Antimicrobial Activity of Korean Medicinal Plants and Herbal Formulations

Lok Ranjan Bhatt, Shin Sun Woo, Byung Soo Yu[1], Seung Hwa Baek*

Department of Herbal Resources, Professional Graduate School of Oriental Medicine, Wonkwang University, 1:Division of Nanobiochemistry, Wonkwang University

In this study, we screened twenty four extracts of eight medicinal plants and three extracts of the commercial product for their antimicrobial and antioxidant properties. Ethanol and ethyl acetate extracts were found active where as aqueous extracts were little or no active. Extracts of *Sophora flavescens*, *Salvia miltiorrhiza* and *Glycyrrhiza uralensis* showed strong activity againsttested organisms and could be the potential antimicrobial agent. The increase of antimicrobial and antioxidant activities of formulations might be due to synergic effect. The results also indicated that the activity of bamboo salt and herbal products can be enhanced by making appropriate formulations.

Key words : Antimicrobial activity, antioxidant activity, bamboo salt, herbal products

Introduction

Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities[1]. Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, much recent attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine[2,3]. Plant based antimicrobials represent a vast untapped source for medicines and therefore further exploration of plant antimicrobials needs to occur. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials[4]. The antimicrobial compounds from plants may inhibit bacterial growth by different mechanisms than those presently used antimicrobials and may have a significant clinical value in the treatment of resistant microbial strains[5].

Traditional Chinese medicine (TCM) prescription is a formula of several single herbs combined at an intrinsic mass ratio. Each herb has its own bioactivities, but when multiple herbs are combined and decocted, there may be chemical changes of active components, resulting in new bioactivities for new clinical indications. In Korea, purple bamboo salt has been used as a folk medicine for the treatment of various ailments. It is processed in traditional furnaces at very high temperature, using common salt and bamboo along with some herbals in specific proportion. It hasbeen reported to be therapeutically effective on diseases like inflammations, viral, diabetes, anti allergic, circulation organ disorders, and cancer[6-9].

In present study antimicrobial activity of eight medicinal plants viz. *Sophora flavescens* Aiton, *Salvia miltiorrhiza* Bunge, *Houttuynia cordata* Thunb, *Ulmus pumila* L., *Glycyrrhiza uralensis* Risch, *Atractylodes macrocephala* Koidz, *Portulaca oleracea* L. and Allium sativum L. that are widely used in traditional Chinesemedicine were evaluated. Although, the plants selected in this study have been widely used in oriental medicine for a long time and their biological activities have been reported previously. Westudied antimicrobial property of individual plants and purple bamboo salt, extracted in three solvent systems (ethanol, ethyl acetate and water) and on the basis of activity of particular plant, made different formulations with purple bamboo salt and compared the activity.

Materials and Methods

1. Preparation of crude extracts

50 g of each dried plant material and 10 g (30 mL of solvents) of herbal formulations and bamboo salt was extracted using ethanol, ethyl acetate and water. Each plant material was macerated in ethanol and ethyl acetate, using 150 mL of each solvent (except *H. cordata*, for which 500 mL of solvent was used for 50 g of sample) for 24 hours at room temperature by continuous stirring. Similarly, 20 g of herbal product was

* To whom correspondence should be addressed at : Seung Hwa Baek, Department of Herbal Resources, Professional Graduate School of Oriental Medicine, Wonkwang University, Iksan 570-749, Korea.
· E-mail : shbaek@wonkwang.ac.kr, · Tel : 063-850-6225
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extracted in 60 mL of each solvent. In all cases, the process was repeated thrice. The extracts were then filtered, usingWhatman No 42 and evaporated under reduced pressure at 35℃. The aqueous extracts were prepared by reflux using same amount of sample and solvent. The reflux was done thrice at 80℃ - 85℃ for 4 hours; suction filtered using Whatman No 42 and vacuum evaporated at 40℃. The samples were then transferred into sterile vials and stored in the refrigerator until use(Table 1).

