

# Evaluation of minimum inhibitory concentrations of plants extracts against several bacterial pathogens

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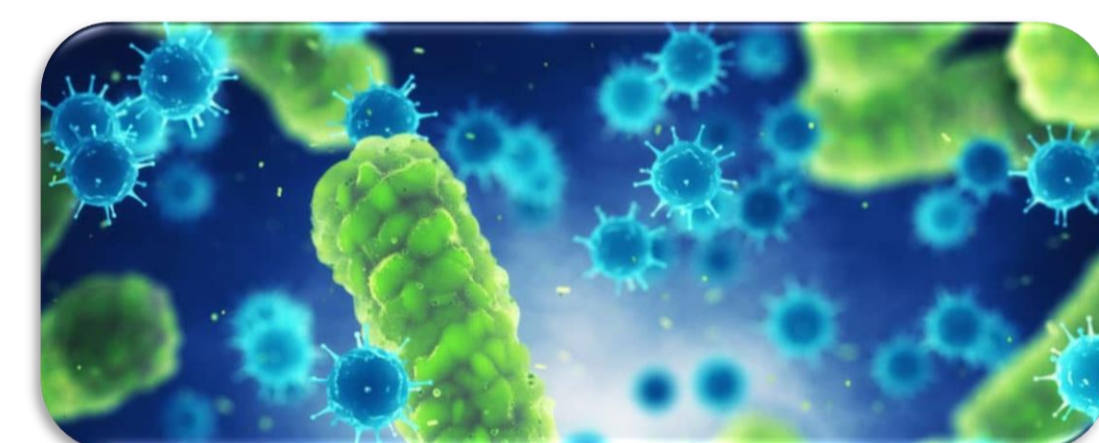
## Introduction

In the context of COVID-19, the use of face masks has been recommended as a preventive measure against the spread of SARS-CoV-2 [1]. Despite their health benefits, usage of single-use masks represents a threat to the environment as they are manufactured from long-lasting plastic materials. Reusable fabric masks are an alternative to reduce the risk of pollution and the amount of plastic wastage [2]. The application of antimicrobial agents in the tissues used to produce masks would be an additional hurdle on the prevention of other respiratory infections and secondary bacterial infections that occur from touching contaminated masks. In this context, impregnation of the fabrics with plant extracts is an attractive approach since they are potentially safe and free of adverse side effects and powerful antimicrobials. The objective of this work was to study twelve plant extracts to select the most efficient against pathogenic microorganisms.

## Methods

Determination of Minimum Inhibition Concentration (MIC) was performed with:

