Functionalized materials with essential oils for eco-friendly antimicrobial nanocoatings

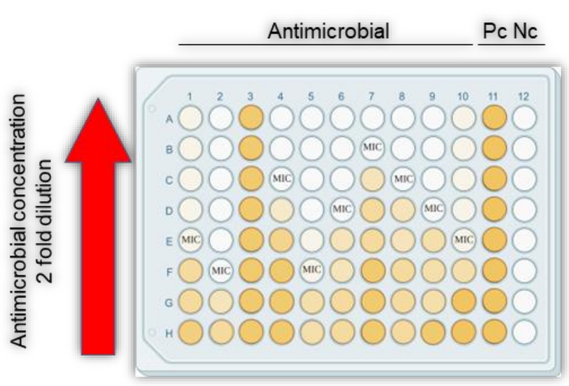
Elisa Recchia[1], Luca Fiore[2], Nathalie Totaro[1], Amaia M. Goitandia[3], Maialen Argaiz[3], Miren Blanco[3], Fabiana Arduini[2], Florigio Lista[1]

[1]*Defence Institute for Biomedical Sciences, Via Santo Stefano Rotondo, 4 - 00184 Rome, Italy*
[2]*Department of Chemical Science and Technologies, University of Rome Tor Vergata, Via Della Ricerca Scientifica 1 - 00133 Rome, Italy*
[3]*Unidad de Química de superficies y Nanotecnología, Fundación Tekniker, Iñaki Goenaga 5 -20600 Eibar, Spain*

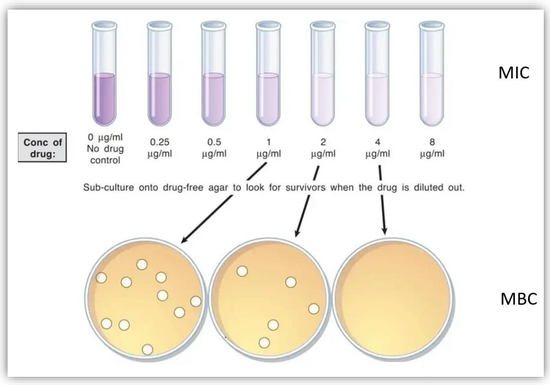
Introduction

Antimicrobial resistance poses a serious threat to global health, leading to increased morbidity, mortality, and economic burden [1, 2]. Addressing this urgent issue requires innovative strategies to prevent bacterial spread, especially through contaminated surfaces. The overriding goal of Horizon Europe RELIANCE project is the development of advanced antimicrobial technologies by creating innovative coatings based on mesoporous silica nanoparticles (MSNs) functionalized with metallic copper and essential oils. These coatings are specifically designed to boost antimicrobial effectiveness beyond existing solutions [3]. Our research aims to: i) evaluate the antibacterial properties of selected essential oils—eugenol, carvacrol, thymol, and menthol—against clinically relevant bacterial strains (*Escherichia coli, Bacillus clausii, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae*); ii) identify the most effective essential oil for nanoparticle functionalization; iii) synthesize and characterize copper-functionalized silica nanoparticles with the selected essential oil to develop broad-spectrum antimicrobial coatings; and iv) assess the antimicrobial activity of these nanomaterials against both Gram-positive and Gram-negative bacteria.

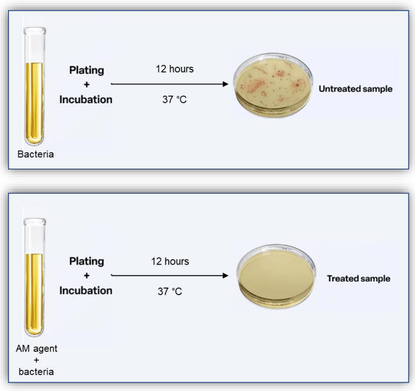
Experimental methods



Minimum Inhibitory Concentration (MIC)



