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In vitro antibacterial activity and major bioactive components of *Cinnamomum verum* essential oils against cariogenic bacteria, *Streptococcus mutans* and *Streptococcus sobrinus*

Okhee Choi^{1,#}, Su Kyung Cho^{1,#}, Junheon Kim^{2,#}, Chung Gyoo Park¹, Jinwoo Kim^{1,2*}¹Division of Applied Life Science (BK21 Plus), Gyeongsang National University, Jinju 52828, South Korea²Institute of Agriculture & Life Science, Gyeongsang National University, Jinju 52828, South Korea

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ABSTRACT

Objective: To evaluate the antibacterial activity of *Cinnamomum verum* (*C. verum*) from 32 different essential oils against cariogenic bacteria, *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*).

Methods: The antibacterial activities of each essential oil were individually investigated against *S. mutans* and *S. sobrinus*. The essential oil of *C. verum* was selected for further evaluation against *S. mutans* and *S. sobrinus*. Gas chromatography mass spectrometry was used to determine the major constituents of *C. verum* essential oil. In addition, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration of the most effective constituent was investigated.

Results: The essential oil from *C. verum* exhibited the greatest antibacterial activity. Gas chromatography mass spectrometry analysis revealed that the major components of *C. verum* essential oil were cinnamaldehyde (56.3%), cinnamyl acetate (7.1%) and β -phellandrene (6.3%). The MIC of cinnamaldehyde was measured using broth dilution assays. The MIC of cinnamaldehyde was 0.02% (v/v) against both bacterial strains tested. The minimum bactericidal concentration of cinnamaldehyde against *S. mutans* and *S. sobrinus* were 0.2% and 0.1% (v/v), respectively.

Conclusions: The essential oil of *C. verum* and its major component cinnamaldehyde possessed considerable *in vitro* antibacterial activities against cariogenic bacteria, *S. mutans* and *S. sobrinus* strains. These results showed that the essential oil of *C. verum* and its bioactive component, cinnamaldehyde, have potential for application as natural agents for the prevention and treatment of dental caries.

1. Introduction

Dental caries is a major oral infectious disease of bacterial origin. The tooth surface can be damaged due to the production of acid by bacterial fermentation of sugars such as sucrose, fructose and glucose in foods or drinks [1,2]. The bacteria

responsible for dental caries are the mutans streptococci including *Streptococcus mutans* (*S. mutans*), *Streptococcus sobrinus* (*S. sobrinus*), *Streptococcus downei*, *Streptococcus rattus*, and *Streptococcus cricetus*, but most prominently *S. mutans* and *S. sobrinus* [3]. *S. mutans* and *S. sobrinus* are the major bacteria associated with dental plaque biofilms, a deposit of proteins and cell-free enzymes. In addition, these bacteria are embedded in exopolysaccharides that adhere firmly to the tooth surface [4–7].

Treatment with antimicrobial agents for the prevention of dental caries has been investigated for over five decades [8–10]. Fluoride has been used as a major agent for prevention of dental caries. Previous reports have shown that fluoride has antibacterial activity against mutans streptococci. In addition, fluoride works by slowing demineralization, which causes calcium and phosphate loss from the tooth enamel [11]. However, the clinical antibacterial effects of fluoride against

*Corresponding author: Jinwoo Kim, Division of Applied Life Science and Institute of Agriculture & Life Science, Gyeongsang National University, Jinju 52828, South Korea.

Tel: +82 55 552 1927

Fax: +82 55 772 1929

E-mail: jinwoo@gnu.ac.kr

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[#]These authors contributed equally to this work.

mutans streptococci have been questioned as the decrease in this disease has not always been correlated with a decline in the numbers of mutans streptococci [12,13]. Moreover, large concentrations and frequent applications of fluoride are required to reduce both the numbers of mutans streptococci and enamel demineralization [14]. Chlorhexidine has broad-spectrum antifungal and antibacterial activity [15]. Although chlorhexidine has been used clinically for mutans streptococci reduction in both saliva and dental plaque, its unpleasant taste and tooth staining have led to the search for appropriate alternatives [15]. Among the alternative therapeutic agents, plant essential oils have become interesting candidates against mutans streptococci. Although over 2000 publications have addressed the antimicrobial activity of essential oils, few studies have evaluated the effectiveness of alternative or complementary treatments with essential oils against mutans streptococci.

