IAFP´S EUROPEAN SYMPOSIUM 2021
Virtual Meeting 27-28 April 2021

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

C. RODRIGUEZ*[1,2], H. MITH [3], B. TAMINIAU [4], N. KORSAK[4], A. CLINQUART[4], G. DAUBE [4]

[1] UGC Aparato Digestivo, Hospital Universitario Virgen de la Victoria, Málaga, Spain
[2] Instituto de Investigación Biomédica de Málaga, Málaga, Spain
[3] Faculty of Chemical and Food Engineering, Institute of Technology of Cambodia, Phnom Penh, Cambodia
[4] University of Liege, Faculty of Veterinary Medicine, Department of Food Science & FARAH, Liège, Belgium

*cris.rdrz@gmail.com

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.

METHODS

- ✓ **Essential oils (n=10)** tested in this study were provided by Pranarom International (**Belgium).**
- ✓ Some **individual constituents** commonly found in these essential oils such as **carvacrol**, t**rans-cinnamaldehyde**, **eugenol**, **linalool** and **thymol** were also studied**.**
- ✓ Fifteen strains of *C. difficile*, including **PCR-ribotypes 014** (n=4), **027** (n=1), **078** (n=6), **UCL57** (n=1), **UCL51** (n=1), **UCL209** (n=1), **UCL5378** (n=1) isolated from feces of hospitalized patients and foods were tested for antimicrobial susceptibilities of the ten essential oils and their individual constituents.
- ✓ The antibacterial activities were determined by **disk diffusion method** and evaluated by measuring the diameter of the inhibitory zones**. Results were expressed as means of triplicates**
- ✓ Six standard antibiotics: **chloramphenicol** (30 µg/disk) , **clindamycin** (2 µg/disk), **erythromycin** (15 µg/disk), **gentamycin** (10 µg/disk), **metronidazole** (80 µg/disk) and **vancomycin** (30 µg/disk), were used as reference controls for the tested bacteria.
- ✓ Data is presented **as a mean value ± standard deviation of triplicates**. The means were analysed by **one way analysis** of variance and **Tukey test** (significant difference at **P values ≤ 0.05**) (SAS version 9.1.).

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

Strain	PCR-Ribotype	Toxin genes	Source
2727	027	$A^+ B^+ CDT^+$	Patient
5063	078	$A^+ B^+ CDT^+$	Patient
5828	078	$A^+ B^+ CDT^+$	Patient
2404	078	$A^+ B^+ CDT^+$	Beef burger
2405	078	$A^+ B^+ CDT^+$	Pork sausage
1246	078	$A^+ B^+ CDT^+$	Meal sample
1003	$UCL57^+$	$A^+ B^+ CDT^-$	Pork sausage
1010	$UCL57^+$	$A^+ B^+ CDT^-$	Patient
0463	VPI 10463*	$A^+ B^+ CDT^-$	Reference
2012	014	$A^+ B^+ CDT^-$	Minced pork
3030	014	$A^+ B^+ CDT^-$	Minced beef
3973	014	$A^+ B^+ CDT^-$	Patient
4455	014	$A^+ B^+ CDT^-$	Patient
5413	UCL209	$A^- B^- CDT^+$	Patient
1703	UCL378	$A^- B^- CDT^-$	Pork sausage

Table 2. List of essential oils and their properties based on their data of gas-chromatography analysis provided by the manufacturer (Pranarom Int.)

