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Synergistic Antimicrobial Activity of Essential Oils and Vitamin C: Mechanisms, Molecular Targets and Therapeutic Potential

Sylvia Stamova [1,*], Neli Ermenlieva [2], Gabriela Tsankova [2], Silviya P. Nikolova [3] and Emilia Georgieva [4]

[1] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Varna, 9000 Varna, Bulgaria
[2] Department of Microbiology and Virusology, Faculty of Medicine, Medical University of Varna, 9000 Varna, Bulgaria; n.ermenlieva@abv.bg (N.E.); gabriela.tsankova@mu-varna.bg (G.T.)
[3] Department of Social Medicine and Health Care Organization, Faculty of Public Health, Medical University of Varna, 9000 Varna, Bulgaria; silviya.p.nikolova@gmail.com
[4] Training Sector "Medical Laboratory Technician", Medical College—Varna, Medical University of Varna, 9000 Varna, Bulgaria; emiliya.georgieva@mu-varna.bg
* Correspondence: sylviastamova@yahoo.com; Tel.: +359-885-78-12-22

Abstract: Antimicrobial resistance (AMR) poses an increasing challenge to modern medicine, necessitating the search for novel therapeutic strategies. This study evaluates the synergistic antimicrobial activity of oregano essential oil (OEO) and thyme essential oil (TEO) in combination with vitamin C against clinically relevant pathogens. The antimicrobial activity was assessed using MIC, MBC and MFC assays, alongside in silico analyses utilizing Swiss Target Prediction, STRING and Reactome to identify potential molecular targets and underlying biological mechanisms. The results demonstrate that the combination of oregano oil and vitamin C significantly reduces MIC and MBC values against *E. coli* and *C. albicans*, indicating a synergistic effect, while an antagonistic interaction was observed against *S. aureus*. Protein interaction analysis revealed that thymol and carvacrol inhibit NF-κB and activate Nrf2, leading to the modulation of inflammatory and antioxidant responses. Additionally, carvacrol suppresses biofilm formation, while vitamin C enhances phagocytosis and the production of reactive oxygen species (ROS). These findings suggest that combining vitamin C with essential oils may serve as an effective adjuvant approach in antimicrobial therapy, particularly for infections associated with oxidative stress and chronic inflammation.

Keywords: antimicrobial activity; essential oils; vitamin C; synergistic effect; molecular targets; adjuvant therapy

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1. Introduction

Antimicrobial agents represent one of the most significant achievements of modern medicine, playing a key role in the treatment of infections and substantially reducing infection-related mortality [1]. They encompass a broad spectrum of compounds—antibiotics, antivirals, antifungals and antiprotozoal agents—whose mechanisms of action target either the inhibition of microbial growth or the elimination of pathogenic microorganisms [2]. Despite their effectiveness, antimicrobial agents face increasing challenges due to the rise of antimicrobial resistance (AMR)—a phenomenon where microorganisms develop resistance mechanisms against traditionally effective drugs [3]. AMR arises from both natural evolutionary processes and human-driven factors, such as inappropriate antibiotic use in healthcare and agriculture, as well as inadequate hygiene and sanitation practices [2]. As the prevalence of multidrug-resistant microorganisms increases, treatment

options become more limited, exacerbating the burden of infectious diseases. Globally, AMR is associated with higher mortality rates, increased treatment complications and prolonged hospital stays, leading to severe public health and economic consequences [4,5]. Current projections indicate that by 2050, AMR will account for approximately 10 million deaths annually, highlighting the urgent need for innovative therapeutic strategies [6]. In recent years, there has been a growing scientific interest in natural compounds as potential alternatives to synthetic antimicrobial agents. An increasing body of evidence suggests that plant extracts and essential oils contain bioactive constituents with notable antimicrobial, antiviral, antifungal and immunomodulatory properties [7–9]. Among them, the essential oils of oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) exhibit particularly strong activity, attributed primarily to the presence of carvacrol and thymol [10–12].

The selection of oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) essential oils in the present study is based not only on their well-established antimicrobial activity but also on their chemical composition, dominated by the phenolic monoterpenes carvacrol and thymol. These compounds are structural isomers that, despite sharing the same molecular formula, differ in hydrophobicity, hydroxyl group positioning and their mode of interaction with microbial cell membranes [13,14].Studies have shown that both isomers disrupt membrane integrity, leading to ion gradient loss and leakage of cellular contents, although with varying degrees of effectiveness depending on the pathogen [14,15]. Moreover, when used in combination, they demonstrate additive or synergistic effects, which increases interest in their potential as co-agents with other biologically active substances such as vitamin C for enhanced antimicrobial efficacy [16,17].

The antimicrobial activity of vitamin C is well-documented [18,19], and its combination with the selected essential oils represents a promising strategy for achieving a synergistic effect.It is hypothesized that high concentrations of vitamin C may lower intracellular pH in microorganisms, potentially amplifying the effects of essential oils. A similar mechanism has been observed with weak organic acids, such as acetate and lactate, which penetrate microbial cells and cause intracellular acidification, compromising their physiological functions [20]. While this specific effect has not been extensively studied in the context of vitamin C, similar mechanisms involving weak organic acids have been described previously [21]. Preliminary studies indicate that short-chain fatty acids (SCFAs) in acidic environments can enhance the efficacy of azole antifungals and antibiotics against *Candida albicans* and *Escherichia coli* [20,22]. This mechanism is likely associated with alterations in ionic homeostasis, membrane permeability and metabolic stress, which can enhance pathogen susceptibility to antimicrobial agents.Furthermore, the efficacy of antibiotics is influenced by environmental factors, including pH, metabolite availability and oxygen tension [20,23]. This highlights the importance of further research into the potential synergistic role of vitamin C in modulating environmental conditions that enhance antimicrobial efficacy.

The selection of *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* in this study was based on their high clinical relevance and increasing resistance to commonly used antimicrobial agents. *E. coli*, while naturally susceptible to many antibiotics, has become a major reservoir of resistance genes through horizontal gene transfer, including genes encoding extended-spectrum β-lactamases (ESBLs), carbapenemases and polymyxin resistance (e.g., mcr genes), making it a critical priority pathogen [24]. *S. aureus* represents a major cause of both hospital- and community-acquired infections and exhibits complex resistance mechanisms to β-lactams (notably methicillin), glycopeptides and other last-line antibiotics, posing significant treatment challenges [25]. *C. albicans*, the most prevalent cause of candidiasis and fungal bloodstream infections, demonstrates adaptive mechanisms such as biofilm formation and efflux pump overexpression, contributing to antifungal resistance, particularly against azoles like fluconazole [26]. These pathogens are frequently associated

with high morbidity and mortality, and their inclusion allows for a representative evaluation of antimicrobial efficacy across both bacterial and fungal species of major public health concern. Alternative treatment strategies, such as the use of chlorine dioxide, have shown promise in vitro for eradicating various antibiotic-resistant bacteria, including *E. coli* and *S. aureus* [27].

This study aims to investigate the synergistic antimicrobial activity of carvacrol and thymol—key components of *Origanum vulgare* and *Thymus vulgaris* essential oils—in combination with vitamin C against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. In addition, the study explores the potential involvement of host-related molecular targets associated with inflammation, oxidative stress and immune modulation, in order to better understand the mechanisms and broader therapeutic potential of these compounds beyond their direct antimicrobial effects.

2. Materials and Methods

2.1. Essential Oils and Ascorbic Acid

The oregano essential oil (OEO) and thyme essential oil (TEO) used in this study were purchased from the commercial supplier *doTERRA*® and are 100% pure, composed of certified organic ingredients. L-Ascorbic acid (≥99%, Product № A5960, CAS: 50-81-7, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) was purchased as a dry substance from Sigma Aldrich (St. Louis, MA, USA).

