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Biological Activities of Essential Oils from *Angelica tenuissima* Nakai

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Abstract – The current study was conducted to evaluate the antibacterial and antioxidant activities of the essential oil fraction from the roots of *Angelica tenuissima* Nakai and its main components. We extracted the essential oil fraction from the roots of *A. tenuissima* using steam distillation and isolated its main components. Their antibacterial activities were determined by broth dilution test against food-borne pathogenic bacteria. Antioxidant activities were evaluated by DPPH-scavenging assay and reducing-power test. Also tested was their ability to inhibit the growth of two gastrointestinal cancer cell lines, Caco-2 and MKN-45. The *A. tenuissima* oil fraction and its main components, ligustilide and butylidene phthalide exhibited marked inhibitory effects against most of the tested antibiotic-susceptible and antibiotic-resistant bacterial strains with minimum inhibiting concentrations (MICs) from 0.21 ± 0.08 to 3.60 ± 0.89 mg/ml. They also showed growth-inhibiting activity against Caco-2 and MKN-45 cells. The oil fraction showed significant antioxidant activities in DPPH radical scavenging assay and reducing-power test. Taken together, *A. tenuissima* essential oil could be used as a safe additive for preventing food contamination by pathogenic bacteria. Additionally, its antioxidative activity and the ability to inhibit gastrointestinal carcinoma cell lines could increase its value for functional foods and prevention of cancer.

Keywords – *Angelica tenuissima,* Essential oil, Ligusitide, Butylidene phthalide, Food-borne, Antioxidant, Anticancer

Introduction

Increasing antimicrobial resistance is an important public health issue worldwide. The development of resistance in both human and animal bacterial pathogens has been associated with the extensive use of antimicrobials for therapeutic applications and as a feed additive for livestock growth promotion (Anderson *et al*., 2003; Devi *et al.,* 2009; Wong *et al*., 2010; Haldar *et al.,* 2011; Sakaridis *et al*., 2011; Dai *et al*., 2012; Da Rocha, 2012). Increased bacterial resistance is thought to have resulted largely from the consumption of processed foods and agricultural products that have been in contact with antibiotics (Roig *et al.,* 2009; Kitiyodom *et al*., 2010; Kitaoka *et al*., 2011).

Antioxidants have been used as food additives to protect humans and food from undesirable oxidative reactions. The antioxidative activity of plant compounds has been increasingly investigated in recent decades for the development of new natural antioxidative agents (Hardin *et al*., 2010; Anthony *et al*., 2012; Prakash *et al*., 2012). Plant essential oils are a promising source of new natural antioxidants (Misharina *et al*., 2009; Mousavizadeh *et al*., 2011; Asensio *et al*., 2012).

Angelica tenuissima Nakai (Umbelliferae), a plant that grows in a deep valley area of Korea, is used in traditional medicine for pain, especially headaches from colds, rheumatic arthralgia, parietal headache and abdominal pain (Zhu, 1998; Jeong and Jung, 2002; Choi *et al*., 2010). The leaves and roots of the plant are edible and used in functional foods for anti-aging and in natural cosmetics.

In this study, we extracted a fraction from an essential oil from the dried roots of *A. tenuissima* using steam distillation. The essential oil was analyzed by gas chromatography-mass spectrometry (GC-MS), and the main components were isolated by column chromatography. The inhibitory activity of the oil was evaluated against ten antibiotic-susceptible and antibiotic-resistant food-borne pathogenic bacteria. In addition, the DPPH-scavenging and reducing activity of the *A. tenuissima* essential oil fraction and its main components were tested. The effects of *A. tenuissima* essential oils on the cell viability of Caco-2, a colon carcinoma cell line, and MKN-45, a human gastric cancer cell line, were determined by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) test (Lee *et al*., 2006: Park *et al*., 2007; Lim *et al*., 2009: Natoli *et al*., 2012; Wang *et al*., 2013).

