Review

Antibacterial Activity of Essential Oils and Their Isolated Constituents against Cariogenic Bacteria: A Systematic Review

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Abstract: Dental caries remains the most prevalent and costly oral infectious disease worldwide. Several methods have been employed to prevent this biofilm-dependent disease, including the use of essential oils (EOs). In this systematic review, we discuss the antibacterial activity of EOs and their isolated constituents in view of a potential applicability in novel dental formulations. Seven databases were systematically searched for clinical trials, in situ, in vivo and in vitro studies addressing the topic published up to date. Most of the knowledge in the literature is based on in vitro studies assessing the effects of EOs on caries-related streptococci (mainly Streptococcus mutans) and lactobacilli, and on a limited number of clinical trials. The most promising species with antibacterial potential against cariogenic bacteria are: Achillea ligustica, Baccharis dracunculifolia, Croton cajucara, Cryptomeria japonica, Coriandrum sativum, Eugenia caryophyllata, Lippia sidoides, Ocimum americanum, and Rosmarinus officinalis. In some cases, the major phytochemical compounds determine the biological properties of EOs. Menthol and eugenol were considered outstanding compounds demonstrating an antibacterial potential. Only L. sidoides mouthwash (1%) has shown clinical antimicrobial effects against oral pathogens thus far. This review suggests avenues for further non-clinical
and clinical studies with the most promising EOs and their isolated constituents bioprospected worldwide.

**Keywords:** natural products; essential oils; monoterpenes; dental caries; *Streptococcus mutans*; preventive dentistry; clinical trials; isolated compounds

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1. **Introduction**

Despite the advances in public policies so far, dental caries remains the most prevalent and costly oral infectious disease worldwide [1,2], representing a global public health problem to be managed by authorities and dental professionals [2,3]. Effective caries-preventive methods have been developed and amended in the last decades. It is well known that the chemical control of plaque is an effective strategy to prevent dental caries development [4]. The main chemical agents currently available are fluoride [5], chlorhexidine [6], triclosan, cetylpyridinium chloride and natural products [4,7].

In this context, natural products (plant extracts, essential oils and isolated compounds, and marine products) have been proposed as novel therapeutic agents against dental caries [8], in order to minimize the adverse effects of synthetics [9] (e.g., altered taste, mucosal desquamation and tooth staining) as well as to provide effective and safer alternatives for dental caries management. Examples of these natural products include propolis, black and green tea, cacao bean husk, oat hulls, cranberry, and shells of crustaceans, among several others [8].

Essential oils (EOs) have aroused attention among the naturally-occurring bioactive agents with promising antimicrobial activity [10,11]. EOs are a mixture of volatile constituents produced by aromatic plants as secondary metabolites, as a protective mechanism against predators, microorganisms or weather adversities [12,13]. Among the 100,000 known secondary metabolites, EOs account for over 3000, of which about 300 have commercial interest and are used by the food, cosmetic and pharmaceutical industries [10]. The diverse chemical structures of EOs encompass two groups with distinct biosynthetic origins [14]: terpenes (monoterpenes and sesquiterpenes) and terpenoids (isoprenoids), and another group of aliphatic and aromatic compounds (e.g., aldehydes, phenols, among others), all characterized by low molecular weight [12]. Monoterpenes are the major compounds found in EOs [12] and have been found to show potent antibacterial activity against caries-related microorganisms [11,15].

Despite the research progress so far, there have been few studies with EOs approaching their potential application in the field of dentistry. Usually, a few substances from this phytochemical class have been used in anti-plaque and anti-gingivitis mouthwash formulations [16–18], hence there is a need for further exploration of EOs with potential use as adjunctive anti-caries chemotherapy.

In this systematic review, we discuss the anti-caries activity of EOs in view of their potential applicability in novel dental formulations. Moreover, the compilation of a vast database from the literature may suggest avenues for further laboratorial and clinical studies with the most promising EOs and their isolated constituents bioprospected worldwide.
2. Results

According to a previously set strategy, literature searches resulted in 1405 articles, of which 25 met the inclusion criteria and were included in the final review after thorough analysis (Figure 1). A total of 22 *in vitro* studies and three clinical trials addressing the anti-caries properties of EOs and their isolated compounds were selected and will be further discussed herein.

![Flow diagram of the search strategy](image)

**Figure 1.** Flow diagram of the search strategy comprising the identification of potentially relevant material, and preliminary screening and final selection of the studies included in this review (based on PRISMA guidelines). * The leading reasons for exclusion of articles were: clinical trials—“score lower than 3 in Jadad’s scale” (see Methods); *in vitro* studies—lack of critical information on chemical profiling, and methodological shortcomings.

2.1. In Vitro Studies

According to the *in vitro* studies analyzed, there was a predominance of tests with planktonic cultures (Tables 1–6) rather than mono- or multi-species biofilm cultures (Table 7). Of the 22 studies, 5 (22.72%) tested the effect of the EO on streptococci and lactobacilli biofilms.
2.1.1. Planktonic Studies

Crude EOs and Planktonic *S. mutans*

Thirty species were found to have very strong or strong antibacterial activity against *S. mutans*, of which the most promising were *Achillea ligustica* All. (ligurian yarrow) [19], *Cryptomeria japonica* D. Don (sugi) [20], *Croton cajucara* Benth (sacaca) [21], *Baccharis dracunculifolia* DC (broom weed), *Coriandrum sativum* L. (coriander), *Lippia sidoides* Cham. (rosemary-pepper), *Mikania glomerata* Sprengel (guaco) and *Siparuna guianenses* Aubl. (wild lemon) [11], with planktonic MIC values equal to or lower than 100 µg/mL (Table 1).

| Plant Species                  | Source          | Microorganism | MIC (µg/mL) | MBC (µg/mL) | Score | Ref.   |
|-------------------------------|-----------------|---------------|-------------|-------------|-------|--------|
| *Achillea ligustica* All.      | Inflorescences  | DSM 20523     | 155         | nt          | +++   | [19]   |
| *Achillea ligustica* All.      | Leaves          | DSM 20523     | 155         | nt          | +++   | [19]   |
| *Achillea ligustica* All.      | flowering aerial parts | DSM 20523 | 38          | nt          | ++++  | [19]   |
| *Achillea ligustica* All.      | Flowers         | DSM 20523     | 155         | 310         | +++   | [22]   |
| *Achillea ligustica* All.      | vegetative parts | DSM 20523   | 39          | 39          | ++++  | [22]   |
| *Ageratum conyzoides*          | Leaves          | ATCC 25175    | 4000        | nt          | –     | [23]   |
| *Aloysia gratissima*           | Leaves          | UA 159        | 125–250     | 250–500     | +++   | [11]   |
| *Aloysia triphylla*            | Leaves          | UA 159        | 125–250     | 125–250     | +++   | [11]   |
| *Alpinia speciosa*             | Root            | UA 159        | 125–250     | 250–500     | +++   | [11]   |
| *Artemisia camphorata* Vill.   | Leaves          | ATCC 25175    | 2000        | nt          | +     | [23]   |
| *Baccharis dracunculifolia*    | Leaves          | UA 159        | 62.5–125    | 250–500     | ++++  | [11]   |
| *Bidens sulphurea*             | Leaves          | ATCC 25175    | 250         | nt          | +++   | [23]   |
| *Cinnamomum zeylanicum*        | Leaves          | UA 159        | 250–500     | 500–1000    | +++   | [11]   |
| *Coriandrum sativum*           | Leaves          | UA 159        | 31.2–62.5   | 62.5–125    | +++   | [11]   |
| *Croton cajucara* Benth        | Leaves          | ATCC 4646     | 40.1        | 13.8        | ++++  | [21]   |
| *Cryptomeria japonica*         | aerial parts    | ATCC 25175    | 100         | 200         | ++++  | [20]   |
| *Cuminum cyminum*              | CS              | PTCC 1601     | 4000        | nt          | –     | [24]   |
| *Cymbopogon citratus*          | Leaves          | UA 159        | 125–250     | 250–500     | +++   | [11]   |
| *Cymbopogon martini*           | leaves          | UA 159        | 125–250     | 250–500     | +++   | [11]   |
| *Cymbopogon winterianus*       | Leaves          | UA 159        | 125–250     | 250–500     | +++   | [11]   |
| *Cyperus articulatus*          | Bulbs           | UA 159        | 125–250     | 250–500     | +++   | [11]   |
| *Elyonurus muticus*            | Leaves          | UA 159        | 125–250     | 250–500     | +++   | [11]   |
| *Eucalyptus radiate*           | CS              | JC-2          | 10,000      | 10,000      | –     | [25]   |
| *Eugenia caryophyllata L.*     | CS              | ATCC 25175    | 200         | 800         | +++   | [26]   |
| *Eugenia caryophyllata L.*     | CS              | ATCC 5175     | 600         | nt          | ++    | [27]   |
| *Eugenia florida*              | Leaves          | UA 159        | 125–250     | 125–250     | +++   | [11]   |
| *Eugenia uniflora*             | Leaves          | UA 159        | 125–250     | 250–500     | +++   | [11]   |
| *Foeniculum vulgare Mill.*     | Leaves          | ATCC 25175    | >4000       | nt          | –     | [23]   |
| *Lavandula officinalis*        | CS              | JC-2          | >10,000     | >10,000     | –     | [25]   |
| *Leptospermum scoparium*        | CS              | JC-2          | 2500        | 2500        | –     | [25]   |
| *Lippia alba*                  | Leaves          | ATCC 25175    | 500         | nt          | +++   | [23]   |
| *Lippia alba*                  | Leaves          | UA 159        | 125–250     | 250–500     | +++   | [11]   |
Table 1. Cont.

