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REVIEW

Antimicrobial activity of compounds from hop (*Humulus lupulus* L.) following supercritical fluid extraction: An overview

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ABSTRACT

Although the female inflorescences of hop (*Humulus lupulus* L.) are mainly used in the brewing industry, they were first used as a medicinal plant. The antimicrobial potential of secondary metabolites, biosynthesized phenolic compounds, and derivatives such as α- and β-acids have been increasingly researched along with cleaner and more efficient extraction practices. Extraction is one of the methodologies used to isolate components from plant-based materials. Supercritical fluid extraction has become the most commonly used method for removing natural chemical components because it is more environmentally friendly, readily available, and non-hazardous. There is interest among researchers to extract bioactive compounds from hops with benefits for human health. This review describes the antimicrobial potential of compounds extracted from hops. Their composition, antibacterial and antifungal properties are explained. The effect of extraction methods on antimicrobial properties is discussed. Finally, the supercritical CO_2 method used to extract the antibacterial and antifungal potential of compounds from hops is reported.

Key words: Antibacterial activity, antifungal activity, hops, supercritical fluid extraction.

INTRODUCTION

Hop (*Humulus lupulus* L.) is a climbing, perennial, and dioecious species. Its female inflorescences are rich in phenolic compounds and bitter acids, such as humulone and lupulone, which are used to produce, preserve, and flavor beer (Zanoli and Zavatti, 2008; Iannone et al., 2022). However, this plant has also been historically used in phytotherapy; it has therapeutic properties for stress, sleep disorders, and gastric activity (Kowalczyk et al., 2013). Hop has also demonstrated antibacterial (Kramer et al., 2015) and antifungal (Nionelli et al., 2018) properties.

The hop plant has biosynthesized phenolic compounds, including phloroglucinol and its derivatives, that is, bitter acids and flavanones, with demonstrated antimicrobial effects against gram-positive bacteria (Kramer et al., 2015; Bocquet et al., 2018b). Although there is limited information on their antimycotic properties, hop acids and prenylchalcones have inhibited fungi (Mizobuchi and Sato, 1984). In an evaluation of bread preparation and preservation, hops significantly inhibited the hyphal growth of some microorganisms (Nionelli et al., 2018).

The antimicrobial and antifungal activity of hop compounds is directly related to the origin of the strain, growing area, harvest year, agronomic factors, drying conditions, and extraction and storage methods (Kowalczyk et al., 2013). Extracts can be produced by organic solvents, such as methylene chloride, trichloroethylene, methanol, ethanol, propanol, butanol, or hexane, or supercritical fluids (Sanz et al., 2019). Supercritical fluids are solvents that can be compressed above their critical point, that is, at a critical pressure (Pc) and critical temperature (Tc). In this state, the fluid is represented

by both its gas and liquid phase properties. In some ways, they are close to a gas but in others are close to a liquid (Uwineza and Waśkiewicz, 2020). Supercritical fluids have high diffusivity and low viscosity, which enables better diffusion into the matrix by improving solvent penetration in the solid (Escobar et al., 2020). Carbon dioxide (CO_2) is the most common supercritical extraction solvent used in food because it is low cost and readily available with a high degree of purity; it is also safe to handle, non-toxic, and its residues are easily disposed of. In addition, it is designated as a safe solvent by different organizations such as the U.S. Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) (Uwineza and Waśkiewicz, 2020).

Therefore, this review evaluates the most recent research on compounds from hop (*Humulus lupulus* L.) obtained under supercritical CO_2 extraction and their potential antibacterial and antifungal properties.

HOP (*Humulus lupulus* L.)

The hop plant has creeping vines up to several meters in height (Bocquet et al., 2018b). It has a main taproot and numerous secondary roots that grow 2 to 3 m deep with radio of more than 2 m.

Hop is extensively cultivated worldwide although it originates from temperate climates that are located between the 35° and 55° latitudes of the Northern and Southern Hemispheres. Germany and USA dominate world hop production (Almaguer et al., 2014). Below the 34-35° latitudes in both hemispheres, yields and demand are lower mainly due to problems with short daylengths (Rossini et al., 2021).

