The Antimicrobial Activity of Essential Oil from *Dracocephalum foetidum* against Pathogenic Microorganisms

Saet Byoul Lee[1], Kwang Hyun Cha[1], Su Nam Kim[1], Shataryn Altantsetseg[2], Sanduin Shatar[2], Oidovsambuu Sarangerel[3], and Chu Won Nho[1,*]

[1]Natural Products Research Center, KIST Gangneung Institute, Gangneung Techno Valley, Gangneung, Gangwon-do, Republic of Korea, [2]Institute of Chemical and Chemical Technology, Ulaanbaatar-51, Mongolia, [3]Mongolian National University, Ulaanbaatar, Mongolia

A number of essential oils from Mongolian aromatic plants are claimed to have antimicrobial activities. The essential oil of *Dracocephalum foetidum*, a popular essential oil used in Mongolian traditional medicine, was examined for its antimicrobial activity. Eight human pathogenic microorganisms including *B. subtilis, S. aureus, M. lutens, E. hirae, S. mutans, E. coli, C. albicans,* and *S. cerevisiae* were examined. The essential oil of *Dracocephalum foetidum* exhibited strong antimicrobial activity against most of the pathogenic bacteria and yeast strains that were tested; by both the agar diffusion method and the minimum inhibitory concentration (MIC) assay (MIC range was 26-2592 μg/ml). Interestingly, *Dracocephalum foetidum* even showed antimicrobial activity against methicilin-resistant *Staphylococcus aureus* (MRSA) strains. We also analyzed the chemical composition of the oil by GC-MS and identified several major components, including *n*-Mentha-1,8-dien-10-al, limonene, geranial, and neral.

Keywords: *Dracocephalum foetidum*, essential oil, antimicrobial activity, volatility, *n*-Mentha-1,8-dien-10-al, limonene

A number of aromatic medicinal plants used for treating infectious diseases have been mentioned in different phyto-therapy manuals due to their availability, fewer side effects, and reduced toxicity. The essential oils of these aromatic plants (Vandendool and Kratz, 1963) are responsible for their fragrance as well as biological properties (Kalemba and Kunicka, 2003). Essential oils are complex mixtures of volatile secondary metabolites that mainly consist of mono- and sesquiterpenes including carbohydrates, alcohols, ethers, aldehydes, and ketones and are responsible for both the fragrant and biological effects of aromatic medicinal plants (Salzer, 1977; Angioni *et al.,* 2003; Senatore *et al.,* 2004). An important characteristic of essential oils and their constituents is their hydrophobicity, which enables them to partition in the lipids of bacterial cell membranes and mitochondria, thus disturbing the structures and rendering them more permeable (Sikkema *et al.,* 1994; Sikkema *et al.,* 1995).

The genus *Dracocephalum foetidum* Bunge, which belongs to the family Labiatae, is characterized as perennial herbs with purple flowers and is distributed in Northeast Asia, including Mongolia, China, Japan, and Korea. *D. foetidum* has been used as a Mongolian traditional medicine for preventing and curing diseases. Traditionally, Mongolian nomads used water extracts of the plant leaves and flowers to wash their faces and hands to prevent bacterial and fungal infections in ancient times. *D. foetidum* is also used to treat pus of the oral cavity, gum disease, rheumatic edema, and

wounds. There are about 300 species of aromatic plants present in Mongolia, and *D. foetidum* is one of the most popular essential oil-bearing plants (Shatar, 2000).

Essential oils are known to have a variety of pharmacological effects, including anti-inflammatory, anti-viral, and antimicrobial activities. Despite the development of antibiotics, bacterial and fungal infections are still a major issue in medicine. Due to the presence and increase of numerous drug-resistant strains an urgent need exists to develop novel antimicrobial agents (Naimi *et al.,* 2001). *Staphylococcus aureus* is a common pathogen causing infections that range from minor skin lesions to life-threatening conditions such as osteomyelitis, bacteremia, endocarditis, and pneumonia (Lindsay and Holden, 2004). MRSA appeared in the 1990s and became a huge problem worldwide for treating nosocomial infections, as a result of their resistance to antibiotics (Naimi *et al.,* 2003). Due to the problems of cost and potential resistance to new antibacterial drugs, we viewed it was important to test *D. foetidum,* a naturally present essential oil, against MRSA strains.