| Plant Species          | Source          | Microorganism | MIC (µg/mL) | MBC (µg/mL) | Score | Ref. |
|------------------------|-----------------|---------------|-------------|-------------|-------|------|
| Lippia sidoides        | Leaves UA 159   | 62.5–125      | 125–250     | ++++        | [11]  |
| Melaleuca alternifolia | CS JC-2         | 10,000        | 10,000      | −           | [25]  |
| Melaleuca alternifolia | Leaves clinical isolates | 0.25–2   | 0.25–2      | *           | [28]  |
| Mentha piperita        | Leaves UA159    | 250–500       | 250–500     | +++         | [11]  |
| Mentha piperita        | CS PTCC 1601    | 6000          | nt          | +           | [24]  |
| Mikania glomerata      | Leaves UA 159   | 62.5–125      | 125–250     | ++++        | [11]  |
| Ocimum americanum L.   | Leaves ATCC 6363| 0.04          | 0.08        | *           | [29]  |
| Ocimum gratissimum L.  | Leaves ATCC 25175| 1000        | nt          | ++          | [23]  |
| Pelargonium graveolens | Leaves ATCC 25175| 1000        | nt          | ++          | [23]  |
| Rosmarinus officinalis L. | Leaves JC-2   | >10,000       | >10,000     | −           | [25]  |
| Rosmarinus officinalis L. | Leaves ATCC 25275| >2000      | nt          | −           | [15]  |
| Rosmarinus officinalis L. | CS PTCC 1601 | 2000          | nt          | −           | [30]  |
| Satureja biflora       | flowering aerial parts | clinical isolates | 640        | nt         | ++   | [31]  |
| Satureja masukensis    | flowering aerial parts | clinical isolates | 570        | nt         | ++   | [31]  |
| Satureja pseudosimensis| Leaves and flowering tops | clinical isolates | 920        | nt         | ++   | [31]  |
| Siparuna guianenses    | Leaves UA 159   | 62.5–125      | 125–250     | ++++        | [11]  |
| Syzygium aromaticum    | Leaves ATCC 25175| 2000        | nt          | +           | [23]  |
| Syzygium aromaticum    | Leaves UA 159   | 250–500       | 250–500     | +++         | [11]  |
| Tagetes erecta L.      | Leaves ATCC 25175| >4000      | nt          | −           | [23]  |
| Thymus eriocalyx       | CS PTCC 1601    | 2000          | nt          | +           | [30]  |
| Ziziphus zoazeiro      | Leaves UA 159   | 250–500       | 500–1000    | +++         | [11]  |

Note: * values are expressed as v/v; CS (commercial source); nt (not tested); Comparative MIC values: (++++) ≤100; (+++) 101 to 500; (++) 501 to 1000; (+) >1001 to 2000; (−) >2001.

Crude EOs and Planktonic S. sobrinus, S. sanguinis and S. salivarius

Four plant species were found to have very strong or strong antibacterial activity against S. sobrinus, as follows: Croton cajucara Benth (sacaca) [21]; Rosmarinus officinalis L. (rosemary) [15]; Eugenia caryophyllata L. (clove) [26] and Cryptomeria japonica (sugi) [20]. Of these, C. japonica also had very strong and strong activity against S. sanguinis and S. salivarius, respectively (Table 2).

Crude EOs and Planktonic Lactobacilli

Achillea ligustica (ligurian yarrow) [19] had strong activity against L. acidophilus. Another species of Lactobacillus, L. casei, was found to be strongly susceptible to Croton cajucara (sacaca) [21], Artemisia camphorata Vill. (camphor), Bidens sulphurea Sch. Bip. (yellow cosmos), Lippia alba Mill. (lemon balm) and Ocimum gratissimum L. (tree basil) [23] (Table 3).

EO-Isolated Compounds against Streptococci and Lactobacilli

Menthol, isolated from Mentha longifolia L., and eugenol, isolated from Eugenia caryophyllata L., were found to be the most promising compounds with strong activity against streptococci and lactobacilli (Tables 4–6).
Table 2. *In vitro* antibacterial activity of essential oils against *S. sobrinus, S. sanguinis* and *S. salivarius.*

| Plant Species                | Source                        | Microorg | S. sobrinus 1 | S. sanguinis 2 | S. salivarius 3 | Ref. |
|-----------------------------|-------------------------------|----------|---------------|----------------|-----------------|------|
| *Achillea ligustica* All    | inflorescences                | IMC104 3 | nt            | nt             | nt              | 1250 nt + | [19] |
| *Achillea ligustica* All    | Leaves                        | IMC104 3 | nt            | nt             | nt              | 1250 nt + | [19] |
| *Achillea ligustica* All    | flowering aerial parts        | IMC104 3 | nt            | nt             | nt              | 625 nt ++ | [19] |
| *Ageratum conyzoides* L.    | Leaves                        | ATCC 33478 1 | >4000 | nt            | >4000          | 4000 nt - | [23] |
|                             |                               | ATCC 10556 2 | >4000 | nt            | >4000          | 4000 nt - | [23] |
|                             |                               | ATCC 25975 3 | >4000 | nt            | >4000          | 4000 nt - | [23] |
| *Artemisia camphorata* Vill.| Leaves                        | ATCC 33478 1 | 2000 | nt            | 2000          | 4000 nt - | [23] |
|                             |                               | ATCC 10556 2 |       | nt            | 4000          | 4000 nt - | [23] |
|                             |                               | ATCC 25975 3 |       | nt            | 4000          | 4000 nt - | [23] |
| *Bidens sulphurea*          | Leaves                        | ATCC 33478 1 | 4000 | nt            | 4000          | 4000 nt - | [23] |
|                             |                               | ATCC 10556 2 |       | nt            | 4000          | 4000 nt - | [23] |
|                             |                               | ATCC 25975 3 |       | nt            | 4000          | 4000 nt - | [23] |
| *Croton cajucara* Benth     | Leaves                        | ATCC 27609 1 | 13.8   | nt            | +++           | nt nt    | [21] |
| *Cryptomeria japonica*      | aerial parts                  | ATCC 27607 1 | 100    | 100           | +++           | 100 nt   | [20] |
|                             |                               | ATCC 10556 2 |       | 100           | +++           | 200 nt   | [20] |
| *Eucalyptus radiate*        | CS                            | ATCC 6715 1 | 10,000 | 10,000        | nt            | nt nt    | [25] |
|                             |                               | ATCC B13 1   |       | nt            | nt            | nt nt    | [25] |
| *Eugenia caryophyllata* L.  | Flowers                       | ATCC 27607 1 | 200    | 800           | +++           | 400 nt   | [26] |
|                             |                               | ATCC 10556 2 |       | 800           | +++           | 800 nt   | [26] |
| *Foeniculum vulgare* Mill.  | Leaves                        | ATCC 33478 1 | >4000 | nt            | >4000         | nt nt    | [23] |
|                             |                               | ATCC 10556 2 |       | nt            | >4000         | nt nt    | [23] |
|                             |                               | ATCC 25975 3 |       | nt            | >4000         | nt nt    | [23] |
| *Lavandula officinalis*     | CS                            | 6715 1 | 10,000 | 10,000        | nt            | nt nt    | [25] |
|                             |                               | B13 1       | 10,000 | 10,000        | nt            | nt nt    | [25] |
| *Leptospermum scoparium*     | CS                            | 6715 1 | 1300   | 2500          | nt            | nt nt    | [25] |
|                             |                               | B13 1       | 2500   | 2500          | nt            | nt nt    | [25] |
Table 2. Cont.