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Experimental

Analysis of essential oil fraction by gas chromatography-mass spectrometry (GC-MS) – *A. tenuissima* was cultivated in Youngju, Korea and harvested in October, 2012. A voucher specimen was deposited at the herbarium of Duksung Women's University (No. UMANTE1). An essential oil fraction (2.4 g) was obtained from the dried roots (1 kg) of *A. tenuissima* by steam distillation for 6 h using a spontaneous distillation and extraction apparatus and analyzed using an Hewlett-Packard 6890 GC (Agilent, USA) and an Agilent 5973 network mass selective detector (280 °C) with a HP-5 MS capillary column (30 m × 250 μm × 0.25 μm). The injector was adjusted to 260ºC and the oven temperature was programmed as: initial temperature 50 ºC for 5 min, heating 2 ºC/min to 180 ºC, heating 3 ºC/min to 280 ºC, and a final temperature of 260 ºC for 10 min.

Isolation of essential oil fraction components – The essential oil fraction (1 g) was subjected to silica gel column chromatography and eluted with hexane-dichloromethane (8 : 2). Rechromatography with a silica gel column with hexane-ethyl acetate (9 : 1 and 30 : 1) of fractions 14-17 and 35-73 yielded butylidene phthalide and ligustilide. Spectral data of the isolated compounds were identical to previously published data (Sim and Shin 2008).

Bacterial strains – Antibiotic-susceptible and antibiotic-resistant bacteria were from the Korea Culture Center of Microorganisms (KCCM) and Culture Collection of Antibiotic Resistant Microbes (CCARM).

Minimum inhibiting concentrations – Minimum inhibiting concentrations (MICs) for oils were determined using a broth dilution method. Two-fold dilutions (16 - 0.125 mg/ml) of essential oils in medium containing 2% Tween-80 were prepared. *A. tenuissima* oil suspensions of 100 μl were added to 96-well plates and 100 μl prepared broth cultures (2×10^5 CFU/ml) of strains cultivated at 37 ºC were added to each well. The MIC was determined by reading the turbidity of the wells after 24 h at 37 ºC (Shin, 2009).

DPPH-scavenging effects of *A. tenuissima* essential oil – Following the method of Blois (1958), a fresh solution of 0.1 mM DPPH and two-fold dilutions of an *A. tenuissima* essential oil fraction (or its main component) (3.2 - 0.05 mg/ml, final concentration) were prepared in ethanol and 900 μl of DPPH was mixed with 100 μl of oil samples. After vortexing for 10 sec, samples were added to five wells in 96-well plates and kept at room temperature for 30 min. Decrease in absorbance was monitored at 540 nm. DPPH-radical scavenging capacity was calculated using the equation:

DPPH scavenging effects (%) = 100 × [1 – {Abs of sample/(Abs of DPPH-Abs of sample)}]

The IC_{50} was determined following the method of Blois (1958).

Determination of reducing power – Reducing power was determined according to the method of Elmastas *et al*. (2007). Various concentrations of *A. tenuissima* essential oil fraction were prepared as two-fold dilutions (12.5 - 200 μg/ml: final concentration) with methyl alcohol. Each sample (0.2 ml) was mixed with 0.6 ml of 0.2 M phosphate buffer (pH 6.6) and 0.6 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50 ºC for 20 min, and 0.6 ml of 10% trichloroacetic acid was added before 10 min of centrifugation at 1000 × *g* (MSE Mistral 2000, UK). The upper layer (1 ml) was mixed with $FeCl_3$ (0.1 ml, 0.1%), and its absorbance was measured at 700 nm. Higher absorbance indicated higher reductive capability.

