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Chemical Composition and Antibacterial Activity of Essential Oil from *Artemisia feddei*

CHA, JEONG-DAN[4], EUN-KYUNG JUNG[2], BONG-SEOP KIL[3], AND KYUNG-YEOL LEE[1]*

[1]*Institute of Oral Bioscience and Department of Oral Microbiology, School of Dentistry, Chonbuk National University, Jeonju 561-756, Korea*
[2]*Department of Dental Hygiene, Ulsan College, Ulsan 682-715, Korea*
[3]*Division of Life Science, College of Natural Science, Wonkwang University, Iksan 570-749, Korea*
[4]*Oral Cancer Research Institute, College of Dentistry, Yonsei University, Seoul 120-749, Korea*

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Abstract The chemical components of the essential oil from *Artemisia feddei* LEV. et VNT. were analyzed using GC-MS. Ninety-nine compounds, accounting for 96.23% of the extracted essential oil, were identified. The main oil compounds were 1,8-cineole (16.86%), chamazulene (9.04%), α-terpineol (8.18%), α-phellandrene (5.78%), α-thujone (5.51%), α-terpinyl acetate (5.07%), borneol (5.08%), β-caryophyllene (4.71%), camphor (4.04%), and terpinen-4-ol (3.04%). The antimicrobial activity of the essential oil and some of its compounds was tested against 15 different genera of oral bacteria. The essential oil from *A. feddei* had a considerable inhibitory effect on all the obligate anaerobic bacteria tested (MICs, 0.025 to 0.05 mg/ml; MBCs, 0.025 to 0.1 mg/ml), whereas the major compounds demonstrated different degrees of growth inhibition.

Keywords: *Artemisia feddei*, essential oil, antimicrobial activity, GC-MS

Artemisia feddei LEV. et VNT. (*A. feddei*) is a perennial plant that is widely distributed across the mountains and fields in Korea [12], and has been used as a folk medicine in Asian countries against a variety of diseases, such as inflammatory complaints and digestive disorders [6, 7, 12]. Coumarins and sesquiterpene lactones have already been isolated and identified from *A. feddei* [7, 10, 11], and the scopoletin isolated from *A. feddei* was found to suppress proinflammatory cytokines, PGE_2, and nitric oxide from an LPS-stimulated cell line, RAW 264.7 macrophages [6, 7].

However, the essential oil from *A. feddei* has not yet been investigated chemically or biologically. Accordingly, this study analyzed the chemical composition of the essential oil from *A. feddei* using GC-MS, and then investigated the antimicrobial activities of the essential oil and some of its major components.

The aerial parts of *A. feddei* were collected in September 2000 from the area of Mt. Mireuk in Korea, and their identity as *A. feddei* confirmed by Dr. Bong-Seop Kil, College of Natural Science, Wonkwang University. A voucher specimen (DJ-100-A21) was deposited at the herbarium of the College of Natural Science, Wonkwang University. The aerial parts of *A. feddei* (about 1 kg) were air-dried, and then distilled for 3 h using a modified Clevenger-type apparatus to obtain the essential oil. Anhydrous sodium sulfate was used to absorb the water contained in the essential oil. The extracted essential oil was stored in a deep freezer (−70°C) to minimize any loss of volatile compounds. The refractive indices, optical rotation, and specific gravity were all ascertained according to methods recommended in the French norms AFNOR. The refractive index was determined using an Abbe refractometer (Atago-3T, Japan), and the optical rotations recorded in 0.5 ml of cells, 1 cm in length, using an ADP 220 polarimeter [Bellingham+Stangley (BS), French] at 589 nm. The GC analysis was performed on a Hewlett Packard model 5890 series II gas chromatograph (HP, Palo Alto, CA, U.S.A.) with a flame ionization detector (FID) and split ratio of 1:35 using two different fused silica capillary columns, Suplecowax 10 (60 m×0.32 mm i.d., 0.25 μm film thickness) and SPB-1 (30 m×0.32 mm i.d., 0.25 μm film thickness). The injector or detector temperature for each analysis was about 250°C, plus the carrier gas was nitrogen at a flow rate of 1.86 ml/min for the Suplecowax 10 column, and nitrogen at a flow rate of 1:20 ml/min for the SPB-1 column. The peak areas were measured by electronic integration, and the relative amounts of the individual components based on the peak areas. The

*Corresponding author
Phone: 82-63-270-4023; Fax: 82-63-270-4037;
E-mail: kyleecnu@chonbuk.ac.kr

Table 1. Chemical composition of the essential oil of *Artemisia feddei*.

