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Chemical composition of essential oil from *Hyptis suaveolens* leaves and antibacterial activity against common skin infections

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ABSTRACT: The most common skin diseases, eczema and acne, require long-term treatment, often leading to the emergence of antibiotic-resistant bacteria. This study aimed to investigate the composition of essential oil from the leaves of *Hyptis suaveolens* (HSEO) from two different locations, Nakhon Ratchasima (NR) and Phitsanulok (PL), and to evaluate their antibacterial activities. HSEO was obtained by steam distillation from fresh leaves, and its chemical composition was determined by gas chromatography coupled with mass spectrometry (GC-MS). Antibacterial activity was assessed using the agar disc diffusion and broth microdilution methods against the common causative agents of eczema and acne. Thirty-eight and thirty-six components were identified in HSEO-NR and HSEO-PL, respectively. The three major components were 1,8-cineole (18.82%, 8.89%), β-caryophyllene (12.95%, 12.81%), and sabinene (11.72%, 18.49%). Antibacterial activity screening using the agar disc diffusion method demonstrated that both HSEOs exhibited antibacterial activity against all tested bacteria, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Escherichia coli*. The minimum inhibitory concentration (MIC) of HSEO-NR ranged from 46.72 to 109.00 mg/ml, while the MIC values for HSEO-PL ranged from 93.45 to 140.18 mg/ml. The minimum bactericidal concentration (MBC) for HSEO-NR ranged from 93.45 to >140.18 mg/ml, and the MBC values for HSEO-PL ranged from 109.00 to >140.18 mg/ml. The antibacterial activity of the HSEO was attributed to 1,8-cineole and other components, but not to β-caryophyllene. Both HSEOs demonstrated potential use for the treatment of eczema and acne.

KEYWORDS: *Hyptis suaveolens*, essential oils, antibacterial, *Staphylococcus aureus*, 1,8-cineole

INTRODUCTION

Thailand is located 15 degrees north of the equator and is characterised by high humidity as well as long periods of warm weather. This climate can lead to various skin problems such as dermatitis, acne, and dermatophytosis, all of which can negatively impact people's quality of life. According to data collected by the Department of Medical Services, Ministry of Public Health, Thailand, on the occurrence of skin diseases from 2023, eczema and acne were ranked as the first and second most common skin diseases affecting the Thai population [1]. Eczema is one of the most common skin diseases, often classified as a psychosomatic condition caused by various factors. It is characterised by symptoms such as redness, swelling, vesicles, and itchy skin. Pathogens associated with eczema include Gram-positive bacteria like *S. aureus* and *S. epidermidis*, as well as Gram-negative bacteria such as *E. coli*. Antibiotics are widely used in their treatment [2]. Acne vulgaris, another prevalent skin condition, affects an estimated 21% to 87% of the population, with approximately 80% of teenagers experiencing it. Treatment typically involves antibiotics such as tetracycline and clindamycin, along with other substances like retinoids, benzoyl peroxide, and azelaic acid, and usually lasts 8 to 12 weeks [3].

A study conducted in a Japanese hospital reported that acne inflammation is often attributed to *S. epidermidis*, a normal skin inhabitant, and *Propionibacterium acnes*, which is frequently isolated from acne lesions. The authors concluded that their findings supported a link between the use of antimicrobial agents and the emergence of clindamycin resistance [4]. The emergence of multidrug-resistant bacteria in dermatology, along with recurrent therapeutic failures, exacerbates the challenges associated with these infections. Beyond physical health issues, skin infections can have a significant psychosocial impact due to their unsightly appearance [5]. An alternative treatment may provide another effective approach and reduce the occurrence of drug resistance. Essential oils (EOs) are considered appropriate alternatives to traditional antimicrobials because they are mixtures of compounds that target multiple sites, making them less susceptible to microbial resistance. A review of 98 EOs recommended for dermatological use showed that 73 EOs are used to treat bacterial infections, while 34 EOs are for fungal infections, and 16 EOs are for viral infections [5].