Effects of *A. tenuissima* essential oil fraction and main components on viability of Caco-2 and MKN-45 cells – Caco-2 and MKN-45 cells from the Korean Cell Line Bank were cultured in RPMI medium (Corning Cellgro, USA) supplemented with penicillin (10 U/ml), streptomycin (10 μg/ml), and 10% fetal bovine serum (FBS) at 37 ºC in a humidified incubator with 5% CO_2. Cells were subcultured for experiments, with 100 μl cell suspension seeded in 96-well plates in triplicate at 5×10^4 cells/well, determined using a hematocytometer. After a 24 h for cell adherence, 5 μl of medium was removed from each well. Cells were treated with 5 μl of *A. tenuissima* essential oil in RPMI with 10% FBS containing 2% Tween 80. After 2 h, MTT solution (25 μl, 5 mg/ml in PBS) was added, and cells were incubated for 4 h at 37 ºC in 5% CO_2. The medium was discarded, and isopropanol containing 0.04 M HCl was added to dissolve the produced formazan. Absorbance was measured at 570 nm with micro-plate reader. Results were expressed as percentage of viable cells compared to untreated control.

Results and Discussion

Analysis of the essential oil fraction from *A. tenuissima* roots – The composition of the essential oil fraction from *A. tenuissima* roots, determined by GC-MS analysis and GC, is in Table 1. Among 55 compounds identified in this oil the most prominent component (56.54%) was ligustilide. The next most common

Table 1. Compounds identified by GC-MS and GC in the essential oil fraction of *A.tenuissima*

Compounds	RI[a]	Area (%)	Identified by
α-pinene	914	0.13	MS[b], GC[c]
sabinene	962	0.07	MS
β-myrcene	986	0.09	MS
2-ethyl-2-hexenal	997	0.13	MS
1-phellandrene	999	0.11	MS
octanal	1001	0.08	MS
α-terpinene	1001	0.05	MS, GC
cymene	1022	0.49	MS, GC
γ-terpinene	1026	8.52	MS, GC
cis-ocimene	1036	0.12	MS
β-ocimene	1046	0.06	MS
terpinolene	1084	0.46	MS
linalool	1099	0.44	MS, GC
camphor	1137	0.05	MS, GC
borneol	1159	0.06	MS
m-cymene	1163	0.07	MS
4-terpineol	1172	0.26	MS
p-cymen-8-ol	1181	0.30	MS
α-terpineol	1185	0.26	MS, GC
anethol	1193	0.31	MS
sabinol	1195	0.06	MS
carveol	1214	0.05	MS
cuminic aldehyde	1234	0.06	MS
δ-3-carene	1255	0.30	MS
phellandral	1269	0.19	MS, GC
bornyl acetate	1282	0.15	MS, GC
thymol	1288	0.06	MS, GC
carvacrol	1297	0.18	MS
4-ethenyl-2-methoxy-phenol	1312	0.76	MS
butyl phenyl ketone	1352	0.59	MS
3-carene	1382	0.05	MS
β-elemene	1386	0.30	MS, GC
β-caryophyllene	1413	0.20	MS
zingiberene	1437	0.32	MS
α-caryophyllene	1455	0.17	MS
β-farnesene	1465	0.14	MS
γ-selinene	1482	0.09	MS
germacrene-D	1488	0.09	MS
α-curcumen	1493	0.30	MS
β-bisabolene	1520	0.30	MS
α-farnesene	1526	0.08	MS
β-sesquiphellandrene	1535	0.55	MS
valencene	1558	0.08	MS
germacrene B	1562	0.05	MS
alloaromadendrene	1565	0.05	MS
δ-selinene	1571	0.12	MS
β-bisabolene	1574	0.14	MS
spathulenol	1585	10.29	MS
ledene	1621	0.11	MS
γ-cadinene	1625	0.06	MS
dehydroaromadendrene	1631	0.41	MS
3-n-butylphthalide	1669	1.40	MS, GC
butylidene phthalide	1693	12.61	MS, GC
ligustilide	1774	56.54	MS, GC
butylidene dihydrophthalide	2111	0.06	MS
in total		98.97	