Peak no.[a]	Compounds	RI[b]	RI[c]	Peak area (%)[d]
Monoterpene hydrocarbons				**(11.35)**
1	Tricyclene	1006	919	0.03
2	α-Pinene	1012	930	0.60
3	α-Thujene	1029	924	0.15
4	Camphene	1071	943	0.76
5	β-Pinene	1111	966	0.41
6	Sabinene	1124	965	0.76
7	α-Phellandren	1167	994	5.78
8	α-Terpinene	1184	1007	0.60
10	Limonene	1196	1021	0.24
12	*cis*-β-Ocimene	1240	1029	0.04
13	γ-Terpinene	1247	1050	1.15
14	*trans*-β-Ocimene	1257	1035	0.18
15	*p*-Cymene	1273	1011	0.39
16	Terpinolene	1283	1078	0.26
Oxygenated monoterpenes				**(58.03)**
9	2,3-Dehydro-1,8-cineole	1193	978	0.11
11	1,8-Cineole	1215	1023	16.86
17	*Artemisia* ketone	1352	1045	t
19	*cis*-3-Hexen-1-ol	1386	836	0.09
20	3-Octanol	1394	979	0.14
21	Yomogi alcohol	1403	984	0.59
22	*trans*-3-Hexen-1-ol	1409	852	0.14
23	α-Thujone	1423	1080	5.51
24	β-Thujone	1440	1091	0.91
26	*trans*-Sabinene hydrate	1465	1052	0.59
28	Chrysanthenone	1508	1096	0.17
30	Camphor	1517	1124	4.04
32	*iso*-Pinocamphone	1536	1118	t
33	Linalool	1546	1106	0.32
34	*cis*-Sabinene hydrate	1550	1130	1.33
35	Pinocarvone	1562	1134	0.16
36	*cis*-*p*-Ment-2-en-1-ol	1566	1104	0.16
37	*cis*-Chrysanthenyl acetate	1570	1243	0.18
38	Bornyl acetate	1578	1265	0.15
39	*iso*-Bornyl acetate	1583	1204	0.13
42	Terpinen-4-ol	1601	1158	3.04
43	Lavandulyl acetate	1612	1275	0.03
44	Myrtenal	1627	1167	0.26
46	Umbellulone	1639	1165	0.07
47	Pulegone	1650	1221	t
48	*trans*-Pinocarveol	1653	1119	0.06
51	*cis*-Piperitol	1671	1187	0.38
52	*trans*-Verbenol	1672	1123	0.20
53	*trans*-Piperitol	1676	1205	1.25
54	α-Terpinyl acetate	1695	1329	5.07
55	α-Terpineol	1701	1169	8.18
56	Borneol	1703	1150	5.08
59	Piperitone	1721	1220	0.09
61	Carvone	1732	1210	0.66
62	*cis*-Chrysanthenol	1754	1150	0.99
67	Myrtenol	1793	1177	0.06
68	*trans*-Carveol	1832	1181	0.31
69	*cis*-Myrtanol	1859	1280	0.21
70	*cis*-Carveol	1862	1196	0.21
71	β-Phenylethyl alcohol	1903	1081	0.13
74	Perillyl alcohol	1997	1277	0.10
84	Thymol	2187	1263	0.07
Sesquiterpene hydrocarbons				**(7.90)**
27	δ-Copaene	1490	1369	0.31
31	α-Gurjunene	1525	1389	0.07
41	β-Caryophyllene	1594	1418	4.71
45	γ-Elemene	1629	–	t
49	α-Humulene	1665	1440	0.67
50	*trans*-β-Farnesene	1668	1454	0.80
57	α-Zingiberene	1715	1490	0.50
58	β-Selinene	1717	1471	t
60	*cis,trans*-α-Farnesene	1726	1447	0.18
63	δ-Cadinene	1762	1505	0.36
64	*ar*-Curcumene	1773	1472	0.24
66	α-Cadinene	1785	1524	0.06
Oxygenated sesquiterpenes				**(4.88)**
72	*iso*-Caryophyllene oxide	1967	1524	0.08
73	Caryophyllene oxide	1979	1556	1.71
75	Eicosane	2000	2000	0.21
76	α-Humulene oxide	2038	1586	0.15
77	*trans*-Nerolidol	2043	1555	0.45
78	Globulol	2058	1568	0.10
79	Elemol	2073	1527	0.14
80	Spathulenol	2125	1551	0.47
81	α-Cedrol	2150	1575	0.21
82	T-Cadinol	2156	1619	0.06
83	α-Cadinol	2173	1628	0.54
85	α-Eudesmol	2221	1622	0.04
86	Carvacrol	2229	1273	0.15
89	*cis,transe*-Farnesol	2298	1695	0.36
97	T-Muurolol	2234	1615	0.14
90	Caryophyllene alcohol	2357	1607	0.07
Others				**(14.07)**
18	*n*-Hexanol	1355	851	0.10
22	1-Octen-3-yl acetate	1383	1096	0.06
25	1-Octen-3-ol	1453	969	1.07
29	*Artemisia* alcohol	1514	1072	1.53
65	Methyl salicylate	1782	1173	0.91
88	Myristicin	2255	1490	1.18
91	Diethyl phthalate	2361	–	0.07
92	Chamazulene	2377	1692	9.04
93	Indole	2444	1248	0.11
Total identified				**(96.23)**

