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Biological Activities of the Essential Oil from *Angelica acutiloba*

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Abstract – *Angelica acutiloba* is one of the most intensively cultivated medicinal plants in Korea. The roots of this plant have been used as an important herbal drug, especially for the treatment of various female disorders, as the traditional therapy in Korea and other Asian countries. Consumption of its fresh leaves as a healthy vegetable has recently increased. In this study, essential oil fractions were extracted from the roots and leaves of this plant by steam distillation. Compositions of the two oils were compared by gas chromatography-mass spectrometry (GC-MS). The antibacterial activities of the essential oil were determined against three strains of *Escherichia coli*. DPPH radical scavenging and reducing power tests were performed to evaluateits antioxidant activities. The cytotoxic activities of the essential oil against a human breast and a uterine cancer cell line were estimated by MTT tests. Additionally, the morphological changes after treatment of the oil fraction were observed under a microscope. The essential oil fraction and its main components, *Z*-ligustilide and butylidene phthalide, inhibited the growth of three *E. coli* strains examined, with minimum inhibiting concentrations (MICs) ranging from 1.0 mg/ml to 8.0 mg/ml. Additionally, the essential oil fraction of *A. acutiloba* exhibited significant DPPH free radical scavenging activity and reducing power. Significant cytotoxic activities of the *A. acutiloba* essential oil were observed for human uterine (Hela) and breast (MCF-7) cancer cell lines.
Keywords – *Angelica acutiloba*, Essential oil, *Escherichia coli,* DPPH, Reducing power, MCF-7, Hela

Introduction

The roots of *Angelica acutiloba* are intensively cultivated as a medicinal plant in Korea and have been used as an important herbal drug, like *A. gigas* and *A. sinensis,* especially for the treatment of various female disorders, as the traditional therapy in Korea and other Asian countries (Chang and But, 1986; Kang *et al.*, 2003; Choi and Yang, 2005; Liu *et al.*, 2005). The consumption of its fresh leaves, having a unique strong aroma, as a healthy vegetable has increased recently. In previous reports studying the essential oil of *A. acutiloba,* various phthalides, including *Z*-ligustilide and butylidene phthalide, as well as monterpenes, sesquiterpenes, and other hydrocarbons, were identified (Du *et al.,* 2002; Park *et al.*, 2003).

In this study, the essential oil fractions were extracted from the roots and leaves of this plant by steam distillation. The compositions of the two oils were compared by gas chromatography-mass spectrometry (GC-MS). Their antibacterial and antioxidant activities were investigated. Additionally, cytotoxic activities of the essential oil were estimated by MTT tests against human uterine (Hela) and breast (MCF-7) cancer cell lines, and the morphological changes after treatment of the oil fractionwere observed under a microscope.

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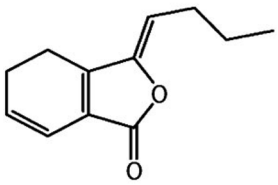
Experimental

Analysis of essential oils from *A. acutiloba* – Essential oils were extracted by steam distillation from the roots (fresh and dried) and fresh leaves of *A. acutiloba* cultivated in Jinbu Pyungchang, Gangwondo, Korea and analyzed for antibacterial activity. The oil (0.54%) obtained was analyzed using a Hewlett-Packard 6890 GC and Hewlett-Packard 5973 MSD apparatus (Agilent 5973 network mass selective detector, 280 °C) with an HP-5 MS (5% phenylmethylsiloxane, 50 m × 200 μm × 0.11 μm) fused silica capillary column. The injector was adjusted to 250 °C, and the oven temperature was regulated as follows: initial temperature at 60 °C for 5 min, 2 °C/min to 230 °C, and 30 min at 180 °C.

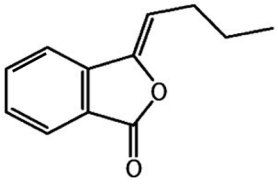
Strains – Three EPEC *E. coli* strains, *E. coli* CCARM 1G149, *E. coli* CCARM 1G207, and *E. coli* KCCM 41290, were subdivided from the Korean Culture Center of Microorganisms (KCCM) and Culture Collection of

Antibiotic Microbes (CCARM). Organisms were subcultured in Müller Hinton Broth (YM, Difco, USA) for 28 h at 37 °C. The turbidity of the cell suspension was measured at 600 nm and adjusted with medium to matchthe 0.5 McFarland standard ($10^5 - 10^6$ colony forming units (CFU)/ml).

