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Thymol, menthol and eucalyptol as agents for microbiological control in the oral cavity: A scoping review

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Summary

Dental plaque is a complex environment that maintains a balance with certain microbial communities; however, this microhabitat can be disturbed by some endogenous species causing disease. An exploratory systematic review was carried out using the PubMed, Scopus, Lilacs, and Science Direct databases, identifying that the thymol, menthol, and eucalyptol compounds present varying antimicrobial activity, intra- and interspecies discordance, and a strong antimicrobial intensity on *Aggregatibacter actinomycetemcomitans*, *Candida albicans*, *Candida dubliniensis*, *Enterococcus faecalis*, *Escherichia coli*, *Lactobacillus plantarum*, and *Streptococcus mutans*, indicating that these phytochemicals can be considered broad-spectrum antimicrobial substances, with an effect on microorganisms linked to oral diseases.

Key words: Oral health, terpenes, phytochemicals.

Resumen

Timol, mentol y eucaliptol como agentes para el control microbiológico en cavidad bucal: una revisión exploratoria

La placa dental es un ambiente complejo que mantiene un equilibrio con determinadas comunidades microbianas; sin embargo, este microhábitat puede ser perturbado por algunas especies endógenas causando enfermedad. Se realizó una revisión sistemática exploratoria empleando las bases de datos Pubmed, Scopus, Lilacs y Science Direct y se identificó que los compuestos timol, mentol y eucaliptol presentan actividad antimicrobiana variable, discordancias intra e inter-especie y una intensidad antimicrobiana fuerte sobre *Aggregatibacter actinomycetemcomitans*, *Candida albicans*, *Candida dubliniensis*, *Enterococcus faecalis*, *Escherichia coli*, *Lactobacillus plantarum* and *Streptococcus mutans*; indicando que estos fitoquímicos pueden ser consideradas como sustancias antimicrobianas de amplio espectro, con efecto sobre microorganismos relacionados con enfermedades bucales.

Palabras clave: Salud bucal, terpenos, fitoquímicos.

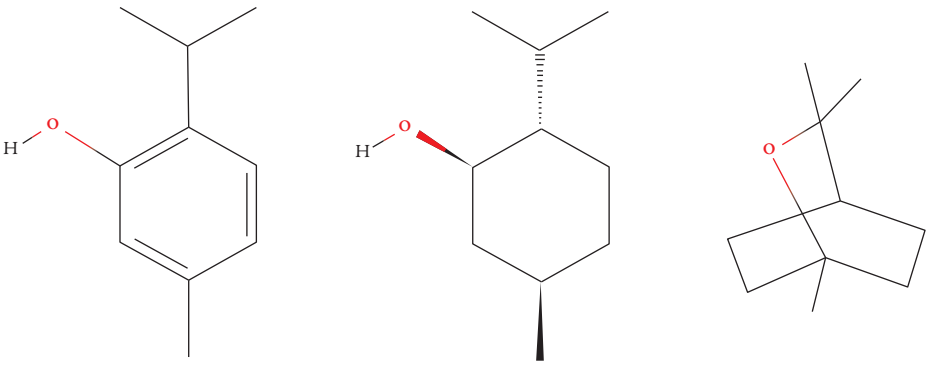
Introduction

The oral cavity is a dynamic complex environment that hosts a wide variety of microorganisms cohabiting in a beneficial way with the host; however, some endogenous species cause disease when environmental changes interfere with the homeostatic balance of this microhabitat [1]. To prevent the progression of these diseases, global and national health promotion programs and oral disease prevention policies have been proposed, including food regulation, traditional dental care programs, and the use of adjuvant products [2, 3]. However, due to the biological complexity and structure of dental plaque [4], it has been necessary to conduct research to identify effective substances not altering the biology of the oral mucosa [5].

As a result of these initiatives, it has been recognized that medicinal plants and their by-products can contribute to the maintenance of human health, with a growing interest in recent years on the identification of biological activity, like the antimicrobial effect [6-9]. Some of these natural substances alter the regular functioning of microbial cells, whether at the level of membranes, molecular interactions such as proton motive force, electron flow and efflux pumps, or the enzymatic activity of some proteins like the dehydrogenases, affecting the synthesis of ATP [6, 10] among other processes. These effects have been evaluated and reported by different techniques, including

dilutions and agar diffusion [11], which are chosen according to the nature or polarity of the substance of interest [11, 12]. The dilution method includes the broth dilution and agar dilution techniques, which in turn can be prepared in micro- and macrodilutions, while the agar or Kirby Bauer diffusion method comprises the techniques of disk diffusion, well diffusion, and antimicrobial gradient (Etest), whose techniques report the results in inhibition diameters [13].