The aim of the present study was to identify essential oils showing antibacterial activity against cariogenic bacteria, *S. mutans* and *S. sobrinus*. Disk diffusion assays showed that the essential oil of *Cinnamomum verum* (*C. verum*) exhibited the greatest activity against both bacterial strains. Using gas chromatography mass spectrometry (GC-MS), we determined the major constituents of *C. verum* essential oil. In addition, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the most effective constituent was investigated.

2. Materials and methods

2.1. Essential oils

Thirty-two essential oils were purchased from HerbMall Co. Ltd., (Seoul, Korea). Table 1 lists names of the essential oils used in this study, extraction methods and plant parts extracted.

2.2. Bacterial strains and culture conditions

S. mutans KCOM 1054 and *S. sobrinus* KCOM 1061 were used in this study. The strains were obtained from the Korean Collection for Oral Microbiology (KCOM) and cultured in Todd-Hewitt (TH, Difco, Lab., USA) broth or agar plates at 37 °C.

2.3. Disk diffusion assay

A bacterial suspension was prepared from an overnight-grown culture, and adjusted to an optical density of 0.5 (600 nm) containing approximately 10⁸ CFU/mL. A sterile swab immersed in the bacterial suspension was used to spread the entire surface of a Todd-Hewitt agar plate. A total of 10 µL of each essential oil or essential oil component was applied to a sterile paper disc aseptically placed on the center of the inoculated plates. After 36 h of incubation at 37 °C, the diameter of the zone of growth inhibition was measured in centimeters. Ampicillin was used as a positive control. All experiments were carried out in triplicate.

The average of inhibition diameters was calculated to classify the essential oils as follows: the strains were termed not sensitive (0) for a diameter smaller than 0.8 cm, moderately sensitive (+) for a 0.8–2.0 cm diameter, sensitive (++) for a 2–3 cm, and very sensitive (+++) for a diameter greater than 3 cm.

Table 1

List of essential oils used in this study.

Plant	Plant species	Plant part used	Extraction
Bergamot	<i>C. bergamia</i>	Zest	P
Bitter orange	<i>Citrus aurantium</i> var. <i>amara</i>	Bud	S
Black pepper	<i>Piper nigrum</i>	Fruit	S
Blue gum	<i>E. globulus</i>	Leaf	S
Cajeput tree	<i>Melaleuca leucadendron</i>	Leaf	S
Cedarwood	<i>Cedrus atlantica</i>	Wood	S
Cinnamon	<i>C. verum</i>	Bark	S
Citronella	<i>Cymbopogon winterianus</i>	Grass in flower	S
Clary sage	<i>Salvia sclarea</i>	Leaf	P
Clove bud	<i>S. aromaticum</i>	Bud	S
Cypress	<i>C. sempervirens</i>	Branch	S
Eucalyptus	<i>Eucalyptus radiata</i>	Leaf	S
Frankincense	<i>B. carteri</i>	Sap	S
Geranium	<i>P. roseum</i>	Aerial part	S
Ginger	<i>Zingiber officinale</i>	Rhizome	S
Grapefruit	<i>C. paradisi</i>	Zest	P
Hyssop	<i>Hyssopus officinalis</i>	Leaf	S
Juniper	<i>J. communis</i>	Fruit	S
Lemon peel	<i>C. limon</i>	Zest	P
Myrrh	<i>Commiphora myrrha</i>	Flower and wood	S
Niaouli	<i>Melaleuca viridiflora</i>	Leaf	P
Patchouli	<i>Pogostemon cablin</i>	Leaf	S
Peppermint	<i>Mentha piperita</i>	Leaf	S
Rosemary	<i>R. officinalis</i>	Leaf and flower	S
Sandalwood	<i>Santalum austrocaledonicum</i>	Wood	S
Scotch pine	<i>Pinus sylvestris</i>	Needle	S
Sweet basil	<i>O. basilicum</i>	Flower and leaf	S
Sweet marjoram	<i>Origanum marjorana</i>	Flower and leaf	S
Sweet orange	<i>C. sinensis</i>	Zest	P
Tea-tree	<i>Melaleuca alternifolia</i>	Leaf	S
True lavender	<i>Lavandula vera</i>	Leading flower	S
Ylang-ylang	<i>Cananga odorata</i>	Flower	S