[a]Numbering refers to the elution order on a Supelcowax 10 column.
[b]Retention index on a polar Supelcowax 10 column.
[c]Retention index on an apolar SPB-1 column.
[d]Peak area percentage is based on a polar Supelcowax 10 column, and values represent the average of three determinations.
t; Trace (<0.05%).

GC-MS was carried out on an HP model 5970 mass spectrometer operating in the EI mode at 70 eV, combined with the GC described above, fitted with an INNOWax column (60 m×0.25 mm i.d., 0.25 μm film thickness) and SPB-1 column (30 m×0.32 mm i.d., 0.25 μm film thickness). The temperature of the column was programmed from 40°C to 230°C at 2°C/min. The injector and ion source temperatures were the same as mentioned above. The carrier gas was helium at a flow rate of 1.25 ml/min for both analyses. The identification of the chemical constituents was based on a comparison of their relative retention times and mass spectra with those obtained from authentic samples and/or the NIST/NBS and Wiley library spectra.

The deep blue essential oil of *A. feddei* yielded a 0.4% dry weight, and the physiochemical properties of the essential oil were d^{21}=0.9612, n_D^{21}=1.5324, and $[\alpha]_D^{23}$= −32.2 ($CHCl_3$, *c* 0.01). The results of the GC and GC/MS analyses for the essential oil are shown in Table 1, where the compounds are listed in order of their elution from the Supelcowax 10 column. Ninety-three constituents, accounting for more than 96.23% of the total oil composition, were identified. Fourteen monoterpene hydrocarbons (11.35%), forty-two oxygenated monoterpenes (58.03%), twelve sesquiterpene hydrocarbons (7.90%), sixteen oxygenated sesquiterpenes (4.88%), and other compounds (14.07%) were identified in the essential oil. The main compounds with concentrations higher than 3% were 1,8-cineole (16.86%), chamazulene (9.04%), α-terpineol (8.18%), α-phellandrene (5.78%), α-thujone (5.51%), α-terpinyl acetate (5.07%), borneol (5.08%), β-caryophyllene (4.71%), camphor (4.04%), and terpinen-4-ol (3.04%). Thus, the *A. feddei* essential oil analysis revealed that the major components were 1,8-cineole, chamazulene, α-terpineol, and camphor (a bornane derivative), in agreement with previous literature on the essential oils from other *Artemisia* species [3–5, 8, 16]. *Artemisia* species generally contain 1,8-cineole and bornane derivatives, which are widely used in the liquor industry across the world. These oils contain a high proportion of oxygenated monoterpenes, indicating that the oils include a strong antifugal and antioxidant activity [4, 5, 8, 16].

