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The Antibacterial Potential of Essential Oils of Oral Care Thai Herbs against *Streptococcus mutans* and *Solobacterium moorei*—In Vitro Approach

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Abstract: Oral malodor, often known as halitosis, is an irritating breath odor that originates in the mouth and can cause significant psychological and social distress. Chlorhexidine, a powerful antimicrobial agent effective against bacteria and fungi, has become the standard treatment for halitosis. However, it has drawbacks including altered taste perception, dry mouth, and more noticeable dental staining. The use of natural essential oils to avoid these unwanted effects has proven to be an attractive strategy. This study aims to evaluate the potential of four essential oils consisting of Ma-kwean fruit (*Zanthoxylum limonella*, MK), clove bud (*Syzygium aromaticum*, CV), star anise fruit (*Illicium verum*, SA) and cinnamon bark (*Cinnamomum aromaticum*, CM) for the purpose of combating bad breath by assessing their antibacterial efficacy against halitosis-associated bacteria (*Streptococcus mutans* and *Solobacterium moorei*). The hydro-distillation process was used to prepare the essential oils, which were obtained as yellowish to colorless liquids with yields of 6.58 ± 0.81, 12.21 ± 2.98, 4.29 ± 0.15 and 1.26 ± 0.09% for MK, CV, SA and CM, respectively. The terpenoid compounds terpinene-4-ol (47.04%), limonene (17.19%), sabinene (13.27%) and alpha-terpineol (6.05%) were found as the main components in MK essential oil, while phenylpropanoids were identified as the primary components of other essential oils, namely trans-cinnamaldehyde (83.60%), eugenol (83.59%) and anethol (90.58%) were identified as the primary components of CM, CV and SA essential oils, respectively. For the antibacterial properties, the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values were investigated. CM essential oil exhibited the greatest capacity to inhibit growth and eradicate *S. mutans*, with MIC and MBC values of 0.039%, followed by CV (MIC of 0.078% and MBC of 0.156%) and MK (MIC and MBC of 0.156%), whereas the MIC of SA was 1.250% without eradication. Both CM and CV essential oils demonstrated exceptional efficacy against *S. moorei*, with MIC and MBC values of 0.019% and 0.033%, respectively. Furthermore, the inhibition of *S. moorei* biofilm formation was investigated and we discovered that the lowest effective concentration necessary to eliminate the *S. moorei* biofilm was one quarter of the MIC for MK, CM and CV, while that for SA essential oil was half of the MIC. These encouraging results suggest that the incorporation of MK, CM and CV essential oils into oral care products could potentially enhance their efficacy in halitosis treatment.

Keywords: star anise (*Illicium verum*); cinnamon (*Cinnamomum aromaticum*); clove (*Syzygium aromaticum*); complementary and alternative medicine; halitosis; Ma-kwean (*Zanthoxylum limonella*); oral malodor; *Solobacterium moorei*; *Streptococcus mutans*

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1. Introduction

Halitosis, sometimes known as oral malodor, is the scientific name for an offensive odor. It is a widespread issue that affects people of all ages and can lead to psychosocial embarrassment [1]. It can be caused by intra- and extra-oral factors. Low salivary flow,

mouth breathing and poor oral hygiene are the main intra-oral contributors to halitosis. This environment is favorable for some bacterial pathogens, allowing them to develop and spread, potentially leading to oral diseases such as dental caries, gingivitis and periodontal disease [2]. The extra-oral factors contributing to halitosis are primarily associated with systemic diseases such as diabetes, gastrointestinal disorders and liver ailments, as well as the use of certain medications and consumption of specific foods. Approximately 90% of halitosis cases are related to intra-oral conditions [3].

The oral anaerobic bacteria that are most related to halitosis are *Porphyromonas gingivalis*, *Tannerella forsythia*, *Fusobacterium nucleatum*, *Prevotella intermedia*, *Treponema denticola* and *Solobacterium moorei* [4]. Among these bacteria, *S. moorei* has been found to have a stronger correlation with oral malodor than the others by creating volatile sulfur compounds (VSCs) through a mechanism involving β-galactosidase activity and an external source of proteases [5–7]. The proteolytic anaerobic bacteria produce VSCs by degrading food proteins into amino acids. Then, sulfur-containing amino acids such as cysteine and methionine are further processed into hydrogen sulfide (H_2S) and methylmercaptan (CH_3SH) by cysteine desulfhydrase and L-methionine-α-deamino-γ-mercaptomethane-lyase (L-methioninase), respectively [8], as shown in Figure 1. Antibacterial substances have been considered for halitosis control as they reduce these microorganisms. In addition, *Streptococcus mutans* has been found to be the primary cause of human dental caries. The crucial virulence characteristic of the bacterium is its capacity to produce dental plaques, a type of biofilm that forms on the surfaces of the teeth, which contributes to dental caries. Moreover, bad odors can occur due to food debris between the teeth and the decay of the exfoliated oral epithelial cells, which increases plaque build-up on the teeth and tongue, escalating the severity of foul breath [9].

