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Chemical composition and antibacterial activity of the essential oil from aerial parts and cell suspension cultures of *Elsholtzia angustifolia*

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In this study, the chemical composition of the essential oil from *Elsholtzia angustifolia*, an aromatic plant, was examined using gas chromatography-mass spectrometry (GC/MS). Thirty-seven components were identified in the essential oil and Elsholtzia ketone (75.7%) was found to be the major compound. The antibacterial activity of the essential oil was assayed against selected bacteria, including foodborne pathogens. The essential oil exhibited remarkable antimicrobial activity against *Salmonella enteritidis*, suggesting that this essential oil has potential applications in food safety. In addition, we established a cell suspension culture of *E*. *angustifolia* to develop a sustainable source of antimicrobial compounds against *S*. *enteritidis*. Taken together, these results confirm the potential use of *E*. *angustifolia* essential oil as an antimicrobial agent to control *S*. *enteritidis* in food.

Keywords: *Elsholtzia angustifolia*; Essential oil; Elsholtzia ketone; Antibacterial activity

INTRODUCTION

Essential oils obtained from medicinal and aromatic plants are volatile, a mixture of various secondary metabolites that belong to different chemical families such as aldehydes, terpenes, esters, alcohols, ethers, phenolic, and ketones (Swamy et al. 2016). Although plants produce essential oils to communicate with herbivores, pathogens, and neighboring plants (Das et al. 2013), essential oils have been employed in food preservation, aromatherapy, and pharmaceutical therapy to treat patients with diseases such as cardiovascular disease, diabetes, Alzheimer disease, and cancer (Ali et al. 2015). Therefore, essential oils lend themselves to many applications as a functional resource for the cosmetic and pharmaceutical industries.

Elsholtzia, a plant genus containing at least 33 species in the family Lamiaceae, is primarily distributed in temperate and tropical Asia, and eastern Asia, particularly China, Korea and Japan (Jang et al. 2010; Guo et al. 2012). The most common of the genus Elsholtzia plants are aromatic plants, and used as folk medicine to treat patients with colds, headaches, digestion disorder, and rheumatic arthritis; they are also used in herbal, tea, cosmetics, and perfumeries (Guo et al. 2012). Previous research on volatile constituents identified more than 550 components from the 21 species of Elsholtzia, suggesting that the volatile oils may potentially be used as additives to food and related products, flavoring agents in cosmetics, and medicinal purposes (Kim and Jung, 2003; Melkani et al., 2005; Xu et al., 2008; Guo et al. 2012). *Elsholtzia angustifolia* is an herbaceous annual plant up to 50 cm high (Figure 1A), and distributed in Korea and China. Although this plant is also known as an aromatic plant, there is no report on chemical composition and bioactivity of *E*. *angustifolia* essential oil.

In this study, we investigated the chemical composition of *E*. *angustifolia* essential oil using GC-MS, and evaluated its antimicrobial activity. In addition, we introduced the callus suspension culture to achieve a stable production of antimicrobial compounds in *E*. *angustifolia*. Taken together, this study is expected to motivate further interest in the use of *E*. *angustifolia* as a natural antimicrobial agent in the food, cosmetic, and pharmaceutical industries.

MATERIALS AND METHODS

Plant materials and essential oil preparation

The seeds of *Esholtzia angustifolia* (Loes.) Kitag were obtained from the Nature Environment Research Park of Gangwon Province, South Korea. The seeds were germinated on soil, and cultivated in the growth chamber under an 8-h dark/16-h light photoperiod at 24°C. The leaves and stems of grown plants were harvested, and subjected to simultaneous stream distillation and extraction (SDE) with n-pentane and diethyl ether (used as an extractant) for 6 hours. The obtained oil layer was dried with anhydrous sodium sulphate and concentrated using N_2 gas. The concentrated extracts were stored at -20 °C in the dark until use.