2.2. Gas Chromatography–Mass Spectrometry Analysis

For this analysis, an Agilent 7890A coupled with a flame ionization detector (FID) and Agilent 5975C mass spectrometric detector (Agilent Technology, Santa Clara, CA, USA) were employed. The separation was conducted using a Stabilwax column (Restek) with dimensions of 30 m in length, 0.25 mm in internal diameter and a film thickness of 0.25 µm. The temperature program commenced at 65 °C, with a ramp to 170 °C at a rate of 1.5 °C/min, resulting in a total analysis time of 70 min. Both the injector and detector temperatures were set to 250 °C, including the FID. The carrier gases used were hydrogen and helium, each at a flow rate of 0.8 mL/min. The mass spectrometric detector operated within a scan range of m/z 40–450. A sample volume of 1.0 µL was injected in split mode with a 100:1 split ratio. Compound identification was achieved by comparing retention times and Kovats retention indices (RI) against reference standards, supplemented by mass spectral data comparison utilizing the NIST'08 (National Institute of Standards and Technology, Gaithersburg, MD, USA) and Adams Library databases.

2.3. Microbial Strains

We performed the antimicrobial tests with three referent microbial strains: Gram-negative *Escherichia coli* (ATCC 25922), Gram-positive *Staphylococcus aureus* (ATCC 29213) and the fungal strain *Candida albicans* (ATCC 10231). For the purposes of our research, we used three methods for testing antimicrobial activity—the serial dilution method for determining the minimum inhibitory concentration (MIC), determining the minimum bactericidal concentration (MBC) and the agar dilution technique.

2.4. Determination of Minimum Inhibitory Concentration (MIC)

The concentrations of essential oils used in this study ranged from 0.035% to 5% (v/v), with sterile DMSO as the diluent. To ensure maximum solubility and stability, all stock solutions of essential oils were initially prepared in DMSO. Following this, two-fold serial dilutions were performed in Brain Heart Infusion (BHI) broth.Sterile distilled water was used to prepare aqueous vitamin C solutions at concentrations ranging from 0.15 to 20 mg/mL. Serial dilutions of thyme and oregano essential oils were prepared in Brain

Heart Infusion broth (BHIb) (HiMedia, Ridacom, Sofia, Bulgaria) at concentrations ranging from 0.035% (v/v) to 5% (v/v) in a final volume of 1 mL. Following dilution, 0.5 mL of an aqueous vitamin C solution at a concentration of 20 mg/mL was added to each tube. Subsequently, 0.1 mL of a standardized microbial suspension (0.5 McFarland standard) was inoculated into all test tubes, which were then incubated under specific conditions. Three positive controls were included, each containing 1 mL of BHI broth and 0.1 mL of a standardized suspension of the three test microorganisms. Additionally, four negative controls were prepared, consisting of 1 mL of BHI broth supplemented with either oregano essential oil, thyme essential oil, vitamin C, or DMSO, respectively. A control sample was included to assess the potential interaction of the DMSO solution with the growth of bacterial and fungal microorganisms. Using the same methodology, separate samples were prepared to evaluate the antimicrobial activity of essential oils alone (0.035% to 5% v/v) and vitamin C alone (0.15 to 20 mg/mL). All tests were conducted in triplicate. The incubation period was set at 24 h at 37 °C for *E. coli* and *S. aureus*, while the test tubes containing *C. albicans* were incubated for 48 h at 35 °C. The minimum inhibitory concentration (MIC) was determined as the lowest concentration (highest dilution) of the antimicrobial agent that prevented visible turbidity.

2.5. Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

To determine the MBC and MFC for the combinations of oregano essential oil and vitamin C, thyme essential oil and vitamin C, and the individual active compounds against *E. coli*, *S. aureus* and *C. albicans*, an inoculum from each sample was streaked onto blood agar plates using a sterile loop. All experiments were performed in triplicate. The agar plates containing *E. coli* and *S. aureus* were incubated at 37 °C for 24 h, while those with *C. albicans* were incubated at 35 °C for 48 h. The lowest concentration of the active compounds at which microbial growth was reduced by 99.9% was considered the MBC or MFC.

2.6. Agar Dilution Method

Muller–Hinton agar (MHA) (HiMedia, Ridacom, Sofia, Bulgaria) was used for this assay. Ten milliliters (mL) of MHA were mixed at 46–48 °C with 1 mL of the active compound in a glass container. The suspension was thoroughly mixed and poured into 60mm Petri dishes. Once the agar solidified completely, 0.1 mL of a standardized microbial suspension (0.5 McFarland standard) was spread onto the surface using a densitometer-calibrated inoculum.The antimicrobial agents were tested at an initial concentration of 5% (v/v) for essential oils and 20 mg/mL for vitamin C. Samples containing *E. coli* and *S. aureus* were incubated aerobically for 24 h at 37 °C, while *C. albicans* cultures were incubated aerobically for 48 h at 35 °C. All tests were performed in triplicate.

2.7. Statistical Analysis

The obtained data were analyzed using one-way analysis of variance (ANOVA) to compare the mean values of MIC, MBC and MFC among different samples. When significant differences between groups were identified, Tukey's post-hoc test was applied for multiple comparisons. To further assess statistical significance between individual sample pairs, Student's *t*-test was conducted. The relationship between essential oil concentration and antimicrobial activity was evaluated using Pearson or Spearman correlation analysis, depending on data distribution.

2.8. Host Modulation Effects Based on Target Prediction

To assess the binding potential of the main components of essential oils (carvacrol and thymol) and vitamin C to human proteins, as well as to investigate their potential associa-

tion with antimicrobial, antioxidant, immunomodulatoryand anti-inflammatory activities, their chemical structures were analyzed using the UniProtKB/Swiss-Prot database and the SwissTargetPrediction platform. For each analyzed compound, the top 15 target proteins were identified and selected for further investigation [28,29].

The selected proteins were input into the STRING network to search for potential protein-protein interactions and to construct a network that provides insights into the biological processes associated with these targets [30].

Reactome analysis of the selected proteins was performed to identify pathways and biological processes associated with these targets. The proteins were input into the Reactome database, which provides information on molecular pathways and cellular interactions [31].

3. Results

3.1. Gas Chromatography–Mass Spectrometry Analysis

In our study, a total of 22 compounds were identified in OEO and 21 compounds in TEO. The predominant constituents of OEO, detected at the highest concentrations, include carvacrol (81.20%), *p*-cymene (3.69%) and ɣ-terpinene (3.52%). In TEO, the major components comprise thymol (41.84%), *p*-cymene (19.85%), ɣ-terpinene (12.64%), β-caryophyllene (2.88%) and carvacrol (2.14%) (Table 1). Given the antimicrobial activity under investigation, the relative abundance of these key constituents is of paramount significance, as it directly influences the bioactivity and efficacy of the essential oils.

Table 1. Chemical composition of thyme and oregano EOs.

No. Compound	Retention Time (Min)	% of Total Ion Current	Retention Time (Min)	% of Total Ion Current
	Thyme EO		Oregano EO	
α-Thujene	9.11	1.38	9.05	0.12
α-Pinene	9.32	1.32	9.27	0.43
Camphene	9.84	1.19	9.79	0.16
β-Pinene	10.75	0.76	10.71	0.08
β-Myrcene	11.23	0.88	11.15	0.64
α-Phellandrene	-	-	11.16	0.11
α-Terpinene	12.06	1.18	12.02	0.90
p-Cymene	12.31	19.85	12.28	3.69
Limonene	12.47	0.80	12.40	0.13
β-Phellandrene	-	-	12.45	0.21
ɣ-Terpinene	13.41	12.64	13.37	3.52
Sabinene hydrate	13.79	0.57	13.73	0.16
β-Linalool	14.74	2.03	14.73	3.76
Camphor	16.12	1.17	-	-
Terpinolen	-	-	14.25	0.10
Borneol	16.90	1.81	16.87	0.71
α-Terpineol	-	-	17.60	0.24
Terpinen-4-ol	17.16	0.84	-	-

Table 1. *Cont.*

No. Compound	Retention Time (Min)	% of Total Ion Current	Retention Time (Min)	% of Total Ion Current
	Thyme EO		Oregano EO	
Thymol methyl ether	18.58	0.31	-	-
Carvacrol, methyl ether	18.84	1.39	-	-
Thymol	20.37	41.84	20.36	1.19
Carvacrol	20.75	2.14	20.75	81.20
β-Caryophyllene	23.77	2.88	23.70	0.97
Aromadendrene	-	-	24.18	0.15
β-Bisabolene	-	-	25.93	1.24
δ-Cadinene	26.19	0.64	-	-
Caryophyllene oxide	27.73	0.58	27.66	0.17

3.2. Dilution Method in Liquid Medium

3.2.1. MIC and MBC Results for the Combination of Oregano Essential Oil and Vitamin C

The minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) of oregano essential oil and its combination with vitamin C were determined using the broth dilution method. The OEO + Vit C combination exhibited MIC values below 0.035% (*v*/*v*) against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. In contrast, the MIC values for pure OEO were 0.31% for *E. coli* and 0.62% for both *S. aureus* and *C. albicans*. The MBC/MFC values for the combination were also lower, measured at <0.035% for *E. coli* and 0.07% for *S. aureus* and *C. albicans*. Pure OEO showed MBC/MFC values of 0.31% for *E. coli*, while for *S. aureus* and *C. albicans* they exceeded 5% (*v*/*v*).