Table 1. Name, extraction and yield of Korean medicinal plants/herbal products

Plant (part)	Solvent used	Weight (g)	Yield (%)	Code
1.*Sophora flavescens* Aiton (Stem)	Ethanol	3.52	7.04	PT 58-1
	Ethyl acetate	2.61	5.22	PT 58-2
	Water	7.75	15.50	PT 58-3
2.*Salvia miltiorrhiza* Bunge (Root)	Ethanol	2.23	4.46	PT 58-4
	Ethyl acetate	1.01	2.02	PT 58-5
	Water	20.16	40.32	PT 58-6
3.*Houttuynia cordata* Thunb (Leaf)	Ethanol	5.82	11.64	PT 58-7
	Ethyl acetate	3.07	6.14	PT 58-8
	Water	15.07	30.14	PT 58-9
4. *Ulmus pumila* L. (Stem bark)	Ethanol	5.05	10.10	PT 58-10
	Ethyl acetate	3.19	6.38	PT 58-11
	Water	9.06	18.12	PT 58-12
5.*Glycyrrhiza uralensis* Risch (Stem)	Ethanol	4.83	9.66	PT 59-1
	Ethyl acetate	3.31	6.62	PT 59-2
	Water	12.3	24.60	PT 59-3
6.A*ctylodes macrocephala* Koidz (Rhizome)	Ethanol	3.88	7.76	PT 59-4
	Ethyl acetate	2.53	5.06	PT 59-5
	Water	27.62	55.24	PT 59-6
7.*Portulaca oleracea* L. (Whole Plant)	Ethanol	11.19	22.38	PT 59-7
	Ethyl acetate	2.30	4.60	PT 59-8
	Water	18.44	36.88	PT 59-9
8. Jasaenghwan	Ethanol	1.06	5.30	PT 59-10
	Ethyl acetate	0.23	1.15	PT 59-11
	Water	13.38	66.90	PT 59-12
9. *Allium sativum* L. (Bulb)	Ethanol	2.55	5.10	PT 60-1
	Ethyl acetate	0.11	0.22	PT 60-2
	Water	16.73	33.46	PT 60-3

2. Microorganisms and media

The test organisms used in this study were as followed: *Staphylococcus aureus* (KCTC 1927), *Staphylococcus epidermidis* (KCTC 1917), *Streptococcus pyogenes* (KCTC 3090) and *Candida albicans* (KCTC 7965), all Korean culture type collection were obtained from…. Nutrient broth, Brain heart infusion and Sabouraud Dextrose broth were purchased from DEFCO Laboratories, USA. *Staphylococcus aureus* and *Staphylococcus epidermidis* were grown in Nutrient broth, *Streptococcus pyogenes* in Brain Heart Infusion and *Candida albicans* in Sabouraud Dextrose broth.

3. Antimicrobial susceptibility testing

Antimicrobial susceptibility of the plant extracts was evaluated by a microdilution method[10,11]. The organisms to be tested were grown in appropriate media at 37℃. The inocula of microorganisms were prepared from 16h broth cultures and suspensions were adjusted to 0.1 OD on UV Vis spectrophotometer at 625 nm. The plant extracts dissolved in dimethylsulfoxide (DMSO) were first diluted to the highest concentration (4,000 μg/mL) to be tested, and then serial two-fold dilutions were made in a concentration range from 62.5 μg/mL to 4,000 μg/ml. However, the active extracts were assayed up to the concentration of 3.9 μg/mL. The final concentration of DMSO in the assay did not exceed 2.5%. Gentamycin and ketoconazole were used as reference standards. The minimum inhibitory concentration (MIC) was the lowest concentration able to inhibit any visible growth of microorganism.

All the wells in the MIC studies that did not show any turbidity of the bacteria were further incubated for Minimum Bactericidal/Fungicidal Concentration (MBC/MFC). An aliquot of 10 μg/ml suspension from the above mentioned wells was transferred onto culture medium agar plate and incubated at 37℃, for 24The MBC/MFC was the lowest concentration of the sample to kill the microorganisms.

