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Antimicribial and Antioxidant Activities of Ethanol Extracts of Medicinal Plants

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Abstract

The objective of this study was to determine the radical scavenging activity, total phenolic content, antimicrobial activity, minimum inhibitory concentration (MIC) of ethanol extracts of 32 medical plant species that have been commonly used in medicinal plants. Total phenolic index of *T. chebula* exhibited the highest value (498.01 mg/g), followed by *R. coreanus miquel* (400.33 mg/g), *Sanguisorba officinalis* (368.25 mg/g), *P. thumbergiana* (259.74 mg/g) and *Eugenia aromaticum* (229.38 mg/g). Radical scavenging activity for the DPPH radical was highest in *T. chebula* (40.91%, $p<0.01$), followed by *C. sappan* (36.50%), *S. officinalis* (32.92%), *R. coreanus miquel* (26.54%) and *P. thumbergiana* (24.50%). The extracts from *T. chebula*, *R. coreanus muquel*, *C. sappan*, *E. aromaticum*, *S. officinalis* and *C. japonica* possessed outstanding antimicrobial activity against *Escherichia coli*, *Salmonella* Typhimurium, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Lactobacillus plantarum*. MIC was determined on those extracts that showed high efficacy against the test organisms. The most potent MIC values were seen for *T. chebula* extract against *P. aeruginosa*, *S. aurusa*, *E. coli*, *B. subtilis*, *L. plantarum* and *S.* Typhimurium at 7.8, 7.8, 15.6, 7.8, 125 and 31.2 μg/mL, respectivley. Furthermore, the total phenolic content and radical scavenging activity were very closely correlated for all samples (r=0.78). The coefficient correlations between total phenolic index and antimicrobial activity were 0.91 (*E. coli*), 0.91 (*B. subtillis*), 0.79 (*P. aeruginosa*), 0.79 (*S.* Typhimurium) and 0.70 (*L. plantarum*).

Key words: antimicrobial activity, antioxidant activity, phenolics, plant, extracts

INTRODUCTION

Spoilage and food-borne illnesses caused by microorganisms are problems that have not yet been brought under adequate control despite the range of robust preservation techniques available. Several antimicrobials have been developed over the years to control these microorganisms. However, the development of antimicrobial resistance and the relatively narrow spectrum of the antimicrobials (1) have resulted in limited success and the microbial contamination of food still poses an important public health and economic challenge. Furthermore, due to the economic impacts of spoiled foods and the consumer's concerns over the safety of foods containing synthetic chemicals, a lot of attention has been paid to naturally derived compounds or natural products (2-4). The preservation of wild indigenous plants is vital because such plants are fully adopted to local environments and conditions compared to any introduced species (5), and natural alternatives are therefore needed to achieve sufficiently long shelf-life of foods and a high degree of safety with respect to food-borne pathogenic microorganisms. In nature there are a large number of different types of antimicrobial compounds that play an important role in the natural defense of all kinds of living organisms. Recently, there has been considerable interest in extracts and essential oils from plants with antimicrobial activities for controlling pathogens and/or toxin producing microorganisms in foods (6,7). Plant extracts have been known since antiquity to process notable biological activity, including antioxidant, antibacterial and antifungual properties. Numerous kinds of metabolites have been isolated from various plants and their chemical structures have been elucidated (8).

This study focused on phenolic compounds. Phenolic compounds constitute a large group of secondary plant metabolites that are ubiquitous among higher plants. The main phenolic compounds found in vergetables and plants extracts are derivatives of phenolic acid (9) which are hydroxycarboxylic acids with phenolic hydroxyl group

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and occur in the forms of their esters, ethers or in their free forms. These compounds include some typical low molecular weight phenolic acids in foods as chlorogenic, caffeic and gallic acids. These compounds not only posses antioxidizing, antiviral activities and stimulate the flowering of plants, but also affect activities of some enzymes (10,11). They are phenolic compounds which generally occur as glycosylated derivatives. The aim of this study was to search for new antimicrobial and antioxidant agents. Therefore, we characterized the ethanol extracts of 32 kinds of medicinal plants and measured antioxidative and antimicrobial activities.