Associated with the leading quality characteristics of beer, hops provide bitterness, flavor, aroma, and stability to the final product and cause a preservative effect due to their antibacterial activity (Iannone et al., 2022). Hop cultivars are almost exclusively destined for the brewing industry; however, hop has attracted a great deal of interest in recent years for its medicinal and pharmacologically active compounds such as flavonones, chalcones, and chloroglucinol derivatives (Zanoli and Zavatti, 2008). Its biosynthesized phenolic compounds have also shown antimicrobial (Natarajan et al., 2008; Bocquet et al., 2018a; 2019) and antifungal properties (Mizobuchi and Sato, 1984; Bocquet et al., 2018a; Nionelli et al., 2018).

Extracts obtained by liquid or supercritical CO_2 offer better yields, fully use all components, and have less environmental impact (Zanoli and Zavatti, 2008; Kowalczyk et al., 2013). The hop compounds with antimicrobial properties are more concentrated in the female cones (Figure 1) and leaves; this occurs because bitter acids are the main compounds in cone extracts in which α- and β-acids form complex mixtures that could have an impact on cell walls and cause membrane permeability (Jacquin et al., 2022). Antifungal properties are mostly found in female cones and essential oils, although they are mediated by environmental factors, area, and growing conditions (Bocquet et al., 2018a).

Figure 1. Hop flower. Photographs were accessed at www.hops-comptoir.com.

Taxonomic classification

The genus *Humulus* belongs to the Cannabaceae family of the Urticales and consists of the *H. lupulus*, *H. japonicus*, and *H. yunnanensis* species (Almaguer et al., 2014). *Humulus lupulus* is further classified into five taxonomic varieties based on their morphological characteristics and geographic locations: *cordifolius* (Miq.) Maxim. in East Asia, *lupulus* in Europe and West Asia, and *lupuloides* E. Small, *neomexicanus* A. Nelson & Cockerell, and *pubescens* E. Small in North America (Alonso-Esteban et al., 2019).

Composition of hops

The mature female inflorescences (hop flowers or cones) are covered with trichome or lupulin glands (Zanoli and Zavatti, 2008), which are formed by cells that secrete essential oils and prenylated phenolic compounds (Bocquet et al., 2018a).

The essential oil, 0.3% of the dry weight of the hop cone trichome glands, consists of more than 70% hydrocarbons and 30% of its compounds are oxygenated. Hydrocarbons can be classified in three groups as aliphatic hydrocarbons, monoterpenes, and sesquiterpenes (Kobus-Cisowska et al., 2019). Previous research on essential oils from *H. lupulus* 'Cascade' in Brazil indicated a high content of humulene, myrcene, β-caryophyllene, and *trans*-β-farnesene (Almeida et al., 2021). An extensive variety of phenolic type compounds, such as protocatechuic acid, isoquercitrin, ferulic acid, caffeic acid, rutin, 4-hydroxymethyl, benzoic acid, naringenin, and taxifolin were also found (Almeida et al., 2021).

These categories of secondary metabolites have shown bioactive effects such as reacting with oxygen, chelating some metals, modifying enzymatic activity, and acting as antimutagenic, anticarcinogenic, and antiaging factors (Kowalczyk et al., 2013). As cones reach maturity, the secondary metabolite concentrations increase, although the final amounts largely depend on the climatic conditions and the cultivation method (Bocquet et al., 2019).

Bitter acids in hops (α- and β-acids), prenylated phenolic compounds, are present in *n*-humulone (co-humulone and ad-humulone) and *n*-lupulone (co-lupulone and ad-lupulone) (Bocquet et al., 2018b). The α-acids are responsible for beer bitterness, foam stability, and controlled bacterial activity (Zanoli and Zavatti, 2008). The β-acids (lupulone) have a less bitter taste, but are reported as having more active antimicrobial activity (Kolenc et al., 2023). In addition, co-humulone has exhibited antifungal properties against some human pathogenic fungi (Bocquet et al., 2018a).

