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Terpenoids as Natural Agents against Food-Borne Bacteria—Evaluation of Biofilm Biomass versus Viability Reduction

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Abstract: This study aimed to analyse the antibacterial potential of limonene, terpineol, and eugenol for the biofilm reduction of food-borne *E. coli*, *S. aureus* and *S. typhimurium*. A microdilution test with resazurin application was used for the minimum inhibitory concentration and a colony plate count was used for the minimum bactericidal concentration. Biofilm biomass was quantified using the crystal violet assay, while biofilm viability was determined using the plate count method. The results show the highest antibacterial potential among terpenoids for eugenol, followed by terpineol and limonene. Both biomass reduction and viability are strongly dependent on the concentration of all terpenoids tested ($p < 0.05$). Moreover, eugenol reduced biofilm biomass most effectively (67% for *E. coli*), while viability was reduced most by terpineol (3.8 log CFU cm^{-2} for *E. coli* and *S. aureus*). The correlation coefficient for the reduction in biomass and viability was highest for eugenol (0.9) and chlorhexidine for all bacteria tested, while the lowest correlation was found for limonene (0.6). Results also demonstrate that tested terpenoids are effective as standard antimicrobial agent chlorhexidine. This suggests that eugenol has potential against food-borne biofilms as it simultaneously reduces both biomass and viability of biofilms.

Keywords: terpenoids; food-borne bacteria; biofilm; minimum inhibitory concentration; viability; biomass

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1. Introduction

Managing the bacterial population on the surface plays a crucial role in medicine, technology and in domestic environments, where we try to keep bacteria to a minimum. Under favourable conditions, bacteria attach themselves to the surface of the material and begin to form biofilms in the presence of nutrients, temperature and water. A biofilm is a community of bacterial cells enclosed in exopolysaccharide substances that adheres to the surface of the material and exhibits sophisticated collective behaviour [1]. Bacterial biofilms pose one of the greatest public health problems, as the bacteria within the biofilm are much better protected from chemical and physical stresses than planktonic cells. From a public health perspective, it is estimated that more than 65% of all human microbial infections are related to biofilm exposure [2]. Therefore, the elimination of biofilms on surfaces requires much higher concentrations of chemical compounds compared to planktonic cells [3]. The most common disinfectants on the market contain chlorine-based active ingredients, quaternary ammonium compounds, triclosan, alcohols and aldehydes, which are effective against bacteria and facilitate good hygiene practices. However, the consumption of these chemicals can have worrying effects on humans, animals and the environment as they are washed into the aquatic environment after the cleaning process [4]. In 2020, sales of disinfectants have doubled worldwide, without evidence of their short- and long-term side effects on the environment [5]. Furthermore, experience shows that increased use of disinfectants is associated with increasing resistance, be it from misuse for chronic infections, misuse for viral prophylaxis, or overuse of antibiotic-based disinfectants [6]. A recent

report on antibacterial resistance indicates that more than 4.95 million deaths worldwide are associated with antibacterial resistance and that *E. coli* and *S. aureus* are two of the leading pathogens for deaths associated with bacterial resistance [7]. Chlorhexidine, for example, is an antiseptic frequently used in hospitals and industrial environments for disinfection. Moreover, in the last decade, concerns have arisen over the increasing resistance of *E. coli* and *S. aureus* to chlorhexidine [8]. An answer to more sustainable cleaning could be natural agents from plants that are effective against bacteria, are generally recognised as safe (GRAS) and for the environment, and have a low potential for resistance due to their non-targeting effect on bacterial cells [9,10]. Natural antimicrobial agents have attracted much attention among researchers in recent years, and essential oils in particular are considered a source of antimicrobial components. Essential oils are complex mixtures that can contain more than 300 compounds and have antibacterial properties due to bioactive volatile components such as terpenoids, alcohols, esters, thujone, carvone and others. However, the vast majority of active components belong to the class of terpenoids, e.g., terpineol, limonene, eugenol, carvacrol, linalool and others [11,12]. Terpenoids represent one of the largest and structurally diverse groups of naturally-occurring compounds derived from the 5-carbon compound isoprene and its derivatives. Many plants synthesize different kinds of terpenoids that have applications in medicine, pharmacy and technology [13,14]. In recent years it has been found that terpenoids play an increasingly important role in the field of antibacterial activity [15]. Upon contact with bacterial cells, terpenoids disrupt the cell membrane to make it more permeable, disrupt ion transfer, interact with membrane proteins and affect cell enzymes and inhibit DNA synthesis [16]. Limonene is a cyclic monoterpene and is the major component in citrus fruit peel oil. It is commonly used in the pharmaceutical, food and perfumes industry due to its safety [17]. For example, Gupta et al. [18] reported that limonene causes the degradation of proteins at the outer membrane of *E. coli*, leading to increased permeability and formation of hydroxyl radicals by Fenton reaction, which in turn leads to oxidative DNA damage. Moreover, Lee et al. [19] reported limonene is effective against *B. cereus, E. coli, S. aureus*, and even against methicillin-resistant *S. aureus*. The antibacterial activity of eugenol and limonene also includes the inhibition of *S. aureus* biofilm [20,21]. Terpineol is a monocyclic terpenoid found in many herbs like marjoram, oregano and rosemary and has a wide range of biological properties [22]. Ding et al. [23] reported that the main mechanism of action of terpineol against *E. coli* is to dissolve the outer membrane of the cells by releasing the lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to ATP. Eugenol is a terpenoid derived mainly from clove oil and has attracted scientific interest due to antibacterial, antiviral and antifungal properties [24]. In addition, eugenol is considered a membrane inhibitor, protease inhibitor and source of reactive oxygen species [24]. Yamaguchi [14] tested different terpenoids and found significant bacteriostatic and bactericidal activities at low concentrations. Moreover, the author found the most effective bactericidal activity against Gram negative bacteria. There are several research studies on the antibacterial potential of terpenoids against pathogenic bacteria, but less is known how terpenoids interact with bacteria within the biofilm, especially synergistic effects on biomass and biofilm viability. Therefore, this study aims to analyse the antibacterial and antibiofilm potential of the terpenoids limonene, terpineol and eugenol against the hygienically-relevant bacteria *E. coli, S. aureus* and *S. typhimurium*, by comparing the effectiveness of selected terpenoids with the standard antibacterial agent chlorhexidine. Furthermore, the removal of biofilm biomass from the polystyrene and the reduction of the viability of the bacteria within the biofilms were analysed. Finally, to evaluate the potential of selected active components for simultaneous removal of biofilm biomass and reduction of cell viability.

