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Evaluation of Antimicrobial Activity of the Methanol Extracts from 8 Traditional Medicinal Plants

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The methanol extract of 12 medicinal plants were evaluated for its antibacterial activity against Gram-positive (5 strains) and Gram-negative bacteria (10 strains) by assay for minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC). The antibacterial activity was determined by an agar dilution method (according to the guidelines of Clinical and Laboratory Standard Institute). All the compounds (12 extracts) of the 8 medicinal plants (leaf or root) were active against both Gram-negative and Gram-positive bacteria. Gram-negative showed a more potent action than Gram positive bacteria. The MIC concentrations were various ranged from 0.6 μg/m*l* to 5000 μg/m*l*. The lowest MIC (0.6 μg/m*l*) and MBC (1.22 μg/m*l*) values were obtained with extract on 4 and 3 of the 15 microorganisms tested, respectively.

***Key words*:** Antimicrobial activity, traditional medicinal plants, minimum inhibitory concentration (MIC), minimum bacterial concentration (MBC), methanol extract, agar dilution method .

INTRODUCTION

From ancients, traditional medicinal plants have been known to possess diverse biological activity as antimicrobial, analgesics, anticancer, antipyrexial, and antihypertensive activity and an important source of many biological active compounds (Inatain *et al*., 1996; Alma *et al*., 2003; Andrade *et al*., 2007; Webster *et al*., 2008). Medicinal plants have been used extensively by a large proportion of the world for their health care and remedy of diseases during the 2000 years and these data have revealed a high degree of correlation between traditional medicinal plants uses and laboratory analysis (Kumar and Roy, 1972; Singh *et al*., 2008). Phytotherapy is based on the use of biological active components contained in plants (Hostettman, 1998; Garza *et al*., 2007). The most interesting area of application for medicinal plant extracts is the inhibition of growth and reduction in numbers of the more serious pathogens (Okolo *et al*., 1995; Kuete *et al*., 2007; Kotzekidou *et al.*, 2008). Recent several studies have been focused on growing interest in plants as a significant source of new pharmaceuticals (Locher *et al.*, 1995; Rabe and Staden, 1997; Rates, 2001).

The 8 traditional medicinal plants used in this study were chosen based on either traditional and ethnobotanical usage suggestive of antibacterial or previous studies that have demonstrated anti-inflammation (Kim *et al.*, 2004), anti-infraction (Ming *et al.*, 2006), antioxidant (Wu *et al.*, 2010), anti-obesity (Miyata *et al.*, 2010), analgesic and anti-fever activity (Cha *et al.*, 1998). However, a little information exists regarding the antimicrobial activity of these medicinal plants. Therefore, the purpose of present study was to screen the antimicrobial activity of 8 different medicinal plants.

MATERIALS AND METHODS

Plant materials and extracts preparation. The medicinal plants were obtained from the Research Institute of Traditional Medicinal Plants of Gyeongnam (Hamyang, the province of Gyeongnam in south-western Korea). The plants obtained from Institute included: *Sedum kamtschaticum* (SK: root or leaf), *Geum japonicum* (GJ), *Geranium sibiricum* (GS), *Saururus chinensis* (SC: root or leaf), *Agrimonia pilosa* (AP: root or leaf), *Houttuynia cordata* (HC:

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Table 1. Plants extract sources and extraction yields of each plants by 80% methanol