Finally, the external parts of the lupulin glands contain chalcones and flavonones. The main chalcone is xanthohumol, which represents 1% of cone dry weight. It also predominates in beer, and is one of the most volatile compounds (Zanoli and Zavatti, 2008; Bocquet et al., 2019).

ANTIMICROBIAL PROPERTIES

Hops were first recognized to prevent spoilage in the 12th century (Natarajan et al., 2008). The current literature identifies the antibacterial, antiviral, antifungal, and antiparasitic properties from compounds in the female cones and leaves (Zanoli and Zavatti, 2008; Bocquet et al., 2019); humulone and lupulone resins and derivatives have values between 10% and 20% dry weight (Kolenc et al., 2023).

Xanthohumol is a prenylflavonoid of hops that has also been attributed with antimicrobial properties (Alonso-Esteban et al., 2019). Although there are drug-resistant strains of some microorganisms, including *Staphylococcus aureus*, parasitic *Trypanosoma brucei*, or *Leishmanis mexicana*, xanthohumol and lupulone have been effective agents in treating infections when administered in synergy with other antibiotics (Bocquet et al., 2019).

Action mechanism of hop extracts against microorganisms

Gram-positive bacteria are much more sensitive to hop extract compounds, including some species such as *Streptococcus*, *Staphylococcus*, *Lactobacillus*, *Micrococcus*, and *Bacillus* (Van Cleemput et al., 2009). Gram-negative microorganisms such as *Escherichia coli* are not significantly affected by hop components. The resistance of some microorganisms to hop compounds originates in the cell membranes (Simpson,

1993) in which lipophilic regions are target sites for bitter hop resin compounds, namely prenyl and acyl chains, whose antibiotic properties are negatively correlated with water solubility (Gerhäuser, 2005) and positively correlated with hydrophobicity (Karabín et al., 2016).

Bocquet et al. (2019) described alterations in primary membranes and cellular homeostasis disturbance. It has been reported that hop β-acids from a supercritical CO_2 hop extract impeded the growth of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella enteritidis*, and *Listeria monocytogenes*; this inhibited the active transport of sugars and amino acids, which disrupted cellular respiration and bacterial replication, transcription, and translation (Tian et al., 2021). Hop compounds are generally weak acids, which can inhibit bacterial growth in undissociated forms (Sakamoto and Konings, 2003).

One of the best ways to evaluate the antimicrobial activity of a compound is the minimum inhibitory concentration (MIC), which is the smallest concentration of a substance that inhibits the growth of organisms in a given time period. The MIC is estimated by the optical density of a culture diluted in a hop extract concentration to estimate the degree of growth. The efficacy of the antibacterial action of compounds and derivatives extracted from hops can be influenced by pH, cations (Simpson, 1993), and the extraction method (Tongnuanchan and Benjakul, 2014). Studies have shown that low intracellular pH interferes with enzymatic reactions and with nutrient absorption, which resulted in the death of hop-sensitive cells (Behr et al., 2006); high concentrations of some monovalent and divalent cations such as Mg^2 inhibited *Lactobacillus brevis* growth (Simpson, 1993).

Although these results suggest the mechanism for antibacterial pathways, further study is required. *Lactobacillus brevis* has exhibited resistance to antibacterial agents, including resistance to hops, which potentially modifies its membrane lipid composition to reduce permeability to hop compounds. The cell walls of gram-positive mycobacteria were reported to be as effective a barrier as the external walls of gram-negative mycobacteria. Hop-resistant microorganisms maintain a higher membrane pH than hop-sensitive strains. In addition, the ATP reserves in the hop-resistant strains were higher than in the sensitive strains (Sakamoto and Konings, 2003).

Antimicrobial activity

When used for long-term beer storage, hops have decreased *Lactobacillus*, which is a primary contaminant that produces undesirable flavors and causes yield losses. The main target of hop compounds are gram-positive bacteria such as *Lactobacillus*, *Streptococcus*, *Staphylococcus*, *Micrococcus*, and *Bacillus*. However, gram-negative bacteria such as *E. coli* can also be affected when hop concentrations are very high (Van Cleemput et al., 2009). Likewise, inhibitory activity has been observed on certain fungi such as *Penicillium* and *Aspergillus* (Alonso-Esteban et al., 2019).