2. Materials and Methods

2.1. Bacterial Strains

For the antibacterial and anti-biofilm assay, standard strains of bacteria that represent hygienically-relevant bacteria were used. The standard strains of *Escherichia coli* ATCC

25922 (*E. coli*), *Salmonella enterica serovar typhimurium* ATCC 14028 (*S. typhimurium*) and *Staphylococcus aureus* ATCC 25923 (*S. aureus*) were obtained from Sigma Aldrich (Virginia, St. Louis, MO, USA). Bacteria from the collection were transferred on nutrient agar (Biolife, Italy) and incubated at 37 °C for 24 h. After that, a single colony of a strain was transferred from nutrient agar to the Mueller Hinton broth (Merck Millipore, Burlington, MA, USA) and incubated under the same conditions.

2.2. Chemicals

Terpenoids active components (R)-(+)- limonene (1-Methyl-4-(prop-1-en-2-yl) cyclohex-1-ene), α-terpineol (2-(4-Methylcyclohex-3-en-1-yl)propan-2-ol), eugenol (2-Methoxy-4-(prop-2-en-1-yl) phenol), standard antimicrobial chlorhexidine digluconate and solvent Tween-80 (Polyoxyethylene (20) sorbitan monooleate) were purchased at Sigma-Aldrich (St. Louis, MO, USA).

2.3. Minimum Inhibitory and Bactericidal Concentration

The minimum inhibitory and bactericidal concentration of limonene, terpineol and eugenol against selected bacteria was tested using microdilution methods according to the standard ISO 20776-1: 2020. Bacterial cultures of *E. coli*, *S. typhimurium* and *S. aureus* were transferred from nutrient agar to a 0.9% NaCl solution to achieve a concentration of 0.5 McFarland. Into sterile flat-bottomed 96-well microplates (Nunc, Denmark), 100 µL of bacterial cells in Mueller-Hinton broth were added at a final concentration of 1×10^6 CFU mL^{-1}. In the second step, twofold dilutions of terpenoid active components were added at concentrations ranging from 15 mg mL^{-1} to 0.03 mg mL^{-1}. The active components were diluted in 0.002% (*v*/*v*) Tween-80 according to the Clinical and Laboratory Standards Institute [25]. The plates were incubated at 37 °C for 24 h. Then, 10 µL of resazurin solution at a concentration of 0.015% was added and incubated again under the same conditions for 4 h. The negative control was 0.9% NaCl with 0.002% Tween-80, while the positive control was chlorhexidine gluconate at a concentration in the range of 0.003 to 0.25 mg mL^{-1}. The minimum inhibitory concentration (MIC) in the resazurin assay was defined according to Sarker et al. [26] as the lowest concentration of the active component that does not convert blue resazurin to pink resorufin. Subsequently, 100 µL of the suspension above the MIC was inoculated onto the nutrient agar and incubated at 37 °C for 24 h. The minimum bactericidal concentration (MBC) was defined as the lowest concentration at which no colonies grew on solid media (Figure 1).