Compounds – *Z*-ligustilide, butylidenephtahlide, and butyl phthalide were isolated from the essential oil fraction of the dried roots of *A. acutiloba* with silicagel column chromatography by the method reported previously (Sim and Shin, 2008). Amphicillin (99%) and norfloxacin



***Z*-Ligustilide**



Butylidenephthalide

(99%) were purchased from Sigma Chemical Co. (USA).

Determination of minimal inhibitory concentration (MIC) – The MIC tests were performed as previously reported (Lim and Shin, 2011). The MIC value was defined as the lowest concentration that inhibited more than 50% of visible bacterial growth after 24 h. Each organism was additionally cultured with blank solution containing Tween 80 at concentrations equivalent to the test solutions.

Evaluation of DPPH scavenging effects of the essential oil from *A. acutiloba* – A fresh solution of 0.1 mM DPPH and a range of two-fold dilution aliquots (3.2 - 0.05 mg/ml, final concentration) of *A. acutiloba* essential oil fraction (or its main component) were prepared in ethanol. Then, 900 μl of DPPH solution was mixed with 100 μl of each concentration of the oil samples. After vortexing the mixtures for 10 sec, they were added to five wells on 96-well plates and kept at room temperature for 30 min. The decrease in absorbance was monitored at 540 nm. DPPH radical scavenging capacity was calculated using the following 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 – The reducing power was determined according to the method of Elmastas *et al.* (2007). Various concentrations of the essential oil fraction from *A. acutiloba* and its main components were prepared by a range of two-fold dilution (0.25 ~ 4.0 mg/ml) with methyl alcohol mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50 °C for 20 min, 2.5 ml of trichloroacetic acid (10%) was then added to the mixture before centrifugation for 10 min at 1,000 g (MSE Mistral 2000, UK, Serial no: S693/ 02/444). The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. A higher absorbance indicated a higher reductive capability.

Results and Discussion

E. coli comprises more than four types of strain, enterotoxigenic (ETEC), enteroinvasive (EIEC), enteropathogenic (EPEC), and enterohemorrhage (EHEC), related with gastrointestinal disorders in humans. Among them, EPEC strains cause diarrheal outbreaks in hospital nurseries and chronic diarrhea in children, as well as in elderly persons (Race *et al.*, 2007; Kiran*et al.*, 2010). The emergence of resistant strains of *E. coli* has increased progressively, particularly due to the consumption of processed food and agricultural products in contact with antibiotics. The quinolones generally used against β-lactam-resistant *E. coli* are relatively safe drugs. However, the adverse effects could be serious in the case of children and in elderly persons because quinolones can induce arthropathies (Scholar and Platt, 2000; Owens and Ambrose, 2005). Essential oils are known as a promising source for new natural antibiotics (Burt, 2004; Shin and Lim, 2004). With a view on developing safe and effective agents, the essential oil from *A. acutiloba* was obtained by steam-distillation and analyzed by GC-MS, and the activity against three antibiotic-resistant EPEC strains of *E. coli* was investigatedby MIC tests.

As listed in Table 1, 27 and 29 compounds were identified from the essential oils in the fresh leavesand roots using GC and GC-MS analysis, respectively. These two oils contained 16 compounds in common. *Z*-ligustilide was identified as the predominant component of the essential oil fraction from the roots (67.97%), as well as from the leaves (46.54%). The relative contents of butylidene phthlide and butyl phthalide were higher in the roots than in theleaves. In the previous report by Park *et al*. (2003) these two compounds were identified only in the essential oil from the leaves but not in the root

Table 1. Compounds identified in the essential oil fractionsfrom fresh leaves and roots of *A. acutiloba* by GC-MS

