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In vitro study of antimicrobial activity of essential oils and their components against the main *Clostridioides difficile* PCR-ribotypes isolated in Belgium

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INTRODUCTION

The repeat use of antibiotics produce increased bacterial resistances and changes in the normal gut ecosystem. Essential oils have been suggested as potential agents to treat intestinal dysbiosis, and as natural preservatives against spoilage in food products. In vitro antimicrobial effects against several foodborne pathogenic bacteria have been previously demonstrated. However, to the present, there is few available data in the literature describing the capacity of some essential oils to inhibit the growth of the intestinal pathogen *Clostridioides difficile* (*C. difficile*).

PURPOSE

The aim of this study was to evaluate the in vitro antibacterial activity of ten commercial essential oils (and some of their main components) against the most common **C.** difficile ribotypes circulating in hospitals and associated with human disease. Additionally, various C. difficile isolates obtained from foods were also tested to compare the susceptibilities between human and food strains.

Table 2. List of essential oils and their properties based on their data of gas-

chromatography analysis provided by the manufacturer (Pranarom Int.)

METHODS

- **Essential oils (n=10)** tested in this study were provided by Pranarom

Table 1. C. difficile bacterial strains and their characteristics. *VPI (Virginia Polytech Institute) *UCL (Catholic University of Louvain)

		a l oils (n = tional (Belg	-	d in this	study we	ere provid	led by Pranar				· ·		Botanical	Common	Family	Part	Main composition (%)
			•		he found in	thaca aca	antial aile auch		Strain	PCR-Ribotype	Ioxin genes	Source	Species	Name	-		
	 Some individual constituents commonly found in these essential oils such as carvacrol, trans-cinnamaldehyde, eugenol, linalool and thymol were also 					2	2727	027	A ⁺ B ⁺ CDT ⁺	Patient	Cinnamomum cassia	Chinese	Lauraceae	Leaf- branch	E-cinnamaldehyde (77.90), trans-o-methoxy- cinnamaldehyde (10.50)		
	studied.			.,,	,				5063	078	A ⁺ B ⁺ CDT ⁺	Patient	Cinnamomum	Ceylon	Lauraceae	Bark	E-cinnamaldehyde (63.56), cinnamyl acetate
	✓ Fifteen strains of C. difficile, including PCR-ribotypes 014 (n=4), 027 (n=1), 078							078	5828	078	A ⁺ B ⁺ CDT ⁺	Patient	verum	cinnamon			(8.33)
	(n=6), UCL57 (n=1), UCL51 (n=1), UCL209 (n=1), UCL5378 (n=1) isolated from							2404	078	A ⁺ B ⁺ CDT ⁺	Beef burger	Coriandrum sativum	Coriander	Apiaceae	Fruit	Linalool (70.07), camphor (5.52), α-pinene (4.86)	
	feces of hospitalized patients and foods were tested for antimicrobial susceptibilities of the ten essential oils and their individual constituents.					bial 2	2405	078	A ⁺ B ⁺ CDT ⁺	Pork sausage	Cymbopogon	Ceylon	Gramineae	Herb grass	Geraniol (24.08), camphene (9.01), geranyl		
	suscepti	ibilities of t	he ten esse	ential oils a	nd their inc	dividual cor	nstituents.	1	1246	078	A ⁺ B ⁺ CDT ⁺	Meal sample	nardus	citronella			acetate (8.81)
					-		sion method a		1003	UCL57 ⁺	A ⁺ B ⁺ CDT ⁻	Pork sausage	Eugenia	Clove	Myrtaceae	Bud	Eugenol (84.75), eugenyl acetate (7.12), β -
	evaluated by measuring the diameter of the inhibitory zones. Results were						ere –			A ⁺ B ⁺ CDT ⁻	Patient	caryophyllus Origanum	Oregano	Laminaceae	Flowering	caryophyllene (4.60) Carvacrol (46.37), thymol (13.70), p-cymene	
	expressed as means of triplicates										A ⁺ B ⁺ CDT ⁻	Reference	compactum	_		plant	(13.33)
				-		-	amycin (2 µg/dis	SK),					Origanum	Greek oregano	Laminaceae	Flowering	Carvacrol (68.14), thymol (7.47), γ-terpinene
	-						netronidazole ce controls for t	tha			A ⁺ B ⁺ CDT ⁻	Minced pork	heracleoticum Origanum	Sweet	Laminaceae	plant Flowering	(6.06) Terpinene-4-ol (24.21), α-terpinene (8.44),
	tested b			με/ αισκ/,		d5 reference					A ⁺ B ⁺ CDT ⁻	Minced beef	majorana	marjoram		plant	sabinene (7.12)
	🗸 Data is	nresented	25 2 maa	n value +	standard	deviation	of triplicates. T		3973	014	A ⁺ B ⁺ CDT ⁻	Patient	Salvia sclarea	Clary sage	Laminaceae	Flowering	Linalyl acetate (62.38), linalool (21.47), $lpha$ -
		•					e and Tukey t		4455	014	A ⁺ B ⁺ CDT ⁻	Patient				plant	terpineol (2.45)
		ant differer		-	-		,		5413	UCL209	A ⁻ B ⁻ CDT ⁺	Patient	Thymus vulgaris thymoliferum	thymol thym		Flowering plant	Thymol (39.74), p-cymene (18.74), γ- terpinene (11.12)
									1703	UCL378	A ⁻ B ⁻ CDT ⁻	Pork sausage					
									DE	SULTS	<u> </u>						
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Antimicrobial activities of essential oils and individual c									onstituents against <i>C. difficile C. difficile</i> PCR-ribotypes					-ribotypes			
								_						_	_		
Та	i ble 3 . Antimi	icrobial activ	ity of essen	tial oils agai	nst bacteria	l strains of C	C. difficile	igure 1 . Ess	sential oil	ls and individu	al constituents	with the highes	t Figure 2	. Different a	ntimicrob	ial activit	ties of essential oils and individual
	i ble 3 . Antimi potypes VPI, (•	•				•			al constituents against <i>C. diffi</i> d	•					ties of essential oils and individual <i>ile</i> PCR-ribotype
L rit	ootypes VPI, (027, UCL209), UCL57 and	d UCL378 us				•				•				e C. diffici	ile PCR-ribotype
L rit		027, UCL209), UCL57 and diameter (d UCL378 us mm)**		sk diffusion	method a	•				•				e C. diffici	
L rit	ootypes VPI, (027, UCL209), UCL57 and	d UCL378 us mm)**	ing paper di	sk diffusion Strains isolate	method a ed from foods	and the low	ver antimic	crobial activity	against <i>C. diffic</i>	•				e C. diffici	diameter >40 Inhibitory diameter <
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CONCLUSIONS

This study is one of the few to report on susceptibilities of human and food C. difficile strains to the essential oils and their components. Our results revealed the efficacy of cinnamon, oregano and thyme essential oils and their main components, trans-cinnamaldehyde, carvacrol and thymol, respectively, against different C. *difficile* PCR-ribotypes. These findings suggest the potential use of these natural antimicrobials as adjuvant o preventive treatment for pathogenic C. difficile.

This work has been performed under the ECVPH resident program (C. Rodriguez, N. Korsak, G. Daube)