Hyptis suaveolens (L.) Poit. (Lamiaceae), commonly known as pignut or "Maeng Lak Kha" in Thai, is an erect, strongly aromatic, branched annual or short-lived perennial herb that grows between 30 and 150 centimetres tall [6]. It is now recognised as

Mesosphaerum suaveolens (L.) Kuntze [7, 8]. This fast-growing herb forms dense clumps along roadsides and emits a characteristic minty smell when crushed. It is naturalised as a weed in open areas and is found in many parts of Thailand [9]. The plant is considered a weed in several countries and continents, including Central America, South America, Africa, Australia, and Asia. Traditionally, *H. suaveolens* has been used to treat respiratory tract infections, pain, fever, colds, cramps, and skin diseases, as well as rheumatism, dermatitis, and eczema. The leaves containing essential oil were mostly studied for their antibacterial activities, with 92.60% (25/27) of research work devoted to the leaves [10]. In Indian ethnomedicine, they have been used to treat skin diseases, boils, cuts, and wounds, among other ailments [11]. The leaves of this plant are well-known among Northern Thais for their anti-itch properties [12].

Various factors influence the composition of EOs, such as temperature, altitude, soil conditions, and the terrain of the country in which the plant is cultivated. The biological activities of EOs are primarily determined by their main components, though occasionally, a group of molecules can significantly modify their effects [10]. A review of the ethnopharmacology and chemical composition of *H. suaveolens* EOs from 16 countries revealed that sabinene, β-caryophyllene, and 1,8-cineole are the three major components [11]. Sabinene was found to be the most abundant in Central America, South America, and several African countries, while β-caryophyllene was predominant in African countries (Tanzania, Nigeria, Benin), and 1,8-cineole was most abundant in India, Australia, and Brazil [11]. A previous study in Thailand also reported sabinene as the major component (25.4%), along with β-caryophyllene (11.69%) or sabinene (21.69%) with 1,8-cineole (12.56%) [10]. The three major components, including sabinene, β-caryophyllene, and 1,8-cineole, were present in the leaves, flowers [13], and fruits, but not in the stem [14]; the volatile oil in the root was not studied. However, from a practical perspective, harvesting leaves is a more sustainable approach compared to other parts of the plant, as it allows the plant to continue growing without significant disruption to its lifecycle. Moreover, the leaves are more readily available year-round compared to the seasonal production of flowers and fruits, further supporting their selection for medicinal and industrial applications.

The essential oil of *H. suaveolens*, with 44.4% of 1,8-cineole, has been reported to exhibit antimicrobial activity against *Candida albicans*, *Aspergillus niger*, *S. aureus*, and *E. coli* with inhibition zones ranging from 25.00–31.00 mm [15]. Another study also reported the same antibacterial activity against these microorganisms at 5 mg/ml [16]. Although some studies have examined the EOs of *H. suaveolens*, no studies have yet compared the antibacterial activity and chemical composition of EOs from this plant in different locations in Thailand. Therefore, this study aimed to evaluate the composition of EOs from the leaves of *H. suaveolens* (HSEO) collected from two locations, including Nakhon Ratchasima (NR) in northeastern Thailand and Phitsanulok (PL) in northern Thailand, and to perform antibacterial testing of the EOs against the common causative agents of eczema and acne, including *S. aureus*, *S. epidermidis*, and *E. coli*.

MATERIALS AND METHODS

Plant collection and identification

The leaf samples were collected from NR Province and PL Province at 9.00 am. The recorded temperature between 8.00–10.00 am at the collection site in NR was 24.9–30.5 °C, with 80% relative humidity in September 2023. The collection site in PL showed a temperature range of 24.5–32.1 °C between 8.00–10.00 am, with 80% relative humidity in October 2023. The plant specimens were identified by the Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation, Bangkok, Thailand, with the following numbers: NR specimen BKF No. 194298, and PL specimen BKF No. 194298.

Extraction of essential oil

Two kilograms of fresh leaves of *H. suaveolens* from each location were reduced in size and subjected to on-site steam distillation in a Clevenger apparatus (JSD Machinery Ltd. Part. Bangkok, Thailand) with 10 l of distilled water for 3 h. The EO was dried over anhydrous sodium sulphate and stored in an amber bottle at 4 °C until use. The EO percentage yield (v/w) was an average from the steam distillation process ($n = 3$).