Biofilm Biomass and Viability

A biofilm test was performed following Fink et al. [27] with some modifications as follows. Bacterial cultures of *E. coli*, *S. typhimurium* and *S. aureus* were prepared in a 0.9% NaCl solution to achieve a concentration of 0.5 McFarland. The bacterial cells were added to Mueller-Hinton broth to achieve a final concentration of 5×10^5 CFU mL^{-1}. Then, 100 µL of the bacterial suspension was added to sterile 96-well flat-bottomed microtitre plates (Nunc, Roskilde, Denmark) and incubated at 37 °C for 24 h. After this, the bacterial suspension was removed, and the biofilms formed on the surface of the microtitre plate were rinsed three times with 100 µL PBS. The biofilms were treated for 15 min at room temperature with 1 MIC, 2 MIC and 3 MIC concentrations of the active components. Samples were then washed three times with 100 µL PBS to neutralise the active components and remove any loosely adhering cells. The biofilm biomass was determined using the crystal violet assay. Cells remaining on the surface were stained with 100 µL 2% crystal violet (Merck Millipore, Darmstad, Germany) and the excess dye removed and washed with PBS. The dye from the cells was remobilised with 100 µL 96% ethanol. The optical density of the solution was measured at a wavelength of 620 nm using the Infinite 200 PRO microplate reader (Tecan, Grödig, Austria). The viability of the biofilm was analysed by counting the bacterial colonies. After neutralisation with PBS, 100 µL 0.9% NaCl was added to each microtitre well and sonicated at 37 kHz and 200 W for 3 min to dissolve the cells from

the well surface into the liquid. After serial dilutions, samples were inoculated onto solid media and incubated at 37 °C for 24 h. Colonies were counted and results were expressed as log CFU cm^2 (Figure 1).

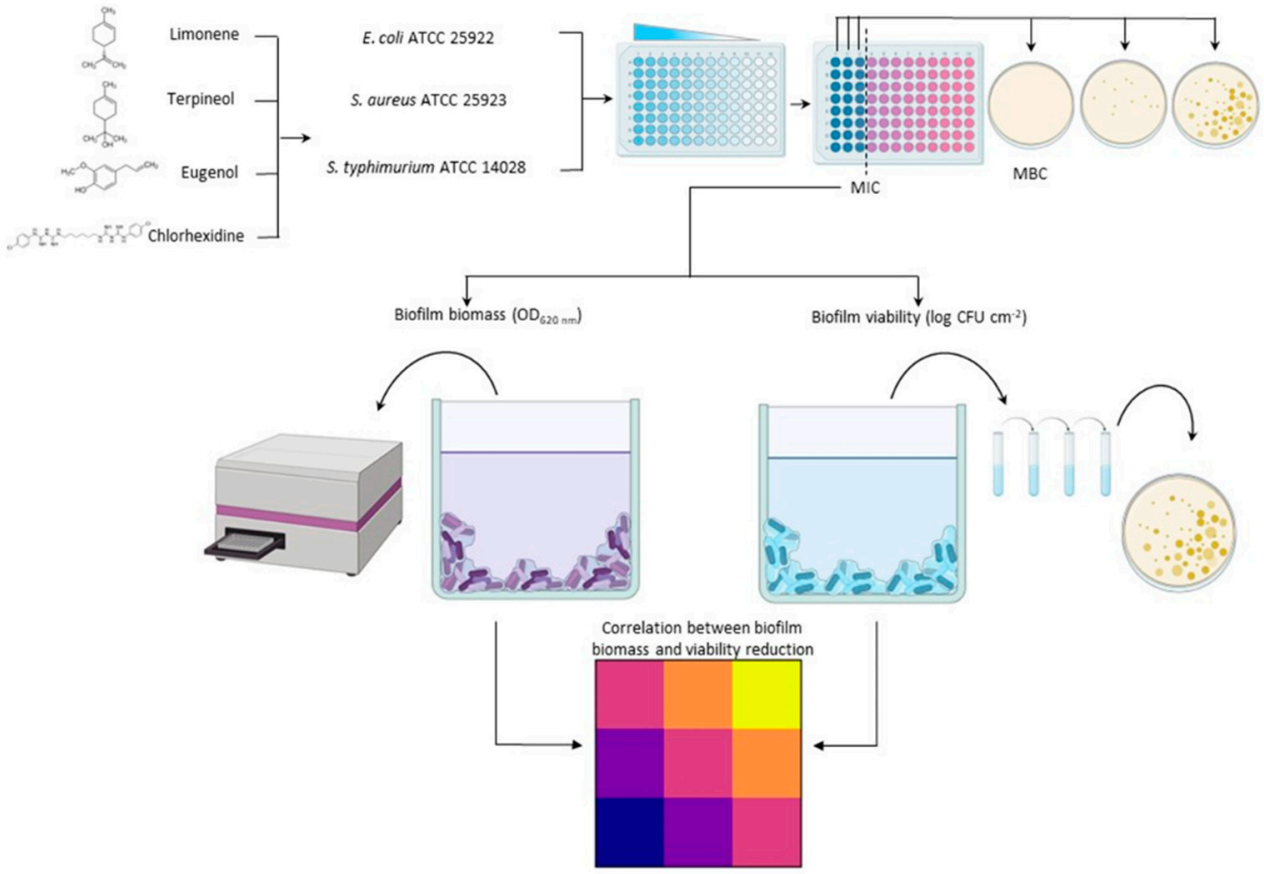

Figure 1. Research design testing antibacterial and antibiofilm potential of limonene, terpineol, eugenol and chlorhexidine against *E. coli*, *S. typhimurium* and *S. aureus* biofilm biomass and viability (Created with BioRender.com).