Due to the incidence and prevalence of oral diseases worldwide [2] and in the country [3], the development of oral hygiene products has been promoted, including toothpastes and mouth rinses with the addition of compounds derived from plants [3, 5, 10], such as thymol, menthol, and eucalyptol (figure 1), which according to the literature have anti-plaque effects [14-19]. However, while bibliographic support is available, it is necessary to clarify some aspects based on a broad literature review, which may be applicable to the experimental design of future research, in order to promote the adequate use of these substances in the formulation of products aimed at oral health care. For this reason, an exploratory systematic review was proposed in order to identify such scientific evidence, analyze it and further clarify the appropriate possible use of these compounds, identifying their origin, procurement techniques, concentrations, and the microorganisms on which they have been tested and effective.



Thymol: $C_{10}H_{14}O$ Menthol: $C_{10}H_{20}O$ Eucalyptol: $C_{10}H_{18}O$

Figure 1. Chemical structure of the thymol, menthol and eucalyptol compounds [20].

Thymol (2-isopropyl-5-methylphenol)

Thymol is a monoterpene isomer with carvacrol, soluble in some organic solvents and alcohols, but with low solubility in water. It is colorless, with unpleasant taste and smell [6, 18-20]. Thymol is found in vegetable oils obtained from the species belonging to the Lamiaceae family and from other plants of the Ranunculaceae, Verbenaceae,

Apiaceae and Scrophulariaceae families. This compound is well accepted among the scientific community because of its wide range of biological properties (antispasmodic, antimicrobial, anticarcinogenic, anti-inflammatory, antiseptic, antioxidant) [18, 19, 21]; in addition, due to its physicochemical properties, it has good absorption, with rapid metabolization and elimination [18, 19, 22]. It is not cytotoxic on human cells derived from a human colon adenocarcinoma Caco-2 at concentrations of ≤250 μM [23], but showed mild toxicity at concentrations of ≥100 μM on the human colorectal carcinoma cell line HT29 [24]; in animals, there was a lethal dose (LD50) above 250 mg/kg [25].

It is classified by the European Commission and the Food and Drug Administration (FDA) as "generally recognized as safe" (GRAS) [26, 27]; however, it should be taken into account that prolonged exposure and intake of amounts higher than those recommended may be toxic [18, 22, 23, 27]. Concerning antimicrobial properties, thymol has effect on cariogenic bacteria [15, 17, 28, 29], periodontal pathogens [29], enterobacteria [17, 28, 30], and fungi of the Candida genus [21, 31, 32] (tables 1 y 2). The mechanisms of action used are exerted on the internal and external cytoplasmic membranes, which alter permeability, inducing their disintegration; in addition, it interferes with the synthesis of proteins of the outer membrane, enzymatic activity of the ATPase, and the citric metabolic pathways [6, 12, 33].

Menthol (2-isopropyl-5-methylcyclohexanol)

Menthol is a white, crystalline phenolic compound very soluble in alcohol, chloroform, ether, petroleum ether, and hexane, which can be isolated from essential oils extracted from some species of the Mentha genus and can also be synthetically manufactured [9, 16, 20]. It is classified by the European Commission and the FDA as GRAS [20, 27]; however, the inhalation of ≥200 mg and intake of ≥ 8000 mg causes a burning sensation in the mucous membranes of the digestive tract, and other discomforts that in rare cases can become serious [20]. Menthol is used in the pharmaceutical and food industries for products such as mouth rinses, toothpastes, candies, and flavoring due to its organoleptic characteristics (refreshing smell and taste), because it has a short half-life that prevents its accumulation in organisms, being metabolized and quickly eliminated by the lungs [9, 20].

It also has antimicrobial properties [9, 16] reported with strong intensity on *A.a* [29], *C. albicans* [34], *E. coli* [35], *L. plantarum* [15], and *S. mutans* [17] (table 3), and its effect is facilitated by its lipophilic characteristics, which allow it to migrate through the aqueous extracellular medium and interact with the phospholipid membranes, causing damage to these structures and even producing permeabilization and the subsequent leakage of intracellular material, thus destabilizing the microbial agent [33].