MATERIALS AND METHODS

Extraction yield of samples

Thirty-two kinds of dried medical plants (Table 1) were purchased from Kungdong market (Seoul, Korea) and were well ground individually by using a grinder. The samples of medical plants were dried for 48 hrs to about 4% moisture (dry basis) in an air drier operating at 40°C. The samples were extracted with 70% ethanol at 70°C in a Soxhlet apparatus for 6 hrs. The extracts were filtered with filter paper (Whatman No 2), concentrated with a evaporator, and after moderate drying, were completely dried in a freeze drier and stored at -10°C until further use.

Table. 1. Parts used and yields of ethanol extracts in the medicinal plants

Medical herbs	Part used	Yield[1)] (%)
Acanthopanax sessiliflorum	Bark	$5.41 \pm 0.30^{op2)}$
Alpinia oxyphylla	Root	9.51 ± 0.37^{mn}
Amomum globosum loureiro	Root	9.85 ± 0.23^{m}
Angelica gigas	Root	27.36 ± 1.21^{d}
Artemisia asiatica	Leaves	12.79 ± 0.25^{jk}
Astragalus membranaceus	Root	22.23 ± 0.30^{f}
Caesalpinia sappan	Body	5.60 ± 0.28^{op}
Cnidium officinale	Root	27.19 ± 0.18^{d}
Coptis japonica	Root	18.24 ± 0.34^{h}
Cornus officinalis	Seeds	39.26 ± 0.90^{a}
Curcuma longa	Root	8.39 ± 0.11^{n}
Dendrobium nobile	Body	3.52 ± 0.34^{q}
Eugenia aromaticum	Seeds	16.57 ± 0.42^{i}
Evodia officinale	Seeds	20.75 ± 0.78^{g}
Fraxinus rhynchophylla	Fruit skin	10.04 ± 0.06^{m}
Glycyrrhiza glabra	Root	21.56 ± 0.49^{fg}
Glycyrrhiza glabra	Bark	28.76 ± 0.91^{c}
Lindera aggregata	Root	4.49 ± 0.39^{pq}
Liriope plathyphylla	Root	32.53 ± 0.58^{b}
Lycium chinense	Seeds	6.38 ± 0.14^{o}
Myristica fragrans	Root	12.43 ± 0.42^{k}
Paeonia albiflora	Root	13.68 ± 0.40^{j}
Phyllostachys nigra	Leaves	3.63 ± 0.31^{q}
Piper longum	Seeds	5.61 ± 0.37^{op}
Polygonum aviculare	Root	18.68 ± 0.74^{h}
Psoralea corylifolia	Seeds	16.87 ± 0.91^{i}
Pueraria thumbergiana	Root	9.48 ± 0.48^{mn}
Rubus coreanus miquel	Fruit	11.29 ± 0.19^{l}
Sanguisorba officinalis	Root	16.78 ± 0.16^{i}
Schizandra chinensis	Seeds	24.97 ± 1.14^{e}
Shining ganodernia	Body	4.53 ± 0.47^{pq}
Terminalia chebula	Seeds	39.56 ± 0.56^{a}

[1)]Lyophilized weight of sample extract/sample weight.
[2)]Values not sharing same superscript are significantly different in the same column from each other ($p<0.01$).

