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Original Articles

Susceptibility of Oral Bacteria to Essential Oil of *Artemisia capillaris Thunb.*

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Objective : The aim of this work is to investigate the antibacterial activity of the essential oil obtained from *Artemisia capillaris* (A. capillaris), as the development of microbial resistance to antibiotics make it necessary to constantly look for new and active compounds effective against pathogenic bacteria.

Methods : The crushed materials of A. capillaris (1 kg) were subjected to steam distillation for 3 h, using a modified Clevenger type apparatus in order to obtain essential oil. Diethyl ether was the extracting solvent kept at 25°…. The essential oil was analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). The essential oil and the composition were tested for antimicrobial activities against 15 different genera of oral bacteria.

Results and Conclusion : The components of the essential oil identified were: β,-pinene (9.36%), camphor (3.32%), 1,8-cineole (4.38%), artemisia alcohol (3.32%), β-caryophyllene (11.08%), γ-cadinene (4.23%), and capillene (32.74%). The essential oil of A. *capillaris* exhibited considerable inhibitory effects against all oral bacteria tested, while their major components demonstrated various degrees of growth inhibition.

Key Words: Essential Oil, Antibacterial Activity, Antibiotics

Introduction

Essential oils of herbs and their components, products from the secondary metabolism of a plant, have many applications in folk medicine, food flavoring, and preservation as well as in the fragrance and pharmaceutical industries[1-3]. The antimicrobial and antioxidant properties of essential oils have been known for a long time, and a number of investigations have been conducted on their antimicrobial activities using various bacteria, viruses, and fungi[4,5].

The genus *Artemisia*, one of the largest genera belonging to the Compositae family consisting of more than 350 species, is predominantly distributed in the world[6,7]. *Artemisia* species are frequently utilized for the treatment of diseases such as ma of laria, hepatitis, cancer, inflammation, and infections by fungi, bacteria, and viruses[8-12]. *A. capillaris* plants are distributed around sandy areas along the seacoast forming

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Table 1. Composition of the Essential Oils of Artemisia Capillaris

Peak no[a]	Components	RI[c]	RI[c]	Peak Area(%)[d]
Monoterpene hydrocarbons (19.47)				
1	Tricyclene	919	1006	t
3	α-Thujene	924	1029	0.06
2	α-Pinene	930	1012	1.36
4	Camphene	943	1071	0.62
6	Sabinene	965	1124	0.39
5	β-Pinene	966	1111	9.36
8	α-Phellandrene	994	1167	1.43
7	δ-3-Carene	1005	1152	t
9	α-Terpinene	1007	1184	1.86
16	ρ-Cymene	1011	1273	0.32
11	Limonene	1021	1196	1.52
13	cis-β-Ocimene	1029	1240	1.11
15	trans-β-Ocimene	1035	1257	0.88
14	γ-Terpinene	1050	1247	0.42
17	Terpinolene	1078	1283	0.14
Oxygenated monoterpenes (16.12)				
10	2,3-Dehydro-1,8-cineole	978	1193	t
20	Yomogi alcohol	984	1403	0.04
12	1,8-Cineole	1023	1215	4.38
19	Artemisia ketone	1045	1352	1.49
24	trans-Sabinene hydrate	1052	1465	0.05
21	α-Thujone	1080	1423	0.07
22	β-Thujone	1091	1440	t
26	Chrysanthenone	1096	1508	t
34	cis-ρ-Ment-2-en-1-ol	1104	1566	t
31	Linalool	1106	1546	0.05
30	iso-Pinocamphone	1118	1536	0.11
28	trans-Pinocarveol	1119	1653	0.10
41	Camphor	1124	1517	3.32
32	cis-Sabinene hydrate	1130	1550	0.08
33	Pinocarvone	1134	1562	0.24
49	Borneol	1150	1703	1.59
57	cis-Chrysanthenol	1150	1754	1.02
38	Terpinen-4-ol	1158	1601	0.89
39	Umbellulone1	1651	649	0.11
39	Myrtenal	1167	1627	0.25
46	α-Terpineol	1169	1701	0.66
61	Myrtenal	1177	1793	0.05
63	trans-Carveol	1181	1832	0.15
42	cis-Piperitol	1187	1671	0.08
66	cis-Carveol	1196	1862	0.06
45	trans-Piperitol	1205	1676	0.17
55	Carvone	1210	1732	0.35
50	Piperitone	1220	1721	0.28
40	Pulegone	1221	1650	0.07
64	Geraniol	1234	1850	0.19
83	Thymol	1263	2178	0.09
70	Perillyl alcohol	1277	1997	0.06
65	cis-Myrtanol	1280	1859	0.05
35	Bornyl acetate	1578	1265	0.07
Sesquiterpene hydrocarbons(23.71)				
25	δ-Copaene	1369	1490	0.25
36	β-Elemene	1381	1589	0.21
29	α-Gurjunene	1389	1525	0.06