Eucalyptol (1, 8-cineole)

Eucalyptol is a liquid, colorless monoterpene soluble with alcohols. It is widely used in the pharmacological, cosmetics, and food industries due to its organoleptic properties (camphor smell and spicy refreshing flavor) [20] and is currently approved by the FDA for food preparation [27, 36]. Eucalyptol is quickly absorbed in the gastrointestinal tract and does not show systemic absorption in the lungs; in addition, it did not produce ocular irritation in bovine corneas, its intake does not cause significant renal toxicity in humans, and an LD50 of 2480 mg/kg has been reported by oral administration in rats; in general, it is considered a safe chemical as long as it is taken in doses indicated as normal [20]. This compound shows therapeutic effects in the treatment of bronchial asthma, chronic obstructive pulmonary disease, and gastric inflammation; it improves blood circulation, causes vasodilation and bronchodilation, and has antitumorigenic, hepatoprotective, anti-inflammatory and antimicrobial effects [36].

The antimicrobial activity has been reported in Gram-positive and Gram-negative bacteria, and such effects occur because the compound alters the permeability and function of the cell membrane, inducing intracellular filtration and morphological alteration of the microbial cell [37]. The effect of eucalyptol is dependent on many variables, like dose, the microbial strain and the presence of other substances; thus, on *S. mutans* it shows MIC/MBC of 250/500 μg/mL, but for *C. albicans, E. faecalis,* and *E. coli* it requires MIC >2001 μg/mL (table 3). However, the mixture of eucalyptol, methyl salicylate and thymol on *C. albicans* decreases the MIC from 125 000 to 62 500 μg/mL, on *S. mutans* from 250 000 to 125 000 μg/mL and on *E. faecalis* from 500 000 to 125 000 μg/mL [16], while an additive effect occurs when eucalyptol is mixed with α-terpineol and linalool respectively, which decreases the MIC on *E. coli* from 7000 to 3000 μg/mL [37].

Methodology

An exploratory scoping review was carried out searching for studies evaluating the antimicrobial (antibacterial and antifungal) activity of the thymol, menthol, and eucalyptol compounds from January 01, 1997 to December 06, 2017 (20 years). A scoping review is a systematic process that helps explore or map the literature reported on a given subject, with results that validate the formulation of hypotheses to respond to research and to guide new studies that complement or strengthen the reported evidence [38, 39].

Databases

A systematic search was carried out in order to find studies related to the antimicrobial activity of the thymol, menthol, and eucalyptol compounds in the PubMed, Scopus, Lilacs, and Science Direct databases.

Search strategy

A search equation was developed in order to filter results in English and Spanish languages using the descriptors registered in two thesauri: Mesh (National Library of Medicine) and the agricultural thesaurus (National Library of Agriculture of the USA and the Inter-American Institute of Cooperation for Agriculture): Thymol (timol); Menthol (mentol); Eucalyptol (eucaliptol); Anti-infective agent (Antimicrobianos); Antimicrobial (antimicrobiana); Antibacterial (antibacterianos, antibacterianas); Antifungal (Antifúngico, fungicida). The title-summary-keywords filter was used.

Search equation in the PubMed database: (((Anti-Infective Agents[MeSH Terms]) OR ((((Anti-Infective Agents[Title/Abstract]) OR antimicrobial[Title/Abstract]) OR antibacterial[Title/Abstract]) OR antifungal[Title/Abstract])) AND (((thymol[MeSH Terms]) OR menthol[MeSH Terms]) OR eucalyptol[MeSH Terms])) OR (((thymol[Title/Abstract]) OR menthol[Title/Abstract]) OR eucalyptol[Title/Abstract])

Criteria of inclusion (by reviewing each record's title and abstract) and exclusion (by reviewing the full text of each article)

- ✓ Language: articles written in English, Spanish, and Portuguese were included.
- ✓ Topic: articles assessing the antimicrobial activity of the thymol, menthol, and eucalyptol compounds were included.
- ✓ Type of text: original articles were included. Reviews, books, chapters, and these were not included.
- ✓ Methodology and results: articles not fully describing the methodology and the respective results were excluded.
- ✓ Articles not reporting the antimicrobial activity of the thymol, menthol, and eucalyptol compounds individually were excluded.
- ✓ Articles evaluating the antimicrobial activity with methods other than dilutions (in broth and agar) and diffusion in agar or Kirby Bauer were excluded.
- ✓ Full text availability: articles with no full text available were excluded.