Determination of total phenolic content using Folin-Ciocalteau method

The Folin-Ciocalteau method was performed as described by Singleton and Rossi Jr (12). An aliquot of 30 μL of the diluted sample solution (10 mg/mL) was mixed with 150 μL of commercial Folin-Ciocalteau reagent and 450 μL of 20% (w/v) sodium carbonate aqueous solution. The final volume was adjusted to 3.0 mL with deionized water. The color intensity generated was measured after about 2 hrs at room temperature at 760 nm using a UV-Vis spectrophotometer from Pharmacia Biotech Ultrospec 2000 connected to a PC using Wavescan software, Tokyo, Japan. A calibration plot of absorbance versus phenolic concentration was made using (+)-catechin as a standard. The polyphenol content in samples was evaluated from the generated absorbance value and results were expressed as (+)-catechin equivalents (mg CE/g of extract powders). The equation of the standard curve was:

$$\text{Total phenolic content} = 12.985 \times \text{O.D} + 0.0721$$

Radical-scavenging activity

The free radical scavenging activity of samples (10 mg/mL) was measured using the modified method of Brand-Williams et al. (13). Two milliliter of a 0.1 mM solution of DPPH (1,1-diphenyl-2-picryl-hydrazyl) in ethanol was prepared, to which was added 0.1 mL of an antioxidant solution in ethanol at different concentrations. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Synthetic antioxidant reagent, L-ascorbic acid, was used in the construction of the standard curve. Estimation of the radical-scavenging activity was carried out in triplicate. The results are expressed as mean values as a percentage of L-ascorbic acid equivalents/10 mg of extract. The standard curve was:

$$\text{Radical-scavenging activity (L-ascorbic acid)} = 15.9457 \times (A_{blank} - A_{sample}) + 0.6716$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compund), and A_{sample} is the absorbance of the test compound.

Antimicrobial activity

The strains of microorganisms employed were the

Gram-positive bacteria *Staphylococcus aureus* (ATCC 12692), *Bacillus subtilis* (ATCC 14593), and *Lactobacillus plantarum* (ATCC 14917), and the Gram-negative bacteria *Escherichia coli* (KCTC 1682), *Salmonella* Typhimurium (ATCC 14028), *Pseudomonas aeruginosa* (ATCC 15522).

The dried plant extracts were dissolved in 1% dimethyl sulfoxide (DMSO) solvent to a final concentration of 100 mg/mL and sterilized through filtration by 0.45 μm Millipore filters. Antimicrobial tests were then carried out by the disc diffusion method (14) using a 100 μL of suspension containing 10^8 CFU/mL of bacteria spread on nutrient agar medium. The 8-mm diameter discs were impregnated with 20 μL of 10 mg/mL extracts (2 mg/disc) and placed on the inoculated agar. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. The inoculated plates were incubated at 37°C for 24 hrs for clinical bacterial strains. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Each assay in this experiment was repeated twice.

Minimum inhibitory concentration (MIC) method

MIC was determined by the micro dilution broth method. The sample extract (1 g) was dissolved in 100 mL of water containing 1% DMSO (10 mg/mL) and serially diluted with sterile nutrient broth medium to obtain the desired concentrations (0, 7.8, 15.6, 31.3, 62.5, 125, 250, 500 and 1,000 μg/20 μL). Each plant extract dilution was inoculated with 20 μL of an individual microorganism present in its log phase. All inoculated dilutions were set at 37°C for 24 hrs. The highest dilution of the plant extract that retained its inhibitory effect resulting in no growth (absence of turbidity) of a microorganism was recorded as the MIC value of the extract. A control experiment was performed in parallel to exam the effect of the solvent itself (without plant components) on the growth of the nine test organisms. DMSO (1%) was diluted in a similar pattern with sterile nutrient broth, as indicated above, and inoculation with microorganisms was performed in the same manner.

Statistical analysis

Data was analyzed using the SAS program. Duncan's multiple range test was performed to verify the significance. Pearson's correlation coefficients were also calculated between the antimicrobial activities and phenolic content and radical-scavenging activity among the 32 kinds of extracts.

RESULTS AND DISCUSSION

Yield of extract

The yields of lyophilized extracts from the 32 medicinal plants are shown in Table 1. From the results, the yield of the following 3 of 32 medicinal plant extracts were above 30%; *Terminalia chebula*, *Cornus officinalis* (39.2639.56%) and *Liriope plathyphylla* (32.56%) ($p < 0.01$). The extract of *C. cassia*, *A. gigas*, *C. officiale*, *S. chinesis* and *A. membranaceus* showed 28.76, 27.36, 27.19, 24.97 and 22.23%, respectively.

