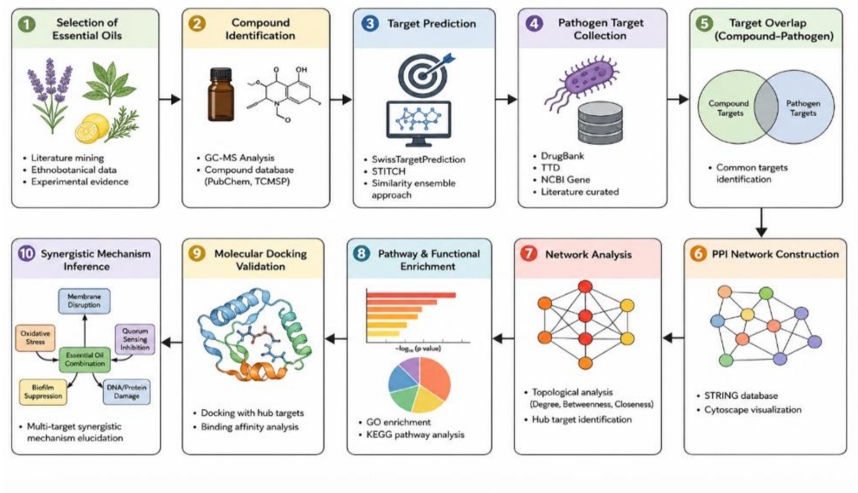

Figure 1. Workflow of the Network Pharmacology Framework for Evaluating Synergistic Essential Oil Combinations

5. MOLECULAR MECHANISMS OF SYNERGISTIC ANTIMICROBIAL EFFECTS

The enhanced antimicrobial effect of essential oils arises from the combined action of multiple compounds acting on different cellular sites. This multi-target mechanism increases effectiveness and lowers the risk of resistance.

5.1 Membrane Disruption

Compounds like thymol, carvacrol, and eugenol compromise bacterial cell membranes by increasing permeability, leading to leakage of cellular contents, loss of structural integrity, and ultimately cell death.

5.2 Induction of Oxidative Stress

Many essential oils promote the accumulation of reactive oxygen species (ROS), causing damage to lipids, proteins, and DNA. When ROS levels exceed bacterial defense capacity, oxidative stress accelerates cell death.

5.3 Inhibition of Quorum Sensing

Essential oils can block bacterial communication systems by interfering with signaling molecules, thereby reducing the expression of virulence factors and disrupting coordinated group behaviors.

5.4 Suppression of Biofilm Formation

These oils hinder both the formation and stability of biofilms by preventing bacterial attachment and breaking down the extracellular matrix, making bacteria more vulnerable to antimicrobial agents.

5.5 Damage to DNA and Proteins

Certain components interact directly with bacterial genetic material and enzymes, impairing DNA replication, transcription, and metabolic functions, which collectively inhibit microbial growth.

5.6 Multi-target Actions Revealed Through Network Pharmacology

Studies using network pharmacology show that essential oil compounds simultaneously affect multiple pathways—such as membrane integrity, stress responses, energy metabolism, and virulence control. This broad mode of action underpins their strong synergistic effects and reduced resistance potential.

Natural Resources for Human Health
eISSN: 2583-1194

6. APPLICATIONS IN FOOD PRESERVATION

Due to their wide-ranging antimicrobial and antioxidant properties, essential oils are increasingly adopted as natural preservatives. Their integration into packaging, edible coatings, and delivery systems improves food safety, quality, and longevity.

6.1 Active Packaging

Incorporating essential oils into packaging films helps suppress microbial growth and oxidation, enhancing food freshness and safety.

6.2 Nanoemulsion Delivery Systems

Nanoemulsions enhance the solubility, stability, and controlled release of essential oils, improving efficacy while minimizing sensory changes in food.

6.3 Edible Coatings

Coatings infused with essential oils form a protective layer on food surfaces, limiting moisture loss, microbial contamination, and oxidative spoilage.