3.2.2. MIC and MBC Results for the Combination of Thyme Essential Oil and Vitamin C

Similar to the oregano combination, the thyme essential oil (TEO) combined with vitamin C also antimicrobial activity. The MIC values of the combination were below 0.035% (*v*/*v*) for all tested strains. For comparison, pure TEO exhibited MIC values of 1.25% for *E. coli*, 0.62% for *S. aureus* and 1.25% for *C. albicans*. The MBC/MFC values of the TEO + vitamin C combination were <0.035% for *E. coli* and 0.07% for *S. aureus* and *C. albicans*. In contrast, the MBC for pure TEO was 1.25% for *E. coli* and exceeded 5% for *S. aureus* and *C. albicans*. Table 2 presents the results of MIC and MBC/MFC of combinations oregano EO–vitamin C and thyme EO–vitamin C against *E. coli*, *S. aureus* and *C. albicans*.

Table 2. Determination of MIC and MBC/MFC of combinations oregano EO–vitamin C and thyme EO–vitamin C against *E. coli*, *S. aureus* and *C. albicans*.

	MIC OEO+ Vit C (*p*-Value)	MIC TEO+Vit C (*p*-Value)	MIC Oregano EO (*p*-Value)	MIC Thyme EO (*p*-Value)	MBC/MFC OEO+ Vit C (*p*-Value)	MBC/MFC TEO+ Vit C (*p*-Value)	MBC/MFC Oregano EO (*p*-Value)	MBC/MFC Thyme EO (*p*-Value)
E. coli	**<0. 035%** *(p < 0.05)*	**<0.035%** *(p < 0.05)*	**0.31%** *(p < 0.05)*	**1.25%** *(p < 0.05)*	**<0.035%** *(p < 0.05)*	**<0.035%** *(p < 0.05)*	**0.31%** *(p < 0.05)*	**1.25%** *(p < 0.05)*
S. aureus	**<0.035%** *(p < 0.05)*	**<0.035%** *(p < 0.05)*	**0.62%** *(p < 0.05)*	**0.62%** *(p < 0.05)*	**<0.07%** *(p < 0.05)*	**<0.035%** *(p < 0.05)*	**>5%** *(p < 0.05)*	**5%** *(p < 0.05)*
C. albicans	**<0.035%** *(p < 0.05)*	**<0.035%** *(p < 0.05)*	**0. 62%** *(p < 0.05)*	**1.25%** *(p < 0.05)*	**<0.07%** *(p < 0.05)*	**<0.035%** *(p < 0.05)*	**>5%** *(p < 0.05)*	**5%** *(p < 0.05)*

3.3. Determination of Antimicrobial Activityby the Agar Dilution Method

The antimicrobial activity of the essential oils (EOs) and their combinations with vitamin C was additionally assessed using the agar dilution method. This allowed for a comparative evaluation of antimicrobial efficacy under solid medium conditions. For *Escherichia coli*, the combination of oregano essential oil (OEO) at 5% (*v*/*v*) with vitamin C at 20 mg/mL resulted in complete growth inhibition. The minimum bactericidal concentration (MBC) for the combination was determined to be 4% (*v*/*v*), compared to the higher values observed for the individual components. In the case of *Candida albicans*, a decrease in the minimum fungicidal concentration (MFC) from 5% (*v*/*v*) for pure OEO to 2% (*v*/*v*) for the combination was observed.In contrast, results obtained for *Staphylococcus aureus* suggested a possible antagonistic interaction. The MBC of pure OEO was 4% (*v*/*v*), while the combination with vitamin C required a higher concentration of 5% (*v*/*v*) to achieve comparable antimicrobial activity.A similar pattern was observed with thyme essential oil (TEO). Against *C. albicans*, the combination with vitamin C reduced the MFC from 5% to 2% (*v*/*v*). However, for *E. coli* and *S. aureus*, no significant difference was noted between the activity of the pure TEO and the combination, with MIC values remaining at 5% (*v*/*v*) in both cases.Control experiments using vitamin C alone (1–20 mg/mL) showed no inhibitory effect on any of the tested strains following 24 h of incubation. These results confirm that, at the tested concentrations, vitamin C does not exhibit independent antimicrobial activity.A summary of the results obtained from the agar dilution method is presented in Table 3.

Table 3. Determination of MBC/MFC of combinations oregano EO–vitamin C and thyme EO–vitamin C against *E. coli*, *S. aureus* and *C. albicans* using the agar dilution method.

	MBC/MFC OEO+ Vit C (*p*-Value)	**MBC/MFC TEO+ Vit C (*p*-Value)**	**MBC/MFC Oregano EO (*p*-Value)**	**MBC/MFC Thyme EO (*p*-Value)**
E. coli	**4%** *(p ≈ 0.05)*	**5%** *(p ≈ 0.05)*	**Lack of activity**	**5%** *(p ≈ 0.05)*
S. aureus	**5%** *(p ≈ 0.05)*	**5%** *(p ≈ 0.05)*	**4%** *(p ≈ 0.05)*	**5%** *(p ≈ 0.05)*
C. albicans	**2%** *(p ≈ 0.05)*	**2%** *(p ≈ 0.05)*	**5%** *(p ≈ 0.05)*	**5%** *(p ≈ 0.05)*

3.4. Statistical Analysis

The analysis of MIC values revealed that the combinations of Oregano EO + Vitamin C and Thyme EO + Vitamin C resulted in a statistically significant reduction in MIC values compared to the individual essential oils ($p < 0.05$). The observed differences in MIC values among the various samples were statistically significant, confirming the synergistic effect of vitamin C when combined with essential oils. Results from the blood agar method further demonstrated significantly lower MBC/MFC values for the combined essential oils compared to the individual oils ($p < 0.05$). In contrast, differences observed using the agar dilution method were less pronounced, showing a general trend toward improved activity of the combinations, though these differences did not reach statistical significance ($p > 0.05$).Correlation analysis indicated a statistically significant positive correlation between the concentration of essential oils and their antimicrobial activity. The combinations with vitamin C exhibited an enhanced effect, further reinforcing the synergistic interaction between essential oils and vitamin C in antimicrobial applications. A summary of the results is presented in Figures 1–3.

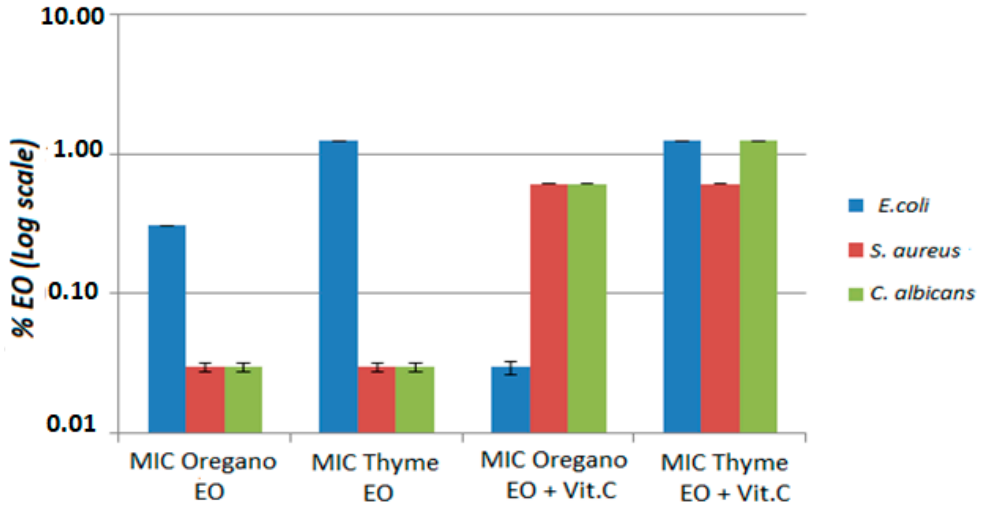

Figure 1. MIC (mean ± SD) of essential oils and vitamin C against *E. coli*, *S. aureus* and *C. albicans*.