The total phenolic content

Total phenolic contents were measured for all the samples (Table 2). The total phenolic contents of plant extracts were analyzed by the Folin-Ciocalteu method and results were expressed as (+)-catechin equivalents (mg CE/g of extract powder). The extract of *T. chebula* showed the highest value (498.01 mg/g), followed by *R. coreanus miquel*, *Sanguisorba officinalis*, *P. thumbergiana* and *Eugenia aromaticum* with 400.33, 368.25, 259.74 and 229.38 CE mg/g, respectively. Therefore, these extracts were used for further antioxidant and antimicrobial studies.

Radical-scavenging activity

The radical scavenging activity was determined by the reduction in the optical absorbance at 517 nm due to scavenging of stable DPPH free radical. The results were expressed as percentage of L-ascorbic acid equivalents/ 10 mg of extract powder. The radical scavenging activitis according to the DPPH radical scavenging assay of all the extracts are shown in Table 2. *T. chebula* extract showed the highest value (40.91%) among 32 extracts ($p < 0.01$), followed by *C. sappan*, *S. officinalis*, *R. coreanus miquel*, and *P. thumbergiana* with the DPPH radical values of 36.50%, 32.92%, 26.54% and 24.50%, respectively. The radical scavenging activities of antioxidants against the DPPH radical are thought to have been due to their hydrogen donation ability. The test samples had numerous phenolic hydroxyl groups in the structure, and phenolic antioxidants have been recognized to function as electron or hydrogen donors (15). Thus, the DPPH radical scavenging activity of these phenolic compounds might be mostly related to their phenolic hydroxyl groups. From these results, the total phenolic content and radical scavenging activity were very closely correlated each other for all sets of samples ($p < 0.01$, $r=0.78$). The key role of phenolic compounds as scavengers of free radicals is emphasized in several reports (16,17). The presence of polyphenols, methoxylated flavonoids in particular, in medical plants was reported elsewhere (18). Moreover, radical-scavenging activity is one of various mechanisms to contribute antioxidant activity.

Antimicrobial activities

The antimicrobial activities of 32 plant extracts aganist

Table 2. Radical-scavenging activity and total phenolic contents of ethanol extracts of medicinal plants

Medical herbs	RSA[1)] (%)	TPC[2)] (mg CE/g)
Acanthopanax sessiliflorum	$10.55 \pm 0.64^{no3)}$	66.60 ± 4.81^{k}
Alpinia oxyphylla	6.43 ± 0.10^{q}	33.68 ± 4.69^{mn}
Amomum globosum loureiro	16.88 ± 0.18^{jk}	137.95 ± 2.90^{g}
Angelica gigas	8.21 ± 0.30^{p}	9.91 ± 0.13^{pq}
Artemisia asiatica	17.95 ± 0.08^{ij}	65.70 ± 6.08^{k}
Astragalus membranaceus	1.94 ± 0.23^{st}	11.81 ± 1.68^{p}
Caesalpinia sappan	36.50 ± 2.12^{b}	195.09 ± 6.95^{f}
Cinnamomum cassia	2.35 ± 0.08^{s}	2.05 ± 1.35^{q}
Cnidium officinale	0.87 ± 0.47^{t}	18.35 ± 2.33^{op}
Coptis japonica	18.81 ± 0.27^{hi}	32.77 ± 3.16^{mn}
Cornus officinalis	6.25 ± 0.35^{q}	25.59 ± 6.24^{no}
Curcuma longa	20.61 ± 0.56^{g}	117.86 ± 3.03^{h}
Dendrobium nobile	19.66 ± 0.49^{gh}	104.85 ± 6.86^{l}
Eugenia aromaticum	18.16 ± 0.48^{ij}	229.38 ± 0.87^{e}
Evodia officinale	24.02 ± 1.39^{ef}	148.25 ± 2.48^{f}
Fraxinus rhynchophylla	20.58 ± 0.59^{g}	48.29 ± 2.42^{l}
Glycyrrhiza glabra	17.60 ± 0.57^{ij}	35.34 ± 6.59^{mn}
Lindera aggregata	22.93 ± 0.11^{f}	129.68 ± 0.45^{g}
Liriope plathyphylla	11.58 ± 0.59^{mn}	17.90 ± 2.97^{op}
Lycium chinense	13.50 ± 0.71^{l}	115.61 ± 6.21^{h}
Myristica fragrans	15.81 ± 0.27^{k}	147.36 ± 3.74^{f}
Paeonia albiflora	3.70 ± 0.71^{r}	79.65 ± 0.49^{j}
Phyllostachys nigra	11.75 ± 0.36^{mn}	69.68 ± 0.45^{k}
Piper longum	0.98 ± 0.31^{t}	28.14 ± 2.63^{n}
Polygonum aviculare	24.50 ± 0.71^{e}	259.74 ± 0.37^{d}
Psoralea corylifolia	19.96 ± 0.06^{gh}	109.92 ± 0.11^{hi}
Pueraria thumbergiana	7.31 ± 0.44^{pq}	113.07 ± 4.14^{hi}
Rubus coreanus miquel	26.54 ± 0.52^{d}	400.33 ± 13.68^{b}
Sanguisorba officinalis	32.92 ± 0.82^{c}	368.25 ± 2.48^{c}
Schizandra chinensis	12.56 ± 0.631^{m}	15.57 ± 6.26^{p}
Shining ganodernia	9.56 ± 0.62^{o}	38.71 ± 1.82^{m}
Terminalia chebula	40.91 ± 1.54^{a}	498.01 ± 2.81^{a}

