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Screening of Antimicrobial Activity against Enterohemorrhagic *Escherichia coli* O157:H7 from Plants in Korea

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Abstract

We screened the methanol extracts from 133 plant species growing in Korea for antimicrobial activity against enterohemorrhagic *Escherichia coli* O157:H7. Those plants are selected from three plant groupings; traditional medicinal herbs, edible plants, and flowers. They were tested by disk diffusion assay. From evaluation of the inhibition zone diameter of microbial growth, we found that the flower extract of *Rhododendron Schlipenbachii* Max had the most significant antimicrobial activity. Among the medicinal herbs, the extracts of *Prunus mume* S. et Z. had the best antimicrobial activity against this bacteria. Extracts from most of the vegetables and plants did not show antimicrobial activity except for the leaves of *Ginkgo biloba* L. and the seeds of *Prunus Sallicina* L.

Key words: antimicrobial activity, *Escherichia coli* O157:H7, Korea, plants

INTRODUCTION

Escherichia coli O157:H7 was first identified during a 1982 outbreak of severe diarrhea and hemorrhagic symptoms in the United States, whose presence was traced back to contaminated hamburgers. Since then, more than 20,000 cases of infection occurred annually in the country, leading to about 250 fatal cases (1). Enterohemorrhagic strains of *E. coli* O157:H7 produce Shiga-like toxins, which put the patients into a spectrum of illnesses, such as milk diarrhea, severe bloody diarrhea, hemolytic uremic syndrome, and death (2), especially among young children and the elderly (1,3). Its occurrence has been closely associated with either the consumption of contaminated ground beef, raw milk and fermented hard salami or person-to-person transmission by the fecal-oral route (1,4-7).

Globally, cases have been reported in more than two dozens countries including Canada, Britain and France (8). The largest outbreak of the *E. coli* O157:H7 infections, with approximately 6,000 patients, was reported in Japan in 1996 (9). In Korea, the O157 bacteria has been recently found in imported beef from the United States (Sep 26, 1997), and in the hamburger patties at a snack bar in Kwangju (May 19, 1998). Also an infection case was reported by the Chungbuk University medical center (May 17, 1998). These cases have driven the Korean government to enforce a stricter guidelines on testing for *E. coli* O157:H7 in imported foods by the National animal quarantine service and Korean food and drug administration.

In onder to reduce and eliminate *E. coli* O157:H7, they have studied the effects of gamma irradiation (10-12), bovine lactoferrin and lactoferricin B (13), bacteriocin (14), vinegar (15), fumaric acid (16), spices (17) as well as several antimicrobial agents (18). Several Korean plants were also put into the antimicrobial screening test against the food-borne pathogens and food spoilage organisms including *E. coli* from Korean plants (19,20). However, Until now, no report has yet appeared on any significant antimicrobial activity against *E. coli* O157:H7 among the Korean medicinal herbs and wild plants.

In this report, we have conducted an antimicrobial screening test against enterohemorrhagic *E. coli* O157:H7 on 142 methanol extracts from 133 plant species growing in Korea which were selected primarily on the basis of their antibacterial activity in traditional oriental medicinal formulas.

MATERIALS AND METHODS

Plant material

The plant materials were collected out of Chungchung-Do district in Korea during March~October in 1996. These materials were identified referring to a botanical encyclopedia (21). The vegetable samples and the medicinal herbs were purchased from several local markets in Korea. The plant materials were dried in the shade and stored at room temperature for a few days until further processing was done.

Preparation of extracts

Preparation of extracts from the dried plant materials (10 g) was done by fine grounding and extraction with 100 mL of methanol for 24 h on a shaker. The plant extract was filtered, and then the filtrate was evaporated to dryness under reduced pressure at 50°C.

Test organisms

We used both *E. coli* O157:H7 and *E. coli* ATCC (American type culture collection, NJ, USA) 25922 as the test organisms.

Antimicrobial activity test

We adopted the disc-plate method used for antibacterial screening developed by Lennette (22) with some modifications:

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One hundred ml of diluted culture was spread on a sterile Muller-Hilton agar (BBL) plate. For the preparation of base plates, we poured the nutrient agar and tryptic soy agar (TSA, Difco) including peptone and beef extracts into sterile Petri-dishes (9 cm), which were then allowed to set. The impregnated discs were then placed on the plates and incubated for 30 min for the ingredients to be diffused homogeneously. Then methanol was introduced in the same amount as the extracts; 20 μl per disk. The plates were incubated for 24~48 h until the resulting zone of inhibition was observed and recorded. The diameter of the inhibitory zone was observation recorded with a resolution of 1 mm. The test was triply duplicated to insure reproducibility.

