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Original Research Article

Chemical Composition and Biological Properties of Essential Oils of Two Mint Species

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Abstract

Purpose: *To analyze the composition of essential oils of two types of mint as well as compare the antimicrobial, antioxidant and anti-inflammatory activities of the two oils.*
Methods: *Peppermint (M. piperita L.) and chocolate mint (M. piperita L.) oils were obtained by steam distillation in a Clevenger-type apparatus. The chemical composition of the essential oils was determined by gas chromatography-mass spectrometry (GC/MS). The minimal inhibitory concentration (MIC) of the essential oils were determined by broth dilution method. The antioxidant activities of the oils were determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH)DPPH radical scavenging assay, β-Carotene-linoleic acid assay, andnitric oxide (NO) radical scavenging assay.*
Results: *The two essential oils contain high levels of alcohol (43.47-50.10%) and terpene (18.55-21.07%) with the major compound being menthol (28.19-30.35%). The antimicrobial activity (minimum inhibitory concentration, MIC) of peppermint oil against E. coli, S. aureus and P. aeruginosa (0.15, 0.08, 0.92 %v/v, respectively) was stronger than that of chocolate mint (0.23, 0.09, 1.22 %v/v, respectively). In the anti-oxidant test including DPPH and β-Carotene-linoleic acid assays, peppermint oil showed superior antioxidant properties to chocolate mint oil (4.45 - 19.86 µl/mL). However, with regard to scavenging NO radical activity, chocolate mint oil exhibited higher activity than peppermint (0.31 and 0.42 µl/mL, respectively). Chocolate mint oil also exhibited higher anti-inflammatory activity than peppermint oil (0.03 and 0.08 µl/mL, respectively).*
Conclusion: *The results obtained should help to clarify the functional applications of these folk herbs and their essential oils for aromatherapeutic healing and other folkloric uses.*

Keywords: *Peppermint, Chocolate mint, Anti-microbial, Anti-oxidant, Anti-inflammatory*

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INTRODUCTION

Essential oils are extracted from aromatic and medicinal plants that are natural concentrated aromatic hydrophobic liquid products obtained by steam distillation or solvent extraction [1,2]. They contain several chemical compounds exhibiting different biological properties and activities. It can reduce foodborne pathogens and decrease the use of synthetic and semisynthetic antimicrobial compounds [3].

Mints (*Mentha* spp.) are famous aromatic and medicinal herb that are used in traditional and folk medicines in the world for the antimicrobial and antioxidant properties. *Mentha* genus contains about 25 species and some hybrids and

belongs to the Lamiaceae family [4]. Mints contain volatile components, flavonoids, organic acids, quinones, such as for the digestive system, central nervous system, respiratory system [5, 6]. It was used in antimicrobial, anti-inflammatory or anesthesia [7]. *M. piperita* is a hybrid of spearmint (*M. spicata* L.) and water mint (*M. aquatica* L.), it grows particularly well in areas with high water-holding capacity soil [8].

In this study, we evaluated the major chemical compositions of the essential oils derived from the peppermint and chocolate mint by gas chromatography-mass spectrometry (GC-MS). We examined the antimicrobial activity of essential oils against some microorganisms. The antioxidant activities of the essential oils were determined by various antioxidant assays, including 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, *β*-Carotene/linoleic acid assay, and nitric oxide (NO) radical scavenging assay. Furthermore, the anti-inflammatory activities of the essential oils were determined by 5-lipoxygenase (5-LOX) inhibition assay.

EXPERIMENTAL

Plant material and isolation of essential oil.

Peppermint (*M. piperita* L.) and chocolate mint (*M. piperita* L.) were purchased from a horticulture shop, Tangshan Herb Gardening, in Puli, Nantou County, Taiwan. The voucher specimens (peppermint no. CHNA MP 11001; chocolate mint no. CHNA MP 11002) were identified and deposited in the herbarium of the Department of Cosmetic Science, Chin Nan University of Pharmacy and Science, Tainan, Taiwan. The whole fresh plants were washed with distilled water (room temperature). The essential oils were obtained by steam distillation in a Clevenger-type apparatus. The essential oils collected were filtered through a 0.45 μm filter and kept at 4 ℃ until further analysis. The steam distilled yields were about 0.3 %.