[1)]The radical-scavenging activity was determined from the standard curve of L-ascorbic acid per g of extract powder.
[2)]The total phenolic content was determined from the standard curve of (+)-catechin mg per 10 mg of extract powder.
[3)]Values not sharing same superscript are significantly different in the same column from each other ($p<0.01$).

Escherichia coli, *Bacillus subtilis*, *Pseudomoas aeruginosa*, *Staphylococcus aureus*, *Salmonella* Typhimurium and *Lactobacillus plantarum* at the concentration of 0.2 mg/mL are shown in Table 3. Of the plants screened for antibacterial activity, fifteen were active. Among them *T. chebula* had the greatest inhibitory activity. It had a very strong inhibitory activity against *S. aureus*, relatively strong inhibitory activities against *B. subtilis* and *P. aeruginosa*, and moderate inhibitory activities against *S.* Typhimurium, *L. plantarum* and *E. coli*. The extract of *C. sappan* had very strong inhibitory activity against *S. aureus*, relatively strong inhibitory activities against *L. plantarum*, and moderate inhibitory activities against *B. subtilis*, *S.* Typhimurium and *P. aeruginosa*. The extract of *R. coreanus muquel* had strong inhibitory activity against *S. aureus*, relative strong inhibitory activity against *P. aeruginoa*, moderate inhibitory activities against *B. subtilis*, *S.* Typhimurium and *E. coli*. The extract of *Sanguisorba officinalis* showed relative strong antimicrobial activitis against *B. subtilis*, *S.* Typhimurium, *P. aeruginoa* and *S. aureus*. Although the plant extracts differed significantly in their activities against the tested microorganisms, most of the extracts showed antimicrobial activities against *B. cereus*, *P. aeruginosa*, *S. aureus*, *E. coli* and *L. plantarum*. From this study we can conclude that the extract of *T. chebula* showed significant inhibitory activity against *B. subtillis*, *P. aeruginosa* and *S. aureus*. It is generally considered that the inhibition of microbial growth by an antioxidant may be due to the free radical scavenging activity of phenolic compounds (10-12). Fig. 1 were showed the correlation between antimicrobial activity and the phenolic content of all samples in the ethanol extracts. From result, as shown in Fig. 2, the coefficient of correlation between phenolic contents and antimicrobial activity against *E. coli*, *B. subtillis*, *P. aeruginosa*, *S.* Typhimurium and *L. plantarum*