| Plant Species               | Source   | Microorg | S. sobrinus | S. sanguinis | S. salivarius | Ref.  |
|-----------------------------|----------|----------|-------------|--------------|---------------|-------|
|                             |          |          | MIC (µg/mL) | MBC (µg/mL) | MIC (µg/mL)  | MBC (µg/mL) | Score | MIC (µg/mL) | MBC (µg/mL) | Score | Ref.  |
|                            |          |          | Score       | Score        | Score         | Score        |       | Score       | Score        |       |       |
| Lippia alba                 | Leaves   | ATCC 33478 | 1000 nt ++ | 1000 nt ++ | 2000 nt + | [23] |
|                            |          | ATCC 10556 | 1000 nt ++ | 1000 nt ++ | 2000 nt + | [23] |
|                            |          | ATCC 25975 | 1000 nt ++ | 1000 nt ++ | 2000 nt + | [23] |
| Melaleuca alternifolia      | CS       | 6715 1    | 10,000 nt – | nt nt          | nt nt          | nt nt          | [25] |
|                            |          | B13 1     | 2500 nt –   | nt nt          | nt nt          | nt nt          |       |
| Mentha piperita             | CS       | Ssb 176 1 | 3000 nt –    | 6000 nt –       | nt nt          | nt nt          | [32] |
|                            |          | Ssg 009 2 | 3000 nt –    | 6000 nt –       | nt nt          | nt nt          |       |
| Ocimum basilicum            | CS       | Ssb 176 1 | 6000 nt –    | 6000 nt –       | nt nt          | nt nt          | [32] |
|                            |          | Ssg 009 2 | 6000 nt –    | 6000 nt –       | nt nt          | nt nt          |       |
| Ocimum gratissimum L.       | Leaves   | ATCC 33478 | 1000 nt ++ | 2000 nt + | 2000 nt + | [23] |
|                            |          | ATCC 10556 | 1000 nt ++ | 2000 nt + | 2000 nt + | [23] |
|                            |          | ATCC 25975 | 1000 nt ++ | 2000 nt + | 2000 nt + | [23] |
| Pelargonium graveolens      | Leaves   | ATCC 33478 | 1000 nt ++ | 2000 nt + | 2000 nt + | [23] |
|                            |          | ATCC 10556 | 1000 nt ++ | 2000 nt + | 2000 nt + | [23] |
|                            |          | ATCC 25975 | 1000 nt ++ | 2000 nt + | 2000 nt + | [23] |
| Rosmarinus officinalis L.   | Leaves   | 6715 1    | 10,000 nt – | nt nt          | nt nt          | nt nt          | [25] |
|                            |          | B13 1     | 10,000 nt – | nt nt          | nt nt          | nt nt          |       |
| Rosmarinus officinalis L.   | Leaves   | ATCC 33478 | 500 nt +++  | >2000 nt –      | 600 nt ++      | 600 nt ++      | [15] |
|                            |          | ATCC 10556 | 500 nt +++  | >2000 nt –      | 600 nt ++      | 600 nt ++      |       |
|                            |          | ATCC 25975 | 500 nt +++  | >2000 nt –      | 600 nt ++      | 600 nt ++      |       |
| Salvia officinalis          | CS       | Ssb 176 1 | 3000 nt –    | 6000 nt –       | nt nt          | nt nt          | [32] |
|                            |          | Ssg 009 2 | 3000 nt –    | 6000 nt –       | nt nt          | nt nt          |       |
| Syzygium aromaticum         | Leaves   | ATCC 33478 | >4000 nt –   | >4000 nt –      | nt nt          | nt nt          | [23] |
|                            |          | ATCC 10556 | >4000 nt –   | >4000 nt –      | nt nt          | nt nt          |       |
|                            |          | ATCC 25975 | >4000 nt –   | >4000 nt –      | nt nt          | nt nt          |       |
| Tagetes erecta L.           | Leaves   | Ssb 176 1 | 6000 nt –    | nt nt          | nt nt          | nt nt          | [32] |
|                            |          | Ssg 009 2 | 6000 nt –    | nt nt          | nt nt          | nt nt          |       |

Note: CS = commercial source; nt (not tested); Comparative MIC values: (++++) ≤100; (+++) 101 to 500; (++) 501 to 1000; (+) >1001 to 2000; (−) >2001; 1 S. sobrinus; 2 S. sanguinis and 3 S. salivarius.
Table 3. *In vitro* antibacterial activity of essential oils against lactobacilli.

| Plant Species | Source | Microorg | MIC (µg/mL) | MBC (µg/mL) | Score | L. acidophilus | Ref. |
|---------------|--------|----------|-------------|-------------|-------|--------------|------|
| *Achillea ligustica* All. | Inflorescences | IMC 101 | 310 | nt | +++ | nt | nt | [19] |
| *Achillea ligustica* All. | Leaves | IMC 101 | 2500 | nt | - | nt | nt | [19] |
| *Achillea ligustica* All. | flowering aerial parts | IMC 101 | 1250 | nt | + | nt | nt | [19] |
| *Ageratsum conyzoides* L. | Leaves | ATCC 11578 | nt | 2500 | nt | ++ | nt | [19] |
| *Ageratsum conyzoides* L. | Leaves | ATCC 4646 | nt | 1250 | nt | ++ | nt | [19] |
| *Croton cajucara* Benth | Leaves | ATCC 25175 | nt | 22.3 | nt | +++ | nt | [21] |
| *Foenicum vulgare* Mill. | Leaves | ATCC 11578 | nt | 500 | nt | +++ | nt | [23] |
| *Lippia alba* | Leaves | ATCC 11578 | nt | 500 | nt | +++ | nt | [23] |
| *Ocimum americanum* L. | Leaves | ATCC 6363 | nt | 0.04 | nt | * | nt | [29] |
| *Ocimum basilicum* | aerial parts | ATCC 4356 | 80,000 | nt | - | nt | nt | [33] |
| *Ocimum gratissimum* L. | Leaves | ATCC 11578 | nt | 500 | nt | +++ | nt | [23] |
| *Oregano vulgare* | aerial parts | ATCC 11578 | nt | 5000 | nt | ++ | nt | [23] |
| *Pelargonium graveolens* | Leaves | ATCC 11578 | nt | 1000 | nt | ++ | nt | [23] |
| *Rosmarinus officinalis* | aerial parts | ATCC 4356 | 80,000 | nt | - | nt | nt | [33] |
| *Salvia officinalis* | aerial parts | ATCC 4356 | 80,000 | nt | - | nt | nt | [33] |
| *Syzygium aromaticum* | Leaves | ATCC 11578 | nt | 1000 | nt | ++ | nt | [23] |
| *Tagetes erecta* L. | Leaves | ATCC 11578 | nt | 4000 | nt | - | nt | [23] |
| *Thymus vulgaris* | aerial parts | ATCC 4356 | 5000 | nt | - | nt | nt | [33] |

Note: * values are expressed as % (v/v); nt (not tested); Comparative MIC values: (++++) <100; (+++) 100 to 500; (+) 501 to 1000; (+) >1001 to 2000; (−) >2001; 1 L. acidophilus; 2 L. casei.

Table 4. Essential oils isolated compounds against *Streptococcus mutans*.

| Compound | Plant Species | Culture Collection | MIC (µg/mL) | MBC (µg/mL) | Score | Ref. |
|----------|---------------|-------------------|-------------|-------------|-------|------|
| 1,8, Cineole | *Achillea ligustica* All | DSM 20523 | 2500 | nt | - | [19] |
| 1,8, Cineole | *Achillea ligustica* All | DSM 20523 | 155 | 1250 | +++ | [22] |
| 1,8, Cineole | *Rosmarinus officinalis* | ATCC 25275 | 1500 | nt | + | [15] |
| Camphor | *Rosmarinus officinalis* | ATCC 25275 | 1500 | nt | + | [15] |
| Caryophyllene oxide | *Satureja species* | clinical isolates | 250 | nt | +++ | [31] |
| Eugenol | *Eugenia caryophyllata* L. | ATCC 25175 | 100 | 200 | ++++ | [26] |
| Linalool | *Achillea ligustica* All | DSM 20523 | 625 | nt | ++ | [19] |
| Linalool | *Achillea ligustica* All | DSM 20523 | 310 | 310 | +++ | [22] |
| Linalool | *Croton cajucara* Benth | ATCC 25175 | no activity | nt | - | [21] |
| Linalool | *Satureja species* | clinical isolates | 370 | nt | +++ | [31] |
| Menthol | *Mentha longifolia* L. | clinical isolates | 15.6 | nt | ++++ | [34] |
| Pulegone | *Satureja species* | clinical isolates | 1750 | nt | + | [31] |
| Sabinene | *Cryptomeria japonica* | ATCC 25175 | 800 | 1600 | ++ | [20] |
| Terpinen-4-ol | *Achillea ligustica* All | DSM 20523 | 1250 | nt | + | [19] |
| Terpinen-4-ol | *Achillea ligustica* All | DSM 20523 | 310 | 625 | +++ | [22] |
| Terpinen-4-ol | *Cryptomeria japonica* | ATCC 25175 | 1600 | 3200 | + | [20] |
| Verbenone | *Rosmarinus officinalis* | ATCC 25275 | 1000 | nt | ++ | [15] |
Table 4. Cont.