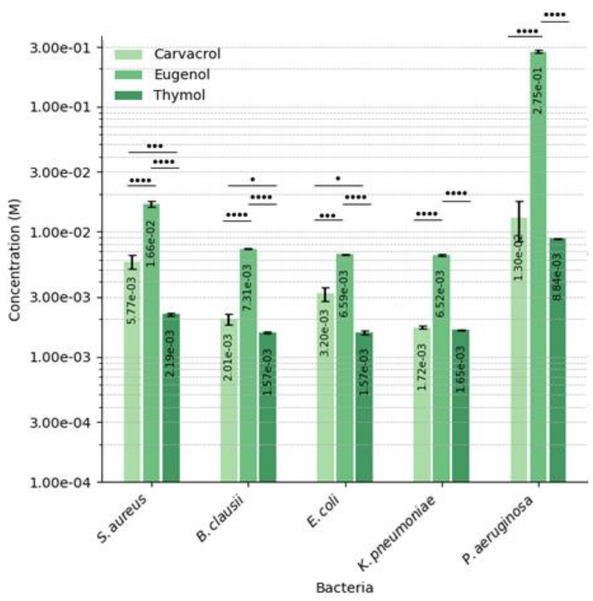
Minimum Bactericidal Concentration (MBC)



Time Killing Assay

Results

We evaluated the efficacy of eugenol, carvacrol, thymol, and menthol against five bacterial strains *(Escherichia coli, Bacillus clausii, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae*) by performing a series of assays, namely MIC, MBC, and Time Killing Assay. Among the four essential oils, thymol and carvacrol exhibit the strongest antibacterial activity, showing the lowest MIC/MBC values and achieving a 99.9% bacterial kill rate (3-log reduction) in just 10 minutes at significantly lower concentrations compared to eugenol and menthol. Eugenol requires the highest concentrations to achieve the same bactericidal effect. On the other hand, due to limited antimicrobial activity exhibited by menthol, and its markedly lower performance compared to the other essential oils tested—combined with issues related to its resuspension—no further experiments were carried out on this compound.

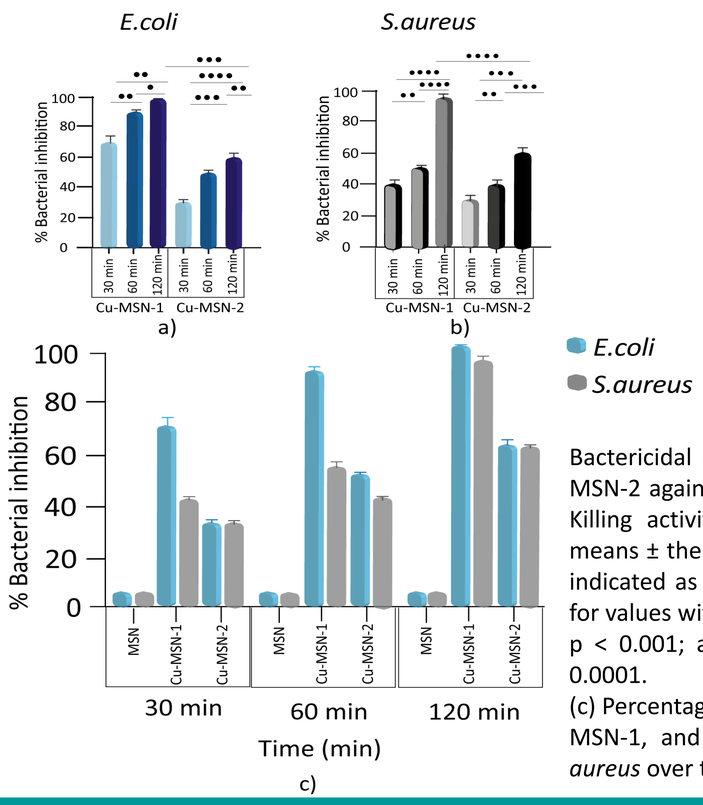


	S. aureus		*B. clausii*		*E. coli*		*K. pneumoniae*		*P. aeruginosa*	
EO	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Carvacrol	0.4 mg/mL	0.8 mg/mL	0.8 mg/mL	1.5 mg/mL	0.2 mg/mL	0.4 mg/mL	0.2 mg/mL	0.2 mg/mL	3.1 mg/mL	> 25.0 mg/mL
Eugenol	0.4 mg/mL	1.5 mg/mL	1.5 mg/mL	6.2 mg/mL	0.8 mg/mL	3.1 mg/mL	3.1 mg/mL	3.1 mg/mL	6.5 mg/mL	25.0 mg/mL
Thymol	$1.5x10^{-4}$ mg/mL	$6.2x10^{-4}$ mg/mL	$3.1x10^{-4}$ mg/mL	$2.5x10^{-3}$ mg/mL	$5.0x10^{-2}$ mg/mL	$5.0x10^{-2}$ mg/mL	$3.1x10^{-4}$ mg/mL	$1.2x10^{-3}$ mg/mL	1.5 mg/mL	3.1 mg/mL
Menthol *	39.0 mg/mL	n.d.	n.d.	n.d.	39.0 mg/mL	39.0 mg/mL	n.d.	n.d.	n.d.	n.d.
Menthol #	0.6 mg/mL	1.2 mg/mL	n.d.	n.d.	4.8 mg/mL	4.8 mg/mL	n.d.	n.d.	n.d.	n.d.
Menthol +	9.7 mg/mL	20.0 mg/mL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