Extraction and analysis of data

The retrieved records were exported to a bibliographic manager. Duplicates were eliminated in the Zotero, EndNote and Colandr programs, in addition to a final manual revision by the operator. To guarantee the quality and reproducibility of the obtained data, two researchers, experts in the field, carried out independent reviews; discrepancies were resolved by means of a third-party's opinion.

After reading the abstracts, full texts were downloaded and saved in the Mendeley bibliographic manager in PDF. Then the following variables were entered in a Microsoft Office Excel 2013 worksheet: year, country, title, authors, compound evaluated, microorganism tested, strain or isolated used, technique used, result, and detail of the result. With this information, a qualitative synthesis was made, and a descriptive analysis was conducted including percentage frequency distribution and its 95% confidence interval (95% CI) in version 22.0 of the IBM SPSS statistical software.

Results

As a result of the exploratory systematic review, a total of 7065 records were retrieved, applying the aforementioned criteria to finally analyze 86 documents related to the antimicrobial activity of the thymol, menthol, and eucalyptol compounds by means of the techniques of dilution in broth and agar diffusion (figure 2).

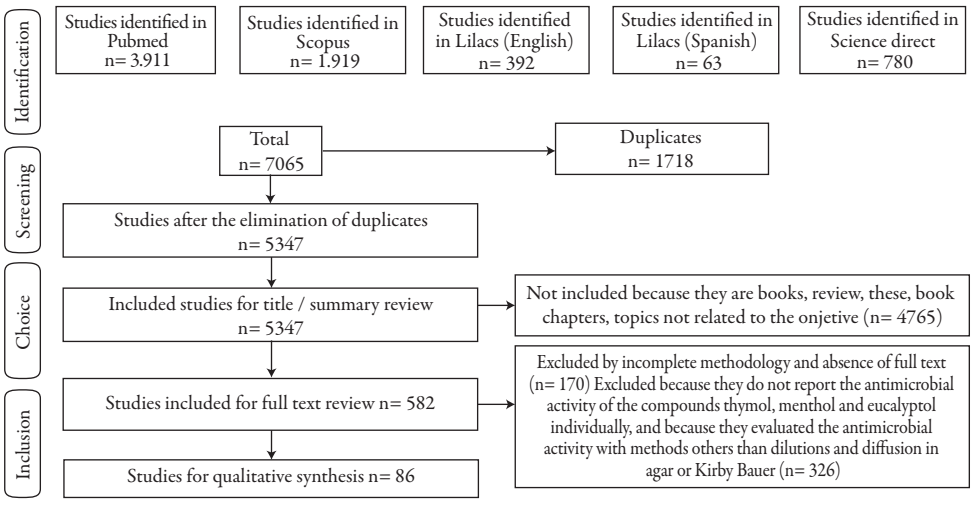


Figure 2. Summary of the number of articles obtained in each phase.

Characteristics of the studies

This exploratory systematic review showed that the thymol, menthol, and eucalyptol compounds have been reported in the literature as possible antimicrobial agents since the 1990's, performing tests on a wide range of microorganisms with nearly 80 species (figure 3) of interest in different disciplines. Concerning the bacterial and fungal agents that alter the homeostasis of the oral cavity, it was found out that the sensitivity of *Aggregatibacter actinomycetemcomitans, Candida albicans, Candida dubliniensis, Enterococcus faecalis, Escherichia coli, Lactobacillus plantarum, Streptococcus mutans, Streptococcus sanguinis,* and *Streptococcus sobrinus* has been evaluated against these compounds (table 1). The reports show inhibition halos diameters of 2-62 mm produced by concentrations ranging from 0.06 μg/mL to 300 000 μg/mL for thymol and menthol (table 1) and varying minimum inhibitory concentration (MIC) (tables 2 and 3).

Thymol was the compound with the largest number of publications (reported in 82.1%, while menthol was reported in 13.4%, and eucalyptol, 4.5%) and the countries with the most studies and publications of these research articles are the United States (14% of articles), Italy (9.3%), India (8.1%), Brazil (7%), Iran (7%), Serbia (5.8%) and Spain (5.8%). 76% of the analyzed reports used the broth dilution technique, 15% used agar diffusion, and 9% used mixed methodologies.