Antibacterial activity

The antibacterial activity of hops is attributed to the compounds in its bitter acids, determined by the degree of hydrophobicity of acylphloroglucinols, and the number and length of the acyl or prenyl side chains (Bocquet et al., 2018a).

Research on the antibacterial activity of hops has been mostly related to food preservation; it has been found that their components can act as effective inhibitors of bacteria such as *Bacillus cereus*, *Listeria monocytogenes*, *Enterococcus faecalis*, *E. coli*, and *Salmonella typhimurium*. This highlights the potential of hop seeds as a source of functional ingredients in extracts or isolated compounds (Alonso-Esteban et al., 2019). These can be even more efficient in acidic foods with low fat content; however, high extract levels could be required, which can affect the sensory quality of applications (Van Cleemput et al., 2009). For example, extracts have been evaluated as a marinade for pork to reduce *S. aureus* and *S. enterica*, which showed an MIC of 6.3 and 12.5 ppm for β-acid and xanthohumol, respectively, whereas antimicrobial activity for α-acids was significantly lower at 200 ppm (Kramer et al., 2015).

Some literature suggests the effectiveness of a hop extract on bacteria associated with human health. Flavonoids in hops have a broad antimicrobial activity against bacteria such as *Streptococcus mutans*, a

causative agent of dental cavities, with an MIC of 12.5 µg mL^{-1}, and *Staphylococcus aureus* with an MIC of 6.25 µg mL^{-1} (Gerhäuser, 2005). Table 1 shows some bacteria that are affected by hop extracts.

Table 1. Microorganisms affected by hop extracts using different extraction methods. MIC: Minimum inhibitory concentration.

Type of activity	Microorganism	MIC (µg mL^{-1})	References
Solid-liquid extraction			
Antibacterial activity	*Bacillus cereus*	0.010	Alonso-Esteban et al., 2019
	Staphylococcus aureus	0.075	
	Listeria monocytogenes	0.150	
	Enterococcus faecalis	0.037	
	Escherichia coli	0.150	
	Staphylococcus typhimurium	0.150	
Antifungal activity	*Aspergillus fumigatus*	0.60	Alonso-Esteban et al., 2019
	A. ochraceus	0.15	
	A. niger	0.30	
	Penicillium ochrochloron	0.15	
	P. funiculosum	0.075	
	P. verrucosum	0.15	
Liquid-liquid extraction			
Antibacterial activity	*Corynebacterium* T25-17	39.00	Bocquet et al., 2019
	Enterococcus faecalis C159-6	39.00	
	Enterococcus sp. 8153	156.00	
	Mycobacterium smegmatis 5003	39.00	
	Staphylococcus aureus 8146	39.00	
	S. aureus 8147	39.00	
	S. epidermidis 5001	39.00	
	S. epidermidis 10282	98.00	
	S. lugdunensis T26A3	156.00	
	S. warneri T12A12	39.00	
	Streptococcus agalactiae T25-7	39.00	
	S. agalactiae T25-7	78.00	
	S. dysgalactiae T46C14	39.00	
Antifungal activity	*Candida albicans* 13203	156.00	Bocquet et al., 2019
	C. albicans ATCC 10231	< 39.00	
Hydrodistillation			
Antibacterial activity	*Corynebacterium* T25-17	39.00	Bocquet et al., 2019
	Enterococcus faecalis C159-6	39.00	
	Enterococcus sp. 8153	156.00	
	Mycobacterium smegmatis 5003	39.00	
	S. aureus 8146	39.00	
	S. aureus 8147	39.00	
	S. epidermidis 5001	39.00	
	S. epidermidis 10282	98.00	
	S. lugdunensis T26A3	156.00	
	S. warneri T12A12	39.00	
	Streptococcus agalactiae T25-7	39.00	
	S. agalactiae T25-7	78.00	
	S. dysgalactiae T46C14	39.00	
	C. albicans 13203	156.00	
	C. albicans ATCC 10231	< 39.00	
		Inhibition (%)	
Antifungal activity	*Penicillium polonicum* CBS 112490	< 20.0	Nionelli et al., 2018
	P. chrysogenum CBS 111214	8.0 to 13.0	
	P. paneum CBS 101032	< 20.0	
	P. albocoremium CBS109582	8.0 to 13.0	
	P. chermesinum CBS117279	< 20.0	
	P. carneum CBS112297	< 20.0	
	Eurotium herbariorum CBS117336	8.0 to 13.0	
	E. rubrum CBS150.92	8.0 to 13.0	
	Aspergillus parasiticus CBS971.97	< 20.0	
	A. versicolor CBS117286	8.0 to 13.0	
	Penicillium bialowiezense CBS110102	8.0 to 13.0	
	P. brevicompactum CBS28997	< 5.0	
	P. roqueforti DPPMAF1	< 20.0	
	P. aethiopicum DPPMAF2	8.0 to 13.0	
	A. niger DPPMAF3	< 20.0	
	Aspergillus penicilloides F1	8.0 to 13.0	
	Wallemia sebi F2	8.0 to 13.0	