Table 1. Table 1 (Continued)

Peak no[a]	Components	RIc	RIc	Peak Area(%)d
37	*β*-Caryophyllene	1418	15941	1.08
43	*α*-Humulene	1440	1665	0.76
54	cis,trans-*α*-Farnesene	1447	1726	1.29
44	trans-*β*-Farnesene	1454	1668	0.45
47	*γ*-Muurolene	1464	1686	0.24
51	Germacrene D	1465	1705	1.64
60	*ar*-Curcumene	1472	1773	2.38
52	*α*-Zingiberene	1490	1715	0.34
48	*γ*-Cadinene	1497	1690	4.23
58	*δ*-Cadinene	1505	1762	0.52
59	*β*-Sesquiphellandrene	1515	1770	0.07
Peak no[a]	Components	RI[c]	RI[c]	Peak Area(%)[d]
56	Germacrene B	1527	1739	0.19
Oxygenated sesquiterpene(3.44)				
79	Eugenol	1323	2146	0.07
68	iso-Caryophyllene oxide	1524	1967	0.03
78	Spathulenol	1551	2125	0.35
73	trans-Nerolidol	1555	2043	0.41
69	Caryophyllene oxide	1556	1979	T
74	Globulol	1568	2058	0.05
75	Viridiflorol	1569	2070	T
77	Guaiol T-Muurolol	1576	2105	0.06
72	*α*-Humulene oxide	1586	2038	0.05
85	**T-Muurolol**	1615	2234	1.42
80	T-Cadinol	1619	2156	0.15
82	Torreyol	1619	2179	T
84	*α*-Eudesmol	1622	2221	0.38
81	*α*-Cadinol	1628	2173	0.19
87	cis,trans-Farnesol	1695	2298	0.19
89	Hexadecanol	1864	2368	0.09
71	Eicosane	2000	2000	T
Others(36.24)				
23	1-Octen-3-ol	969	1453	0.07
27	Artemisia alcohol	1072	1514	3.32
67	*β*-Phenylethyl alchol	1081	1903	0.11
86	Capillene	1457	2252	32.74
Total identified	98.98			

[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 average of three determinations .

perennial plant communities. *A. capillaris* has been used in traditional medicines from many cultures for a variety of indications, including liver ailments, and a number of studies in animal models showed hepatoprotective effects[13, 14]. Recently, it is used for the treatment of hypertension, respiratory disease, and chronic cervicitis[15, 16]. However, there are no reports on the antibacterial activity of the essential oil against oral bacteria of *A. capillaris*.

In this study, the essential oil of *A. capillaris* was extracted and their chemical compositions were analyzed by GC and GC-MS. The antimicrobial activities of the essential oil against oral bacterial were investigated by broth dilution method[17].

Materials and Methods

1. Plant material

The aerial parts of *A. capillaris* were collected from the Medicinal Herbs Experiment Station of Jinan, Korea, in September, 2003. The identity was confirmed by the College of Oriental Medicine, Woosuk University. The voucher specimen (JS-98-A22) was at the department of the Herbarium of the College of Oriental Medicine, WooSuk University.

2. Extraction of Essential oil

The crushed materials of *A. capillaris* (1 kg) were subjected to steam distillation for 3 h, using a modified Clevenger type apparatus in order to obtain essential oil. Diethyl ether was the extracting solvent kept at 25℃. The ether extract was dried over anhydrous sodium sulfate and concentrated at 38℃. The essential oil was stored on deep freezer (-70℃) to minimize the loss of volatile compounds.

3. Analysis of physicochemical properties of the essential oil

Refractive indices, optical rotation, and specific gravity were determined according to methods recommended in the French norms AFNOR (The Association Francaise de Normalisation). Optical rotations were recorded in a 0.5 mL cell of 1 cm length using a ADP 220 polarimeter (Bellingham+Stangley (BS), French) at 589 nm. The refractive index was determined using an Abbe refractometer (Atago-3T, Japan).

