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Screening of Korean Traditional Medicines and Medicinal Herbs for Antimicrobial Activity Against Pathogenic Bacteria

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Abstract

This study screened 76 Korean traditional patent medicines and 58 medicinal herbs for antimicrobial activity against pathogenic bacteria. Among the prescriptions methanol and hot water extracts of Samsoŭm, Chujunghwang-yont'ang, and Sashinhwan showed antimicrobial activities against *E. coli* and Chujunghwang-yont'ang showed the strongest antimicrobial activity. Among the medicinal herbs, hot water and methanol extracts of *Terminalia chebula*, *Caesalpinia sappan*, and *Coptis chinensis* showed the strongest antimicrobial activities against *Escherichia coli* O157, *Staphylococcus aureus*, *Shigella flexnery*, *Shigella sonnei* and *Salmonella typhimurium*. An interesting characteristic of the traditional medicines such as Samsoŭm, Chujunghwang-yont'ang, and Sashinhwan was that both methanol and hot water extracts were active. Chujunghwang-yont'ang exhibited the strongest antimicrobial activities against the microorganisms tested. Water and methanol extracts of *Terminalia chebula*, *Caesalpinia sappan*, and *Coptis chinensis* showed the strong of inhibitory effects on the growth of all microorganisms tested.

Key words: antimicrobial activity, Korean medicinal herbs

INTRODUCTION

Enterohemorrhagic *Escherichia coli* (EHEC) is highly toxic with the potential to cause high fever, liquid-form-diarrhea, and a abdominal pain. In advanced infections, the symptoms are life-threatening giving rise to diarrhea with bleeding and ultimate fatality, if unsuccessfully treated (1,2). The public health risk for food poisoning is illustrated by the serious mass food poisoning due to *E. coli* O157 contaminated hamburger in USA in 1982. About 70% to 90% of EHEC belongs to serotype O157, which is sometimes called Verotoxin-producing *Escherichia coli* (VTEC) since most EHEC produce cytotoxins to verocell (3). While other EHECs come up with mainly verotoxin 1 (VT1), serotype O157 produces both VT1 as well as VT2 (4). Every year, mass food poisoning caused by *E. coli* O157 has been a serious problem somewhere in the world. Pathogenic microbes including *E. coli* O157 did harm to not only human health but also various social fields. Therefore, strong antibiotic agents have been required for those pathogens (5). Many antibiotic agents are chemically synthesized, resulting in weak stability (6,7). Therefore, natural antimicrobial agents such as plant-extracted compounds, specific proteins or enzymes, organic acids, and bacteriocin has been sought (8-14). There have been many reports of antioxidant agents from plants, however it turns out that plant flavonoids also have potent antimicrobial activity (15). Flavonoids are phenolic antioxidants that also exhibit growth inhibition and antimicrobial activity against many bacteria (16,17).

Korean traditional herbal medicine has a long history with classical textbooks, such as Uibangyuchui in the Korea dynasty and Tonguibogam in the Chosun dynasty, documenting the medicinal effects of many plant extracts. Although considerable effort has been directed toward isolating antimicrobial compounds from plant extracts, since they contain many stable bioactive compounds, very few have included botanicals used in Korean traditional medicine. Recent developments in analytical instrumentation and automation make it possible to perform high throughput analysis with easy extraction and high resolution separation of antimicrobial compounds (18). Therefore, it now feasible to utilize the new technologies for screening large numbers of natural materials for new anti-microbial compounds that are more stable and offer superior efficacy to medicines produced by chemical synthesis (19,20). We previously reported on the effectiveness of methanol extracts from 133 plant species growing in Korea for anti-

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ı iicrobial activity against EHEC O157:H7 (21).

The purpose of this study was to screen for potential ; ntimicrobial compounds, with emphasis on intestinal path- ‹ gens, from Korean traditional patent medicines and me- ‹ icinal herbs based on Hanbangbangjeron.

MATERIALS AND METHODS

Materials

A total of 58 Korean medicinal herbs from 76 Korean ı 'aditional prescriptions including 11 Tonguibogam (22), ‹ Tonguisusebowon prescriptions (23), 18 Tonguiimsang- l angjehak prescriptions (24), and 41 Pangyakappyon pre- : criptions (25) were purchased at a Kyongdong market in . 'ebruary 2001. The lists of those herbs are shown in Table and Table 2.

Table 1. Korean traditional medicines screened for antimicrobial activity

Name of prescriptions	Name of prescriptions
Samsoŭm	Kwak'yangjŏnggisan
Kungsosan I	Wip'ŭngt'ang
Kungsosan II	Sasŭpt'ang
Hyangsosan I	Sambaekt'ang
Hyangsosan II	Manbyŏng-oryŏngsan
Shinch'ult'ang	Oryŏngsan
Gjŏnggisan	Ijungt'ang
Hyangsosan III	Ch'untaekt'ang
Sogalt'ang	Yukchusan
Ch'angch'ulsan	Chijungt'ang
Kumiganghwalt'ang	Yuryŏngt'ang
Ogap'ijangch'okt'ang	Hyang-yusan
Kunggiwhyangsosan	Sŭngmagalgŭnt'ang
Pojung-ikkit'ang I	Shiryŏngt'ang
P'almulgunjat'ang	Ch'ŏngsŏyuk'wat'ang
Hyongbangjihwangt'ang	Igwonsan
Mahwangch'ont'ang	Ch'ŏngsŏikkit'ang
Taech'ongnyongt'ang	Sach'ŏnghwan
Mahwangbujaseshint'ang	Sŭng-yangjesŭpt'ang
Soshihot'ang I	Kullyŏngt'ang
Shihogagyejit'ang	Sagunjat'ang
Soshihot'ang II	Chŏnssiigongsan
Sanggugŭm	Chŏnssibaekch'ulsan
Un-gyosan	Samnyongbaekch'ulsan
Hyang-yuŭm	Ijint'ang
Gamnosodoksan	Yukkunjat'ang
Tarwonŭm	P'aljusan
T'ongmaeksayŏkt'ang	Pojung-ikkit'ang II
Kumbulch'osan	Chujunghwang-yont'ang
Hyongsot'ang	Ch'angch'ulbangp'ungt'ang
Mahwangt'ang	Odŏkwan
Kamihyansosan	Sashinhwan
Hyanggalt'ang	Shinkihwan
Chasoŭm	Ojŏksan
Soch'ŏngnyongt'ang	Ishinhwan
Insamwiryŏngt'ang	Samshinhwan
Wiryŏngt'ang	Wigwanjŏn
P'yŏng-wisan	Hwanggigŏnjungt'ang

Preparation of extracts

Samples were extracted by using methanol or hot water. When methanol was used, the herbs were dissolved in 500 mL of methanol, precipitated at 60°C for 18 hrs, then extracted. The extract was centrifuged at 8,000×g for 15 min, evaporated, and lyophilized. The powdered extracts were then dissolved in 0.9% ethanol and filtered (pore size 0.45 μm). When water was used, the herbs were dissolved in 1300 mL of deionized water, boiled for 2.5 hrs, and filtered with gauze. The extract was centrifuged at 8,000×g for 15 min, evaporated, lyophilized, redissolved in 0.9% ethanol, and filtered (pore size 0.45 μm).