Compounds	RI	Area (%)		Identification
		Leaves	Roots	
α-pinene	981	0.17	–	GC, MS
camphene	1001	0.41	0.09	GC, MS
sabinene	1026	–	0.05	MS
β-myrcene	1047	1.68	0.61	MS
octanal	1059	–	0.12	MS
α-terpinene	1070	–	0.05	MS
1-methyl-4-(1-methylethyl)-benzene	1080	12.13	4.68	MS
limonene	1083	1.42	0.58	MS
cis-ocimene	1096	0.39	0.58	MS
γ-terpinene	1116	10.76	10.71	MS
linalool	1161	0.17	0.18	GC, MS
neo-allo-ocimene	1192	–	0.11	MS
pentyl-benzene	1219	0.44	–	MS
borneol	1227	0.14	–	GC, MS
terpinene-4-ol	1240	0.18	0.34	GC, MS
thymyl methyl ether	1300	–	0.09	MS
bornyl acetate	1356	0.24	0.41	GC, MS
lavandulyl acetate	1365	0.45	–	MS
thymol	1375	–	0.07	GC, MS
phthalic anhydride	1381	1.52	–	GC, MS
1-phenyl-1-pentanone	1430	–	0.07	MS
β-elemene	1473	–	0.22	MS
β-caryophyllene	1502	0.23	0.25	MS
khusimone	1518	–	0.07	MS
aromadendrene	1542	–	0.08	MS
trans-β-farnesene	1546	0.92	0.51	MS
cyclodecane	1562	0.13	–	MS
α-cedrene	1620	–	0.02	MS
α-bisabolene epoxide	1660	0.11	0.00	MS
nerolidol	1668	–	0.17	MS
caryophyllene oxide	1689	0.86	0.19	GC, MS
carotol	1705	–	0.19	MS
t-cadinol	1754	0.14	–	MS
1,4-benzenedicaboxaldehyde	1759	0.31	–	MS
butylphthalide	1765	4.57	2.41	GC, MS
α-guaiene	1779	0.23	–	MS
butylidene phthalide	1785	10.12	5.79	GC, MS
Z-ligustilide	1867	46.54	67.97	GC, MS
clovene	1933	0.04	–	MS
vulgarol B	1993	0.35	–	MS
In total		**95.64**	**95.98**	

***RI*:** GC retention indices calculated against C_9 to C_{24} *n*-alkanes on an HP-5MS capillary column.

essential oil. However, they were identified as main components of the essential oil from the roots of *A. acutiloba* in the study by Du *et al.* (2002).

Furthermore, 1-methyl-4-(1-methylethyl)-benzene and

Table 2. MICs of the essential oil fractions from the dried roots and the fresh leaves of *A. acutiloba* against three EPEC strains of *E.coli*

Samples (mg/ml)	Strains of *E. coli*		
	CCARM 1G149	CCARM 1G207	KCCM 41290
Root essential oil fraction	4.00	2.00	2.00
Leaf essential oil fraction	2.00	1.00	1.00
Butylidene phthalide	4.00	4.00	4.00
Z-ligustilide	8.00	8.00	8.00
Amphicillin*	> 512	512	> 512
Norfloxacin*	0.12	0.12	0.02

* μg/ml

γ-terpinene showed relatively higher content in both oils. However, the activity of these two components was not assessed in further experiments, as they are non-oxygenated hydrocarbons, which are generally inactive.

As demonstrated in Table 2, the *A. acutiloba* essential oil fractions and its main components, butylidene phthalide and *Z*-ligustilide, inhibited the three ampicillin-resistant EPEC strains of *E. coli,* with MIC values in the range of 1.00 - 8.00 mg/ml. There were no significant differences in the results between the *E. coli*strains. The essential oil fraction of the leaves showed two-fold stronger inhibiting activity than the root essential oil against all three tested strains. *Z*-ligustilide, the predominant compound of this oil, and butylidene phthalide exhibited generally lower activity than the total essential oil, indicating a higher contribution of other constituents in the essential oil fractions to the activity.

In view of the increasing consumption of the fresh parts of this plant as healthy foods in recent time, the antioxidant activity has been an important target of study concerning the physiological activities of plant essential oils (Base *et al.,* 2010). Kim *et al.* (2009) studied the antioxidant activities of various solvent extracts from this plant and confirmed especially high activity in the hexane extract. In the present study, the essential oil fraction was obtained from the fresh leaves and roots, and their composition was analyzed by GC-MS. The antioxidant activities of the essential oil fraction and main components isolated by column chromatography were evaluated and compared.