Results of the evaluations of the antimicrobial activity of thymol, menthol and eucalyptol on microorganisms that affect the homeostasis of the oral cavity

The phytochemicals used in the 86 articles were purchased in commercial versions from Sigma-Aldrich, BDH Laboratory Reagents, Quinar®, Difco, HiMedia Chem. Ltd, Acros Organics, Fluka, S.D. Fine Chemicals, Kurt Kitzing. They have also been isolated from the essential oils of *Satureja thymbra*, *S. laxiflora, Thymus algeriensis, T. vulgaris, T. zygis, T. capitatus* and *T. herba-barona, T. kotschyanus, Trachyspermum ammi, Origanum dictamnus, O. syriacum, O. vulgare, Melissa officinalis, Dianthus caryophyllus, and Lavandula officinalis,* and others have been provided by institutions like the Laboratory for Phytobiochemistry and Medicinal Plants Studies of the University of Yaoundé, the Barij Essence Biological Center, and the Institute for Medicinal Plants Research "Dr. Josif Pančić".

The compounds analyzed in this review show antimicrobial activity at variable intensities, as well as intra- and interspecies discrepancies (tables 2 and 3); it should be noted that these variations may depend on the phytochemicals' mechanism of action [6, 10], the technical variations implemented by each research group, and the biological characteristics of the strains used (tables 2 and 3). On *C. albicans,* thymol at a concentration of 31 μg/mL can alter the morphogenesis of strain ATCC 90028 [49, 50], while in exerting such an effect on the same specimen, menthol requires concentrations of 500 μg/mL [50].

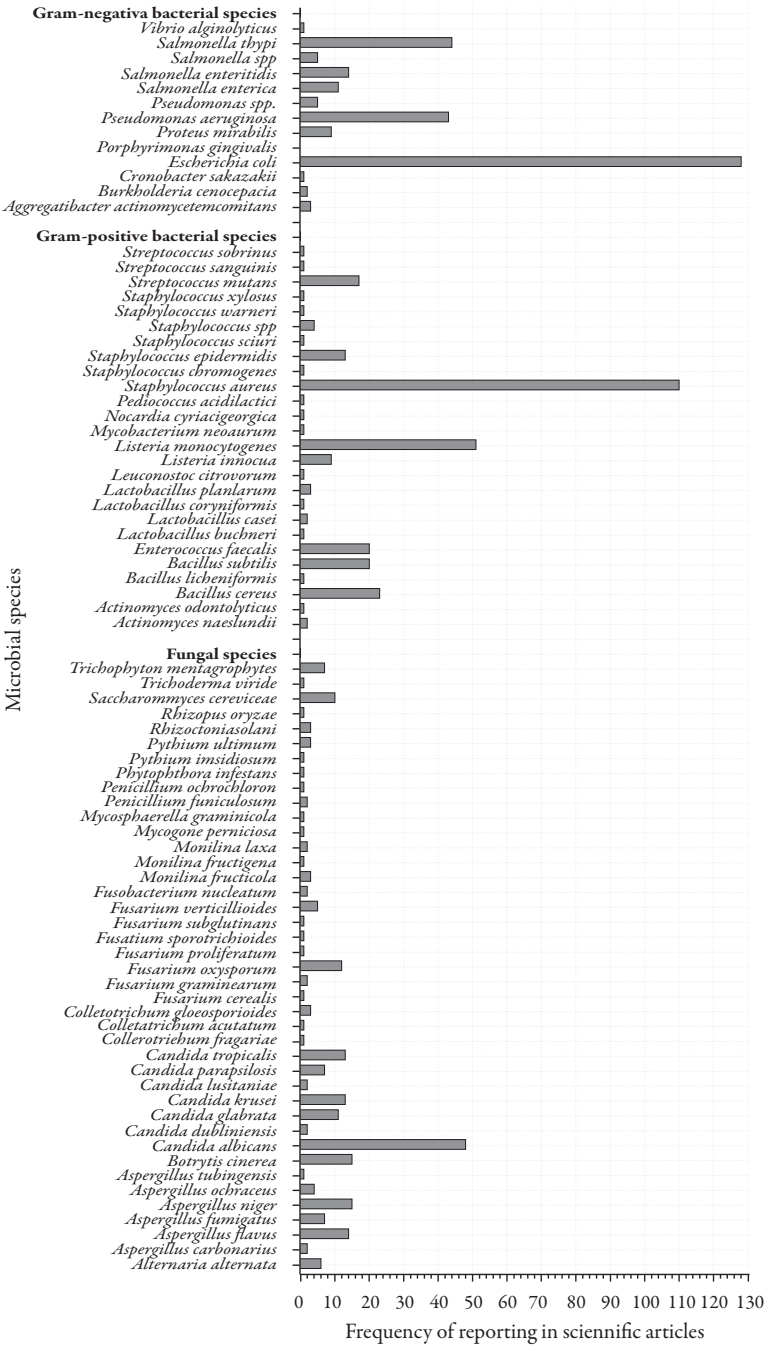


Figure 3. Frequency of use of microorganisms in the evaluations of thymol, menthol and eucalyptol compounds.