Table 2. Name, extraction and yield of herbal formulations

Formulations	Solvent used	Weight (g)	Yield (%)	Code
[a] 3:3:3:1	Ethanol	0.38	3.8	HF 60-1
	Ethyl acetate	0.11	1.1	HF 60-2
	Water	5.18	51.8	HF 60-3
[b] 4:2:2:2	Ethanol	0.33	3.3	HF 60-4
	Ethyl acetate	0.15	1.5	HF 60-5
	Water	5.31	53.1	HF 60-6
[c] 3:3:3:1	Ethanol	0.65	6.5	HF 65-1
	Ethyl acetate	0.32	3.2	HF 65-2
	Water	2.75	27.5	HF 65-3
[d] 4:2:2:2	Ethanol	0.54	5.4	HF 65-4
	Ethyl acetate	0.22	2.2	HF 65-5
	Water	2.66	26.6	HF 65-6
Bamboo salt	Ethanol	0.06	1.2	HF 65-7
	Ethyl acetate	0.018	0.36	HF 65-8
	Water	3.06	60.6	HF 65-9
Bamboo salt in water	Water	1.0	100	HF 65-10

[a] 3:3:3:1(Jasenghwan: *Sophora flavescens: Glycyrrhiza uralensis: Salvia miltiorrhiza*). [b] 4:2:2:2 (Jasenghwan: *Sophora flavescens: Glycyrrhiza uralensis: Salvia miltiorrhiza*). [c] 3:3:3:1(Bamboo salt: *Sophora flavescens: Glycyrrhiza uralensis: Salvia miltiorrhiza*). [d] 4:2:2:2(Bamboo salt: *Sophora flavescens: Glycyrrhiza uralensis: Salvia miltiorrhiza*).

Results and Discussion

In the present study, twenty four extracts of eight medicinal plants, four extracts of bamboo salt along with its six formulations three extracts of commercial product and its six formulations were evaluated for their antimicrobial properties. Ethanol and ethyl acetate extracts from medicinal plants and herbal formulations were more activewhere as water extracts showed little or no activity at tested concentrations. Among the tested organisms, *Streptococcus pyogenes* was the most susceptible and *Candiia albicans*, the

least. Extracts of *Salvia miltiorrhiza* and *Glycyrrhiza uralensis* showed strong activity against all tested organisms(Table 3).

Table 3. Antimicrobial activity of medicinal plants

Sample	*S. aureus*		*S. epidermidis*		*S. pyogenes*		*C. albicans*	
	MIC (μg/mL)	MBC (μg/mL)	MIC (μg/mL)	MBC (μg/mL)	MIC (μg/mL)	MBC (μg/mL)	MIC (μg/mL)	MFC (μg/mL)
PT 58-1	31.25	62.5	31.25	31.25	15.62	15.62	>4000	>4000
PT 58-2	15.62	15.62	15.62	15.62	7.8-15.62	15.62	>4000	>4000
PT 58-3	1000-2000	>4000	>4000	>4000	>4000	>4000	>4000	>4000
PT 58-4	62.5	500	62.5	250	15.62	31.25	1000	>4000
PT 58-5	62.5	250	62.5	62.5	3.9	7.8-15.62	500	1000
PT 58-6	>4000	>4000	>4000	>4000	>4000	>4000	>4000	>4000
PT 58-7	4000	>4000	2000	>4000	2000-4000	>4000	>4000	>4000
PT 58-8	4000	>4000	2000	>4000	1000-2000	>4000	>4000	>4000
PT 58-9	4000	>4000	2000-4000	>4000	>4000	>4000	4000	>4000
PT 58-10	2000	>4000	>4000	>4000	2000-4000	>4000	>4000	>4000
PT 58-11	>4000	>4000	>4000	>4000	2000-4000	>4000	>4000	>4000
PT 58-12	4000	>4000	>4000	>4000	>4000	>4000	>4000	>4000
PT 59-1	62.5	62.5	31.25	31.25	7.8	15.62	250	250
PT 59-2	15.62	15.62	7.81-15.62	15.62	3.9-7.8	15.62	125-250	250
PT 59-3	4000	>4000	4000	>4000	4000	4000	>4000	>4000
PT 59-4	>4000	>4000	>4000	>4000	62.5	62.5	>4000	>4000
PT 59-5	>4000	>4000	>4000	>4000	31.25	31.25	>4000	>4000
PT 59-6	>4000	>4000	>4000	>4000	>4000	>4000	>4000	>4000
PT 59-7	>4000	>4000	>4000	>4000	31.25	62.5	>4000	>4000
PT 59-8	>4000	>4000	>4000	>4000	31.25	62.5	>4000	>4000
PT 59-9	>4000	>4000	2000-4000	>4000	>4000	>4000	4000	>4000
PT 59-10	>4000	>4000	>4000	>4000	62.5-125	250	>4000	>4000
PT 59-11	>4000	>4000	1000-2000	>4000	62.5	125	500-1000	>4000
PT 59-12	>4000	>4000	>4000	>4000	>4000	>4000	>4000	>4000
PT 60-1	>4000	>4000	>4000	>4000	>4000	>4000	>4000	>4000
PT 60-2	>4000	>4000	>4000	>4000	2000	>4000	>4000	>4000
PT 60-3	>4000	>4000	>4000	>4000	>4000	>4000	>4000	>4000
Gentamicin	0.08	0.08	0.08	0.08-0.156	1.25	1.25-2.5	NT	NT
Ketoconazole	NT	NT	NT	NT	NT	NT	15.62-31.25	62.5-125