| Compound    | Plant Species         | Culture Collection | MIC (μg/mL) | MBC (μg/mL) | Score | Ref. |
|-------------|-----------------------|--------------------|-------------|-------------|-------|------|
| Viridiflorol| *Achillea ligustica* All | DSM 20523         | 2500        | nt          | −     | [19] |
| α-Pinene    | *Cryptomeria japonica* | ATCC 25175       | 6400        | 28,000      | −     | [20] |
| α-Pinene    | *Rosmarinus officinalis* | ATCC 25275   | 2000        | nt          | +     | [15] |
| α-Terpineol | *Cryptomeria japonica* | ATCC 25175       | 1600        | 3200        | +     | [20] |
| β-Caryophyllene | *Eugenia caryophyllata* L. | ATCC 25175 | 1600        | 3200        | +     | [26] |
| β-Caryophyllene | *Rosmarinus officinalis* | ATCC 25275 | 300         | nt          | +++   | [15] |
| β-Myrcene   | *Rosmarinus officinalis* | ATCC 25275       | 400         | nt          | +++   | [15] |
| β-Pinene    | *Achillea ligustica* All | DSM 20523   | 1250        | nt          | +     | [19] |
| β-Pinene    | *Achillea ligustica* All | DSM 20523   | 625         | 1250        | ++    | [22] |
| γ-Terpineol | *Achillea ligustica* All | DSM 20523   | 2500        | nt          | −     | [19] |

Note: CS (commercial source); nt (not tested); Comparative MIC values: (++++) < 100; (+++) 100 to 500; (+) 501 to 1000; (−) > 1001 to 2000; (−) > 2001.

Table 5. Essential oils isolated compounds against lactobacilli.

| Compound          | Source                        | Culture Collection | MIC (μg/mL) | MBC (μg/mL) | Score    | L. acidophilus | L. casei |
|-------------------|-------------------------------|--------------------|-------------|-------------|----------|---------------|---------|
| 1,8-Cineole *     | *Achillea ligustica* All      | IMC101 1           | 5000        | nt          | −        | nt            | nt      | [19] |
| Linalool *        | *Croton cajucara* Benth       | ATCC 4646 2        | nt          | nt          | no activity | nt            | −       | [21] |
| Linalool *        | *Achillea ligustica* All      | IMC101 1           | 5000        | nt          | −        | nt            | nt      | [19] |
| Menthol           | *Mentha longifolia* L.        | clinical isolates  | 31.2        | nt          | +++      | nt            | nt      | [34] |
| Terpinen-4-ol *   | *Achillea ligustica* All      | IMC101 1           | 5000        | nt          | −        | nt            | nt      | [19] |
| β-Pinene *        | *Achillea ligustica* All      | IMC101 1           | 2500        | nt          | −        | nt            | nt      | [19] |
| γ-Terpineol *     | *Achillea ligustica* All      | IMC101 1           | 5000        | nt          | −        | nt            | nt      | [19] |

Note: * standard from Sigma-Aldrich® (St. Louis, MO, USA); nt (not tested); Comparative MIC values: (++++) < 100; (−) > 2001; 1 L. acidophilus; 2 L. casei.
Table 6. Essential oils isolated compounds against *S. sobrinus*, *S. sanguinis* and *S. salivarius*.

| Compound          | Plant Species          | Culture Collection | *S. sobrinus* MIC (µg/mL) | *S. sanguinis* MIC (µg/mL) | *S. salivarius* MIC (µg/mL) | Ref. |
|-------------------|------------------------|--------------------|---------------------------|---------------------------|-----------------------------|------|
| 1,8-cineole       | *Achillea ligustica* All | IMC104             | nt                        | nt                        | 1250                        |      |
| Camphor           | *Rosmarinus officinalis* | ATCC 33478         | 1500                      | 400                       | 400                         | [15] |
| 2                  |                        | ATCC 10556         |                           |                           |                             |      |
| 3                  |                        | ATCC 25975         |                           |                           |                             |      |
| Eugenol           | *Eugenia caryophyllata* L. | ATCC 27607         | 200                       | 400                       | 400                         |      |
| 2                  |                        | ATCC 10556         |                           |                           |                             | [26] |
| Linalool           | *Achillea ligustica* All | IMC104             | nt                        | nt                        | 625                         |      |
| Linalool           | *Croton cajucara* Benth | ATCC 27609         | no activity               | nt                        | nt                          |      |
| Sabinene           | *Cryptomeria japonica* | ATCC 27607         | 200                       | 400                       | 400                         |      |
| 2                  |                        | ATCC 10556         |                           |                           |                             | [20] |
| Terpinen-4-ol     | *Achillea ligustica* All | IMC104             | nt                        | nt                        | 625                         |      |
| Verbenone          | *Rosmarinus officinalis* | ATCC 33478         | 1000                      | 400                       | 400                         |      |
| 2                  |                        | ATCC 10556         |                           |                           |                             | [15] |
| 3                  |                        | ATCC 25975         |                           |                           |                             |      |
| Viridiflorol       | *Achillea ligustica* All | IMC104             | nt                        | nt                        | 625                         | [19] |
| α-Pinene           | *Cryptomeria japonica* | ATCC 27607         | 6400                      | 6400                      |                             |      |
| 2                  |                        | ATCC 10556         |                           |                           |                             | [20] |
| α-Pinene           | *Rosmarinus officinalis* | ATCC 33478         | 1000                      | 400                       | 400                         |      |
| 2                  |                        | ATCC 10556         |                           |                           |                             | [15] |
| 3                  |                        | ATCC 25975         |                           |                           |                             |      |
| α-Terpineol        | *Cryptomeria japonica* | ATCC 27607         | 1600                      | 1600                      |                             |      |
| 2                  |                        | ATCC 10556         |                           |                           |                             | [20] |
| Compound   | Plant Species                                      | Culture Collection |   | S. sobrinus ¹ | S. sanguinis ² | S. salivarius ³ | Ref. |
|------------|---------------------------------------------------|--------------------|---|--------------|---------------|----------------|------|
|            |                                                   |                    |   | MIC  (µg/mL) | MBC  (µg/mL) | Score  | MIC  (µg/mL) | MBC  (µg/mL) | Score | MIC  (µg/mL) | MBC  (µg/mL) | Score |      |
| β-Caryophyllene | *Eugenia caryophyllata* L.                          | ATCC 27607 ¹     |   | 12,800       | 12,800        | −       | 1600           | 3200       | +     | nt             | nt             |       | [26] |
|            |                                                   | ATCC 10556 ²     |   |              |               |         |                |            |       |                |                |       |      |
| β-Caryophyllene | *Rosmarinus officinalis*                            | ATCC 33478 ¹     |   | 400          | nt            | +++    | 400            | nt         | +++   | 400            | nt            | +++   | [15] |
|            |                                                   | ATCC 10556 ²     |   |              |               |         |                |            |       |                |                |       |      |
|            |                                                   | ATCC 25975 ³     |   |              |               |         |                |            |       |                |                |       |      |
| β-Myrcene  | *Rosmarinus officinalis*                            | ATCC 33478 ¹     |   | 1500         | nt            | +      | 1500           | nt         | +     | 400            | nt            | +++   | [15] |
|            |                                                   | ATCC 10556 ²     |   |              |               |         |                |            |       |                |                |       |      |
|            |                                                   | ATCC 25975 ³     |   |              |               |         |                |            |       |                |                |       |      |
| β-Pinene   | *Achillea ligustica* All                            | IMC104 ³         |   | nt           | nt            | nt     | nt             | nt         | nt    | 625            | nt            | ++    | [19] |
| γ-Terpinene | *Achillea ligustica* All                            | IMC104 ³         |   | nt           | nt            | nt     | nt             | nt         | nt    | 625            | nt            | ++    | [19] |

Note: nt (not tested); Comparative MIC values: (+++) 100 to 500; (++) 501 to 1000; (+) >1001 to 2000; (−) >2001; ¹ S. sobrinus; ² S. sanguinis and ³ S. salivarius.
2.1.2. Biofilm Studies

Crude EOs and Biofilms of Streptococci and Lactobacilli

A total of eight species were tested against biofilm cultures of *S. mutans*, *S. sobrinus* and/or *L. casei* using different assays (Table 7). Interestingly, bioactive fractions of *C. sativum* and *B. dracunculifolia* inhibited 90% of *S. mutans* biofilm formation at concentrations as low as 31.2 μg/mL. Moreover, *C. cajucara* EO (100 μg/mL) and *O. americanum* EO (3%) inhibited *S. mutans* and *L. lactis* biofilms as effectively as chlorhexidine, used as positive control.