Analysis of essential oil

The essential oils were analyzed by GC/MS using a Hewlett-Packard GC systems (HP6890 series Ⅱ) coupled with a mass detector (MSD5973) equipped with a HP-5MS capillary column (5 % phenyl methylsiloxane, 30 m × 0.25 mm id., 0.25 μm film thickness) (Agilent Technologies, Palo Alto, CA). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used over a scan range of 40–400 amu. Helium was used as a gas carrier at a flow rate of 1 mL/min. Split ratio was adjusted at 25:1. The column temperature was initially kept at 60 °C for 4 min, then gradually increased up to 240 °C at an increment of 3 °C/min, and finally held isothermal for 10 min. The percentage of components was calculated from total ion chromatograms. Identification of the primary component was assigned by matching mass spectral data with those detailed in Wiley 7n.1, and NIST02.L libraries [9].

Bacterial strains and culture conditions

The following were used as test bacteria: *E. coli* ATCC 2592, *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 9027, *P. ovale* ATCC 12078, *C. albicans* ATCC 10231, and *P. acnes* ATCC 6919. All of the microorganisms were purchased from the Bioresource Collection and Research Center (BCRC, Hsinchu, Taiwan, ROC), and were employed in antimicrobial.
E. coli, S. aureus, and *P. aeruginosa* were incubated at 37 ℃ in TSB, and *P. acnes* was incubated anaerobically at 37 ℃ in RCM. *P. ovale* and *C. albicans* were cultured at 30 ℃ in Dixon medium (MEB with 2 % ox-bile, 1 % Tween-40, and 0.25 % glycerol mono-oleate) and YMPD medium (0.3% yeast extract, 0.3% malt extract, 0.5 % peptone, and 1% dextrose), respectively [10].

Determination of antimicrobial activity

The minimal inhibitory concentration (MIC) of the essential oils for the inhibition of six bacterial strains were determined by the broth dilution method with some modifications. Each test was executed in corresponding media supplemented with 0.5% Tween 80. Serial dilutions of the essential oils were prepared in a 96-well microtiter plate over a range 0.02 to 49 mg/mL. Overnight broth cultures of the various strains were prepared, and the final concentration in the various wells was adjusted to 2×10^4 CFU/mL. Plates were incubated at the corresponding culture temperatures, with incubation time of 24 h for *E. coli, S. aureus, P. aeruginosa,* and *C. albicans*, 48 h for *P. acnes*, and 72 h for *P. ovale*. MIC was defined as the lowest concentration of the essential oil at which the microorganism does not exhibit visible growth, as indicated by the turbidity of the medium. MIC was defined as the lowest concentration of the essential oil at which inoculated microorganism was completely killed [9].

Determination of antioxidant activity by DPPH radical scavenging assay

Each concentration of essential oils was mixed

individually into a methanolic solution containing DPPH radicals (0.1mM), and the final volume was 1 mL. These solution mixtures were kept in dark for 30 minutes (incubation period) at room temperature. After thirty minutes, the absorbance was measured at 517 nm [11]. Each test was carried out in three times. Finally, DPPH radical scavenging activity (DPPH) was determined as in Eq 1.

DPPH (%) = $\{(A_b - A_s)/A_b\}100$ ..... (1)

where A_b is the absorbance of blank and A_s the absorbance of the sample.

Determination of antioxidant activity by β-Carotene-linoleic acid assay

β-Carotene was dissolved in 0.2 mL of chloroform (1 mg/mL) and this was added to a solution of linoleic acid (20 mg) in 200 mg of Tween 40. Chloroform was evaporated using a rotary evaporator under vacuum at 40℃ for 5min. Distilled water (50 mL) was added to the flask and the mixture was stirred in a sonicator. Each concentration of essential oils was added individually to 4.8 mL of the emulsion and this was then incubated at 50℃ for 3h. The absorbance was measured at 470 nm [9]. Reading of all samples were done immediately (t = 0 h) and after 3 h of incubation. The antioxidant activities of the essential oils were evaluated in term of inhibition of β-Carotene (inhibition) as in Eq 2.