The DPPH scavenging and reducing powers are important indicators of the potential antioxidant activity. As demonstrated in Fig. 1, the essential oil of *A. acutiloba* and its main components showed significant DPPH scavenging activities in a dose-dependent manner. In the DPPH test, both the essential oil fraction and *Z*-ligustilide

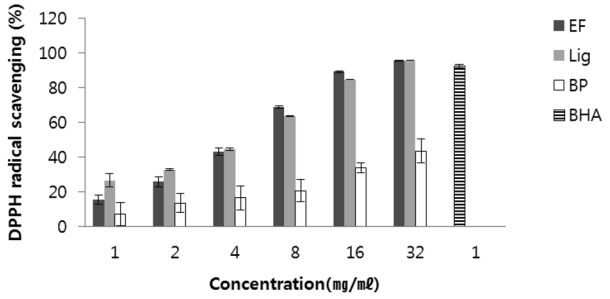


Fig. 1. DPPH free radical scavenging effects (%) of the essential oil fraction, *Z*-ligustilide, butylidene phthalide, and BHC from the roots of *A. acutiloba*. Values are mean ± SD of triplicate tests. Statistical analysis was performed with Student's *t*-test. BHA (1 mg/ml) was used as a control. EF: the essential oil fraction, Lig: *Z*-ligustilide, BP: butylidene phthalide.

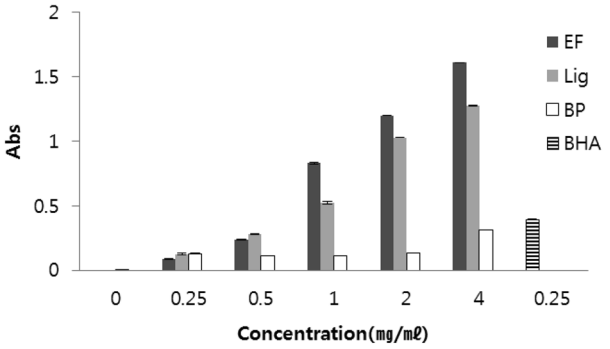


Fig. 2. Reducing power of the essential oil fraction (EF), *Z*-ligustilide (Lig), butylidene phthalide (BP), and BHC from the roots of *A.acutiloba*. Values (absorbance at 700 nm) are mean ± SD of triplicate tests. Statistical analysis was performed with Student's *t*-test. BHA (0.12 mg/ml) was used as a control. EF: the essential oil fraction, Lig: *Z*-ligustilide, BP: butylidene phthalide.

(95.38 ± 0.14% and 95.41 ± 0.07%, respectively) showed higher activity than butylidene phthalide (43.44 ± 7.09%) at a 32 mg/ml sample concentration. BHA, the control compound, exhibited a similar scavenging rate (92.27 ± 0.83%) at 1 mg/ml.

In reducing power assays, the essential oil fraction exhibited the highest activity among the three tested samples (Fig. 2). A higher absorbance indicated a higher reducing power in this test. At a concentration of 4 mg/ml, its absorption was 1.60 ± 0.002, while those of *Z*-ligustilide and butylidene phthalide were 1.27 ± 0.001 and 0.31 ± 0.001, respectively. BHA showed a greater than three times higher absorbance than the *A. acutiloba* essential oils.

Though the antioxidant activity of the *A. acutiloba* essential oils appeared weaker than BHA in this study, it could still be a promising natural antioxidant source if the toxicity of BHA is considered (Liu *et al.*, 2011).