Overall, the majority of studies in this review tested the effectiveness of EO against *S. mutans* (35 out of 40 studies), followed in lower proportions by *S. sobrinus*, *S. salivarius*, *S. sanguinis* and *Lactobacillus* spp. As seen in Table 8, just a few studies carried out a comprehensive analysis of the effect of EO against a broad panel of caries-related species.

2.2. In Vivo Studies

Randomized Clinical Trials

Three high quality randomized, double-blind clinical trials of herbal interventions with low risk of bias were included in this review (Figure 2). The EOs from *L. sidoides* [35,36] and a multi-herbal formulation including *Melaleuca alternifolia* and *Leptospermum scoparium* oils (combined with *Calendula officinalis* and *Camellia sinensis* extracts) [37], were tested in humans for their effectiveness in reducing the amount of cariogenic biofilm, measured by means of plaque indexes. The experimental period of studies ranged from 1 week to 12 weeks, with different assessment checkpoints and dosing protocols. As seen in Table 9, only individuals treated with 1% *L. sidoides* EO mouthwash had a statistically significant reduction in their supragingival biofilm levels compared to chlorhexidine group (positive control) and to their baseline condition.

2.3. Chemical and Botanical Characterization and Georeferencing of the most Promising Bioactive EOs

Viridiflorol, terpinen-4-ol and β-pinene are found in the EO from all parts [19,22] of *A. lingustica*; however, important terpenes such as linalool, 1,8-cineole and germacrene D have also been identified in specific parts of the plant. Elemol, terpinen-4-ol, sabinene, 10(15)-cadinen-4-ol, α-terpineol and α-pinene are the major compounds identified in *C. japonica* [20]. Linalool is the most abundant compound of *C. cajucara* Benth [21]. Trans-nerolidol, spathulenol and trans-caryophyllene are found in *B. dracunculifolia* [11]. 1-decanol, trans-2-decen-1-ol and 2-dodecen-1-ol are the most abundant compounds of *C. sativum* [11]. Thymol is the major compound of *L. sidoides* [11]. Camphor, verbenone, α-pinene, β-myrcene, 1,8-cineole and β-caryophyllene are found in *R. officinalis* [15]. Eugenol and β-caryophyllene are the major compounds of *E. caryophyllata* [26].
Table 7. Essential oils, fractions or isolated compounds against in vitro oral biofilm formation.

| Ref. | Essential Oil/Fraction or Isolated Compound | Strain | Test(s) Performed | Biofilm Age Conditions | Outcomes |
|------|-------------------------------------------|--------|------------------|-----------------------|----------|
| [11] | *Aloysia gratissima* (Ag), *Coriandrum sativum* (Cs) and *Baccharis dracunculifolia* (Bd) fraction | *S. mutans* UA159 | Formation of *S. mutans* biofilm, the samples were placed in the wells of sterile polystyrene U-bottom microtiter plates, previously treated with saliva | *S. mutans* cells (1.0 × 10^7 cells/mL in BHI medium) were added to wells containing BHI medium with 2% sucrose and the samples were incubated at 37 °C for 18 h | Biofilm of Cs4 and Bd2 fractions presented a better performance since they inhibited more than 90% of biofilm formation at lower concentrations (31.2 μg/mL). |
| [21] | *Croton cajucara* Benth leaves | *S. mutans* ATCC 25175 | Macro technique using microbial disks subjected to the action of the essential oil and controls | The biofilms were exposed to controls and essential oil for 3 min and incubated for 72 h at 37 °C | Growth inhibition: EO 70%–75% Chlorhexidine 65%–70% |
| [21] | *Croton cajucara* Benth leaves | *S. sobrinus* ATCC 27609 | Macro technique using microbial disks subjected to the action of the essential oil and controls | The biofilms were exposed to controls and essential oil for 3 min and incubated for 72 h at 37 °C | Growth inhibition: EO 75%–80% Chlorhexidine 50%–55% |
| [21] | *Croton cajucara* Benth leaves | *L. casei* ATCC 4646 | Macro technique using microbial disks subjected to the action of the essential oil and controls | The biofilms were exposed to controls and essential oil for 3 min and incubated for 72 h at 37 °C | Growth inhibition: EO 80%–85% Chlorhexidine 65%–70% |
| [38] | *Curcuma longa* root | *S. mutans* ATCC 25175 | Technique using 24-well plates containing resin teeth. | After cultivating *S. mutans* for 24 h at 37 °C, the supernatant was removed, and the wells were rinsed with distilled H2O. Biofilm formation in the wells was measured by staining with 0.1% safranin | Biofilm formation was decreased in the presence of *C. longa* essential oil at concentrations higher than 500 μg/mL. |
| [39] | *Mentha piperita* and *Rosmarinus officinalis* | *S. mutans* PTCC 1601 | Biofilm formation (SBF) assay | The biofilms were exposed to controls and essential oil and incubated for 17 ± 1 h at 37 °C | *M. piperita* and *R. officinalis* oils effectively inhibited *S. mutans* biofilm at 6000 and 2000 ppm, respectively. |
| [29] | *Ocimum americanum* L. leaves | *S. mutans* KPSK2 | Microtiter technique Protocol using saliva. | The biofilms were exposed to controls and essential oil (0.3% and 3% v/v) for 5 min and incubated for 24 h | EO 0.3% (v/v) 7.2 × 10^4 CFU/mL; EO 3% (v/v) 2.9 × 10^3 CFU/mL; 0.2% Chlorhexidine: 1.7 × 10^5 CFU/mL; Saline solution 8.5 × 10^5 CFU/mL |
| [29] | *Ocimum americanum* L. leaves | *L. casei* ATCC 6363 | Microtiter technique Protocol using saliva. | The biofilms were exposed to controls and essential oil (0.3% and 3% v/v) for 5 min and incubated for 24 h | EO 0.3% (v/v) 5.1 × 10^5 CFU/mL; EO 3% (v/v) 6.3 × 10^3 CFU/mL; 0.2% Chlorhexidine: 2.5 × 10^5 CFU/mL; Saline solution 6.0 × 10^5 CFU/mL |
Table 8. Framework of studies. Distribution of promising EOs and their isolated constituents tested against caries-related bacteria.

| Plant Species or Chemical Constituent | Antibacterial Efficacy | Clinical Trial |
|---------------------------------------|------------------------|----------------|
|                                       | Planktonic Cells        | Biofilms       |                  |
|                                       | Smu Ssob Ssan Ssal Lc  | Smu Ssob Ssal Lc |                  |
| **A. ligustica**                      | +                      |                |                  |
| **B. dracunculifolia**                | +                      |                |                  |
| **C. cajucara**                       | + + + +               | +             | +               |
| **C. japonica**                       | + + +                 | +             |                  |
| **C. sativum**                        | +                      |                |                  |
| **E. caryophyllata**                  | + + +                 | +             | +               |
| **L. sidoides**                       | +                      |                | Plaque reduction |
| **O. americanum**                     | + + + +               | +             | +               |
| Menthol                               | +                      |                |                  |
| Eugenol                               | + + +                 |                |                  |

Note: (+): MIC <100 µg/mL or correspondent; Smu: *S. mutans*; Ssob: *S. sobrinus*; Ssan: *S. sanguinis*; Ssal: *S. salivarius*; Lc: *L. casei*; La: *L. acidophilus*.