NT : Not tested

Salvia miltiorrhiza is one of the most widely used medicinal plants in the clinics either by themselves or in combination with other herbs in TCM. It has been used widely in TCM for the treatment of various kinds of disorders such as coronary artery disease and angina pectoris[12,13]. The major bioactive constituents of *S. miltiorrhiza* are phenolic compounds and abietane-type diterpenes[13,14]. So, higher antimicrobial activity of *Salvia miltiorrhiza* extract might be due to these phenolic components and abietane-type diterpenes. The main constituents of *Glycyrrhiza uralensis* include triterpene saponins[15], flavonoids[16], coumarins[17]. Flavonoids from this plant have been reported to have antimicrobial activity against methicillin sensitive and methicillin resistant *S. aureus*, along with other gram positive and negative bacteria[18].

Extracts of *Sophora flavescens* showed strong activity against all bacterial strains but not active against yeast at tested concentrations. The root of *S. flavescens* is a widely used traditional Chinese herbal drug. Flavonoids, from the roots of this plant exhibited significant antimicrobial activities against the Gram-positive bacteria[19,20] and less active against *C.albicans*[20].

A wide range of microorganisms including bacteria, fungi, protozoa and viruses have been shown to be sensitive to crushed garlic preparations[21]. Garlic extract was reported to exhibit considerable antibacterial and anti-candidal activity[22]. However, in present study none of the garlic extracts showed activity at tested concentration. This might be due to less concentration of the extract or due to the type of garlic extracts used[23]. In earlier studies also activity of essential oil[23] and allicin[24] of garlic extract was found to inhibit bacterial and mould growth at higher concentrations.

Houttuynia cordata one of the important plant in TCM has a wide range of pharmacological activities including antiviral[25,26], antileukemic[27], antioxidative and antimutagenic effects[28].

In the present study the different solvent extracts of the plant showed activity against the microorganisms only at high concentration. Ethyl acetate extract of bamboo salt showed strong activity against *Candida albicans* and was also active against bacterial strains. However, ethanol and aqueous extracts were not active against the microorganisms at tested concentrations. Similarly, ethanol and ethyl acetate extracts of Jasenghwan were active against Streptococcus pyogenes at low concentration but were not active against other organisms.