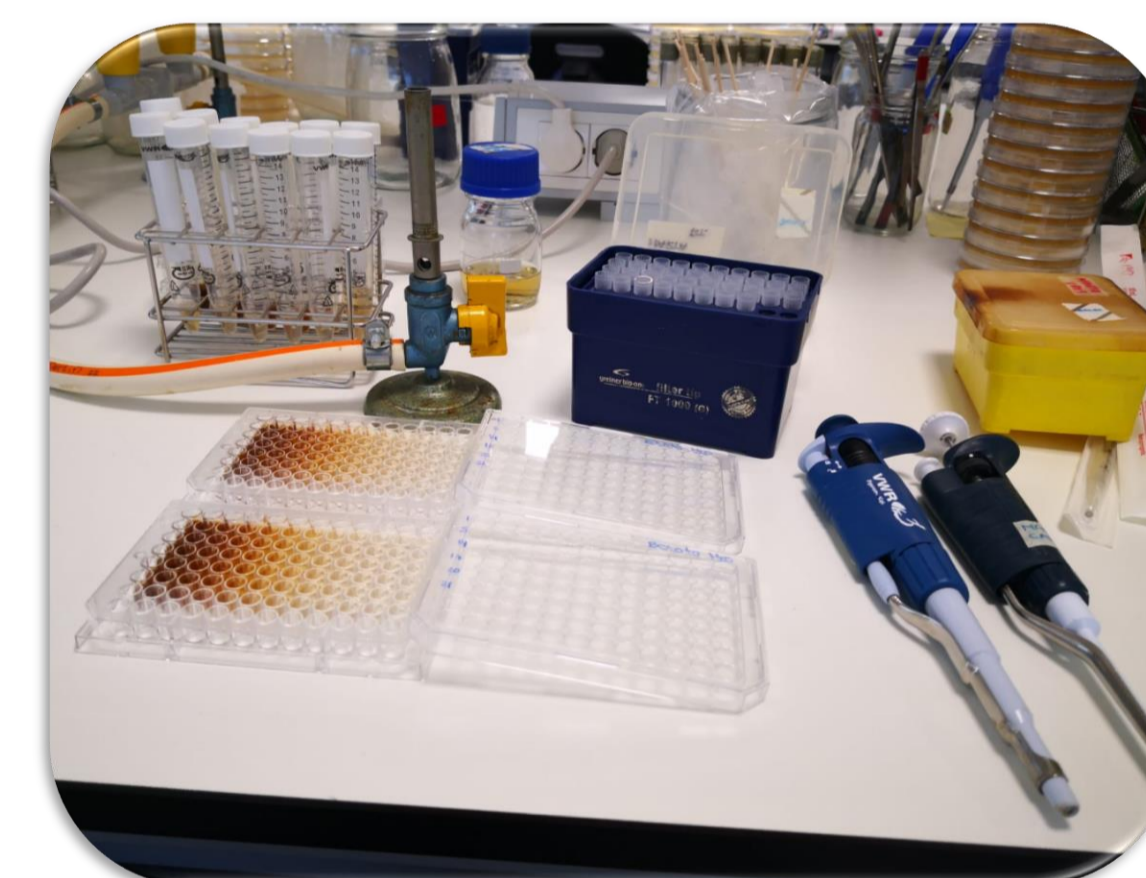
40 pathogenic strains



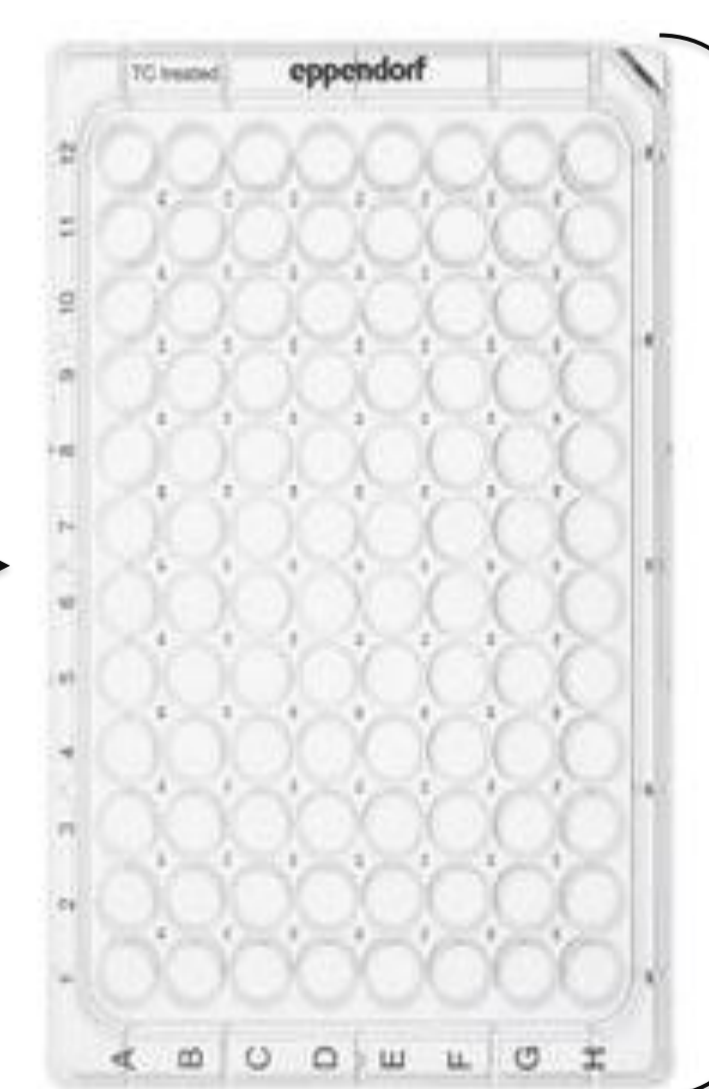
- 6 *Campylobacter* spp.
- 6 *Clostridium* spp.
- 5 *Enterococcus* spp.
- 5 *Salmonella* spp.
- 4 *Bacillus* spp.
- 3 *Listeria* spp.
- 3 *Candida* spp.
- 2 *E. coli*
- 2 *Staphylococcus aureus*.
- 1 *Pseudomonas aeruginosa*
- 1 *Acinetobacter* spp.
- 1 *Corynebacterium xerosis*
- 1 *Geobacillus stearothermophilus*