6.4 Application in Meat and Dairy

In meat and dairy products, essential oils help manage pathogens and spoilage organisms, maintaining product quality and extending shelf life.

6.5 Use in Fruits and Vegetables

Sprays or coatings based on essential oils delay microbial decay, reduce postharvest losses, and preserve the freshness of produce.

6.6 Shelf-Life Extension

By slowing microbial proliferation and oxidative degradation, essential oils significantly prolong the usable life of various foods and reduce reliance on synthetic additives.

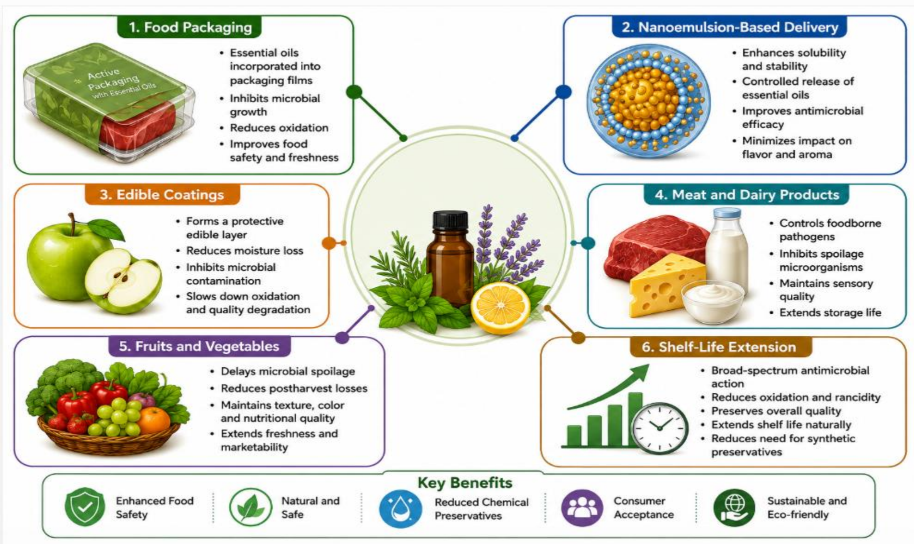

Figure 2. Applications of Synergistic Essential Oil Combinations in Food Preservation

7. CURRENT CHALLENGES AND FUTURE PERSPECTIVES

Despite their potential, broader use of synergistic essential oil blends is hindered by inconsistent composition, lack of standardized formulations, and regulatory barriers. Progress in artificial intelligence, multi-omics, and network pharmacology is expected to drive innovation in developing safe and effective preservation solutions.

7.1 Variability in Essential Oil Composition

The chemical profile of essential oils varies depending on plant species, growing conditions, climate, and extraction techniques, resulting in inconsistent antimicrobial performance.

7.2 Standardization Issues

Without uniform extraction protocols, quality controls, or dosage recommendations, reproducibility and commercial scalability remain limited.

7.3 Regulatory Considerations

Differing food safety regulations and approval requirements across countries complicate the industrial adoption of essential oil-based preservatives.

7.4 Integration of AI and Machine Learning

AI and machine learning tools can accelerate the discovery of synergistic combinations, refine formulations, and identify new antimicrobial candidates more efficiently.

7.5 Multi-omics and Network Pharmacology

Integrating genomics, transcriptomics, proteomics, metabolomics, and network modeling offers deeper understanding of complex antimicrobial mechanisms and synergies.

7.6 Clinical and Industrial Translation

Future efforts should prioritize large-scale testing, formulation stability, safety evaluation, and commercial feasibility to support real-world implementation.

8. CONCLUSION

Synergistic combinations of essential oils offer a viable natural alternative for controlling foodborne pathogens through simultaneous effects on multiple microbial targets. Innovations in network pharmacology, artificial intelligence, and multi-omics technologies open new avenues for optimizing these mixtures, improving food preservation, extending shelf life, and decreasing dependence on synthetic chemicals. Widespread application will depend on greater standardization and rigorous industrial validation.

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