Analysis of essential oils

The compositions of the SDE prepared essential oils of *E. angustifolia* were analyzed using gas chromatography-mass spectrometry (GC/MS). The analytical GC was carried out on an Agilent 6890 GC (Agilent Technologies, Santa Clara, CA, USA) equipped with an Agilent 5973i inert mass selective detector. The compounds were separated on a DB-5 fused silica capillary column (30 m × 250 µm, 0.25 µm film thickness). The column was held at 50ºC for 2 min, and the temperature was increased to 250ºC at a rate of 10ºC/min, followed by holding at 250ºC for 10 min. Ultra-high purity (99.999%) helium was used in constant pressure mode as the carrier gas with a flow rate of 1.0 mL/min. MS parameters were as follows: EI mode, with ionization voltage 70 eV, ion source temperature, 230 ºC; electron scanning ranges was 15500 amu. The peaks were tentatively identified based on GC retention time on a DB-5 capillary column relative to computer matching of mass spectra using the W10N11-Full library and Wiley/7n mass spectral database (Hewlett Packard, Palo Alto, CA, USA).

Callus induction

The seeds were first rinsed in 70% ethanol for 1 minute, and subsequently surface-sterilized with 1% sodium hypochlorite containing two to three drops of tween-20 for 30 minutes, and washed five times with sterilized distilled water. The sterilized seeds were germinated on MS agar medium containing 3% sucrose and 0.7% plant agar at 24°C under an 8-h dark/16-h light photoperiod. The cotyledon of germinated seeds was excised from the 7 day-old seedlings, and were cultivated on MS medium supplemented with 0.5 mg/ml NAA (α-naphthalene acetic acid) and 2 mg/mL BAP (6-benzylaminopurine) to induce callus. All cultures were kept in a dark condition at 24 °C.

Establishment of cell suspension cultures

To establish suspension cultures, friable calli were transferred into the liquid MS medium containing 0.5 mg/mL 2,4-D (2,4-dichlorophenoxyacetic acid), 2 mg/mL BAP (6-benzylaminopurine), and 3% sucrose. All culture flasks were incubated on a rotary shaker at 120 rpm and 24°C in a dark condition, and suspension-cultured cells were sub-cultured every week.

Determination of antimicrobial activity

The bacterial test strains used in this study were *Kocuria rhizophila* (KACC 14744) and *Micrococcus luteus* (KACC 14819), *Salmonella enteritidis* (KCTC 12021), *Listeria monocytogenes* (ATCC 19115), *Staphylococcus aureus* (KCTC 1916), *Pseudomonas aeruginosa* (KACC 10186), and *Enterobacter cloacae* KACC 11958. To determine the minimum inhibitory concentration (MIC) of essential oils, the degree of antimicrobial activity was assayed by the serial twofold dilution method as described by Jang et al. (2016).

RESULTS AND DISCUSSION

Chemical composition of the essential oil

The essential oil of fresh aerial parts of *E*. *angustifolia* was obtained using SED. The chemical composition analyzed using GC-MS is listed in Table 1. A total of 37 constituents, representing 95.9% of the total oil, have been identified from the essential oil. Elsholtzia ketone (75.7%) was found to be a major compound, followed by cycloprop [a] indene, 1,1a, 6,6a-tetrahydro- (5.96%), humulene (2.21%), and trans-β-farnesene (1.57%). Differences in composition between *E*. *angustifolia* and other species of Elsholtzia were also observed,

Table 1. Composition of essential oil extracted from aerial parts of *Elsholtzia angustifolia*.