Inhibition (%) = $\{(As_{3h} - Ab_{3h})/(Ab_{0h} - Ab_{3h})\}100$ ..... (2)

where As_{3h} is sample absorbance at the 3rd hour, Ab_{3h} blank absorbance at the 3rd hour, Ab_{0h} blank absorbance at 0 h, and Ab_{3h} blank absorbance at the 3rd hour

Determination of antioxidant activity by NO radical scavenging

Each concentration of essential oils was mixed individually with 0.1 mL of sodium nitroprusside (100 mM) and phosphate buffer solution (pH 7.4), yielding a final volume of 1 mL. After 2.5 h of incubation at 20℃, 0.05 mL of the mixture was added to 0.05 mL of Griess reagent over 10 min at 25℃. The absorbance was determined at 540 nm [12], and NO radical scavenging activity (NO) was obtained as in Eq 3

NO (%) = $\{(A_b - A_s)/A_b\}100$ ..... (3)

where A_b is the absorbance of blank and A_s the absorbance of the sample.

Determination of anti-inflammatory activity

Linoleic acid was used as substrate for 5-LOX. Various concentrations of 30 µL aliquots of essential oil with 30 µL of linoleic acid and potassium phosphate buffer (0.1 M, pH 6.3) containing 5-LOX (25 U), yielding a final volume of 3 mL. The mixture was incubated at 25℃ for 10 min, and the absorbance was determind at 234 nm [13]. Because linoleic acid is enzymatically converted to a conjugated diene by 5-LOX, which results in a continuous increase in absorbance at 234 nm, inhibition activity (Infl) was calculated as in Eq 4.

Infl (%) = $\{(A_b - A_s)/A_b\}100$ ..... (3)

where A_b is the absorbance of blank and A_s the absorbance of the sample.

Statistical analysis

All determinations were performed at least in triplicate. The results were analyzed by Student's *t*-test using Microsoft Excel 2007, and expressed as mean ± standard deviation (SD) for each measurement. Differences were recognized as significant at $p < 0.05$.

RESULTS

Based on GC/MS analysis, the major components of peppermint essential oil were menthol (30.35 %), menthone (21.12 %), and trans-carane (10.99 %). In addition, we also found some other components of peppermint essential oil, including isomenthol, isopulegol, camphor. For the chocolate mint essential oil, the major components were menthol (28.19 %), menthone (15.53 %), 1,8-cineole (11.89 %), as shown in Table 1.

The essential oils of peppermint and chocolate mint have as their major compounds alcohols, terpenes and ketones. The essential oil of peppermint contained alcohols (43.47 %), ketones (25.9 %), and terpenes (18.55 %) while the essential oil of chocolate mint contained alcohols (50.1 %), terpenes (21.07 %), and ketones (9.68 %), as Table 4 shows.

The results of the antioxidant assays of the two essential oils are stated in Table 3. The IC_{50} value of peppermint essential oil were 4.45, 0.37 and 0.42 µl/mL for DPPH radical scavenging assay, *β*-Carotene-linoleic acid assay, and NO radical scavenging assay, respectively while the IC_{50} values of chocolate mint essential oil were 19.86, 5.07, and 0.31 µl/mL, respectively. The antioxidant activities of peppermint essential oil were stronger than those of the essential oil of chocolate mint with regard to DPPH radical scavenging and *β*-Carotene-linoleic acid assays.

Table 1: Major compounds (%) of essential oils

	Peppermint		Chocolate mint	
S/No.	***Compound***	***Peppermint***	***Compound***	***Chocolate mint***
1	menthol	30.35	menthol	28.19
2	menthone	21.12	menthone	15.53
3	trans-carane	10.99	1,8-cineole	11.89
4	isomenthol	6.26	menthomenthene	8.29
5	(+)-carvone	5.60	isomenthol	7.08
6	1,8-cineole	5.33	(+)-carvone	5.33
7	mint furanone	2.49	bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-	2.75
8	pulegone	2.12	D-(+)-camphor	1.75
9	mono-(2-ethyl hexyl) ester	0.85	β-pinene	1.37
10	menthyl acetate	0.81	mint furanone	1.30

Table 2: The minimal inhibitory concentration (MIC, %v/v) of the essential oils.