RESULTS AND DISCUSSION

The plants used in the traditional Korean medicines and foods were selected from three plant groupings; flowers, traditional medicinal herbs, and edible plants. The antibacterial activities of the 24 methanol extracts of the flowers were screened by modified disc plate diffusion assay. Table 1 presented the results of antibacterial activity of 24 flowers. Out of the 24 samples, as shown in Table 1 the antibacterial activity against *E. coli* ATCC 25922 was observed in 13 extracts. Against *E. coli* O157, however, only the extracts from *Rhododendron Schlipenbachii* Max showed any effective antimicrobial activity.

Table 2 presents the antibacterial activity of 64 Korean traditional medicinal herbs, mostly used against bacterial infections, among which 28 herbs are also used as food (23). Even though these plants have been reported to have an antimicrobial effect against clinical and food pathogenic microorganisms (24,25), they showed only minor antimicrobial activities against *E. coli* O157:H7 in this study. Among the 64 medicinal herbs, the highest antimicrobial activity against the bacterial was observed from the extracts of *Prunus mume* S. et Z., *Lonicera japonica, Paeonic albiflora* and *Euenia caryophyllata* Thunb. It is worth mentioning that the addition of *Euenia caryophyllata* Thunb and *Cinnamomum cassia* Blume into cooked meals can exhibit antibacterial activity against *E. coli* O157:H7 (17). Against *E. coli* O157:H7, the excellent antibacterial activity of *Prunus mume* S. et Z. may be closely related to its high activity. However, considering that *E. coli* O157:H7 can easily survive in acidic condition (26), we cannot exclude the possibility of the presence of some unknown compounds in *Prunus mume* S. et Z., which may effectively act against the bacteria.

The antibacterial activity results of vegetables and plants are presented in Table 3. Against *E. coli* O157, most extracts from vegetables and plants had only minor antimicrobial activity compared with the medicinal herbs, except the leaves of *Ginkgo biloba* L. and the seeds of *Prunus sallicina* L. In one hamburger study, *Allium satium* var. *pekinense* (garlic) is found to have the excellent inhibitory effect against the *E. coli* O157:H7 (17); however, its methanol extract did not show any activity in our study. It suggests that *Allium satium* var. *pekinense* may have some antimicrobial active compounds that cannot be extracted by methanol.

CONCLUSION

In this report, we present the antimicrobial screening result of the selected 133 plant species growing in Korea against *E.*

Table 1. Antimicrobial activity of the flowers extracts by disk diffusion assay

Botanical name	English name	Inhibition zone (mm) per microorganism	
		E. coli O157:H7	*E. coli* ATCC 25922
Allium fistulosum L. var. *Giganteum* Makino	Welsh onion	12	14
Castanea crenata Sieb. et. Zucc.	Chestnut	12	14
Celosia cristata L.	Cockscomb	11	12
Dianthus caryophyllus L.	Carnation	-	-
Hibiscus syriacus L.	Rose of sharon	10	10
Hosta plantaginea Ascher	Hosta	10	10
Hydrangea macrophylla sering var. *Otaksa* Makino	Japanese hydrangea	17	17
Koelreuteria paniculata Lax	Golden rain tree	14	15
Lespedeza bicolor Turcz var. *Japanica* Nakai	Bush clover	-	-
Lilium lancifolium Thunb	Lily	-	-
Magnolia kobus A.P.DC.	Kobus Magnolia	-	-
Magnolia liliflora Desr.	Lily Magnolia	14	14
Malus Pumila Mill	Apple	-	-
Nicotiana Tabacum L.	Tabacco	-	11
Prunus serrulata L. var. *Spontanea* Makino	Japanese flowering cherry	-	-
Rhododendron schlipenbachii Max	Rhododendron	15	13
Robinia pseucoacacia L.	Black locust	-	-
Rosa centifolia L.	Rose	17	16
Rudbeckia laciniata L.	Chrysanthemum	-	-
Syringa Vulgaris L.	Lilac	10	10
Tagates Patula L.	French marigold	-	-
Taraxacum platycarpum Dahlst	Dandelion	-	-
Trifolium repense L.	Clover	14	15
Wistaria Floribunda De Candolle	Japanese Wistaria	-	-

Table 2. Antimicrobial activity of the medicinal herbs extracts by disk diffusion assay