RI[a]: retention indices calculated against C_9 to C_{24} *n*-alkanes on a HP-5MS column.
MS[b]: Mass Spectrum, GC[c]: Co-GC with a corresponding standard compound

components were butylidene phthalide (12.61%) and spathulenol (10.29%). These results generally agreed with previous data of similar analysis of this oil qualitatively. (Kim and Chi, 1989; Park *et al*., 1997). However, the relative percentage of the individual components varies in different reports. As already known there are variations in the composition of metabolites in the same species of a plant resulted from several factors, such as chemical races, cultural conditions and etc. (Lee *et al*., 2008).

The main components of the oil fraction, ligustilide (228 mg) and butylidene phthalide (4 mg), were isolated by silica gel column chromatography. Their structures were elucidated by UV, IR, ^{1}H-NMR and ^{13}C-NMR compared with those reported by Sim and Shin (2008).

Antimicrobial activities of the *A. tenuissima* essential oil fraction and its main components – The activities of the *A. tenuissima* essential oil fraction and its main components, ligustilide and butylidene phthalide, to inhibit the growth of ten food-borne pathogenic bacteria were determined as MICs (Table 2). The susceptibilities of the tested bacterial strains to antibiotics are also listed in the table. Most of the bacteria were resistant to amphicillin or oxacillin but susceptible to norfloxacin or trimethoprim/sulfamethoxazole (T/S, 1 : 19). However, *Listeria monocytogenes* strains were resistant to T/S. The activities of the essential oil fraction of *A. tenuissima* (EOAT) depended on the bacterial species, with MICs from 0.21 ± 0.08 to 3.60 ± 0.89 mg/ml. The oil fraction showed the strongest inhibition against *Shiegella sonnei* strains. Ligustilide showed similar or stronger activity than the total essential oil fraction against most of the tested bacterial strains except *Escherichia coli*. These results suggested that the first main component, ligustilide might significantly affect the antibacterial activity of the essential oil fraction with contributions from other, minor compounds. The MICs of butylidene phthalide were higher than MICs for EOAT or ligustilide.

Antioxidant activities – *A. tenuissima* roots has been used in functional health foods and in functional natural cosmetics for anti-aging. Anti-aging activity is closely related to antioxidative components. Ka *et al,* (2005) reported the antioxidant activity of *A. tenuissima* volatile extracts using an aldehyde/carboxylic acid assay, and the determination of the potential to protect against H_2O_2-induced cytotoxicity and lipid peroxidation. However, many antioxidative mechanisms are known and therefore various other methods are also needed to fully determine the antioxidative activity of *A. tenuissima* oil.

To evaluate *A. tenuissima* oil as an antioxidant, DPPH free radical-scavenging activity and reducing power were

Table 2. MICS of the essential oil of *A tenuissima* against food-borne pathogenic strains

