

In vitro antimicrobial activity of various essential oils against *Clostridioides difficile*

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Introduction

Clostridioides difficile is a Gram-positive, endospore-forming, and toxin-producing anaerobic organism responsible for mild-to-severe forms of gastrointestinal infections, ranging from asymptomatic intestinal colonization, self-limiting mild diarrhea to severe or life-threatening pseudomembranous colitis, toxic megacolon, sepsis, and death.^[1,2] Since *C. difficile* infection is associated with the antibiotics use and hypervirulent strains have shown antibiotic resistances, alternative therapeutics are urgently required. Plant-derived essential oils are natural antimicrobials found in many plants and could be capable of inhibiting the growth and survival of some microorganisms.^[3] Essential oils in aromatic plants are among the most significant active compounds of herbs and spices. Various biological characteristics, such as digestive, anti-inflammatory, sedative, antioxidant, antimicrobial, antiviral. Cytotoxic activities have also been attributed to the essential oils.^[4]

Methods

1. Essential oils and target *C. difficile*

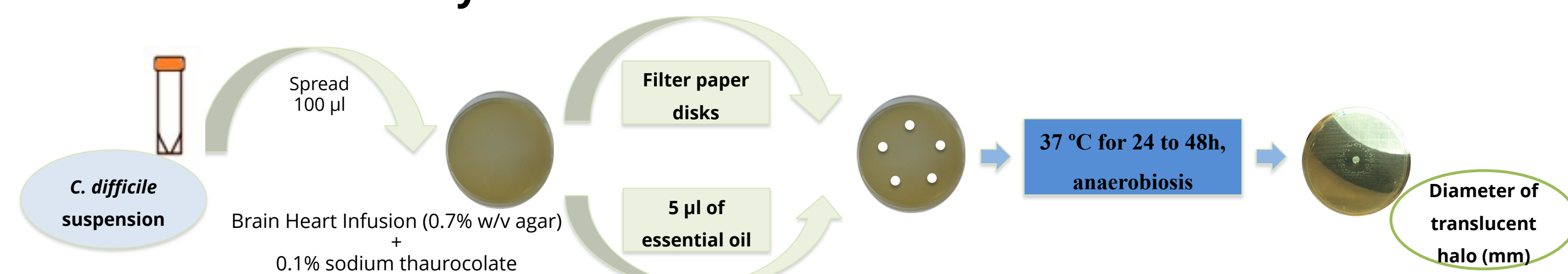
23 plant essential oils

Anise (*Pimpinella anisum*), basil (*Ocimum basilicum*), bay (*Laurus nobilis* L.) and fennel (*Foeniculum vulgare*), carrot (*Daucus carrot* L.), cloves (*Syzygium aromaticum*), coriander (*Coriandrum sativum*), cumin (*Cuminum cyminum*), nutmeg (*Myristica fragrans*), parsley (*Petroselinum crispum*), oregano (*Origanum vulgare* L.), rosemary (*Rosmarinus officinalis*), pepper mint (*Mentha piperita* L.) and sage (*Salvia officinalis*) lemon (*Citrus limon*), garlic (*Allium sativum*), ginger (*Zingiber officinale*), oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*)