Statistical analysis was provided using R software version 4.1.1. (Bell Laboratories, Holmdel, NJ, USA). One-way analysis of variance (ANOVA) and the Duncan test were used to determine the significant differences at a significance level of $p < 0.05$. Pearson correlation coefficient (r) was calculated to correlate biofilm biomass and cell viability reduction ($p < 0.05$). The correlation was interpreted as weak (0.1–0.3), moderate (0.4–0.6) or string (0.7–0.9).

Figure 1 was created using BioRender.com (accessed on 25 November 2022).

3. Results and Discussion

One of the principles of green chemistry is to replace classic cleaning and disinfecting agents with less hazardous substances and to offer consumers ingredients that are safer for human health and the environment [28]. Terpenoids have been ported to be GRAS, but to also exert antimicrobial activities against both antibiotic-susceptible and -resistant bacteria at the same time. Notably, carvacrol, eugenol, carvone, geraniol and thymol are among the terpenoids that show antibacterial potential against *S. aureus* [29]. Our study aims to evaluate the potential of the terpenoids limonene, terpineol and eugenol for managing the biomass and viability of biofilms in the food industry. The results show that the lowest MIC for all tested terpenoids and bacteria is 0.1 mg mL^{-1} for eugenol, followed by terpineol (0.1–0.2 mg mL^{-1}) and limonene (0.9–1.9 mg mL^{-1}) (Table 1). In a study by Zhao et al. [30], which investigated the exposure of eugenol to *S. typhimurium*, the MIC was 0.125 mg mL^{-1},

which is consistent with our results. Furthermore, the authors reported that treatment with eugenol deformed the morphology of *S. typhimurium*. As with eugenol, the MIC for terpineol was 0.1 mg mL^{-1} for *E. coli* and *S. aureus*, and 0.2 mg mL^{-1} for *S. typhimurium* (Table 1). Similarly, Huang et al. [31] have shown that *S. typhimurium* and *E. coli* seem to be sensitive to terpineol at a concentration of 0.153 mg mL^{-1}. Limonene appears to be the least antibacterial with an MIC of 0.9 mg mL^{-1} for *E. coli* and 1.9 mg mL^{-1} for *S. typhimurium*. In contrast to our findings, Costa et al. [32] found the MIC of limonene for *S. aureus* at 0.256 mg mL^{-1}, which is lower than the figures reported in our study. One of the reasons for this could be the fact that the researchers used the solvent DMSO. This is particularly important as Van de Vel et al. [33] report that the choice of solvent can have a significant impact on the MIC of essential oils. Meanwhile, the results for chlorhexidine show MIC for *E. coli* and *S. aureus* at 0.02 mg mL^{-1}, which corresponds to Kampf's findings [34] that reported MIC for *E. coli* at 0.0117 mg mL^{-1} and *S. aureus* at 0.02 mg mL^{-1}.

Table 1. MIC and MBC of limonene, terpineol, eugenol and chlorhexidine against *E. coli*, *S. aureus* and *S. typhimurium*.

	Limonene		**Terpineol**		**Eugenol**		**Chlorhexidine**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
E. coli ATCC 25922	0.9	3.7	0.1	0.4	0.1	0.4	0.02	0.03
S. aureus ATCC 25923	1.9	3.7	0.1	0.3	0.1	0.6	0.02	0.06
S. typhimurium ATCC 14028	0.9	2.8	0.2	0.7	0.1	0.6	0.03	0.09

Legend: MIC—minimal inhibitory concentration (mg mL^{-1}); MBC—minimal bactericidal concentration (mg mL^{-1}).

The results of the efficacy of removing biofilm biomass from the polystyrene surface show for all tested bacteria that increasing the concentration of terpenoid agents and chlorhexidine leads to a decrease in biomass ($p < 0.05$) (Figure 2, Table 2). More detailed analysis shows that 2 MIC will significantly decrease the biofilm biomass for all bacteria and all tested compounds, while 3 MIC of limonene will not have a significant effect on *S. typhimurium*, nor will terpineol on *E. coli* and *S. aureus*, or chlorhexidine on *E. coli* (Table 2). We have shown that eugenol can remove up to 67% of *E. coli* biofilm biomass, followed by *S. aureus* (53%) and *S. typhimurium* (46%). A similar trend is observed for limonene (56%, 46% and 27% respectively), while terpineol removes up to 44% of *E. coli* biofilm biomass, followed by *S. typhimurium* (38%) and *S. aureus* (26%) (Figure 2). A study by Yadav et al. [21] investigated the effects of eugenol on *S. aureus* biofilm and found that 0.2 mg mL^{-1} reduced biofilm by 50%, which is comparable to our results. Ding et al. [23] tested 0.3 mg mL^{-1} terpineol against *E. coli* biofilm and found that a 40% reduction was possible with a short exposure time; increasing the exposure time increased the removal of the biofilm. Chlorhexidine can remove up to 57% of *E. coli* biofilm biomass, followed by *S. aureus* (51%) and *S. typhimurium* (37%). Comparable to eugenol, chlorhexidine at 3 MIC removes about 8% less biofilm biomass. Cota et al. [35] tested about ten times higher concentrations of chlorhexidine on wild strains of *S. typhimurium* to demonstrate biofilm eradication.