Figure 2. Risk-of-bias summary of the clinical trials included in this systematic review. Red (−) stands for high risk of bias, green (+) stands for low risk of bias and yellow (?) stands for unclear risk of bias. Overall, the studies are compliant with the CONSORT guidelines for clinical trials of herbal interventions, showing low risk of bias.
Table 9. Characteristics of the Randomized Clinical Trials included in this systematic review.

| Plant Species | Essential Oil Formulation | Study Design          | Sample Size                          | Country | Age (Mean ± SD)/Gender (Fem) * | Sample Loss/Reasons                                                                 | Control Group | Dosing Protocol                          | Assessment Checkpoints | Assessment Instruments of Interest | Outcome ** | Ref. |
|---------------|---------------------------|-----------------------|-------------------------------------|---------|--------------------------------|----------------------------------------------------------------------------------|---------------|------------------------------------------|------------------------|------------------------------------|-------------|------|
| Lipia sidoides| 1% *L. sidoides* mouthrinse | Phase II, randomized, double-blind, crossover | \( n = 55 \) (\( n = 27 \) L. sidoides group; \( n = 28 \) control group) | Brazil  | 31 ± 10.90/55.6% F           | 16 individuals (no gender distinction)/lack of compliance or could not be reached for follow-up visits. | 0.12% CHX | Rinsing approx. 15 mL for 30 s, twice a day (once after breakfast and once in the late afternoon) during a 7-day period. | Baseline, 1 week | Plaque index (PI) measured at four sites per tooth (Ainamo & Bay, 1975) | +/-         | [35] |
| Lipia sidoides| 10% *L. sidoides* gel       | Phase II, randomized, double-blind, crossover. Partial mouth experimental model | \( n = 26 \) (\( n = 13 \) L. sidoides group; \( n = 13 \) control group) | Brazil  | 22 ± 4.24/50.0% F           | 4 individuals (no gender distinction)/third molar extraction                     | Placebo gel | Filling a toothshield with the gel prior to insertion in the mouth and seating it over the experimental teeth 3 times a day for at least 1 min. | Baseline, 3 weeks | Plaque index (PI) measured at six sites per tooth (Turesky et al., 1970) | -/+         | [36] |
Table 9. *Cont.*

| Plant Species                  | Essential Oil Formulation                                                                 | Study Design                          | Sample Size | Country | Age (Mean ± SD)/Gender (Fem) * | Sample Loss/Reasons                                                                                                                                   | Control Group | Dosing Protocol | Assessment Checkpoints | Assessment Instruments of Interest | Outcome ** | Ref. |
|-------------------------------|------------------------------------------------------------------------------------------|---------------------------------------|-------------|---------|-------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|---------------|-----------------|------------------------|-----------------------------------|-------------|------|
| Melaleuca alternifolia,        | Multi-herbal mouthrinse containing 0.67% \((v/v)\) *M. alternifolia* oil, 0.33% \((v/v)\) *Leptospermum scoparium* oil, 1% \((v/v)\) *Calendula officinalis* flower extract (1:2) liquid extract \([90\% E/W]\), 0.5% \((w/v)\) *Camellia sinensis* extract\((dry extract, 80\% polyphenols) and 12.8\% ethanol in water.\) | Phase I and II, randomized, double-blind | Phase I: \(n = 8\) (experimental group) | Phases I and II: \(n = 20\) (\(n = 10\) experim. group, \(n = 10\) control group) | 31.88 ± 7.51 | Phase I: 62.5% F \(\text{Phase I: 1 female reported mild 'hay fever'-like symptoms. Her symptoms were judged to be unrelated to the mouthrinse.}\) Phases I and II: 82.3% F \(\text{Phase II: 3 female/One reported lightheadedness (possibly related to the test rinse); One dropped out to participate in another study; and one was excluded because she required treatment with antibiotics for an unrelated condition.}\) | Per day, oral rinse, twice a day for 6 weeks | Placebo mouth rinse, 15 mL for 30 s, twice a day during a 6-week period. | Baseline, 6 weeks and 12 weeks | Plaque index (PI) measured at six sites per tooth \(\text{per tooth}\) | −/− | [37] |

Note: CHX (chlorhexidine) mouthrinse; * Age and gender of individuals assigned to the experimental group; ** Statistically significant reduction (+) or not (−) in the amount of cariogenic biofilm compared to CHX or placebo (first sign) and to the baseline condition (second sign, after slash) \(p < 0.05\).
The EO from *A. camphorata*, *B. sulphurea*, *L. alba*, *M. glomerata*, *O. gratissimum* and *S. guianenses* were not chemically characterized by the studies included in this review. Therefore, 21.7% of the selected studies had no chemical control regarding the EO under test. Furthermore, only 60.8% of the studies proceeded with a botanical identification of the aromatic plants that served as source for the EO. Finally, only 56.52% of the studies showed any piece of information about georeferencing of the plant species and 47.82% reported the period of plant collection.

3. Discussion

Essential oils have stood out as a promising source of bioactive molecules with potential application in the management of dental caries [40,41]. The data presented in this review suggest potential EO and constituents to be further tested as bioactive ingredients of anti-caries formulations. Moreover, the results of the reported chemical assessments of EO-isolated compounds could lead them to be used as chemical markers in future screening. Surprisingly, 20% and 60% of the studies do not provide any chemical or botanical information, respectively, which inevitably results in a biased and inconclusive analysis with reproducibility and traceability issues. Also, despite an understanding of the biological and physicochemical processes associated with the aetiopathogenesis of dental caries [8], great part (88%) of the current evidence on the anti-caries potential of EO is based on *in vitro* studies rather than clinical trials (see Section 3.3 in this Discussion). Altogether, the benefits and issues related to EO research suggest wide avenues for scientists to work on more comprehensive and trustworthy bioprospection studies.

According to our searches, the majority of *in vitro* studies have evaluated the effect of EO or isolated compounds against *S. mutans*, as expected. Considered the most cariogenic of the oral streptococci, *S. mutans* colonizes the tooth surfaces and produces significant amounts of extra- and intra-cellular polysaccharides [42], being responsible for the initial stage of oral biofilm formation and carious lesions [43]. Nevertheless, other streptococci and lactobacilli species are also implicated on the onset [44] and progression [45] of caries, respectively, thus playing a role in the aetiopathogenesis of this biofilm-dependent disease. An EO of interest to be included in a formulation should be that able to affect bacterial virulence without suppressing the resident oral species, as a more specific therapeutic approach [8]. However, most studies provide just preliminary evidence of anti-caries activity without further assessing the effects of EO on putative virulence factors in cariogenic bacteria (e.g., glycosyltransferase and F-ATPase activity). In addition, the cariogenic biofilm is composed of a multi-species microbial community, in which the predominance of different microorganisms is changed as a function of host, diet and microorganism factors [46]. These aspects are not considered in most studies evaluating only planktonic cultures and, at most, monospecies biofilm cultures.

Next, we provide a brief summary of the plant species whose EO and their isolated compounds were found to have significant *in vitro* anti-caries potential. Attention is given to the ethnopharmacological knowledge, biological properties and chemical composition. Despite our attempts to make inter-study comparisons, there are underlying distinctions related to extraction methods, georeferencing, seasonality, which should be taken into account.
3.1. Promising Essential Oils against Cariogenic Bacteria

*Achillea ligustica* (Asteraceae) is a small herbaceous plant rich in terpenes that grows in the Mediterranean region and has been used in folk medicine mainly for the treatment of gastrointestinal disorders [47]. The EO from different parts of this plant (inflorescences, leaves and flowers) is also found to have antimicrobial activity, particularly against *S. mutans* [19,22]. However, as it can be seen in this review, when the major compounds of *A. ligustica* EO are tested alone (e.g., γ-terpinene, β-pinene, 1,8-cineole, terpinen-4-ol), there is a decrease in their antimicrobial activity, which suggests a synergistic effect of the compounds present in the whole EO. Different EOs from the genus *Achillea* have been used in the cosmetic and liqueur industry as fragrances and flavoring agents, demonstrating commercial and economic relevance [22].

*Baccharis dracunculifolia* (Asteraceae) a native plant from Brazil, is widespread in the tropical areas of South America and is the botanical source of Southeastern (or green) propolis [48]. It has been widely used in folk medicine as febrifuge, anti-inflammatory, antiseptic and in the treatment of skin sores and gastrointestinal disorders [49]. The trans-nerolidol- and spathulenol-rich EO from *B. dracunculifolia* and its active fractions are bacteriostatic and have an *in vitro* anti-cariogenic activity by disrupting *S. mutans* biofilm at concentrations as low as 31.25 µg/mL [11].

*Croton cajucara* (Euphorbiaceae) is a common shrub growing in the Amazonian region commonly used in folk medicine as a tea for ailments such as diarrhea, diabetes and gastrointestinal disorders [50]. Alviano *et al.* [21] found that the EO of *C. cajucara* has significant antibacterial activity against *S. mutans*, *S. sobrinus* and *L. casei* in planktonic and monospecies biofilm cultures, unlike its isolated major compound linalool. This result disagrees with others reported in this review showing that linalool is considerably active against *S. mutans* [19,22,31]; however, it remains controversial.