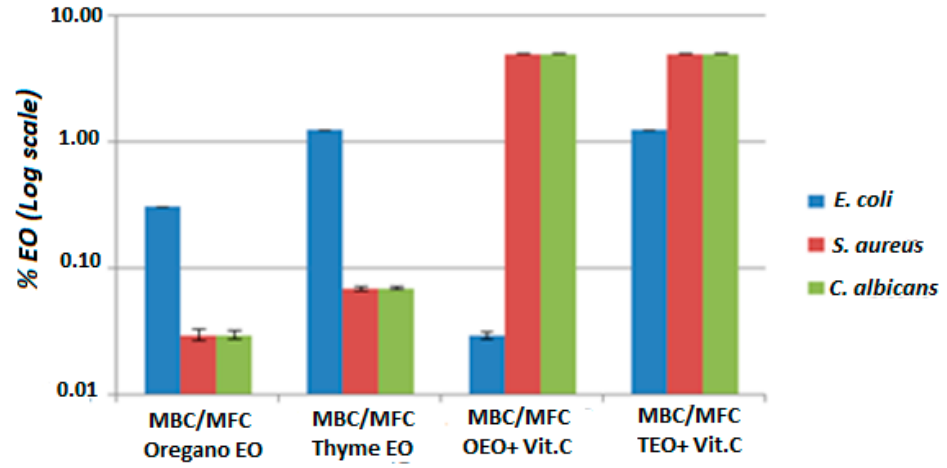

Figure 2. MBC/MFC (mean ± SD) of essential oils and vitamin C against *E. coli*, *S. aureus* and *C. albicans*.

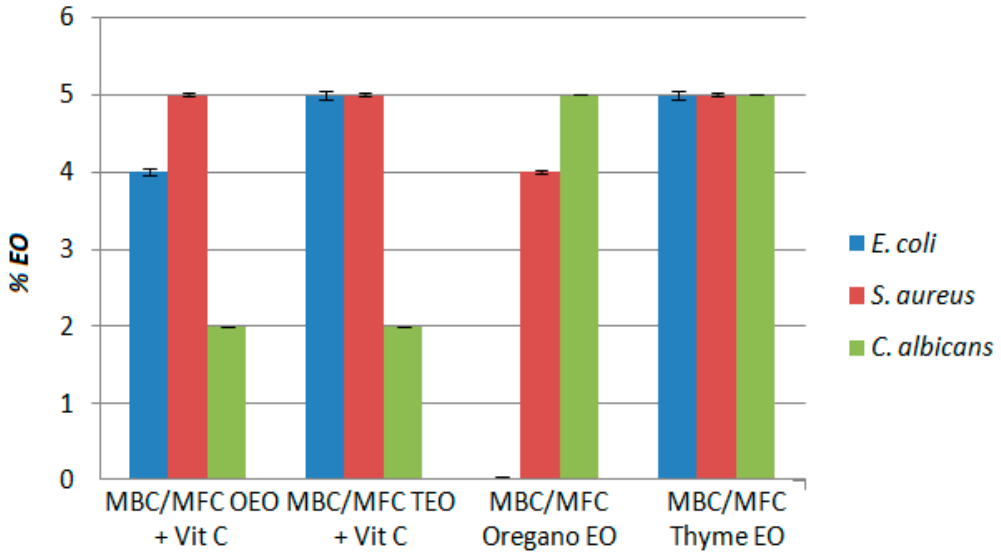

Figure 3. MBC/MFC (mean ± SD) of essential oils and vitamin C against *E. coli*, *S. aureus* and *C. albicans* using the agar dilution method.

The experimental results from the combination of oregano and thyme essential oils with vitamin C demonstrated a significant synergistic effect against certain pathogens, while exhibiting an antagonistic effect against others. To complement the observed antimicrobial effects, we performed in silico analyses to identify potential human protein targets and related biological pathways modulated by the active compounds. We analyzed the interactions between the target proteins, mapped their biological function networksand identified the biochemical pathways influenced by these molecules to better understand how these interactions contribute to their antimicrobial effects.

3.5. Host Modulation Effects Based on Target Prediction

To complement the in vitro findings, an in silico analysis was conducted to predict potential interactions between carvacrol, thymol and vitamin C and human proteins. The SwissTargetPrediction platform [28] was used to identify the 15 most probable targets for each compound, based on structural similarity and probability scores derived from the UniProtKB/Swiss-Prot database [29]. The predicted targets and their corresponding UniProt identifiers are shown in Figure 4.

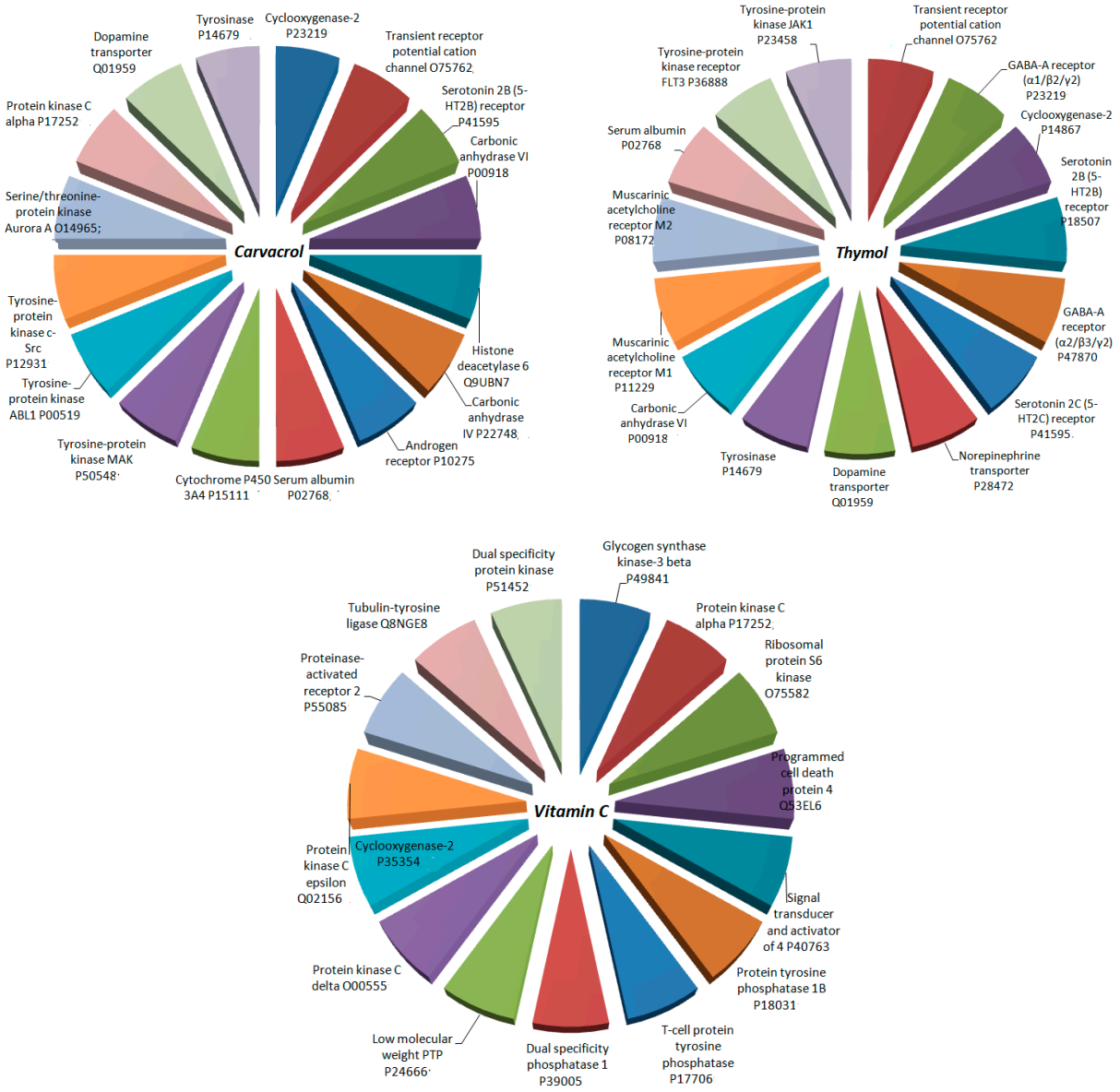

Figure 4. ProteinTargets and UniProt IDs of selected proteins [28,29].