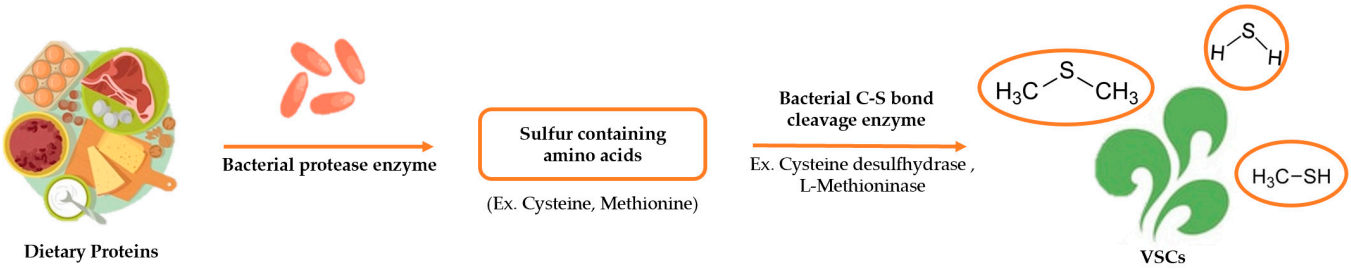

Figure 1. The formation of VSCs via the catalytic action of bacterial enzymes.

There are four distinct types of halitosis treatments, including mechanical cleaning, the use of masking agents, the chemical reduction of oral bacteria and the chemical neutralization of odorous substances. The most fundamental technique to decrease oral bacteria and their substrates is mechanical cleaning with toothbrushes or tongue scrapers [10]. Masking items, such as mouth sprays, mouth rinses and chewing gum, are marketed commercially and aim to control halitosis with pleasant flavors and scents. This method is not fully effective in treating halitosis because the underlying cause is not removed. In addition, masking agents without antibacterial ingredients have a time limit regarding their efficacy [11]. Oral care products with antibacterial activity can reduce oral microbes, and the chemical neutralization of VSCs, which change volatile gases into non-volatile components, has been demonstrated to significantly and completely alleviate halitosis [12,13].

Chlorhexidine has long been considered as the most effective remedy for bad breath. Its strong antibacterial properties have been shown to drastically reduce VSC levels. However, it has drawbacks such as altered taste perception, dry mouth and increased tooth staining [14]. Furthermore, it has been reported by the World Health Organization (WHO) that approximately 80% of the global population incorporates herbal treatments into their healthcare regimens [15]. The use of natural essential oils, which are complex combinations of volatile secondary metabolites, has shown promise as a novel approach to avoiding these unwanted effects. In addition, essential oils have long been incorporated into den-

tal care products for their ability to reduce bad breath [16]. Numerous research teams have previously studied the antimicrobial effects of essential oils and their chemical constituents [14,16]. Choi et al. [17] used the disc diffusion method to screen the antimicrobial properties of 32 essential oils against *S. mutans* and *S. sobrinus*, two common oral infection agents. The results showed that the most of the tested essential oils had positive effects. Strong inhibition was observed for cinnamon; a sensitive clear zone was provided by citronella, sweet basil and geranium; and moderate inhibition was observed for ylang-ylang, cedarwood, lavender, hyssop, niaouli, peppermint, clove bud, sweet marjoram, scotch pine, black pepper, patchouli, bitter orange, myrrh, tea tree and cajuput tree. The primary mechanism by which essential oils exhibit antibacterial activity appears to involve the disruption of the cell membrane. Empirical evidence has shown the antibacterial efficacy of many essential oils against oral bacteria that produce volatile sulfur compounds (VSCs) [18]. Additionally, a number of advantageous qualities of essential oils in relation to oral malodor are being taken into account. The aforementioned qualities include anti-inflammatory and antioxidant properties, which may have an impact on relevant clinical metrics related to gingival inflammation and dental health status. Furthermore, essential oils exhibit significant breath-masking properties, hence augmenting their total efficacy. Consequently, there has been an increasing inclination towards the utilization of natural essential oils as complementary and alternative medicine (CAM) [15,16].