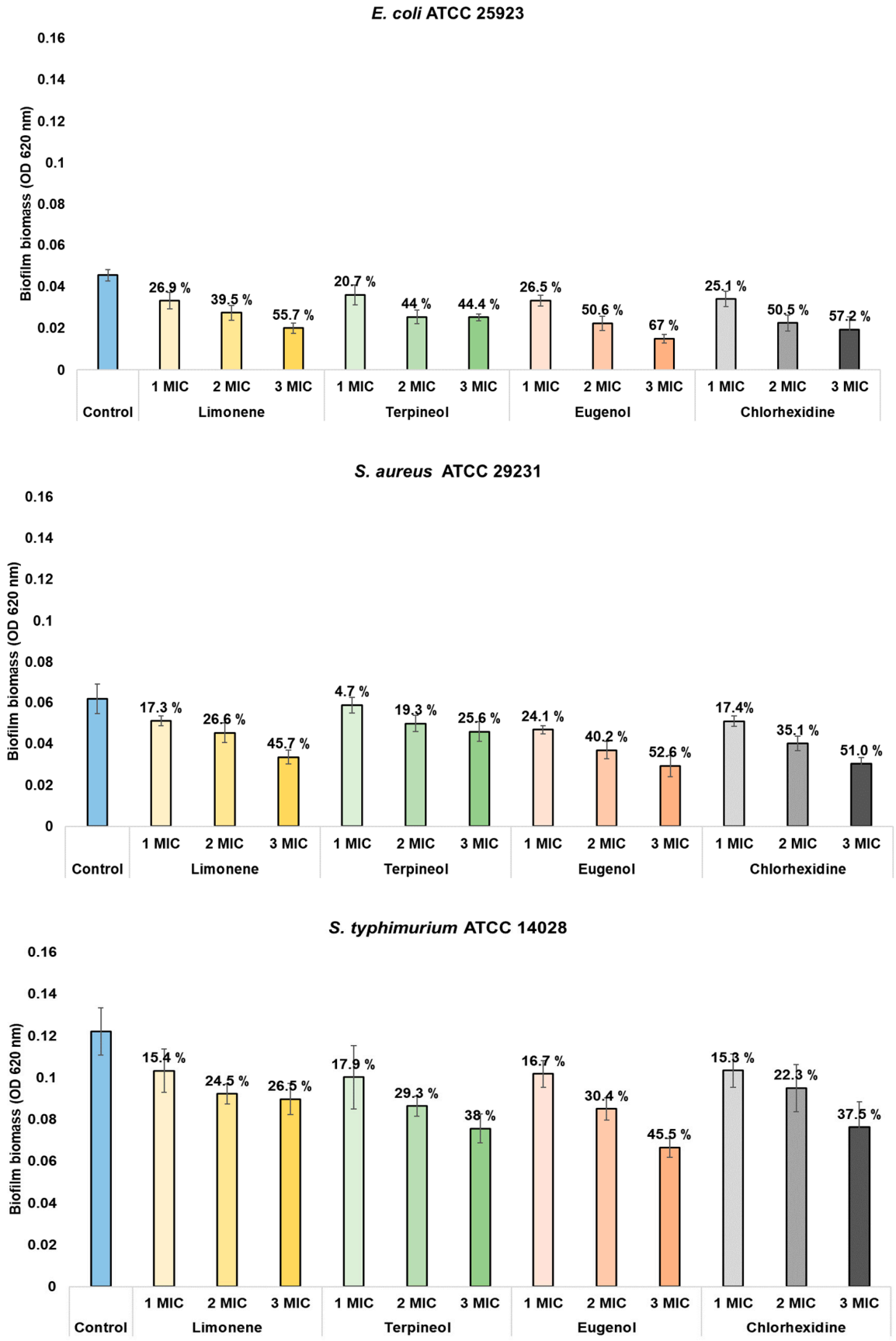

Figure 2. Biofilm biomass (OD620 nm and %) of *E. coli*, *S. aureus* and *S. typhimurium* after exposure to 1 MIC, 2 MIC and 3 MIC limonene, terpineol eugenol and chlorhexidine.

Table 2. Analysis of variance and post hoc Duncan test for biofilm biomass and viability in comparison to limonene, terpineol, eugenol and chlorhexidine concentration.