Botanical Species	Common Name	Family	Part	Main composition (%)
Cinnamomum cassia	Chinese cinnamon	Lauraceae	Leaf-branch	E-cinnamaldehyde (77.90), trans-o-methoxy-cinnamaldehyde (10.50)
Cinnamomum verum	Ceylon cinnamon	Lauraceae	Bark	E-cinnamaldehyde (63.56), cinnamyl acetate (8.33)
Coriandrum sativum	Coriander	Apiaceae	Fruit	Linalool (70.07), camphor (5.52), α-pinene (4.86)
Cymbopogon nardus	Ceylon citronella	Gramineae	Herb grass	Geraniol (24.08), camphene (9.01), geranyl acetate (8.81)
Eugenia caryophyllus	Clove	Myrtaceae	Bud	Eugenol (84.75), eugenyl acetate (7.12), β-caryophyllene (4.60)
Origanum compactum	Oregano	Laminaceae	Flowering plant	Carvacrol (46.37), thymol (13.70), p-cymene (13.33)
Origanum heracleoticum	Greek oregano	Laminaceae	Flowering plant	Carvacrol (68.14), thymol (7.47), γ-terpinene (6.06)
Origanum majorana	Sweet marjoram	Laminaceae	Flowering plant	Terpinene-4-ol (24.21), α-terpinene (8.44), sabinene (7.12)
Salvia sclarea	Clary sage	Laminaceae	Flowering plant	Linalyl acetate (62.38), linalool (21.47), α-terpineol (2.45)
Thymus vulgaris thymoliferum	Common thymol thym	Laminaceae	Flowering plant	Thymol (39.74), p-cymene (18.74), γ-terpinene (11.12)

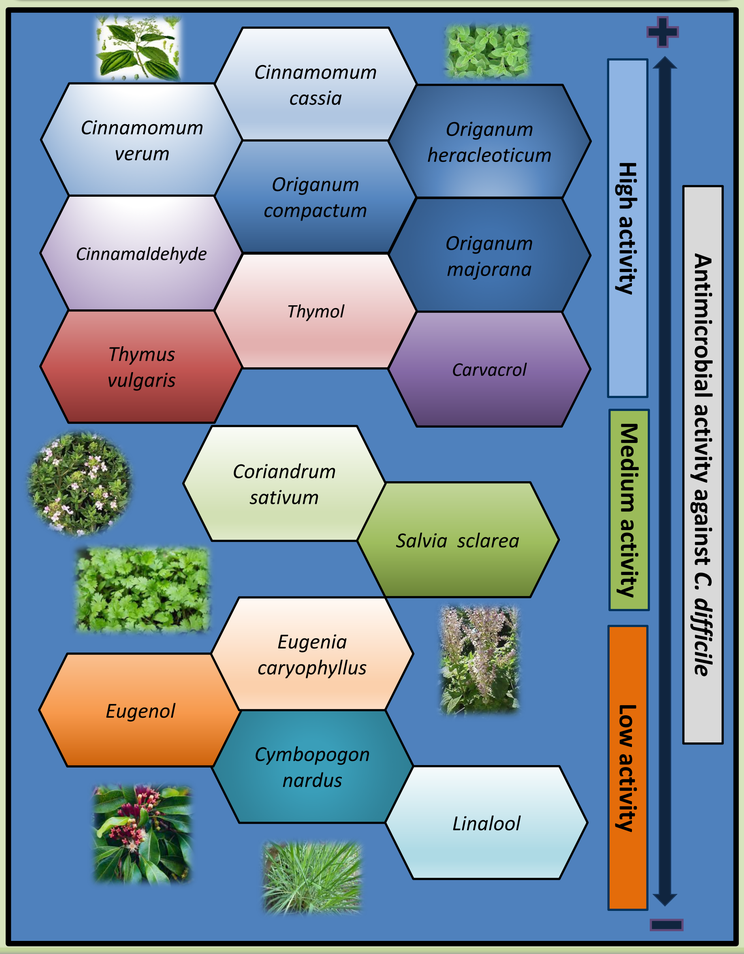
RESULTS

Antimicrobial activities of essential oils and individual constituents against *C. difficile*

Table 3. Antimicrobial activity of essential oils against bacterial strains of *C. difficile* ribotypes VPI, 027, UCL209, UCL57 and UCL378 using paper disk diffusion method