Determination of chemical composition by gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analysis was performed according to the method in previous research [9] with modifications for the Agilent 7890A GC system and Agilent 7000D mass spectrometre system (Agilent Technologies). The column used was an HP-5 MS capillary column (30 m × 0.25 mm, 0.25 µm). The carrier gas was helium, with a flow rate of 1.2 ml/min. The injector and detector temperatures were set at 230 °C. The HSEO sample (1.0 µl) was injected in the split mode at 20:1, and the oven temperature was maintained at 50 °C for 1 min, which was then increased linearly to 230 °C at a rate of 4 °C/min, then held at 230 °C for 6 min. The MS conditions were as follows: ionisation energy, 70 eV, and mass range of m/z 40–400 amu. Identification of chemical components was

based on analysis of the chromatograms obtained for each EO, through evaluating the retention indices (RI) in comparison with the standards of alkane mixtures (C_8-C_{20}), and the mass spectral data for each peak using the computer library (Wiley-14 and NIST-17 Mass Spectral Libraries) and comparison with literature references [17].

Antibacterial activity

Microorganisms

The bacterial strains were clinical strains obtained from the Culture Collection for Medical Microorganisms, Department of Medical Sciences, Thailand. Microorganisms included two Gram-positive strains: *S. aureus* DMST 8840 and *S. epidermidis* DMST 15505, and one Gram-negative strain: *E. coli* DMST 4212. The selected strains were cultured on Mueller Hinton Agar (MHA, HiMedia Laboratories, Maharashtra, India) medium at 37 °C for 18 h. Subsequently, a single colony of bacteria was inoculated into a Mueller Hinton Broth (MHB, HiMedia Laboratories) at 37 °C with 180 rpm for 4 h. After that, the culture broth was adjusted to the 0.5 McFarland standard turbidity, corresponding to an approximate concentration of 1.5×10^8 CFU/ml. McFarland Standards are used to standardise the approximate number of bacteria in a liquid suspension by comparing the turbidity of the test suspension with that of the McFarland Standard (fine precipitate of barium sulphate). The density of McFarland Standards can be checked using spectrophotometry; a 0.5 McFarland Standard has an absorbance reading of 0.08 to 0.1 at 625 nm, corresponding to an approximate concentration of 1.5×10^8 CFU/ml.

Determination of antibacterial activity by the agar disc diffusion method

This procedure was performed according to the method by the Clinical and Laboratory Standards Institute [18]. Petri plates (90.0 mm) were prepared by pouring 25 ml MHA for *S. aureus*, *S. epidermidis*, and *E. coli*, allowing the medium to solidify. The plates were then dried and uniformly spread. Paper discs (6.0 mm diameter) were impregnated with 20 µl of each test sample, including HSEO-NR, HSEO-PL, the major compounds 1,8-cineole and β-caryophyllene (Sigma-Aldrich, St. Louis, Missouri, USA). Ampicillin (10 µg/disc, Oxoid Ltd., Basingstoke, UK) was used as the positive control. Each test paper disc was placed on the test medium plate using forceps, with slight pressure applied to ensure firm contact between the disc and the medium. After incubation at 37 °C for 24 h, zones of inhibition (mm) were measured by an electronic Vernier caliper. Each sample was tested three times on separate occasions, and mean values were calculated.

Determination of antibacterial activity by the broth microdilution method

The antibacterial activity of EO was determined by MIC and MBC using the broth microdilution method [19]. HSEOs were dissolved in a mixture of dimethyl sulfoxide (DMSO) and MHB at a concentration of 700 mg/ml to prepare a stock solution. Briefly, 96-well microplates were filled with 50 µl of MHB, while 50 µl of sample oil was diluted two-fold with a final concentration range of 350 to 1.36 mg/ml, and 50 µl of the bacterial suspension with 0.5 McFarland standards. Then, plates were incubated at 37 °C for 24 h. The MIC was determined by observing changes in opacity or colour. The MIC was the lowest concentration of an antibacterial that inhibited visible growth (absence of turbidity). Ampicillin was used as a positive antibacterial control. DMSO was used as a solvent control. MBC was determined by subculturing 10 µl from each well showing no visible bacteria growth on MHA plates and incubating at 35 °C for 24 h. The concentration at which there was no visible bacterial growth after 24 h incubation was regarded as the MBC.

Determination of MBC/MIC ratio

The ratio of MBC/MIC was calculated to determine the efficacy and bactericidal/bacteriostatic effect of EO on the bacterial growth of strains tested. If the MBC/MIC is $\leqslant 4$, then the effect is bactericidal, while the effect is bacteriostatic if the MBC/MIC is >4 [20].

Statistical analysis

All data were reported as mean ± standard deviation of the mean from 3 determinations. The one-way ANOVA followed by Tukey's test was used, and the significance between the test samples was at a 95% confidence level.