Thymol and carvacrol exhibited a comparable number of predicted targets. Among the most frequently identified protein classes for thymol were serotonin receptors (5-HT2B, 5-HT2C), GABA-A receptors, the norepinephrine transporter, cyclooxygenase-2 (COX-2) and tyrosine–protein kinases. Carvacrol shared several of these targets but also showed interactions with TRP channels and enzymes involved in metabolic pathways. In contrast, vitamin C was associated with a distinct set of targets, including oxidoreductases and proteins related to cell cycle regulation and antioxidant activity.

The STRING analysis revealed that the predicted targets of thymol, carvacrol and vitamin C were functionally associated with key biological processes, including inflammatory signaling, antioxidant defense and components of the innate immune response. The resulting interaction network revealed three distinct functional clusters, each associated with specific biological processes, as shown by the color-coded groupings in Figure 5. The largest cluster comprises proteins involved in inflammatory signaling and immune regulation, including GSK3B, JAK1 and STAT3—key modulators of pathways such as NF-κB, IL-6 and TNF-α. The second cluster is primarily associated with antioxidant responses, while the third includes targets related to neurotransmission and cellular homeostasis.

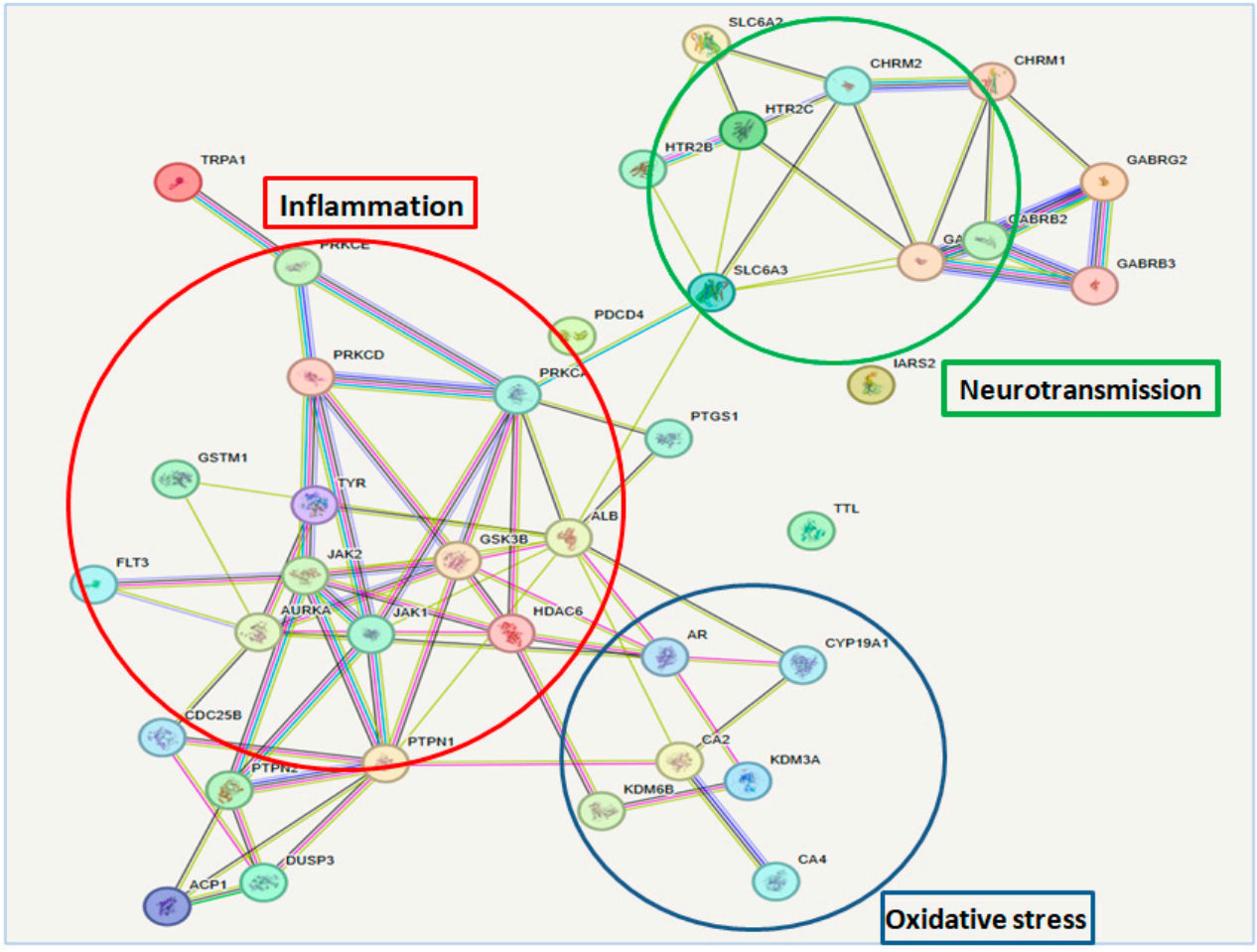

Figure 5. Protein–protein interaction (PPI) network of predicted human targets of carvacrol, thymoland vitamin C, generated using STRING v11.5 [30]. Nodes represent proteins; edges indicate predicted functional associations. Line colors reflect the source of interaction evidence: pink (experiments), blue (databases), green (neighborhood), black (co-expression), yellow (text mining) and light blue (co-occurrence). The three functional clusters—inflammation (red circle), neurotransmission (green circle) and oxidative stress (blue circle)—are labeled based on biological roles of the included proteins. Key nodes such as JAK1, STAT3and GSK3B appear centrally within the inflammatory cluster.

Subsequent pathway analysis using the Reactome [31] database identified a total of 136 signaling pathways associated with at least one of the predicted targets. These included pathways involved in cytokine signaling, transcriptional regulation, redox homeostasis, cellular stress responses and general metabolic processes. A summary of the enriched pathways linked to the three compounds is provided in Figure 6.

This analysis offers a closer look at the predicted interactions between protein targets, drawing attention to key hubs such as JAK1, STAT3 and GSK3B—known regulators of inflammatory pathways involving NF-κB and IL-6. To further interpret these interactions, Reactome analysis was performed, identifying 136 signaling pathways associated with at least one predicted target, including cytokine signaling, redox homeostasis, mitochondrial function and cell cycle regulation. Although descriptive, the findings provide valuable insight into potential host-mediated mechanisms through which the studied compounds might exert effects related to oxidative stress, immune responses and cellular signaling.

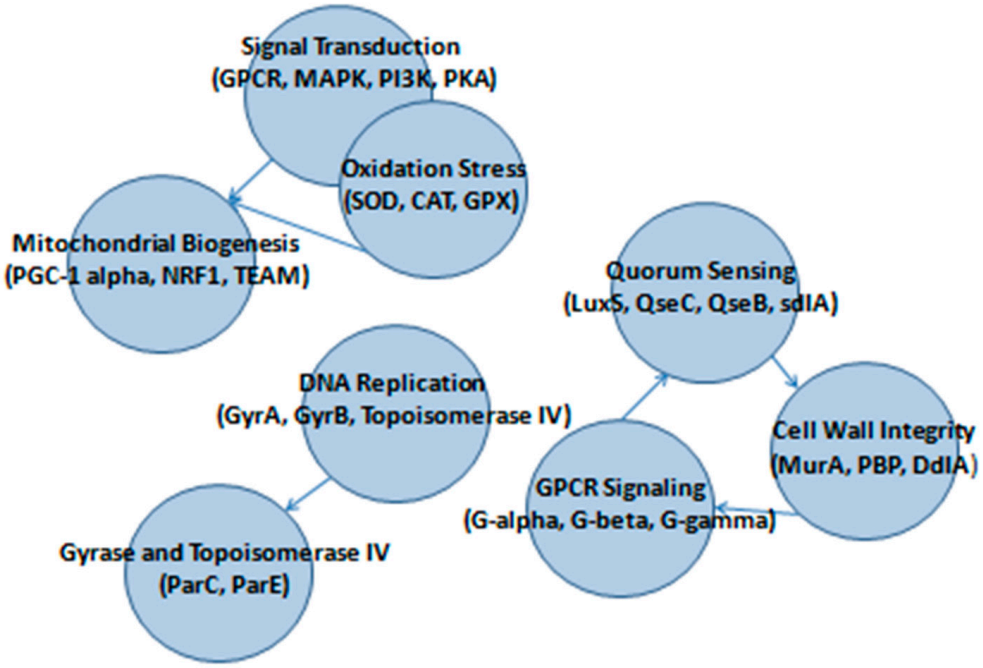

Figure 6. Functional clustering of predicted molecular targets modulated by carvacrol, thymol and vitamin C. Each node represents a biological process, with key proteins or genes indicated in parentheses. Lines denote functional associations. The clusters highlight processes relevant to antimicrobial action and host modulation, including oxidative stress, signal transduction, mitochondrial biogenesis, DNA replication, quorum sensing and cell wall integrity.