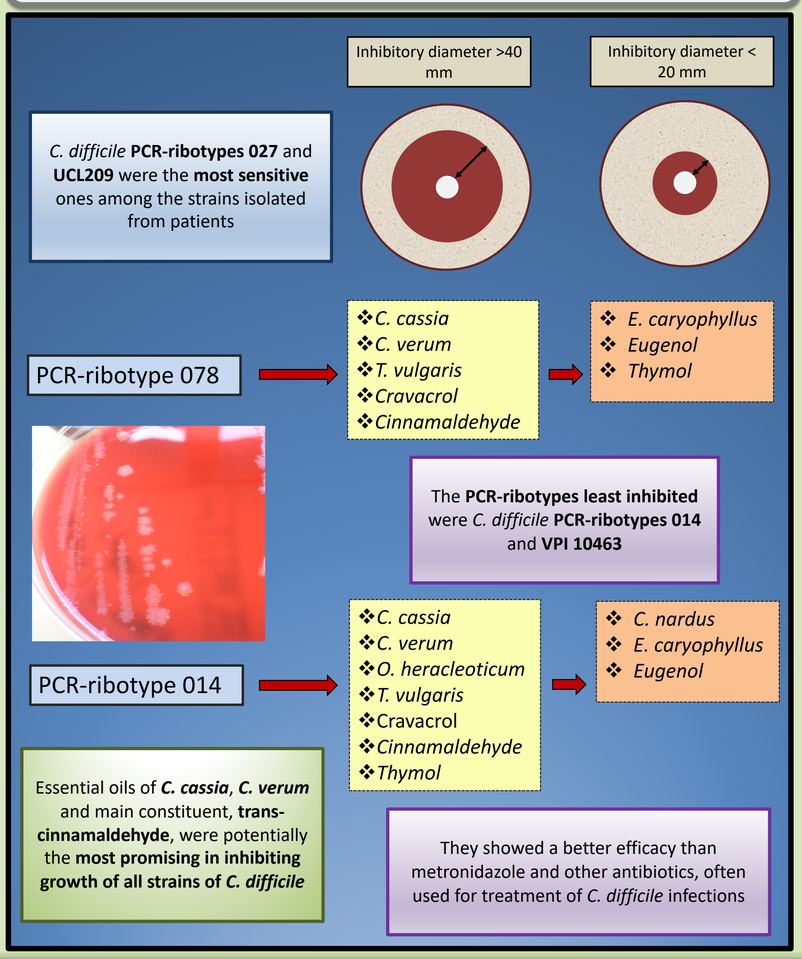
Samples	Inhibitory diameter (mm)**					
	Strains isolated from patients				Strains isolated from foods	
	VPI 10463	027	UCL209 (5413)	UCL57 (1010)	UCL57[6] (1003)	ULC378[7] (1703)
C. cassia	47.3 ± 0.3[a]	57.8 ± 0.1[a]	54.1 ± 1.3[a]	46.1 ± 1.2[a]	45.8 ± 1.3[a]	51.4 ± 0.5[a]
C. nardus	18.2 ± 0.5[b]	26.1 ± 0.8[b]	18.8 ± 0.2[b]	15.4 ± 0.6[b]	15.7 ± 0.7[b]	19.7 ± 1.0[b]
C. sativum	29.4 ± 0.9[ci]	31.2 ± 0.7[ck]	31.4 ± 0.5[cg]	29.2 ± 0.1[c]	27.1 ± 0.6[c]	29.6 ± 0.7[c]
C. verum	47.3 ± 1.6[a]	57.2 ± 0.9[a]	55.0 ± 0.4[a]	46.0 ± 1.4[a]	49.7 ± 0.4[d]	51.0 ± 0.6[a]
E. caryophyllus	15.6 ± 0.4[d]	20.3 ± 0.3[d]	22.2 ± 0.3[d]	17.9 ± 0.2[d]	17.4 ± 0.6[e]	19.7 ± 0.2[b]
O. compactum	31.0 ± 0.3[cf]	39.8 ± 0.7[e]	40.1 ± 1.3[e]	32.9 ± 0.1[e]	35.7 ± 0.7[f]	39.5 ± 1.4[de]
O. heracleoticum	33.9 ± 0.8[efl]	42.7 ± 1.4[fh]	43.4 ± 0.7[f]	36.8 ± 0.8[f]	40.7 ± 0.9[gl]	41.6 ± 0.5[eh]
O. majorana	32.1 ± 0.1[fl]	35.9 ± 0.8[g]	31.3 ± 0.6[cg]	33.3 ± 0.3[e]	33.9 ± 0.4[h]	36.0 ± 0.9[f]
S. sclarea	24.4 ± 1.1[g]	30.8 ± 0.8[ck]	32.7 ± 0.8[gk]	27.1 ± 0.3[g]	32.7 ± 1.2[h]	33.9 ± 0.5[g]
T. vulgaris thymoliferum	34.9 ± 0.9[e]	42.6 ± 0.2[f]	41.1 ± 1.3[ef]	35.9 ± 0.8[f]	38.1 ± 0.9[i]	42.6 ± 0.8[h]
Carvacrol	34.8 ± 0.5[e]	44.7 ± 1.3[h]	41.0 ± 0.5[ef]	41.2 ± 0.5[h]	38.4 ± 0.5[gi]	38.6 ± 0.5[d]