Strains and media

Verotoxin producing *E. coli* O157 ATCC 43895 (V1+V2) and non-verotoxin producing *E. coli* O157 ATCC 3515 were evaluated. Also, *S. aureus* ATCC 29213, *S. flexnery* ATCC 9403, *S. sonnei* ATCC 25931 and *S. typhi* ATCC 13311 were used for comparison. All strains and media are listed in Table 3.

Preparation of McFarland turbidity standards

McFarland No. 0.5 was prepared from 99.5 mL of 0.36 NH_2SO_4 (1% V/V) and 0.5 mL of 0.048 M $BaCl_2$ (1.175% W/V $BaCl_2$ $2H_2O$), and stored at room temperature in the dark. McFarland No. 0.5 with equal volumes of culture media was used for turbidity measurement after shaking.

Measurement of antibacterial activity

Initially, each bacteria strain was cultured in media broth while incubated at 37°C for 18 hrs. When the turbidity of the cultured strain was the same as that of the McFarland, the strain was transferred to an agar plate. Ampicillin was employed as the positive control for *E. coli* O157 and *S. typhi*. Kanamycin was used for *S. aureus*, *S. sonnei* and *S. flexnery*. Antimicrobial activity was measured by Disc diffusion method (26).

Measurement of minimum inhibitory concentration (MIC)

MIC was measured by the tube dilution method (26). A 0.5 mL of tryptic soy broth for *E. coli* O157 and of Muller Hinton broth for *S. aureus*, *S. sonnei*, *S. flex*, and *S. typhi* was placed in each glass tube. Lyophilized samples were dissolved in 0.9% ethanol, and then diluted successively to make the half-concentration with the final volume of 0.5 mL. The cultured strains with the turbidity equilvalent to that of the McFarland were diluted 100 times and 0.5 mL of each strain was mixed with the diluted herbal samples. The mixture was then cultured for 18 hrs at 37°C in a shaking incubator, and growth evaluated by turbidity and the Mueller Hinton agar plate test, MIC was set at the concentration which showed no bacterial growth.

Table 2. Korean medicinal herbs screened antimicrobial activity

Vernacular name(s)	Family	Scientific names	Portions
Kaja I	Combretaceae	*Terminalia chebula*	Fruit
Kaja II	Combretaceae	*Terminalia chebula*	Fruit
Kaja III	Combretaceae	*Terminalia chebula*	Fruit
Kamcho	Leguminosae	*Glycyrrhiza uralensis*	Root and rhizome
Kŏngang	Zingiberaceae	*Zingiber officinale*	Rhizome
Kyeji	Lauraceae	*Cinnamomun cassia*	Stem
Kyepi	Lauraceae	*Cinnamomum cassia*	Bark
Kyomaek	Saururaceae	*Houttuynia cordata*	Aerial part
Kwakhang	Labiatae	*Agastache rugosus*	Aerial part
Taehwang	Polygonaceae	*Rheum undulatum*	Rhizoma
Mahwang	Eupedraceae	*Ephedra simica*	Herbaceous twig
Manhyongja	Verbenaceae	*Vitex trifolia.*	Ripe fruit
Maeshil	Amygdalaceae	*Prunus mume*	Fructus
Mok kwa	Rosuceae	*Chaenomeles specosa*	Ripe fruit
moktong	Lardizabalaceae	*Akebia trifoliata*	Stem
Pakha	Labiatae	*Mentha haploclyx*	Aerial part
Panha	Araceae	*Pinellia ternata*	Tuberous
Panggi	Menispermaceae	*Stephania tetrandra*	Tuberous root
Paektugu	Zingiberaceae	*Amomum kravah*	Fruit juice
Paekbokryŏng	Polyporaceae	*Poria cocos*	Sclerotium
Chagyak I	Ranunculaceae	*Paeonia japonica*	Root
Chagyak II	Ranunculaceae	*Paeonia japonica*	Root
Paekp'yŏndu	Leguminosae	*Dolichos lablab*	Seed
Binrang	Palmae	*Areca catechu*	Seed
Sangyŏp	Moraceae	*Morus alba*	Leaf
Sang-gihwang	Scrophulariaceae	*Rehmannia glutinosa*	Tuberous root
Sŏnbokhwa	Compositae	*Innia japonica*	Flour-head
Saeshin	Aristolochiaceae	*Asaruon heterotropoides*	Entire plant
Somok	Leguminosae	*Caesalpinia sappan*	Heart-wood
Soyŏp	Labiatae	*Perilla frutescens*	Herba
Sŭngma	Ranunculaceae	*Cimicifuga hetracleifolia*	Rhizome
Yŏngyo	Oleaceae	*Frosythia suspensa*	Fruit
Yonyuk	Nymphaceae	*Nelumbo nucifera*	Semen
OmijaI	Magnoliaceae	*Schizandra chinensis*	Fructus
OmijaII	Magnoliaceae	*Schizandra chinensis*	Fructus
Osuyu	Rutaceae	*Evodia officinalis*	Fructus
Yukke	Lauraceae	*Cinnamomum cassia*	Bark juice
Yuktugu	Myristicaceae	*Myristica fragrans*	Semen
Injin	Compositae	*Artemisia scoparia*	Entire plant
Chasoyŏp	Labiatae	*Perilla frutescens*	Leaf juice
Chŏryŏng	Polyporaceae	*Polyporusumbellatus*	Polyporus
Chŏkchagyak	Ranunculaceae	*Paeonia albiflora*	Root
Chinkyo	Gentianaceae	*Gentiana crassicaulis*	Root juice
Changchul	Compositae	*Atractylodes japonica*	Rhizome
Ch'ongsongjŏl	Pinaceae	*Pinus densiflora*	Tewig
Chogwa	Zigiberaceae	*Amomum tgao-ko*	Fruit
Ch'ija	Rubiaceae	*Gardenia jasminoides*	Fructus
P'agoji	Leguminosae	*Psoralea corylifolia*	Semen
Haengin	Rosaceae	*Prunus armeniaca*	Seed
Hyangbuja	Cyperaceae	*Cyperus rotundus*	Tuberous
Hyangyu	Labiatae	*Elsholtzia splendens*	Aerial part
Hyong-gae	Labiatae	*Schizonepeta tenuefolia*	Aerial part
Hwang-gŭm	Labiatae	*Scultellaria baicalensis*	Root
Hwangbaek I	Rutaceae	*Phellodendron amurense*	Cortex
Hwangbaek II	Rutaceae	*Phellodendron amurense*	Cortex
Hwangryŏn I	Ranunculaceae	*Coptis chinensis*	Rhizome
Hwangryŏn II	Ranunculaceae	*Coptis chinensis*	Rhizome
Hubak	Magnoliaceae	*Machilus rimosa*	Stem bark