Botanical name	English name	Plant parts	Inhibition zone (mm) per microorganism	
			E. coli O157:H7	*E. coli* ATCC 25922
Acanthopanax gracilistylus W. W. Smith	Cordex Acanthopanacis	fruits	-	-
Achyranthes bidenta var. japonica M.	Niuxi	roots	-	-
Acorus tatarinowii Schott.	Rhizoma Acori tatarinowii	roots	-	-
Adenophoratriphylla D.C.	Ginseng	roots	-	-
Alisma orientalis (Sam.) Juzeep.	Rhizoma alismtis	stems	-	-
Aloe vera L.	Aloe	leaves	-	-
Angelica sinensis Oliv Diels	Radix Angelicae sinensis	roots	-	-
Astragalus membranaceus Bunge	Astragalus	roots	-	-
Aucklandia lappa Decue	Radix Aucklandiae	roots	-	-
Biota orientalis	Oriental arborviae	leaves	-	-
Carthamus tinctorium L.	Flos carthami	flowers	-	-
Cassia obtusifolia L.	Semen cassiae	fruits	-	-
Chaenomeles sinensis Koehne	Chinese Quince	fruits	-	-
Chrsanthmum indicum L.	Chrysanhemum	stems, leaves	-	-
Cibotium barometz L. J. Sm.	Rhizoma Cibotii	roots	12	10
Cimicifuga foetada L.	Rhizoma cimicifugae	leaves	-	-
Cinnamomum cassia Blume	Cinnamon	barks	13	13
Crataegus pinnatifida Bunge	Hawthorn	fruits	-	-
Cuscuta chinensis Lam.	Semen cuscutae	seeds	-	-
Cyathula officinalis Kuan	Chuanniuxi	roots	-	-
Cyperus rotundus L.	Rhizoma cyperi	roots	-	-
Dioscorea japonica Thunb.	Throatwort	roots	10	15
Epimedium brevicornum Max.	Herba epimedii	leaves	-	-
Epimedium koreanum Nakai	Barberry	leaves	-	-
Eucommia ulmoides Oliv	Cordex ecommiae	stems	-	-
Euenia caryophyllata Thunb	Clove	buds	15	10
Forsythia suspensa Thunb Vahi	Fructs forsythiae	fruits	-	-
Ganoderma lucidum (Fr) Karsten	Lingzhi Cao	body	10	12
Gastorodia elata Blume	Tian Ma	roots	-	-
Ginkgo biloba L.	Ginkgo	nuts	-	-
Gleditsia sinenis Lam.	Spaina gleditsiae	spine	-	-
Glycyrrihiza glabla L.	Licorice	bark	-	-
Houttuynia cordata Thunb	Herba houttuyniae	stems	-	-
Kalopanax pictum Nakai	Kalopanax	bark	-	-
Leonurus sibiricus L.	Motherwort	whole	-	-
Ligusticum chuanxiong Hort.	Rhizoma chuanxiong	roots	-	15
Lonicera japonica	Honeysuckle	flowers	30	20
Lycium chinense Miller	Night shade	roots	-	-
Morus alba L.	Cortex mori	roots	-	-
Paeonia albiflora	Peony	roots	20	20
Perillae herva	Perilla herb	leaves	-	-
Phyllostachys bambusoides Siebold	Bamboo	leaves	-	-
Plantago asiatica L.	Plantain seed	seeds	-	-
Platycodon grandiflorum Jacq. A. D.C.	Radix Platycodi	roots	-	-
Polygala tenuifolia Wild	Chalk milk wort	body	-	-
Polygomun nultiflorum Thunb	Radix polygoni multiflori	roots	-	-
Poria cocos wolr	Polyporaceae	body	-	-
Prunus armeniaca var. Ansu Max.	Apricot	seeds	-	-
Prunus mume S. et Z.	Ume	fruits	34	20
Prunus persica L. Batsch	Semem persicae	seeds	-	-
Pueraria lobata Ohwi	Radix puerariae	roots	-	-
Rehmannia glutinosa	Radix Rehmanniae	roots	14	11
Rehmannia glytinosa Libosch.	Radix Rehmanniae	roots	-	-
Rheum officinale Baill.	Radix et Rhizomarei	roots	-	-
Rubus chingii Hu.	Fructus Rubi	fruits	-	-
Saposhnikovia divaricata Turcz. Schischk	Radix Saposhinkoviae	roots	-	-
Saururus chinensis Lour. Baill.	Rhizoma saururi	stems	-	-
Schizandra chinensis (Turcz.) Baill.	Fructus schisandrae	fruits	10	9
Scrophularia ningpoensis Hemsl.	Radix scrophulariae	roots	-	-
Scutellaria baicalensis Georgi	Radix Scutellariae	roots	-	-
Sophora flavescens Ait	Radix sophorae	roots	-	-
Trigonella foenumgraecum L.	Semen trignellae	seeds	-	-
Ulmus parvifolia Jaca. var. *Coreana* Uyeki	Elm	roots	-	-
Vitex trifolia L. var. *Simplicifolia* Cham.	Fructus viticis	fruits	-	-