	Major compounds	Molecular formula	RT	SI [1)]	Area %
1	3-Hexenal	$C_6H_{10}O$	5.345	9684	0.04
2	Hexanal	$C_6H_{12}O$	5.396	11525	0.07
3	2-Hexenal, (E)-	$C_6H_{10}O$	7.124	9800	0.76
4	alpha-Pinene	$C_{10}H_{16}$	10.096	48166	0.07
5	Camphene	$C_{10}H_{16}$	10.828	48064	0.08
6	sabinene	$C_{10}H_{16}$	11.862	48388	0.12
7	beta-Pinene	$C_{10}H_{16}$	12.077	48726	0.04
8	β-Myrcene	$C_{10}H_{16}$	12.632	48617	0.05
9	Eucalyptol	$C_{10}H_{18}O$	14.682	83101	0.44
10	gamma-Terpinene	$C_{10}H_{16}$	15.906	48867	0.09
11	Rosefuran	$C_{10}H_{14}O$	17.584	72612	0.64
12	beta-Linalool	$C_{10}H_{10}$	18.076	40432	0.9
13	Cycloprop [a] indene, 1,1a, 6,6a-tetrahydro-	$C_{10}H_{18}O$	20.581	40477	5.96
14	Elsholtzia ketone	$C_{10}H_{14}O$	23.546	108317	75.731
15	Benzene, 1,3-bis (1,1-dimethylethyl) -	$C_{14}H_{22}$	25.319	171484	0.39
16	Citral	$C_{10}H_{16}O$	26.183	78033	0.09
17	Cinnamaldehyde	C_9H_8O	26.479	42334	0.17
18	Dehydroelsholtzia ketone	$C_{10}H_{12}O_2$	27.646	103222	1.22
19	1,5,5-Trimethyl-6-methylenecyclohexene	$C_{10}H_{16}$	28.839	48107	0.06
20	beta-caryophyllene	$C_{15}H_{24}$	32.605	212245	0.96
21	Aromadendrene	$C_{15}H_{24}$	33.337	212012	0.15
22	alpha-guaiene	$C_{15}H_{24}$	33.652	212323	0.09
23	trans-β-Farnesene	$C_{15}H_{24}$	34.031	212069	1.57
24	Humulene	$C_{15}H_{24}$	34.163	6753	2.21
25	alloaromadendrene	$C_{15}H_{24}$	34.264	212002	0.04
26	(-)-Germacrene D	$C_{15}H_{24}$	35.116	211899	0.2
27	Viridiflorene	$C_{15}H_{24}$	35.513	212658	0.15
28	bicyclogermacrene	$C_{15}H_{24}$	35.747	212614	1
29	2,6-Bis(1,1-dimethylethyl)-4-methylphenol	$C_{15}H_{24}O$	35.999	261738	0.15
30	dihydro-beta-agarofuran	$C_{15}H_{26}O$	36.138	268966	0.18
31	2,6-Di-tert-butylphenol	$C_{17}H_{30}OSi$	36.251	218455	0.32
32	delta-Cadinene	$C_{15}H_{24}$	36.598	211732	0.2
33	alpha-Cadinol	$C_{15}H_{26}O$	41.986	269075	0.12
34	alpha-gurjunene	$C_{15}H_{24}$	42.718	212509	0.04
35	Neophytadiene	$C_{20}H_{38}$	48.332	448963	0.07
36	Hexadecanoic acid	$C_{16}H_{32}O_2$	52.843	377795	1.11
37	Octadecanoic acid	$C_{18}H_{36}O_2$	58.861	467829	0.41
Total identified compounds				95.891 %	

[1)] SI: Library search purity value.

although alpha-pinene and beta-pinene were identified from *E*. *angustifolia* essential oil, like other 15 species in the genus (Gou et al. 2012). In the essential oil of *E*. *ciliate* aerial parts, dehydroelsholtzia ketone, Elsholtzia ketone, carvacrol, and thymol were found to be the main constituents (Zhao et al. 2016). In addition, carvacrol was identified from the essential oils of *E*. *densa*, *E*. *bodinieri*, *E*. *penduliflora*, *E*. *splendens*, *E*. *frulicosa*, *E*. *strobilifera*, *E*. *ciliate*, and *E*. *cypriani* (Gou et al. 2012). Such differences may be due to differences among species, and differences of the plant source, growth conditions, harvesting time, and extraction method. The relatively increased level of Elsholtzia ketone in the flowers and leaves was observed, when the plants were damaged by herbivorous beetles (Peng and Yang, 2005; Zhang et al. 2016). In addition, Elsholtzia ketone exhibited acute toxicity against insects (Zhao et al. 2016), indicating that Elsholtzia ketone is the resisting characteristic of *E*. *angustifolia* against the herbivorous insects.