	Active Component	Concentration	Bacteria *E. coli*			Bacteria *S. aureus*			Bacteria *S. typhimurium*		
			$\bar{x}$	F-Value	*p*-Value	$\bar{x}$	F-Value	*p*-Value	$\bar{x}$	F-Value	*p*-Value
Biofilm biomass (OD 620 nm)	Limonene	Control	0.0454 ^{a}	90.28	<0.0000 *	0.0616 ^{a}	47.75	<0.0000 *	0.1221 ^{a}	22.21	<0.0000 *
		1 MIC	0.0332 ^{b}			0.0510 ^{b}			0.1033 ^{b}		
		2 MIC	0.0275 ^{c}			0.0452 ^{c}			0.0922 ^{c}		
		3 MIC	0.0201 ^{d}			0.0335 ^{d}			0.0897 ^{c}		
	Terpineol	Control	0.0454 ^{a}	69.35	<0.0000 *	0.0616 ^{a}	16.66	<0.0000 *	0.1221 ^{a}	29.73	<0.0000 *
		1 MIC	0.0360 ^{b}			0.0587 ^{a}			0.1001 ^{b}		
		2 MIC	0.0254 ^{c}			0.0497 ^{b}			0.0863 ^{c}		
		3 MIC	0.0252 ^{c}			0.0458 ^{b}			0.0756 ^{d}		
	Eugenol	Control	0.0454 ^{a}	190.4	<0.0000 *	0.0616 ^{a}	61.3	<0.0000 *	0.1221 ^{a}	81.89	<0.0000 *
		1 MIC	0.0334 ^{b}			0.0467 ^{b}			0.1017 ^{b}		
		2 MIC	0.0224 ^{c}			0.0368 ^{b}			0.0849 ^{c}		
		3 MIC	0.0150 ^{d}			0.0292 ^{d}			0.0665 ^{d}		
	Chlorhexidine	Control	0.0421 ^{a}	27.75	<0.0000 *	0.0649 ^{a}	124.5	<0.0000 *	0.1192 ^{a}	17.36	<0.0000 *
		1 MIC	0.0340 ^{b}			0.0509 ^{b}			0.0103 ^{b}		
		2 MIC	0.0225 ^{c}			0.0400 ^{c}			0.0949 ^{b}		
		3 MIC	0.0194 ^{c}			0.0301 ^{d}			0.0762 ^{c}		
Biofilm viability (CFU cm^{-2})	Limonene	Control	8.0261 ^{a}	1530	<0.0000 *	8.3507 ^{a}	493.5	<0.0000 *	8.5591 ^{a}	348.3	<0.0000 *
		1 MIC	5.8995 ^{b}			6.4502 ^{b}			7.4750 ^{b}		
		2 MIC	4.7727 ^{c}			5.3065 ^{c}			6.5878 ^{c}		
		3 MIC	4.5634 ^{d}			5.2276 ^{c}			5.8535 ^{d}		
	Terpineol	Control	8.0261 ^{a}	471.6	<0.0000 *	8.3507 ^{a}	366.6	<0.0000 *	8.5591 ^{a}	869.6	<0.0000 *
		1 MIC	5.9338 ^{b}			5.9054 ^{b}			6.7936 ^{b}		
		2 MIC	5.3726 ^{c}			4.6700 ^{c}			5.9604 ^{c}		
		3 MIC	4.3589 ^{d}			4.5578 ^{c}			5.6755 ^{d}		
	Eugenol	Control	8.0261 ^{a}	2073	<0.0000 *	8.3507 ^{a}	1,965	<0.0000 *	8.5591 ^{a}	470.3	<0.0000 *
		1 MIC	5.9214 ^{b}			6.6092 ^{b}			6.9335 ^{b}		
		2 MIC	4.8198 ^{c}			5.7823 ^{c}			6.5252 ^{c}		
		3 MIC	4.5893 ^{d}			5.0638 ^{d}			6.3395 ^{d}		
	Chlorhexidine	Control	8.1222 ^{a}	109.2	<0.0000 *	8.2914 ^{a}	475.1	<0.0000 *	8.6001 ^{a}	163	<0.0000 *
		1 MIC	3.3453 ^{b}			3.8277 ^{b}			2.6214 ^{b}		
		2 MIC	3.3193 ^{b}			3.1963 ^{c}			2.3027 ^{c}		
		3 MIC	3.2561 ^{c}			1.9623 ^{d}			1.5898 ^{d}		

Legend: $\bar{x}$ mean value; * significant difference at $p < 0.05$; Means (a–d) sharing a common letter are not significantly different at $p < 0.05$.

The viability of the biofilm for all tested bacterial strains and the selected terpenoid agents show an increasing logarithmic decrease with increasing concentration (Figure 3, Table 2). More detailed analysis shows that double concentration will significantly decrease the biofilm viability for all bacteria and for all tested compounds, while for *S. aureus*, 3 MIC of limonene and terpineol will not have a significant effect (Table 2). In contrast to the biomass assessment, biofilm viability shows the highest logarithmic reduction at 3 MIC for terpineol (3.8 log CFU cm^{-2} for *E. coli* and *S. typhimurium* and 2.9 for *S. aureus*). Ulhag et al. [36] tested the extract of *Citrus hystrix* against *S. typhimurium* and found that terpineol was the major antibacterial component acting against bacterial cells. In addition, we found that eugenol and limonene showed similar results against *E. coli*, while limonene reduced more cells at an MIC of 3 (2.9 log CFU cm^{-2}) compared to eugenol (2.2 log CFU cm^{-2}) in the case of *S. aureus* (Figure 3). One study by Umagiliyage et al. [37] tested limonene against *E. coli* and found that 1 mg mL^{-1} limonene can reduce bacterial cells by 1.6 log CFU. Results of chlorhexidine show similar reduction for *E. coli* as for eugenol (3.5 log CFU cm^{-2}) or terpineol for *S. aureus* (3.8 log CFU cm^{-2}), while it was least effective for *S. typhimurium*. Condell et al. [38] reported that *Salmonella* spp., when exposed to sub-lethal concentrations of chlorhexidine, can respond with modification of the cell wall, virulence and a shift in cellular metabolism.