Antifungal activity

Various individual hop compounds are known for their antifungal activity. Bocquet et al. (2018a) stated that bacteria were more sensitive to hop extracts than fungi, and related research is mainly limited to fungi involving humans. Evaluations of fungi such as *Aspergillus fumigatus*, *Penicillium ochrochloron*, *P. funiculosum*, and *P. verrucosum* have shown remarkable inhibitory results for hop resins compared with tested controls (Alonso-Esteban et al., 2019). In addition, evaluations of human pathogen fungi found that xanthohumol exhibited some inhibitory properties against *Trichophyton mentagrophytes*, *T. rubrum*, *Candida albicans*, *Fusarium oxysporum*, and *Mucor rouxianus* (Mizobuchi and Sato, 1984). The 6-prenylnaringenin compound extracted from hops was the most potent antifungal agent against *T. rubrum* and *T. mentagrophytes* with an MIC of 6.25 µg mL^{-1} (Gerhäuser, 2005).

The antifungal activity of hop extracts on wheat-borne fungi such as *Alternaria alternata*, *Epicoccum nigrum*, *Botrytis cinerea*, *Fusarium oxysporum*, and *Fusarium poae* has been evaluated in food; the supercritical hop extracts affected *B. cinerea*, which suggests potential as an antifungal agent in specific crops (Schoss et al., 2022). Table 1 lists some of the fungi that are affected by hop extracts.

EFFECT OF EXTRACTION METHODS ON ANTIMICROBIAL PROPERTIES

Common methods to extract plant substrates include steam distillation or extraction by organic solvents; however, these involve high temperatures that can decrease extract quality, and separation processes are required to remove the potentially toxic solvent from the final product (Kupski et al., 2017). Methyl chloride is the most common solvent to extract hops, but hexane and methanol are also used. These essential oils contain a high proportion of α- and β-acids (Del Valle et al., 2003), which provide antimicrobial properties to hops. However, the acid content of these extracts depends on the level of α-acids, age of the hops, storage conditions, solvent used, and extraction process temperatures (Bocquet et al., 2018a). Table 2 shows some techniques that have been applied to extract antimicrobial compounds from hops.

Table 2. Methods of extraction for specific hop compounds and their microbiological activity.

Extracted compound	Extraction method	Microbiological activity	References
α-Acids (humulin)	Supercritical extraction	Antibacterial/Antifungal	Van Cleemput et al., 2009; Karabín et al., 2016
β-Acids (lupulin)	Supercritical extraction	Antibacterial/Antifungal	Natarajan et al., 2008; Van Cleemput et al., 2009; Kramer et al., 2015; Karabín et al., 2016
Xanthohumol	Supercritical CO_2 extraction, conventional ethanol and methanol extraction, ultrasonic extraction, microwave extraction	Antibacterial/Antifungal	Mizobuchi and Sato, 1984; Gerhäuser, 2005; Magalhaes et al., 2007; Natarajan et al., 2008; Kramer et al., 2015; Zhang et al., 2021; Karabín et al., 2016; Klimek et al., 2021
Isoxanthohumol	Conventional extraction with methanol	Antifungal	Mizobuchi and Sato, 1984; Magalhaes et al., 2007; Yan et al., 2021
6-Prenylnaringenin	Supercritical extraction with CO_2, conventional extraction with ethanol and methanol	Antifungal	Mizobuchi and Sato, 1984
8-Isopentenyl naringenin	Conventional extraction with methanol	Antifungal	Mizobuchi and Sato, 1984