*Cryptomeria japonica* (Cupressaceae) is an endemic and widely distributed coniferous plant in Japan, normally used for forestry, whose EO has been reported to have several pharmacological properties including larvicidal [51], antiulcer [52], antifungal [53] and antibacterial [20]. *C. japonica* EO is another example of how the complex mixture of chemical molecules plays a synergistic role in the antibacterial power of the EO over its isolated major compounds (sabinene, terpinen-4-ol, α-pinene and α-terpineol) [20]. In this review, we found significant inhibitory effects of the leaf EO against caries-related streptococci, warranting further investigation.

*Coriandrum sativum* (Apiaceae) popularly known as coriander, is an annual small plant whose leaves and seeds are widely used in folk medicine as anti-hypertensive, cholesterol-lowering and digestive stimulant [54], and also as food condiment. Moreover, other biological properties of *C. sativum* EO have also been reported: antifungal [55,56] antibacterial [11,56], antioxidant [57] and hepatoprotective [58]. The EO from *C. sativum* leaves contains mostly decanal, trans-2-decenal and 2-decen-1-ol [55], and has been shown to have *in vitro* anti-cariogenic potential against *S. mutans* biofilms and to be more active than its chemical fractions [11].

*Eugenia caryophyllata* (Myrtaceae) is widely cultivated in Indonesia, Sri Lanka, Madagascar, Tanzania and Brazil. *E. caryophyllata* EO (clove) has been described as having useful antiseptic, analgesic and anaesthetic effects. In community medicine, it serves as a topical pain-relieving and healing agent and in the industry as a fragrance and flavoring substance [59]. The main compounds of clove oil are phenylpropanoids such as eugenol and β-caryophyllene. According to our findings,
eugenol was proven to be more active than the EO against *S. mutans*, *i.e.*, showed lower MIC values. Nevertheless, the crude EO of *E. caryophyllata*, in general, showed strong antimicrobial activity against streptococci.

*Lippia sidoides* (Verbenaceae) is a typical shrub commonly found in the Northeastern Brazil, popularly used as topical skin and mucosal antiseptic [60]. *L. sidoides* EO also has anti-inflammatory, antioxidant and gastroprotective properties [61]. Its antimicrobial activity against cariogenic bacteria has been correlated with the presence of the phenolic monoterpenes thymol and carvacrol [62], and it may be considered of the most scientifically explored medicinal plants in Brazil, whose studies have reached the clinical phase. According to this review, *L. sidoides* EO showed both strong *in vitro* antibacterial activity and clinical efficacy as a mouthwash (see Section 3.3 in this Discussion), thus being considered a promising anti-plaque and anti-gingivitis phase II agent [37].

*Ocimum americanum* (Lamiaceae) popularly known as hoary basil, is an annual herbaceous plant native to Asia and Africa. *O. americanum* EO is reported to have anti-inflammatory, antinociceptive [63], antibacterial and insecticidal properties [64], and it is considered valuable for the cosmetic industry of soups and perfumes. The findings of this review showed that the leaf EO has strong antimicrobial activity against *S. mutans* and *L. casei*, either planktonic or biofilm cultures. The study by Thaweboon and Thaweboon [29] indicated that the 3% leaf EO is as effective as 0.2% chlorhexidine in reducing the bacterial counting of cariogenic biofilm cultures of *S. mutans* and *L. lactis*, thus highlighting its potential as an antiseptic agent for oral care. Other studies *in vitro* and *in vivo* are now encouraged to elucidate its effects on other aspects related to the aetiopathogenesis of tooth decay (e.g., glucosyltransferase activity, acid production, enamel demineralization, among others).

*Rosmarinus officinalis* (Lamiaceae) is a culinary evergreen shrub native to the Mediterranean region that has also been used for medicinal purposes to treat bacterial and fungal infections [65]. Unlike the other cases presented thus far, the major compounds of *R. officinalis* EO (camphor, verbenone, α-pinene, β-myrcene, 1,8-cineole and β-caryophyllene) showed better activity (lower MIC value) against cariogenic bacteria—particularly *S. sobrinus* and *S. salivarius*—than the crude EO.

### 3.2. Promising Compounds Isolated from Essential Oils against Cariogenic Bacteria

Generally, the major phytochemical compounds determine the biological properties of EOs [66]. In these cases, the study of isolated compounds is meaningful to concentrate the active principle, enable industrial scale production and allow improvements in the chemical structure using molecular engineering approaches. Here, we provide a summary on menthol and eugenol as the most outstanding compounds isolated from EOs that possess an anti-caries potential.

Menthol is a compound that has raised interest of the pharmaceutical and food industry in the last decades. It is a terpenoid that can be found in the EO of the *Mentha* spp. genus, such as peppermint, with a crystalline, clear or white-colored aspect (Figure 3). Although there are several isomers of menthol available, only (−)−menthol occurs in nature [34].

*In vitro* [34,67] and *in situ* [68] studies have demonstrated that menthol inhibits the growth of both Gram-positive and -negative bacteria and yeasts, and that its mechanism of action may be related to membrane disruption leading to cell leakage. A number of clinical trials [18] have also supported the use of this compound as an ingredient of mouthwash formulations; some of which are already
commercially available worldwide. Although menthol has been used more as a flavoring agent than an active principle, it has been proven to have a considerable antimicrobial activity and is considered as GRAS (Generally Regarded as Safe) by the FDA (US Food and Drug Administration).

Eugenol is an amphipathic phenolic compound (Figure 4) representing the major constituent of EO from clove (Eugenia caryophillis) and cinnamon (Cinnamomum zeylanicum) leaves [12]. Eugenol has been reported to have antiseptic, antimicrobial, anesthetic, analgesic, antioxidant, anti-inflammatory, and cardiovascular activities [69]. In dentistry, it is used as component of a cement containing zinc oxide for provisional sealing of cavities or as base for definitive fillings [70]. According to our review, eugenol has a promising antimicrobial activity against streptococci, particularly S. mutans, and should be considered as an anti-cariogenic agent to further clinical testing. It is an interesting source of new drugs as it is classified as GRAS by the FDA. This compound has been commercially marketed.

In addition to these three compounds, some others indicated in this review arouse attention for their antibacterial power with MIC values lower than 500 µg/mL, as follows: 1,8, cineole, terpinen-4-ol, linalool, β-myrcene, β-caryophyllene and caryophyllene oxide. As such, the presence of these compounds in the EO of a plant could predict its antibacterial properties.

3.3. Rational Clinical Use of Essential Oils and Isolated Compounds

Despite the large number of in vitro studies on the antimicrobial activity of EOs, just a few reach the clinical phase and even fewer lead to a commercial product. Indeed, there is a small number of clinical trials reported in the literature aiming at the development of an EO-containing dental formulation.
The most effective way to use the majority of EOs is by external application, such as mouthwashes for dental care. Topical application is generally safe [66] because most compounds are considered as GRAS by the FDA and have been long used in food preparation in several cultures. In case of eventual oral administration of a mouthwash, for instance, most EO compounds (such as (−)-menthol, thymol, carvacrol and eugenol) would be excreted renally or exhaled via the lungs [71,72], and their fast metabolism and short half-life highlight a minimal risk of accumulation in the organism [73]. However, although EOs have the advantage of being usually devoid of long-term cytotoxicity and genotoxic risks [12], the high volatility and chemical instability of some of their compounds in the presence of heat, humidity, light, or oxygen, may negatively impact their clinical use [74].

At the present time, the most popular EO-based formulation used in dental care in Western society is composed of a fixed combination of four EO-derived active ingredients: thymol (0.064%), eucalyptol (0.092%), methyl salicylate (0.060%) and menthol (0.042%). It is considered effective against cariogenic bacteria and relatively safe, although its 21%–27% alcoholic formula used to keep the constituents in solution is still controversial [75]. In some cases, such as with *A. ligustica* [19,22], *C. japonica* [20] and *C. sativum* [56], the synergism of compounds in the EO is critical for its biological properties as opposite to its isolated constituents. Such chemical complexity may favor solubility in vehicles other than ethanol (e.g., propylene glycol), with less likelihood of adverse effects.