4. Analysis of the chemical composition of the essential oils.

The oils were analysed on a Hewlett Packard model 5890 series II gas chromatograph (HP, Palo Alto, CA, USA) equipped with a flame ionization detector (FID), a split ratio of 1:40 using two different fused silica capillary column, SPB?1 (polydimethylsiloxane 30 m × 0.32 mm, i.d., film thickness 0.20㎛) and Supleco wax 10 (polyethyleneglycol 30m × 0.32mm. i.d., film thickness 0.25㎛). Oven temperature was programmed to 50℃ for 5 min, and then increased to 250℃ at a rate of 2℃/min. The injector and detector temperatures for both analysis were 250℃, respectively. The carrier gas, helium, was adjusted to a linear velocity of 30 cm/s. The GC-MS was carried out on an Hewlett-Packard 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㎛ film thickness) and SPB-1 column (polydime thylsiloxane 30 m × 0.32 mm, i.d., film thickness 0.20 ㎛). The temperature of the column was programmed from 40℃ to 230℃ at 2℃/min. The injector and ion source temperatures were the same as 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 comparisons of their relative retention times and mass spectra with those obtained from authentic sample and/or the NIST/NBS and Wiley

libraries spectra.

5. Microbial strains

The antimicrobial activity of the essential oil against the facultative anaerobic bacteria: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus pyogenes* (ATCC 21059), *Streptococcus mutans* (ATCC 25175), *Streptococcus sanguinis* (ATCC 10556), *Streptococcus sobrinus* (ATCC 27607), *Streptococcus ratti* (KCTC 3294), *Streptococcus criceti* (KCTC 3292), *Streptococcus anginosus* (ATCC 31412) and *Streptococcus gordonii* (ATCC 10558), microaerophilic bacteria: *Actinobacillus actinomycet emcomitans* (ATCC 43717) and obligate anaerobic bacteria: *Fusobacterium nucleatum* (ATCC 51190), *Prevotella intermedia* (ATCC 49046) and *Porphylomonas gingivalis* (ATCC 33277) was determined by the broth dilution method.

Brain heart infusion (BHI; Difco Laboratories, Detroit, MI, USA) was used for the facultative anaerobic bacteria. For A. *actinomycetemcomitans*, *F. nucleatum* and *P. intermedia*, brain heart infusion broth supplemented with 1% yeast extract (Difco) was used. For the obligate anaerobic bacteria, *P. gingivalis*, brain heart infusion broth containing hemin and menadione was used.

6. Microbiological methods

The minimum inhibitory concentrations (MICs) were determined for the oil and its components by the broth dilution method, and were carried out in triplicate. The antibacterial activities were examined after incubation at 37℃ for 18 h (facultative bacteria), for 24 h (microaerophilic bacteria), and for 1-2 days (obligate anaerobic bacteria) under anaerobic conditions. MICs were determined as the lowest concentration of test samples that resulted in a complete inhibition of visible growth in the broth. Ampicillin and Gentamicin were used as standard antibiotics in order to compare the sensitivity with the oil and its components against test bacteria.

Results and Discussion

The yield of the essential oil obtained of *A. capillaris* was about 0.9% on fresh weight; basis oil density d^{21}=0.9560, n_d^{21}=1.5134, $[\alpha]_D^{23}$= -10.9 (*c* 0.12, $CHCl_3$). The chemical compounds of the essential oil identified by GC and GC-MS analysis were given in Table 1. Eighty-nine compounds were identified in the oil, representing 99.05% of the total oil. Fifteen monoterpene hydrocarbons (19.47%), thirty-four oxygenated monoterpenes (16.12%), fifteen sesquiterpene hydrocarbons (23.71%), and seventeen oxygenated sesquiterpenes (3.44%) were identified in the oil. The main compounds with concentrations higher than 3% as percentage peak area of GC analysis were β-pinene (9.36%), camphor (3.32%), 1,8-cineole (4.38%) artemisia alcohol (3.32%), β-caryophyllene (11.08%), γ-cadinene (4.23%), and capillene (32.74%). The essential oil of *A. capillaris* showed that the major components were β-pinene (9.36%) and capillene (32.74%), in difference with literature reports on the essential oils of other Artemisia species[5,17,18].