Essentail oil		***E. coli***	***S. aureus***	***P. aeruginosa***	***P. ovale***	***P. acnes***	***C. albicans***
Mentha piperita L., peppermint	MIC_{90}	0.15±0.00	0.08±0.00	0.92±0.00	0.22±0.00	0.03±0.00	0.08±0.00
Mentha piperita L., chocolate	MIC_{90}	0.23±0.02	0.09±0.00	1.22±0.00	0.21±0.00	0.03±0.00	0.09±0.00

Table 3: IC_{50} (*μ*l/mL) values for antioxidant and anti-inflammatory activities of essential oils

Essentail oil	Antioxidant activity			Anti-inflammatory activity
	DPPH radical scavenging activity	*β*-Carotene-linoleic acid assay	NO radical scavenging activity	5-LOX inhibition assay
Mentha piperita L., peppermint	4.45±0.75	0.37±0.05	0.42±0.01	0.08±0.01
Mentha piperita L., chocolate	19.86±1.25	5.07±0.17	0.31±0.06	0.03±0.01

Table 4: Classification of content of essential oil components (%)

Class of compund	**Peppermint**	**Chocolate mint**
Alcohols	43.47	50.10
Ketones	25.90	9.68
Terpenes	18.55	21.07
Others	9.27	15.90
Esters	2.81	3.25

but lower with regard to NO radical scavenging assay. For anti-inflammatory activity.test, IC_{50} values were 0.08 and 0.03 μl/mL for essential oils of peppermint and chocolate mint, respectively (Table 3). The anti-inflammatory activity of chocolate mint essential oil was higher than that of peppermint essential oil.

MIC results (Table 2) show the essential oils of peppermint and chocolate mint exhibited the strongest bactericidal activity at a concentration

of 0.03 % (v/v) against *P. acnes*. The MIC of peppermint essential oil decreased in the order: *P. acnes* (0.03 % v/v) > *S. aureus* (0.08 %v/v) = *C. albicans* > *E. Coli* (0.15 %v/v) > *P. ovale* (0.22 %v/v) > *P. aeruginosa* (0.92 %v/v). The MIC of chocolate mint essential oil decreased in the order: *P. acnes* (0.03 %v/v) > *S. aureus* (0.09 % v/v) = *C. albicans* > *P. ovale* (0.21 %v/v) > *E. Coli* (0.23 %v/v) > *P. aeruginosa* (1.22 %v/v). The essential oil of peppermint demostrated stronger antimicrobial activity than that of chocolate mint.

DISCUSSION

The essential oils from peppermint and chocolate mint were analyzed to determine their chemical components. They were also tested for antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Pitrosporum ovale*, *Candida albicans*, and *Propionibacterium acnes*. Besides, the essential oils were used in antioxidant, and anti-inflammatory assays.

Khan and Abourashed reported that peppermint yields 0.1 – 1.0 % of volatile oil that is composed mainly of menthol (29 – 48 %), menthone (20 – 31 %), and menthyl acetate (3 – 10 %) [14]. This result is similar to ours. In our study, menthol and menthone were major compositions in both peppermint and chocolate mint essential oils. Menthyl acetate content was 0.81 % in peppermint but was practically absent from chocolate mint.

The components of mint oils vary with plant maturity, variety, geographical region and processing conditions [8,15,16].

Many studies have assessed the antibacterial [17] and antifungal [8] activities of peppermints. Our results showed that Gram-positive *S. aureus* and *P. acnes* were more sensitive to the essential oils than Gram-negative *E. coli* and *P. aeruginosa.* The results were similar to Djenane *et al* (2012) [18]. In previous studies, the mint essential oils had the ability to inhibit *P. acnes* [19]. Therefore, this property could be used for developing new anti-acne or antimicrobial ingredients from peppermint oils for cosmetic or personal products.

The antioxidant activity had different results by different assay systems [9]. The antioxidant activities of antioxidants have been attributed to various mechanisms, including the prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging [20].

CONCLUSION

This study showed the two mint essential oils contain more alcohol and terpene and the major compound was menthol. The antimicrobial activity of peppermint against *E. coli*, *S. aureus* and *P. aeruginosa* was stronger than that of chocolate mint. For the anti-oxidation test using DPPH radical and *β*-Carovtene-linoleic acid assay, peppermint showed better properties than chocolate mint. However, for the scavenging NO radical activity, chocolate mint was superior to peppermint. Chocolate mint also showed stronger anti-inflammatory activity than peppermint.

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