According to our analysis, the mouthwash of thymol- and carvacrol-rich *L. sidoides* EO (ethanol-free) rinsed twice a day is an effective agent to prevent/disrupt the accumulation of cariogenic biofilm [36]. Furthermore, in a previous systematic review [76] we also found that such experimental mouthwash was effective against biofilm-induced gingivitis in adults. Altogether, these findings highlight the therapeutic potential of *L. sidoides* EO for dental care, but it is important to note that further studies are needed to investigate its effects on other aspects related to tooth decay, such as bacterial acid production, biofilm formation, enamel de- and remineralization, inhibition of glycosyltransferase production/activity, among others. Furthermore, the 10% gel of thymol- and carvacrol-rich *L. sidoides* EO was not effective to reduce the amount of biofilm in adults compared to a placebo [37], suggesting that the pharmaceutical preparation plays a crucial role in this clinical outcome.

The synergistic association of EOs with other topical agents, e.g., fluoride, should also be considered for the management of dental caries, combining both antimicrobial and remineralization properties. A study by Zero *et al.* [77] showed that an EO mouthrinse with 100 parts per million fluoride should be effective in promoting enamel remineralization and fluoride uptake, thus providing anti-caries efficacy.

In dentistry, EOs could be useful as preoperative rinses, in periodontal procedures (e.g., sub-gingival irrigation), post-treatment applications, as a conventional mouthwash *etc.* Nevertheless, the majority of studies in the literature up to date fail to indicate robust and translational data to support the clinical use of novel EOs as ingredients of dental formulations, particularly against dental caries. With that said, this review suggests further research on the EOs and their constituents described earlier due to their favorable potential against streptococci and lactobacilli. In addition, it is important to determine the effects of EO on bacterial virulence factors related to dental caries, such as synthesis of extracellular polysaccharides and ability to survive in and produce acidic environments [8]. The scientific validation of the anti-caries activity of EOs and isolated compounds could provide not only patentable preparations and advances in preventive dentistry, but also commercial value.
4. Methods

4.1. Focused Question

The aim of the present review was to answer the specific question: “Based on the current literature, which essential oils and/or isolated compounds are promising anti-caries agents warranting further investigation for clinical use?”

4.2. Search Strategy and Selection of the Studies

This systematic review of scientific studies followed the guidelines of the Transparent Reporting of Systematic Reviews and Meta-Analyses (PRISMA statement) [78]. Seven databases were systematically searched for clinical trials and in situ, in vivo and vitro studies (Table 10).

Table 10. Search strategy and bibliographic databases used to retrieve the articles falling into the scope of this systematic review.

| Bibliographic Databases (Primary Sources) | Search Strategy (Descriptors and Boolean Operators) |
|-------------------------------------------|---------------------------------------------------|
| SciVerse Scopus (Since 1995 until December 2014) | • (oils, volatile OR essential oil) AND (anti caries OR anti caries agents)  
  • (oils, volatile OR essential oil) AND (mouthwashes OR dentifrice OR gel) AND anti plaque  
  • (oils, volatile OR essential oil) AND (oral pathogens OR cariogenic bacteria)  
  • (oils, volatile OR essential oil) AND antimicrobial AND oral cavity  
  • essential oils AND oral |
| Web of Science (Refine: article or review) (Since 1990 until December 2014) | • (oils, volatile OR essential oil) AND (anti caries OR anti caries agents)  
  • (oils, volatile OR essential oil) AND (mouthwashes OR dentifrice OR gel) AND anti plaque  
  • (oils, volatile OR essential oil) AND (oral pathogens OR cariogenic bacteria) |
| Medline via Pubmed (Since 1966 until December 2014) | • (oils, volatile OR essential oil) AND (anti caries OR anti caries agents)  
  • (oils, volatile OR essential oil) AND (mouthwashes OR dentifrice OR gel) AND anti plaque  
  • (oils, volatile OR essential oil) AND (oral pathogens OR cariogenic bacteria) |
| SciELO (Scientific Electronic Library Online) (Since 1998 until December 2014) and LILACS (Latin American and Caribbean Health Sciences Literature) (Since 1982 until December 2014) | • aceites esenciales  
  • aceite volatile  
  • essential oil AND caries  
  • óleo essencial AND Streptococcus mutans  
  • óleo essencial AND Lactobacillus  
  • óleo essencial AND oral  
  • óleo essencial AND antibacteriano  
  • essential oil AND caries  
  • essential oil AND anti-caries agents  |
| Cochrane Library | • óleo essencial AND Lactobacillus  
  • óleo essencial AND oral  
  • óleo essencial AND antibacteriano  |
| Google Scholar | • Manual searches according to the reference lists of the articles |
4.3. Eligibility Criteria

A systematic selection of the articles was carried out by three independent examiners based on the following inclusion criteria: (1) Biological activity: anti-caries activity against oral microorganisms involved in the etiology and progression of dental caries; (2) Plant material and chemical assessment: essential oils and/or isolated compounds from aromatic plants (their chemical assessment was not a restricted inclusion criteria, instead, it served as a point for discussion); (3) Study design: *In vitro*, *in situ* and/or *in vivo* laboratorial studies (planktonic and biofilm assays); randomized controlled clinical trials (outcome of interest: reduction in the amount of cariogenic biofilm); (4) Methodological quality: For clinical trials, *Jadad* scale [79] equal to or greater than 3, meeting high quality standards (see Section 4.4 for details); accuracy of outcomes; internal and external validity; (5) Language: Articles written in English, Spanish or Portuguese; (6) Novelty: Novel essential oils-containing dental formulations were included, if not currently marketed. Examiners agreed that in cases of inconsistency the final verdict on which articles should be included in this review would be reached by consensus.

4.4. Data Pooling and Analysis

The data were allocated into worksheets to proceed with exploratory analysis according to the study design. For *in vitro* studies, in order to standardize the susceptibility patterns of microorganisms to essential oils or isolated compounds, we used their minimum inhibitory concentration (MIC) range as a parameter to determine the intensity of antibacterial activity, based on the literature [80] and on our research experience (Table 11). The retrieved data were expressed according to the bacterial species related to different types of tooth decay, in terms of selectivity to specific surfaces: *Streptococcus mutans* (sulcus and fissure, smooth surface caries—main etiological agent of dental caries) [81]; *S. sanguinis*, *S. sobrinus*, *S. salivarius* play a secondary role and may be recovered from sulcus, fissure and smooth surface caries [82]; *Lactobacillus* spp. (dentin and root surface caries) [45], either in planktonic or biofilm assays.

Table 11. Established parameters based on Minimum Inhibitory Concentrations of essential oils or related chemical constituents.

| MIC Range     | Intensity of Antibacterial Activity | Score |
|---------------|-------------------------------------|-------|
| ≤100 µg/mL    | very strong activity                | (++++)|
| 101–500 µg/mL | strong activity                     | (+++) |
| 501–1000 µg/mL| moderate activity                   | (++)  |
| 1001–2000 µg/mL| weak activity                       | (+)   |
| >2001 µg/mL   | no activity                         | (−)   |

For clinical trials, the data were analyzed based on the CONSORT guidelines for reporting randomized, controlled trials of herbal interventions [83]. Jadad Scale [79] has also been adopted in this review as it checks the validity of evidence on interventions and evaluates methodological quality (randomization, blinding and loss of follow-up). Based on these criteria, we assigned scores to the studies ranging from 0 to 5. Studies reaching a score <3 were considered of poor quality and thus excluded from this review. Several studies, including systematic reviews, have already embraced
this validated evaluation tool [84–87]. Furthermore, we used the risk-of-bias table proposed by Cochrane [88] to check the presence of selection, performance, detection, attrition and reporting biases in the selected clinical trials.

5. Conclusions

This review attempted to shed light on the anti-caries activity of EOs and their isolated constituents. Certainly, EOs extracted from a variety of aromatic plants worldwide can be considered promising sources of bioactive molecules effective against caries-related microorganisms, particularly S. mutans; however, most of the knowledge in the literature is based on in vitro studies and on a limited number of clinical trials. Overall, the studies have assessed the effects of EO and isolated compounds on microbial growth rather than virulence factors (e.g., bacterial EPS synthesis), which play a key role in the aetiopathogenesis of dental caries. Attention is also drawn to the fact that a number of studies do not provide any chemical or botanical characterization data, raising concern about the reproducibility and accuracy of their findings. Scientific journals should be more stringent in the adoption of criteria for the publication of studies with natural products, particularly EOs. Due to the gap between the in vitro biological properties identified in EOs and their clinical use for the prevention of dental caries, future researches should focus on translational approaches to advance the development of effective anti-caries products containing EO, given that most of them are considered as GRAS by the FDA.

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Author Contributions

All authors contributed equally to this work.

Conflicts of Interest

The authors declare no conflict of interest.

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