RESULTS AND DISCUSSION

Previous studies revealed variations in EO yields and compositions of *H. suaveolens* from different countries or locations within the same country [10, 11, 21]. These variations were attributed to intrinsic and extrinsic factors such as climate, soil, and water [8]. Hin Dat Sub-district, Dan Khun Thot District, NR Province is located at coordinates 15°07′41.6″ North and 101°33′47.8″ East. This area is a plateau at an altitude of 214 m above sea level. The physical and geological features are mostly small hills. The soil is mostly sedimentary rocks, with the lower layer being red sandstone. Wang Thong District, PL Province, has GPS coordinates 16°50′35.9″ North and 100°32′23.7″ East. This area has a topography of plateaus and mountains at an altitude of 49 m above sea level. Most of the area is forest and mountains, representing a distinct environment. The method of sample preparation may also contribute to these differences. For example,

a study in India found that drying leaf samples for 10 days in the shade led to a loss of monoterpenoid content, while sesquiterpenoid and diterpene compounds increased [8]. Another study showed that storing oil at 45 °C for six months resulted in reduced monoterpenes, including sabinene, 1,8-cineole, and α-terpinolene, while β-caryophyllene content increased by 105.56% [22].

Essential oil yield from fresh *H. suaveolens* leaves

In this study, fresh leaves were collected from two locations, comprising NR and PL, provinces in northeastern and northern Thailand, respectively. Fresh *H. suaveolens* EO (HSEO) obtained through on-site steam distillation yielded 0.078 ± 0.002% and 0.080 ± 0.003% (v/w) for NR and PL samples, respectively, with a density of 0.89, similar to the 0.88 density reported in a previous study [23]. Another study using hydrodistillation provided only a 0.03% (v/w) yield [21]. Hydrodistillation of sun-dried samples over three days resulted in lower yields compared to fresh samples (0.0004% vs. 0.0057% v/w) [9]. This could be due to long exposure to sunlight, which could be higher than 45 °C, and distillation was not carried out on-site. The temperature difference experienced by the sample in hydrodistillation would be at the boiling point of water (100 °C), while the temperature would be less than 100 °C in steam distillation, and the sample was not immersed in water during distillation of the volatile oil. A study on the effect of distillation temperature on EO components such as sabinene, α-pinene, and β-pinene revealed that the contents of these compounds were higher at 80, 90, and 95 °C, respectively, than at 100 °C. Notably, the content of sabinene was twice as much at 80 °C compared to 100 °C [24]. These results confirm that the on-site steam distillation of fresh leaf samples is the most effective method for obtaining HSEO.

Chemical composition of EOs from *H. suaveolens* leaves

The chemical composition of HSEO was determined by GC-MS analysis (Table 1). A total of 39 compounds representing 99.71% of HSEO-NR and 36 compounds representing 99.67% of HSEO-PL were identified. The highest content was hydrocarbon monoterpenes (NR 51.77%, PL 54.45%), followed by hydrocarbon sesquiterpenes (NR 33.37%, PL 29.54%). Both oils had similar diterpene content (NR 8.08%, PL 8.26%), and oxygenated compounds were less than 4% in both samples.

Both oils shared three major components: sabinene, 1,8-cineole, and β-caryophyllene. However, there was a major difference in 1,8-cineole content: 18.82% in HSEO-NR compared to 8.89% in HSEO-PL, which could be due to the difference in the altitudes of the collection sites, at 214 m (NR) vs. 49 m (PL) above sea level. A previous study on leaf flavonoids in Chinese sea-buckthorn revealed that quercetin and isorhamnetin increased with rising altitude [25]. On the other hand, sabinene content was higher in HSEO-PL than in HSEO-NR (18.49% vs. 11.72%). The content of β-caryophyllene was nearly identical in both oils (NR 12.95%, PL 12.81%). Minor components of HSEO-NR included α-terpinolene (6.17%), β-pinene (6.14%), trans-α-bergamotene (5.39%), and 8,12-abietadiene (4.95%). The minor components of HSEO-PL included α-terpinolene (6.87%), β-pinene (6.61%), 8,12-abietadiene (4.95%), and trans-α-bergamotene (4.40%). Caryophyllene oxide was detected in both oils (NR 0.98%, PL 1.05%). Furthermore, differences in minor compounds were identified: fenchone, α-gurjunene, and γ-cadinene were absent in HSEO-PL, while spathulenol was absent in HSEO-NR.