Santoyo et al. (2005) demonstrated supercritical fluid extraction (SFE) of *R. officinalis* for its potential use as a natural preservative in food due to its antimicrobial properties, in which produced concentrated and solvent-free compounds.

The SFE is an effective alternative for obtaining antimicrobial compounds from plant matrices because of its high productivity, which provides quality yields, and is suitable for extracting volatile and thermolabile compounds (Uwineza and Waśkiewicz, 2020). The quality of supercritical extracts is superior to extracts obtained by organic solvent extraction or those extracted by steam distillation (Santoyo et al., 2005). This is because fluid extraction preserves compounds such as hydrocarbons and mono- and sesquiterpene oxygenates, which are the main components of essential oils (Santoyo et al., 2005), and plays a key role in the antimicrobial properties of hop compounds (Bocquet et al., 2019).

The acids in hops can behave as bacteriostatic or bactericidal substances, although the profile and bioactivity of the hop essential oil depends on genetics, environmental factors, and the extraction process. The antiseptic potential of hops has increased with low pH, which generates changes in the permeability of the bacterial cell wall (Van Cleemput et al., 2009). Sanz et al. (2019) used low temperatures (7 to 8 °C) and pressures ranging from 1 to 1.2 MPa. These authors reported that supercritical CO_2 extraction of hop essential oils provided purer extracts of α-acids, which were free of resins, polyphenols, and pigments, and produced yields of 82.5% essential oils and 92.0% α-acids. However, it could be possible to obtain better extraction yields under stricter conditions. Table 1 shows the effect of different extraction methods on some microorganisms.

Supercritical extracts and hops

The SFE emerged from industrial applications in the mid-1980s as a response to reduce the use of organic solvents; it was effectively adopted in different countries (Uwineza and Waśkiewicz, 2020). Currently, SFE is recognized as one of the most important green technologies to produce solvent-free extracts, preserve bioactive components, and promote non-toxic solvents such as CO_2 and water (Pantoja et al., 2017).

Compounds can become supercritical when subjected to pressure and temperature conditions above their critical point at which they behave as a gas with the density of a liquid and function as a solvent (Uwineza and Waśkiewicz, 2020). When a compound is in a supercritical state, its high diffusivity and low viscosity enable it to penetrate a solid matrix, thus facilitating the extraction process and optimizing extraction times. The variations in pressure and temperature conditions provide extracts with different compositions (Escobar et al., 2020).

The SFE technology is used extensively in the food industry, especially with CO_2, which has enormous advantages over other solvents. This is because CO_2 is low cost and readily available, safe to handle, non-toxic, and its residues are easily disposed of. Supercritical CO_2 extraction is already widely applied in coffee and tea decaffeination, separation of spices and essential oils, lipid and cholesterol extraction from meat and meat products, and hop extraction (Uwineza and Waśkiewicz, 2020).

Supercritical fluid applications can also prioritize the extraction of certain components. By adjusting the process temperature and pressure, previous studies have obtained better diluted extracts, higher extraction rates, more bitter extracts (Kupski et al., 2017), improved compound concentrations (Van Opstaele et al., 2012), and have more effectively isolated bitter acids (Formato et al., 2013). Del Valle et al. (2003) found that 40 °C at 200 bar pressure is the ideal condition for optimal yields of soft resins from hop compounds.