Antimicrobial activities of *E. angustifolia* essential oil

Since it has been demonstrated that the essential oils are related to plant defense against insects and pathogens, the antimicrobial activities of plant essential oils have been determined, suggesting that plant essential oils are effective natural antimicrobial agents (Solórzano-Santos and Miranda-Novales, 2012). Unlike the action of chemical antibiotics, antimicrobial activity of essential oil is related with their hydrophobicity, which allows them to penetrate the lipids of the bacterial cell membrane, and results in distribution of the cell structure and functionality (Nazzaro et al. 2013). To investigate the antimicrobial activity of *E*. *angustifolia*, we determined the MIC of its essential oil using the serial twofold dilution method and results are expressed as MIC values (Table 2). Overall, the essential oil exhibited antimicrobial activity against *M*. *luteus* (MIC = 250 µg/ml), *K*. *rhizophila* (MIC = 250 µg/ml), *S*. *enteritidis* (MIC = 125 µg/ml), and *E*. *cloacae* (MIC = 250 µg/ml). Because of the outer membrane containing hydrophilic lipopolysaccharides, Gram-negative bacteria are considered to be less susceptible to essential oils than Gram-positive bacteria (Trombetta et al. 2005). However, *E*. *angustifolia* essential oil exhibited good antimicrobial activity against two Gram-negative bacteria (*S*. *enteritidis*, and *E*. *cloacae*). This may indicate that some constituents of *E*. *angustifolia* essential oil gain access to the periplasm of Gram-negative bacterial via the porin proteins localized on their outer membrane (O'Bryan et al. 2015; Abed 2017). Since *S*. *enteritidis* has been recognized as a major cause of human foodborne illness, the increasing antimicrobial resistance in *S*. *enteritidis* has become a major public health problem (Firoozeh et al. 2011). The antimicrobial activity of *E*. *angustifolia* essential oil against *S*. *enteritidis* suggested that this essential oil could be potential candidate to be used as natural alternatives for food preservation.

Table 2. Antibacterial activity of essential oil extracted from the aerial parts and suspension cells of *Elsholtzia angustifolia*.

	MIC (µg/ml) [1)]						
Extract and fractions	**M.l[2)]**	**K.r.[2)]**	**L.m[2)]**	**S.a[2)]**	**S.e[2)]**	**P.a[2)]**	**E.c[2)]**
Essential oil	250	250	500	>1000	125	>1000	250
Suspension cells	1000	>1000	>1000	>1000	500	>1000	1000
Ampicillin	15.625	7.8125	62.5	31.25	7.8125	62.5	125

[1)] MIC values against bacteria and yeast were determined by the serial twofold dilution method.
[2)] K.r.: *Kocuria rhizophila* KACC 14744; M.l.: *Micrococcus luteus* KACC 14819; S.e.: *Salmonella enteritidis* KCTC 12021; L.m.: *Listeria monocytogenes* ATCC 19115; S.a.: *Staphylococcus aureus* KCTC 1916; P.a.: *Pseudomonas aeruginosa* KACC 10186; E.c.: *Enterobacter cloacae* KACC 11958