Antibacterial activity

Agar disc diffusion method

HSEOs and their major components, 1,8-cineole and β-caryophyllene, were evaluated for antibacterial activity against clinical strains of *S. aureus* DMST 8840, *S. epidermidis* DMST 15505, and *E. coli* DMST 4212, using ampicillin (10 µg per disc) as the positive control in disc diffusion tests ($n = 3$) (Table 2). Categories of bacterial growth response based on inhibition zone diameter (IZ) were considered as follows: weak IZ $\leqslant$ 5 mm; moderate 6 $\leqslant$ IZ $\leqslant$ 10 mm; strong 11 $\leqslant$ IZ $\leqslant$ 20 mm; and very strong IZ $\geqslant$ 21 mm [26]. Both HSEOs demonstrated inhibitory activity against all tested bacterial strains, with IZ ranging from 10.17 to 14.83 mm. HSEO-NR and HSEO-PL showed strong activity against *S. aureus* and *S. epidermidis*, with IZ of 14.83 ± 1.04 mm and 13.42 ± 0.38 mm, respectively, for HSEO-NR and 13.65 ± 1.44 mm and 12.33 ± 1.89 mm, respectively, for HSEO-PL. Both oils exhibited moderate activity against *E. coli*, with IZ of 10.17 ± 0.28 and 10.67 ± 0.94 mm, respectively. Among test samples, 1,8-cineole demonstrated the strongest activity against *E. coli* (IZ of 15.25 ± 2.59 mm) but weaker activity against *S. aureus* and *S. epidermidis* (10.83 ± 0.28 mm and 10.50 ± 1.50 mm, respectively). In contrast, β-caryophyllene showed the weakest antibacterial activity against *S. epidermidis* (IZ 7.33 ± 0.14 mm) and no activity against *S. aureus* or *E. coli*. Ampicillin exhibited very strong activity against *S. aureus* and *S. epidermidis*, with IZ of 25.33 ± 1.55 mm and 22.67 ± 0.78 mm, respectively, but showed strong activity against *E. coli* (IZ of 11.50 ± 0.50 mm). Statistical analysis revealed no significant difference between HSEO-NR and HSEO-PL against all tested bacterial strains, but HSEO-NR showed slightly better activity than HSEO-PL. However, both oils exhibited significantly better activity than 1,8-cineole against *S. aureus* and *S. epidermidis*, but 1,8-cineole demonstrated

Table 1 Chemical composition of the essential oils from leaves of *H. suaveolens*.

Sample no.	Compound[a]	RI[b]	RI[c]	Relative area[d] (%)		Identification[e]
				NR	PL	
1	α-Thujene	932	932	0.46	0.80	RI, MS
2	α-Pinene	936	939	2.80	3.49	RI, MS
3	Sabinene	973	980	11.72	18.49	RI, MS
4	β-Pinene	978	983	6.14	6.61	RI, MS
5	Myrcene	987	991	0.43	0.29	RI, MS
6	α-Phellandrene	1002	1007	0.36	0.54	RI, MS
7	δ-3-Carene	1012	1012	0.24	0.26	RI, MS
8	α-Terpinene	1013	1022	1.00	1.82	RI, MS
9	p-Cymene	1015	1028	0.63	0.79	RI, MS
10	Limonene	1025	1034	1.37	2.73	RI, MS
11	1,8-Cineole	1024	1038	18.82	8.89	RI, MS
12	γ-Terpinene	1049	1065	1.63	2.87	RI, MS
13	α-Terpinolene	1082	1095	6.17	6.87	RI, MS
14	Fenchone	1086	1102	0.31	ND	RI, MS
15	Terpinen-4-ol	1164	1186	2.73	3.91	RI, MS
16	α-Cubebene	1337	1359	0.57	0.39	RI, MS
17	α-Copaene	1379	1386	3.28	2.30	RI, MS
18	β-Bourbonene	1386	1395	0.76	0.54	RI, MS
19	α -Gurjunene	1398	1398	0.28	ND	RI, MS
20	β -Elemene	1389	1402	0.60	0.93	RI, MS
21	β-Caryophyllene	1421	1432	12.95	12.81	RI, MS
22	trans-α-Bergamotene	1434	1445	5.39	4.40	RI, MS
23	α-Humulene	1459	1466	1.87	1.66	RI, MS
24	allo-Aromadendrene	1460	1470	0.48	0.36	RI, MS
25	Germacrene D	1477	1493	3.26	2.14	RI, MS
26	β-Selinene	1483	1499	0.99	0.82	RI, MS
27	Bicyclogermacrene	1498	1508	1.24	2.20	RI, MS
28	α-Bulnesene	1517	1517	0.53	0.32	RI, MS
29	γ-Cadinene	1518	1524	0.38	ND	RI, MS
30	δ Cadinene	1519	1537	0.79	0.67	RI, MS
31	Spathulenol	1571	1588	ND	0.38	RI, MS
32	Caryophyllene oxide	1583	1597	0.98	1.05	RI, MS
33	Selin-11-en-4-α-ol	1659	1670	1.01	1.00	RI, MS
34	(Z)-trans- α-Bergamotol	1690	1706	1.46	1.07	RI, MS
35	Phyllocladene	2004	2022	0.22	0.27	RI, MS
36	8,12-Abietadiene	2022	2037	4.95	5.40	RI, MS
37	Abitatriene	2056	2085	1.69	1.31	RI, MS
38	Kaur-16-ene	2061	2096	0.42	0.45	RI, MS
39	Abitadiene	2087	2130	0.80	0.83	RI, MS
	Hydrocarbon monoterpenes			51.77	54.45	
	Oxygenated monoterpenes			3.04	3.91	
	Hydrocarbon sesquiterpenes			32.13	27.34	
	Oxygenated sesquiterpenes			4.69	5.70	
	Diterpene			8.08	8.26	
	Total identified			99.71	99.66	