Pharmacopeia scientific name	Common name	Korean name	Yield[1]	Plant part used[2]
Sedum kamtschaticum	Stonecrop	Gilin weed	49.46 ± 1.8[a]	R
Sedum kamtschaticum	Stonecrop	Gilin weed	26.65 ± 1.3[c]	L
Geranium sibiricum	Siberian geranium	Illjeel	41.45 ± 1.3[b]	L
Perilla frutescens	Chinese Basil	Deulggae	23.47 ± 2.2[c]	L
Geum japonicum	Geum macrophyllum	BamnnMoo	47.54 ± 1.6[a]	L
Saururus chinensis	Spathium Chinense Lour	Samback glass	40.17 ± 1.1[b]	R
Saururus chinensis	Spathium Chinense Lour	Samback glass	29.22 ± 1.4[c]	L
Agrimonia pilosa	Hairy Agrimony	Sunhaek weed	32.96 ± 1.5[c]	L
Agrimonia pilosa	Hairy Agrimony	Sunhaek weed	9.97 ± 1.2[d]	R
Houttuynia cordata	Chameleon	Uaesung weed	32.02 ± 1.6[c]	L
Houttuynia cordata	Chameleon	Uaesung weed	14.92 ± 2.4[d]	R
Agastache rugosaia	Wrinkled giant hyssop	Baechohang	11.94 ± 2.3[d]	L

[1]: % W/W, dry base.
[2]: Plant parts used are indicated as follows; L-leaf, R-root.
[abcd]: Means ± SD with different superscript in the same column are significantly different ($p < 0.01$).

root or leaf), *Perilla frutescens* (PF), *Agastache rugosa* (AR). The plants with their common names are listed in Table1.

Table 1 also provides a description of the parts of the plants used and extraction yields (%, w/w. dry base) of the plants. Each voucher specimens were deposited in the same Research Institute. The identification of the plants material was conducted entirely in the same Research Institute. The plant materials (leaf and root) were air dried under shade and cut into small pieces and stored at 4°C until use. Each plant materials 300 g were extracted with 80% 900 m*l* methanol in a shaking incubator at 80°C for 12 hr. The residue was re-extracted under the same condition three times. The extracts obtained were pooled and filtered. The combined methanol specimen was evaporated to dryness using a vacuum rotary evaporator and weighted (9.97 to 49.46%; W/W, dry base) to determine the yield of soluble constituents. The extract obtained was subject to evaluate the antimicrobial activity on against fifteen bacteria by preliminary bioassay screening. Results of previous research in our laboratory with various doses of herbal plants were used to determine the levels of herbal plants that used in the experiments reported here. The plant extracts dissolved in 2.5% dimethyl sulfoxide (DMSO), which is maximum volume of DMSO that could be used to dissolve solid extracts, were first dilution to the final concentration (2500 μg/m*l*) for each extract and then serial two fold dilution was made in concentration range 0.6~2500 μg/m*l* in 10 m*l* sterile test tube containing 2.5% DMSO. The solvent DMSO (2.5%) that would not inhibit growth of the microorganisms (Zgoda and Porter, 2001) was used as the negative control for all the experiments.

Microbial strains. The bacterial test strains used in this study were *Salmonella typhimurium* (ATCC14028), *Pseudomonas aeruginoas* (ATCC 9027), *E. coli* (ATCC 31165), *Salmonella enteritidis* (ATCC 4931), *Klebsiella pneumonae* (ATCC 13883), *E. coli O157 :H7* (ATCC 43894), *Enterobacte aerogenes* (ATCC 29010), *Shigella dysenteriae* (ATCC 29026), *Bacillus subtillis* (ATCC 31091), *Bacillus cereus* (ATCC 11778), *Staphylococcus epidermidis* (ATCC 1228), and *Staphylococcus aureus* (ATCC 29213), all of them obtained from the American Type Culture Collection (ATCC) and *Proteus mirabilis* (CDC S 17), *Proteus vulgaris* (CDC 527 C), and *Listeria monocytogenes* (EGD) were kindly provided by Department of Microbiology, College of Veterinary Medicine, Gyeongsang National University, Korea. The bacterial strains were maintained on agar slant at 4°C in the same above- mentioned laboratory where the antimicrobial tests were performed.

Culture media. Nutrient Agar (NA) containing Bromocresol purple was used for the activation of Bacillus species while NA was used for the other bacteria for minimum bactericidal concentration (MBC). Mueller Hinton Agar (MHA) was used for minimum inhibitory concentration (MIC).

Agar dilution method assays: (According to the guidelines of Clinical and Laboratory Standard Institute).