P: Pressure extraction; S: Steam distillation; *C. bergamia*: *Citrus bergamia*; *E. globulus*: *Eucalyptus globulus*; *S. aromaticum*: *Syzygium aromaticum*; *C. sempervirens*: *Cupressus sempervirens*; *B. carteri*: *Boswellia carteri*; *P. roseum*: *Pelargonium roseum*; *C. paradisi*: *Citrus paradisi*; *J. communis*: *Juniperus communis*; *C. limon*: *Citrus limon*; *R. officinalis*: *Rosmarinus officinalis*; *O. basilicum*: *Ocimum basilicum*; *C. sinensis*: *Citrus sinensis*.

2.4. Gas chromatography – flame ionization detector and GC-MS analyses and chemical synthesis

The active chemical constituents of the *C. verum* essential oil were determined using gas chromatography – flame ionization detector, a GC-17A (Shimadzu, Japan) fitted with a DB-5MS [30 mm × 0.25 mm (inner diameter) × 0.25 µm, J & W Scientific Co., USA] non-polar column and GC-MS, a GC puls-2010 coupled with GCMS-QP2010 (Shimadzu, Japan), which was fitted with a HP-Innowax polar column [30 mm × 0.25 mm (inner diameter) × 0.25 µm, J & W Scientific Co., USA]. The temperature program started at 40 °C for 1 min and increased to 250 °C at 6 °C/min, and then held for 4 min. Split injection (1:5 ratio) was performed with 5 mg sample diluted with 1 mL of hexane. The mass detector was fitted with an electron ionization source operated at 70 eV with a source temperature of 230 °C.

Helium was the carrier gas at a flow rate of 1 mL/min. Identification of essential oil compositions was based on the mass spectral information in a mass spectra library (Wiley Registry of Mass Spectra Data, 2000), and sample peaks were confirmed by comparison with the retention indices (RI) and mass spectra of authentic standards.

For the identification of the essential oil components, α -pinene, camphene, 3-carene, α -terpinene, (+)-limonene, (-)-limonene, *p*-cymene, linalool, α -terpineol, and β -caryophyllene oxide were purchased from Aldrich. (+)- β -Pinene, (-)- β -pinene, α -phellandrene, α -terpinolene, and α -humulene were purchased from Fluka. β -Caryophyllene and benzyl benzoate were purchased from TCI (Tokyo Chemical Industry Co., Ltd.). Eugenol and cinnamyl alcohol were purchased from Alfa Aesar. β -Phellandrene was prepared as described previously [16].

To determine the MBC, 10 μ L of bacterial inoculum were removed from tubes that had not presented visible turbidity and spread onto Todd-Hewitt agar. These plates were incubated at 37 °C for 48 h. The MBC was considered as the lower concentration that showed no bacterial growth on Todd-Hewitt agar plates. Each MIC and MBC value was obtained from three independent experiments.

3. Results

3.1. Screening of antibacterial activity

Antibacterial activities of plant essential oils against *S. mutans* and *S. sobrinus* strains were presented in Figure 1.