ND: Not detected; NR: Nakhon Ratchasima; PL: Phitsanulok.
[a] Compounds are listed in order of elution on HP-5 MS column.
[b] RI experimentally calculated against straight-chain n-alkane series (C_8-C_{20}).
[c] RI obtained from the literature series (C_8-C_{20}).
[d] The percentage of relative peak area is the average value of three measurements.
[e] Identification: RI = tentative identification based on linear retention indices; MS = tentative identification based on MS.

Table 2 Inhibition zone diameter of *H. suaveolens* essential oils and their major components.

Test sample	Diameter of inhibition zone (mm)[1]		
	S. aureus (DMST 8840)	*S. epidermidis* (DMST 15505)	*E. coli* (DMST4212)
HSEO-NR	14.83 ± 1.04^{a}	13.42 ± 0.38^{a}	10.17 ± 0.28^{a}
HSEO-PL	13.65 ± 1.44^{a}	12.33 ± 1.89^{a}	10.67 ± 0.94^{a}
1,8-Cineole	10.83 ± 0.28^{b}	10.50 ± 1.50^{b}	15.25 ± 2.59^{b}
β-Caryophyllene	NA	7.33 ± 0.14^{c}	NA
Ampicillin	25.33 ± 1.15^{c}	22.67 ± 0.76^{d}	11.50 ± 0.50^{a}

[1] Disc diameter (6 mm) included. Data are mean ± standard deviation ($n = 3$). Mean values followed by the same lower-case letters indicate no statistically significant difference. Different lower-case letters in column indicate statistically significant differences by the Tukey's test. NA: not active; HSEO-NR: *H. suaveolens* EO from Nakhon Ratchasima; HSEO-PL: *H. suaveolens* EO from Phitsanulok.

Table 3 Antibacterial activities of HSEOs and their major components.

Test sample	Concentration (mg/ml)								
	S. aureus (DMST 8840)			*S. epidermidis* (DMST 15505)			*E. coli* (DMST 4212)		
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
HSEO-NR	46.72	93.45	2.00	109.00	124.60	1.14	70.09	>140.18	−
HSEO-PL	93.45	109.00	1.16	109.00	124.60	1.14	140.18	>140.18	−
1,8-Cineole	45.54	91.17	2.00	80.60	184.20	2.28	54.51	>109.02	−
β-Caryophyllene	135.15	157.67	1.16	157.67	180.20	1.14	140.18	>140.18	−
Ampicillin	>2.5	>2.5	−	>2.5	>2.5	−	0.004	0.20	>4

HSEO-NR: *H. suaveolens* EO from Nakhon Ratchasima; HSEO-PL: *H. suaveolens* EO from Phitsanulok; MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; −: not calculated.

significantly stronger activity than ampicillin against *E. coli*. These results suggest that HSEOs possess stronger antibacterial properties against Gram-positive bacteria than Gram-negative bacteria, with HSEO-NR displaying slightly stronger antibacterial activity than HSEO-PL.