The oral bacterial strains used in this study were *Streptococcus mutans* (ATCC 25175), *Streptococcus sanguinis* (ATCC 10556), *Streptococcus sobrinus* (ATCC 27607), *Streptococcus ratti* (KCTC; Korean collection for type cultures 3294), *Streptococcus criceti* (KCTC 3292), *Streptococcus anginosus* (ATCC 31412), *Streptococcus gordonii* (ATCC 10558), *Actinobacillus actinomycetemcomitans* (ATCC 43717), *Fusobacterium nucleatum* (ATCC 10953), *Prevotella intermedia* (ATCC 25611), and *Porphylomonas gingivalis* (ATCC 33277). The antibacterial activities were examined after incubation at 37°C for 18 h (facultative anaerobic bacteria), 24 h (microaerophilic bacteria), and 1–2 days (obligate anaerobic bacteria) under anaerobic conditions. The antibacterial activities of the essential oil and some of its major compounds against certain oral bacteria and reference strains were determined based on the minimum inhibitory concentrations (MICs) using the broth dilution method performed in triplicate [9, 15]. The MICs were determined as the lowest concentration of the test samples that resulted in a complete inhibition of visible growth in the broth. Following anaerobic incubation of the MIC plates, the minimum bactericidal concentrations (MBCs) were determined based on the lowest concentration of the essential oil that killed 99.9% of the test bacteria after plating onto appropriate agar plates. Ampicillin or gentamicin were used to compare the antibacterial sensitivity of antibiotics with that of the essential oil and some of its major compounds against the test bacteria.

Some oils have been shown to have applications in food preservation and aromatherapy, and exhibit pharmacological properties, such as antibacterial, antifungal, antioxidant, spasmolytic, antiplasmodial, anti-inflammatory, and anticancer activities [1, 3, 5, 8, 13, 16]. The essential oils of *Artemisia* have also been found to have antibacterial, antifungal, and antioxidant activities [3–5, 8, 16]. The present results of the antibacterial activities of the essential oil and some of its major compounds are shown in Table 2. The essential oil of *A. feddei* exhibited antimicrobial activities against all the bacteria tested (MICs, 0.025 to 3.2 mg/ml; MBCs, 0.05 to 3.2 mg/ml), a strong antimicrobial activity against all the facultative bacteria and microaerophilic bacteria (MICs, 0.05 to 0.8 mg/ml; MBCs, 0.1 to 1.6 mg/ml), except for *E. coli* and *S. epidermidis* (MICs/MBCs values, 3.2 and 3.2 mg/ml, respectively), and a strong antimicrobial activity against the obligate anaerobic bacteria (MICs, 0.025 to 0.1 mg/ml; MBCs, 0.05 to 0.1 mg/ml). The oxygenated monoterpene borneol showed a strong antimicrobial activity against all the bacteria (MICs, 0.2 to 1.6 mg/ml; MBCs, 0.4 to 3.2 mg/ml), *S. ratti* and *S. criceti* appeared to be less sensitive, and the oxygenated monoterpenes camphor, 1,8-cineole, α-terpineol, terpinen-4-ol (MICs, 0.05 to 12.8 mg/ml; MBCs, 0.1 to 12.8 mg/ml), and sesquiterpene hydrocarbon β-caryophyllene (MICs, 0.4 to 12.8 mg/ml; MBCs, 0.8 to 12.8 mg/ml) showed moderate antimicrobial activities against all the bacteria tested. In general, there was a correlation between the antifungal and antibacterial activities and the percentage of some of the major components. This observation was consistent with a previous report, where 1,8-cineole and borneol were found to have a moderate antibacterial activity [2, 3, 12]. Camphor, 1,8-cineole, borneol, α-terpineol, terpinen-4-ol, bornyl acetate, and chrysanthenol as the major constituents of the oils of *Artemisia* have previously been reported to exhibit antibacterial, antifungal, and antioxidant activities [2, 3, 8]. Yet, the essential oil of *A. feddei* exhibited more antibacterial activity than some of its major compounds.

Table 2. MICs and MBCs (mg/ml) of the essential oil and its major components of *Artemisia feddei* for some oral bacteria with a few reference strains.

