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ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS EXTRACTED FROM CITRUS FRUITS

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ABSTRACT

A comprehensive literature review was conducted on the antibacterial activity of essential oils extracted from citrus fruits, such as lemon, orange, mandarin and grapefruit. The chemical composition of these oils is complex and can vary due to factors such as temperature, altitude and growing conditions. Essential oils are known for their antimicrobial properties and are used in various industries. The antimicrobial action is specific and it is suggested to combine them with other preservation methods. Citrus peel is rich in essential oils, the extraction of which has been of interest to the pharmaceutical and cosmetic industries. These oils contain compounds such as limonene and terpenes, known for their antibacterial properties. The concentration of essential oils varies in different citrus fruits, and those with thicker skins tend to have higher concentrations. The extraction of essential oils is carried out using emerging, more efficient and environmentally friendly technologies. The evaluation of antimicrobial activity is based on the Minimum Inhibitory Concentration (MIC), which stops bacterial growth. The essential oils of lemon and eucalyptus showed bactericidal effect on various strains, while those of orange and mandarin showed less activity. The relationship between antibacterial activity and the chemical composition of essential oils is crucial, for example compounds such as monoterpenes for Salmonella control are essential for antimicrobial activity. The reviewed literature evidenced that citrus essential oils are a promising alternative to conventional food preservatives due to their antimicrobial properties.

Keywords: antibacterial activity, essential oils, citrus fruits, Minimum Inhibitory Concentration (MIC).

1 Introduction

Citrus fruits include various species belonging to the genus "Citrus", such as lemon (*Citrus limon L.*), orange (*Citrus sinensis L.)*, tangerine (*Citrus reticulata White*) and grapefruit (*Citrus paradisi Macfie*.). The antimicrobial efficacy of citrus fruits can vary due to the complexity of their chemical components. (Bozkurt, Aka, & Gulnaz, 2017)
Throughout history, more efficient and better quality essential oil extraction methods have been sought and their chemical composition is influenced by factors such as temperature, altitude, growing conditions, soil origin, and plant age. . (Jiménez, Zamora, Campoverde, Mariscal, & Walter, 2022)Essential oils (ECs) have natural antimicrobial properties and are obtained from a variety of aromatic plants and fruits. They exhibit a wide range of biological activities, which has made them widely used ingredients in food chemistry, pharmacology, pharmacy, and related fields. (Song, y otros, 2020)
The antimicrobial action of essential oils is highly specific and it is generally recommended to combine essential oils with other preservation methods to achieve biopreservation. (Denkova, y otros, 2020)
The use of emerging technologies for essential oil extraction focuses on sustainable methods that reduce environmental impact. Two notable examples of these technologies are supercritical fluid-assisted extraction, which uses carbon dioxide in a supercritical state to obtain essential oils efficiently and without generating harmful residues, and ultrasonic extraction, which uses sound waves to release aromatic compounds from plants. These innovations play an important role in preserving biodiversity by preventing overexploitation of natural resources. (Wong, Zárate, Veana, & Muñiz, 2021)
Evaluations of oils from different sources show that grapefruit and lemon essential oil are effective against pathogenic bacteria (*E. coli*), but less effective against beneficial bacteria (*L. plantarum* ATCC 8014 and *L. plantarum* ES147) in the gastrointestinal tract, which may be beneficial. (Raspo, Vignola, Andreatta, & Juliani, 2020)
Essential oils offer natural antimicrobial properties and diverse applications in different industries, but their use must be complemented with other preservation methods and factors that affect their chemical composition must be considered. The aim of this article is to investigate the antibacterial activity of essential oils extracted from citrus fruits.

2 Citrus fruits

Citrus fruits are in predominant demand in the food industry, especially in the beverage sector, due to their high juice content that is used in the production of items such as juices and nectars. The various citrus varieties used include oranges (*Citrus sinensis*), tangerines (*C. unshi, C. tangerine, C. reticulate, C. clementine*), lemons (*C. lemon*), limes (*C. aurantifolia/latifolia*), grapefruit (*C. paradisi*) and grapefruits (*C. grandis*). (Wong, Aguilar, Veana, & Muñiz, 2020)

1. Waste

Citrus fruits have different chemical compositions as reported in Table 1.