Strains	Sample						
	EOAT	Ligustilide	Butylidene Phthalide	Ampicillin	Norfloxacin	Oxacillin	T/S*
Bacillus cererus (n = 4)	1.00 ± 0.00	0.07 ± 0.03	1.00 ± 0.00	> 32.00	0.07 ± 0.03	> 32.00	0.20 ± 0.07/ 3.84 ± 1.25
E. coli (n = 3)	1.83 ± 0.37	> 4.00	> 4.00	> 32.00	0.16 ± 0.06	> 32.00	0.25 ± 0.00/ 4.75 ± 0.00
Listeria monocytogenes (n = 2)	0.58 ± 0.19	0.46 ± 0.09	1.80 ± 0.45	> 32.00	0.46 ± 0.09	> 32.00	> 1.00 / > 19.00
Shigella boydii (n = 3)	0.40 ± 0.14	0.46 ± 0.09	2.00 ± 0.00	> 32.00	0.04 ± 0.02	> 32.00	0.12 ± 0.00/ 2.85 ± 1.06
S. flexneri (n = 3)	0.22 ± 0.06	0.21 ± 0.08	2.00 ± 0.00	> 32.00	0.06 ± 0.00	> 32.00	0.22 ± 0.06/ 2.37 ± 0.00
S. sonnei (n = 3)	0.21 ± 0.08	0.25 ± 0.00	0.22 ± 0.06	> 32.00	0.02 ± 0.01	> 32.00	0.12 ± 0.00/ 3.80 ± 1.30
Staphyllococcus aureus (n = 5)	3.60 ± 0.89	1.00 ± 0.00	4.00 ± 0.00	> 32.00	> 32.00	> 32.00	0.25 ± 0.00/ 4.75 ± 0.00
Vibrio harveyi (n = 2)	0.60 ± 0.22	0.25 ± 0.00	1.83 ± 0.37	> 32.00	0.50 ± 0.00	4.00 ± 0.00	0.20 ± 0.07/ 4.75 ± 0.00
V. parahamolyticus (n = 3)	1.00 ± 0.00	0.25 ± 0.00	1.17 ± 0.37	> 32.00	0.25 ± 0.00	> 32.00	0.12 ± 0.00/ 2.37 ± 0.00
V. vulnicus (n = 2)	3.60 ± 0.89	0.21 ± 0.08	1.00 ± 0.00	1.80 ± 0.45	0.22 ± 0.06	3.60 ± 0.89	0.08 ± 0.03/ 1.18 ± 0.00

EOAT: Essential oil fraction of *A. tenuissima*, T/S: trimethoprim/sulfamethoxazole. n: number of tested strains. Values are means ± SD of triplicate tests. Statistical analysis were by Student's *t*-test.

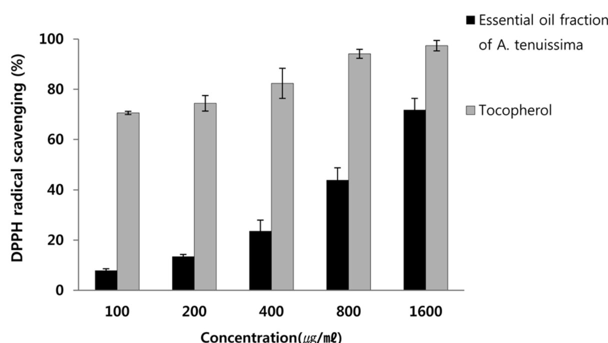

Fig. 1. DPPH Free-radical scavenging activity of essential oil fractions from *A.tenuissima* roots and tocopherol (control). Values are means ± SD of triplicate measurements.

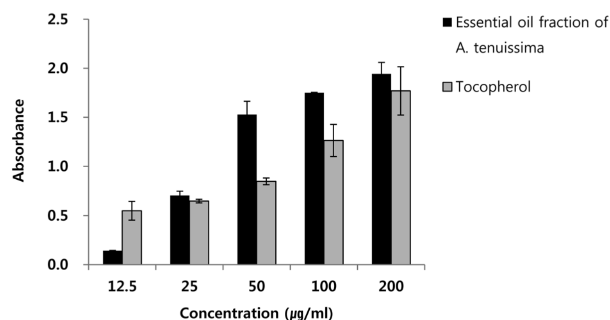

Fig. 2. Reducing power of essential oil fractions from *A. tenuissima* roots and tocopherol (control). Values are means ± SD of triplicate measurements.

studied. As shown in Fig. 1, *A. tenuissima* essential oil showed significant, dose-dependent DPPH-scavenging activity in serially diluted samples (100 - 1600 μg/ml). However, the results showed lower scavenging activity than the control, α-tocopherol. When compared at a final concentration of 1.6 mg/ml, the DPPH-scavenging activity of the oil was 73.7% the activity of α-tocopherol.