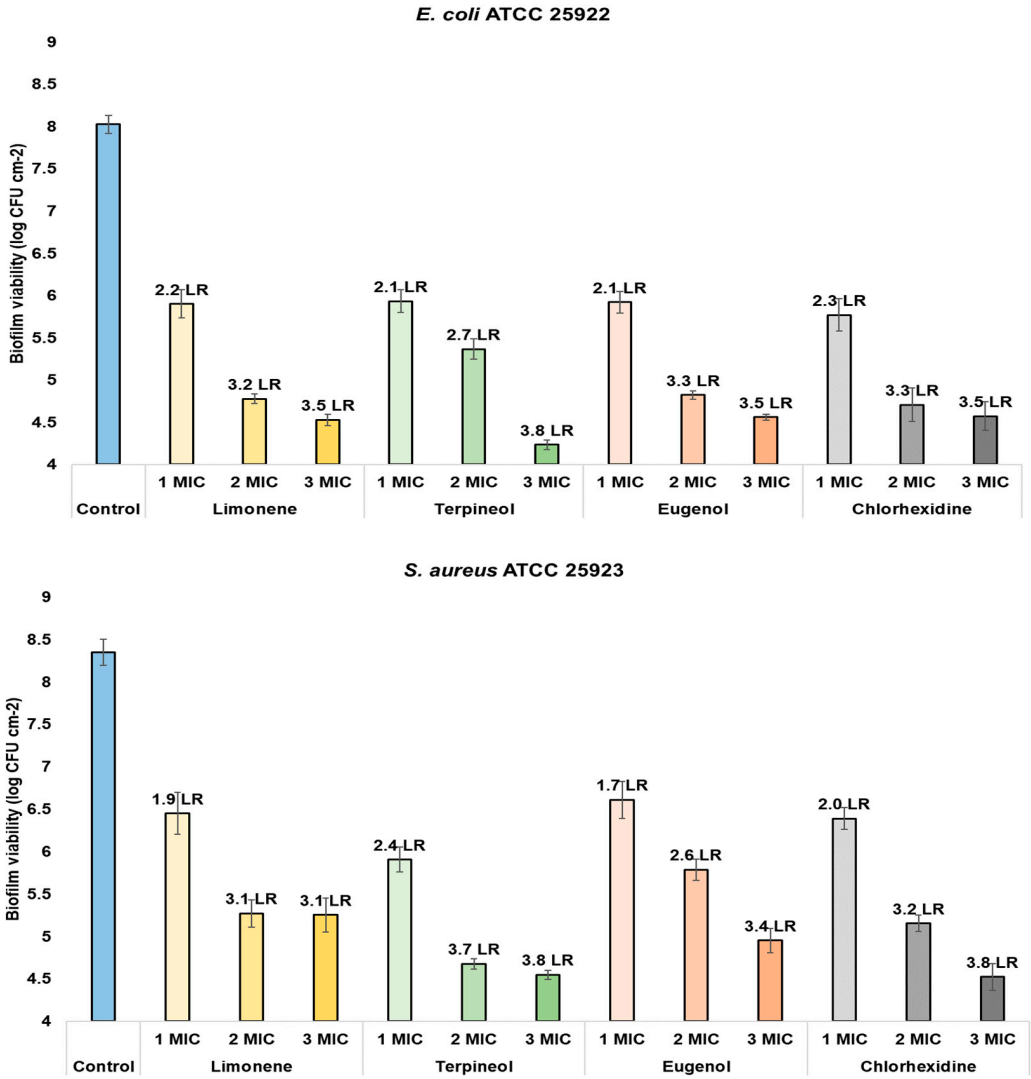

Figure 3. *Cont.*

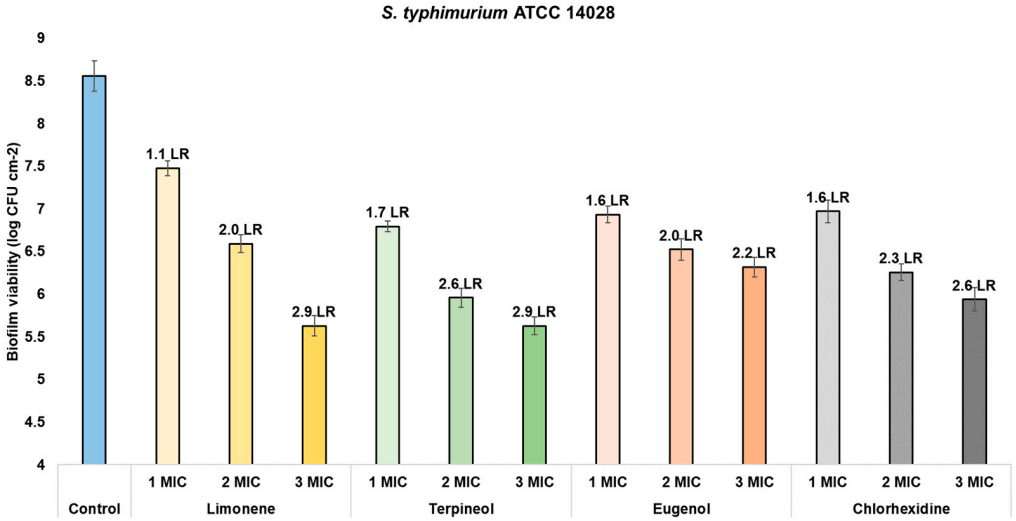

Figure 3. Biofilm viability (log CFU cm^{-2} and LR) of *E. coli*, *S. aureus* and *S. typhimurium* after exposure to 1 MIC, 2 MIC and 3 MIC limonene, terpineol, eugenol and chlorhexidine.

Results of the Pearson correlation coefficient show a strong correlation between the reduction of biofilm biomass and viability for all three tested bacteria against the eugenol (0.835–0.915), while for terpineol, we observed a strong correlation for *S. typhimurium* (0.756) and *S. aureus* (0.790), but moderate (0.612) for *E. coli*. Contrary to those results, limonene shows a moderate correlation between biomass and viability reduction for *S. aureus* and *S. typhimurium*, and only a strong one for *E. coli* (Figure 4). Results for chlorhexidine show a strong correlation for *S. aureus* (0.919) and *E. coli* (0.772), and moderate for *S. typhimurium* (0.643). All this indicates that eugenol is a good antibacterial agent, effectively reducing biofilm biomass and viability, and is comparable to chlorhexidine.

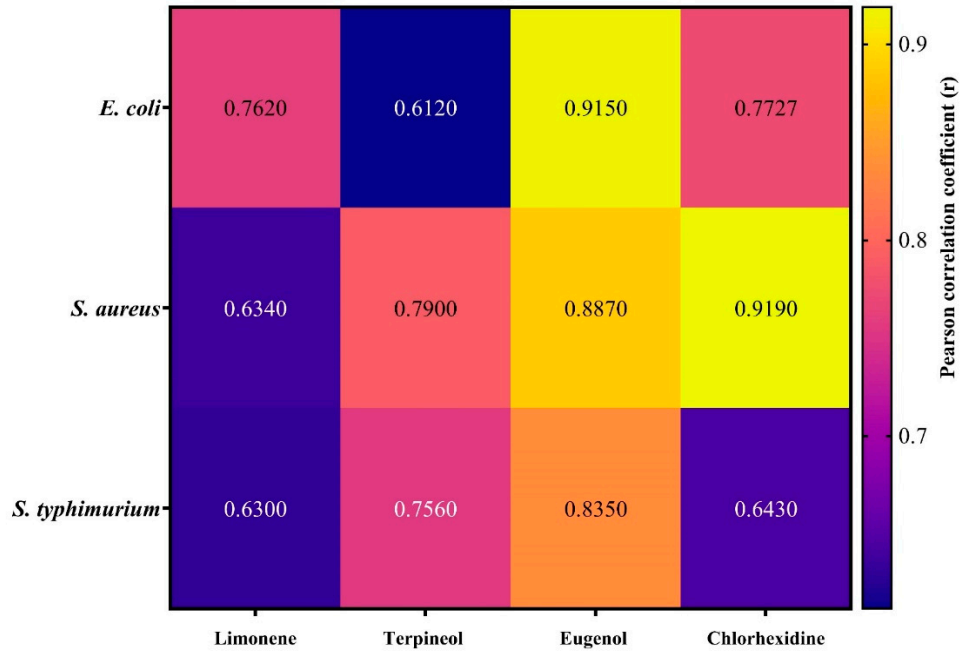

Figure 4. Pearson correlation coefficient between biofilm biomass (OD in %) and biofilm viability (LR in log CFU cm^{-2}) for all three tested bacterial strains and active components.

4. Conclusions

Reducing the environmental impact of cleaning and disinfection products while maintaining their effectiveness is a green chemistry priority. New antibacterial agents

must achieve comparable efficiency to classical ones, while reducing the pressure on the environment. Our research has shown that the terpenoids eugenol and terpineol have the strongest antibacterial activity against food-borne *E. coli*, *S. aureus* and *S. typhimurium*, while limonene has less antibacterial potential. Double and triple concentrations of MIC showed significant reductions in both biomass and biofilm viability, with eugenol being most effective in removing biomass, while terpineol was most effective in reducing biofilm viability. We also demonstrated that chlorhexidine is effective against *E. coli* and *S. aureus*, but less so against *S. typhimurium*. A comparison of the correlation of biomass and viability shows that eugenol has the greater potential to simultaneously reduce the biomass and viability of the food-borne biofilms tested. The anti-biofilm properties of terpineol and eugenol are comparable to chlorhexidine and therefore represent a good candidate for substitution in practice.

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Data Availability Statement: https://zenodo.org/record/3543000#.Y7QVpRVBy3A (accessed on 25 November 2022).

Conflicts of Interest: The author declares no conflict of interest.

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