The aim of this study was to evaluate the antimicrobial activities of the essential oil based on its traditional Mongolian use. The major constituents for the oil's activity were also investigated by gas chromatography-mass spectroscopy (GC-MS). To the best of our knowledge, we are the first to report that *D. foetidum* essential oil showed antimicrobial activity against the pathogenic microorganisms that were tested.

Materials and Methods

Plant material
Dracocephalum foetidum Bunge was collected in August 2003

* To whom correspondence should be addressed.
(Tel) 82-33-650-7102; (Fax) 82-33-650-7199
(E-mail) cwnho@kist.re.kr

Table 1. The pathogenic microorganisms submitted for antimicrobial activity tests

Group	Strain	Cultivation condition
Gram (+)	*Bacillus subtilis* KCTC 1021[a]	Nutrient broth/30°C
Gram (+)	*Staphylococcus aureus* KCTC 1916[a]	Nutrient broth/37°C
Gram (+)	Methicillin resistance *S. aureus* MRSA 2659[c]	Nutrient broth/37°C
Gram (+)	Methicillin resistance *S. aureus* MRSA 9561[c]	Nutrient broth/37°C
Gram (+)	*Micrococcus luteus* KCCM 11548[b]	Nutrient broth/37°C
Gram (+)	*Enterococcus hirae* KCCM 11768[b]	Nutrient broth/37°C
Gram (+)	*Streptococcus mutans* KCTC 3065[a]	BHI broth/37°C
Gram (−)	*Escherichia coli* KCTC 2593[a]	Nutrient broth/37°C
Yeast	*Candida albicans* KCTC 7965[a]	YM broth/30°C
Yeast	*Saccharomyces cerevisiae* KCCM 50113[b]	YM broth/30°C

[a]KCTC strains were purchased at the Korean Collection for Type Cultures.
[b]KCCM strains were purchased at the Korean Culture Center of Microorganisms.
[c]MRSA strains 2659, 9561 were isolated from the clinical specimens of Wonju Christian Hospital, Korea, from June to July 1994.

in the Gobi-Altains region of Mongolia. The voucher specimen (specimen number UB-7819) has been deposited at the herbarium in the Institute of Botany of the Mongolian Academy of Sciences, Ulaanbaatar. *Dracocephalum foetidum* Bunge was identified by Dr. Shine Shatar in the Institute of Chemistry and Chemical Technology, Ulaanbaatar.

Isolation of the essential oil
The aerial parts (1.2 kg) of the freshly collected plants were finely chopped and hydrodistilled for 3 h using a Clevenger-Adams type apparatus (Adams, 1991). The yield of the essential oil produced during the steam distillation was 0.97% (v/w).

Gas chromatography-mass spectroscopy analysis
GC-MS analyses were carried out using an HP-5890 gas chromatograph coupled with a Finnigan MAT 800 ion Trap detector a J&W DB-1 column (30×0.25 film thickness 0.25 μm) was used with helium as carrier gas (linear velocity 30 cm/s). The temperature programming was performed as follows: 60°C isothermal for 10 min then increased from 60°C to 220°C at 4°C/min, and finally isothermal at 220°C for 5 min. The injector temperatures were 250°C. The EI/MS spectra were recorded at 70 eV (ion source temperature: 220°C). The mass range was 35 to 425 m/z. The essential oil components were identified on the basis of their mass data and the comparison of their relative retention index (RRI) (Vandendool and Kratz, 1963) that was obtained using various series of *n*-alkanes. Their EI-mass spectra were either compared with the NIST/NBS and Wiley library spectra found in the literature (Massada, 1976), or were confirmed by comparison with data published in a reference book (Adams, 2001). The percentages of the individual components were obtained from the relative GC peak areas as shown in Table 2.