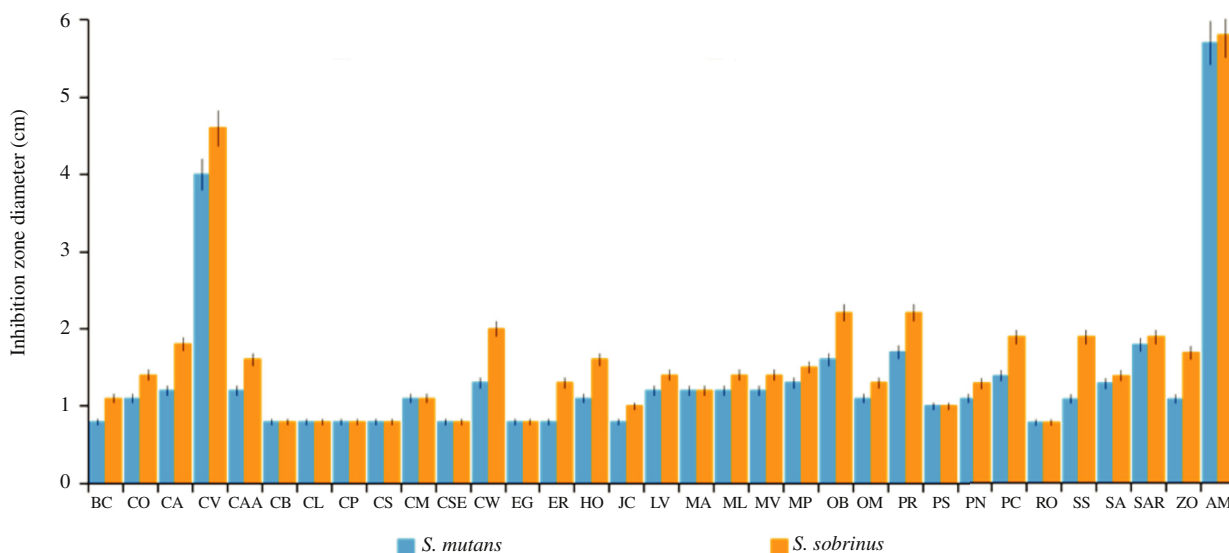


Figure 1. Antibacterial effect of essential oils against cariogenic bacteria *S. mutans* and *S. sobrinus*. Average inhibition diameters were calculated to classify the essential oils as follows: the tested strains were not sensitive for a diameter smaller than 0.8 cm (0), moderately sensitive for a 0.8–2 cm diameter (+), sensitive for a 2–3 cm (++), and very sensitive for a diameter larger than 3 cm (+++).

BC: *B. carteri*; CO: *Cananga odorata*; CA: *Cedrus atlantica*; CV: *C. verum*; CAA: *Citrus aurantium* var. *amara*; CB: *C. bergamia*; CL: *C. limon*; CP: *C. paradisi*; CS: *C. sinensis*; CM: *Commiphora myrrha*; CSE: *C. sempervirens*; CW: *Cymbopogon winterianus*; EG: *E. globulus*; ER: *Eucalyptus radiata*; HO: *Hyssopus officinalis*; JC: *J. communis*; LV: *Lavandula vera*; MA: *Melaleuca alternifolia*; ML: *Melaleuca leucadendron*; MV: *Melaleuca viridiflora*; MP: *Mentha piperita*; OB: *O. basilicum*; OM: *Origanum marjorana*; PR: *P. roseum*; PS: *Pinus sylvestris*; PN: *Piper nigrum*; PC: *Pogostemon cablin*; RO: *R. officinalis*; SS: *Salvia sclarea*; SA: *Santalum austrocaledonicum*; SAR: *S. aromaticum*; ZO: *Zingiber officinale*; AM: Ampicillin.

Benzaldehyde, hydrocinnamic aldehyde and cinnamaldehyde were synthesized from the corresponding alcohol by pyridinium chlorochromate oxidation [17]. Hydrocinnamyl acetate and cinnamyl acetate were obtained by acetylation of the corresponding alcohol. Hydrocinnamyl alcohol was synthesized by hydrogenation of cinnamyl alcohol with Pd on the carbon.

2.5. Determination of MIC and MBC

MIC of cinnamaldehyde was determined using the broth dilution method in Todd-Hewitt broth as described by Sfeir *et al.* [18]. Briefly, each compound was first diluted to 40% (v/v) in dimethyl sulfoxide. Serial dilutions were carried out in sterile distilled water at concentrations of 0.01%–0.50% (v/v). One milliliter of bacterial suspension (10^6 CFU/mL) and 0.1 mL of each compound showing antibacterial activity were added to 2.9 mL of Todd-Hewitt broth. Controls without test compounds were prepared. After 24 h of incubation at 37 °C under agitation in culture tubes, the MIC was determined as the lowest concentration that visibly inhibited bacterial growth.