Table 3. Antimicrobial effects of ethanol extracts from medicinal plants (n=3)

Medical herbs	Ethanol extracts					
	Gram positive			Gram negative		
	BS	ST	LP	EC	PA	SA
Acanthopanax sessiliflorum	~[1)]	~	~	-[2)]	-	~
Alpinia oxyphylla	-	~	-	-	-	~
Amomum globosum loureiro	~	~	-	-	~	-
Angelica gigas	-	~	-	-	~	-
Artemisia asiatica	-	~	-	-	-	-
Astragalus membranaceus		~	-	-	~	~
Caesalpinia sappan	+[3)]	+	++[4)]	-	+	+++
Cinnamomum cassia	-	-	-	-	-	-
Cnidium officinale	~	~	-	-	~	~
Coptis japonica	+	~	+	-	+	++
Cornus officinalis	~	~	-	-	-	-
Curcuma longa	+	+	+	-	~	~
Dendrobium nobile	-	+	+	-	+	+
Eugenia aromaticum	++	+	-	+	+	++
Evodia officinale	+	~	~	-	++	-
Fraxinus rhynchophylla	-	~	-	-	-	-
Glycyrrhiza glabra	+	~	-	-	+	+
Lindera aggregata	-	-	~	-	~	-
Liriope plathyphylla	~	~	-	-	-	~
Lycium chinense	-	~	-	-	~	-
Myristica fragrans	++	~	-	-	~	~
Paeonia albiflora	+	++	-	+	+	+
Phyllostachys nigra	-	~	-	-	-	~
Piper longum	-	~	-	-	~	~
Polygonum aviculare	+	++	-	-	+	+
Psoralea corylifolia	+	~	-	-	+	+
Pueraria thumbergiana	+	~	-	-	~	~
Rubus coreanus miquel	++	+	-	+	++	++
Sanguisorba officinalis	++	++	-	+	++	++
Schizandra chinensis	-	~	-	~	~	~
Shining ganodernia	-	~	-	-	-	-
Terminalia chebula	+++[5)]	++	++	++	+++	++++[6)]

[1)]Slight antimicrobial activity, inhibition zone (i.z) of sample 1~5 mm>i.z of control.
[2)]No antimicrobial activity.
[3)]Moderate antimicrobial activity, i.z. of sample 5~10 mm>i.z. of control.
[4)]Relatively strong antimicrobial activity, i.z. of sample 10~15 mm>i.z. of control.
[5)]Strong antimicrobial activity, i.z. of sample 15~20 mm>i.z. of control.
[6)]Very strong antimicrobial activity, i.z. of sample>i.z. of 20 mm.
BS=*Bacillus substillis* ATCC 14593, ST=*Salmonella* Typhimurium ATCC 14028, LP=*Lactobacillus plantarum* ATCC 14917, EC=*Escherichia coli* KCTC 1682, PA=*Pseudomonas aeruginosa* ATCC 15522, SA=*Staphylococcus aureus* ATCC 12692.

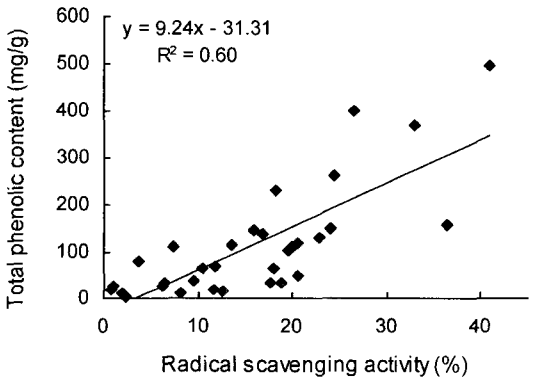

Fig. 1. The correlation between total phenolic content and radical-scavenging activity in the ethanol extracts of plants.

were of 0.91, 0.91, 0.79, 0.79 and 0.70, respectively.