Bacterial and fungal cultures
The bacterial and fungal strains used in this study are listed in Table 1. The *Streptococci* were grown in BHI broth (Difco, USA) without aeration at 37°C. The *B. subtilis* were

grown in nutrient broth (Difco, USA) with aeration at 30°C. Other microorganisms were grown in nutrient broth with aeration at 37°C. The yeasts (*C. albicans* and *S. cerevisiae*) were grown in YM broth (Difco, USA) with aeration at 30°C.

Agar diffusion method
The essential oil of *D. foetidum* was tested for antimicrobial activity using the agar diffusion method on solid media. BHI agar (Difco, USA) plates were used for *Streptococci*, and Nutrient Agar (Difco, USA) plates were used for *B. subtilis* and the other microorganisms. The yeasts (*C. albicans* and *S. cerevisiae*) were grown in YM agar (Difco, USA) plates. Sterile 5 mm diameter paper discs were placed on the agar plates of the appropriate media, which had been surface spread with 0.1 ml of a logarithmic phase bacteria at a density adjusted to approximately 10^8 CFU/ml. After 2-200 μl of the essential oil was applied to each paper disc, the agar plates were further incubated for 24 h at 37°C or 30°C. The results were recorded by measuring the zones of growth inhibition around the discs. To evaluate the effects of oil volatility on the antimicrobial activity, the discs containing the oil were placed inside the upper lids of petri dishes. The paper discs containing the oil were at a distance of approximately 4 mm from the growth surface of the test organism. After 24 h of incubation, the zones of growth inhibition by the essential oil were measured against the pathogenic microorganisms tested.

Determination of the minimal inhibitory concentration (MIC)
The initial test concentration of the essential oil was made in broth media at 1% (v/v) by vortexing at room temperature. Two-fold serial dilutions were prepared in broth media to obtain a concentration range of 26-8280 ppm (μg/ml). The plates were inoculated with the bacterial suspension (50 μl per well) and incubated with gentle aeration at 37°C for 24 h. *p*-INT (*p*-iodonitrotetrazolium violet Sigma, USA) solution was added to each well and the plates were further incubated for at least 30 min to ensure adequate color development. The MIC^{INT} was determined as the lowest concentration at

Table 2. The chemical composition of the essential oil from *Dracocephalum foetidum* Bunge analyzed by GC-MS

No	RI	Compounds	Percentage (in oil)	Identification method
1	104	α-thujene	0.51	a
2	136	α-pinene	0.20	a
3	149	Sabinene	1.85	a
4	178	Myrcene	0.20	a
5	196	*p*-cymene	0.66	a, b
6	212	Limonene	17.00	a
7	244	γ-terpinene	1.71	a
8	288	Linalool	1.58	a
9	337	Camphor	0.80	a
10	343	Trans-sabinene hydrate	1.20	a, b
11	369	Borneol	1.45	a
12	390	Terpin-4-ol	1.05	a
13	400	α-terpineol	1.60	a
14	423	*n*-Mentha-1,8-dien-10-al	39.19	a
15	429	Myrtenol	1.00	a, b
16	435	Trans-carveol	0.10	a, b
17	450	Nerol	0.63	a
18	457	Neral	3.20	a
19	480	Geraniol	2.68	a
20	491	Geranial	4.56	a
21	501	Citronellyl formiate	0.20	a, b
22	522	*n*-Mentha-1,8-dien-10-ol	2.87	a
23	535	Bornyl acetate	0.20	a
24	560	Geranyl formiate	0.30	a
25	572	Neryl acetate	2.10	a
26	623	Geranyl acetate	0.84	a
27	659	β-caryophyllene	0.60	a
28	761	Bisabolene	0.40	a
29	780	γ-cadinene	0.20	a
30	852	Caryophyllene oxide	1.10	a, b
31	886	α-cadinol	0.90	a

The components of the oil were identified by comparisons of their mass spectra with those in a computer library[a] or confirmed by comparisons of their retention indices with data published in a reference book[b] (Adams, 2001).

which no red color (signifying) appeared (Langfield *et al.*, 2004).