Table 3. List of strains and media used for screening of antimicrobial compounds

	Strain	Media
Gram negative	*Escherichia coli* O157 ATCC 43895	Muller Hinton agar (Difco) & Tryptic soy broth (Difco)
	Escherichia coli O157 ATCC 3515	Muller Hinton agar (Difco) & Tryptic soy broth (Difco)
	Shigella flexnery ATCC 9403	Muller Hinton agar (Difco) & Muller Hinton broth (Difco)
	Salmonella typhi ATCC 13311	Muller Hinton agar (Difco) & Muller Hinton broth (Difco)
	Shigella sonnei ATCC 25931	Muller Hinton agar (Difco) & Muller Hinton broth (Difco)
Gram positive	*Staphylococcus aureus* ATCC 29213	Muller Hinton agar (Difco) & Muller Hinton broth (Difco)

RESULTS AND DISCUSSION

Antimicrobial activity of water and methanol extracts of Korean traditional patent medicines

Table 4 shows the antimicrobial activities of the extracts by hot water from 76 Korean traditional patent medicines. A strong antimicrobial activity was observed in Samsoŭm and Chujunghwang-yont'ang against *E. coli* O157 (V1+V2), *S. sonnei*, *S. flexnery*, and *S. typhi*. Some antimicrobial activity was exhibited by Samsoŭm, Gamnosodoksan, and Chujunghwang-yont'ang against *E. coli* O157 (none). Among the 51 extracts with antimicrobial activity against *S. aureus*, Samsoŭm and Chujunghwang-yont'ang were more effective.

Table 5 shows the antimicrobial activities of hot water extracts against 6 bacteria. Fifty eight different species of plants were classified according to which plant part exhibited activity. The water extract of *Caesalpinia sappan* showed wide and strong antimicrobial activity against *E. coli* O157 (V1+V2). Against *E. coli* O157 (none), *Cinnamomum cassia*, *Prunus mume*, *Caesalpinia sappan*, *Coptis chinensis* I, and *Coptis chinensis* II exhibited good antimicrobial activity. Especially, *Caesalpinia sappan* and *Coptis chinensis* I were most potent among the 41 plants in inhibiting *S. aureus* growth. Some antimicrobial activities were found for *Terminalia chebula* I · II · III, *Prunus mume*, *Paeonia japonica* I, *Caesalpinia sappan*, and *Coptis chinensis* I against *S. flexnery*; for *Prunus mume*, *Caesalpinia sappan*, and *Coptis chinensis* I against *S. sonnei*; and for *Terminalia chebula*I · II · III, *Rheum undulatum*, *Prunus mume*, *Caesalpinia sappan*, *Coptis chinensis* against *S. typhi*.

Table 4 shows the antimicrobial activities of methanol extracts from 76 Korean traditional patent medicines against bacteria. The extracts from Samsoŭm, Mahwangch'ont'ang, Chujunghwang-yont'ang, and Shinkihwan exhibited a modest activity against *E. coli* O157 (V1+V2), and the extracts from Samsoŭm, Hyangsosan I, Soshihot'ang II,

Table 4. Antimicrobial activities of water and methanol extracts from Korean traditional medicines

Prescriptions	Clear zone on plate (mm)											
	E. coli O157 (V1+V2)		*E. coli* O157 (none)		*S. aureus*		*S. flexnery*		*S. sonnei*		*S. typhi*	
	CW	CM	CW	CM	CW	CM	CW	CM	CW	CM	CW	CM
Samsoŭm	9.0	10.0	10.0	16.0	22.5	26.5	11.0	20.0	8.0	12.0	13.5	21.0
Kungsosan I	-	-	-	-	7.0	-	-	-	-	-	-	-
Kungsosan II	-	-	-	-	9.0	-	-	-	-	-	-	-
Hyangsosan I	-	8.0	-	10.0	12.3	11.0	-	-	-	-	-	-
Hyangsosan II	-	-	-	-	9.3	8.0	-	-	-	-	-	-
Shinch'ult'ang	-	-	-	-	-	-	-	-	-	-	-	-
Gjŏnggisan	-	-	-	-	8.3	-	-	-	-	-	-	-
Hyangsosan III	-	-	-	-	10.0	7.0	-	-	-	-	-	-
Sogalt'ang	-	-	-	-	9.5	10.0	-	-	-	-	-	-
Ch'angch'ulsan	-	-	-	-	8.0	11.0	-	-	-	-	-	-
Kumiganghwalt'ang	-	-	-	-	-	8.0	-	-	-	-	-	-
Ogap'ijangch'okt'ang	-	-	-	-	-	9.0	-	-	-	-	-	-
Kunggiwhyangsosan	-	-	-	-	-	-	-	-	-	-	-	-
Pojung-ikkit'ang I	-	-	-	-	-	-	-	-	-	-	-	-
P'almulgunjat'ang	-	-	-	-	-	8.0	-	-	-	-	-	-
Hyongbangjihwangt'ang	-	-	-	-	-	8.0	-	-	-	-	-	-
Mahwangch'ont'ang	-	-	-	-	12.0	12.0	-	-	-	-	-	-
Taech'ongnyongt'ang	-	-	-	-	11.8	12.0	-	-	-	-	-	-
Mahwangbujaseshint'ang	-	-	-	-	10.0	12.5	-	-	-	-	-	-
Soshihot'ang I	-	-	8.0	-	11.0	11.0	-	-	-	-	-	-
Shihogagyejit'ang	-	-	-	9.0	14.0	10.0	-	-	-	7.0	-	-