MIC and MBC values of carvacrol, eugenol, thymol, and menthol against *S. aureus, B. clausii, E. coli, K. pneumoniae,* and *P. aeruginosa.*
*: menthol dissolved in H2O, # menthol dissolved in ethanol, + menthol dissolved in DMSO. n.d.: not determined.

Concentrations of carvacrol, eugenol, and thymol (expressed as means ± the SD) required to kill 99.9% of *S. aureus, B. clausii, E. coli, K. pneumoniae,* and *P. aeruginosa* after 10 minutes of exposure. Statistical significance was indicated as • for values with $p < 0.05$; •• for values with $p < 0.01$; ••• for values with $p < 0.001$; and •••• for values with $p < 0.0001$.

Based on comprehensive bactericidal and virucidal performance, carvacrol was selected as the most promising essential oil for nanoparticle functionalization. Two versions of copper-modified mesoporous silica nanoparticles, namely Cu-MSN-1, without calcination, and Cu-MSN-2, with calcination, were synthesized and functionalized with copper hydroxide ($Cu(OH)_2$) and carvacrol. Comparative antimicrobial evaluation using MIC, MBC, and time killing assays demonstrated that Cu-MSN-1 exhibited superior bactericidal activity against both *E. coli* and *S. aureus*. In fact, MIC values of 2.5 mg/mL were obtained for both Cu-MSNs preparations and both bacterial strains. However, an MBC value of 2.5 mg/mL was produced exclusively by Cu-MSN-1 against both bacteria. Moreover, after 120 minutes of incubation, Cu-MSN-1 achieved complete bacterial eradication (100% killing) against *E. coli*, while Cu-MSN-2 demonstrated only 60.4% inhibition ($p = 0.0009$). Against *S. aureus*, Cu-MSN-1 showed 95.1% reduction compared to 60.5% for Cu-MSN-2 ($p < 0.0001$), confirming the superior broad-spectrum antimicrobial efficacy of the non-calcined formulation (Cu-MSN-1).



Bactericidal activity of Cu-MSN-1 and Cu-MSN-2 against *E. coli* (a) and *S. aureus* (b). Killing activity values were expressed as means ± the SD. Statistical significance was indicated as • for values with $p < 0.05$; •• for values with $p < 0.01$; ••• for values with $p < 0.001$; and •••• for values with $p < 0.0001$.

(c) Percentage of killing activity of MSN, Cu-MSN-1, and Cu-MSN-2 on *E. coli* and *S. aureus* over time (30, 60, and 120 min).

Conclusions

The results of this study highlight the potential of copper-functionalized nanoparticles combined with carvacrol as effective antimicrobial coatings, offering promising applications in reducing pathogen transmission and raising the antimicrobial resistance on frequently touched surfaces, surpassing current surface-coating technologies.

References

[1] P.S. Stewart, J.W. Costerton, Antibiotic resistance of bacteria in biofilms, *Lancet*, **2001**, *14*, 135-8.
[2] World Health Organization. Global Action Plan on Antimicrobial resistance. Geneva, Switzerland: World Health Organization; **2015**.
[3] B. Xu, S. Li, R. Shi, H. Liu, Multifunctional mesoporous silica nanoparticles for biomedical applications, *Signal Transduct Target Ther*, **2023**, *24*, 435.

Acknowledgements

The RELIANCE consortium consists of 15 partners spanning 8 EU and 2 non-countries. Partners include research institutions, universities, SMEs, and large industries.

Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Health and Digital Executive Agency (HADEA). Neither the European Union nor the granting authority can be held responsible for them.

This project has received funding from the European Union's Horizon Europe research and innovation program, grant agreement No 101058570 (RELIANCE).