Since ancient times, Thailand's culinary fragrant herbs have also been employed for the purpose of maintaining dental health. Clove (*Syzygium aromaticum*, CV), Ma-kwean (*Zanthoxylum limonella*, MK), star anise fruit (*Illicium verum*, SA) and cinnamon (*Cinnamomum aromaticum*, CM) are among the most commonly mentioned [11,19–22]. CV essential oil, derived from the dried flower buds of the clove tree, has been utilized in clinical dentistry for many centuries. The key constituents include eugenol, caryophyllene and eugenol acetate, which provide a unique refreshingly clean sensation and have been demonstrated to be antibacterial by disrupting the cell membrane of the common oral infection agent *S. mutans* [23,24]. MK is an aromatic herb that is commonly found in the northern part of Thailand. Its fruit essential oils are utilized in Thai folk medicine to prevent dental caries and halitosis [25]. Sabinene, terpinene-4-ol and limonene have been identified as the three main components of MK essential oil, and sabinene was found to be the primary factor that suppressed the growth of *S. mutans* in a dose-dependent manner [26]. Terpinen-4-ol also showed antibacterial activity against *S. aureus* and good antibiofilm activity [27]. Limonene reduced the amount of biofilm developed by *Streptococcus pyogenes* (SF370) [28]. Moreover, MK crude oil had a higher level of microbial inhibition than the pure substance, indicating a synergistic effect with other components in the crude oil [29]. SA essential oil is obtained from dried fruit and anethole considered as the active component in its essential oil. Although there are many reports about its antimicrobial activity against *Agrobacterium tumefaciens*, *Bradyrhizobium japonicum*, *Bacillus subtilis*, *Bacillus megatarium*, *Bacillus licheniformis*, *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Klebsiella aerogenes*, *Sarcina lutea* and *Rhizobium leguminosarum*, its effectiveness against oral pathogens has not yet been thoroughly studied [30,31]. CM is also named *Cinnamomum cassia*, which is found wild and cultivated in Southeast Asia. Its bark essential oil has been found to be a powerful tool against *S. mutans* and *S. moorei* [18,32]. The cinnamaldehyde was found as the major component and was responsible for its antibacterial properties.

To the best of our knowledge, the inhibitory effects of CV, MK and SA crude oils on the growth and biofilm formation of halitosis-associated bacteria have not been previously examined. Therefore, this study aims to investigate the antibacterial effects of these essential oils in comparison to CM against bacteria linked with halitosis: *Streptococcus mutans* and *Solobacterium moorei*.

2. Materials and Methods

The dried plant materials consisted of the whole fruit of MK, bark of CM, bud of CV and fruit of SA were purchased from Samunpai Tharpajan Co., Ltd. (Bangkok, Thailand). Agar powder, glucose, Todd Hewitt Broth (THB) and yeast extract were purchased from

Huankai Microbial Sci & Tech (Guangdong, China). Hemin and Vitamin K1 were purchased from HR Chemical, (Shandong, China). Anhydrous sodium sulfate and Tween 80 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dimethylsulfoxide (DMSO) was purchased from RCI Labscan (Bangkok, Thailand). Crystal violet and phosphate-buffered saline (pH 7.2) were purchased from HiMedia Laboratories Pvt. Ltd., (Mumbai, India). The analytical grade gas mixture (N_2:H_2:CO_2/75:10:15) was prepared by Labgaz (Thailand) Co., Ltd. (Bangkok, Thailand). *S. moorei* JCM 10645 was purchased from the RIKEN Bioresource Research Centre (Japan Collection of Microorganisms, Ibaraki, Japan) and *S. mutans* DMST 1877 was purchased from the Department of Medical Sciences (DMST, Nonthaburi, Thailand).

2.1. Essential Oil Extraction

Each dried plant material (100 g) was extracted by the hydro-distillation method using a Clevenger-type apparatus for 4 h [33]. The oils were dried over anhydrous sodium sulfate and filtrated through Whatman No.1 filter paper. The weight of the extracted oils was recorded and they were sealed before being placed in a light-protected bottle at 4 °C for future analysis. The extraction of each sample was done in triplicate and reported as the mean value of the percentage yield of essential oil based on the weight of dried plant material.

$$\text{Yield (\%)} = (\text{weight of extracted oil/weight of dried plant}) \times 100$$

2.2. Analysis of Essential Oil Composition

The essential oils were analyzed by the Hewlett Packard Model HP6890 with an HP model 5973 mass-selective detector system (Agilent Technologies, Santa Clara, CA, USA), via the gas chromatography–mass spectroscopy (GC-MS) technique, following the method of Gong et al. [34] with some modifications. Briefly, a HP-5MS column (30 m × 0.25 mm (5% phenyl)-methtylpolyxiloane) with a 0.25 µm film thickness was used (Agilent Technologies, Santa Clara, CA, USA). A split/splitless injector was heated to 220 °C. The oven temperature was programmed as follows: initial temperature of 60 °C for 1 min, increase of 3 °C per minute up to 240 °C and held at 240 °C for 5 min. Then, 99.999% purity helium gas was used as a carrier gas at a flow rate of 1 mL/min. The injection volume was 1.0 µL in spitless mode. For electron ionization, mass spectra were used with ionization energy of 70 eV and ionization voltages over the range of m/z 29–300. The electron multiplier voltage was 1150 V. The ion source and quadrupole temperatures were set to 230 °C and 150 °C, respectively. The identified components were assigned by matching their mass spectra with the reference mass spectra via Wiley and the National Institute of Standards and Technology (NIST) database library. The results were also confirmed by the comparison of their Kovát retention indices (RI), relative to C_8–C_{20} n-alkanes assayed under the same conditions. The percentage compositions of individual components were expressed as percentages of the peak area relative to the total peak area.