Although other sources have provided valuable information, antimicrobial compounds extracted from hops by SFE have been evaluated. Flavonoid xanthohumol was tested by the agar diffusion method against microorganisms such as *S. aureus*, *E. coli*, *Alternaria* sp., *Rhodotorula rubra*, and *C. albicans* in a study on hop compounds from SFE. Stompor and Zarowska (2016) demonstrated that all these compounds were highly effective on gram-positive bacteria such as *S. aureus*, which had a marked 3.57 mm inhibition zone at concentrations of 5×10^5 CFU mL^{-1}. Moreover, all microorganisms,

except *E. coli*, were susceptible in different proportions to these compounds (Stompor and Zarowska, 2016). In another study, Rój et al. (2015) prepared extracts using supercritical CO_2 (50 °C at 30 MPa) that yielded 41% α-acids (*vs*. 0.7% in Soxhlet extractions). Additionally, the tested concentrations of these supercritical hop extracts effectively inhibited microorganisms such as *L. monocytogenes*, *B. cereus*, *Lactobacillus* sp., and *S. aureus* without affecting organoleptic properties. The *E. coli* isolated from intestinal flora from that study was unaffected by supercritical hop extracts (Rój et al., 2015). Table 3 shows other microorganisms affected by supercritical hop extracts.

Table 3. Microorganisms affected by supercritical hop extracts. MIC: Minimum inhibitory concentration.

Extraction with Supercritical Fluid with CO_2			
Type of activity	Microorganism	MIC μg mL^{-1}	References
Antibacterial activity	*Listeria monocytogenes*	80.0	Rój et al., 2015
	Bacillus cereus	20.0	
	B. cereus	2.5	
	Lactobacillus sp.	1280.0	
	Staphylococcus aureus MRSA ATCC 43300	5.0	
	S. aureus TCC 29213	5.0	
	Escherichia coli	> 2560.0	
	Propionibacterium acnes 199	3.1	Weber et al., 2019
	P. acnes 201	3.1-6.2	
	P. acnes 209	3.10	
	P. acnes ATCC 6919	3.11	
	S. aureus ATCC 29213	6.25-12.50	
	S. aureus ATC 25923	6.25-12.50	
	S. aureus 2407	6.25	
	S. aureus MRSA 4810	12.50	
	S. epidermidis CCM 7221	7.5-125	Bogdanova et al., 2018
	S. epidermidis 15895	30.0	
	S. capitis ssp. *ureolyticus* 16300	15.0-125.0	
	S. aureus CCM 4223	15.0-60.0	
	S. aureus MRSA 4591	15.0-60.0	
Antifungal activity	*Alternaria alternata*	72.32	Schoss et al., 2022
	Epicoccum nigrum	81.18	
	Fusarium poae	21.46	
	F. oxysporum	67.10	
	Botrytis cinerea	76.87	

Finally, research with notable biomedical interest (Bocquet et al., 2018b) has evaluated supercritical gel hop extracts against the microorganisms *Propionibacterium acnes* and *S. aureus* at 50% humulone and lupulone. Results were MIC 3.1 μg mL^{-1} for *P. acnes* and 9.4 μg mL^{-1} for *S. aureus* with inhibition zones of 5.5 and 3 mm, respectively. The supercritical hop compounds also exhibited anti-inflammatory and antioxidant properties (Weber et al., 2019).

CONCLUSIONS

It has been demonstrated that supercritical CO_2 extraction of hop compounds is effective and readily available; it is also safe to handle, non-toxic, and its residues are easily eliminated. The method shows better yields and higher concentrations compared with other techniques, which makes this technology an attractive process for hop extracts and research to effectively inhibit microorganisms.

Although food science has been using supercritical extracts of hop compounds for decades, knowledge about some specific antimicrobial pathways remains underexplored. Future research could specifically aim at gaps in the literature regarding antifungal activity and gram-negative microorganisms, needed concentrations of hop extracts, and the potential alterations in sensory properties that could result.

Author contribution

Writing-original draft: M.B.D. Historical and scientific search of literature: M.B.D. Structured the review and analyzed the main papers: M.B.D., J.L. Writing-review & editing: J.L. Conceived the research idea: J.L. Supervision: F.S. Project administration: F.S. Funding acquisition: F.S. All co-authors reviewed the final version and approved the manuscript before submission.

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