Strains	Essential oil	Borneol	α-Terpineol	Camphor	1,8-Cineole	Terpinen-4-ol	β-Caryophyllene	Ampicillin	Gentamicin
Escherichia coli ATCC 25922	3.2/3.2	0.4/0.8	1.6/3.2	12.8/12.8<	3.2/6.4	1.6/1.6	12.8/12.8<	$256/256\times10^{-3}$	$8/16\times10^{-3}$
Staphylococcus aureus ATCC 29213	0.8/1.6	1.6/3.2	1.6/3.2	12.8/12.8<	12.8/12.8	1.6/3.2	12.8/12.8<	$16/16\times10^{-3}$	$2/4\times10^{-3}$
Staphylococcus epidermidis ATCC 12228	3.2/3.2	0.8/1.6	1.6/3.2	12.8/12.8<	0.8/1.6	1.6/3.2	12.8/12.8<	$32/64\times10^{-3}$	$1/2\times10^{-3}$
Streptococcus pyogenes ATCC 21059	0.2/0.4	0.8/0.8	1.6/1.6	12.8/12.8<	12.8/12.8	1.6/1.6	12.8/12.8	$4/8\times10^{-3}$	$8/16\times10^{-3}$
Streptococcus mutans ATCC 25175	0.4/0.8	0.8/1.6	1.6/3.2	6.4/12.8	12.8/12.8	1.6/3.2	1.6/3.2	$4/4\times10^{-3}$	$8/8\times10^{-3}$
Streptococcus sanguinis ATCC 10556	0.8/1.6	1.6/3.2	1.6/3.2	12.8/12.8	12.8/12.8	1.6/3.2	1.6/3.2	$32/32\times10^{-3}$	$8/16\times10^{-3}$
Streptococcus sobrinus ATCC 27607	0.2/0.4	0.4/0.8	1.6/1.6	12.8/12.8	12.8/12.8	1.6/3.2	12.8/12.8	$2/2\times10^{-3}$	$4/8\times10^{-3}$
Streptococcus ratti [a]KCTC 3294	0.2/0.4	3.2/6.4	1.6/3.2	12.8/12.8<	12.8/12.8	1.6/3.2	12.8/12.8<	$4/4\times10^{-3}$	$4/8\times10^{-3}$
Streptococcus criceti KCTC 3292	0.4/0.8	3.2/6.4	3.2/3.2	12.8/12.8	12.8/12.8	3.2/3.2	12.8/12.8<	$4/4\times10^{-3}$	$8/8\times10^{-3}$
Streptococcus anginosus ATCC 31412	0.8/1.6	0.8/0.8	1.6/3.2	12.8/12.8	3.2/6.4	1.6/3.2	12.8/12.8<	$4/4\times10^{-3}$	$16/16\times10^{-3}$
Streptococcus gordonii ATCC 10558	0.05/0.1	0.8/0.8	0.05/0.1	12.8/12.8	6.4/6.4	0.05/0.1	1.6/3.2	$1/2\times10^{-3}$	$2/4\times10^{-3}$
Actinobacillus actinomycetemcomitans ATCC 43717	0.8/0.8	0.8/1.6	1.6/3.2	6.4/12.8	6.4/12.8	1.6/3.2	1.6/1.6	$64/64\times10^{-3}$	$2/2\times10^{-3}$
Fusobacterium nucleatum ATCC 10953	0.025/0.05	0.2/0.4	0.4/0.8	6.4/6.4	3.2/6.4	0.2/0.4	12.8/12.8	$0.25/0.25\times10^{-3}$	$16/32\times10^{-3}$
Prevotella intermedia ATCC 25611	0.025/0.05	0.2/0.4	0.2 /0.4	1.6/3.2	1.6/3.2	0.2/0.4	0.8/1.6	$32/32\times10^{-3}$	$0.5/1\times10^{-3}$
Porphylomonas gingivalis ATCC 33277	0.1/0.1	0.8/0.8	0.2/0.8	6.4/12.8	6.4/6.4	0.4/0.8	0.4/0.8	$0.5/1\times10^{-3}$	$256/512\times10^{-3}$

[a]Korean Collection for Type Cultures.

The present results also indicate the possibility of exploiting the essential oil of *A. feddei* as an effective inhibitor of oral bacteria; for example, as a toothpaste or gargling solution component. However, for medicinal purposes, the safety and toxicity of this essential oil still need to be addressed.

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