Table 3. Antimicrobial activity of the vegetables and plants extracts by disk diffusion assay

Botanical name	English name	Plant parts	Inhibition zone (mm) per microorganism	
			E. coli O157:H7	*E. coli* ATCC 25922
Acer palmatum Thunb.	Japanese Maple (red)	leaves	10	10
Acer palmatum Thunb.	Japanese Maple (blue)	leaves	11	-
Actinidia arguta Planch	Cotton ball	whole	-	-
Albizzia julibrissin Purazzini	Silk tree	leaves	-	-
Allium cepa L.	Onion	roots	20	15
Allium satium var. pekinense	Garlic	burb	20	16
Allium monanthum Max.	Wild rocambole	roots	-	-
Allium monanthum Max.	Wild rocambole	leaves	-	-
Allium tuberosum Roth	Leek	leaves	-	-
Amaranthus mangostanus L.	Amaranthus	leaves	-	-
Amorpha fruticosa L.	Bastard Indigo	leaves	20	15
Arachis hypogaea L.	Peanut	nuts	-	-
Aretium lappa L.	Burdock	roots	-	-
Artemisia aciatica Nakai	Mugwort	leaves	-	-
Berchemia berchemiaefolia Mak. Koidzumi	Korean Berchemia	leaves	20	19
Capsella bursa-pastoris L. Medicus	Shepherd's purse	roots	-	-
Capsella bursa-pastoris L. Medicus	Shepherd's purse	leaves	-	-
Capsicum annnuum L.	Red pepper	fruits	-	-
Capsicum annnuum L.	Unripe pepper	fruits	-	-
Chelidonium majus var. *asiticum* (HARA) Ohwl	Chelidonium majus	leaves	-	-
Codonopsis lanceolata S. et. Z. T.	Codonopsis lanceolara	roots	-	-
Colocasia antquorum Schott. var. *esculentum*	Taro	roots	-	-
Colocasia antquorum Schott. var. *esculentum*	Taro	leaves	-	-
Diospyros Kaki L. fil	Persimmon	leaves	12	13
Diospyros Kaki L. fil	Persimmon	fruits	-	-
Doncirus trifoliata Rafinesque	Trifoliate orange	seeds	-	-
Gardenia jasminoides for. *grandiflora* Makino	Gardenia seed	fruits	-	-
Ginkgo biloba L.	Ginkgo	leaves	22	20
Hemerocallis longituba Miq	Day lily	leaves	-	-
Juglans sinensis DC. Dode	Chinese walnut	leaves	-	-
Juniperus chinensis L.	Chinese juniper	leaves	-	-
Lactuca indica L.	Agave	leaves	10	11
Lilium lancifolium Thunb	Lily	seeds	10	9
Morus alba L.	White mulberry	seeds	-	-
Oenanthe javanica BL. DC.	Dropwort	leaves	-	-
Perilla frutescens Brit var. *japonica* Hara	Green perilla	leaves	-	-
Persicaria hydropiper L.	Persicaria	leaves	8	10
Persicaria senticosa Nakai	Persicaria senticosa	leaves	-	-
Petasites japonicus S. et. Z Maxim	Butterbur	fruits	10	-
Pinus densiflora S. et Z.	Japanese red pine	bark	-	-
Pinus densiflora S. et Z.	Japanese red pine	leaves	12	13
Pinus densiflora S. et Z.	Japanese red pine	flowers	13	10
Pinus rigida Miller	Pitch pine	leaves	-	-
Polygonatum odoratum var. *pluriflorum* OHWL	Redoute	roots	-	-
Prunus Salicina L.	Japanese plum	fruits	20	15
Prunus Salicina L.	Japanese plum	seeds	15	13
Ricinus communis L.	Castor oil plant	fruits	-	-
Rorippa nasturtium Beck	Shepherd's purse	leaves	-	-
Sedum sarmentosum Bunge	Sedum	leaves	-	-
Semiaquilegia adoxoides Makino	Semiaquilegia adoxoides	whole	-	-
Solanum tuberosum L.	Potato	peel	-	-
Taraxacum platycarpum Dahlst	Dandelion	seeds	-	-
Xeris dentata Thunb. Nakai	Lettuce	leaves	-	-
Zingiber officinale ROSC	Ginger	roots	-	-

coli O157:H7. Out of the 133 plant species, five species were found to have the significant antibacterial activities; *Rhodo dendron Schlipenbachii* Max, *Prunus mume* S. et Z., *Lonicera japonica, Ginkgo biloba* L. and *Prunus Sallicina* L.

We hope that this report may serve as a primary guide for future works on the isolation and elucidation of antibacterial compounds against *E. coli* O157:H7.

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