0,61 Chemical Components of Some Citrus Fruits

Fruit	**Energy (kcal)**	**Water (g)**	**carbohydrates (g)**	**Dietary fiber (g)**	**Fats (g)**	**Proteins (g)**
Citrus sinensis	38	86	8,6	2,3	0,3	0,8
citrus lemon	39	87	9	1	0,3	0,7
Citrus reticulata	39	86	9	Fruit	Energy (kcal)	Water (g)
Carbohydrates (g)	Dietary Fiber (g)	Fat (g)	Protein (g)	Citrus sinensis	38	86

Fountain: (Cazar, 2016)

It is relevant to note that the chemical structure of citrus fruits can change depending on the species, however, citrus fruits are a remarkable source of essential nutrients and beneficial elements for well-being.

3 Essential oils

Essential oils are highly concentrated, aromatic and volatile extracts that can be obtained from the bark, leaves, flowers and fruits of various plants. (López, 2014)

The extraction of essential oils from waste has attracted the attention of the pharmaceutical and cosmetic industry due to its rich chemical composition, which includes alcohols, aldehydes, ketones, phenols, esters, ethers, and terpenes in different proportions. Many of these components are generally considered "safe" for use. (Mitidieri, Barbieri, Brambilla, & Piris, 2021)

It is possible to obtain these essential oils from the peel of the lemon, as it contains components such as monoterpene hydrocarbons, limonene, pinene and gamma-terpinene, which have been shown to have outstanding antibacterial properties. (Alvarez, Rodriguez, & Polo, 2022)

The components of oils are classified into terpenoids and non-terpenoids:

Terpenoids are essential and widely marketed in the industry due to their complex structure and presence in plants, especially citrus species.

Non-terpenoid compounds include aliphatic, aromatic, sulphur and nitrogenous substances, although they are less important in terms of utility. (Sharmeen, Mahomoodally, Zengin, & Maggi, 2021).

Table 2 shows the percentage yield of essential oil from citrus peels.

0,21%2 *Yield Percentage of Citrus Peel Essential Oil*

8,6	**2,3**
0,3	0,8
Lemon Citrus	39
87 Meski	9
1 Risso	0,3
0,7	Citrus reticulata
39	86
9	1,9

Fuete: (Urrunaga, Carpio, Gutierrez, & Tomaylla, 2022)

Citrus rinds harbor significant amounts of essential oils (0.5 to 3.0 kg per ton of fruit). Citrus fruits with thicker skins, such as sour orange (*Citrus aurantium*), grapefruit (*Citrus paradisi*) and bergamot (*Citrus bergamia*), show a high concentration of essential oils in contrast to thin-skinned citrus varieties. (Urrunaga, Carpio, Gutierrez, & Tomaylla, 2022)

2. Essential Oil Extraction

Extraction methods are known as emerging or unconventional technologies, those that have the potential to improve and optimize the process of obtaining essential oils from plants and natural products, in addition, they seek to be more efficient, environmentally friendly and, in some cases, more economical than traditional extraction methods. (Biamonte, 2017)

According to previous research, these approaches have the ability to improve yields in obtaining bioactive compounds. They are more accessible and easier to operate, generating high-quality compounds in significant quantities without requiring large amounts of solvents or high energy consumption. This is due to the short times required for extraction, which typically range from 5 to 40 minutes. (Caputo, Quintieri, Cavalluzi, & Lentini, 2018)

****Conventional extraction, **Unconventional extraction.3****MAE 2: 8 min at 100 °C*

0,19	**0,8**	**Citrus paradisi**	**32**
90	8,1	1.1 Extraction time: 8 h Temperature: 17°C to 20°C	0,1 Ethanol: 23.65% Water: 6.53%
	Ultrasound assisted extraction**	Solvents: ethanol:water (1:1), ethanol and water Extraction time: ultrasound power at 50%, 5 min and ultrasound power at 100% for 30 min. Temperature: 25°C to 30°C	Ethanol/water (50%): 36.92%; Ethanol/water (100%): 41.38% Ethanol (50%): 32.74%; Ethanol (100%): 40.90% Water (50%): 43.20%; Water (100%): 44.94%
	Microwave assisted extraction**	Solvents: ethanol:water (1:1), ethanol and water Extraction time: 3s to 20s Temperature: 80°C to 85°C	Ethanol/water: 9.52% Ethanol: 30.21% Water: 22.73%
C. aurantifolia Swingle	Maceration*	Solvents: 120 mL of 1% citric acid solution. Extraction time: 1 hour. Temperature: 90°C	Total phenols = 34.00 ± 0.04 – 37.67 ± 0.05 mg/g extract
	Yield (g oil/g sample)	Citrus jambhiri Extraction time: 5 min, 800 W. Temperature: 120°C	0,72%*
	1,06%	Citrus sinensis var.	2.31%