Evaluation of the antimicrobial activity of medicinal plants extracts was conducted according to the agar dilution method with some modification (Pottumarthy *et al.*, 2006). Gentamycin (Sigma) and bacteria-free solvent were used as a positive and negative control, respectively.

Inoculation preparation: At least four well isolated colonies of the same type from a culture agar plate were selected and touched the top of colony with a loop and transferred to a tube containing 4 m*l* of a suitable broth such as tryptic soy broth (TBS). The suspension was incubated at 37°C and the size was adjusted to the 0.5 MacFarland standard turbidity (NCCLS, 1999), approximately 1.5×10^6 CFU/m*l*.

Preparation of antimicrobial plates: The diluted methanol extracts were added to the melted and cooled medium in a ratio of 1 part extract sample agent to 9 parts medium

(2 m*l* of plant extract to 18 m*l* of Mueller Hinton agar for each petri dishes plate) with most susceptibility test. Gentamycin (0.62~5 μg/m*l*) was used as control for the 15 microorganisms assay. The reference antibiotic and its concentration were chosen because they are often employed as first line antibiotic in the respective bacterial infections.

Inoculation of test organisms: Full each well of multiple-inoculator with inoculums test organisms and dip the tip of multiple-inoculator on Mueller Hinton Agar plates and incubate at 37°C for 24 hr. At least three repetitions were run for each assay.

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC): The MIC value of the extract was determined as the lowest concentration that completely inhibited bacterial growth after 48 hr of incubation at 37°C. For the determination of MBC, a portion of liquid (5 μ*l*) from each plates well that exhibited no growth were taken and then incubating 37°C for 24 hr. The lowest concentration that revealed no visible bacterial growth after sub-culturing was taken as MBC. Positive and negative cultures were also prepared.

RESULTS AND DISCUSSION

The methanol extracts from the root or leaf of 8 traditional medicinal plants showed various degrees of the inhibition against 15 bacterial strains using the agar dilution method (Table 2). The antibacterial activity of the methanol extracts tested was found mainly against Gram negative bacteria. In agreement with this finding, Nikaido (1996) reported that the Gram negative bacteria have hydrophilic out membrane owing to the consist of lipopolysaccharide molecular, thus, small hydrophilic molecules pass the outer membrane, on the other hand, this outer membrane have property passing the lipophilic compounds and macromolecules and permeating outer membrane of the microorganisms is prerequisite condition for any solute having antibacterial activity. Thus, despite methanol extracts used in this study are limited solubility in water, it penetrate the outer membrane of Gram negative bacteria and disturbed cellular function, metabolism, and loss of cellular constituents, leading their death. Similar results have also been reported in other previous studies (Rajeshwar *et al.*, 2005; Kuete *et al.*, 2007). In contrast, other reports did not consistent with our results (Rabe and Staden, 1997; Rezende *et al*., 2006). This difference may be due probably to the composition of samples used and the extraction process (water or solvents). Most microorganisms tested, except 5 strains, were found to be susceptive to the methanol extracts with MICs 0.6~ -5000 μg/m*l* and its growth was completely inhibited by the extracts. The *S. aureus* was found to be the most resistant microorganism against methanol extracts and was inhibited by only 7 of 12 extracts tested followed by *S. enteritidis* which were inhibited by 10 of 12 extracts and *P. aeruginosa, S. epidermidis* and *L. monocytogenes* were inhibited by 11 of 12 extracts (Table 2, 3). This result was agreed

Table 2. Antimicrobial activity of the methanol extracts from the leaves or root of 8 medicinal plants