Minimum inhibitory concentration (MIC)

Five out of 32 plant extracts showed an outstanding inhibitory effect against the microorganism with micro dilution broth method: These selected plant extracts were from *T. chebula*, *R. coreanus miquel*, *C. sappan*, *S. officinalis*, *E. aromaticum*, and *C. japonica*. The MIC value of each extract against each microorganism is shown in Table 4. The MIC values of the extracts ranged between 7.8 and 1,000 μg/mL. Results obtained by measurements indicated that the gram negative microorganisms of *P. aeruginosa* and *S. aureus* were the most sensitive microorganism tested, with the lowest MIC

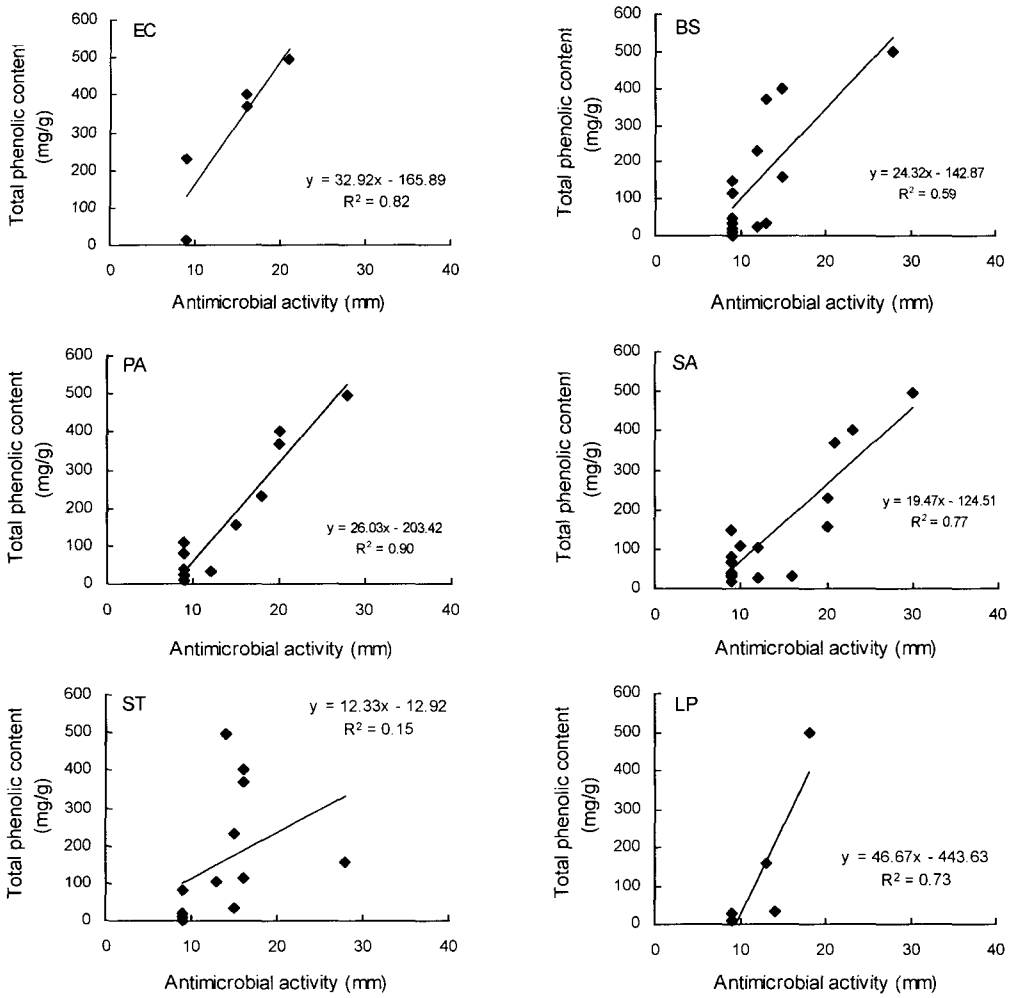

Fig. 2. The correlation between total phenolic content and antimicrobial activity zone in the water extracts. EC=*Escherichia coli* KCTC 1682, BS=*Bacillus substillis* ATCC 14593, PA=*Pseudomonas aeruginosa* ATCC 15522, SA=*Staphylococcus aureus* ATCC 12692, ST=*Salmonella* Typhimurium ATCC 14028, LP=*Lactobacillus plantarum* ATCC 14917.