Table 1. Results of the antimicrobial effect of thymol and menthol obtained by the agar diffusion technique (Kirby Bauer).

M.O.	Strain/isolate	Comp.	Inhibition diameter (mm per µg/mL)	Ref.
C. albicans	ATCC 10231	T	25: 0.6 µg/mL	[28]
	ATCC 1023	T	62.6 +/- 2.9: 200 000 µg/mL	[40]
	Clinical isolates sensitive to fluconazole	T	2.20 ±0.1 mm: 400-500 µg/mL	[41]
	Clinical isolates resistant to fluconazole	T	2.176±0.152: 400-600 µg/mL	[41]
	ATCC 44829, 10261, 90028	T	3.27±0.88: 1000-1500 µg/mL	[42]
	Isolates resistant and sensitive to fluconazole	T	2.22±0.22-2.28±0.14: 1000-1500 µg/mL	[42]
	Human pathogen	M	12: 1µg/mL	[43]
	ATCC 10231	T	8.1 ± 0.09: 1 000 000 µg/mL	[44]
E. faecalis	ATCC 0157:H7	T	20: 1µg/mL	[30]
	ATCC 0157:H7	M	16: 1µg/mL	[30]
	Human pathogen	T	27: 1µg /mL	[43]
	BCRC 10675, 15374; O157:H7	T	17.9 ± 2.8-23.9 ± 2.8: 20 000 µg/mL	[45]
	NCIMB 8879	T	0: 6250 µg/mL 12.7 ±0.6: 12 500 µg/mL 13.8 ±0.2: 25 000 µg/mL 20.78 ±0.8: 50 000 µg/mL 33.8 ±0.6: 100 000 µg/mL	[46]
	ND	T	10: 1502.2 µg/mL	[47]
	ATCC 8739	T	12: 3000 µg/mL	[48]

M.O.: microorganisms; Comp.: component; ND: not described T: thymol; M: menthol. Note: Articles related to eucalyptol did not present the required data and were therefore not included in the table.

Similarly, menthol has an MBC of 2 µg/mL for *E. coli* ATCC 0157:H7 [30] and an MBC >2500 µg/mL for ATCC 10798 [29]; eucalyptol has an MBC of 500-1000 µg/mL for the strain ATCC 25175 of *S. mutans* [51, 52], but an MBC of 10 000 µg/mL for menthol [29]. However, when the cells are clustered as biofilms, the effects of antimicrobial agents can vary, as in the case of thymol, which requires concentrations of 250-1000 µg/mL to inhibit cell adhesion and the formation of biofilms in strains ATCC 66396, 90028 and 3153 of *C. albicans*, while menthol needs

concentrations higher than 2000 µg/mL [50]. In *S. mutans* ATCC 25175 with 100 µg/mL reduces the viability and biofilm formation [53], but for *E. faecalis* ATCC 4083 concentrations between 25 000 and 100 000 µg/mL are necessary [54, 55].

Table 2. Intensity of the antimicrobial activity of thymol per the minimum inhibitory concentration reported in the studies according to reported standards [9].