4. Discussion

The present study demonstrates significant differences in antimicrobial activity when essential oils are combined with vitamin C, compared to their individual application. Notably, the combination of oregano essential oil (OEO) and vitamin C resulted in a pronounced decrease in MIC, MBC and MFC values against *E. coli* and *C. albicans*, suggesting a synergistic effect. The MIC of OEO combined with vitamin C was several-fold lower than that of pure OEO, indicating that vitamin C enhances antimicrobial efficacy. However, a potential antagonistic effect was observed against *S. aureus*, where the combination was less effective than the essential oil alone. A similar pattern was observed for thyme essential oil (TEO) combined with vitamin C. While no synergy was detected against *E. coli* and *S. aureus*, enhanced antifungal activity was noted against *C. albicans*. Importantly, vitamin C alone did not exhibit antimicrobial activity at the tested concentrations (1–20 mg/mL), which aligns with previous studies reporting its lack of direct antimicrobial effects in neutral

conditions [32,33]. This observation supports the idea that vitamin C may modulate the physicochemical environment rather than act as a direct antimicrobial agent [34–36]. Moreover, vitamin C, being a weak organic acid, may reduce the pH of the surrounding medium, thereby influencing the ionization and diffusion of essential oil constituents [34,37,38]. The observed antagonistic effect against *S. aureus* may reflect interference between the molecular targets or competitive interactions among active compounds, which has been documented in previous studies on essential oil combinations [39]. These findings support the hypothesis that vitamin C may act as a synergistic adjuvant, enhancing the antimicrobial effects of certain plant-derived compounds. This is consistent with evidence that vitamin C and related redox-active compounds can influence immune signaling pathways such as NF-κB and Nrf2, which are also known to modulate inflammatory responses and cellular defense mechanisms [40–42]. However, the effects are strain-specific, highlighting the complexity of phytochemical interactions. Similar synergistic, additive and antagonistic interactions have been reported in the literature [43,44]. For instance, Semenius et al. reported antagonistic effects when thyme oil was combined with parsley, lovage and basil oils [44]. Likewise, the combination of amoxicillin with methanolic extracts of *Tetraclinis articulata* showed reduced activity against *S. aureus* [45]. In fact, true synergy is relatively rare, whereas additive or even antagonistic effects are more commonly observed among plant-derived compounds [46,47]. One possible explanation for the antagonism observed against *S. aureus* is the competition between bioactive components of essential oils for shared molecular targets, or chemical interactions that reduce efficacy [35,43]. Additionally, vitamin C might alter the ionization state of essential oil constituents through pH modulation, which can influence their biological activity. This mechanism has been discussed in relation to acidic shifts in the medium that affect essential oil solubility and reactivity [37,38]. The absence of a pH-matched control in this study limits our ability to fully separate pH effects from compound-specific interactions [39]. This limitation has now been acknowledged. Discrepancies between broth and agar dilution methods were also observed, particularly for *S. aureus*. Several factors may account for these differences, including diffusion limitations, medium composition and the physicochemical nature of essential oils. In solid media, the dilution of active compounds occurs in 10 mL of moltenagar, compared to only 1 mL of broth in the microdilution method. This ten-fold increase in volume can significantly lower the final concentration of active components, especially for volatile and hydrophobic substances. Additionally, the solid matrix impedes uniform diffusion of essential oils, which maylead to the underestimation of their actual antimicrobial potential. The selimitations are particularly relevant for lipophilic compounds with low solubility, suchas thymol and carvacrol [37–39].

In silico analysis provided additional insight into possible molecular mechanisms underlying the observed effects. Using Swiss Target Prediction, STRING and Reactome, we identified putative human protein targets of thymol, carvacrol and vitamin C. Thymol was associated with neuroreceptors and COX-2, indicating potential anti-inflammatory and sedative properties [48,49]. Carvacrol interacted with TRP receptors and metabolic enzymes, suggesting immunomodulatory and stress response effects [50,51]. Vitamin C primarily targeted oxidoreductases and enzymes involved in cell cycle regulation and antioxidant defense [52,53]. The STRING analysis indicated that these compounds influence pathways related to inflammation, oxidative stress and immune modulation. Specifically, suppression of NF-κB and activation of Nrf2 may contribute to reduced inflammation and enhanced cellular protection [54–56]. Vitamin C has also been associated with increased phagocytic activity and ROS production, mechanisms that play a critical role in innate immunity and are modulated under oxidative stress conditions [37,38,50,56]. Previous studies have associated thymol and carvacrol with strengthening of the epithelial barrier

and inhibition of biofilms [57,58], although such effects were not directly investigated in this study. While in silico analyses do not confirm antimicrobial mechanisms directly, they support the hypothesis that combined agents act through complementary pathways. Previous reports have also highlighted the potential of natural antioxidants such as vitamin C to support epithelial integrity and immune resilience under stress conditions, adding an additional layer to the interpretation of these results [36,50,56]. The data align with previous findings indicating that multi-component natural mixtures exert broader antimicrobial activity and are less prone to resistance development compared to single-target agents [59]. The observed effects may also involve the enhanced penetration of essential oil components facilitated by vitamin C, leading to oxidative and metabolic stress within microbial cells. This mechanism is supported by previous reports on the ability of antioxidants to modulate microbial redox balance, indirectly contributing to membrane destabilization and intracellular damage [37,38]. Thymol and carvacrol have been shown to disrupt bacterial membrane integrity and interfere with quorum-sensing pathways, both of which are critical for biofilm formation and antimicrobial resistance [60,61]. For instance, thymol has been reported to inhibit the MexAB-OprM efflux pump and act as a quorum-sensing inhibitor in *Pseudomonas aeruginosa*, enhancing susceptibility to antibiotics [39,61].

Finally, although the test strains used in this study (ATCC reference strains) do not carry known resistance genes, future work should involve multidrug-resistant clinical isolates to better evaluate therapeutic potential. In conclusion, our findings confirm that vitamin C can modulate the antimicrobial activity of essential oils, enhancing their effect in a strain-dependent manner. The combined antimicrobial and host-modulating properties of these compounds present a promising approach, though further in vivo validation and more rigorous pH-controlled studies are necessary to substantiate their use in clinical or food preservation settings.

5. Conclusions

The present study highlights the significance of combined antimicrobial strategies, exploring the potential of essential oils and vitamin C as adjuvants in therapy. The findings reveal that the effectiveness of these combinations depends on the interactions between the active compounds and the characteristics of the target microorganisms.The observed regulation of inflammatory and antioxidant mechanisms draws attention to the role of these substances not only as direct antimicrobial agents but also as modulating factors of the immune response. While the results demonstrate promising trends, further research is needed to gain a deeper understanding of the mechanisms of interaction and to assess clinical applicability.This study presents promising prospects for the development of innovative and more effective antimicrobial strategies, combining natural compounds with conventional therapies.