Table 4. Minnimum inhibitory concentrations (MIC) obtained with micro dilution broth method

Microbial species	Gram positive			Gram negative		
	BS	LP	ST	EC	PA	SA
T. chebula	7.8	125	31.2	15.6	7.8	7.8
R. coreanus miquel	15.6	-	31.2	62.5	31.2	31.2
C. sappan	15.6	15.6	125	-	62.5	15.6
S. officinalis	62.5	-	61.5	62.5	62.5	62.5
E. aromaticum	-	-	61.5	62.5	62.5	31.2
C. japonica	125	250	-	-	125	62.5

BS=*Bacillus substillis* ATCC 14593, LP=*Lactobacillus plantarum* ATCC 14917, ST=*Salmonella* Typhimurium ATCC 14028, EC=*Escherichia coli* KCTC 1682, PA=*Pseudomonas aeruginosa* ATCC 15522, SA=*Staphylococcus aureus* ATCC 12692.

values (7.8 μg/mL) in the presence of ethanol extracts from *T. chebula*. The MIC value of *T. chebula* extract against *E. coli*, *B. subtilis*, *L. plantarum* and *S.* Typhimurium was 15.6, 7.8, 125 and 31.2 μg/mL, respectivley. The value of *R. coreanus miquel* extract against *B. subtillis*, *S.* Typhimurium, *E. coli*, *P. aeruginosa* and *S. aureus*, was 15.6, 31.2, 62.5, 31.2 and 31.2 μg/mL, respectively. These differences could be due to the nature and level of the antimicrobial agents present in the extracts and their mode of action on the different test microorganisms. *T. chebula* extract showed the highest efficacy against 6 kinds of miroorganisms at 20 μL per disk (Table 3). This growth inhibitory activity of the sample extract against different test microorganisms suggests its potential as an antimicrobial agent. Actually, it was shown that the *T. chebula* activity against mi-

croorganisms was mediated by phenolic compounds such as phloroglucinol, pyrogallol, protocaechuic acid, p-hydroxy benzoic acid, catechins, vanillic acid and similar compounds (19-22).

Discussion and conclusions

Medicinal plants have been used by a large proportion of the Orient population. The reasons for this include the improvement of disease condition after herbal treatment, low harmful side effects and relatively low cost of the other forms of treatment. In the present study the results were encouraging as 25 out of 32 plants appeared to contain substances with antimicrobial activitis. Several kinds of plant extracts with high phenolic content, antioxidant activity and consistent phenolic profile were identified and characterized. The high phenolic and antioxidant activities correlated well with antimicrobial activity against *E. coli*, *B. subtillis*, *P. aeruginosa*, *S.* Typhimurium and *L. plantarum*. In general, the extracts of *T. chebula* had highest the phenolic content, antioxidant activity and antimicrobial activity. The main phenolic compound found in vegetables and plants extracts containing phenolic acid as hydroxycarboxylic acids with phenolic hydroxyl group occur in the forms of their esters, ethers or in their free forms. These compounds include some typical low molecular weight phenolic acids as chlorogenic, caffeic and gallic acids. These compounds not only posses antioxidant and aniviral activities and stimulate the flowering of plants function, but also affect activities of some enzymes. They are phenolic compounds which generally occur as glycosylated derivatives. Further work is needed to identify the active principle from the various extracts and their phyto-pharmaceutical properties. It is possible that better therapeutic activities for many microbial diseases can be found in the bark, leaves and the other parts of hitherto neglected plants. These preliminary results appear to indicate that a number of plants have a high potential for antimicrobial activity.

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