대한안이비인후피부과학회지 제17권 제3호(2004년 12월)
The Journal of Korean Oriental Medical Ophthalmology & Otolaryngology & Dermatology 2004;17(3):26-32

Analyzing of the Essential Oil Chemical Constituents in *Artemisia lavandulaefolia* and its Pharmacological Property on Antibacterial Activity

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

Method : The aerial parts of A. lavandulaefolia (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 were 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. Ninety-nine compounds accounting for 94.74% of the oil were identified. The main compounds in the oil were 1,8-cineole (5.63%), yomogi alcohol (4.49%), camphor (4.92%), â-caryophyllene (16.10%), trans-â-farnesene (5.09%), á-terpineol (3.91%), borneol (5.27%), cis-chrysanthenol (6.98%), and á-humulene oxide (3.33%). The essential oil and its compounds were tested for antimicrobial activity against 10 different genera of oral bacteria.

Conclusion : The essential oil of A. lavandulaefolia exhibited considerable inhibitory effects against all obligate anaerobic bacteria (MICs, 0.025 ~ 0.05 mg/ml) tested, while their major compounds demonstrated various degrees of growth inhibition

Key words : A. lavandulaefolia, Antibacterial activity

Introduction

The essential oils extracted of aromatic plant species are used in industries for the production of soaps, perfumes, and toiletries[1,2]. Many of them are also used in traditional medicine as fortifying, tonic, relaxing and postnatal medications. Essential oils have been found to be have antibacterial[3], antifungal[4],

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antioxidant[5], and anticancer activities[6]. Some oils have been shown to have applications in food preservation, pharmacological properties, and aromatherapy[7].

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[8,9]. Artemisia species are frequently utilized for the treatment of diseases such as malaria, hepatitis, cancer, inflammation, and infections by fungi, bacteria, and viruses[10-14].

A. lavandulaefolia is a perennial plant which is distributed widely around mountains and fields in Korea. A. lavandulaefolia has been used in traditional medicines from many cultures for a variety of indications, including allelopatic effects[15] and cancer [16].

In this study, the essential oil obtained from A. lavandulaefolia was analyzed for its chemical composition, and then the antimicrobial activities of the essential oil and its components were investigated.

Materials and Methods

1. Plant material

The aerial parts of A. lavandulaefolia were collected from the in October 2002 from the area of Jinan, Jeonbuk, Korea, in October 2002. A voucher specimen (JS 02 A21) was deposited at the herbarium of the College of Oriental Medicine, Woosuk University.

2.. Essential oil extraction

The aerial parts of A. lavandulaefolia (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 (4℃) to minimize the loss of volatile compounds.

3. Analysis of the chemical composition of the essential oils

GC analysis was performed on a Hewlett Packard model 5890 series II gas chromatograph (HP, Palo Alto, CA, USA), with a flame ionization detector (FID), a split ratio of 1:35 using fused silica capillary column SPB 1 (30 m × 0.32 mm, i.d., 0.25㎛ film thickness). The injector and detector temperatures for both analysis were 250℃, respectively. 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. Peak areas were measured by electronic integration. The relative amounts of the individual components are based on the peak areas. The 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 SPB 1 column (30 m × 0.32 mm, i,d., 0.25 ㎛ film thickness). 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.

4. 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), 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 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.

5. Microbiological methods

The minimum inhibitory concentrations (MICs) was 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 12 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

A light green essential oil of A. lavandulaefolia was obtained in a yield of 0.6 % dry weigh. Results of the GC and GC MS analysis of the oil were shown in Table 1, where the components were listed in order of their elution from SPB 1 column. Ninety eight constituents, representing 94.74% of the total oil composition, were identified.. The main compounds with concentrations higher than 3% as percentage peak area of GC analysis were 1,8 cineole (5.63%), yomogi alcohol (4.49%), Camphor (4.92%), trans β farnesene (5.09%), α terpineol (3.91%), borneol (5.27%), cis chrysnathenol (6.98%), α humulene oxide (3.33%), and β caryophyllene (16.10%).