- 7 extracted with 1:1 ethanol:water
- 3 Extracted with 8:2 ethanol:water
- 2 Extracted with water

12 Plant Extract



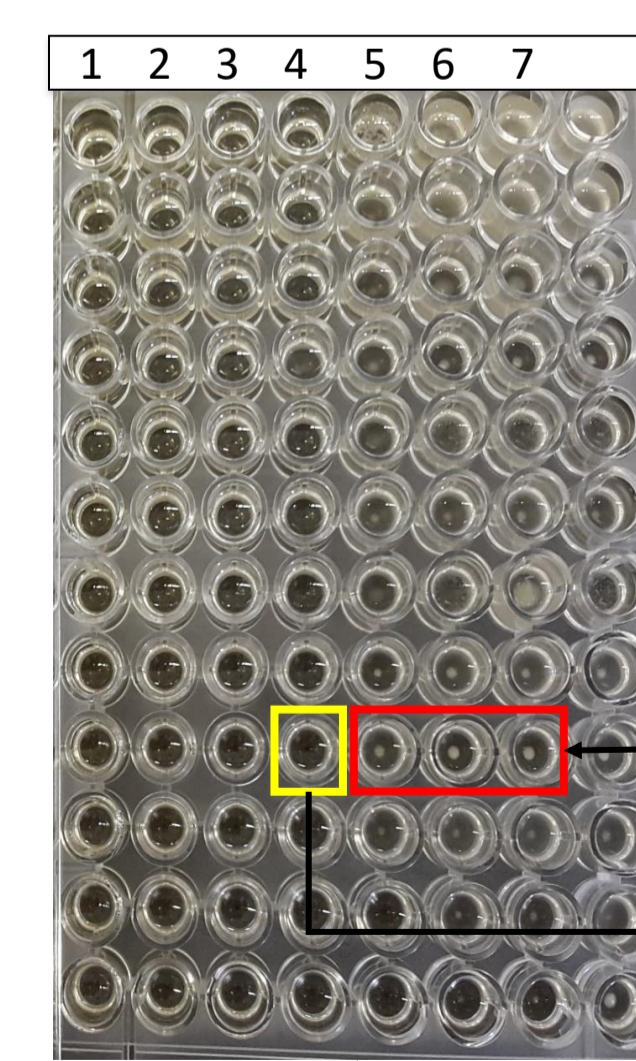
Dilution 1:1 in Mueller-Hinton Broth of each Plant Extract + selected microorganism



Final concentration in each well:

- Plant Extract:**
- 1- 50.00%
  - 2- 25.00%
  - 3- 12.50%
  - 4- 6.25%
  - 5- 3.13%
  - 6- 1.56%
  - 7- 0.78%

**Pathogens:** 10<sup>6</sup> UFC/ml



Bacterial growth

MIC: 6.25%

Incubation during 18-24 h at 37 °C (*Clostridium* strains were incubated 48 h at 37 °C in anaerobiosis and *Campylobacter* spp. were incubated during 48 h at 44 °C in microaerophilia).