Table 4. Continued

Prescriptions	Clear zone on plate (mm)											
	E. coli O157 (V1+V2)		*E. coli* O157 (none)		*S. aureus*		*S. flexnery*		*S. sonnei*		*S. typhi*	
	CW	CM	CW	CM	CW	CM	CW	CM	CW	CM	CW	CM
Soshihot'ang II	-	-	-	10.0	15.0	17.0	-	7.0	-	7.0	-	-
Sanggugŭm	-	-	-	-	12.5	-	-	-	-	-	-	-
Un-gyosan	-	-	-	-	12.0	-	-	-	-	-	-	-
Hyang-yuŭm	-	-	-	-	-	6.5	-	-	-	-	-	-
Gamnosodoksan	-	-	-	-	17.0	12.0	-	-	-	-	10.0	-
Tarwonŭm	-	-	11.0	9.0	14.0	15.0	-	-	-	-	-	-
T'ongmaeksayŏkt'ang	-	-	-	-	-	14.0	-	-	-	-	-	-
Kumbulch'osan	-	-	-	-	14.0	11.5	-	-	-	-	-	-
Hyongsot'ang	-	-	-	-	15.0	7.0	-	-	-	-	-	-
Mahwangt'ang	-	12.0	-	-	11.0	12.5	-	-	-	-	-	-
Kamihyansosan	-	-	-	-	13.0	11.0	-	-	-	-	-	-
Hyanggalt'ang	-	-	-	-	12.0	7.0	-	-	-	-	-	-
Chasoŭm	-	-	-	-	12.0	9.0	-	-	-	-	-	-
Soch'ŏngnyongt'ang	-	9.0	-	-	-	-	-	-	-	-	-	-
Insamwiryŏngt'ang	-	-	-	-	9.0	10.0	-	-	-	-	-	-
Wiryŏngt'ang	-	-	-	-	-	8.5	-	-	-	-	-	-
P'yŏng-wisan	-	-	-	-	9.0	8.5	-	-	-	-	-	-
Kwak'yangjŏnggisan	-	-	-	-	9.0	11.0	-	-	-	-	-	-
Wip'ŭngt'ang	-	-	-	-	-	8.0	-	-	-	-	-	-
Sasŭpt'ang	-	-	-	-	9.0	9.0	-	-	-	-	-	-
Sambaekt'ang	-	-	-	-	-	8.5	-	-	-	-	-	-
Manbyŏng-oryŏngsan	-	-	-	-	-	10.0	-	-	-	-	-	-
Oryŏngsan	-	-	-	-	-	-	-	-	-	-	-	-
Ijungt'ang	-	-	-	-	8.0	10.0	-	-	-	-	-	-
Ch'untaekt'ang	-	-	-	-	-	-	-	-	-	-	-	-
Yukchusan	-	-	-	-	9.0	10.0	-	9.0	-	-	-	-
Chijungt'ang	-	-	-	-	8.0	9.0	-	-	-	-	-	-
Yuryŏngt'ang	-	-	-	-	9.0	14.0	-	-	-	-	-	-
Hyang-yusan	-	-	-	-	8.0	9.0	7.0	-	-	-	-	-
Sŭngmagalgŭnt'ang	-	-	-	-	8.5	11.0	8.0	-	-	-	-	-
Shiryŏngt'ang	-	-	-	-	9.0	9.0	-	-	-	-	-	-
Ch'ŏngsŏyuk'wat'ang	-	-	-	-	-	-	-	-	-	-	-	-
Igwonsan	-	-	-	-	-	13.5	-	-	-	-	-	-
Ch'ŏngsŏikkit'ang	-	-	-	-	8.0	-	-	-	-	8.0	-	-
Sach'ŏnghwan	-	-	-	8.0	7.0	-	-	-	-	-	-	-
Sŭng-yangjesŭpt'ang	-	-	-	-	11.0	-	-	-	-	-	-	-
Kullyŏngt'ang	-	-	-	-	9.0	11.0	-	-	-	-	-	-
Sagunjat'ang	-	-	-	-	11.0	10.0	-	-	-	-	-	-
Chŏnssiigongsan	-	-	-	-	9.0	7.5	-	-	-	-	-	-
Chŏnssibaekch'ulsan	-	-	-	-	10.0	10.5	-	-	-	-	-	-
Samnyongbaekch'ulsan	-	-	-	-	-	11.5	-	-	-	-	-	-
Ijint'ang	-	-	-	-	10.0	7.5	-	-	-	-	-	-
Yukkunjat'ang	-	-	-	-	-	10.0	-	-	-	-	-	-
P'aljusan	-	8.0	-	9.0	11.0	13.0	-	10.0	-	9.5	-	-
Pojung-ikkit'ang II	-	-	-	-	11.0	9.0	-	-	-	-	-	-
Chujunghwang-yont'ang	10.0	12.0	15.0	15.0	18.5	21.0	11.5	14.0	13.5	18.0	11.0	11.0
Ch'angch'ulbangp'ungt'ang	-	-	-	-	-	11.0	-	-	-	-	-	-
Odŏkwan	-	-	-	-	7.5	7.0	-	-	-	-	-	-
Sashinhwan	8.0	10.0	8.0	10.0	9.0	8.5	7.5	10.0	7.5	8.5	-	7.0
Shinkihwan	-	-	-	-	-	9.0	-	-	-	-	-	-
Ojŏksan	-	-	-	-	10.0	9.0	-	-	-	-	-	-
Ishinhwan	7.0	-	7.0	9.0	-	-	-	-	-	-	-	-
Samshinhwan	7.0	-	7.0	-	-	-	-	-	-	-	-	-
Wigwanjŏn	7.0	-	7.0	-	8.0	9.0	-	-	-	-	-	-
Hwanggigŏnjungt'ang	-	-	-	-	-	8.0	-	-	-	-	-	-

Concentration of each disc is 10 mg/mL. CW: water extract of Korean traditional medicines, CM: methanol extract of Korean traditional prescriptions, *E. coli* O157 (V1+V2): *E. coli* O157 which produce Vero toxin 1 and 2, *E. coli* O157 (none): *E. coli* O157 which do not produce Vero toxin 1 and 2.