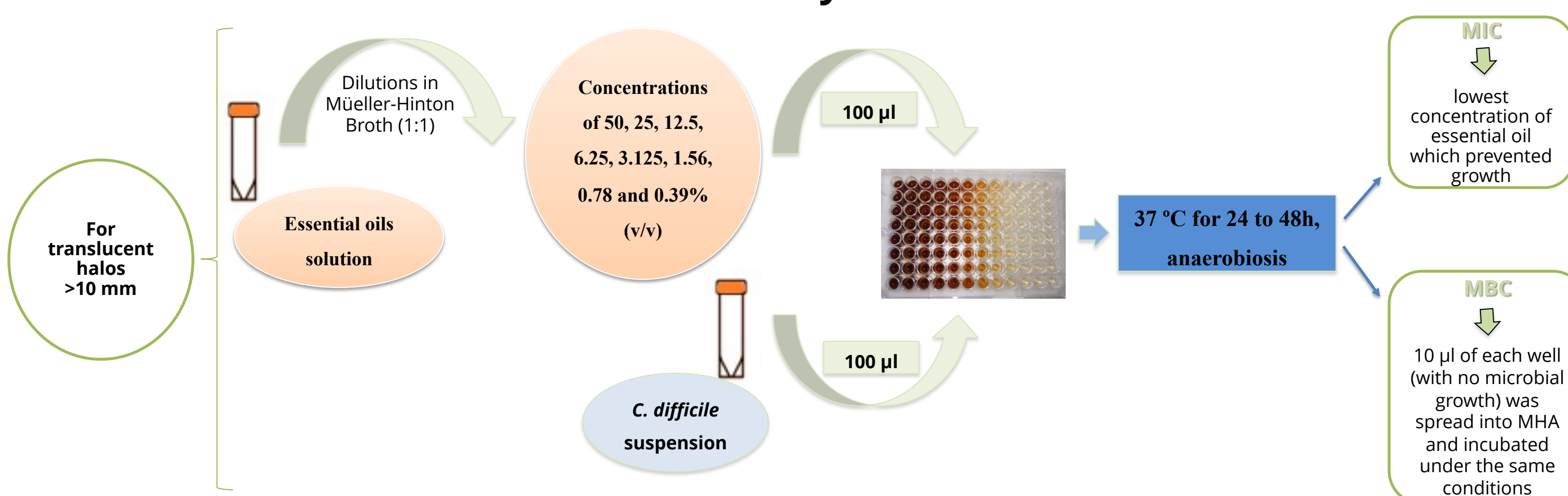
4 clinical *C. difficile* strains

C. difficile H63866
C. difficile 1805452
C. difficile 1805937
C. difficile V315638 (gently provided by Hospital de S. Marcos, Braga)

2. Disk diffusion assay



3. Determination of minimum inhibitory and bactericidal concentrations



References

- [1] Kachrimanidou M., Malisiovas N. (2011). *Clostridium difficile* infection: a comprehensive review. Crit Rev Microbiol, 37: 178-187.
- [2] Rupnik M., Wilcox M.H., Gerding D.N. (2009). *Clostridium difficile* infection: new developments in epidemiology and pathogenesis. Nat Rev Microbiol 7: 526-536.
- [3] Calo J.R., Crandall P.G., O'Bryan C.A., Ricke S.C. (2015). Essential oils as antimicrobials in food systems – A review. Food Control, 54: 111-119.
- [4] Raut J.S., Karuppaiyl S.M. (2014). A status review on the medicinal properties of essential oils. Ind Crops Prod, 62: 250-264.

Conclusions

- The greatest antiostridial activity was obtained for essential oils of oregano (oregano V. and oregano C.E) and thyme, being this last one the best, with translucent halo zones with diameters higher than 85 mm. Also good inhibitions were achieved for several essential oils, such as of basil, bay, cumin, nutmeg or parsley. In contrast, no inhibition was obtained for essential oils of garlic and anise against all *C. difficile* isolates.
- The highest inhibitions observed in the disk diffusion assay were in accordance with the lowest MIC and MBC values obtained. Essential oils of oregano and thyme presented MIC and MBC values ranging from 0.39 to 3.13% (v/v). Also essential oils of basil, bay, coriander, parsley and rosemary allowed inhibiting *C. difficile* isolates with low MIC and MBC values.
- The evaluation of antimicrobial activity of essential oils observed against *C. difficile* isolates revealed that several essential oils are potentially useful as alternative to antibiotics on the treatment and/or prevention of *C. difficile* infection.

Acknowledgements

This work was supported by funding from the National Funds from the Fundação para a Ciência e a Tecnologia (FCT) through project UIDB/50016/2020. Financial support for author J. Barbosa was provided by a post-doctoral fellowship SFRH/BPD/113303/2015 (FCT).



FCT Fundação para a Ciência e a Tecnologia

Objectives

The objective of this study was to determine in vitro antimicrobial activity of various essential oils against *C. difficile* isolates, aiming for a future anti-*C. difficile* strategy.