In reducing-power assays, the *A. tenuissima* essential oil fraction exhibited higher activity than α-tocopherol at concentrations of 12.5 - 200 μg/ml (Fig. 2). Effects of the oil fraction and α-tocopherol were dose dependent. At 50 μg/ml, *A. tenuissima* essential oils had 1.8-times higher reducing activity than α-tocopherol.

Activities on Caco-2 and MKN-45 carcinoma cell lines- Caco-2 is a colon carcinoma cell line and MKN-45 is a gastric carcinoma cell line. We determined the effects of *A. tenuissima* essential oil on the viability of these human gastrointestinal cancer cell lines by MTT test. Fig. 3 shows that the essential oil fraction of *A. tenuissima* and ligstilide and butylidene phthalide dose-dependently inhibited Caco-2 cell growth. The essential oil fraction of *A. tenuissima* and its two main components showed

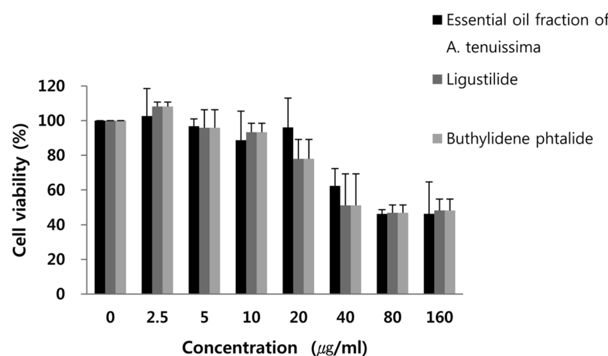

Fig. 3. Effects of essential oil fraction from *A. tenuissima* roots, ligustilide and butylidene phthalide on viability of Caco-2 cells. Values are means ± SD of triplicate measurements.

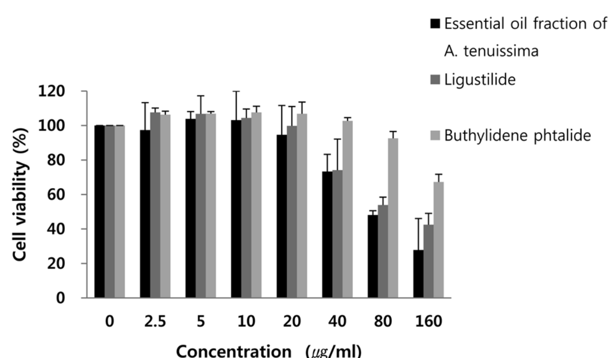

Fig. 4. Effects of essential oil fraction from *A. tenuissima* roots, ligustilide and butylidene phthalide on viability of MKN-45 cells. Values are means ± SD of triplicate measurements.

similar inhibition of cell proliferation. At 80 μg/ml of the oil fraction or its main components, Caco-2 cell growth was more than 50% inhibited compared to untreated cells. However, as shown in Fig. 4, the viability of MKN-45 cells was most strongly decreased by treatment with the essential oil fraction at 160 μg/ml compared to the individual components, resulting in a 27.82% ± 4.87% of cell survival rate. Butylidene phthalide treatment resulted in relatively little growth inhibition.

Conclusion

The results of this study indicated that *A. tenuissima* essential oil and its main components, ligustilide and butylidene phthalide, could be useful agents for treating food-borne pathogenic bacterial infections. *A. tenuissima* essential oil could also be used as a safe additive for preventing food contamination by pathogenic bacteria. The antioxidative activity of *A. tenuissima* essential oil and its ability to inhibit gastrointestinal carcinoma cell lines could increase its value for functional foods and prevention of cancer. However, further studies are required.

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References

Anderson, A.D., Nelson, J.M., Rossiter, S., and Angulo, F.J., Public health consequences of use of antimicrobial agents in food animals in the United States. *Microb. Drug Resist.* **9**, 373-379 (2003).

Anthony, K.P., Deolu-Sobogun, S.A., and Saleh, M.A., Comprehensive assessment of antioxidant activity of essential oils. *J. Food Sci*. **77**, 839-843 (2012).