Citrus grandis		meski Temperature: 40°C	80% ethanol= 18.46% 50% ethanol= 15.64% 100% ethyl acetate= 5.12%
	1,75%	Citrus aurantium Linn. Extraction time: 60 min Temperature: 45 °C Frequency: 35 kHz	1.29% 80% ethanol= 19.24% 100% methanol= 13.84%
Citrus paradisi	0,79%	Citrus limon (L.) Burm. Extraction time: 24 hours. Temperature: 50°C	0,21%
	Microwave assisted extraction**	Solvent: water Power: 10 W MAE 1: 20 min at 80 °C MAE 2: 8 min at 100 °C MAE 3: 20 min at 100 °C	Interval between treatments between 18.0% to 21.5%

*extracción convencional, **extracción no convencional.
Fountain: (Wong, Aguilar, Veana, & Muñiz, 2020)

4 Bacteria

Bacteria require a minimal amount of nutrients for their growth, including water, a carbon source, a nitrogen source, and some mineral salts. These nutrients are used to carry out the biosynthesis of new cellular components, which requires energy from the environment. (Caycedo, Corrales, & Trujillo, 2021)

At present, a wide variety of microorganisms have been identified, comprising thousands of species. Some of these microorganisms are able to survive in extreme environments. In nature, they perform vital functions, such as decomposing organic matter and participating in biogeochemical cycles. (Rios & Bardales, 2022) (Molina, Paños, Reyes, & Ruiz, 2021)

These microorganisms, which are less than 1.0 mm in size according to our anthropocentric perception based on the resolving capacity of the human eye, are single-celled organisms that cannot be seen with the naked eye, but can be detected by using conventional light microscopes or, in greater detail, by scanning and transmission electron microscopes. (Sanchez, 2018)

Microbiological causes generate negative changes in food, however, there is a perception about the role of microorganisms, often relating them to unhygienic environments and diseases. (Ballesteros, Paños, & Ruiz, 2018)

Microorganisms obtain food from the substrate where they grow and secrete enzymes to break down complex compounds into simpler soluble foods. Enzyme activity is affected by temperature, pH, the presence of salts, and time. Microorganisms can survive in a wide temperature range and some require oxygen to grow, while others can thrive in the absence of oxygen. pH also influences growth, with some bacteria and fungi preferring low pH, but the optimal range for bacteria is 6.0 to 8.5. (Cervantes, Orihuela, & Rutiaga, 2017)

c. Different letters in the same column indicate significant differences between the strains tested (P<0.05)4 Diameters of inhibition halos (mm) of AE on strains tested

	Citric	**Type of extraction**	**Conditions**
Yield **ES147**	C. aurantium	Maceration*	Solvents: ethanol: water (1:1), ethanol and water
Ethanol/Water: 29.28% **Ethanol: 23.65%**	7,17 ± 2,56 b	Ultrasound-assisted extraction**	Solvents: ethanol: water (1:1), ethanol and water
Ethanol/water (50%): 36.92%; **Ethanol/water (100%): 41.38%**	14,50 ± 3,15 c	Microwave-assisted extraction**	Solvents: ethanol: water (1:1), ethanol, and water
Ethanol/Water: 9.52% **Ethanol: 30.21%**	C. aurantifolia Swingle	Maceration*	Solvents: 120 mL of 1% citric acid solution.

Total phenols = 34.00 ± 0.04 \u2012 37.67 ± 0.05 mg/g extract
b. ni: no inhibió
Microwave-assisted extraction**
(Vignola, Serra, & Andreatta, 2020)

3. Determination of the minimum inhibitory concentration (MIC)

Assessment of the susceptibility of bacteria to antimicrobials can be carried out using phenotypic (such as dilution and diffusion), biochemical, or genetic methods. Among them, phenotypic methods are the most commonly used initially due to their simplicity when it is desired to determine the sensitivity of microorganisms to a new antimicrobial compound. While diffusion techniques provide us with a preliminary assessment of the antimicrobial capacity of the compounds we are studying, dilution techniques allow us to determine the Minimum Inhibitory Concentration (MIC). (Pinheiro, y otros, 2022) (Muthular, y otros, 2019)

The Minimum Inhibitory Concentration (MIC) is the minimum amount of an antimicrobial (measured in µg/mL) needed to stop the visible growth of a microorganism after 24 hours of incubation at 37°C. MIC is considered the most reliable method for assessing antimicrobial susceptibility, outperforming other methods. In addition to detecting rare resistances, it provides definitive answers when other methods are inconclusive. (Horna, Silva, Vicente, & Tamariz, 2005)