2.3. Determination of Antibacterial Activity

The culture conditions were followed the report of Lebel et al. [4] with some modifications. Briefly, each bacterium was separately grown in THB supplemented with 0.001% hemin, 0.0001% vitamin K, 0.5% Tween 80, 0.2% yeast extract and 1% glucose, at 37 °C, under anaerobic conditions (N_2:H_2:CO_2/75:10:15) for 24 h before further experiments.

The MIC and MBC were determined by a microplate dilution assay following the method of Tanabe et al. [7] with some modifications. Briefly, *S. moorei* JCM 10645 and *S. mutan* MST 18777 were subcultured in fresh supplemented THB medium for 24 h. Then, this was diluted with fresh medium to obtain an optical density at 660 nm (OD_{660}) of 0.1. An equal volume of 100 µL bacterial suspension and two-fold serial dilution of essential oil (ranging from 5% to 0.0195%) in the culture medium were added to the 96-well microplate and incubated at 37 °C under anaerobic conditions for 24 h. The bacterial growth was

monitored by recording the optical density at 660 nm in a UV–visible microplate reader (MR-1000S Bioplate Reader, Wilmington, DE, USA). A well with no bacteria suspension was used as the control, and the lowest concentration of essential oil with no bacterial growth was recorded as the MIC. The MBC value was determined by taking aliquots of 20 µL from all the tubes that showed no visible growth into a supplemented THB agar plate and incubated for 48 h anaerobic incubation period at 37 °C, the lowest concentration at which no colonies formed indicated the MBC value. The experiments were performed in triplicate.

2.4. Determination of Antibiofilm Qualities

The method to evaluate biofilm formation followed the methods of Yanti et al. [35]. Briefly, *S. moorei* broth cultures were adjusted with fresh supplemented THB medium to obtain an OD_{660} of 0.1. Then, 100 µL diluted essential oil at concentrations of MIC, 1/2 MIC, 1/4 MIC and 1/8 MIC were added into a 96-well microplate and mixed with 100 µL of bacterial suspension and then incubated at 37 °C under anaerobic conditions for 24 h. After this, each well of the microplate was washed twice with a phosphate buffer solution that had a pH of 7.2, and then were allowed to air dry at ambient temperature for 1 h. Then, the biofilms were stained with 200 µL of 1% crystal violet for 30 min. Excessive staining was removed by washing with 200 µL deionized water 4 times. Then, 200 µL of DMSO was added and the absorbance at 590 nm was measured. As a control, 1% DMSO was employed. All experiments were performed in triplicate and data were presented as means ± standard deviation. The percentage of biofilm inhibition was calculated with the equation below.

$$\text{Biofilm inhibition (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

where $A_{control}$ is the absorbance of the control (without essential oil) and A_{sample} is the absorbance in the presence of the test sample.

2.5. Statistical Analysis

The obtained data were statistically analyzed using SPSS (SPSS Inc., Chicago, IL, USA) by using a paired sample *t*-test and one-way analysis of variance (ANOVA). A significant difference was considered when $p < 0.05$.

3. Results and Discussion

3.1. Essential Oil Extraction

Following the hydro-distillation technique, the essential oils obtained from MK and CV exhibited a transparent, yellowish appearance. The CM essential oil was acquired in the form of a yellowish to reddish brown liquid, whereas the SA essential oil was a colorless liquid, as depicted in Figure 2. Furthermore, it is worth noting that all essential oils possessed distinct aromatic properties. The mean percentage yield of the essential oils was determined by calculating the percentage yield using the weight of dried plant material (100 g) as the basis. The results for MK fruit, CV bud, SA fruit and CM bark were 6.58 ± 0.81, 12.21 ± 2.98, 4.29 ± 0.15 and 1.26 ± 0.09%, respectively. The results indicated that CV exhibited the highest yield of essential oil, with MK, SA and CM following in descending order.

3.2. Analysis of Essential Oil Composition

A GC-MS analysis was conducted to determine the chemical components in each essential oil, and the results are displayed in Table 1. The phenylpropanoid compounds eugenol (83.59%) and eugenol acetate (13.78%) were found as major compounds in CV essential oil. This result corresponded with the previous reports of Alfikri et al. [36], the concentration of eugenol ranged from 70 to 80% and eugenol acetate was found at 4–15% depending on the phenological stage of the bud.

Ten compounds were found in MK essential oil, including two monoterpenes (D-limonene 17.19% and *E*-sabinene 13.27%) and eight oxygenated monoterpenes (terpinene-4-ol 47.04%,

α-terpineol 6.05%, *E*-sabinene hydrate 2.52%, *Z*-sabinene hydrate 4.17%, *E*-2-menthenol 2.50%, *Z*-2-menthenol 4.31%, *Z*-carveol 1.75% and L-carvone 1.20%). Terpinene-4-ol (47.04%) emerged as the predominant chemical compound among those that were identified. D-limonene (17.19%), *E*-sabinene (13.27%) and α-terpineol (6.05%) were found as minor constituents.