Results and Discussion

Chemical composition of the essential oil

The essential oil composition and the relative component percentages are listed in Table 2. A total of 31 compounds were identified, representing 90.88% of the total oil. The terpenes made up the largest component of the oil and had many representative volatiles. The monoterpenes (87.68%) were the dominant group and the sesquiterpenes (3.2%) represented the second largest group. These groups have high enough vapor pressures at normal atmospheric conditions to allow for their significant release into the air (Dudareva *et al.*, 2004). The main constituents were found to be *n*-Mentha-1,8-dien-10-al (39.19%), limonene (17%), geranial (4.56%), and neral (3.2%) (Table 2). *n*-Mentha-1,8-dien-10-al is one of the menthol related monoterpenes, and is made via biogenesis from geranial and neral. Since *n*-Mentha-1,8-dien-10-al was a dominant compound in the essential oil, the amounts of geranial and neral present in *D. foetidum* could also affect the percentage of *n*-Mentha-1,8-dien-10-al found in the plant (personal communication with S. Shatar). A primary physical characteristic of the *D. foetidum* essential oil that we observed was a strong volatility, which could be related to the *n*-Mentha-1,8-dien-10-al content.

Table 3. The quantitation of antimicrobial activity for *D. foetidum* essential oil measured by the agar diffusion method. The effectiveness of the essential oil is demonstrated by the size of the microorganism growth inhibition zone around the filter paper disc, which is typically expressed as the diameter of the zone in mm. The strength of the activity is presented as +.

Conc. of essential oil (%)	Volume used (µl/disc)	C. albicans	S. cerevisiae	E. coli	B. subtilis	S. mutans	S. aureus	E. hirae	M. luteus
100	20	++[a]	+++++	++	+++++	+++++	+++++	+++++	+++++
100	10	+++++	+++++	+	+++++	+++++	+++++	+++++	+++++
100	2	+++	+++	+	+++++	+++++	+++++	+++++	+++++
Volatility	2	+++	++++	−	+++++	+++++	+++++	+++++	+++++

[a]Diameter of theinhibition zone: no inhibition: (−) 7~20 mm; (+) 21~40 mm; (++) 41~60; (+++) 61~80; (++++) no cell growth; (+++++).

Table 4. The minimal inhibitory concentration (MIC) of *D. foetidum*essential oil on pathogenic microorganisms. Antibiotics (ampicillin and hygromycin B) were used as the positive controls. The minimal inhibitory concentration is defined as the lowest broth concentration of essential oil that resulted in no visible microorganism growth changes. The assay was performed in duplicate and repeated three times separately. This table shows one of the three independent experiments that were performed.

	E. coli	S. mutans	S. aureus	M. luteus	C. albicans	S. cerevisiae	B. subtilis	E. hirae
Antibiotics (µg/ml)	25[a]	0.78125[a]	0.78125[a]	1.5625[a]	6.23[b]	3.125[c]	0.78125[d]	1.5625[a]
Essential oil (µg/ml)	1035	1035	1035	2070	2592	2592	1633	26

[a]ampicillin, [b]hygromycin B, [c]amphotericin B, [d]gentamycin.