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References

1. Murugaiyan, J.; Kumar, P.A.; Rao, G.S.; Iskandar, K.; Hawser, S.; Hays, J.P.; Mohsen, Y.; Adukkadukkam, S.; Awuah, W.A.; Jose, R.A.M.; et al. Progress in Alternative Strategies to Combat Antimicrobial Resistance: Focus on Antibiotics. *Antibiotics* **2022**, *11*, 200. [CrossRef] [PubMed]
2. The New EU One Health Action Plan Against Antimicrobial Resistance. 2017. Available online: https://health.ec.europa.eu/system/files/2020-01/amr_2017_summary-action-plan_0.pdf (accessed on 10 April 2025).
3. Abushaheen, M.A.; Muzaheed; Fatani, A.J.; Alosami, M.; Mansy, W.; George, M.; Acharya, S.; Rathod, S.; Divakar, D.D.; Jhugroo, C.; et al. Antimicrobial resistance, mechanisms and its clinical significance. *Dis. Mon.* **2020**, *66*, 100971. [CrossRef] [PubMed]
4. Read, A.F.; Woods, R.J. Antibiotic resistance management. *Evol. Med. Public Health.* **2014**, 147. [CrossRef]
5. Khameneh, B.; Diab, R.; Ghazvini, K.; Fazly Bazzaz, B.S. Break throughs in bacterial resistance mechanisms and the potential ways to combat them. *Microb. Pathog.* **2016**, *95*, 32–42. [CrossRef]
6. O'Neil, J. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. Review on Antimicrobial Resistance. 2016. Available online: https://amr-review.org (accessed on 10 April 2025).
7. Rossiter, S.E.; Madison, H.F.; Wuest, W.M. Natural products as platforms to overcome antibiotic resistance. *Chem. Rev.* **2017**, *117*, 12415–12474. [CrossRef]
8. Boccolini, P.M.M.; Boccolini, C.S. Prevalence of complementary and alternative medicine (CAM) use in Brazil. *BMC Complement. Med. Ther.* **2020**, *20*, 51. [CrossRef]
9. Vaou, N.; Stavropoulou, E.; Voidarou, C.; Tsigalou, C.; Bezirtzoglou, E. Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. *Microorganisms* **2021**, *9*, 2041. [CrossRef]
10. Inouye, S.; Yamaguchi, H.; Takizawa, T. Screening of the antibacterial effects of a variety of essential oils on respiratory tract pathogens, using the modified dilution assay method. *J. Infect. Chemother.* **2001**, *7*, 251–254. [CrossRef]
11. Stamova, S.; Mihaylova, S.; Ermenlieva, N.; Georgieva, E. In vitro study of antimicrobial activity of essential oils of *Origanum vulgare* against *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922. *Scripta Sci. Pharm.* **2021**, *8*, 34–37.
12. Ermenlieva, N.; Georgieva, E.; Mihaylova, S.; Stamova, S.; Laleva, K.; Tsankova, G.; Tsvetkova, A. Synergistic interaction between *Lamiaceae* essential oils and antifungal drugs against *Candida albicans* ATCC10231. *Farm. J.* **2022**, *70*, 720–772.
13. Cristani, M.; D'Arrigo, M.; Mandalari, G.; Castelli, F.; Sarpietro, M.G.; Micieli, D.; Venuti, V.; Bisignano, G.; Saija, A.; Trombetta, D. Interaction of four monoterpenes contained in essential oils with model membranes: Implications for their antibacterial activity. *J. Agric. Food Chem.* **2007**, *55*, 6300–6308. [CrossRef] [PubMed]
14. Xu, J.; Zhou, F.; Ji, B.; Pei, R.; Xu, N. The antibacterial mechanism of carvacrol and thymol against *Escherichia coli*. *Lett. Appl. Microbiol.* **2008**, *47*, 174–179. [CrossRef]
15. Lambert, R.J.W.; Skandamis, P.N.; Coote, P.J.; Nychas, G.J.E. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J. Appl. Microbiol.* **2001**, *91*, 453–462. [CrossRef] [PubMed]
16. Heckler, C.; Lemos, G.; Andrade, G.; Ribeiro, A.; Rocha, T.; Silva, C.; Rosa, C. Combined effect of carvacrol, thymol and nisin against pathogens. *Ann. Braz. Acad. Sci.* **2021**, *93* (Suppl. 4), e20210550. [CrossRef]
17. Mantzourani, I.; Plessa, S.; Alexopoulos, A.; Bekatorou, A. The antimicrobial effect of thymol and carvacrol in combination with organic acids against foodborne pathogens in chicken and beef meat fillets. *Microorganisms* **2025**, *13*, 182. [CrossRef] [PubMed]
18. Hong, J.M.; Kim, J.H.; Kang, J.S.; Lee, W.J.; Hwang, Y.I. Vitamin C is taken up by human T cells via sodium-dependent vitamin C transporter 2 (SVCT2) and exerts inhibitory effects on the activation of these cells in vitro. *Anat. Cell Biol.* **2016**, *49*, 88–98. [CrossRef]
19. Mousavi, S.; Bereswill, S.; Heimesaat, M.M. Immunomodulatory and antimicrobial effects of vitamin C. *Eur. J. Microbiol. Immunol.* **2019**, *9*, 73–79. [CrossRef]
20. Lund, P.A.; De Biase, D.; Liran, O.; Scheler, O.; Pereira, M.N.; Cetecioglu, Z.; Noriega, E.F.; Bover-Cid, S.; Hall, R.; Sauer, M. Understanding how microorganisms respond to acid pH is central to their control and successful exploitation. *Front. Microbiol.* **2020**, *11*, 556140. [CrossRef]
21. Liu, Y.; Yan, H.; Yu, B.; He, J.; Mao, X.; Yu, J.; Zheng, P.; Huang, Z.; Luo, Y.; Luo, J. Protective effects of natural antioxidants on inflammatory bowel disease: Thymol and its pharmacological properties. *Antioxidants* **2022**, *11*, 1947. [CrossRef]