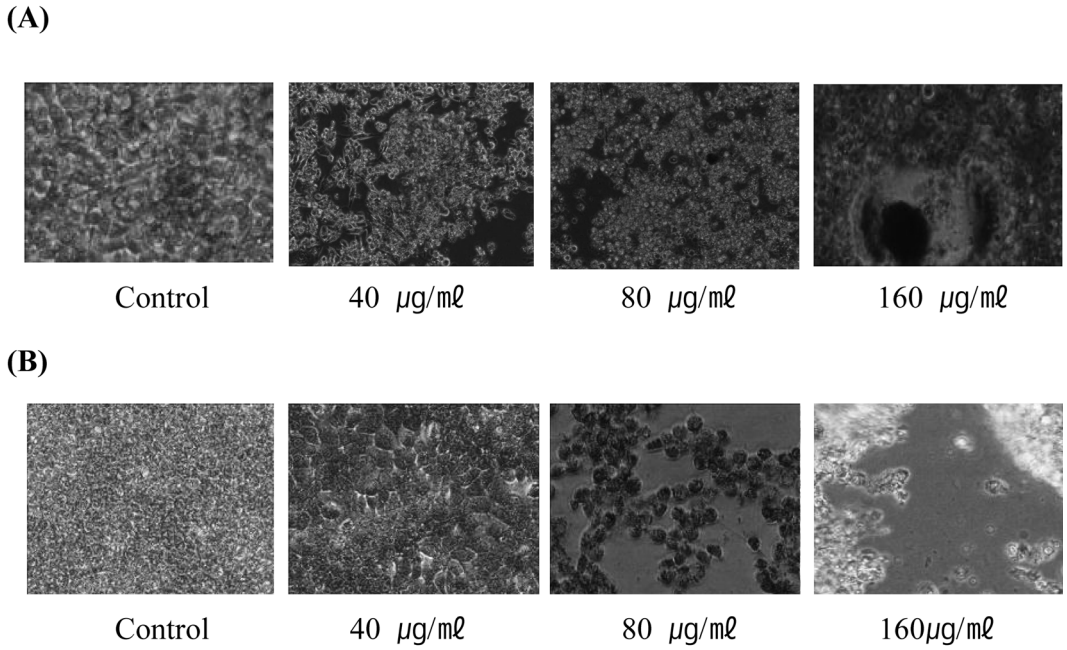


Fig. 3. Changes of cell morphology of Hela (A) and MCF-7 (B) cells after treatment with various concentrations (0 - 160 ug/ml) of the essential oil fraction from the roots of *A. acutiloba* for 24 h were observed by light microscope. Magnification, × 200.

Table 3. Effects of the essential oil fraction from the roots of *A. acutiloba*, *Z*-ligustilide, and butylidene phthalide on the proliferation of Hela cells

μg/ml	EF	Lig	BP
0	100.000 ± 6.519	100.000 ± 2.819	100.000 ± 2.093
10	93.295 ± 6.133	102.408 ± 6.981	95.533 ± 3.760
20	76.831 ± 6.263	94.967 ± 2.013	87.809 ± 1.148
40	45.812 ± 4.464	66.409 ± 4.600	54.755 ± 3.569
80	16.440 ± 1.151	25.100 ± 0.481	25.245 ± 0.173
160	16.225 ± 0.724	31.244 ± 3.265	16.599 ± 0.449

EF: the essential oil fraction, Lig: *Z*-ligustilide, BP: butylidene phthalide.
The values (% of live cells) are mean ± SD (n = 3). Significant differences ($^{*}p < 0.05$) between the means were determined by Student's *t*-test.The values represent mean values from experiments performed in triplicate. *μg/ml.

Table 4. Effects of the essential oil fraction from the roots of *A. acutiloba*, *Z*-ligustilide, and butylidene phthalide on the proliferation of MCF-7 cells

μg/ml	EF	Lig	BP
0	100.000 ± 1.125	100.000 ± 2.765	100.000 ± 4.509
10	103.983 ± 1.815	100.157 ± 8.862	104.571 ± 3.257
20	99.868 ± 1.853	96.770 ± 4.765	100.773 ± 5.658
40	78.327 ± 3.286	73.179 ± 9.208	93.597 ± 3.860
80	31.187 ± 1.639	10.063 ± 0.558	56.392 ± 6.679
160	11.138 ± 0.214	9.652 ± 0.373	11.747 ± 1.424

EF: the essential oil fraction, Lig: *Z*-ligustilide, BP: butylidene phthalide.

The roots of *A. acutiloba* have been used as an important drug in oriental medicine, especially for the treatment of various gynecological diseases. To test for the possibility of developing a natural anticancer agent from this plant, various concentrations of the essential oil fraction were treated in culture suspensions of Hela and MCF-7 cells. The survival rates of the cell were determined by MTT tests, and the morphological changes were observed after treatment of the essential oil fraction of *A. acutiloba*. As listed in Table 3 and Table 4, the *A. acutiloba* essential oil fraction affected the cell growth of the two tested cancer cell lines in a mostly dose-dependent manner. At a concentration of 40 μg/ml, the cultured suspension of Hela cells showed an absorbance of 45.81 ± 4.46, indicating an inhibition of cell proliferation greater than 50%, while only 21.68% of MCF-7 cell growth was inhibited. However, at 160 μg/ml, the activity of the treated sample on MCF-7 cells was higher than that of the Hela cells. The changes of cell morphology observed 24 hours after treatment of the *A. acutiloba* essential oil fraction are demonstrated in Fig. 3. Significant changes of cell morphology were confirmed from the sample concentration of 20 μg/ml. At this sample concentration, most of the cells were floating dead in the culture media.

In conclusion, the antibacterial activities against *E. coli,* the anti-oxidant activities, and the inhibition of cell proliferation of cancer cell lines were studied for the essential oil of *A. acutiloba* in order to develop a novel source of a natural drug. This oil showed significant effects in all of the studied tests, and its antioxidant activity was highly significant. However, further studies are necessary to assess their potential for practical application.

Acknowledgement

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