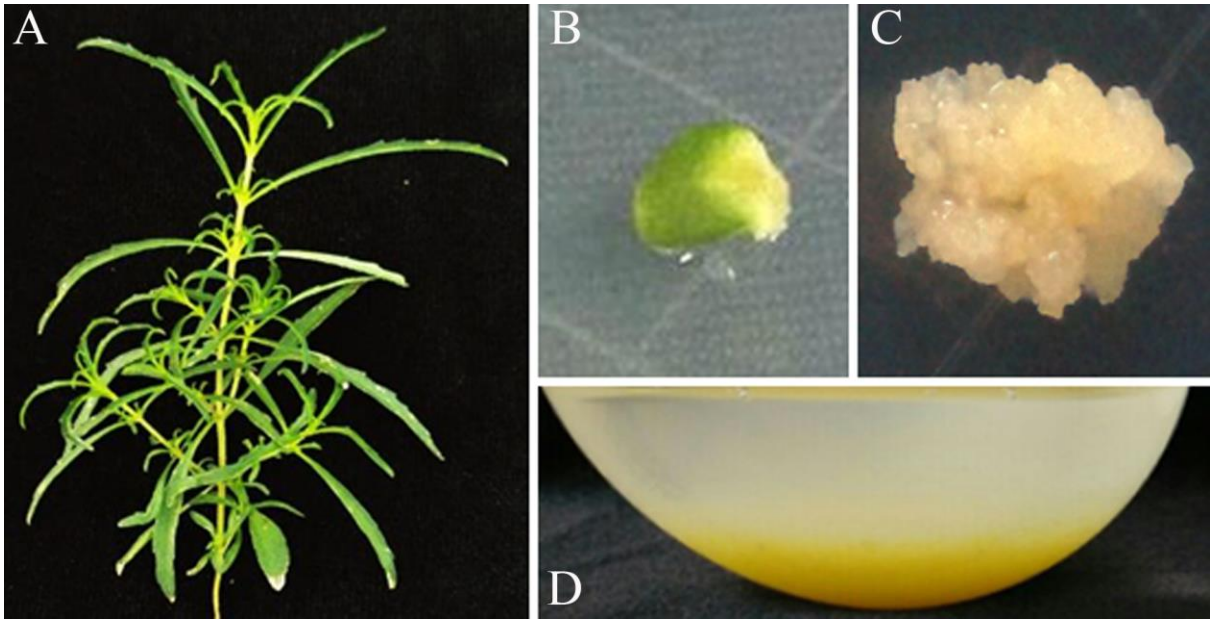

Figure 1. *Elsholtzia angustifolia* (A) and in vitro culture. Cotyledon of germinated seeds (B), callus induction at the cotyledon (C), and cell suspension cultures (D) of *Elsholtzia angustifolia*.

The composition and yield of essential oil in higher plants are affected by environmental conditions. Therefore, the development of environmentally sustainable source for essential oil production remains a challenge. Recently, it has been shown that *in vitro* culture is a useful tool for the production of essential oil (Jawdat et al. 2016). To achieve a stable production of essential oil in *E*. *angustifolia*, we have induced callus formation from cotyledon of *E*. *angustifolia* seedlings (Figure 1B and 1C), and friable calli were used to establish suspension culture (Figure 1D). In addition, the essential oil obtained from suspension cultured cells exhibited antimicrobial activity against *S*. *enteritidis* (Table 2). Although its antimicrobial activity was lower, compared with the essential oil obtained from the *E*. *angustifolia* aerial part. In addition, the presence of detectable antimicrobial activity suggested that *E*. *angustifolia* suspension culture should be useful for the stable production of antimicrobial compounds against *S*. *enteritidis*

CONCLUSION

Overall, we analyzed the chemical composition and antimicrobial activity of the essential oil obtained from the *E*. *angustifolia* aerial part, and found that it has the potential to be applied as natural food preservatives. In addition, we have introduced callus suspension culture to achieve a stable production of antimicrobial compounds. Further research on the optimization of culture and elicitation conditions will be necessary to increase the production yield of essential oil from *E*. *angustifolia* suspension culture.

CONFLICT OF INTEREST

The present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

H.J. Kwon, S. J, Lee, S. H. Eom and T.K. Hyun contributed to the experimental design and management and data analysis. S. H. Eom and T.K. Hyun prepared manuscript. All authors have read and approved the final manuscript.

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