Polyherbal formulations are frequently prescribed in traditional medicine. In these complex formulations, the plant constituents may not only enhance the activity of compounds or counteract the toxic effect of compounds, from other plants but may also act synergistically with other constituents from the same plants[29]. Considering this fact, formulations of most active plants (*Sophora flavescens,Glycyrrhiza uralensis* and *Salvia miltiorrhiza*) were made with purple bamboo salt and Jasenghwan and their activity was evaluated. Interestingly formulations of both purple bamboo salt and Jasenghwan showed better activity. Formulations of purple bamboo salt, especially ethanol and ethyl acetate extracts showed strong activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* but less active against *C. albicans*(Table 5). Likewise, formulations of Jasenghwan showed strong activity against all tested organisms(Table 4). Ethyl acetate extract of 4:2:2:2 (Jasenghwan: *Sophora flavescens*: *Glycyrrhiza*

uralensis: *Salvia miltiorrhiza*) herbal formulations showed strong activity against all tested organisms. Furthermore, the aqueous extract of 4:2:2:2 formulation of Jasenghwan, the only water extract was active against *Candida albicans* in present study.

Table 4. Antimicrobial activity of herbal formulations

Sample	*Staphylococcus aureus*		*Staphylococcus epidermidis*		*Streptococcus pyogenes*		*Candida albicans*	
	MIC (μg/mL)	MBC (μg/mL)	MIC (μg/mL)	MBC (μg/mL)	MIC (μg/mL)	MBC (μg/mL)	MIC (μg/mL)	MFC (μg/mL)
[a] HF60-1	31.25	62.5	31.25	62.5	31.25	62.5	2000-4000	4000
[a] HF60-2	15.62	31.25	15.62-31.25	31.25	15.62	31.25	2000-4000	>4000
[a] HF60-3	>4000	>4000	>4000	>4000	>4000	>4000	>4000	>4000
[b] HF60-4	62.5-125	500	125-250	500	62.5	125	2000-4000	4000
[b] HF60-5	31.25	125	62.5	125	31.25	31.25	1000	4000
[b] HF60-6	>4000	>4000	>4000	>4000	>4000	>4000	1000-2000	4000
Gentamicin	0.08	0.08	0.08	0.08-0.156	1.25	1.25-2.5	NT	NT
Ketoconazole	NT	NT	NT	NT	NT	NT	5.62-31.25	62.5-125

[a] 3:3:3:1(Jasenghwan: *Sophora flavescens*: *Glycyrrhiza uralensis*: *Salvia miltiorrhiza*). [b] 4:2:2:2 (Jasenghwan : *Sophora flavescens*: *Glycyrrhiza uralensis*: *Salvia miltiorrhiza*). NT: Not tested

Table 5. Antimicrobial activity of Bamboo salt and its formulations

Sample	Staphylococcus aureus		Staphylococcus epidermidis		Candida albicans	
	MIC (μg/mL)	MBC (μg/mL)	MIC (μg/mL)	MBC (μg/mL)	MIC (μg/Ml)	MFC (μg/mL)
[c] HF65-1	125	250	125	250	>4000	>4000
[c] HF65-2	15.62-31.25	250	15.62	62.5-125	4000	4000
[c] HF65-3	4000	>4000	>4000	>4000	>4000	>4000
[d] HF65-4	125	250	125	250-500	4000	>4000
[d] HF65-5	31.25	62.5	15.62-31.25	62.5	4000	>4000
[d] HF65-6	4000	>4000	4000	>4000	>4000	>4000
HF 65-7	>4000	>4000	>4000	>4000	>4000	>4000
HF 65-8	1000	2000	1000	2000	500	500-1000
HF 65-9	>4000	>4000	>4000	>4000	>4000	>4000
HF 65-10	>4000	>4000	>4000	>4000	>4000	>4000
Ketoconazole	NT	NT	NT	NT	15.62-31.25	62.5-125
Gentamicin	0.08	0.08	0.08	0.08-0.156	NT	NT

[c] 3:3:3:1 (Bamboo salt: *Sophora flavescens*: *Glycyrrhiza uralensis*: *Salvia miltiorrhiza*). [d] 4:2:2:2 (Bamboo salt: *Sophora flavescens*: *Glycyrrhiza uralensis*: *Salvia miltiorrhiza*). NT: Not tested

In conclusion, the active antimicrobial components of plants and herbal formulations were present in the soluble components of the ethanol and ethyl acetate extracts. The increase of antimicrobial activity of formulations might be due to synergic effect. The results also indicated that the activity of bamboo salt and herbal products can be enhanced by making appropriate formulations.

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