M.O.	Strain/isolate	MIC (µg/mL)	MBC/MFC (µg/mL)	Antimicrobial intensity	Ref.
A.a	ATCC 33384	101-500	200	Strong	[29]
C. albicans	Isolates from blood, sterile body fluids, deep tissue, genital tract, gastrointestinal tract, respiratory tract, vulvovaginal candidiasis (M1, H37), mouth, urinary tract, vaginal and cerebrospinal fluid of patients with HIV/AIDS; ATCC 10231, 18804, 76615, 90028; 10261, CBS 562	<100	0.125-50	Very strong	[21, 28, 31, 32, 40, 56-64]
	Isolates sensitive and resistant to fluconazole; ATCC 10231, 3153A, MYA2876; 90028, 11006	101-500	6500	Strong	[35, 41, 42, 49, 50, 63, 65-71]
	ATCC 10261	1001-2000	4000	Weak	[72]
	ATCC 90028	>2001	62 500	Absent	[16]
C. dubliniensis	Isolates from vulvovaginal candidiasis (M1, H37)	<100	0,32	Very strong	[32]
	Isolate resistant to fluconazole	101-500	400	Strong	[70]
E. faecalis	Clinical isolates	<100	-	Very strong	[28]
	ATCC 29212, CCM 4224; Isolates from boar semen	101-500	256	Strong	[73,74]
	ATCC 4083	501-1000	-	Moderate	[54, 55]
	Isolates from raw sheep's milk and cheese mixture (cows and sheep); ATCC 11700	1001-2000	4000	Weak	[75, 72]
	Isolate T9	>2001	250 000	Absent	[16]

(*Continúa*)

Table 2. Intensity of the antimicrobial activity of thymol per the minimum inhibitory concentration reported in the studies according to reported standards [9].

M.O.	Strain/isolate	MIC (µg/mL)	MBC/MFC (µg/mL)	Antimicrobial intensity	Ref.
E. coli	ATCC 8739, 43895, 35150; 35210, BCRC 10675, BCRC 15374; Clinical isolates, human pathogen, F. T. Jones	<100	2	Very strong	[28, 30, 43, 45, 60, 76-78]
	NCTC 9001, ATCC 10231; 35218, 25922, 700728, 43889, 43894, 8739, 10536, 35150, 10798, CCM 3954, NRRL B-3008, TUV 93-0, CGMCC 1.487, 380-94, J21, CO1, CO2, DPC6054, DPC6053; clinical isolates, O157:H7 •M 370, OPS: EQAS-2003 non-toxigenic, from boar semen, fecal specimens from domestic poultry; Crops Collection of the Food Research Institute (IFR), of chicken CVCC1553 and CVCC1490 (serotype O78), VTEC fago type 34	101-500 µg/mL	128-512	Strong	[26, 29, 37, 61, 62, 66, 73, 74, 79-93]
	ATCC 25922, 35218, 35860, 25922, NCIMB 8879; Isolates from broilers affected by avian colibacillosis (serotype O45, strain 184049/2014), DPC6055, O26, O111, O103, O145	501-1000	625-2000	Moderate	[24, 46, 54, 88, 94, 95]
	ATCC 25922, O157, 15221, 700728; Clinical isolates from bovine mastitis (DTSL-2, 39 and 40), isolates from milk and meat products	>2001	4800	Absent	[33,72, 96-98]
L. plantarum	Isolate SA-1	501-1000	-	Moderate	[15]
	Isolate SA-1	1001-2000	-	Weak	[15]

(*Continúa*)

Table 2. Intensity of the antimicrobial activity of thymol per the minimum inhibitory concentration reported in the studies according to reported standards [9].

M.O.	Strain/isolate	MIC (µg/mL)	MBC/MFC (µg/mL)	Antimicrobial intensity	Ref.
S. mutans	ATCC 25175, 446, 35668	101-500	-	Strong	[17, 29, 51, 52, 54, 72]
	ATCC 25175	501-1000	400-1000	Moderate	[15]
	ATCC 25175	1001-2000	-	Weak	[15]
	DSM 20523	>2001	250 000	Absent	[16]
S. sanguinis	ATCC 10556	>2001	8000	Absent	[72]
S. sobrinus	ATCC 27607	>2001	8000	Absent	[72]

M.O.: microorganisms.

Table 3. Antimicrobial effect of menthol and eucalyptol and some combinations.

M.O.	Strain/isolate	MIC range (µg/mL)	MBC/MFC (µg/mL)	Intensity of antimicrobial activity	Comp./mixtures	Ref.
A.a	ATCC 33384	101-500	1000	Strong	M	[29]
C. albicans	ATCC 10 261, 44 829, 90 028	101-500	1000	Strong	M	[34]
	ATCC 90 028	>2001	125 000	Absent	M; E+M, E; E+T	[16, 50]
E. faecalis	Isolate T9	>2001	1000 000	Absent	M, E, E+M	[16]
E. coli	ATCC 0157: H7; Isolate	<100	2	Very strong	M	[30, 43]
	ATCC 8739	101-500		Strong	M	[35]
	F'lac K12 LE140 (tsx, str, Δlac, su−, λr, mal−)	501-1000		Moderate	M	[99]
	ATCC 15221, 10798	>2001	>2500	Absent	M	[29, 33]
	NRRL B-3008; ATCC 700728	>2001		Absent	E	[37, 66]

(*Continúa*)

Table 3. Antimicrobial effect of menthol and eucalyptol and some combinations.