CMI, was determined in applications of essential oils that act as antimicrobial agents that are frequently studied for their ability to protect food against pathogenic microorganisms (Gonzalez, Salazar, & Fuertes, 2022)

The antimicrobial activity of different ECs (lemon, orange, tangerine, bay leaf, and eucalyptus) in beneficial bacteria such as*Lactobacillus plantarum*ES147 and ATCC 8014, pathogens such as*Escherichia coli*ATCC 25922 and a food-spoilage bacterium,*Leuconostoc mesenteroides*MS1. Oils with significant inhibition halos were tested for minimal inhibitory and bactericidal concentrations. Lemon and eucalyptus showed bactericidal effect on all strains. Orange and tangerine had little antimicrobial effect. Laurel, tangerine, and orange had little impact on beneficial bacteria and generated large zones of inhibition in pathogenic or food spoilers. (Vignola, Serra, & Andreatta, 2020)

4. Relationship of antibacterial activity

The antimicrobial activity of citrus peel essential oils, with antimicrobial properties, may be an alternative to preservatives in food, taking advantage of a by-product of the juice industry. Its effectiveness varies according to the chemical composition, influenced by cultivation and extraction. (Perez, y otros, 2017)

Fruits have been found to contain a wide variety of nutritional compounds, including antioxidants such as phenolic acids, anthocyanins, carotenoids, flavonoids, vitamin C, and vitamin E. Therefore, incorporating more of these fruits into the diet helps prevent chronic diseases

(Mora, Cabrera, Alarcón, & Garcia, 2022)

5 Assessment of the antibacterial activity of citrus essential oils

(Torrenegra, Pajaro, & Leon, 2017)According to them, monoterpenes are the most abundant volatile metabolites in EAs. The main compound found is limonene for both HD and MWHD methods in all the samples evaluated, the identification of the components, retention times and abundance percentages are reported in theTable 5.

Relative abundance (%) of essential oils5 Major components detected in the AEs obtained by the method of steam entrainment hydrodistillation (HD) and microwave-assisted hydrodistillation (MWHD).

Solvent: 60 mL of 1% citric acid solution.	**Total phenols = 38.00 ± 0.02 \u2012 41.43 mg/g extract**							
	*Maceration**		*Solvents: ethanol, methanol, acetone, ethyl acetate (50%, 80%, 100%) Material/solvent ratio: 1:15 w/v*		*The highest yield was using ethanol.*		*C. paradisi*	
	Solvents: ethanol, methanol (50%, 80%, 100%) Material:solvent ratio: 1:20 w/v	**The highest yield was using ethanol.**	**C. medica L. C. sinensis L. C. lemon L**	**Hot Water Extraction***	**Solvent: preheated water**	**Range from 18.0% to 21.5%**	**HD**	**Microwave-assisted extraction****
Solvent: water Power: 10 W	Interval between treatments between 18.0% to 21.5%	*Conventional extraction, **Unconventional extraction.	1.68 (9.09)	1.77 (9.00)	0.70 (9.91)	0.90 (9.91)	0.45 (9.98)	0.65 (9.88)
β-mireno	1.17 (12.01)	1.39 (12.45)	Lemon	Orange	Tangerine	Lactobacillus plantarum	10.00 ± 3.40 b	7.17 ± 0.98 A

7.67 ± 0.52 A	Lactobacillus plantarum	7.17 ± 2.56 b	nor	6.67 ± 0.82 A	Escherichia coli	14.50 ± 3.15 C	5.50 ± 0.63 A	6.50 ± 0.55A
Leuconostoc mesenteroides	7.00 ± 2.28 to	nor	nor	to. Diameter of inhibition halo including 5 mm disk, expressed as the average of 3 replicates	B. Ni: Did not inhibit	c. Different letters in the same column indicate significant differences between the strains tested (P<0.05)	0.54 (15.90)	0.66 (16.09)

Tiempo de retención (t_R)

Abundancia relativa (%) de los aceites esenciales

On the other hand, the results of the evaluation of the antibacterial susceptibility of the essential oils obtained by the HD and MWHD methods (Table 1) allowed us to identify the extracts capable of inhibiting the growth of the bacterial strains, taking as a selection criterion, those that were able to inhibit the three strains by more than 90% (Table 6). Therefore, the MIC was determined (Table 7).
98.00±0.336 Antibacterial sensitivity of essential oils.