Asensio, C.M., Nepote, V., and Grosso, N.R., Sensory attribute preservation in extra virgin olive oil with addition of oregano essential oil as natural antioxidant. *J. Food Sci.* **77**, 294-301 (2012).

Blois, M.S., Antioxidant determination by use of a stable free radical. *Nature.* **181**, 1199-1200 (1958).

Choi, U.K., Lee, O.H., Lim, S.I., and Kim, Y.C., Optimization of antibacterial activity of *Angelica tenuissima* leaf against *Pseudomonas aerunginosa* using the evolutionary operation-factorial design. *Int. J. Mol. Sci.* **11**, 3922-3932 (2010).

Da rocha, L.S., Gunathilaka, G.U., and Zhang, Y., Antimicrobial-resistant *Listeria* species from retail meat in Metro Detroit. *J. Food Protect.* **75**, 2136-2141 (2012).

Dai, M., Lu, J., Wang, Y., Liu, Z., and Yuan, Z., Press the escape key to close In vitro development and transfer of resistance to chlortetracycline in *Bacillus subtilis*. *J. Microbiol.* **50**, 807-812 (2012).

Devi, R., Surendran, P.K., and Chkraborty, K., Antibiotic resistance and plasmid profiling of *Vibrio parahaemolyticus* isolated from shrimp farms along the southwest coast of India. *World J. Microb. Biot.* **25**, 2005-2012 (2009).

Elmastas, M., Isildak, O., Turkekul, I., and Temur, N., Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. *J. Food Compos Anal.* **20**, 337-345 (2007).

Haldar, S., Mody, K.H., and Jha, B., Abundance, diversity and antibiotics resistance pattern of Vibrio spp. In coral ecosystem of Kursdai island. *J. Basic Microb.* **51**, 162-163 (2011).

Hardin, A., Crandall, P.G., and Stankus, T., Essential oils and antioxidants derived from Citrus by-products in food protection and medicine: an introduction and review of recent literature. *J. Agric. Food Inform.* **11**, 99-122 (2010).

Jeong, C.S. and Jung, K.H., Protective effects of *Angelica tenuissima* NaKai on Hepatotoxicity by Carbon tetrachloride in rats. *J. Appl. Pharmacol.* **10**, 211-217 (2002).

Ka, M.H., Choi, E.H., Chun, H.S., and Lee, K.G., Antioxidative activity of volatile extracts isolated from *Angelica tenuissimae* roots, Peppermint leaves, Pine needles, and Sweet flag leaves. *J. Agr. Food Chem.* **53**, 4124-4129 (2005).

Kim, H.S. and Chi, H.J., Studies on essential oils of plants of Angelica genus in Korea (II). Essential oils of the root of *Angelica tenuissima*. *Kor. J. Pharmacogn.* **20**, 13-20 (1989).

Kitiyodom, S., Khemtong, S., Wongtavatchai, J., and Chuanchuen, R., Characterization of antibiotic resistance in *Vibrio* spp. Isolated from farmed marine shrimps (*Penaeus monodon*). *FEMS. Microbiol. Ecol.* **72**, 219-227 (2010).

Kitaoka, M., Miyata, S.T., Unterweger, D., and Pukatzki, S., Antibiotic resistance mechanisms of *Vibrio cholerae*. *J. Med. Microbiol.* **60**, 397-

407 (2011).

Lee, H.W., Choi, J.H., Park, S.Y., Choo, B.K., Chun, J.M., Lee, A.Y., and Kim, H.K., Constituents comparison of components in native and cultivated species of *Angelica tenussima* NaKai. *Kor. J. Med. Crop. Sci.* **16**, 168-172 (2008).