The results of the antibacterial activity (Table 2) showed that the essential oil of *A. capillaris* exhibited moderate activities against all the tested bacteria (MICs, 0.1 to 12.8 mg/ml). The oil showed the antimicrobial activity against the facultative bacteria: *S. mutans, S. ratti, S. sanguinis*, and *S. gordonii* (MICs, 0.4 to 0.8 mg/ml) and also the essential oil showed the strong antimicrobial activity against obligate anaerobic bacteria: *F. nucleatum*, *P. intermedia*, and *P. gingivalis* (MICs, 0.1 to 0.2 mg/ml), while *E. coli*, *S. aureus*, and *S. epidermidis* appeared to be less sensitive. The

oxygenated monoterpenes camphor and 1,8-cineole and sesquiterpene hydrocarbon β-caryophyllene showed moderate antimicrobial activity against all bacteria tested (MICs, 0.4 to 12.8 mg/ml). The active volatile components are probably responsible for the antibacterial activity of the essential oils. Cariogenic and periodontopathic bacterial strains were killed completely by exposure for 30s to 0.2% manuka oil, tea tree oil or eucalyptus oil [19]. It has been reported that phenolic components of essential oils showed strong antibacterial activity[20, 21,22, 23] and also 5-phenyl-1,3-pentadiyne and capillarin isolated from *A. capillaris* showed antifeedant activity against cabbage butterfly larva *Pieris rapae crucivora* by the leaf disk assay [24,25,26, 27]. Recent data suggest that essential oils may disrupt the permeability barrier of cell membranes and inhibit respiration[28].

Conclusion

In conclusion, the results presented in this paper further support the antimicrobial activities of essential

Table 2. Antibacterial Activity (MIC/MBC) of Essential Oil of from *Artemisia Capillaris*

Strains	Essential oil	Camphor	1,8-cineole	β-caryophyllene	Ampicillin	Gentamicin
Escherichia coli ATCC 25922	12.8/12.8 a	12.8/12.8 ↑	3.2/6.4	12.8/12.8 ↑	256/256	8/16
Staphylococcus aureus ATCC 29213	12.8/12.8	12.8/12.8 ↑	12.8/12.8	12.8/12.8 ↑	16/16	2/4
Staphylococcus epidermidis ATCC 12228	12.8/12.8	12.8/12.8 ↑	0.8/1.6	12.8/12.8 ↑	32/64	1/2
Streptococcus pyogenes ATCC 21059	1.6/3.2	12.8/12.8	12.8/12.8	12.8/12.8	4/8	8/16
Streptococcus mutans ATCC 25175	1.6/3.2	6.4/12.8	12.8/12.8	1.6/3.2	4/4	8/8
Streptococcus sanguinis ATCC 10556	0.8/1.6	12.8/12.8	12.8/12.8	1.6/3.2	32/32	8/16
Streptococcus sobrinus ATCC 27607	3.2/3.2	12.8/12.8	12.8/12.8	12.8/12.8	2/2	4/8
Streptococcus ratti KCTC 3294	0.8/1.6	12.8/12.8 ↑	12.8/12.8	12.8/12.8 ↑	4/4	4/8
Streptococcus criceti KCTC 3292	6.4/6.4	12.8/12.8	12.8/12.8	12.8/12.8 ↑	4/4	8/8
Streptococcus anginosus ATCC 31412	1.6/1.6	12.8/12.8	3.2/6.4	12.8/12.8 ↑	4/41	6/16
Streptococcus gordonii ATCC 10558	0.4/0.8	12.8/12.8	6.4/6.4	1.6/3.2	1/2	2/4
Actinobacillus actinomycetemcomitans ATCC 43717	6.4/6.4	6.4/12.8	6.4/12.8	1.6/1.6	64/64	2/2
Fusobacterium nucleatum ATCC 10953	0.1/0.4	6.4/6.4	3.2/6.4	12.8/12.8	0.25/0.25	16/32
Prevotella intermedia ATCC 25611	0.025/0.1	1.6/3.2	1.6/3.2	0.8/1.6	32/32	0.5/1
Porphylomonas gingivalis ATCC 33277	0.2/0.4	6.4/12.8	6.4/6.4	0.4/0.8	0.5/1	256/512

[a]MIC/MBC: mg/ml (Essential oil and its compounds), MIC/MBC: μg/ml (Antibiotics)

oils from other sources [3,5,10] and extend these findings. These results also indicate the possibility of exploitation of the essential oil of A. capillaries as an effective inhibitor of oral bacteria. However, for medicinal purposes, the safety and toxicity of this essential oil need to be researched.

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