## Results

**Table 1.** Determination of Minimum Inhibitory Concentration (MIC) (results are expressed in %) of twelve Plant Extract against different pathogenic microorganisms – NI: No inhibition

	Plant Extract 1	Plant Extract 2	Plant Extract 3	Plant Extract 4	Plant Extract 5	Plant Extract 6	Plant Extract 7	Plant Extract 8	Plant Extract 9	Plant Extract 10	Plant Extract 11	Plant Extract 12
<i>B. atrophaeus</i> ATCC 9372	12.50	50.00	25.00	50.00	12.50	12.50	12.50	NI	12.50	6.25	12.50	3.13
<i>B. cereus</i>	25.00	50.00	6.25	50.00	50.00	25	50.00	NI	3.13	1.56	12.50	1.56
<i>B. subtilis</i>	25.00	12.50	3.13	3.13	50.00	3.13	50.00	NI	50.00	NI	50.00	1.56
<i>B. stearothermophilus</i>	50.00	50.00	25.00	50.00	50.00	NI	50.00	NI	NI	1.56	12.50	12.50
<i>Corynebacterium xerosis</i> ATCC 7711	12.50	3.13	6.25	6.25	6.25	12.50	6.25	NI	NI	NI	NI	NI
<i>Clostridium perfringens</i> 1.16	NI	NI	NI	50.00	NI	NI	NI	NI	NI	NI	NI	NI
<i>Clostridium perfringens</i> 1.19	50.00	12.50	NI	50.00	50.00	50.00	NI	NI	NI	NI	NI	NI
<i>Clostridium perfringens</i> 1.22	50.00	50.00	50.00	50.00	50.00	NI	NI	NI	NI	NI	NI	NI
<i>Clostridium sporogenes</i> 1.31	50.00	12.50	50.00	50.00	NI	50.00	NI	NI	NI	NI	NI	NI
<i>Clostridium sporogenes</i> 1.34	50.00	50.00	NI	50.00	50.00	50.00	NI	NI	NI	NI	NI	NI
<i>Clostridium sporogenes</i> 1.61	50.00	50.00	NI	50.00	50.00	NI	NI	NI	NI	NI	NI	NI
<i>E. faecalis</i> ATCC 29212	50.00	3.13	3.13	6.25	50.00	3.13	50.00	NI	50.00	6.25	50.00	1.56
<i>E. faecium</i> DSMZ 13590	25.00	50.00	NI	50.00	50.00	50.00	NI	NI	50.00	NI	50.00	50.00
<i>E. flavescens</i> DSMZ 7370	12.50	3.13	25.00	3.13	3.13	25.00	50.00	NI	25.00	50.00	50.00	25.00
<i>E. gallinarum</i> DSMZ 20628	25.00	50.00	6.25	50.00	50.00	50.00	50.00	NI	50.00	50.00	50.00	25.00
<i>E. casseliflavus</i> DSMZ 20680	25.00	25.00	12.50	50.00	50.00	50.00	50.00	NI	25.00	NI	50.00	25.00
<i>Geobacillus stearothermophilus</i> ATCC 12980	1.56	1.56	50.00	1.56	3.13	6.25	6.25	50.00	25.00	50.00	50.00	25.00
<i>L. innocua</i> 2030c	12.50	25.00	12.50	25.00	50.00	25.00	50.00	NI	25.00	50.00	50.00	3.13
<i>L. monocytogenes</i> 2542	25.00	3.13	6.25	12.50	50.00	25.00	50.00	NI	25.00	50.00	50.00	6.25
<i>L. monocytogenes</i> SCOTT A	25.00	25.00	12.50	6.25	50.00	12.50	50.00	NI	50.00	50.00	50.00	3.13
<i>St. aureus</i> 18N (MRSA)	12.50	3.13	12.50	50.00	50.00	25.00	25.00	NI	25.00	50.00	50.00	12.50
<i>St. aureus</i> ATCC 6538	6.25	1.56	3.13	1.56	25	50.00	12.50	NI	50.00	25.00	25.00	3.13
<i>Acinetobacter</i> spp. 260	25.00	25.00	25.00	12.50	25.00	25.00	25.00	NI	NI	50.00	NI	12.50
<i>A. baumannii</i>	12.50	25.00	25.00	12.50	12.50	12.50	25.00	NI	NI	50.00	50.00	12.50
<i>A. calcoaceticus</i>	25.00	6.25	25.00	12.50	12.50	12.50	25.00	NI	NI	50.00	50.00	12.50