22. Li, Y.; Sun, L.; Lu, C.; Gong, Y.; Li, M.; Sun, S. Promising antifungal targets against *Candida albicans* based on ion homeostasis. *Front. Cell Infect. Microbiol.* **2018**, *8*, 286. [CrossRef]
23. Yang, J.H.; Bening, S.C.; Collins, J.J. Antibiotic efficacy—Context matters. *Curr. Opin. Microbiol.* **2017**, *39*, 73–80. [CrossRef] [PubMed]
24. Poirel, L.; Madec, J.Y.; Lupo, A.; Schink, A.K.; Kieffer, N.; Nordmann, P.; Schwarz, S. Antimicrobial Resistance in *Escherichia coli*. *Microbiol. Spectr.* **2018**, *6*, 10–1128. [CrossRef]
25. Brdová, D.; Ruml, T.; Viktorová, J. Mechanism of staphylococcal resistance to clinically relevant antibiotics. *Drug Resist. Updat.* **2024**, *77*, 101147. [CrossRef] [PubMed]
26. Costa-de-Oliveira, S.; Rodrigues, A.G. *Candida albicans* antifungal resistance and tolerance in bloodstream infections: The triad yeast–host–antifungal. *Microorganisms* **2020**, *8*, 154. [CrossRef] [PubMed]
27. Georgiou, G.; Kotzé, A. Eradication of antibiotic-resistant *E. coli*, *S. aureus*, *K. pneumoniae*, *S. pneumoniae*, *A. baumannii*, and *P. aeruginosa* with chlorine dioxide *in vitro*. *Med. Res. Arch.* **2023**, *11*, 4218. [CrossRef]
28. Swiss Target Prediction. Available online: http://www.swisstargetprediction.ch (accessed on 10 April 2025).
29. UniProt Database. Available online: https://www.uniprot.org (accessed on 10 April 2025).
30. STRING Database. Available online: https://string-db.org (accessed on 10 April 2025).
31. Reactome Pathway Database. Available online: https://reactome.org (accessed on 10 April 2025).
32. Tajkarimi, M.; Ibrahim, S.A. Antimicrobial activity of ascorbic acid alone or in combination with lactic acid on *Escherichia coli* O157:H7 in laboratory medium and carrot juice. *Food Control* **2011**, *22*, 801–804. [CrossRef]
33. Kallio, J.; Jaakkola, M.; Mäki, M.; Kilpeläinen, P.; Virtanen, V. Vitamin C inhibits *Staphylococcus aureus* growth and enhances the inhibitory effect of quercetin on growth of *Escherichia coli* in vitro. *Planta Med.* **2012**, *78*, 1824–1830. [CrossRef]
34. Evangelista-Martínez, Z.; Reyes-Vázquez, N.; Rodríguez-Buenfil, I. Antimicrobial evaluation of plant essential oils against pathogenic microorganisms: *In vitro* study of oregano oil combined with conventional food preservatives. *Acta Univ.* **2018**, *28*, 10–18. [CrossRef]
35. Gutierrez, J.; Barry-Ryan, C.; Bourke, P. The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *Int. J. Food Microbiol.* **2008**, *124*, 91–97. [CrossRef]
36. Burt, S. Essential oils: Their antibacterial properties and potential applications in foods—A review. *Int. J. Food Microbiol.* **2004**, *94*, 223–253. [CrossRef]
37. Cárcamo, J.M.; Pedraza, A.; Bórquez-Ojeda, O.; Golde, D.W. Vitamin C suppresses TNFα-induced NF-κB activation by inhibiting IκBα phosphorylation. *Biochemistry* **2002**, *41*, 12995–13002. [CrossRef] [PubMed]
38. Wu, L.; Xu, W.; Li, H.; Dong, B.; Geng, H.; Jin, J.; Han, D.; Liu, H.; Zhu, X. Vitamin C attenuates oxidative stress, inflammation, and apoptosis induced by acute hypoxia through the Nrf2/Keap1 signaling pathway in gibel carp (*Carassius gibelio*). *Antioxidants* **2022**, *11*, 935. [CrossRef] [PubMed]
39. Vilchèze, C.; Hartman, T.; Weinrick, B.; Jacobs, W.R., Jr. *Mycobacterium tuberculosis* is extraordinarily sensitive to killing by a vitamin C–induced Fenton reaction. *Nat. Commun.* **2013**, *4*, 1881. [CrossRef]
40. Hatano, T.; Tsugawa, M.; Kusuda, M.; Taniguchi, S.; Yoshida, T.; Shiota, S.; Tsuchiya, T. Enhancement of antibacterial effects of epigallocatechin gallate, using ascorbic acid. *Phytochemistry* **2008**, *69*, 3111–3116. [CrossRef] [PubMed]
41. McCarrell, E.M.; Gould, S.W.J.; Fielder, M.D.; Kelly, A.F.; ElSankary, W.; Naughton, D.P. Antimicrobial activities of pomegranate rind extracts: Enhancement by addition of metal salts and vitamin C. *BMC Complement. Altern. Med.* **2008**, *8*, 64. [CrossRef]
42. Chen, Y.; Gao, Y.; Wu, X.; Wang, L.; Zhao, M.; Yang, L. Ascorbic acid enhances the inhibitory effect of theasaponins against *Candida albicans*. *Int. J. Mol. Sci.* **2024**, *25*, 10661. [CrossRef]
43. Altun, M.; Yapici, B.M. Determination of chemical compositions and antibacterial effects of selected essential oils against human pathogenic strains. *An. Acad. Bras. Cienc.* **2022**, *94*, e20210074. [CrossRef]
44. Semeniuc, C.A.; Pop, C.R.; Rotar, A.M. Antibacterial activity and interactions of plant essential oil combinations against Gram-positive and Gram-negative bacteria. *J. Food Drug Anal.* **2017**, *25*, 403–408. [CrossRef]
45. Abderrahmane, D.; Boualem, S.; Boudarene, L.; Benseradj, F.; Aberrane, S.; Aitmoussa, S.; Chelghoum, C.; Lamari, L.; Sabaou, N.; Baaliouamer, A. *In vitro* synergistic/antagonistic antibacterial and anti-inflammatory effect of various extracts/essential oil from cones of *Tetraclinis articulata* (Vahl) Masters with antibiotic and anti-inflammatory agents. *Ind. Crops Prod.* **2014**, *56*, 60–66. [CrossRef]
46. Buldain, D.; Buchamer, A.V.; Marchetti, M.L.; Aliverti, F.; Bandoni, A.; Mestorino, N. Combination of cloxacillin and essential oil of *Melaleuca armillaris* as an alternative against *Staphylococcus aureus*. *Front. Vet. Sci.* **2018**, *5*, 177. [CrossRef]
47. Sumera, W.; Goc, A.; Niedzwiecki, A.; Rath, M. L-lysine and vitamin C work better in synergy against *Escherichia coli* and *Acinetobacter baumannii*. *J. Cell. Med. Nat.* **2023**, *1*, 1.
48. Mortazavi, A.; Mohammad, P.K.H.; Beheshti, F.; Anaeigoudari, A.; Vaezi, G.; Hosseini, M. The effects of carvacrol on oxidative stress, inflammation, and liver function indicators in a systemic inflammation model induced by lipopolysaccharide in rats. *Int. J. Vitam. Nutr. Res.* **2023**, *93*, 111–121. [CrossRef]

49. Imran, M.; Aslam, M.; Alsagaby, S.A.; Saeed, F.; Ahmad, I.; Afzaal, M.; Arshad, M.U.; Abdelgawad, M.A.; El-Ghorab, A.H.; Khames, A.; et al. Therapeutic application of carvacrol: A comprehensive review. *Food Sci. Nutr.* **2022**, *10*, 3544–3561. [CrossRef] [PubMed]
50. Padayatty, S.J.; Katz, A.; Wang, Y.; Eck, P.; Kwon, O.; Lee, J.H.; Chen, S.; Corpe, C.; Dutta, A.; Dutta, S.K.; et al. Vitamin C as an antioxidant: Evaluation of its role in disease prevention. *J. Am. Coll. Nutr.* **2003**, *22*, 18–35. [CrossRef] [PubMed]
51. Bechara, N.; Flood, V.M.; Gunton, J.E. A systematic review on the role of vitamin C in tissue healing. *Antioxidants* **2022**, *11*, 1605. [CrossRef] [PubMed]
52. Brasier, A.R. The nuclear factor-kappaB–interleukin-6 signalling pathway mediating vascular inflammation. *Cardiovasc. Res.* **2010**, *86*, 211–218. [CrossRef]
53. Liu, T.; Zhang, L.; Joo, D.; Sun, S.-C. NF-κB signaling in inflammation. *Signal Transduct. Target. Ther.* **2017**, *2*, 17023. [CrossRef]
54. Ebrahimi, N.; Abdulwahid, A.H.; Mansouri, R.R.; Karimi, N.; Bostani, R.J.; Beiranvand, S.; Adelian, S.; Khorram, R.; Vafadar, R.; Hamblin, M.R. Targeting the NF-κB pathway as a potential regulator of immune checkpoints in cancer immunotherapy. *Cell. Mol. Life Sci.* **2024**, *81*, 106. [CrossRef]
55. Hayes, J.D.; Dinkova-Kostova, A.T. The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. *Trends Biochem. Sci.* **2014**, *39*, 199–218. [CrossRef]
56. Carr, A.C.; Maggini, S. Vitamin C and immune function. *Nutrients* **2017**, *9*, 1211. [CrossRef]
57. Omonijo, F.A.; Liu, S.; Hui, Q.; Zhang, H.; Lahaye, L.; Bodin, J.-C.; Gong, J. Thymol improves barrier function and attenuates inflammatory responses in porcine intestinal epithelial cells during LPS-induced inflammation. *J. Agric. Food Chem.* **2019**, *67*, 615–624. [CrossRef] [PubMed]
58. Barak, T.H.; Eryilmaz, M.; Karaca, B.; Servi, H.; Ertekin, S.K.; Dinc, M.; Ustuner, H. Antimicrobial, anti-biofilm, anti-quorum sensing and cytotoxic activities of *Thymbra spicata* L. subsp. *spicata essential oils. Antibiotics* **2025**, *14*, 181. [CrossRef]
59. Yap, P.; Yiap, B.; Ping, H.; Lim, S. Essential oils, a new horizon in combating bacterial antibiotic resistance. *Open Microbiol. J.* **2014**, *8*, 6–14. [CrossRef]
60. Basavegowda, N.; Baek, K.H. Synergistic antioxidant and antibacterial advantages of essential oils for food packaging applications. *Biomolecules* **2021**, *11*, 1267. [CrossRef] [PubMed]
61. Teixeira, B.; Marques, A.; Ramos, C.; Neng, N.R.; Nogueira, J.M.F.; Saraiva, J.A.; Nunes, M.L. Chemical composition and antibacterial and antioxidant properties of commercial essential oils. *Ind. Crops Prod.* **2013**, *43*, 587–595. [CrossRef]

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