Results

Table 1. Zones of growth inhibition (mm; mean±standard deviation) showing antiostridial activity of tested

Essential oil	CdH63866	Cd1805452	Cd1805937	CdV315639
Anise	NI	NI	NI	NI
Basil	30.5 ± 2.1	28.5 ± 2.1	26.5 ± 2.1	29.5 ± 0.7
Bay	26.5 ± 3.5	41.5 ± 12.0	40.0 ± 0.0	41.0 ± 7.1
Carrot	11.5 ± 0.7	18.0 ± 2.8	19.0 ± 5.7	16.0 ± 5.7
Cloves	35.0 ± 2.8	25.0 ± 5.7	22.0 ± 0.0	27.0 ± 7.1
Coriander	31.0 ± 8.5	25.5 ± 7.8	30.0 ± 2.8	19.0 ± 1.4
Cumin	36.5 ± 4.9	35.0 ± 1.4	36.0 ± 5.7	43.5 ± 9.2
Fennel	10.0 ± 2.8	10.0 ± 1.4	13.5 ± 4.9	16.0 ± 5.7
Garlic	NI	NI	NI	NI
Ginger	19.0 ± 1.4	19.0 ± 1.4	18.0 ± 2.8	11.0 ± 1.4
Lemon	21.0 ± 1.4	21.5 ± 0.7	11.0 ± 1.4	11.5 ± 0.7
Nutmeg	27.0 ± 7.1	19.5 ± 2.1	36.0 ± 5.7	30.5 ± 0.7
Oregano V.	63.0 ± 7.1	77.5 ± 10.6	75.0 ± 7.1	79.0 ± 8.5
Oregano C.E.	60.5 ± 7.8	55.0 ± 4.2	62.5 ± 3.5	66.0 ± 2.8
Parsley	26.5 ± 7.8	36.5 ± 4.9	39.0 ± 4.2	33.0 ± 7.1
Pepper mint	13.5 ± 3.5	17.5 ± 3.5	11.5 ± 2.1	11.0 ± 1.4
Rosemary	29.0 ± 4.2	11.0 ± 1.4	13.0 ± 4.2	35.5 ± 7.8
Sage	34.5 ± 7.8	27.0 ± 4.2	30.5 ± 7.8	20.5 ± 2.1
Thyme	71.0 ± 0.0	>85 ± 0.0	>85 ± 0.0	>85 ± 0.0

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of essential oils tested (results are expressed in % v/v)

Essential oil		CdH63866	Cd1805452	Cd1805937	CdV315639
Basil	MIC	25	12.5	25	25
	MBC	25	6.25	50	25
Bay	MIC	3.13	6.25	6.25	6.25
	MBC	6.25	6.25	6.25	6.25
Carrot	MIC	50	50	50	50
	MBC	>50	50	>50	>50
Cloves	MIC	50	50	50	50
	MBC	>50	>50	>50	>50
Coriander	MIC	12.5	6.25	6.25	12.5
	MBC	12.5	12.5	12.5	12.5
Cumin	MIC	25	25	25	25
	MBC	50	50	50	50
Fennel	MIC	50	25	25	50
	MBC	>50	50	50	>50
Ginger	MIC	>50	>50	>50	>50
	MBC	>50	>50	>50	>50
Lemon	MIC	50	50	50	>50
	MBC	>50	>50	>50	>50
Nutmeg	MIC	50	50	25	25
	MBC	>50	>50	50	50
Oregano V.	MIC	3.13	0.39	0.39	0.39
	MBC	0.39	0.39	0.39	0.39
Oregano C.E.	MIC	0.39	0.39	0.39	0.39
	MBC	0.39	0.39	0.39	0.39
Parsley	MIC	3.13	6.25	6.25	6.25
	MBC	6.25	6.25	6.25	6.25
Pepper mint	MIC	50	50	25	50
	MBC	50	50	25	50
Rosemary	MIC	25	12.5	50	6.25
	MBC	50	25	50	12.5
Sage	MIC	25	12.5	12.5	12.5
	MBC	25	25	25	25
Thyme	MIC	3.13	0.39	0.39	0.39
	MBC	0.39	0.39	0.39	0.39