The results of the antimicrobial activity (Table 2.) showed that the essential oil of A. lavandulae exhibited antimicrobial activities against all the bacteria tested (MICs, 0.025 ~ 3.2 mg/ml). The essential oil showed the strong antimicrobial activity against all the facultative bacteria and microaerophilic bacteria (MICs, 0.05 to 0.8 mg/ml), excepted E. coli (MIC values; 3.2 mg/ml), and also the essential oil showed the strong antimicrobial activity against obligate anaerobic bacteria (MICs, 0.025 to 0.05 mg/ml). 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 antibacterial property of the essential oil is known to be associated with the high percentage of α pinene and 1,8 cineole[17]. The monoterpene ketones in the essential oils of M. longifolia and M. piperita, 1,8 cineole in the essential oil of M. aquatica[18] and α pinene, p cymene, and β pinene of Juniper oil have been investigated for antimicrobial activity[4]. The

differences in the susceptibility of test organisms to monoterpenes and the differences in the efficacy of different monoterpenes may be explained by the variations in the rate of monoterpene penetration through the cell wall and cell membrane structure as suggested[19].

The results presented in this paper further support the antimicrobial activities of essential oils from other sources[19], and extends these findings. These results also indicate the possibility of exploitation of the essential oil of A. lavandulaefolia as an effective inhibitor of oral bacteria, for example, a component of tooth paste and/or gargling solution. However, for medicinal purposes, the safety and toxicity of this essential oil need to be addressed. The difference in susceptibility may allow formulation of products that will selectively kill or inhibit certain organisms while having a minimal effect on the commensal microorganisms.

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Table 1. Chemical composition of the essential oil of Artemisia lavandulaefolia

Peak noa	Compounds	RIb	Peak Area(%)c
1	cis 3 Hexen 1 ol	836	0.06
2	n Hexanol	851	0.10
3	α Thujene	924	0.00
4	α Pinene	930	2.18
5	1,4 Dimethylbenzene	936	0.06
6	Camphene	943	1.22
7	1,3 Dimethylbenzene	946	0.14
8	Sabinene	965	0.14
9	β Pinene	966	1.69
10	1 Octen	969	0.85
11	2,3 Dehydrocineole	978	0.05
12	3 Octanol	979	0.07
13	Yomogi alcohol	984	4.49
14	α Phellandren	994	0.51
15	α Terpinene	1007	0.23
16	p Cymene	1011	0.73
17	Limonene	1021	0.45
18	1,8 Cineole	1023	5.63
19	cis β Ocimene	1029	0.18
20	trans β Ocimene	1035	0.10
21	Artemisia ketone	1045	1.43
22	γ Terpinene	1050	0.42
23	Artemisia alcohol	1072	2.90
24	Terpinolene	1078	0.14
25	α Thujone	1080	1.83
26	β Phenylethyl alcohol	1081	0.50
27	β Thujone	1091	0.34
28	1 Octenyl acetate	1096	0.06
29	cis p Ment 2 en 1 ol	1104	0.94
30	iso Pinocamphone	1118	0.05
31	trans Pinocarveol	1119	0.33
32	trans Verbenol	1123	0.35
33	Camphor	1124	4.92
34	cis Sabinene hydrate	1130	0.13
35	Pinocarvone	1134	0.10
36	Borneol	1150	5.27
37	cis Chrysanthenol	1150	6.98
38	Terpinen4 ol	1158	0.87
39	Umbellulone	1165	0.22
40	Myrtenal	1167	0.19
41	α Terpineol	1169	3.91
42	Methyl salicylate	1173	0.22