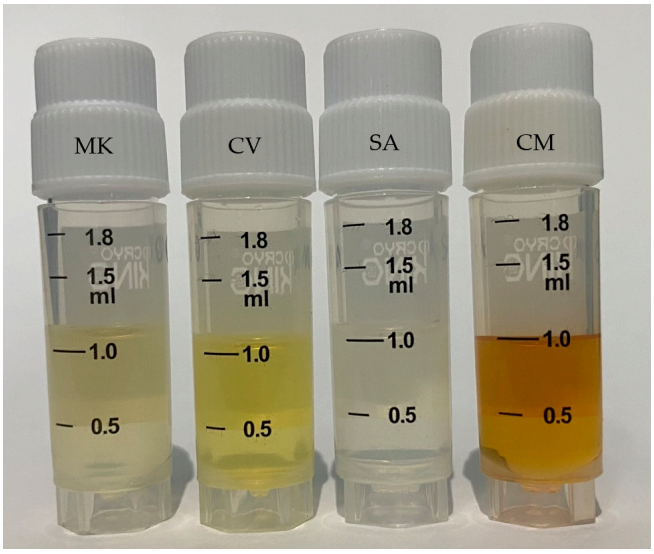

Figure 2. The physical appearance of each essential oil.

In the context of the compounds found in SA, it was observed that a significant proportion of phenylpropanoids, specifically anethol (90.58%), was detected. Additionally, minor compounds like oxygenated monoterpene (estragole, 2.00%) and monoterpene (isocarvestrene, 1.71%) were identified. These results corresponded with Matos et al. [37], who found anethole (88.85%) and estragole (5.10%) as the major component in *I. verum* fruit essential oil that was extracted by the steam distillation method.

A phenylpropanoid (*E*-cinnamaldehyde) was identified in the CM essential oil as the main constituent with 83.59%, along with a terpene compound (α-pinene 1.18%), two oxygenated monoterpene compounds (eucalyptol 3.06% and α-terpineol 1.78%) and three other phenylpropanoid compounds (cinnamyl acetate 1.17% and *Z*-cinnamaldehyde 1.00%). These findings were in agreement with those reported by Deng et al. [38] and Firmino et al. [39], who found *E*-cinnamaldehyde in the range of 72.23–82.42%. The minor components were identified as α-terpineol (1.181%), eucalyptol (3.06%) and α-terpineol (1.78%).

Table 1. Chemical composition analysis of dried MK, CV, SA and CM essential oils by GC-MS.

Compound	Molecular Formula	RI_1 [a]	RI_2 [b]	Relative Content (%) [c]			
				MK	CV	SA	CM
α-2-Pinene [d]	$C_{10}H_{16}$	938	939				1.181
Camphene [d]	$C_{10}H_{16}$	950	951				0.860
Benzaldehyde [h]	C_7H_6O	961	961				0.524
E-Sabinene [d]	$C_{10}H_{16}$	979	975	13.269 *			
β-Myrcene [d]	$C_{10}H_{16}$	991	992			0.077	
α-Phellandrene [d]	$C_{10}H_{16}$	1007	1007			0.122	
3-Carene [d]	$C_{10}H_{16}$	1012	1012			0.210	
p-Cymene [d]	$C_{10}H_{14}$	1026	1026			0.191	
Isocarvestrene [d]	$C_{10}H_{16}$	1030	1027			1.710	
Eucalyptol [f]	$C_{10}H_{18}O$	1034	1033				3.064
D-Limonene [d]	$C_{10}H_{16}$	1033	1030	17.189 *			
Sabinene hydrate [f]	$C_{10}H_{18}O$	1079	1075	2.517			

Table 1. *Cont.*

Compound	Molecular Formula	RI_1 [a]	RI_2 [b]	Relative Content (%) [c]			
				MK	CV	SA	CM
Terpinolene [d]	$C_{10}H_{16}$	1090	1093			0.119	
Linalool [f]	$C_{10}H_{18}O$	1102	1104			0.224	0.257
E-2-Menthenol [f]	$C_{10}H_{18}O$	1110	1106	4.173			
Norbornane [d]	$C_{10}H_{16}$	1132	-	4.313			
Z-2-Menthenol [f]	$C_{10}H_{18}O$	1149	1139	2.497			
Terpinen-4-ol [f]	$C_{10}H_{18}O$	1178	1177	47.036 *			
α-Terpineol [f]	$C_{10}H_{18}O$	1196	1190	6.052 *			1.781
Estragole [f]	$C_{10}H_{12}O_2$	1203	1196			2.003	
Hydrocinnamyl alcohol [h]	$C_9H_{12}O$	1234	1233				1.000
Z -Carveol [f]	$C_{10}H_{16}O$	1229	1225	1.752			
L-Carvone [f]	$C_{10}H_{14}O$	1254	-	1.200			
Chavicol [h]	$C_9H_{10}O_2$	1257	1254		0.404		
E-Cinnamaldehyde [h]	C_9H_8O	1317	1266				82.804 *
Anethol [h]	$C_{10}H_{12}O$	1284	1283			90.575 *	
Eugenol [h]	$C_{10}H_{12}O_2$	1375	1378		83.588 *		
Caryophyllene [e]	$C_{15}H_{24}$	1430	1428		1.933		
Cinnamyl acetate [h]	$C_{11}H_{12}O_2$	1456	1445				1.169
Humelene [e]	$C_{15}H_{24}$	1460	1452		0.272		
Eugenol acetate [h]	$C_{12}H_{14}O_3$	1532	1524		13.075 *	0.312	
Caryophylleneoxide [g]	$C_{15}H_{24}O$	1590	1581		0.187		
Foeniculin [h]	$C_{14}H_{18}O$	1683	1684			0.477	
Total				99.846	99.998	99.998	99.901