Results obtained from disk diffusion assay showed that essential oil of *C. verum* was the most active against the tested bacterial strains, with zones of inhibition ranging from 4.0 to 4.6 cm (+++). *S. sobrinus* was sensitive (++) to essential oils from *O. basilicum* and *P. roseum*. Most essential oils tested showed moderate inhibitory activities (+) against both tested strains. Seven essential oils (*C. bergamia*, *C. limon*, *C. paradisi*, *C. sinensis*, *C. sempervirens*, *E. globulus* and *R. officinalis*) showed no activity (0) against both test strains. Additionally, two essential oils (*B. carteri* and *J. communis*) showed no activity (0) against *S. mutans*. The zone of inhibition of essential oils were smaller than that of the positive control, ampicillin.

3.2. Chemical composition of the essential oil of *C. verum*

The results of the chemical analysis of *C. verum* essential oil were presented in Table 2. The compounds are listed according to their elution order, which was in agreement with their RI on HP-

Table 2Chemical composition of the essential oil of *C. verum*.

No.	Compound	RI ^a on DB-5MS		RI ^a on HP-Innowax		Relative area ^b (%)	Source of standard
		Standard	<i>C. verum</i> essential oil	Standard	<i>C. verum</i> essential oil		
1	α -Pinene	934	933	1018	1015	0.5	Aldrich
2	Camphene	950	950	1061	1061	2.7	Aldrich
3	β -Pinene	979	978	1105	1103	0.7	Fluka
4	3-Carene	1009	1010	1145	1144	0.2	Aldrich
5	α -Phellandrene	1007	1007	1165	1161	2.1	Fluka
6	α -Terpinene	1018	1018	1180	1176	1.8	Aldrich
7	Limonene	1031	1032	1193	1195	2.0	Aldrich
8	β -Phellandrene	1032	1032	1205	1205	6.3	Synthetic ^d
9	<i>p</i> -Cymene	1027	1026	1271	1270	4.1	Aldrich
10	α -Terpinolene	1087	1087	1283	1281	0.2	Fluka
11	Unknown	–	–	–	1470	0.3	–
12	Benzaldehyde	964	967	1526	1525	1.2	Synthetic ^d
13	Linalool	1102	1100	1550	1550	4.1	Aldrich
14	β -Caryophyllene	1425	1425	1595	1595	3.2	TCI
15	Unknown	–	–	–	1602	1.0	–
16	Humulene	1463	1462	1669	1670	0.6	Fluka
17	α -Terpineol	1200	1200	1702	1697	1.1	Aldrich
18	Hydrocinnamic aldehyde	1167	1171	1782	1782	0.2	Synthetic ^d
19	Unknown	–	–	–	1897	0.3	–
20	Hydrocinnamyl acetate	1375	1373	1944	1944	0.2	Synthetic ^d
21	Caryophyllene oxide	1591	1591	1980	1982	0.5	Aldrich
22	Cinnamaldehyde	1279	1280	2041	2042	56.3	Synthetic ^d
23	Cinnamyl acetate	1453	1453	2155	2155	7.1	Synthetic ^d
24	Eugenol	1357	1357	2170	2171	2.0	Alfa Aesar
25	Cinnamyl alcohol	1313	1316	2286	2287	0.3	Alfa Aesar
26	Methoxycinnamaldehyde ^c	–	–	–	2441	0.2	–
27	Benzyl benzoate	1777	1776	2627	2627	0.5	TCI

^a: Retention index [19], according to *n*-alkanes (C9–C25); Components were identified by comparison of RI on two columns and mass spectrum with authentic standards. ^b: Obtained from GC-FID analysis. ^c: Identified by GC-MS library. ^d: Purities of synthetic compounds was >99.0%.

Innowax columns [19]. Of the 27 components of *C. verum* essential oil, 24 were identified (Table 2 and Figure 2). Three peaks showed no match with the MS library. Cinnamaldehyde (56.3%) was the main compound in *C. verum* essential oil, followed by cinnamyl acetate and β -phellandrene (Table 2 and Figure 2).