<i>Campylobacter</i> spp. C9	50.00	25.00	25.00	12.50	12.50	12.50	25.00	50.00	NI	12.50	12.50	50.00
<i>Campylobacter</i> spp. C1305	6.25	6.25	6.25	3.13	1.56	1.56	12.50	50.00	25.00	25.00	12.50	25.00
<i>Campylobacter</i> spp. DFVF 1099	25.00	12.50	25.00	12.50	12.50	6.25	12.50	25.00	25.00	50.00	12.50	12.50
<i>Campylobacter</i> spp. NCTC 11168	25.00	25.00	25.00	12.50	6.25	12.50	25.00	25.00	50.00	50.00	50.00	50.00
<i>Campylobacter</i> spp. C3	25.00	25.00	25.00	12.50	12.50	25.00	25.00	25.00	25.00	12.50	12.50	50.00
<i>Campylobacter</i> spp. C21A	25.00	25.00	25.00	25.00	6.25	12.50	12.50	25.00	12.50	50.00	25.00	50.00
<i>E. coli</i> ATCC 25922	NI	NI	NI	50.00	50.00	50.00	NI	NI	NI	NI	NI	50.00
<i>E. coli</i> ATCC 8739	NI	50.00	NI	50.00	50.00	50.00	NI	NI	NI	NI	NI	50.00
<i>S. Braenderup</i>	NI	50.00	NI	50.00	50.00	50.00	NI	NI	NI	3.13	NI	50.00
<i>S. Enteritidis</i>	NI	50.00	NI	50.00	50.00	50.00	NI	NI	NI	NI	NI	50.00
<i>S. infantis</i> (M2016)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	50.00
<i>S. seftenberg</i>	NI	50.00	NI	50.00	50.00	50.00	NI	NI	NI	NI	NI	50.00
<i>S. Tiphymurium</i>	50.00	50.00	NI	50.00	50.00	50.00	NI	NI	NI	NI	NI	50.00
<i>Pseudomonas aeruginosa</i> ATCC 15442	50.00	50.00	NI	25.00	25.00	50.00	25.00	NI	NI	NI	NI	50.00
<i>Yersinia enterocolitica</i> NCTC10406	NI	50.00	NI	50.00	50.00	50.00	NI	NI	NI	NI	NI	NI
<i>Candida albicans</i>	NI	NI	NI	NI	NI	50.00	NI	NI	NI	NI	NI	NI
<i>Candida albicans</i> 1386	12.50	50.00	NI	50.00	50.00	50.00	NI	NI	25.00	NI	NI	NI
<i>Candida tropicalis</i>	6.25	25.00	NI	3.13	3.13	12.50	3.13	50.00	50.00	50.00	50.00	50.00

➤ Based on the results presented in **Table 1**, two extracts showed a potential antimicrobial effect to be explored in future studies: plant extract 2 and 4 were the most effective against most of the pathogens tested, with MICs between 50.00 and 1.56%.

➤ It is important to highlight that Gram-positive bacteria were eliminated more easily than Gram-negative bacteria – *Salmonella* spp. and *E. coli* were the most resistant.

## Conclusion

Although this was a preliminary work and more tests are needed, two out of twelve plant extracts studied could be good candidates to be impregnated into tissues in order to eliminate pathogens and avoid health-associated problems.

## References

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- Silva, A., Prata, J., Barceló, C., Duarte, A., Rocha-Santos, T. (2021) Risks of Covid-19 face masks to wildlife: Present and future research needs, *Science of The Total Environment*, Volume 792, 148505, ISSN 0048-9697

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