Table 5. Antimicrobial activities of water and methanol extracts from Korean medicinal herbs

Herbs	Clear zone on plate (mm)											
	E.coli O157 (V1+V2)		*E.coli* O157 (none)		*S. aureus*		*S. flexnery*		*S. sonnei*		*S. typhi*	
	SW	SM	SW	SM	SW	SM	SW	SM	SW	SM	SW	SM
Kaja I	7.0	7.0	8.0	7.0	14.5	14.0	18.0	22.0	7.0	8.0	14.5	14.0
Kaja II	8.0	8.0	9.0	9.0	17.0	16.0	21.0	23.0	8.0	11.0	15.0	17.0
Kaja III	-	-	-	-	19.0	15.0	19.0	22.0	8.0	9.0	17.0	15.0
Kamcho	-	-	-	-	13.0	16.0	-	-	-	-	-	-
Kŏngang	-	-	-	-	11.0	-	-	-	-	-	-	-
Kyeji	-	-	-	-	-	11.0	-	-	-	-	-	-
Kyepi	-	7.0	12.0	7.0	-	-	-	-	-	-	-	-
Kyomaek	-	-	-	-	-	9.0	-	-	-	-	-	-
Kwakhang	-	-	-	-	14.0	8.0	-	-	-	-	-	-
Taehwang	-	-	-	-	12.0	10.0	8.0	9.0	-	-	12.0	11.5
Mahwang	-	-	-	-	14.0	13.0	-	-	-	-	-	-
Manhyongja	-	-	-	-	9.0	-	-	-	-	-	-	-
Maeshil	9.0	9.0	10.0	9.0	9.0	9.0	15.0	17.0	10.0	11.0	10.0	11.5
Mokkwa	-	-	-	-	11.0	11.0	-	-	-	-	-	-
Moktong	-	-	-	-	11.0	9.0	-	-	-	-	-	-
Pakha	-	-	-	-	13.0	-	-	-	-	-	-	-
Panha	-	-	-	-	-	-	-	-	8.0	-	-	-
Panggi	-	-	-	-	10.0	18.0	-	-	-	-	-	-
Paektugu	-	-	-	-	9.0	-	-	-	-	-	-	-
Paekbokryŏng	7.0	-	-	-	-	-	-	-	-	-	-	-
Chagyak I	-	-	-	-	13.0	13.0	13.0	-	-	-	-	-
Chagyak II	-	8.0	-	7.5	-	-	-	-	-	-	-	-
Paekp'yŏndu	-	7.5	-	8.0	-	-	-	-	-	-	-	-
Binrang	-	-	-	-	14.0	15.5	-	8.0	-	-	-	7.0
Sangyŏp	-	-	-	-	9.0	-	-	-	-	-	-	-
Sang-gihwang	-	-	-	-	9.0	-	-	-	-	-	-	-
Sŏnbokhwa	-	-	-	-	11.0	15.0	-	-	-	-	-	-
Saeshin	-	-	-	-	-	12.0	-	-	-	-	-	-
Somok	11.0	11.0	11.0	12.0	27.0	25.0	19.0	18.0	12.0	13.0	19.0	19.0
Soyŏp	-	-	-	-	10.5	-	-	-	-	-	-	-
Sŭngma	-	-	-	-	9.5	-	-	-	-	-	-	-
Yŏngyo	-	-	-	-	13.0	9.0	-	8.0	-	-	-	7.5
Yonyuk	-	-	-	-	10.0	8.5	7.5	-	-	7.0	8.0	-
Omija I	7.5	9.0	9.5	9.0	-	-	-	10.0	-	8.0	-	8.0
Omija II	9.0	9.0	9.0	9.0	-	8.0	-	14.0	-	9.0	7.0	9.5
Osuyu	-	-	-	-	-	-	-	-	-	6.5	7.0	7.0
Yukke	-	-	-	-	10.5	13.0	-	-	-	-	-	-
Yuktugu	-	-	-	-	7.0	8.0	7.0	-	-	-	-	-
Injin	-	-	-	-	12.0	-	-	-	-	-	-	-
Chasoyŏp	-	-	-	-	10.0	10.0	-	-	-	-	-	-
Chŏryŏng	-	-	-	-	-	8.0	-	-	-	-	-	-
Chŏkchagyak	-	-	-	-	10.0	12.0	-	8.0	-	-	-	-
Chinkyo	-	-	-	-	12.0	18.0	-	-	-	-	-	-
Changchul	-	-	7.0	-	-	-	-	-	-	-	-	-
Ch'ongsongjŏl	-	-	-	-	11.0	-	-	-	-	-	-	-
Chogwa	-	-	-	-	9.0	-	-	-	-	-	-	-
Ch'ija	7.0	-	7.0	-	-	-	-	-	-	-	-	-
P'agoji	-	-	-	-	-	9.0	-	-	-	-	7.0	-
Haengin	-	-	-	-	8.0	8.0	-	-	-	-	-	-
Hyangbuja	9.0	-	9.0	-	-	-	-	-	-	-	-	-
Hyangyu	-	-	-	-	9.0	-	-	-	-	-	-	-
Hyong-gae	-	-	-	-	13.0	11.0	-	7.0	-	8.0	-	8.0
Hwang-gŭm	-	-	7.0	-	13.0	12.0	8.0	8.0	9.0	-	10.0	7.0

Table 5. Continued

Herbs	Clear zone on plate (mm)											
	E.coli O157 (V1+V2)		*E.coli* O157 (none)		*S. aureus*		*S. flexnery*		*S. sonnei*		*S. typhi*	
	SW	SM	SW	SM	SW	SM	SW	SM	SW	SM	SW	SM
Hwangbaek	7.5	-	8.0	-	12.0	14.0	8.5	7.0	8.5	7.0	8.0	6.5
Hwangbaek	-	-	-	-	12.0	15.0	-	9.0	-	8.0	8.0	6.5
Hwangryŏn I	8.0	12.0	10.0	12.0	21.0	20.0	13.0	13.0	13.0	12.0	8.0	9.0
Hwangryŏn II	8.5	9.0	12.0	11.0	-	-	-	-	-	-	-	-
Hubak	7.0	7.0	8.0	8.0	13.0	14.0	-	-	-	-	-	-

Concentration of each disc is 10 mg/mL. SW: water extract of Korean traditional herbs, SM: methanol extract of Korean traditional herbs, *E. coli* O157 (V1+V2): *E. coli* O157 which produce Vero toxin 1 and 2, *E. coli* O157 (none): *E. coli* O157 which do not produce Vero toxin 1 and 2.