3.3. Antibacterial activity of the *C. verum* essential oil components

The antibacterial activities of the chemical constituents of *C. verum* essential oil against *S. mutans* and *S. sobrinus* strains were presented in Figure 2. Results obtained from the disk diffusion assay showed that cinnamaldehyde was the most active

against the tested bacterial strains, with zones of inhibition ranging from 4.2 to 5.7 cm (+++). *S. sobrinus* was sensitive (++) to eugenol and cinnamyl alcohol, whereas *S. mutans* was moderately sensitive (+). Both tested strains were moderately sensitive (+) to 3-carene, α -terpinene, benzaldehyde, linalool, β -caryophyllene, α -humulene, α -terpineol, hydrocinnamic aldehyde, hydrocinnamyl acetate and cinnamyl acetate. No activity (0) was detected against either strain in 11 compounds: α -pinene, camphene, (+)- β -pinene, (–)- β -pinene, α -phellandrene, α -terpinene, (+)-limonene, (–)-limonene, *p*-cymene, β -caryophyllene oxide and benzyl benzoate (Figure 3). The zone of inhibition of all the essential oils were smaller than that of the positive control, ampicillin.

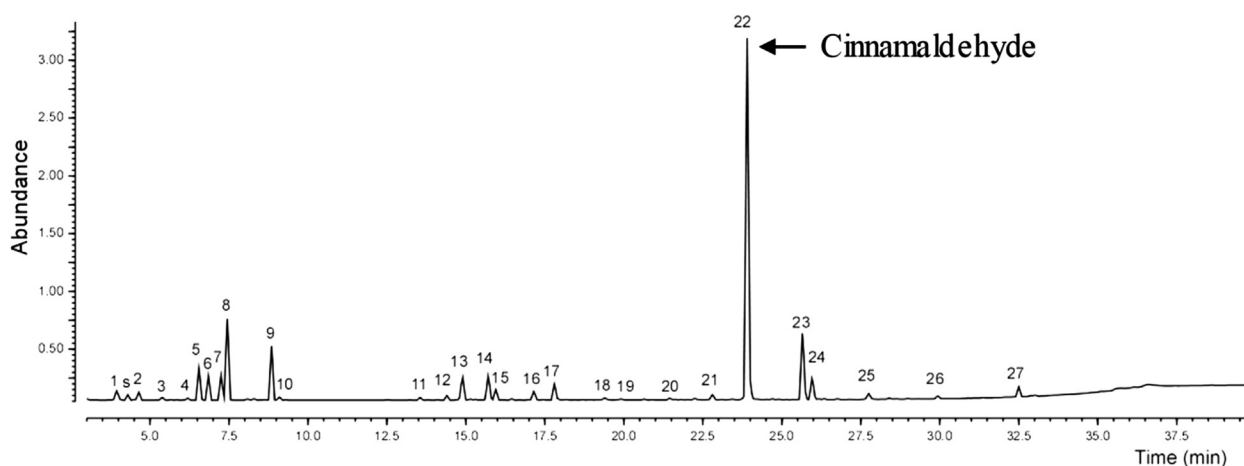


Figure 2. GC-MS chromatograms showing chemical compositions of cinnamon essential oil.

Total ion chromatogram of cinnamon oil on HP-Innowax column; Numbers of peaks are the same as those in Table 2; s: Toluene from solvent (hexane).