Antimicrobial activity of the essential oil

The *D. foetidum* essential oil was tested for antimicrobial activity using the agar diffusion method against 8 different pathogenic microorganisms, including Gram-positive bacteria such as *Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus, Enterococcus hirae, Streptococcus mutans*; Gram-negative bacteria such as *Escherichia coli* and fungal strains such as *Candida albicans*, and *Saccharomyces cerevisiae*. As little as 2 µl of the 100% *D. foetidum* essential oil completely inhibited growth of all of Gram-positive bacteria (no cell growth) and partially inhibited the growth of the fungal strains. However, the essential oil showed little antibacterial effect on the Gram-negative bacteria, *E. coli*, with a 20 µl treatment applied to a paper disc (Table 3). Generally, the Gram-positive strains of bacteria that were tested seemed to be more sensitive to the essential oil, which is attributed to the absence of an outer lipopolysaccharide layer in Gram-negative bacteria that provides a resistant barrier (Inouye *et al.*, 2001; Delaquis *et al.*, 2002). It has been proposed that the mode of action for the antimicrobial effects can be attributed to the inhibition of microbial respiration and an increase in plasma membrane permeability, followed by ion leakage from the cells (Lambert *et al.*, 2001; Walsh *et al.*, 2003). The different effects we observed between the Gram-positive and Gram-negative bacteria could be due to the hydrophobicity of the essential oil that may have increased the membrane permeability of the cells (Knobloch *et al.*, 1986).

The most interesting observation was that the strong volatility of the *D. foetidum* essential oil affected its total antimicrobial effects (Table 3). To assess the influence of the oil's volatility on microbial growth, a paper disc containing *D. foetidum* essential oil was placed inside the upper lid of a Petri dish and was then inverted onto the bottom lid containing an agar medium inoculated with the test organism. Although the oil did not diffuse through the agar, an equal potency of antimicrobial activity was observed using this agar diffusion method due to the oil's strong volatility (Table 3).

This suggests that the volatility of the chemical composition of the oil can influence the potency of its antimicrobial activity. However, further research is needed to test this hypothesis and to identify the specific volatile compounds responsible for the antimicrobial effects.

The MIC of the *D. foetidum* essential oil was detected in a range of 26-2592 µg/ml against 8 pathogenic microorganisms (Table 4). The oil showed equal antimicrobial effects on both Gram-positive and Gram-negative bacteria, which is inconsistent with the results from the agar diffusion method. This anomaly indicates that the evaluation techniques we used for the examination significantly affected the antibacterial activity of the essential oil, as was also reported by Nguefack *et al.* (2004). Since the major compounds of the *D. foetidum* essential oil are non-polar, such as *n*-Mentha-1,8-dien-10-al and limonene, they may have had limited diffusion through the polar matrix (agar). In the MIC assay system the essential oil is present as an emulsion form, and the less dense oil is partitioned from the liquid culture system. This may enhance the antibacterial effects in the MIC assay.

Antimicrobial activity of the essential oil against MRSA strains

We tested the antibacterial activity of the essential oil on 2 different MRSA strains, MRSA2659 and MRSA9561. The *D. foetidum* essential oil showed marked antibacterial effects on the MRSA strains that were tested via the agar diffusion method and MIC assay (Table 5). The oil showed similar antibacterial effects on the MRSA strains as compared to the normal *S. aureus* strains (Table 3 and 4).

In conclusion, *D. foetidum* essential oil had strong antimicrobial effects on a variety of human pathogenic microorganisms, including MRSA. We found that the degree of volatility in the oil also affected its antimicrobial activities. Therefore, we suggest that *D. foetidum* essential oil may be a potential candidate as a therapeutic agent, and could be further developed as a constituent for antiseptic products

Table 5. The antibacterial effects of *D. foetidum* essential oil on MRSA strains analyzed by both the agar diffusion method and MIC assay. Ten μl of the oil was used for the agar diffusion method. The results represent one of three independent experiments that were performed.

		MRSA2659[a]	MRSA9561
Inhibition zone (mm)	Essential oil	+++++[b]	+++++
	Volatility	+++++	+++++
MIC (μg/ml)	Essential oil	572	1035

[a]MRSA, methicillin-resistant *S. aureus*
[b]Diameter of the inhibition zone: no inhibition: (−) 7~20 mm; (+) 21~40 mm; (++) 41~60; (+++) 61~80; (++++) no cell growth; (+++++).

such as antibacterial cleansing formulas and disinfectants.

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