Chujunghwang-yont'ang, and Shinkihwan addressed some activity in *E. coli* O157 (none). Some anti-microbial activity was obtained from Samsoŭm, Chujunghwang-yont'ang, and Shinkihwan against *S. flexnery*, and Samsoŭm and Chujunghwang-yont'ang against both *S. sonnei* and *S. typhi*. Samsoŭm and Chujunghwang-yont'ang had the strongest activity against *S. aureus* among the 59 plants.

Table 5 shows the antimicrobial activities of the methanol extracts of by methanol of 58 different herbs against 6 different bacteria. A great activity was shown in the extracts from *Caesalpinia sappan* and *Coptis chinensis* extracts were highly active against *E. coli* O157 (V1+V2), and extracts from *Caesalpinia sappan* and *Coptis chinensis* I · II were active against *E. coli* O157 (none). Some activity was detected in 33 plant extracts against *S. aureus*, with expecially high activities in extracts of *Caesalpinia*

Table 6. Minimum inhibitory concentration (MIC) of water and methanol extracts from Korean traditional medicines

Prescriptions	MIC (mg/mL)											
	E. coli O157 (V1+V2)		*E. coli* O157 (none)		*S. aureus*		*S. flexnery*		*S. sonnei*		*S. typhi*	
	CW	CM	CW	CM	CW	CM	CW	CM	CW	CM	CW	CM
Samsoŭm	2.50	2.50	2.50	1.25	0.15	0.07	0.78	0.31	0.78	0.31	0.78	0.31
Kungsosan I	-	-	-	-	3.13	-	-	-	-	-	-	-
Kungsosan II	-	-	-	-	3.13	-	-	-	-	-	-	-
Hyangsosan I	-	-	-	-	3.13	3.13	-	-	-	-	-	-
Hyangsosan II	-	-	-	-	6.25	3.13	-	-	-	-	-	-
Shinch'ult'ang	-	-	-	-	-	-	-	-	-	-	-	-
Gjŏnggisan	-	-	-	-	12.5	-	-	-	-	-	-	-
Hyangsosan III	-	-	-	-	12.5	6.25	-	-	-	-	-	-
Sogalt'ang	-	-	-	-	3.13	6.25	-	-	-	-	-	-
Ch'angch'ulsan	-	-	-	-	3.13	3.13	-	-	-	-	-	-
Kumiganghwalt'ang	-	-	-	-	-	6.25	-	-	-	-	-	-
Ogap'ijangch'okt'ang	-	-	-	-	-	6.25	-	-	-	-	-	-
Kunggiwhyangsosan	-	-	-	-	-	-	-	-	-	-	-	-
Pojung-ikkit'ang I	-	-	-	-	-	-	-	-	-	-	-	-
P'almulgunjat'ang	-	-	-	-	-	-	-	-	-	-	-	-
Hyongbangjihwangt'ang	-	-	-	-	-	3.13	-	-	-	-	-	-
Mahwangch'ont'ang	-	-	-	-	3.13	3.13	-	-	-	-	-	-
Taech'ongnyongt'ang	-	-	-	-	1.56	1.56	-	-	-	-	-	-
Mahwangbujaseshint'ang	-	-	-	-	1.56	-	-	-	-	-	-	-
Soshihot'ang I	-	-	-	-	1.56	-	-	-	-	-	-	-
Shihogagyejit'ang	-	-	-	-	3.13	1.56	-	-	-	-	-	-
Soshihot'ang II	-	-	-	-	1.56	0.78	-	-	-	6.25	-	-
Sanggugŭm	-	-	-	-	3.13	-	-	-	-	-	-	-
Un-gyosan	-	-	-	-	3.13	-	-	-	-	-	-	-
Hyang-yuŭm	-	-	-	-	-	-	-	-	-	-	-	-
Gamnosodoksan	-	-	-	-	12.5	1.56	-	-	-	-	-	-
Tarwonŭm	-	-	-	-	3.13	0.78	-	-	-	-	-	-
T'ongmaeksayŏkt'ang	-	-	-	-	-	6.25	-	-	-	-	-	-
Kumbulch'osan	-	-	-	-	1.56	3.13	-	-	-	-	-	-
Hyongsot'ang	-	-	-	-	-	3.13	-	-	-	-	-	-
Mahwangt'ang	-	-	-	-	1.56	3.13	-	-	-	-	-	-
Kamihyansosan	-	-	-	-	3.13	3.13	-	-	-	-	-	-

