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Citrus Essential Oils' Antibacterial Effects against Food-Borne Bacteria

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Abstract
Seven citrus essential oils (CEOs) and four terpenes were tested against eleven bacterial pathogenic strains isolated from food to evaluate the antimicrobial effects. All CEOs presented antibacterial activities. In particular, CEOs were very active against *Staphylococcus Epidermidis*, which was found to be the most sensitive toward all oils, showing high growth inhibition.

Keywords: Antimicrobial properties; Citrus essential oils; Food-borne bacterial pathogenic strains.

1. INTRODUCTION

The *Rutaceae* family comprises more than 2000 species across 160 genera, inside to this family there is the genus *Citrus*, important food crops, and some are grown as garden ornamentals. The *Citrus* species, widely used in the preparation of food, both sweet and savory, are spread all over the world but originally coming from Northern India, Northern Myanmar, Southern China, and Southeast Asia [1]. The citrus genus, and subsequently, the *Citrus* fruits have many compounds that give them unique flavor and aroma and that have beneficial effects on health, such as phenols, flavonoids, limonoids, essential oils (EOs), and vitamins, particularly, vitamin C and carotenoids [2]. Citrus essential oils (CEOs) are extracted and extensively studied for their potential uses in the food industry [3]. In fact, CEOs, thanks to their properties (i.e. antifungal, antibacterial, etc.), can be used in various industries from cosmetic to food [4-10]. CEOs consist of a mixture of t 400 compounds that are made up of two parts, volatile and non-volatile, of which the largest are volatile compounds (85-99%) [11]. The volatile fraction consist of mono terpenes such as α-/β-pinene, sabinene, β-myrcene, d-limonene, linalool, α-humulene, and α-terpineol and sesquiterpenes such as alcohols, acids, chetones, aldehydes, and esters [12]. Limonene is the major chemical component of CEOs. The difference in the composition of CEOs varies based on a number of factors such as season, geographic location, and a fruit's stage of maturity [13]. Thanks to their chemical composition, these oils can be used in the food industry, to counteract the growth of pathogenic bacteria and to lengthen the freshness of food, ensuring a high quality of food by replacing chemical preservatives [14]. This study aimed to test nine CEOs and four terpenes against 11 food-borne pathogenic bacteria isolated from various types of food to verify whether the CEOs have antibacterial activities against these pathogens and whether they can be considered as natural preservatives or as substances to add to food packaging.

2. METHOD(S)

Bergamot, lemon, bitter orange, sweet orange, blood orange, yellow tangerine, and clementine oils and bergamot, lemon, orange, and yellow tangerine terpenes, kindly furnished by Simone Gatto srl (Messina, Italy), coming from Southern Italy (Calabria and Sicily), were tested in this work.

Samples were were stored at 4°C in the dark until the analysis.

Tests were carried out at the IRBIM – CNR laboratory. The food-borne pathogenic strains were kindly furnished by Biomedical and Dental Sciences and Morphofunctional Imaging Department of the University of Messina; they are *Staphylococcus epidermidis*, *Vibrioharvey*, two strains of *Staphylococcus aureus*, two *Klebsiella sp*. strains, *Klebsiella pneumoniae*, and four strains of *Escherichia hermanii* and the food from which they were taken is listed in Table 1.

The essential oils' antimicrobial activity was performed *in vitro* by the disk diffusion method on agar plates, measuring the diameter of the inhibition zone as reported in [5].

Each experiment was carried out in triplicate.

3. RESULTS AND DISCUSSION

Staphylococcus epidermidis was the one that proved to be the most sensitive of all the strains tested, showing high sensitivity toward all essential oils. The highest halo was with bergamot terpene (39 mm).

Vibrio harvey showed a middle sensitivity for five EOs (bergamot and its terpene, lemon and its terpene, and bitter orange) and *S. aureus* 788 showed middle sensitivity for four EOs (i.e. bergamot and its terpene, lemon, bitter orange).

The other strains were sensitive to only a few oils and with an average sensitivity, as shown in Table 2. Among the CEOs, the bergamot essential oil and its terpene showed the best antibacterial action (against 7 out of 11 strains), followed by lemon EO (5 out of 11 strains), and bitter orange (4 out of 11 strains).

Bora *et al*. (2020) and Pandey *et al*. (2017) [2,15] highlighted that CEOs, thanks to their antimicrobial properties, could be used in the food industry. In fact, the antimicrobial activity of CEOs against human pathogenic bacteria has been reported from several authors [5,16,17].

Moreover, CEOs could be applied in the food packaging to protect food from environmental conditions such as humidity, light, and temperature, or from other factors such as dust, microorganisms, shocks, and vibrations [18], enhancing in this way the quality of food and to extend the shelf-life of food [19,20].

Our results suggested that these oils, in particular, Bergamot and Lemon, can be used as natural preservatives to combat food-bourne bacterial pathogens. The use of natural substances could be a good solution as natural alternatives to prolong the food shelf life and to prevent the development of most common bacterial pathogens linked to foods.

Table 1: Essential oil extraction.

EO	T	Extractor
L	Cold	Type brown
YT	Cold	Faso press
SO	Cold	FMC in line
BLO	Cold	FMC in line
BO	Cold	Sfumatrice
B	Cold	Peeler
EO	T	Extractor
L	Cold	Type brown
YT	Cold	Faso press
SO	Cold	FMC in line

L: Lemon, YT: yellow tangerine, SO: sweet orange, BLO: blood orange, BO: bitter orange, B: bergamot, C: clementine.

Table 2: Inhibition halos (mm).

	EO	T	EO	T	EO	T	EO	T	EO	EO	EO
Strain	B	B	L	L	BO	BO	YT	YT	SO	BLO	C
1	32	39	35	25	27	35	20	20	28	25	20
2	13	14	15	0	14	0	0	12	0	0	0
3	12	17	15	0	19	0	0	0	0	0	0
4	0	12	0	12	0	0	0	13	0	0	0
5	0	15	0	0	0	15	0	0	0	0	0
6	10	0	15	0	0	0	0	0	0	0	0
7	0	17	0	0	0	14	0	0	0	0	0
8	12	0	25	0	0	0	0	0	0	0	0
9	12	18	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	13	0	0	0	0	0
11	0	0	0	0	0	0	0	12	0	0	0

1: *S. epidermidis*; 2: *V. harvey*; 3: *S. aureus* 788; 4: *Klebsiella sp.*; 5: *K. pneumoniae 94 A*; 6: *S. aureus* 766; 7: *Klebsiella sp.* 2A; 8: *E. hermanii* 78; 9: *E. hermanii* 275; 10: *E. hermanii*; 11: *E. hermanii* 1015. B: bergamot, L: lemon, BO: bitter orange, YT: yellow tangerine, SO: sweet orange, BLO: blood orange, C: clementine, T: terpene.

4. CONCLUSION

In this article, the authors highlighted the potentiality of CEOs as natural alternatives to chemical and synthetic food preservatives, in fact, they can add to edible film and coatings to preserve and prolong the food shelf life. Further studies are necessary to better understand the real possibilities to add the bergamot and lemon oils to food antimicrobial packaging.

Author Contributions

Both authors contributed equally to this study.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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