Table 1. - continued

Peak noa	Compounds	RIb	Peak Area(%)c
43	Myrtenol	1177	0.10
44	trans Carveol	1181	0.09
45	cis Carveol	1196	0.05
46	trans Piperitol	1205	0.12
47	Carvone	1210	0.74
48	cis Chrysanthenyl acetate	1243	0.94
49	Thymol	1263	0.38
50	Bornyl acetate	1265	0.32
51	Carvacrol	1273	0.12
52	Perillyl alcohol	1277	0.17
53	cis Myrtanol	1280	0.00
54	Eugenol	1323	0.09
55	α Terpinyl acetate	1329	0.35
56	α Ylangene	1362	0.15
57	Decanoic acid	1362	0.12
58	δ Copaene	1369	0.26
59	α Gurjunene	1389	0.82
60	β Caryophyllene	1418	16.10
61	Aromadendrene	1447	0.87
62	cis,trans Farnesene	1447	0.54
63	trans β Farnesene	1454	5.09
64	γ Muurolene	1464	0.36
65	β Selinene	1471	1.33
66	ar Curcumene	1472	0.40
67	α Zingiberene	1490	0.32
68	Myristicin	1490	0.87
69	γ Cadinene	1497	0.15
70	α Cadinene	1505	1.15
71	β Sesquiphellandrene	1515	0.13
72	δ Cadinene	1524	0.08
73	iso Caryophyllene oxide	1524	0.09
74	Germacrene B	1527	0.08
75	Elemol	1527	0.22
76	Spathulenol	1551	0.69
77	trans Nerolidol	1555	0.48
78	Caryophyllene oxide	1556	2.69
79	Globulol	1568	0.36
80	Viridiflorol	1569	0.06
81	α Cedrol	1575	0.11
82	Guaiol	1576	0.08
83	α Humulene oxide	1586	3.33
84	Caryophyllene alcohol	1607	0.25
85	T Muurolol	1615	0.67
86	Torreyol	1619	0.54
87	α Eudesmol	1622	0.14
88	β Eudesmol	1627	0.12

Table 1. - continued

Peak noa	Compounds	RIb	Peak Area(%)c
89	α Cadinol	1628	0.42
90	Chamazulene	1692	0.22
91	cis,trans Farnesol	1695	0.32
92	Eicosane	2000	0.09
· Total identified			(94.74)

a Numbering refers to the elution order on an SPB 1column.

b Retention index on an apolar SPB 1 column.

c Peak area percentage is based on an apolar SPB 1 column, and values represent average of three determinations .

t ; Trace(<0.05%)

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

Strains	1	2	3	4	5
Escherichia coli ATCC 25922	3.2	12.8	3.2	256	8
Staphylococcus aureus ATCC 29213	1.6	12.8	12.8	16	2
Staphylococcus epidermidis ATCC 12228	1.6	12.8	0.8	32	1
Streptococcus pyogenes ATCC 21059	0.4	12.8	12.8	4.0	8
Streptococcus mutans ATCC 25175	0.4	6.4	12.8	4.0	8
Streptococcus sanguinis ATCC 10556	0.4	12.8	12.8	32	8
Streptococcus sobrinus ATCC 27607	0.4	12.8	12.8	2	4
Streptococcus ratti KCTC 3294	0.4	12.8	12.8	4	4
Streptococcus criceti KCTC 3292	0.4	12.8	12.8	4	8
Streptococcus anginosus ATCC 31412	0.4	12.8	3.2	4	16
Streptococcus gordonii ATCC 10558	0.05	12.8	6.4	1	2
Fusobacterium nucleatum ATCC 10953	0.025	6.4	3.2	0.25	16
Prevotella intermedia ATCC 25611	0.025	1.6	1.6	32	0.5
Porphylomonas gingivalis ATCC 33277	0.05	6.4	6.4	0.5	256

1 : Extracted essential oil of Artemisia lavandulaefolia,

2: Camphor, 3: 1,8 cineole, 4: Ampicillin, 5: Gentamicin.