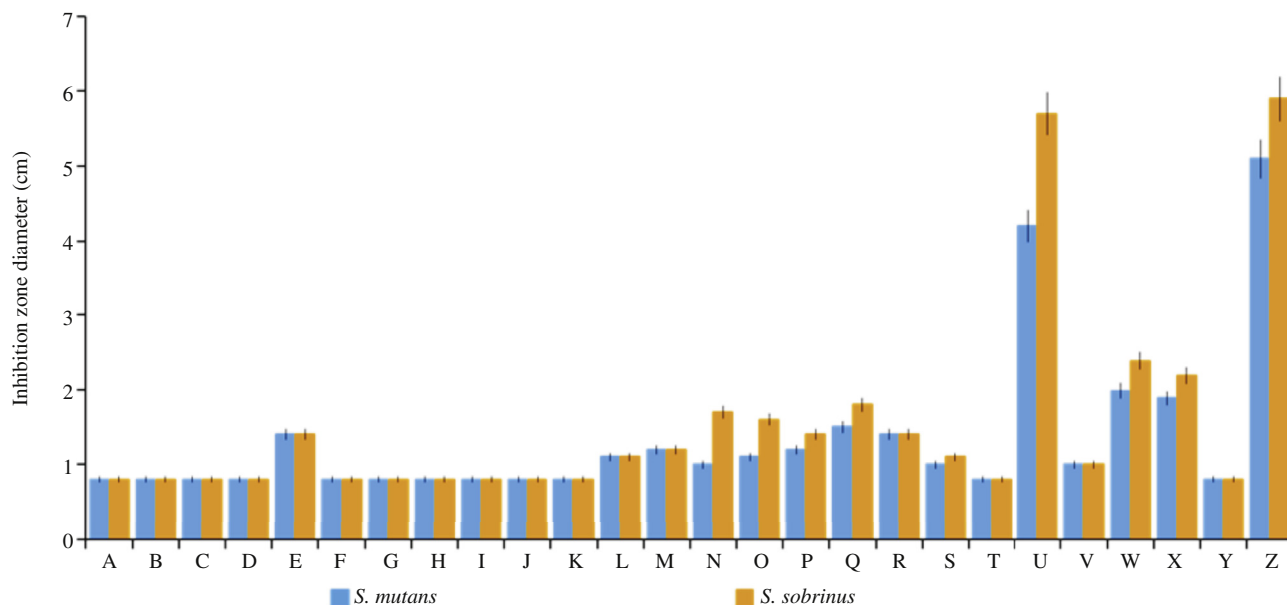


Figure 3. Chemical constituents of *C. verum* essential oil and their antibacterial effect against cariogenic bacteria *S. mutans* and *S. sobrinus*.

Average of inhibition diameters were calculated to classify the essential oils as follows: the tested strains were not sensitive for a diameter smaller than 0.8 cm (0), moderately sensitive for a 0.8–2.0 cm diameter (+), sensitive for a 2–3 cm (++), and very sensitive for a diameter greater than 3 cm (+++); A: α -Pinene; B: Camphene; C: (+)- β -Pinene; D: (-)- β -Pinene; E: 3-Carene; F: α -Phellandrene; G: β -Phellandrene; H: α -Terpinene; I: (+)-Limonene; J: (-)-Limonene; K: *p*-Cymene; L: α -Terpinolene; M: Benzaldehyde; N: Linalool; O: β -Caryophyllene; P: α -Humulene; Q: α -Terpineol; R: Hydrocinnamic aldehyde; S: Hydrocinnamyl acetate; T: β -Caryophyllene oxide; U: Cinnamaldehyde; V: Cinnamyl acetate; W: Eugenol; S: Cinnamyl alcohol; Y: Benzyl benzoate; Z: Ampicillin.

3.4. MIC and MBC values

Disk diffusion assays for cinnamaldehyde were used to determine the most effective compound, and the MIC values were determined by means of broth dilution assays. The MIC of cinnamaldehyde was 0.02% (v/v) against both bacterial strains tested (Figure 4). The MBC values of cinnamaldehyde against

S. mutans and *S. sobrinus* were 0.2% and 0.1% (v/v), respectively (Figure 4).

4. Discussion

Plant-derived essential oils are ideal for use in oral care formulations, including toothpastes, mouthwashes, sprays and gels because they are non-toxic and have antiseptic properties [20]. Scientific investigations to evaluate the antimicrobial activity of essential oils are needed.

The present work evaluated the antibacterial activities of essential oils specifically against the *S. mutans* and *S. sobrinus* strains responsible for dental caries. We analyzed the chemical composition of *C. verum* essential oil showing the greatest antibacterial activity against the tested bacteria. To our knowledge, no previous publications have reported the antibacterial activity of cinnamaldehyde, the major bioactive component of *C. verum* essential oil, against cariogenic bacteria.

In this study, we used a disk diffusion assay to identify essential oils with the highest inhibitory activity against *S. mutans* and *S. sobrinus* strains among 32 essential oils tested. The essential oil of *C. verum* exhibited the highest inhibitory activity against both bacterial strains compared. These results were consistent with previous work. In the literature, the essential oil of *C. verum* inhibited the growth of fungi including *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., *Fusarium* spp. and *Mucor* spp. It also inhibited the growth of various bacterial taxa, including *Lactobacillus* sp., *Bacillus thermoacidurans*, *Salmonella* sp., *Corynebacterium michiganense*, *Pseudomonas striarfacines* and *Streptococcus pyogenes* [21–24]. Cinnamaldehyde has been proposed to inhibit the growth of food-spoilage bacteria, including *Escherichia coli* O157:H7 and *Salmonella typhimurium* [25].