Determination of MIC and MBC of *H. suaveolens* EOs *and their major components*

The MIC and MBC of HSEO-NR, HSEO-PL, and their major components were evaluated using the broth microdilution method to determine their bacteriostatic and bactericidal properties (Table 3). HSEO-NR showed MIC values of 46.72 mg/ml, 109.00 mg/ml, and 70.09 mg/ml against *S. aureus*, *S. epidermidis*, and *E. coli*, respectively, and displayed bactericidal activity against *S. aureus* and *S. epidermidis* with MBC values of 93.45 mg/ml and 124.60 mg/ml, respectively. The MBC against *E. coli* was greater than 140.18 mg/ml. HSEO-PL demonstrated MIC values of 93.45 mg/ml, 109.00 mg/ml, and 140.18 mg/ml against *S. aureus*, *S. epidermidis*, and *E. coli*, respectively, with bactericidal activity against *S. aureus* and *S. epidermidis* (MBC values of 109 mg/ml and 124.60 mg/ml, respectively). The MBC against *E. coli* was greater than 140.18 mg/ml. MIC values of 45.54 mg/ml, 80.60 mg/ml, and 54.51 mg/ml were shown by 1,8-cineole against *S. aureus*, *S. epidermidis*, and *E. coli*, respectively, while the MBC values were 91.17 mg/ml, 184.20 mg/ml, and >109.02 mg/ml, respectively. In contrast, β-caryophyllene had MIC values of 135.15 mg/ml, 157.67 mg/ml, and 140.18 mg/ml against *S. aureus*, *S. epidermidis*, and *E. coli*, respectively, with corresponding MBC values of 157.67 mg/ml, 180.20 mg/ml, and >140.18 mg/ml. The higher activity of HSEO-NR was primarily attributed to its higher 1,8-cineole content. Another study reported the antibacterial activity of HSEO containing 44.4% 1,8-cineole against Gram-negative bacteria, including *E. coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi* [15]. Potent antimicrobial and antiviral activities were also displayed by 1,8-cineole, which may aid in treating skin infections [27]. From the above results, *S. aureus* was more sensitive to HSEO-NR than *E. coli* (MICs of 46.72 vs. 70.09 mg/ml). The same results were observed with HSEO-PL (MICs 93.45 vs. 140.18 mg/ml), while *S. epidermidis* was equally sensitive to both HSEO-NR and HSEO-PL (MICs of 109.00 mg/ml). The classification of antibacterial activity based on the MBC/MIC ratio [20] showed that both oils, 1,8-cineole and β-caryophyllene, exhibited bactericidal effects. Additionally, sabinene, another major constituent, demonstrated antimicrobial activity against *S. aureus* with a MIC value of 0.4 mg/ml [28]. Other than the three major compounds sabinene, 1,8-cineole, and β-caryophyllene, another minor component, caryophyllene oxide, has also shown antibacterial properties stronger than β-caryophyllene [29].

Other components over 2% have also been reported to have antibacterial activity, including α-pinene, β-pinene [30], terpinen-4-ol [31], α-copaene [32], and germacrene D [33]. Moreover, sabinene has been found to reduce pro-inflammatory cytokines, such as IL-1β, IL-6, IL-27, and IL-1ra, thereby preventing lipopolysaccharide-induced macrophage activation [34]. A recent study also showed that β-caryophyllene and HSEO could inhibit LPS-induced inflammation in RAW 264.7 macrophages by reducing the production of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α [8]. Therefore, the incorporation of HSEO in dermatological products would provide a positive outcome. A study on the development of antimicrobial skin ointment from an herbal extract has been reported previously [35].

CONCLUSION

This study presents a comparative evaluation of the chemical composition of HSEOs from two different locations in Thailand and their antibacterial activities against bacteria that cause eczema and acne. Fresh leaves should be used for EO extraction via on-site steam distillation. The oils contain two major monoterpenes, 1,8-cineole, and sabinene, along with the sesquiterpene β-caryophyllene. In this study, HSEO from NR Province exhibited slightly stronger antibacterial activity than that from PL Province, likely due to its higher 1,8-cineole content. The specific location of plant material collection is important, as it can influence the chemical composition of the oil. These findings provide a basis for further research on HSEO as a potential antibacterial agent for treating bacterial infections. Moreover, HSEO also contains anti-inflammatory agents like sabinene and β-caryophyllene, which are beneficial in treating skin conditions such as eczema and acne.

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