Cinnamaldehyde	54.9 ± 0.9[h]	59.5 ± 1.0[i]	57.2 ± 0.6[h]	45.3 ± 0.4[a]	49.1 ± 0.8[do]	50.3 ± 0.5[a]
Eugenol	18.5 ± 1.1[b]	21.3 ± 0.5[d]	21.6 ± 0.2[i]	18.7 ± 0.6[d]	20.9 ± 0.8[j]	20.2 ± 0.7[bi]
Linalool	27.9 ± 0.3[i]	30.1 ± 0.3[c]	32.6 ± 0.9[gk]	29.5 ± 0.6[c]	30.8 ± 0.3[k]	30.0 ± 0.2[c]
Thymol	41.0 ± 0.3[j]	52.1 ± 1.0[j]	47.4 ± 0.1[j]	40.4 ± 0.8[h]	40.8 ± 1.2[l]	43.5 ± 0.9[h]
*Chloramphenicol**	23.8 ± 0.6[g]	32.4 ± 0.5[k]	33.6 ± 0.6[k]	29.4 ± 1.2[c]	19.2 ± 0.1[m]	21.8 ± 0.9[i]
*Clindamycin**	9.6 ± 0.9[k]	-	11.6 ± 0.5[l]	-	-	8.2 ± 0.0[j]
*Erythromycin**	28.0 ± 0.7[i]	-	30.8 ± 0.9[c]	29.0 ± 0.8[c]	21.1 ± 0.3[j]	26.0 ± 0.4[k]
*Gentamicin**	15.0 ± 0.6[d]	10.9 ± 0.2[l]	13.3 ± 0.4[m]	-	9.3 ± 0.1[n]	14.8 ± 0.6[l]
*Metronidazole **	43.0 ± 1.6[j]	52.1 ± 0.6[j]	49.0 ± 0.9[n]	50.0 ± 0.1[i]	47.2 ± 0.7[ao]	45.5 ± 0.1[m]
*Vancomycin**	33.0 ± 0.7[l]	30.2 ± 0.6[c]	29.7 ± 0.2[c]	30.2 ± 0.3[c]	26.6 ± 0.2[c]	27.9 ± 0.7[n]

Figure 1. Essential oils and individual constituents with the highest and the lower antimicrobial activity against *C. difficile*



C. difficile PCR-ribotypes

Figure 2. Different antimicrobial activities of essential oils and individual constituents in function of the *C. difficile* PCR-ribotype



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)