Note: [a] Retention index values from experiment, [b] retention indices from literature of Adams Libraries [40], [c] the percentage compositions of individual components were expressed as percentages of the peak area relative to the total peak area, [d] monoterpene, [e] sesquiterpene, [f] oxygenated monoterpenes, [g] oxygenated sesquiterpene, [h] phenylpropanoid, * major component.

3.3. Determination of Antibacterial Activity

S. moorei is a Gram-positive anaerobic bacterium that has been identified as being uniquely connected with oral malodor. This association is supported by the much greater incidence of *S. moorei* in individuals with halitosis compared to control participants [4,6,7]. Moreover, *S. mutans* is a bacterium that plays a significant role in the development of dental caries. The pathogenicity of this bacterium may be enhanced as a result of its capacity to initiate biofilm formation on the tooth surface. Subsequently, the presence of other bacteria contributes to the development of dental plaques, thus creating a conducive environment to anaerobic bacterial growth [41]. In this study, *S. moorei* and *S. mutans* were used as our test organisms to determine the antibacterial efficacy (as measured by MIC and MBC) of the four most frequently mentioned fragrant herbs in traditional Thai oral care: MK, CV, SA and CM.

The MIC and MBC values of each essential oil against *S. moorei* and *S. mutans* are shown in Table 2. The most powerful inhibition and eradiation of both bacteria were found in CM essential oil, with the MICs of 0.019% for *S. moorei* and 0.039% for *S. mutans*, while the concentration of 0.039% was found to be the MBC in both *S. moorei* and *S. mutans*. The MIC and MBC of CV essential oil against *S. moorei* compared favorably to those of CM essential oil; however, CV essential oil was less effective against *S. mutans*, with an MIC of 0.078% and MBC of 0.156%. MK essential oil demonstrated effectiveness against *S. moorei* and *S. mutans*, with higher MIC and MBC values of 0.156% than the CM and CV essential oils, which indicated its lower ability. However, MK essential oil was more effective than SA essential oil, which only had the capacity to restrict bacterial growth, with MIC values ten-times higher on both bacteria at 1.250% and no capacity to eradicate the bacterium entirely.

Table 2. The MIC and MBC values of each essential oil against *S. moorei* and *S. mutans*.

Essential Oil	*S. moorei*		*S. mutans*	
	MIC (%)	MBC (%)	MIC (%)	MBC (%)
MK	0.156	0.156	0.156	0.156
CV	0.019	0.039	0.078	0.156
SA	1.250	>5.000	1.250	>5.000
CM	0.019	0.039	0.039	0.039

These findings are consistent with prior studies that have demonstrated the superior efficacy of CM essential oil against *S. moorei* [4]. Trans-cinnamaldehyde has been identified as the primary component in the essential oil derived from CM, which is responsible for its antibacterial activity [39,42]. In a separate study conducted by Alexa et al. [42], CM essential oil that contained cinnamyl alcohol (88.45%) as a major compound and trans-cinnamaldehyde (0.39%) as a minor compound failed to exhibit antibacterial properties against *S. mutans*. Hence, it is imperative to ensure that the utilization of CM essential oil as an active element in the therapy of halitosis is accompanied by a substantial concentration of trans-cinnamaldehyde. Nevertheless, it is crucial to acknowledge that the utilization of cinnamaldehyde in cosmetic products may elicit allergic responses on human skin. Consequently, it is advisable to limit its concentration to a maximum of 0.05%, as recommended by the International Fragrance Association (IFRA) [43,44]. Moreover, a considerable number of reports have emerged regarding oral sensitivities linked to toothpaste formulations containing cinnamaldehyde at concentrations below the recommended limit set by the IFRA [43–46]. The incorporation of CM essential oil in oral hygiene products warrants cautious consideration due to this aspect. Furthermore, the essential oil obtained from CV showed similar effectiveness against *S. moorei*, as observed with CM essential oil, which is reported for the first time in this investigation. The antibacterial activity of CV essential oil is attributed to eugenol, its primary constituent. It is hypothesized that these qualities are associated with the rupture of the bacterial cellular membrane [47]. Moreover, it possesses an added advantage as an analgesic drug owing to its capacity to reduce dental pain [48]. The application of these substances has the ability to induce a thermogenic response upon contact with the tongue, mediated by the transient receptor potential channel (TRPV3), which is a calcium-permeable cation channel sensitive to warmth [49]. Nevertheless, the efficacy of this phenomenon might be constrained among certain individuals who exhibit intolerance to high temperatures, particularly children, older adults or individuals with oral conditions, including mouth ulcers and stomatitis. The efficacy of MK essential oil against the bacterial strains *S. moorei* and *S. mutans* was demonstrated by assessing the MIC and MBC values, which were determined to be 0.156%. The essential oil derived from MK exhibited a comparatively lower capacity to inhibit and eradicate the growth of the two bacterial strains in comparison to the essential oils obtained from CM and CV. The main active ingredients found in MK essential oil, including sabinene, terpinene-4-ol and limonene, contribute to the refreshing aroma and more enjoyable taste for a wide range of oral care product consumers than CM essential oil, providing a woody scent [18]. The essential oil derived from MK showed greater efficacy compared to the essential oil derived from SA, since it possessed the capacity to restrict bacterial growth without achieving the complete eradication of the organism. The main constituent responsible for the antibacterial activity of SA essential oil has been discovered as anethole. Moreover, the combination of anethole with mupirocin has been found to have enhanced efficacy against methicillin-resistant *Staphylococcus aureus* (MRSA) strains that are resistant to mupirocin. The observed improvement is thought to be caused by the interaction between anethole and the lipids present in the bacterial cell wall. This contact leads to an increase in the permeability of hydrophilic antibiotics [50]. Therefore, an opportunity for additional inquiry lies in exploring the synergistic effect of SA essential oil with another essential oil.