Table 6. Continued

Prescriptions	MIC (mg/mL)											
	E. coli O157 (V1 + V2)		*E. coli* O157 (none)		*S. aureus*		*S. flexnery*		*S. sonnei*		*S. typhi*	
	CW	CM	CW	CM	CW	CM	CW	CM	CW	CM	CW	CM
Hyanggalt'ang	-	-	-	-	6.25	3.13	-	-	-	-	-	-
Chasoŭm	-	-	-	-	3.13	3.13	-	-	-	-	-	-
Soch'ŏngnyongt'ang	-	10.0	-	10.0	-	3.13	-	-	-	-	-	-
Insamwiryŏngt'ang	-	-	-	-	-	2.50	-	-	-	-	-	-
Wiryŏngt'ang	-	-	-	-	-	1.25	-	-	-	-	-	-
P'yŏng-wisan	-	-	-	-	-	1.25	-	-	-	-	-	-
Kwak'yangjŏnggisan	-	-	-	-	1.25	-	-	-	-	-	-	-
Wip'ŭngt'ang	-	-	-	-	-	1.25	-	-	-	-	-	-
Sasŭpt'ang	-	-	-	-	-	-	-	-	-	-	-	-
Sambaekt'ang	-	-	-	-	-	-	-	-	-	-	-	-
Manbyŏng-oryŏngsan	-	-	-	-	-	1.25	-	-	-	-	-	-
Oryŏngsan	-	-	-	-	-	-	-	-	-	-	-	-
Ijungt'ang	-	-	-	-	2.50	0.62	-	-	-	-	-	-
Ch'untaekt'ang	-	-	-	-	-	-	-	-	-	-	-	-
Yukchusan	-	-	-	-	2.50	0.62	-	-	-	-	-	-
Chijungt'ang	-	-	-	-	-	-	-	-	-	-	-	-
Yuryŏngt'ang	-	-	-	-	2.50	0.31	-	-	-	-	-	-
Hyang-yusan	-	-	-	-	1.25	2.5	-	-	-	-	-	-
Sŭngmagalgŭnt'ang	-	-	-	-	2.50	0.31	-	-	-	-	-	-
Shiryŏngt'ang	-	-	-	-	1.25	2.50	-	-	-	-	-	-
Ch'ŏngsŏyuk'wat'ang	-	-	-	-	-	-	-	-	-	-	-	-
Igwonsan	-	-	-	-	-	0.31	-	-	-	-	-	-
Ch'ŏngsŏikkit'ang	-	-	-	-	-	-	-	-	-	2.50	-	-
Sach'ŏnghwan	-	-	-	-	-	-	-	-	-	-	-	-
Sŭng-yangjesŭpt'ang	-	-	-	-	-	-	-	-	-	-	-	-
Kullyŏngt'ang	-	-	-	-	-	1.25	-	-	-	-	-	-
Sagunjat'ang	-	-	-	-	2.50	1.25	-	-	-	-	-	-
Chŏnssiigongsan	-	-	-	-	-	-	-	-	-	-	-	-
Chŏnssibaekch'ulsan	-	-	-	-	-	-	-	-	-	-	-	-
Samnyongbaekch'ulsan	-	-	-	-	-	1.25	-	-	-	-	-	-
Ijint'ang	-	-	-	-	-	-	-	-	-	-	-	-
Yukkunjat'ang	-	-	-	-	-	1.25	-	-	-	-	-	-
P'aljusan	-	-	-	-	0.62	0.62	-	1.25	-	2.50	-	-
Pojung-ikkit'ang II	-	-	-	-	-	2.50	-	-	-	-	-	-
Chujunghwang-yont'ang	5.0	5.0	5.0	5.0	0.01	0.01	0.31	0.07	0.15	0.15	0.31	0.31
Ch'angch'ulbangp'ungt'ang	-	-	-	-	-	1.25	-	-	-	-	-	-
Odŏkwan	-	-	-	-	-	2.50	-	-	-	-	-	-
Sashinhwan	-	-	-	-	1.25	1.25	1.25	1.25	2.5	1.25	-	1.25
Shinkihwan	-	-	-	-	-	1.25	-	-	-	-	-	-
Ojŏksan	-	-	-	-	-	-	-	-	-	-	-	-
Ishinhwan	-	-	-	-	-	-	-	-	-	-	-	-
Samshinhwan	-	-	-	-	-	-	-	-	-	-	-	-
Wigwanjŏn	-	-	-	-	2.50	-	-	-	-	-	-	-
Hwanggigŏnjungt'ang	-	-	-	-	-	1.25	-	-	-	-	-	-

sappan and *Coptis chinensis* I among those plants. Furthermore, good activities were observed from *Terminalia chebula* I · II · III, *Prunus mume*, *Caesalpinia sappan*, *Schizandra chinensis* I · II, and *Coptis chinensis* I against *S. flexnery*, from *Prunus mume*, *Caesalpinia sappan*, and *Coptis chinensis* I against *S. sonnei*, and from *Terminalia chebula* I · II · III, *Rheum undulatum*, *Prunus mume*, and *Caesalpinia sappan* against *S. typhi.*

Both hot water and methanol extracts of Samsoŭm and Chujunghwang-yont'ang by both hot water and methanol showed strong activities against all bacteria tested in this study. Further study for these interesting plants is under way. Higher sensitivity was observed in Gram-negative bacteria such as *S. aureus* than in Gram-positive bacteria in the antimicrobial tests. *Terminalia chebula* II, *Caesalpinia sappan*, and *Coptis chinensis* I exhibited strong antimicrobial activity for both Gram-positive and Gram-negative bacteria, and *Caesalpinia sappan* had the highest overall antimicrobial activity.

Minimum inhibitory concentration (MIC)

Table 6 shows the minimum inhibitory concentrations of 76 Korean patent medicines against bacteria. As for

overall activity as shown in Table 5, the methanol extracts from Samsoŭm and Chujunghwang-yont'ang also showed the strongest activity based on MIC.

The MIC of the extract from Samsoŭm was 0.07 mg/mL in *S. aureus*, the bacteria in which it was the lowest of all those tested. The MICs of the extract from Samsoŭm were 2.5 mg/mL and 1.25 mg/mL against *E. coli* O157 (V1 + V2) and *E. coli* O157 (none), respectively. The MICs of the methanol extract of Chujunghwang-yont'ang were 0.01 mg/mL (lowest), 0.15 mg/mL, 0.07 mg/mL, and 0.31 mg/mL in *S. aureus, S. sonnei, S. flex* and *S. typhi*, respectively, and had the same MIC against both *E. coli* O157 and *E. coli* O157 (none) at 5.0 mg/mL. It was reported that the MICs of the extract of *Ulmus pumila* L. extract against five Gram-positive bacteria such as *S. aureus* and for five Gram-negative bacteria such as *S. typhi* were between 2.5 mg/mL and 3.0 mg/mL (18). Our study clearly shows that the extracts from Samsoŭm and Chujunghwang-yont'ang also exhibited high antimicrobial activities against many bacteria including *E. coli* O157.

Table 7 shows the minimum inhibitory concentrations of 58 herbs against intestinal microflora. As shown in Table 7, the methanol extracts of *Caesalpinia sappan* extract *Coptis chinensis* by methanol had the highest antimicrobial activities. The MIC for *Caesalpinia sappan* against other bacteria were 0.39 mg/mL for *S. aureus*, 0.078 mg/mL for *S. flexnery*, 0.15 mg/mL for *S. sonnei*, and 0.15 mg/mL for *S. typhi*.