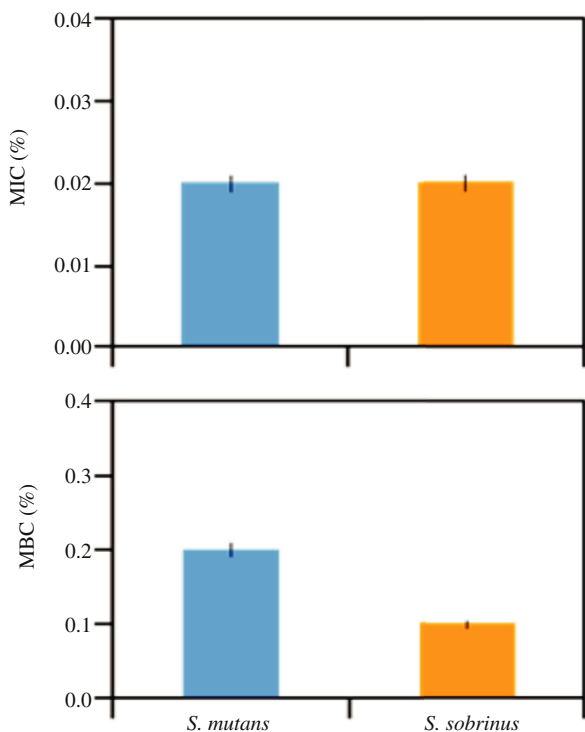


Figure 4. MICs and MBCs of cinnamaldehyde against *S. mutans* and *S. sobrinus* (means \pm SD).

Using GC-MS analysis, we showed that cinnamaldehyde (56.3%) was the major component of *C. verum* essential oil; and other components identified were cinnamyl acetate (7.1%) and β -phellandrene (6.3%). The chemical components of *C. verum* essential oil used this study was very similar to that used in previous reports [26,27]. The three principal components, eugenol, cinnamaldehyde, and linalool, were detected in both bark and leaf essential oils of *Cinnamomum* sp. [26]. According to Wang et al. [27], the essential oil of *C. osmophloeum* was classified into 9 types: cassia, cinnamaldehyde, coumarin, linalool, eugenol, camphor, 4-terpineol, linalool-terpineol, and a mixed-type oil. Our present GC-MS results indicated that the essential oils of *C. verum* were of the cinnamaldehyde type.

Among the major components, cinnamaldehyde exhibited the greatest antibacterial activity against *S. mutans* and *S. sobrinus* strains (Figure 2). Therefore, the essential oil of *C. verum* containing cinnamaldehyde showed the highest activity. Essential oils containing mainly aromatic phenols or aldehydes have been reported to exhibit considerable antimicrobial activity, whereas essential oils containing terpene ether, ketone or oxide have weaker activity [22,28]. Eugenol exhibited sensitive and moderately sensitive activities against *S. sobrinus* and *S. mutans*, respectively (Figure 2). Therefore, essential oils from *O. basilicum* and *S. aromaticum*, which are known to contain 70%–85% eugenol, showed antibacterial activities against *S. sobrinus* and *S. mutans*. Xu et al. [29] have shown that the inhibitory effects of eugenol on dental caries development caused by *S. mutans*.

Essential oils have a complex mode of antibacterial action [30]. However, the antibacterial actions are intimately attached to their major characteristic, hydrophobicity, which produces an increase in the bacterial membrane permeability and the consequent loss of their cellular elements [31–34]. In *Staphylococcus aureus*, cells treated with the essential oil of *C. verum* showed a considerable decrease in the metabolic activity and replication capacity, leading to a viable but non-cultivable state [35]. The essential oil of *C. verum* damages the cellular membrane of *Pseudomonas aeruginosa*, which leads to the collapse of membrane potential and loss of membrane-selective permeability [35]. Cinnamaldehyde exposure causes morphological changes in foodborne pathogenic bacteria, including *Staphylococcus aureus*, *Staphylococcus anatum*, and *Bacillus cereus* [36].

The essential oil of *C. verum* was not harmful when consumed in food additives and may be used as an antibacterial agent. However, there are occasional case reports of allergic contact dermatitis and stomatitis among the consumers [37,38].

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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