However, it is important to mention that thyme, eucalyptus and peppermint essential oils are frequently utilized as active components in mouth rinse solutions in the commercial sector. The primary factor driving the selection of these essential oils is their odor, despite their comparatively weaker antibacterial efficacy. The essential oils that are most preferred among individuals are those that possess invigorating and refreshing scents [18,51–53]. Therefore, the essential oils of MK and CV exhibit promising potential as natural alternatives for incorporation as active ingredients in oral care products, namely for the purpose of enhancing their odor-masking capabilities in addition to managing bacterial halitosis.

3.4. Determination of Antibiofilm Qualities

S. moorei is recognized as the predominant bacterial species responsible for the development of halitosis via the conversion of sulfur-containing amino acids into VSCs. Additionally, this bacterium possesses the capability to create biofilms within the oral cavity. The establishment of these biofilms is hypothesized to represent the primary and pivotal phase in the ecological progression within the oral cavity, ultimately resulting in the initiation of halitosis [5]. In a study conducted by Conceição et al. [54], the efficacy of a mouthwash containing antibiofilm components was assessed in terms of reducing tongue coating. The results indicated that the antibiofilm agent effectively decreased oral malodor by inhibiting bacterial colonization through the disruption of their colonization tools.

This study aimed to explore the antibiofilm efficacy of four aromatic herbal essential oils. The effects of individual essential oils on the development of biofilms by *S. moorei* at MIC and sub-MIC levels (1/2, 1/4, 1/8) are summarized in Table 3 and illustrated in Figure 3. The results of the study indicated that all essential oils exhibited a dose-dependent capacity to decrease the production of biofilms by *S. moorei*. At the MIC level, the essential oils showed considerable inhibition of biofilm formation. The inhibition percentages were determined to be 90.50 ± 3.65% for CM, 86.78 ± 5.75% for CV, 75.55 ± 2.78% for MK and 82.28 ± 8.28% for SA. These values did not exhibit statistically significant differences. The results of this study indicate that the essential oils of MK, CV and SA have the potential to serve as alternative antibiofilm agents to CM essential oil, which demonstrated strong antibiofilm activity at the MIC level. When we diluted the concentration to half of the MIC, all essential oils showed a noteworthy capacity to decrease biofilm development in comparison to the control group. When comparing CM (70.29 ± 4.85%) to CV, there was no statistically significant difference in terms of biofilm inhibition, as CV exhibited biofilm inhibition of 64.45 ± 7.73%. However, MK and SA essential oils showed a lesser ability to prevent biofilm formation compared to CM and CV essential oils, with values of 22.87 ± 3.23% and 12.82 ± 1.88%, respectively.

Table 3. The inhibition effects of essential oils against *S. moorei* biofilm formation.

Concentration	% Biofilm Formation Inhibition			
	MK	CV	SA	CM
1/8 of MIC	4.774 ± 3.45% ^{bA}	5.44 ± 3.50% ^{bA}	5.29 ± 5.10% ^{bA}	13.15 ± 10.60% ^{bA}
1/4 of MIC	15.15 ± 1.17% ^{aB}	27.52 ± 10.90% ^{aA}	6.28 ± 3.67% ^{bC}	34.12 ± 6.51% ^{aA}
1/2 of MIC	22.878 ± 3.23% ^{aB}	64.45 ± 7.73% ^{aA}	12.82 ± 1.88% ^{aC}	70.29 ± 4.85% ^{aA}
MIC	75.55 ± 2.78% ^{aA}	86.78 ± 5.75% ^{aA}	82.28 ± 8.28% ^{aA}	90.50 ± 3.65% ^{aA}
Control	0.00 ± 8.73% ^{b}			

The different lowercase letters indicate a significant difference between sample and control, and different capital letters indicate a significant difference among the samples at the same diluted MIC concentration at $p < 0.05$.