Samsoŭm and Chujunghwang-yont'ang have strong antimicrobial activities against a wide variety of bacteria as demonstrated by their minimum inhibitory concentrations. Therefore, the isolation of their antimicrobial compounds and subjecting them to rigorous experimentation seems warranted. It is an interesting fact is that the extracts from *Terminalia chebula*, *Caesalpinia sappan*, and *Coptis chinensis* that have the colors such as yellow, orange, or brown showed some antimicrobial activities and relatively low MICs. Their anti-microbial activities might be attributed to the phenolic compounds, which have aromatic rings that may also have antioxidant activities. These results identify traditional Korean herbs that have antimicrobial potential and may serve as a guide which may help us select sources with antimicrobial activity for further work on the isolation and elucidation of the active compounds. Continuation of this research for the development of chemical, pharmacological and neutraceutical applications is in progress.

Table 7. Minimum Inhibitory Concentrations (MIC) of the water and methanol extracts from Korean medicinal herbs

Prescriptions	MIC (mg/mL)											
	E.coli O157 (V1+V2)		*E.coli* O157 (none)		*S. aureus*		*S. flexnery*		*S. sonnei*		*S. typhi*	
	SW	SM	SW	SM	SW	SM	SW	SM	SW	SM	SW	SM
Kaja I	-	-	-	-	1.56	1.56	1.56	0.78	-	-	-	-
Kaja II	-	-	-	-	1.56	1.56	1.56	0.78	-	-	-	-
Kaja III	-	-	-	-	1.56	1.56	1.56	0.78	-	-	-	-
Kamcho	-	-	-	-	6.25	0.39	-	-	-	-	-	-
Kŏngang	-	-	-	-	25.0	-	-	-	-	-	-	-
Kyeji	-	-	-	-	-	-	-	-	-	-	-	-
Kyepi	-	-	-	-	-	-	-	-	-	-	-	-
Kyomaek	-	-	-	-	-	-	-	-	-	-	-	-
Kwakhang	-	-	-	-	1.56	0.78	-	-	-	-	-	-
Taehwang	-	-	-	-	1.56	1.56	25.0	-	-	-	25.0	-
Mahwang	-	-	-	-	-	-	-	-	-	-	-	-
Manhyongja	-	-	-	-	12.50	-	-	-	-	-	-	-
Maeshil	10.0	10.0	10.0	5.0	3.13	3.13	6.25	6.25	3.13	6.25	3.13	3.13
Mokkwa	-	-	-	-	-	-	-	-	-	-	-	-
Moktong	-	-	-	-	3.13	1.56	-	-	-	-	-	-
Pakha	-	-	-	-	1.56	-	-	-	-	-	-	-
Panha	-	-	-	-	-	-	-	-	-	-	-	-
Panggi	-	-	-	-	-	25.0	-	-	-	-	-	-
Paektugu	-	-	-	-	25.0	-	-	-	-	-	-	-
Paekbokryŏng	-	-	-	-	-	-	-	-	-	-	-	-
Chagyak I	-	-	-	-	3.13	3.13	6.25	-	-	-	-	-
Chagyak II	-	-	-	-	-	-	-	-	-	-	-	-
Paekp'yŏndu	-	-	-	-	-	-	-	-	-	-	-	-
Binrang	-	-	-	-	-	-	-	-	-	-	-	-
Sangyŏp	-	-	-	-	3.13	-	-	-	-	-	-	-
Sang-gihwang	-	-	-	-	-	-	-	-	-	-	-	-
Sŏnbokhwa	-	-	-	-	-	-	-	-	-	-	-	-
Saeshin	-	-	-	-	25.0	-	-	-	-	-	-	-

Table 7. Continued

Prescriptions	MIC (mg/mL)											
	E.coli O157 (V1+V2)		*E.coli* O157 (none)		*S. aureus*		*S. flexnery*		*S. sonnei*		*S. typhi*	
	SW	SM	SW	SM	SW	SM	SW	SM	SW	SM	SW	SM
Somok	1.25	1.25	1.25	1.25	0.078	0.39	0.15	0.078	0.63	0.15	0.15	0.15
Soyŏp	-	-	-	-	6.25	-	-	-	-	-	-	-
Sŭngma	-	-	-	-	6.25	-	-	-	-	-	-	-
Yŏngyo	-	-	-	-	0.78	3.13	-	1.56	-	-	-	-
Yonyuk	-	-	-	-	12.50	3.13	-	-	-	-	-	-
Omija I	10.0	10.0	10.0	10.0	-	6.25	-	6.25	-	6.25	-	6.25
Omija II	10.0	10.0	10.0	10.0	-	6.25	-	3.13	-	3.13	-	6.25
Osuyu	-	-	-	-	-	-	-	-	-	-	-	-
Yukke	-	-	-	-	-	-	-	-	-	-	-	-
Yuktugu	-	-	-	-	-	-	-	-	-	-	-	-
Injin	-	-	-	-	1.56	-	-	-	-	-	-	-
Chasoyŏp	-	-	-	-	-	25.0	-	-	-	-	-	-
Chŏryŏng	-	-	-	-	-	-	-	-	-	-	-	-
Chŏkchagyak	-	-	-	-	6.25	6.25	-	6.25	-	-	-	-
Chinkyo	-	-	-	-	0.039	3.13	-	-	-	-	-	-
Changchul	-	-	-	-	-	-	-	-	-	-	-	-
Ch'ongsongjŏl	-	-	-	-	-	-	-	-	-	-	-	-
Chogwa	-	-	-	-	-	-	-	-	-	-	-	-
Ch'ija	-	-	-	-	-	-	-	-	-	-	-	-
P'agoji	-	-	-	-	-	-	-	-	-	-	25.0	-
Haengin	-	-	-	-	-	-	-	-	-	-	-	-
Hyangbuja	-	-	-	-	-	-	-	-	-	-	-	-
Hyangyu	-	-	-	-	-	-	-	-	-	-	-	-
Hyong-gae	-	-	-	-	12.50	-	-	-	-	-	-	-
Hwang-gŭm	-	-	-	-	1.56	1.56	-	-	-	-	6.25	12.50
Hwangbaek	10.0	-	-	-	1.56	0.78	-	-	-	25.0	-	-
Hwangbaek	-	-	-	-	1.56	1.56	-	-	-	12.5	-	-
Hwangryŏn I	10.0	-	-	-	0.156	0.078	1.25	0.63	2.50	1.25	2.50	2.50
Hwangryŏn II	-	-	-	-	-	-	-	-	-	-	-	-
Hubak	-	-	-	-	0.78	0.39	-	-	-	-	-	-

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