Microorganisms	Methanol extracts												
	SK-R[a]	SK-L	GJ	GS	SC-R	SC-L	AP-L	AP-R	HC-L	HC-R	PF	AR	RA[b]
Gram-negative bacteria													
S. typhimurium	+	+	+	+	+	+	+	+	+	+	+	+	+
E. coli	+	+	+	+	+	+	+	+	+	+	+	+	+
P. aeruginosa	+	+	+	+	+	+	+	+	+	+	+	-	+
S. enteritidis	+	+	+	+	+	+	+	+	-	-	+	+	+
K. pneumonae	+	+	+	+	+	+	+	+	+	+	+	+	+
E. coli-O 157:H7	+	+	+	+	+	+	+	+	+	+	+	+	+
E. aerogenes	+	+	+	+	+	+	+	+	+	+	+	+	+
P. mirabilis	+	+	+	+	+	+	+	+	+	+	+	+	+
S. dysenteriae	+	+	+	+	+	+	+	+	+	+	+	+	+
P. ulgaris	+	+	+	+	+	+	+	+	+	+	+	+	+
Gram-positive bacteria													
B. subtillis	+	+	+	+	+	+	+	+	+	+	+	+	+
S. epidermidis	+	+	+	+	+	+	+	+	+	+	+	-	+
S. aureus	+	+	+	+	+	-	+	-	-	-	+	-	+
L. monocytogenes	+	+	+	+	+	+	+	+	+	+	+	-	+
B. cereus	+	+	+	+	+	+	+	+	+	+	+	+	+

[a]: SK-R; *Sedum kamtschaticum* root, SK-L; *Sedum kamtschaticum* leaf, GJ; *Geum japonicum*, GS; *Geranium sibiricum*, SC-R; *Saururus chinensis* root, SC-L; *Saururus chinensis* leaf, AP-R; *Agrimonia pilosa* root, AP-L; *Agrimonia pilosa* leaf, HC-R; *Houttuynia cordata* root, HC-L; *Houttuynia cordata* leaf, PF; *Perilla frutescens*, AR; *Agastache rugosaia*.
[b]: RA, reference antibiotics (Gentamycin), (-); no inhibition.

Table 3. Minimum inhibition concentration (μg/m*l*) for the methanol extracts from leaves or root of 8 medicinal plants

Microorganisms	Methanol extracts												
	SK-R[a]	SK-L	GJ	GS	SC-R	SC-L	AP-L	AP-R	HC-L	HC-R	PF	AR	RA[b]
Gram-negative bacteria													
S. typhimurium	9.76	19.53	78.12	1.22	156.2	78.12	0.6	1250	4.88	312.5	1250	156.2	0.31
E. coli	9.76	9.76	78.12	9.76	312.5	78.12	0.6	1250	9.76	1250	1250	156.2	0.62
P. aeruginosa	39.0	9.76	2500	0.6	156.2	1250	4.88	2500	9.76	1250	5000	-	0.31
S. enteritidis	78.12	19.53	78.12	625	1250	1250	1250	1250	-	-	1250	1250	1.25
K. pneumoniae	78.12	78.12	78.12	4.88	39	1250	4.88	1250	9.76	312.5	312.5	156.2	0.62
E. coli-O 157:H7	19.53	1250	1250	4.88	156.2	1250	1.22	1250	9.76	1250	78.1	1250	1.25
E. aerogenes	9.76	1250	625	0.6	0.6	1250	0.6	78.12	4.88	4.88	78.1	156.2	1.25
P. mirabilis	4.88	156.2	78.12	0.6	0.6	1250	0.6	9.76	2.44	1.22	1250	1.22	0.62
S. dysenteriae	9.76	1250	625	0.6	0.6	1250	0.6	19.53	1.22	1.22	19.53	0.6	0.62
P. vulgaris	156.2	78.12	78.12	4.88	19.53	1250	9.76	156.2	156.2	9.76	4.88	156.2	0.31
Gram-positive bacteria													
B. subtillis	156.2	9.76	625	4.88	1250	1250	19.53	2500	1250	1250	625	1250	0.62
S. epidermidis	312.5	9.76	1250	9.76	2500	625	78.12	1250	1250	2500	625	-	1.25
S. aureus	1250	78.12	2500	78.1	2500	-	156.2	-	-	-	5000	-	2.5
L. monocytogenes	78.12	156.2	78.12	0.6	0.6	1250	4.88	312.5	19.53	156.2	2500	-	2.5
B. cereus	1250	156.2	156.2	39	78.12	1250	78.12	1250	312.5	312.5	1250	1250	0.62