Lee, K.H., Lee, Y.C., Kim, T.I., Noh, S.H., Kim, J.Y., Paik, H.D., and Kim, C.H., Inhibitory effect of Capsaicin on interleukin–8 production by Helicobacter pylori-infected MKN-45 cells. *J. Microbiol. Biotechnol.* **16**, 1078-1083 (2006).

Lim, S.M., Ahn, D.H., and Im, D.S., Inhibitory effect of *Lactobacillus plantarum* K11 on the Adhesion of *Escherichia coli* 0157 to Caco-2 cells. *Food Sci. Biotechnol.* **18**, 343-349 (2009).

Misharina, T.A., Terenina, M.B., and Krikunova, N.I., Antioxidant properties of essential oils. *Appl. Biochem. Microbiol.* **45**, 642-647 (2009).

Mousavizadeh, S.J. and Sedaghathoor, S., Peroxidase activity in response to applying natural antioxidant of essential oils in some leafy vegetables. *Aust J Crop Sci.* **5**, 494-499 (2011).

Natoli, M., Leoni, B.D., D'Agnano, I., Zucco, F., and Felsani, A., Good Caco-2 cell culture practices. *Toxicol in Vitro.* **26**, 1243-1246 (2012).

Park, H.K., Lee, S.I., Lee, S.H., Park, H.M., and Rhee, J.S., A study on the qualitative and quantitative analysis of essential oil in *Angelicae tenuissimae* radix of Ligustici rhizoma. *Kor. J. Food Sci. Tech.* **29**, 189-193 (1997).

Park, S.H., Kim, Y.A., Chung, M.J., Kang, B.Y., and Ha, N.J., Inhibition of proliferation by Anti-microbial peptide isolated from *Pediococcus pentosaceus* and *Lactobacillus* spp. on colon cancer cell line (HT-29, SW 480 and Caco-2). *J. Environ. Toxicol.* **22**, 65-71 (2007).

Prakash, B., Singh, P., Kedia, A., Singh, A., and Dubey, N.K., Efficacy of essential oil combination of *Curcuma longa* L. and *Zingiber officinale* ROSC. as a postharvest fungitoxicant, aflatoxin inhibitor and antioxidant agent. *J. Food Safety.* **32**, 279-288 (2012).

Roig, F., Llorens, A., Fouz, B., and Amaro, C., Spontaneous quinolone resistance in the zoonotic serovar of *Vibrio vulnificus. Appl. Environ. Microb.* **75**, 2577-2580 (2009).

Sakaridis, I., Soultos, N., Iossifidou, E., Papa, A., Ambrosiadis, I., and Koidis, P., Prevalence and antimicrobial resistance of *Listeria monocytogenes* isolated in chicken slaughterhouses in Northern Greece. *J. Food Protect.* **74**, 1017-1021 (2011).

Shin, S., Antibacterial effects of vulgarone B from *Artemisia iwayomogi* alone and in combination with oxacillin. *Arch. Pharm. Res.* **32**, 1711-1719 (2009).

Sim, Y. and Shin, S., Combinatorial anti-*Trichophyto*n effects of *Ligusticum chuanxiong* essential oil components with antibiotics. *Arch. Pharm. Res.* **31**, 497-502 (2008).

Wang, Y.C. and Huang, K.M., In vitro anti-inflammatory effect of apigenin in the *Helicobacter pylori*-infected gastric adenocarcinoma cells. *Food Chem. Toxicol.* **53**, 376-383 (2013).

Wong, M.R., Reddy, V., Hanson, H., Johnson, K.M., Tsoi, B., Cokes, C., Gallagher, L., Lee, L., Plentsova, A., Dang, T., Krueger, A., Joyce, K., and Balter, S., Antimicrobial resistance trends of *Shigella* Serotypes in New York City, 2006-2009. *Microb. Drug Resist.* **16**, 151-154 (2010).

Zhu, Y.P., *Chinese Materia Medica,* Amsterdam. Nederland: Harwood Academic Publishers, pp. 54-55 (1998).

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