	Bacterial growth inhibition percentage				
Samples	*S. aureus*	*S. epidermidis*	*K. pneumontae*	*P. aeruginosa*	*E. coli*
C. sinensis HD	Compound	Percentage relative abundance (tR, min)*	92,50±0,33	C. sinensis	C. aurantifolia
*C. reticulata*MWHD	C. paradisi	91,03±0,30	HD	MWHD	HD
*MWHD*HD	HD	MWHD	HD	MWHD	α-pinene
*0.38 (9.89)***MWHD	0.59 (9.90)	1.68 (9.09)	1.77 (9.00)	0.70 (9.91)	0.90 (9.91)
*0.45 (9.98)*HD	0.65 (9.88)	β-Mireno.	1.17 (12.01)	1.39 (12.45)	1.00 (12.01)
*1.09 (12.45)*MWHD	0.90 (12.10)	1.00 (12.05)	6.11 (11.89)	6.25 (11.99)	Limonene
*88.80 (13.05)*HD	0.20 (13.01)	69.28 (13.36)	78.80 (13.61)	92.22 (13.34)	93.55 (13.55)
*72.11 (12.90)*MWHD	75.22 (12.91	Linalool	0.60 (15.09)	0.89 (15.56)	14.60 (18.14)
17.89 (18.09)	3.03 (14.79)	3.89 (14.88)	0.54 (15.90)	0.66 (16.09)	Retention Time (tR)

(Ambrosio, y otros, 2019)According to the IMC, it is determined with the use of inoculated and standardized broth, to which solutions of the essential oil at different concentrations are added, causing dilution. The values are presented in theboard
6007 Minimum inhibitory concentration (MIC)

Relative abundance (%) of essential oils	**Essential oil**	**Extraction method**	**CMI (µg/mL)**
Percentage of Bacterial Growth Inhibition	*Samples*	S. aureus	S. epidermidis
		E. coli	C. sinensis
	90.00±1.00	92.50±0.33	92.98±0.25
		90.90±0.50	91.03±0.30
	93.55±0.66	92.80±0.11	C. paradisi
		92.50±0.33	91.98±0.25
	C. paradisi	91.00±0.33	90.33±0.11
		92.78±0.11	C. aurantifolia
90.10±0.89	*90.23±0.05*	91.09±0.78	91.07±0.78
		90.99±0.25	90.47±.33
	92.98±0.33	92.17±0.67	C. reticulata
		92.08±0.15	92.00±0.22
	C. reticulata	91.00±0.33	91.00±0.25
		92.22±0.35	Gentamicin (control)
	98.00±0.50	Phone: 97.00±0.55	98.00±0.25
		MWHD	900
Bacterial strains	*Essential oil*	Extraction Method	MIC (µg/mL)
		HD	900
	C. paradisi	MWHD	900
		HD	1000
	C. aurantifolia	MWHD	1000
		HD	1000
	C. reticulata	MWHD	1000
		HD	900
P. aeruginosa	*C. sinensis*	MWHD	900
		HD	1000
	C. paradisi	MWHD	900
		HD	1000
	C. aurantifolia	MWHD	1000
		HD	1000
	C. reticulata	MWHD	1000
		HD	900
E. coli	*C. sinensis*	MWHD	900
		HD	700

	C. paradisi	MWHD	600
		HD	800
	C. aurantifolia	MWHD	700
		HD	900
	C. reticulata	MWHD	800
		HD	800

6 Conclusions

In conclusion, citrus fruits of the genus "Citrus" have a diverse chemical composition that includes essential components such as limonene, alcohols, aldehydes and terpenoids, which the essential oils extracted from these fruits demonstrate antimicrobial and preservative properties, presenting a potential as a promising alternative to conventional preservatives in the food industry.

The antimicrobial activity of these oils is linked to their chemical composition and varies according to the bacterial strains studied, the extraction methods, Although some citrus essential oils have a selective effect on pathogenic bacteria without significantly affecting beneficial bacteria, it is essential to balance their use to maintain the health of the microbiota, however research on the antibacterial activity of citrus essential oils highlights their potential as natural antimicrobial agents in the food industry, requiring a deeper understanding of their action on different strains and their Interaction with other preservation methods for optimal application.

7 Recommendation

It is advisable to conduct research that focuses on optimizing these methods. This could include evaluating different extraction techniques to maximize the yield of bioactive compounds and ensure efficient and sustainable production of citrus essential oils.

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