Determination of minimum inhibitory concentrations of several plants extracts against different 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 several plant extracts to select the most efficient against pathogenic microorganisms.

Methods

60 plant extracts tested against 55 microorganisms → Plants extracts were tested at 50% → The MIC was only determined for extracts that demonstrated antimicrobial activity

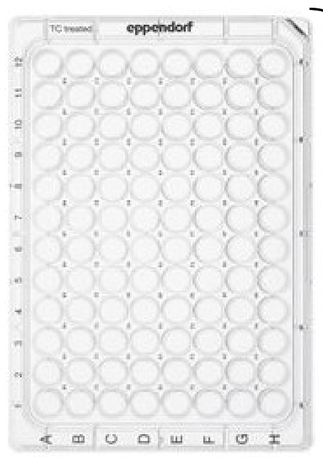
40 strains

- *Campylobacter* spp. (6)
- *Clostridium* spp. (6)
- *Enterococcus* spp. (5)
- *Salmonella* spp. (5)
- *Bacillus* spp. (4)
- *Listeria* spp. (3)
- *Candida* spp. (3)
- *Escherichia coli* (2)
- *Staphyloccocus aureus* (2)
- *Pseudomonas aeruginosa* (1)
- *Acinetobacter* spp. (1)
- *Corynebacterium xerosis* (1)
- *Geobacillus stearotermophilus* (1)

12 Plant Extract

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

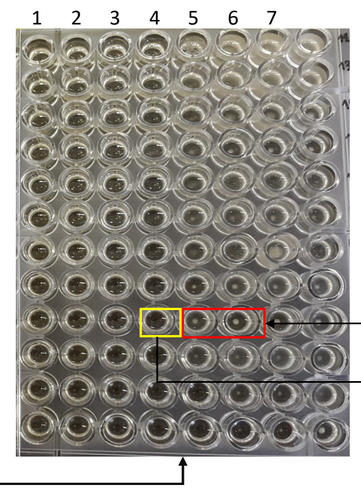
Dilution 1:1 in Mueller-Hinton Broth of each Plant Extract + selected microorganism

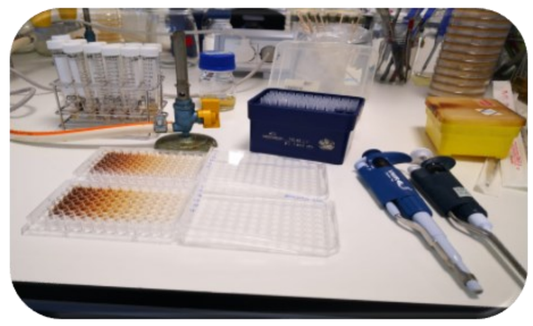


Final concentration in each well:

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

Pathogens: 10^6 UFC/ml





Incubation during 18-24 h at 37 ºC (*Clostridium* strains were incubated 48 h at 37 ºC in anaerobiosis and *Campylobacter* spp. were incubated during 48 h at 44 ºC 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
B. atrophaeus 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
B. cereus	25.00	50.00	6.25	50.00	50.00	25	50.00	NI	3.13	1.56	12.50	1.56
B. subtilis	25.00	12.50	3.13	3.13	50.00	3.13	50.00	NI	50.00	NI	50.00	1.56
B. stearothermophilus	50.00	50.00	25.00	50.00	50.00	NI	50.00	NI	NI	1.56	12.50	12.50
Corynebacterium xerosis ATCC 7711	12.50	3.13	6.25	6.25	6.25	12.50	6.25	NI	NI	NI	NI	NI
Clostridium perfringens 1.16	NI	NI	NI	50.00	NI	NI	NI	NI	NI	NI	NI	NI
Clostridium perfringens 1.19	50.00	12.50	NI	50.00	50.00	50.00	NI	NI	NI	NI	NI	NI
Clostridium perfringens 1.22	50.00	50.00	50.00	50.00	50.00	NI	NI	NI	NI	NI	NI	NI
Clostridium sporogenes 1.31	50.00	12.50	50.00	50.00	NI	50.00	NI	NI	NI	NI	NI	NI
Clostridium sporogenes 1.34	50.00	50.00	NI	50.00	50.00	50.00	NI	NI	NI	NI	NI	NI
Clostridium sporogenes 1.61	50.00	50.00	NI	50.00	50.00	NI	NI	NI	NI	NI	NI	NI
E. faecalis 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
E. faecium DSMZ 13590	25.00	50.00	NI	50.00	50.00	50.00	NI	NI	50.00	NI	50.00	50.00
E. flavescens 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
E. gallinarum 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
E. casseliflavus DSMZ 20680	25.00	25.00	12.50	50.00	50.00	50.00	50.00	NI	25.00	NI	50.00	25.00
Geobacillus stearothermophilus ATTC 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
L. innocua 2030c	12.50	25.00	12.50	25.00	50.00	25.00	50.00	NI	25.00	50.00	50.00	3.13
L. monocytogenes 2542	25.00	3.13	6.25	12.50	50.00	25.00	50.00	NI	25.00	50.00	50.00	6.25
L. monocytogenes 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
St. aureus 18N (MRSA)	12.50	3.13	12.50	50.00	50.00	50.00	25.00	NI	25.00	50.00	50.00	12.50
St. aureus ATCC 6538	6.25	1.56	3.13	1.56	25	50.00	12.50	NI	50.00	25.00	25.00	3.13
Acinetobacter spp. 260	25.00	25.00	25.00	12.50	25.00	25.00	25.00	NI	NI	50.00	NI	12.50
A. baumannii	12.50	25.00	25.00	12.50	12.50	12.50	25.00	NI	NI	50.00	50.00	12.50
A. calcoaceticus	25.00	6.25	25.00	12.50	12.50	12.50	25.00	NI	NI	50.00	50.00	12.50
Campylobacter 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
Campylobacter spp. CJ305	6.25	6.25	6.25	3.13	1.56	1.56	12.50	50.00	25.00	25.00	12.50	25.00
Campylobacter 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
Campylobacter 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
Campylobacter 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
Campylobacter 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
E. coli ATCC 25922	NI	NI	NI	50.00	50.00	50.00	NI	NI	NI	NI	NI	50.00
E. coli ATCC 8739	NI	50.00	NI	50.00	50.00	50.00	NI	NI	NI	NI	NI	50.00
S. Braenderup	NI	50.00	NI	50.00	50.00	50.00	NI	NI	NI	3.13	NI	50.00
S. Enteritidis	NI	50.00	NI	50.00	50.00	50.00	NI	NI	NI	NI	NI	50.00
S. infantis (M2016)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	50.00
S. seftenberg	NI	50.00	NI	50.00	50.00	50.00	NI	NI	NI	NI	NI	50.00
S. Tiphymurium	50.00	50.00	NI	50.00	50.00	50.00	NI	NI	NI	NI	NI	50.00
Pseudomonas aeruginosa ATCC 15442	50.00	50.00	NI	25.00	25.00	50.00	25.00	NI	NI	NI	NI	50.00
Yersinia enterocolitica NCTC10406	NI	50.00	NI	50.00	50.00	50.00	NI	NI	NI	NI	NI	NI
Candida albicans	NI	NI	NI	NI	NI	50.00	NI	NI	NI	NI	NI	NI
Candida albicans 1386	12.50	50.00	NI	50.00	50.00	50.00	NI	NI	25.00	NI	NI	NI
Candida tropicalis	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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