[a]: SK-R; *Sedum kamtschaticum* root, SK-L; *Sedum kamtschaticum* leaf, GJ; *Geum japonicum*, GS; *Geranium sibiricum*, SC-R; *Saururus chinensis* root, SC-L; *Saururus chinensis* leaf, AP-R; *Agrimonia pilosa* root, AP-L; *Agrimonia pilosa* leaf, HC-R; *Houttuynia cordata* root, HC-L; *Houttuynia cordata* leaf, PF; *Perilla frutescens*, AR; *Agastache rugosaia*.
[b]: RA; reference antibiotics (Gentamycin), (-): no inhibition.

with observation of the Dorman and Deans (2000) and Kotzekidou *et al*. (2008). The other results, However, did not in accord with the reports of Al-Bakri and Afifi (2007), Kuete *et al*. (2007) and Kotzekidou *et al*. (2008). This discrepancy may be also due to the difference of samples used and the extraction process. The antimicrobial spectra of the SK-R, SK-L, GJ, SC-R, AP-L and PF methanol extracts were 100% and GS, SC-L, AP-R, HC-L, HC-R and AR were showed selective activity at 93, 86 and 73% against the tested pathogens, respectively (Table 2). Similar results have also been reported in the other previous studies (Dordevic *et al.*, 2007; Kuete *et al*., 2007, 2008; Kukic *et al.*, 2008; Kotzekidou *et al*., 2008).

The results of Table 3 showed that the MIC values varied from 0.6~5000 μg/m*l*, respectively for the 12 extracts. The lowest MIC values (0.6 μg/m*l*) obtained with GS, SC-R, AP-L, and AR were recorded on the 7 of the 15 microorganisms tested. The MIC values of 0.6 μg/m*l* obtained with extracts GS, SC-R and AP-L against *Enterobacte aerogenes* were 2 fold greater than that of reference antibiotic on the corresponding microorganisms and showed the same MIC values to reference antibiotic against *Proteus mirabilis* and *Shigella dysenteriae*. Also, the MIC values of 0.6 and 1.22 μg/m*l* obtained with extract AP-L exhibited the same potency to reference antibiotic for *E. coli*, and *E. colic*-O157:H7, respectively. Especially, the *Listeria monocytogenes* exhibited 4 times susceptibility for extracts GS and SC-R When compared to reference antibiotic (Table 3).

Minimum bactericidal effects were exhibited with various degrees in all the methanol extracts. These effects were also observed on 15/15 microorganisms for SK-R, SK-L, GJ, GS, SC-R and AP-L. The AR had only eleven (11/15) bactericidal potency. Mims *et al.* (1993) reported that the value of the lowest MBC obtained was not more four times higher than that of MIC's on the corresponding pathogens, It seems possible that the sample tested was possessed the antimicrobial activity. The lowest MBC (1.22 μg/m*l*) was obtained in this study. This value not more than four times greater than that of the MIC's on the corresponding microorganisms (Table 4). This data was supported by the other studies (Meyer and Lall, 2007; Kuete *et al.*, 2007, 2008).

The known antibacterial mechanisms of medicinal plants against microorganisms were inhibit cell wall synthesis (Cowan, 1999; Marcucci *et al.*, 2001), accumulate in bacterial membranes causing energy depletion (Conner, 1993), or interfere the permeability of cell membrane which had a consequence a permeability increase and loss of cellular constitutes, membrane disruption and changes the structure and function of key cellular constituents, resulting in mutation, cell damage, and death (Kim *et al.*, 1995). Lin and Tang (2007) reported that phenolic and flavonoids contents in various fruits and vegetables help immune-modulator organs, killing the microorganisms. Medicinal plants used in the present study were known to possess various phenolic and flavonoids contents (Cha *et al*., 1997; Wu *et al*., 2010). Although, antibacterial mechanisms of medicinal plants

Table 4. Minimum bactericidal concentration (μg/m*l*) for the methanol extracts from leaves or root of 8 medicinal plants