M.O.	Strain/isolate	MIC range (µg/mL)	MBC/ MFC (µg/mL)	Intensity of antimicrobial activity	Comp./ mixtures	Ref.
L. plantarum	Isolate SA-1	101-500		Strong	M	[15]
	Isolate SA-1	501-1000		Moderate	M	[15]
S. mutans	ATCC 25175	101-500	500	Strong	M; E	[17, 51, 52]
	ATCC 25175	501-1000	1000	Moderate	M, E	[15, 17, 29]
	DSM 20523	>2001	250 000	Absent	M, E, E+M, E+T	[16]

M.O.: microorganisms; Comp.: components; T: thymol; M: menthol; E: eucalyptol.

Conclusions

Worldwide, natural products provide benefits thanks to their large chemical diversity; therefore, plants and their derivatives are gaining greater attention, encouraging and promoting scientific support for safe use [6, 7, 12]. The scientific articles retrieved and included in this scoping review using the described methodology show that the greatest interest exists in developed countries like the United States, Italy, Spain, and Brazil; however, other countries with smaller economies, like Serbia, India, and Iran, also invest on this area of research, as the aromatic, medicinal, and seasonal species are some of the most important natural resources in such territories, obtaining essential oils and various compounds that are terpenic in nature [100]. In Latin America, Brazil is one of the countries with the richest flora diversity and a population that generally accepts the pharmacopoeia; this country is also a leader in the publication of scientific studies on natural products and their derivatives, with tropical conditions similar to those of Colombia, meaning that both countries share the cultivation of certain species because of their geographic location [6, 100].

The obtained results validate the implementation of studies with a biotechnological approach for a better use of the antimicrobial properties of the thymol, menthol, and eucalyptol compounds in the control of microbial agents that alter the homeostasis of the oral cavity; however, it is extremely important to take into account a few aspects that maximize the effectiveness and safety of the supplies, such as equivalence of the in vitro and in vivo antimicrobial activity, toxicity, mechanisms of action, and the specificity of the application pathway [12]; also, it should be kept in mind that while this study aimed to analyze each compound to determine their potential as active

ingredients [15, 34, 86], it is important to conduct mixes with other substances and to evaluate the synergistic, neutral, antagonistic, and additive associations that may arise, taking into account the proportions and nature of the mixed substances [12, 68], as phytochemicals can enhance their effect in the presence of other substances [64, 77, 90], but the effect can also be reduced [16, 26] or even certain combinations may prove to be unnecessary [16].

These remarks should be considered and may even be essential for effective and safe products like mouth rinses and toothpastes mixed with active compounds that are terpenic in nature; for this reason, the scientific committees of several institutions have consolidated their agreements with research centers, in order to manufacture efficient, effective, safe products that can contribute to oral health by adequately controlling dental plaque without affecting patient's health.

This exploratory systematic review showed that the thymol, menthol, and eucalyptol compounds can be considered as broad-spectrum antimicrobial substances, with effect on microorganisms linked to various human diseases, including some that are closely related to oral diseases. This effect is dependent on a number of variables, such as species and used microbial strain, and highlights the need to standardize parameters related to the concentrations used in research, as the analyzed studies use diverse units for reporting the antimicrobial activity, complicating both the comparison and the analysis of results.

This scoping review showed that the thymol compound does not have significant synergies with the menthol and eucalyptol compounds in the evaluated concentrations; however, in other quantities and in the presence of other substances, with specific containers and carriers, this combination may be more relevant. *S. sanguinis* and *S. sobrinus* were the most resistant microorganisms, and eucalyptol was the least effective compound; however, it was important to identify that these compounds have a strong antimicrobial intensity on *A.a, C. albicans, C. dubliniensis, E. faecalis, E. coli, L. plantarum,* and *S. mutans*, showing that concentrations of 0.06-100 μg/mL are required for an antimicrobial effect, validating the possibility of formulating products with these compounds, as the FDA indicates that the lower the necessary amount of a substance to exert an effect, the lower the toxicity risks, and therefore the greater the safety for the exposed organisms.

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No potential conflict of interest was reported by the authors.

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