At a quarter of the MIC level, MK, CV and CM exhibited film reduction activity in comparison to the control group. Only SA essential oil was determined to be inactive. Furthermore, the essential oil derived from CV exhibited a biofilm inhibition rate of 34.12 ± 6.51%, which was not significantly different from the biofilm inhibition rate of CM essential oil (34.12 ± 6.51%). On the other hand, MK essential oil demonstrated a lower biofilm inhibition capacity, with a rate of 15.15 ± 1.17%. Nevertheless, the capacity

of all critical components to inhibit biofilm formation was compromised when diluted to one eighth of their MICs. The present investigation elucidated the potential of CV, MK and SA essential oils in mitigating biofilm development by *S. moorei*. The aforementioned discoveries enhance our understanding of the antibacterial characteristics of essential oils and their prospective utilization in oral hygiene products.

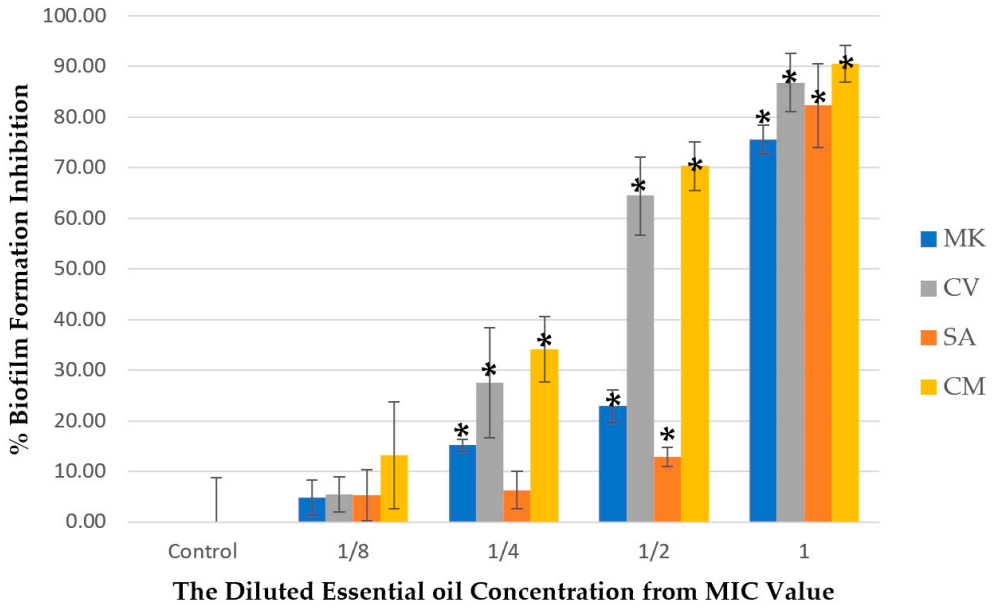

Figure 3. The effect of essential oils on *S. moorei* biofilm formation, *: significantly different from control at $p < 0.05$.

4. Conclusions

The yields of MK, CV and SA essential oils were determined to be 6.58 ± 0.81, 12.21 ± 2.98 and 4.29 ± 0.15%, respectively. These values were found to be greater compared to CM (1.26 ± 0.09%), suggesting stronger potential for utilization in commercial products in terms of yield. The primary constituents identified in MK essential oil were limonene (17.19%), sabinene (13.27%) and terinene-4-ol (47.04%), while CV, SA and CM essential oils were found to include eugenol (83.59%), anethol (90.58%) and trans-cinnamaldehyde (82.80%), respectively. The antibacterial activity of CV essential oil was determined to be similar to that of CM essential oil in terms of its ability to limit the growth of and eliminate *S. moorei*, while the essential oils MK and SA had lower effectiveness. The ability to suppress biofilm development was observed in all essential oils, and this suppression was found to be dependent on the dosage administered. The researchers observed that the smallest concentration of the essential oils needed to prevent the formation of *S. moorei* biofilms was one quarter of the MIC for MK, CM and CV and half of the MIC for SA. The findings of our research have confirmed the significant potential of CV, along with the MK and SA essential oils, as active components to enhance the antibacterial and antibiofilm abilities of dental care products. To the best of our knowledge, this study represents the first exploration that compares the antibacterial and antibiofilm properties of CV, MK and SA in relation to CM essential oil. Clinical trials should be conducted in the future to assess the halitosis control effectiveness of the essential oils in oral care formulations. Additionally, it is necessary to assess the acceptance of these formulations among subjects based on taste and odor in order to validate the findings and provide evidence for the effectiveness of essential oils in managing halitosis.

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