Microorganisms	Methanol extracts												
	SK-R[a]	SK-L	GJ	GS	SC-R	SC-L	AP-L	AP-R	HC-L	HC-R	PF	AR	RA[b]
Gram-negative bacteria													
S. typhimurium	78.1	156.2	625	9.76	2500	625	2.44	5000	78.12	2500	>5000	1250	0.62
E. coli	78.1	78.12	312.5	78.12	2500	625	4.88	5000	156.2	2500	>5000	1250	1.25
P. aeruginosa	156.2	78.12	5000	2.44	1250	5000	39.0	>5000	156.2	5000	nt	nt	0.62
S. enteritidis	312.5	156.2	625	2500	>5000	5000	5000	5000	nt	nt	5000	5000	2.5
K. pneumonae	312.5	625	1250	78.12	625	5000	39	2500	78.12	2500	2500	2500	1.25
E. coli-O 157:H7	156.2	5000	2500	39	1250	>5000	4.88	2500	78.12	5000	625	5000	2.5
E. aerogenes	78.12	5000	2500	1.22	2.44	2500	2.44	1250	39	39	1250	2500	1.25
P. mirabilis	39	1250	312.5	1.22	1.22	5000	1.22	312.5	19.57	9.76	>5000	9.76	1.25
S. dysenteriae	156.2	2500	5000	2.44	1.22	5000	2.44	312.5	4.88	9.76	156.2	9.76	1.25
P. vulgaris	625	625	625	78.12	625	2500	78.1	1250	1250	78.12	39	1250	0.62
Gram-positive bacteria													
B. subtillis	625	78.12	2500	39	5000	2500	625	5000	5000	>5000	500	5000	2.5
S. epidermidis	1250	78.12	5000	156.2	>5000	2500	1250	2500	2500	>5000	500	nt	2.5
S. aureus	2500	1250	>5000	1250	>5000	nt	39	nt	nt	nt	nt	nt	5
L. monocytogenes	625	625	1250	2.44	2.44	5000	19.53	2500	312.5	2500	500	nt	5
B. cereus	2500	625	625	625	1250	5000	625	2500	2500	2500	500	5000	2.5

[a]: SK-R; *Sedum kamtschaticum* root, SK-L; *Sedum kamtschaticum* leaf, GJ; *Geum japonicum*, GS; *Geranium sibiricum*, SC-R; *Saururus chinensis* root, SC-L; *Saururus chinensis* leaf, AP-R; *Agrimonia pilosa* root, AP-L; *Agrimonia pilosa* leaf, HC-R; *Houttuynia cordata* root, HC-L; *Houttuynia cordata* leaf, PF; *Perilla frutescens*, AR; *Agastache rugosaia*.
[b]: RA; reference antibiotics (Gentamycin), nt; not tested because the MIC was not determined.

used in this study against various microorganisms were not fully illustrate, we suggest that one of the mechanisms above mentioned play an important role in their antibacterial activity. From these findings, we suggest that plant extracts used in this study may be become source for discovery of novel antibiotics agent from plant sources. But we also should consider the other reports that determination of MIC values and antibacterial activity were influenced by technical methods in each laboratory and bacterial inherent virulence and susceptibility and the result of *in vitro* should not apply directly to clinical field without *in vivo* study (Nikaido, 1996). Thus, it is important to keep in mind that *in vitro* and *in vivo* research must be conducted to evaluate the biological effects and the application in clinics of using different compounds formulations. Also, It is necessary that we must be consider the methods of plant extract because traditionally plant extract were prepared with water such as poultices, decoction and infusions. The plant extracts, However, used in current study were extracted with methanol.

In conclusion, We found that the methanol extracts from the 8 medicinal plants (leaf or root) had significant antimicrobial activity, Especially, GS, SC-R and AP-L which have the lowest MIC (0.6 μg/m*l*) on 7 microorganisms strains and showed antibacterial activity against all of the 15 microorganisms used in present study and would be interesting source for discovery of novel antibiotics agent from plant sources.

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